Associations between dietary factors, lung function and bronchial responsiveness in middle-aged and older Australians

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Submitted in total fulfilment of the requirements of the degree of

Doctor of Philosophy

October 2020

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Dedication

To Fotis and Elena

Thesis Abstract

Studies of relationships between dietary factors, lung function and bronchial responsiveness (BR) are limited and findings inconsistent. My aim was to investigate these relationships in middle-aged and older adults. A secondary aim was to identify a more suitable statistical method to assess factors associated with BR and compare its findings to those from the common regression model of the log-transformed dose-response slope (logDRS).

I used data from two cross-sectional studies – the Tasmanian Longitudinal Health Study (TAHS) 2010 follow-up and the Chronic Obstructive Pulmonary Disease (COPD) Study. The TAHS is a respiratory study of Tasmanian school children born in 1961. In 2010-2012, an asthma and bronchitis enriched subsample completed spirometry, a methacholine challenge and a questionnaire. I used a linear mixed model (LMM) to examine "known" predictors of BR and compared the findings to those from regression of the logDRS. I used multivariable linear regression to investigate associations between fruit and vegetable intakes and lung function and LMMs to examine associations with BR.

The COPD study is a population-based cross-sectional study of adults aged 45-69 years living in inner south-east Melbourne. A random subsample completed spirometry, a methacholine challenge, and questionnaires including a semi-quantitative food frequency questionnaire. I derived dietary patterns from nutrient intakes using principal component analysis and calculated an energy-adjusted dietary inflammatory index (E-DII) as a measure of the inflammatory potential of the diet. I examined associations between these dietary factors and lung function and BR using linear regression and LMMs, respectively.

I explored sex, BMI, smoking, asthma status and atopy as effect modifiers of these associations.

Results from the LMM differed to those from regression of the logDRS. In particular, sex predicted BR in the regression model but not the LMM.

I found relationships between several dietary factors and lung function in those with current asthma only. In this group, higher vegetable intake, higher intakes of a "high potassium & magnesium" dietary pattern, indicating a diet high in fruits, vegetables and wholegrains, and higher intakes of a "low calcium & sugars" dietary pattern, indicating a diet high in vegetables and low in sugar and dairy products, were associated with better lung function. A higher E-DII, indicating a more proinflammatory diet high in animal products and low in fruits and vegetables, was associated with poorer lung function.

I also found higher fruit intake was associated with increased BR. Conversely, in those with current asthma, higher scores for several dietary patterns were associated with less BR.

In conclusion, I demonstrated results from an LMM can differ to those from regression of the logDRS, and recommend using the LMM to investigate factors associated with BR. My findings suggest a diet low in animal products and high in fruit, vegetables and wholegrains may be beneficial for lung function in adults with asthma. Therefore, a dietary modification program in this group may improve lung function and reduce the prevalence and severity of asthma and COPD. However, further studies are needed to establish causality of the diet-lung function associations and clarify relationships with BR.

Declaration

This is to certify that

I. The thesis comprises only my original work towards the PhD, except where indicated in the Preface and Acknowledgements.
II. Due acknowledgement is made in the text to all other materials used.
III. The thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

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Stephanie Byrne

30th October 2020

Preface

The analyses presented in this thesis utilise data collected as part of the Tasmanian Longitudinal Health Study (TAHS) 2010 follow up and the Chronic Obstructive Pulmonary Disease (COPD) study. The TAHS is an ongoing cohort study conducted by researchers within the Allergy and Lung Health Unit at the University of Melbourne. A large multi-disciplinary team work on the study including epidemiologists, respiratory physicians, respiratory scientists, statisticians, project coordinators and research assistants. Professor Shyamali Dharmage is the Principal Investigator of the TAHS. The 2010 follow up was financially supported by grants from the National Health and Medical Research Council (Grant ID APP1021275); Clifford Craig Foundation, Tasmania; Royal Hobart Hospital Research Foundation; and Asthma Australia. The study protocol was approved by the Human Research Ethics Committee at the University of Melbourne.

The COPD study was a cross-sectional study conducted in 2000-2002 by researchers within the Department of Epidemiology and Preventive Medicine at Monash University. Again, there was a large multi-disciplinary team involved. Professor Michael Abramson was the Principal Investigator of the study and remains the custodian of the data. The study was funded by the National Health and Medical Research Council and approval was obtained from the Human Research Ethics Committee at Monash University.

Both the TAHS 2010 follow up and the COPD study were designed, and data collection completed, prior to my involvement. Most of the data cleaning had also been performed. Under the guidance of my PhD supervisors Professor Mark Jenkins, Associate Professor Allison Hodge, Dr John Burgess and Dr Elasma Milanzi, I performed the following activities as part of my doctoral work:

- I performed all literature searches reported in this thesis
- I collated and cleaned the COPD study food frequency questionnaire data, following electronic scanning by Cancer Council Victoria
- I performed data checks and further cleaning where necessary
- I developed the statistical analysis plans for all chapters
- I performed all statistical analyses
- I interpreted all the results
- I drafted all chapters and the manuscript included in this thesis

Chapter 5 includes a manuscript entitled “Measuring bronchial responsiveness using the linear mixed effects model” that has been submitted for review to the European Respiratory Journal. I identified the limitations of the current methods used to analyse data from a provocation challenge; I searched for other methods that have been used in the literature; and I assessed the linear mixed model as a potential method for analysing data from a bronchial provocation challenge. I then

performed the statistical analysis, interpreted the results; drafted the manuscript and edited its contents in response to comments from co-authors; and I submitted the manuscript to the journal for review. The work for this manuscript was performed largely under the guidance of my PhD supervisor Dr Elasma Milanzi. All co-authors reviewed and approved the manuscript.

Acknowledgements

Wow, what a rollercoaster my PhD journey has been! At the outset, I imagined some challenges along the way, but I did not foresee anything like the hurdles that lay ahead. I have learnt much about myself during this journey. It would have been easy to give up at any one of the difficulties I experienced, but I was determined to push on, encouraged by the support around me and motivated by my findings. Hence, there are several people I am extremely grateful for, without whom this work would not have been possible.

First and foremost, I would like to thank my Principal PhD supervisor, Professor Mark Jenkins, for encouraging me to question existing methods and explore my own ideas. Your support has allowed me to grow as a researcher. I would also like to thank all my PhD supervisors for their invaluable encouragement, support and guidance. Specifically, I would like to thank Associate Professor Allison Hodge, for your expert insights into analysing and interpreting research with diet-related exposure variables, and for your prompt and thorough review of my work and timely response to all my questions; Dr John Burgess, for always making the time to discuss all things research, from study samples to biologically plausible mechanisms, and for your expert views as a respiratory epidemiologist; and Dr Elasma Milanzi, who sparked a passion and interest in statistics in me that I never knew was there. Without that, and your help, guidance and support exploring and learning new statistical techniques, I know I would not have made it this far. Thank you!

I would also like to thank my former supervisors, Professor Shyamali Dharmage and Associate Professor Melanie Matheson, for encouraging me to undertake a PhD and for your guidance and support in the early stages. Professor Dharmage generously supported my work financially with a PhD top-up scholarship, for which I am very grateful. Professor Dharmage is also the Principal Investigator on the TAHS study. I thank her for her hard work keeping the study going and for granting me access to the data for this research.

The TAHS is a large, long running cohort study that began many years ago in 1968. I would like to thank the founders and previous TAHS study investigators Heather Gibson, Bryan Gandevia, Harold Silverstone and Norelle Lickiss for their work in the early years of the study's inception. I thank Professor Michael Abramson, the Principal Investigator of the COPD study, for his work on the study and for granting me access to the data. I would also like to acknowledge the investigators, clinicians, respiratory scientists, and research and administrative staff who worked on both the TAHS and COPD studies for their contribution to the study designs and data collection, and the study participants and funding bodies of both studies, without whom this work would not have been possible.

Lastly, I would like to thank my amazing family. Specifically, my parents Nellifer and James, and my brother Chad, for their continual love and support; my husband Fotis, for his love, support and sacrifices he has made to enable me to continue on this journey; and my sister Mandy who is my biggest cheerleader! And last but not least, my beautiful daughter Elena who came along mid-PhD! Elena has brought so much love and cuddles into my life and has helped me keep it all in perspective.

Publications, presentations, and research contributions

Publication submitted for review

Byrne S, Milanzi E, Burgess J, Hodge A, Matheson M, Erbas B, Perret J, Thompson B, Abramson M, Walters E, Dharmage S, Jenkins M. Measuring bronchial responsiveness using the linear mixed effects model. Submitted to the European Respiratory Journal for review.

Published conference abstracts

Byrne S, Milanzi E, Burgess J, Jenkins M, Abramson MJ, Walters EH, Shivappa N, Hebert JR, Hodge A. Associations between the dietary inflammatory index and lung function in middle-age differ by asthma status. *Respirology*. 2018;23(s1): 21–103. https://doi.org/10.1111/resp.13267

Byrne S, Milanzi E, Burgess J, Abramson MJ, Walters EH, Johns D, Jenkins M, Hodge A. Assessing the relationship between diet and lung function in middle-aged Australians using nutrient-based dietary patterns. *Proceedings.* 2018;2(12):573. https://doi.org/10.3390/proceedings2120573

Byrne S, Milanzi E, Burgess J, Abramson MJ, Walters EH, Johns D, Jenkins M, Hodge A. Associations between nutrient-based dietary patterns and lung function in middle-aged and older Australian adults. *Respirology.* 2017;22(s3):56.

Byrne S, Milanzi E, Burgess J, Hodge A, Matheson MC, Erbas B, Perret J, Thompson BR, Abramson MJ, Walters EH, Dharmage S, Jenkins M. Measuring bronchial hyperresponsiveness using a more sophisticated biostatistical method: the linear mixed effects model. *Respirology*. 2017;22(s2):18-100.

Byrne S, Burgess JA, Wood L, Abramson MJ, Erbas B, Johns D, Perret J, Lowe A, Walters EH, Dharmage SC, Matheson MC. Lung function in middle-age is not associated with dietary fat intake: a cross-sectional analysis. *Respirology.* 2016;21(s2):108–115.

Byrne S, Burgess J, Wood L, Abramson M, Erbas B, Perret J, Morrison S, Lodge C, Lowe A, Walters E, Dharmage S, Matheson M. Associations of fruit intake and lung function in middle-age are modified by obesity. *European Respiratory Journal.* 2015;46(s59). doi: 10.1183/13993003.congress-2015.PA5078.

Byrne S, Burgess J, Wood L, Abramson M, Erbas B, Perret J, Morrison S, Lodge C, Lowe A, Walters E, Dharmage S, Matheson M. BMI modifies associations between fruit intake and measures of lung function in middle-age: cross-sectional results from a cohort study. *Respirology.* 2015;20(s2):63–69.

Other conference presentations

"Is consumption of high-fat dairy foods associated with lung function in middle-aged smokers?" Oral Presentation, Nutrition Society of Australia (NSA) Annual Scientific Meeting 2016,

Melbourne, November 2016.

"Intake of high-fat dairy foods is associated with poorer lung function in middle-aged smokers" Oral Presentation, Thoracic Society of Australia and New Zealand (TSANZ) Victorian Branch Annual Scientific Meeting 2016, Melbourne, November 2016.

Other research contributions

Byrne S, Brindal E, Williams G, Anastasiou KM, Tonkin A, Battams S and Riley MD (2018), E-cigarettes, smoking and health. A Literature Review Update. CSIRO, Australia. https://www.csiro.au/~/media/BF/Files/E-cigarettes/E-cigarettes-Consolidated-Final-Report240618-pdf.pdf?la=en&hash=F03466E531949D4A93E61B03FA730F45347A3919

Teaching

Tutor, computer demonstrator and marker – Introduction to Biostatistics, The University of Melbourne

Marker – Epidemiology 1, The University of Melbourne

Computer demonstrator - Survival Analysis and Regression for Rates, The University of Melbourne

Abbreviations used in this thesis

95%CI	95% confidence interval
ACO	Asthma-chronic obstructive pulmonary disease overlap
ACQ	Asthma control questionnaire
AHEI	Alternate healthy eating index
AIHW	Australian Institute of Health and Welfare
aMDS	Alternate mediterranean diet score
ARIC	Atherosclerosis Risk in Communities
ATS	American Thoracic Society
BD	Bronchodilator
BHR	Bronchial hyperresponsiveness
BMI	Body mass index
BOLD	Burden of Obstructive Lung Disease
BR	Bronchial responsiveness
CCV	Cancer Council of Victoria
CG	Control group
CNSLD	Chronic non-specific lung disease
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CT	Computed tomography
CVD	Cardiovascular disease
DAG	Directed acyclic graph

DASH	Dietary Approaches to Stop Hypertension
DHA	docosahexaenoic acid
DII	Dietary Inflammatory Index
DNA	Deoxyribonucleic acid
DPA	Docosapentaenoic acid
DRS	dose-response slope
ECLIPSE	Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints
EDDC	Emergency department data collection
E-DII	Energy-adjusted dietary inflammatory index
EPA	Eicosapentaenoic acid
ERS	European Respiratory Society
$FEV_{0.75}$	Forced expired volume in 0.75 seconds
FEV1	Forced expired volume in one second
FFQ	Food frequency questionnaire
FVC	Forced vital capacity
GA^2 LEN	Global Asthma and Allergy Network of Excellence
GOLD	Global Initiative for Chronic Obstructive Lung Disease
HAO	High antioxidant diet
HEI	Healthy eating index
HI	High intervention
HR	Hazard ratio
HRCT	High resolution computed tomography
HREC	Human research ethics committee

HSC	Higher school certificate
IG	Intervention group
IL	Interleukin
ILD	Interstitial lung disease
IPF	Idiopathic pulmonary fibrosis
LAO	Low antioxidant diet
LI	Low intervention
LMM	Linear mixed effects model
logDRS	log-transformed dose response slope
MDS	Mediterranean diet score
MeSH	Medical subject headings
MUFA	Monounsaturated fatty acids
n-3 PUFA	Omega 3 polyunsaturated fatty acid
n-6 PUFA	Omega 6 polyunsaturated fatty acid
NHANES	National Health and Nutrition Examination Survey
NHMD	National hospital morbidity database
NHS	National Health Survey
OR	Odds ratio
PCA	Principal component analysis
PD_{20}	Provocative dose causing a 20% fall in FEV_1
PUFA	Polyunsaturated fatty acids
RCT	Randomised controlled trial
RE	Retinol equivalents

RR	Relative risk
SD	Standard deviation
SDAC	Survey of Disability, Ageing and Carers
SEM	Standard error of the mean
SES	socio-economic status
SFA	Saturated fatty acids
SFQ	Short fat questionnaire
SPT	Skin prick test
TAHS	Tasmanian Longitudinal Health Study
TEI	Total energy intake
TLco	Transfer factor of the lung for carbon monoxide
TNF	Tumour necrosis factor
VIF	Variance inflation factor
WHO	World Health Organisation

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Chapter 1 – Introduction

Lung function is an objective measure of severity of two highly prevalent and related chronic lung diseases, asthma and chronic obstructive pulmonary disease (COPD). Bronchial responsiveness (BR) is an objective measure of airway sensitivity and hyper-sensitivity is a risk factor for both these diseases. Despite extensive research, the determinants of lung function and bronchial responsiveness are not yet well understood, but if identified, could reveal important causes of respiratory disease.

Over the last 50 years, research has identified several dietary factors that contribute to risk of chronic diseases such as cardiovascular disease and various types of cancer, but the relevance of diet to lung disease is less certain. Firstly, although several studies have observed a relationship between diet and lung function, the findings are inconsistent, possibly due to these relationships varying between population subgroups, or because the associations are modified by other factors. Few studies have assessed relevant effect modifiers. Secondly, research into the relationship between diet and bronchial responsiveness (BR) is sparse. Therefore, the overall aim of my doctoral work is to examine the relationship between dietary factors, lung function and bronchial responsiveness in middle-aged and older adults and explore potential effect modifiers of these relationships.

Chapter 2 provides a detailed description of lung function, its measurement via spirometry testing, and diseases affecting lung function. Asthma and COPD are the most prevalent of these diseases, the other chronic lung conditions are relatively rare. Therefore, asthma and COPD are described in greater depth, with information on trends in prevalence rates; burden on people with the disease, their families, society, and the healthcare system; and mortality. BR is also defined and its measurement via a bronchial provocation challenge is described. Finally, the use of measures obtained from spirometry and provocation tests in epidemiological research is discussed. These measures are obtained through objective clinical tests and are, therefore, appealing as outcomes in research on relationships of diet, lung function and BR.

Chapter 3 explores diet as a potential risk factor for reduced lung function and increased BR and describes potential biological mechanisms that may underlie any relationships. The methods of measuring diet in epidemiological studies are also described, along with their advantages and limitations. Although there are several methods available, a validated semi-quantitative food frequency questionnaire over 12 months is the preferred method of assessing usual dietary intake in large population-based epidemiological studies. Through the comprehensive literature review included in this chapter, an inconsistency in findings for relationships of diet and lung function is noted, as well as the limited research on the relationship between diet and BR. Limited assessment of potential effect modifiers of these relationships is also identified. Effect modification may contribute to the inconsistent results. As previous research has typically focussed on asthma or COPD, there is also a lack of research in middle-aged adults, or subgroups of this population, in whom a definitive

singular diagnosis may be difficult.

Chapter 4 describes in detail the methodology used in my thesis research. Data from two Australian population-based cross-sectional studies are utilised - the 2010 follow-up of the Tasmanian Longitudinal Health Study cohort and the COPD study. The design and data collection of both studies are described, along with an explanation of the confounders included in the analyses and justification of the potential effect modifiers assessed. Several limitations of the statistical methods typically used to examine associations of exposures of interest and BR are identified. Thus, an alternative method is proposed in Chapter 5 using the linear mixed effects model, a well-established statistical method that is suitable for BR data; however, it has not previously been used for this purpose. Results from examination of known predictors of BR using the linear mixed model and the frequently used linear regression of the log-transformed dose-response slope are compared and discussed.

In Chapters 6-8, my investigations of associations between dietary factors, lung function and BR, and potential effect modifiers of these relationships, are presented. The dietary factors examined are fruit and vegetable intakes (Chapter 6), dietary patterns defined using principal component analysis (Chapter 7), and the overall inflammatory potential of the diet estimated using the Dietary Inflammatory Index (Chapter 8).

Chapter 9 contains a summary of the key findings of my doctoral work, along with a discussion of the strengths and limitations of this body of work, and the implications of, and recommendations from, this research in relation to public health and directions of future studies in this area.

Chapter 2 - Background: Lung function and bronchial responsiveness

2.1 Lung function

2.1.1 Anatomy and function of the respiratory system

The respiratory system is a group of bodily organs and tissues that work together to perform ventilation, or breathing, and gas exchange between the air and the blood stream. All the cells in the body require oxygen to generate energy. A biproduct of generating energy is carbon dioxide. The respiratory system transfers oxygen from the air into the blood stream for transport around the body, and removes carbon dioxide from the blood stream, which is then exhaled.

A diagram of the respiratory system is provided in Figure 2.1 below. This diagram details the structure of the lungs and surrounding tissues which will be referred to in upcoming sections of this chapter.

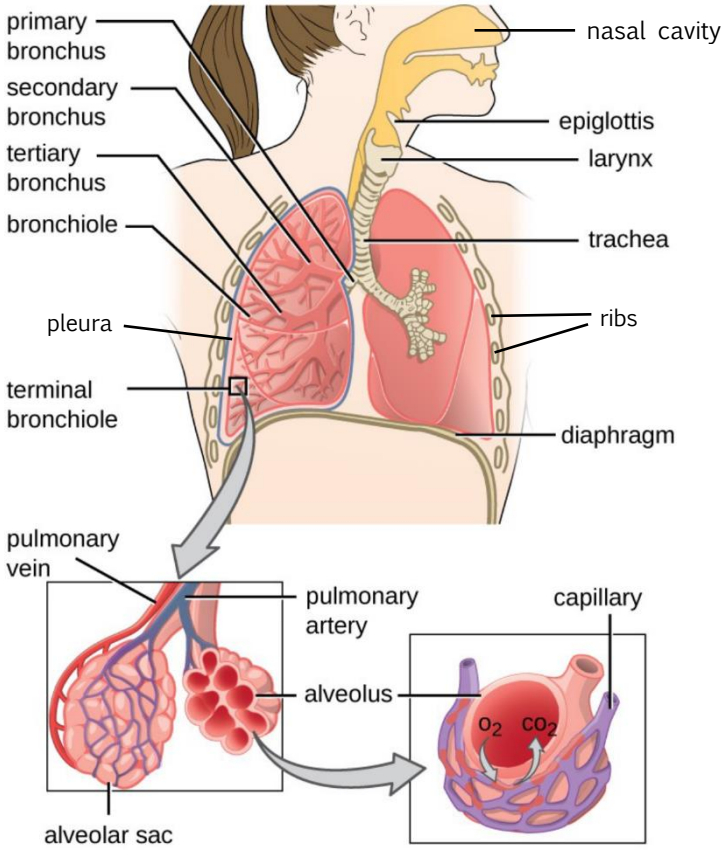


Figure 2.1 - The respiratory system (modified from Lumen Learning (1))

The process of ventilation occurs as follows. The diaphragm contracts and moves downward, drawing air through the nose or mouth, down the trachea and into the lungs, causing the lungs to expand (also known as inhalation). Inside the lungs, the air travels through the bronchial tubes to the

alveoli or air sacs. Oxygen passes through the alveoli walls into the blood in the surrounding capillaries and, at the same time, carbon dioxide passes from the blood into the alveoli. The diaphragm then relaxes, moving upward and forcing the carbon dioxide and remaining gases from the air out of the lungs, back up the trachea and out of the nose or mouth (also known as exhalation).

2.1.2 What is lung function and how is it measured?

The term lung function is used to describe how completely and efficiently the lungs can be filled and emptied with air. It is typically measured via a spirometry test using a device called a spirometer.

During a spirometry test the test subject inhales as completely as possible, seals their mouth around a mouthpiece connected to the spirometer, and forcibly exhales as quickly and completely as possible. The measures obtained include the forced expired volume in one second, or FEV_1, which is the volume of air exhaled in the first second of forced maximal exhalation following maximal inhalation; and the forced vital capacity, or FVC, which is the maximum volume of air exhaled with maximal force (2). The spirometer uses all the data obtained to produce a volume-time curve called a spirogram, shown below (Figure 2.2).

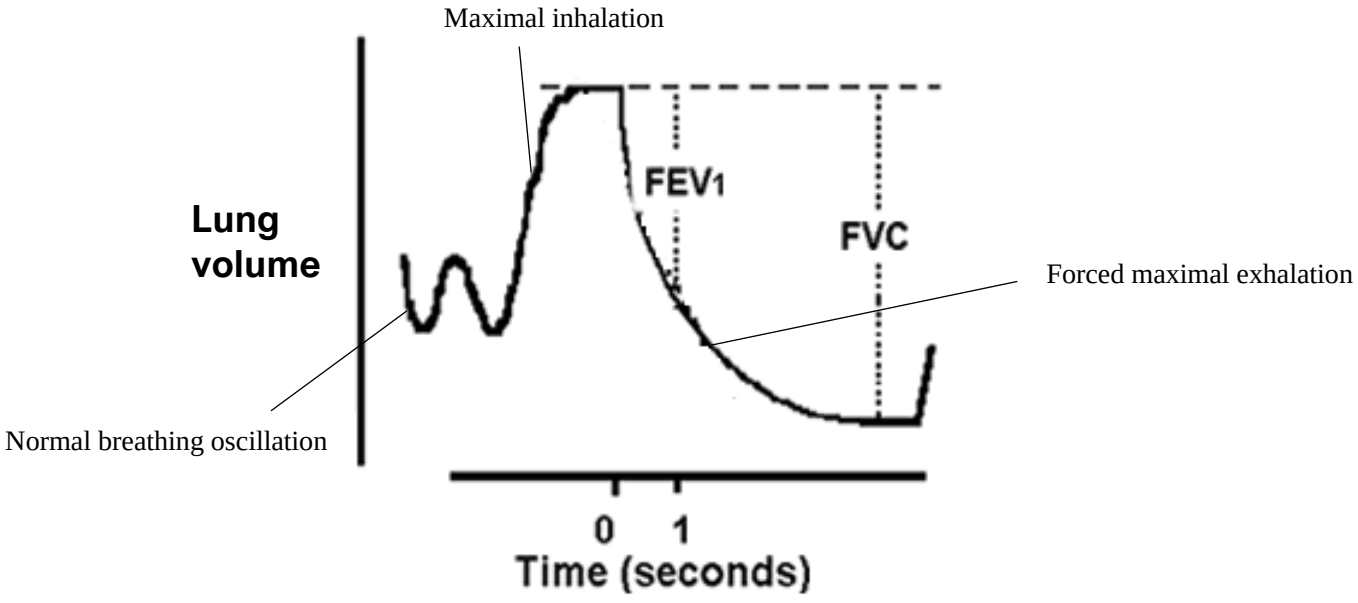


Figure 2.2 - Spirogram produced from spirometry testing (modified from Johns and Pierce (2)).

The FEV_1, FVC and FEV_1/FVC ratio are then compared to predicted values based on the individual's age, sex, height and ethnicity to determine how well the lungs are functioning (2, 3). These predicted values have been determined from a sample of almost 100,000 non-smoking healthy males and females, aged 2-95 years of age, from 33 countries. A range of ethnicities were represented in the sample including Caucasian, African-American, and Asian (3).

2.1.3 Diseases of lung function

There are many diseases affecting lung function. These diseases are collectively categorised as either obstructive or restrictive lung diseases.

2.1.3.1 *Obstructive lung diseases*

Obstructive lung diseases are conditions in which the airway is narrowed, and exhalation of air from the lungs is hindered. Airway obstruction is determined by comparing spirometry test results to predicted values based on an individual's age, sex, height and ethnicity. Lower than predicted FEV_1 and FEV_1/FVC ratio are indicators of airflow obstruction (2).

Obstructive lung diseases include chronic obstructive pulmonary disease (COPD), asthma, bronchiectasis and cystic fibrosis. A brief description of these conditions is given below.

COPD (see 2.1.3.3 for full description) is a chronic respiratory condition defined by airflow limitation that is not fully reversible. Sufferers may also have a persistent cough due to excess mucus production in the airways (also known as chronic bronchitis) or deterioration of lung tissue resulting in enlarged air sacs and further reductions in lung function (also known as emphysema) (4). It is estimated that approximately 15% of Australians aged 40 years and older have COPD, the majority of which are undiagnosed (5). COPD is the fifth leading cause of death in Australia and the third leading cause of death globally (6, 7).

Asthma (see 2.1.3.4 for full description) is a chronic inflammatory condition of the airways which causes recurrent episodes of wheezing, breathlessness and chest tightness. It is defined by variable symptoms and reversible airflow limitation, as demonstrated during a spirometry test. Bronchial provocation testing may also be used when investigating asthma as a cause of respiratory issues. Asthma affects people of all ages and is estimated to affect 339 million people globally and 11% of Australians (8-12).

Bronchiectasis is a chronic respiratory condition characterised by abnormal and irreversible widening of the airways. This in turn leads to pooling of mucus in the airways, chronic infection due to the excess mucus, chronic inflammation, airway obstruction, and further lung damage (13-15). Clinical diagnosis is based on symptoms such as recurrent chest infections, signs of systemic inflammation and lung disease on examination, and a high resolution computed tomography (HRCT) scan demonstrating dilation of the bronchial wall (13). Bronchiectasis can affect people of any age; however, it is more common in the elderly. Females and indigenous peoples are also at greater risk (13). The prevalence of bronchiectasis in Australia is unknown, however it is considered a relatively rare condition. It has been estimated to affect 4.2 per 100,000 U.S. adults aged 18-34 years and 272 per 100,000 elderly (16). In the UK, estimates are higher at 486 per 100,000 in adult men, 566 per

100,000 in adult women, and around 1% in the elderly (17). The prevalence of bronchiectasis in Australia has only been investigated in Aboriginal children in central Australia with a prevalence of 1,470 per 100,000 reported in 2003 (18).

Cystic fibrosis is a serious recessively-inherited genetic condition affecting the secretory glands. It causes the body to produce thick, sticky mucus. In the airways, this thick mucus causes airflow obstruction, chronic infection and inflammation, and lung damage (19-21). Cystic fibrosis also affects the digestive and reproductive systems. Symptoms such as chronic respiratory infections or gastrointestinal issues typically lead to investigative tests. Diagnosis of cystic fibrosis is then confirmed with genetic testing or a sweat chloride test (sufferers usually have abnormally salty sweat) (19, 20). In 2016 there were 3,422 Australians with cystic fibrosis (22). It affects over 70,000 people worldwide (21). The life expectancy of a person with cystic fibrosis in Australia is currently 33 years (22).

Restrictive lung diseases

Restrictive lung diseases involve difficulty, or restriction, fully expanding the lungs upon inhalation. Restrictive lung diseases are also detected by spirometry testing, with a lower than predicted FVC an indicator of lung restriction (2).

Restrictive lung diseases include interstitial lung diseases (e.g. idiopathic pulmonary fibrosis and pneumoconiosis), sarcoidosis, and neuromuscular disease. A brief description of these conditions is given below.

Interstitial lung disease (ILD) is an umbrella term for a diverse group of chronic respiratory diseases characterised by inflammation and fibrosis of the area surrounding the alveoli or air sacs in the lungs, known as the interstitium (23, 24). This accumulation of fibrous tissue makes the lungs stiff causing shortness of breath, also known as dyspnea. ILD is usually progressive and can lead to respiratory failure (23, 24). The cause is often unknown; however, known causes include infection, environmental/occupational exposures, radiation, and medications. ILD can also develop as a consequence of other diseases (25, 26). Diagnosis of ILD is based on symptoms (e.g. dyspnea, dry cough), medical history including exposure to possible causes of ILD, physical examination (crackling sounds may be heard at the base of the lungs on inspiration), spirometry testing (most have reduced lung volume), and HRCT scan (various abnormal patterns may be observed in the lungs such as a honeycomb or mosaic appearance). Bronchoscopy or a lung biopsy may also be required to investigate the cause and thereby identify the type of ILD present (27). ILD is considered a rare condition. It has been estimated to affect 80.9 per 100,000 men and 67.2 per 100,000 women in the United States (28). The prevalence of ILD in Australia is currently unknown.

Idiopathic pulmonary fibrosis (IPF) is the most common form of ILD. The cause of IPF is unknown. Diagnosis is made based on the radiologic pattern observed in the HRCT scan and exclusion of known causes of ILD (23, 29). It typically affects individuals aged 60 years and older and is more common in men and those with a history of smoking. The estimated prevalence of IPF is

2-29 per 100,000 in the general population (23, 29). The prevalence of IPF in the Australian population is not known. IPF has a poor survival rate with a median survival of only 2-3 years following diagnosis (23).

Pneumoconiosis is a form of ILD caused by exposure to mineral or organic dusts or fibres. It includes asbestosis, silicosis, and black lung disease, which are caused by exposure to asbestos, silica dust and coal dust, respectively (24, 30). Pneumoconiosis is very uncommon. Therefore, prevalence is very difficult to determine; however, according to the National Occupational Health and Safety Commission, there were approximately 750 new cases of pneumoconiosis reported in Australia between 2001 and 2003. Whilst this suggests a low incidence of the disease, it is likely that pneumoconiosis is underdiagnosed (30).

Sarcoidosis is a systemic inflammatory disease in which small nodules called sarcoid granulomas develop in various tissues in the body. These granulomas appear in or near lung tissue in 90% of sufferers (31). When this occurs lung function can be affected resulting in dyspnea, cough and oxygen deficiency in the blood (hypoxemia) although many cases are symptom free particularly in young women. Diagnosis of the condition is based on symptoms and microscopic analysis of affected tissue collected by biopsy. Most cases of pulmonary sarcoidosis stabilise or regress without the need for therapy. However, some cases progress, requiring medical intervention, and for those with multiple organ involvement can be fatal (23, 32-34). The estimated prevalence of sarcoidosis is 20-40 per 100,000 of the population. It can occur at any age but is most common between the ages of 20 and 40. African-Americans are at greater risk of developing sarcoidosis compared to those of Caucasian, Asian or Aboriginal decent, suggesting a genetic link (31, 35).

In this thesis I will be looking at lung function in two population-based samples of middle-aged and older Australians. Given the low prevalence of many respiratory diseases, poor lung function will predominantly represent asthma or COPD at various stages. Therefore, I will discuss the prevalence and mortality rates, burden and risk factors of these diseases in more detail.

2.1.3.3 *Chronic obstructive pulmonary disease (COPD)*

COPD is a serious, progressive, chronic respiratory condition. It is a heterogeneous disease with multiple clinical phenotypes. The key characteristic shared by these phenotypes is airflow limitation that is not fully reversible with the use of bronchodilator medication (4, 36, 37). Symptoms may include breathlessness (initially on exertion and eventually at rest), chronic cough and excess mucus production. Wheezing, chest tightness and fatigue are also common. Diagnosis of COPD is confirmed when post-bronchodilator FEV_1/FVC measured by spirometry is less than 0.70, indicating fixed airflow obstruction (38). Well-recognised clinical phenotypes of COPD include chronic bronchitis, which is characterised by a chronic cough with sputum, and emphysema, in which damage to lung tissue occurs resulting in enlarged air sacs and further reductions in lung function (4, 36, 39).

COPD is a significant health issue in Australia and internationally. According to the Global Burden of Disease Study, COPD is estimated to affect 251 million people worldwide (39). The prevalence of COPD in Australia is estimated at 598,800 cases based on self-reported data from the 2017-18 National Health Survey. This equates to 2.5% of the Australian population (10). COPD is typically underdiagnosed, particularly in the early stages. Therefore, the prevalence of COPD in the Australian population is likely to be much higher than this. In 2006-10 the Australian arm of the Burden of Obstructive Lung Disease (BOLD) study tested lung function in a population-based sample of Australians aged 40 years and over. This study estimated the prevalence of COPD to be 7.5% in those aged 40 and over and 30% in those aged 75 and over based on spirometry diagnostic criteria (5). It also demonstrated the large proportion of COPD that is undiagnosed in the Australian community, with almost two thirds of those meeting COPD spirometry diagnostic criteria being undiagnosed in this study (5).

COPD is also a major cause of death in Australia and globally. In 2015, COPD was the cause of 3.17 million deaths which equates to 5% of all deaths worldwide (39). In 2006 the World Health Organisation predicted COPD would become the 3rd leading cause of death globally by 2030 (36, 40). At that time, it was the 5th leading cause of death. By 2016, COPD was already the 3rd leading cause of death globally, according to WHO estimates (7). In the same year, COPD was the 5th leading cause of death in Australia, accounting for 7,212 deaths, corresponding to 4.6% of all deaths (6).

COPD usually progresses into a debilitating condition, reducing quality of life, affecting families and substantially burdening health-care systems. The symptoms of breathlessness, chronic cough and excessive sputum production can make daily activities challenging. As the condition progresses, breathlessness worsens and is experienced at rest, and basic activities such as walking, showering, eating and sleeping become increasingly difficult (4, 39, 41). Exacerbations (acute episodes in which symptoms worsen for a period of days or weeks) can also occur more frequently, increasing the need for hospitalisation, general medical care, and assistance with daily routines. Frequent exacerbations are also associated with an increased risk of death (37, 39).

Given the severity of symptoms, level of disability, and impact on quality of life COPD can bring, it is not surprising that COPD is associated with significant burden for sufferers. In fact, a total burden of disease analysis, which combines the burden of living with a disease and the burden of premature death from the disease, found COPD was the 4th leading cause of total burden of disease in Australia in 2011(6). The 2014-15 National Health Survey, reported that, in participants aged 45 years and over, those with COPD were almost four times as likely to rate their health as poor compared to non-COPD sufferers (4).

COPD is a major cause of hospitalisation and disability in Australia. In 2014-15, COPD was responsible for 66,540 hospitalisations for those aged 45 and over, corresponding to a hospitalisation

rate of 690 per 100,000 in this age group (24). According to data from the New South Wales (NSW) Emergency Department Data Collection (EDDC), 79% of those aged 55 and over who visited a hospital emergency department in NSW for a COPD exacerbation in 2009 were admitted to hospital. Admitted COPD patients had a median length of stay of 5-6 days (42). The 2003 Survey of Disability, Ageing and Carers (SDAC) found 34% of COPD sufferers had some level of disability because of their condition and 12.1% had severe or profound disability, defined as difficulty with core activities including communication, mobility (e.g. getting in and out of bed or a chair), and self-care (e.g. bathing, dressing, eating and going to the toilet) (43). The total direct and indirect costs of COPD in Australia have not been estimated, however, in the United States these costs were estimated to be US$32.1 billion in 2003 by the National Heart, Lung and Blood Institute, US$18.0 billion of which was attributed to direct costs to healthcare services (44).

Comorbidities are also common in COPD due to ageing and risk factors that are common with other diseases. These comorbidities can complicate treatment and contribute to further ill health, with studies showing that hospitalisation for non-respiratory reasons is more common in those with COPD, their hospital stay is longer and they have an increased risk of death (45, 46).

COPD develops over many years and therefore mainly affects middle-aged and older people with prevalence increasing with age (4). Smoking is considered the main modifiable risk factor for COPD in high- and middle-income countries (39). With no cure, and increasing smoking rates and ageing populations observed in a number of countries, prevalence rates and deaths from COPD worldwide have increased in recent years and are predicted to continue to rise (39, 40). Although smoking rates have been declining in Australia since the 1970s, population ageing will likely maintain COPD's position as one of the leading causes of death, disability and burden in the years to come (43, 47).

Three quarters of the total burden of COPD in Australia in 2011 was attributed to smoking, including passive smoking (24). Other known modifiable risk factors include indoor and outdoor air pollution, occupational exposure to dusts and chemical fumes, vapours and irritants, and frequent childhood respiratory infections. Asthma is also a major risk factor for COPD. Use of biomass fuels for cooking and heating is considered the main cause of COPD in low-income countries (24, 39, 48).

The known non-modifiable risk factors for COPD are age and genetic predisposition (4, 46).

2.1.3.4 *Asthma*

Asthma is a chronic inflammatory condition of the airways causing recurrent episodes of wheezing, breathlessness, chest tightness, and cough. In asthma, the airway walls become thickened and inflamed, the muscles around the airways contract, and excess mucus is produced in response to inhaled stimuli, resulting in narrowing of the airways (8, 9, 49). Asthma is characterised by variable airflow obstruction, airway inflammation and airway hyperresponsiveness. Although spirometry testing is recommended in the diagnosis of asthma, the condition is often diagnosed based on

symptoms alone (42, 50). Asthma episodes vary widely in severity and frequency between, and sometimes within, individuals. A sudden or severe flare-up of symptoms is referred to as an asthma attack (9, 49). Asthma is similar to COPD in that it is a heterogeneous disease, principally characterised by airflow limitation. However, with asthma the airflow limitation is reversible, resolving either spontaneously or with the help of bronchodilator medication. Unlike COPD, asthma affects people of all ages (42).

Asthma is a highly prevalent disease, both in Australia and globally. It is estimated to affect 339 million people worldwide, based on 2016 data from the Global Burden of Disease Study (12). Australia has one of the highest asthma prevalence rates in the world, with 2.7 million people (11.2%) suffering from asthma, according to results from the 2017-18 National Health Survey (10). The prevalence of asthma in Australia has increased over the last decade from 9.9% in 2007-08 to 11.2% in 2017-18, with middle-aged and older women having the highest asthma prevalence rates of 14.7%, 14.8% and 15.6% in the 45-54, 55-64 and 65-74 age groups respectively (10).

Whilst asthma can be life threatening in severe cases, death due to asthma is fairly uncommon. According to the Global Burden of Disease Study, there were an estimated 420,000 deaths globally from asthma in 2016 (12). In Australia, asthma was the cause of 419 deaths in 2014, corresponding to a mortality rate of 2 per 100,000 population (24). These deaths could have been prevented with timely medical intervention as the airflow limitation in asthma is reversible with medication. Although asthma can affect people of any age, the majority of deaths from asthma occur in middle-aged and older adults (12). In Australia, over 92% of fatalities from asthma in 2006 were among adults aged 45 years and over (51).

Asthma can greatly impact an individual's physical, mental and social wellbeing. Symptoms can often disrupt sleep; cause daytime fatigue; limit ability to perform exercise, daily activities, or participate in social sport activities; and increase school and work absenteeism (9, 42, 49). According to the 2017-18 National Health Survey, people with asthma are half as likely to rate themselves as having excellent health and are more likely to report having fair or poor health compared to the total population (10). Studies have also shown that asthma sufferers are more likely to have anxiety, depression and higher levels of psychological distress compared to those without asthma (42, 52-54). According to data from the 2007-08 Australian National Health Survey, those with asthma were significantly more likely to suffer from high or very high psychological distress compared those without asthma; men with asthma being 1.6 times more likely and women with asthma being 2.1 times more likely, compared to their asthma free counterparts (42).

Asthma also substantially burdens the community and the healthcare system. In 2015 the total direct and indirect costs of asthma in Australia were estimated to be $28 billion, $24.7 billion of which was attributable to disability and premature death. The direct costs to healthcare services were estimated to be $1.2 billion, a large portion of which was attributable to prescription medication (55, 56).

Hospitalisation for asthma should be rarely required, as symptoms are usually not life-threatening and can be managed at home or with the help of a general practitioner, particularly when appropriate medications are taken as directed and asthma action plans are followed. However, results from an Australian study conducted in 2012 indicate that asthma is not well-controlled in 45% of asthma sufferers aged 16 years and over and most of those whose asthma is not well-controlled were not using medications appropriately (57). The 2017-18 National Health Survey (NHS) found less than a quarter of Australian adults with asthma had a written asthma action plan (10). Hence hospitalisations are not that rare. In 2008-09, twenty percent of asthma expenditure was attributed to hospitalisation (55). According to data from the AIHW National Hospital Morbidity Database (NHMD), asthma was the primary diagnosis for 39,502 hospitalisations in 2014-15, corresponding to an annual hospitalisation rate of 171 per 100,000 population (24). Whilst hospitalisations were less common in adults (98 per 100,000 in those aged 15 and over), 2010-11 data from the AIHW NHMD suggests hospital stays are usually longer for adults compared to children, averaging 2.9 days compared to 1.5 days (55). Asthma is also a common concern discussed in the rooms of general practitioners, with asthma being managed in 2 of every 100 general practice encounters in 2015-16 (58).

There are also indirect costs from asthma through days off work, school and study for asthma sufferers and their carers. According to data from the 2007-08 NHS, 10% of people aged 15 and over with asthma had at least one day off work, school or study in the previous 12 months because of asthma (42). Clearly asthma is a significant health issue in Australia and globally and further research is needed to reduce the burden of asthma.

The causes of asthma are currently not fully understood; however, a number of risk factors have been identified. Genetic predisposition plays a role in asthma development (9, 12). Environmental exposure to inhaled substances and particles can irritate the airways and trigger an asthma episode in those who are susceptible (9, 12). Examples of such substances and particles include:

- tobacco smoke
- indoor allergens and pollutants such as house dust mite, mould, pet dander, heating and cooking fumes, and particles from cosmetics and aerosols
- outdoor allergens and pollutants such as pollens, moulds and traffic exhaust fumes
- exposure to allergens, animals, fine particles and chemical irritants in the workplace

Upper respiratory tract infections, exercise, cold dry air, extreme emotional reactions and certain medications (e.g. aspirin) can also trigger an asthma episode. Allergic and non-allergic rhinitis and obesity have been found to be associated with asthma (8, 9, 12, 37). Indigenous Australians are almost twice as likely to have asthma compared to non-Indigenous Australians, with a prevalence rate of 18% observed among Indigenous Australians in 2012-13 (59). Higher prevalence rates are also observed among lower socioeconomic status populations (42).

2.1.3.5 *Asthma-COPD Overlap (ACO)*

Asthma and COPD can be difficult to distinguish, particularly in middle-aged and older adults who may exhibit a portion of fixed airflow obstruction and a portion of reversible airflow obstruction, as well as a mixture of other characteristics of both asthma and COPD (60-62). Studies looking at asthma or COPD often exclude middle-aged persons, or subgroups of this population, instead recruiting younger people with classical asthma traits for studies on asthma and older people with obvious features of COPD for studies on COPD, in an effort to obtain a study population with a clear diagnosis. However, asthma-COPD overlap (ACO), is increasingly recognised by the respiratory medicine and research communities. Because research studies have often excluded participants with ACO, little is known about the phenotypes within ACO, the underlying mechanisms involved in the development of ACO, and appropriate treatment for ACO sufferers (61). Few studies have even looked at the prevalence of ACO, defined using spirometric criteria, among those with asthma and COPD. A Finnish study conducted by Kauppi and colleagues suggests that approximately 15% of adults with asthma or COPD have ACO, whilst another study conducted in Wellington, New Zealand, found 55% of adults over 50 years of age with COPD have ACO (63, 64). Evidence suggests that those with ACO are at a higher risk of adverse outcomes compared to those with asthma or COPD alone including more frequent exacerbations and hospitalisations, poorer quality of life, increased rate of lung function decline, and greater risk of death (61, 62, 65). Research that includes patients with ACO is urgently needed to fill the gaps in knowledge regarding ACO (and, more broadly, obstructive lung disease), and improve the outcomes experienced by ACO sufferers. Using lung function measures as outcomes in research studies on airflow limitation removes the necessity to allocate a diagnosis of asthma or COPD to participants and enables the inclusion of individuals with ACO. This is the recommendation for research moving forward, particularly in middle-aged and older populations (61).

2.1.4 The use of spirometry testing in epidemiological studies

Spirometry testing is commonly used in epidemiological studies to confirm a diagnosis of asthma or COPD. It may also be used to define eligibility in case-control or intervention studies, or to create a dichotomous outcome variable in population-based studies. Spirometry measures can also be used as continuous outcome measures, the benefits of which are discussed in this section. There are many studies of obstructive lung diseases that do not use spirometry due to the cost and time required, opting instead for self-reported information on respiratory symptoms and doctor diagnoses. The use of respiratory symptoms and doctor diagnosis as outcomes, however, has limitations which are also described here.

2.1.4.1 *Why use spirometry measures as outcome measures*

Spirometry measures are excellent to use as outcomes in epidemiological studies because they are

objective measures of lung function that are associated with adverse health outcomes in COPD, asthma, and in the general population.

Lung function and adverse health outcomes in COPD

In COPD, spirometry measures are used to assess the degree of airflow limitation and, therefore, disease severity. The severity of COPD, as defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD), is based solely on post-bronchodilator FEV_1 cut-off points (Table 1 below) (38).

Table 2.1 - Classification of severity of COPD using post-bronchodilator FEV_1 (38)

Stage of disease	Disease Severity	Post-bronchodilator FEV_1
1	Mild	$FEV_1 \geq 80\%$ predicted
2	Moderate	$50\% \leq FEV_1 < 80\%$ predicted
3	Severe	$30\% \leq FEV_1 < 50\%$ predicted
4	Very severe	$FEV_1 < 30\%$ predicted

Note: At all stages $FEV_1/FVC < 0.70$ (COPD diagnostic criteria)

Increasing severity based on these cut-off points has been shown to be associated with increased risk of future exacerbations, hospitalisation and death (66-68). Investigators for the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) study followed 2138 persons with COPD, recruited from 46 centres in 12 countries. Participants, aged 40-75 years with a history of smoking, were followed for a 3-year period (67, 69). Increasing severity of COPD, as defined by GOLD, was associated with increased risk of frequent exacerbations, with 22% of those with stage 2 COPD, 33% of those with stage 3, and 47% of those with stage 4, having 2 or more exacerbations in the first year of follow up. Severity of disease was also associated with hospitalisation, with 7% of those with stage 2 COPD, 18% of those with stage 3, and 33% of those with stage 4 requiring hospitalisation in the same 1-year period (67).

Another study conducted by Mannino and colleagues investigated if severity of COPD at baseline, defined by a modified version of the GOLD classification using pre-bronchodilator FEV_1 and FEV_1/FVC, predicted mortality. This study utilised data from the Atherosclerosis Risk in Communities (ARIC) cohort study and involved 15,440 participants aged 43-66 years at baseline followed for up to 11 years. After adjusting for potential confounders including age, race, sex, smoking status, pack-years of smoking, BMI, and education level, this study found that as severity of COPD increased, risk of death increased, with stages 1, 2, and 3 or 4 having hazard ratios for death of 1.4 (95%CI 1.1, 1.6), 2.4 (2.0, 2.9) and 5.7 (4.4, 7.3) respectively relative to those with no

respiratory symptoms or evidence of lung disease (68).

Lung function and adverse health outcomes in asthma

Research into the predictors of poor health outcomes in asthma sufferers has demonstrated low FEV_1 is a strong predictor of future risk of adverse outcomes such as future exacerbations, development of fixed airflow limitation (and therefore COPD), and death (70-74). One such study, conducted by Osborne and colleagues in Oregon, USA, aimed to identify risk factors for future acute asthma exacerbations using a cohort of 554 adults with asthma followed up over a 2 ½ year period (70). After adjusting for a range of confounders including age, education, allergies to animals, and history of medical care for asthma, this study found those with an FEV_1 of 60-80% of the predicted value at baseline had a 2.5-fold increased risk of future exacerbations requiring hospital care (95%CI 1.6 – 3.7) compared to those with an FEV_1 greater than 80% of the predicted value. Those with an FEV_1 less than 60% of the predicted value had an even greater 4.6 fold increased risk of requiring hospital care (95%CI 3.2 – 6.8) (70).

Another study, conducted in the Netherlands by Vonk and colleagues, assessed irreversible airway limitation at follow-up in 228 patients with asthma and bronchial hyperresponsiveness (BHR) at baseline (see section 2.2 for a full description of BHR). Patients were 13 to 44 years of age at baseline. Follow-up occurred 21 to 33 years after baseline testing (median follow-up = 26 years) at which time patients were 35 to 74 years old (median age 49.5 years)(72). In those demonstrating airflow limitation at follow-up (n=130), higher pre-bronchodilator percent predicted FEV_1 at baseline was associated with reduced odds of irreversible airflow limitation at follow up (OR (95%CI) = 0.95(0.92-0.98)). This association was observed after adjusting for confounders including age, sex, BHR and reversibility at baseline, and smoking (pack-years) and corticosteroid use at follow-up (72).

Lung function and adverse health outcomes in the general population

A number of studies have also demonstrated that lung function is a predictor of future risk of death in population-based samples (71, 74). One such study, conducted by Hole and colleagues, investigated the relationship between FEV_1 and mortality 15 years later in a large longitudinal study of over 15,000 men and women living in Scotland (71). Participants were 45-64 years of age at the initial assessment. After adjusting for potential confounders including age, smoking, diastolic blood pressure, serum cholesterol levels, BMI and socioeconomic status, this study found that men and women with a percent predicted FEV_1 in the lowest quintile were at increased risk of all-cause mortality (Hazard Ratio (HR) (95%CI) men: 1.9 (1.7-2.2); women: 1.9 (1.6-2.2)), mortality from respiratory diseases (HR (95%CI) men: 9.4 (4.9-18.0); women: 6.5 (3.2-13.2), and mortality from other causes including heart disease, lung cancer and stroke compared to those with an FEV_1 in the highest quintile (71). Lange and colleagues also found lower percent predicted FEV_1 was associated with an increased risk of all-cause mortality, death caused by obstructive lung disease, and death in

which obstructive lung disease was a contributing factor, 10 years later (74). This cohort study involved a sample of 13,756 men and women aged 20 years and over living in Copenhagen. Percent predicted FEV_1 was categorised as greater than or equal to 80; 60-79; 40-59; and less than 40. Risk increased with decreasing categories of FEV_1 for all outcomes (relative to ≥80% FEV_1), after adjusting for relevant confounders including age, smoking, BMI, and bronchial asthma at baseline (74).

2.1.4.2 *Using spirometry measures as outcomes in statistical analyses*

When spirometry testing is performed, the measures obtained are not commonly modelled as continuous outcomes in statistical analyses. They are typically used to create a binary variable (diseased/healthy) or to categorise participants according to level of disease severity using pre-determined cut-off points. When used as continuous variables, spirometry measures are often converted to a percentage of the predicted value for each individual rather than using the raw spirometry values. Using raw spirometry values as continuous outcomes, however, has a number of advantages. Firstly, use of a continuous outcome provides greater power to detect associations because information is not lost by reducing the variable to a small number of categories. Secondly, when including the variables that are used to calculate percentage of predicted values (i.e. age, sex and height) in the statistical model as covariates, using raw spirometry values allows straight forward interpretation of the results. Hence raw spirometry values should be used, provided the sample is large enough to adjust for age, sex, height, and other relevant confounders.

2.2 Bronchial Responsiveness (BR)

2.2.1 What is BR and how is it measured?

The term bronchial responsiveness (BR) describes how sensitive the airways are to inhaled stimuli that can cause bronchoconstriction (i.e. narrowing of the airways) (75). BR is measured via a bronchial provocation challenge. This involves repeated spirometric measures of airway narrowing with gradually increasing doses of a bronchoconstricting stimulus up to a pre-determined maximum dose. Bronchial hyperresponsiveness, or BHR, is diagnosed when the bronchoconstricting stimuli induces a predetermined percentage fall in FEV_1 from baseline during the test. This predetermined value depends on the stimuli used (Table 2.2). A positive result from the bronchial provocation challenge is often expressed as the provocative dose required to produce a fall in FEV_1 that meets the cut-off (PD_{15} or PD_{20}) (61, 75-77).

Table 2.2 - Bronchial provocation challenge – stimuli used, and corresponding cut points applied in adults (61)

Bronchoconstricting stimulus	BHR cut-off point
Methacholine	≥20% fall in FEV_1 from baseline
Histamine	
Hypertonic saline	≥15% fall in FEV_1 from baseline
Mannitol	
Eucapnic voluntary hyperventilation	
Exercise	Fall in FEV_1 of >10% and >200 ml from baseline

Bronchial provocation tests can be classed as direct or indirect challenge tests depending on the mechanism by which the inhaled stimulus produces a reaction. Methacholine and histamine challenge tests are considered direct challenge tests as both agents act directly on the bronchial smooth muscle cells to cause bronchoconstriction. Tests involving the other stimuli listed in Table 2.2 are considered indirect challenge tests as these agents act through an intermediate pathway to induce bronchoconstriction. The methacholine challenge test is the most commonly performed chemical challenge test (75-77).

2.2.2 BHR as a clinical indicator of disease

BHR is a characteristic of asthma. Bronchial provocation challenges are not often carried out as a means of clinical testing. However, they can be used to confirm a diagnosis of asthma, when symptoms are suggestive of asthma and variable airflow limitation is not observed during a spirometry test. Bronchial provocation challenges can also be used to confirm a diagnosis of exercise-induced asthma (61, 77).

2.2.3 The use of bronchial provocation challenge data in epidemiological studies

Bronchial provocation challenges are typically used as part of eligibility criteria in case-control studies of asthma or to define an outcome in cross-sectional studies of asthma. When data from the bronchial provocation challenge is used to define an outcome, the outcome is typically a dichotomous variable classified as BHR if the relevant percentage fall in FEV_1 is achieved during the test, or as a continuous variable in the form of the dose-response slope, often log-transformed due to a strongly skewed distribution.

2.2.3.1 *Why use bronchial provocation challenge data to define the outcome*

As mentioned previously, BHR is considered a characteristic of asthma, however, it has also been shown to be associated with ACO, COPD, accelerated lung function decline and future development of obstructive lung disease (62, 78-81). It may also be seen in apparently normal persons. One study conducted in Switzerland by Brutsche and colleagues, assessed the relationship between BHR in asymptomatic adults and lung function decline and development of asthma and COPD 11 years later. This was a large population-based cohort study involving 4852 participants aged 18-60 years at baseline (79). In those who were asymptomatic at baseline (n=3931), BHR at baseline was associated with a 3-fold increased risk of newly diagnosed asthma (Adjusted OR (95%CI): 3.0 (1.8-5.0)) and a 4-fold increased risk of new COPD determined by spirometry (Adjusted OR (95%CI): 4.0 (2.9-5.6)). Confounders included in the analysis were sex, age, height, education level, BMI and atopy at baseline, and smoking (status and pack-years) and occupational exposures at follow up (79). The magnitude of these associations was quite large indicating BHR is a strong predictor of future asthma and COPD and the association is not likely to be due to residual confounding. This study also found an association between BHR and lung function decline in those who were asymptomatic at baseline and this relationship was modified according to smoking status. After adjusting for confounders, asymptomatic individuals with BHR had a mean decline in FEV_1 12 ml/year (95%CI: 5-18), 11 ml/year (95%CI: 5-16) and 4 ml/year (95%CI: 2-8) greater for current, former and never smokers respectively, compared to asymptomatic individuals without BHR (79). The results from this study demonstrate that BHR is a predictor of future asthma and COPD and is associated with accelerated lung function decline.

Some individuals with asthma, COPD and ACO demonstrate BHR and others do not. This suggests BHR may be a characteristic of certain phenotypes of these diseases. BHR has been shown to be associated with a higher number of certain inflammatory cells in the lungs such as mast cells and eosinophils which have long been associated with early-onset allergic asthma phenotypes and, more recently, late-onset asthma that is less allergic (82-86). BHR has also been associated with less eosinophilic asthma phenotypes, including asthma with high neutrophilia and non-allergic obesity-related asthma (82, 86). BHR is associated with ACO and COPD, both of which are typically less eosinophilic than asthma. However, research into the phenotypes of ACO and COPD is in relatively early stages and, the relationship between BHR and potential phenotypes of these diseases is still unknown (62, 87, 88). Therefore, it is important to assess associations between potential risk factors for obstructive lung disease and BHR as this information may assist in characterising these phenotypes and provide useful information that will help identify at risk individuals or inform such individuals on how to prevent or delay the development or progression of these diseases once the phenotypes of these diseases are fully defined.

2.2.3.2 *Using data from a bronchial provocation challenge in statistical analyses*

There are some significant limitations in the way in which data obtained during a bronchial

provocation challenge are currently utilised. As mentioned previously, the data is typically used to create a dichotomous variable or is converted to a continuous log-transformed dose-response slope. The main limitations of both of these methods are briefly discussed here.

The first major limitation is loss of information. Both methods use only two FEV_1 measurements, however, the majority of individuals tested would have had more than two measurements collected. The second major limitation is an inherent bias in the outcome because of the way in which the outcome is defined. Both outcomes are determined using a percentage fall in FEV_1. However, this means that individuals with a lower baseline FEV_1 require less of an absolute fall in FEV_1 to achieve the same percentage fall in FEV_1 compared to those with a higher baseline FEV_1. These individuals are, therefore, more likely to have a higher dose-response slope and be classified as having BHR. This means those with airflow obstruction, smokers, women, smaller statured people, elderly and children are all more likely to be classified as having BHR simply because of the way in which these measures are defined. Using the dichotomous BHR variable has an additional limitation in that it uses an arbitrary cut-off point, while using the log-transformed dose-response slope also has further limitations including producing regression estimates that cannot be easily interpreted. These and other limitations are discussed in more detail in Chapter 5. Due to these limitations, I have investigated alternative statistical methods for assessing factors associated with BR as part of this PhD thesis, and report a more accurate, reliable method of analysis with fewer limitations in Chapter 5.

2.3 Advantages of using spirometry and BR measures as outcomes over other commonly used measures of obstructive lung disease

The most frequently used outcome measures of obstructive lung disease are created from spirometry measures, self-report of respiratory symptoms, or doctor diagnosed respiratory disease from a questionnaire. The most obvious advantage of using spirometry data or data from a bronchial provocation challenge is that these are objective measures. They are, therefore, not affected by biases that impact self-reported retrospectively collected measures such as recall bias.

There is one significant limitation that is specific to the use of respiratory symptoms - respiratory symptoms are not a good measure of disease severity. This is because symptoms are affected by a number of factors including whether the patient is receiving appropriate medical care, treatment adherence, adequacy of inhaler technique, existence of comorbidities which can complicate treatment, and frequency of exposure to triggers (61, 89). Asthma phenotype also influences the severity of symptoms experienced as some phenotypes are less responsive to current asthma medications and can be poorly controlled despite treatment adherence (85). Studies have also shown that asthma symptoms and lung function measures are not strongly correlated (90-92). Research has demonstrated that lung function measures and BHR are associated with a number of adverse health outcomes and death (discussed previously), suggesting these measures are more suitable outcomes

than respiratory symptoms.

The use of doctor diagnosis of asthma or COPD also has some significant limitations. Firstly, research has demonstrated that a large proportion of COPD in Australia is undiagnosed (5). This will lead to misclassification of the outcome. A portion of this misclassification is likely to be differential where the reason the condition remains undiagnosed is due to individuals not seeking medical advice for their respiratory issues. This is likely to be more common in low SES populations who are also more likely to smoke, be overweight and eat poorly (6). The effect of differential misclassification on the direction and magnitude of the estimate of association is difficult to determine and results should be interpreted with caution.

Asthma and COPD can be difficult to distinguish in middle-aged and older populations, as discussed previously. This will also lead to misclassification of the outcome, a portion of which is also likely to be differential. Without adequate testing, smokers and older adults are more likely to be diagnosed with COPD, and non-smokers and younger adults are more likely to be diagnosed with asthma because of the known risk factors of these diseases. Again, the effects of differential misclassification on the estimates of association are difficult to predict. Therefore, lung function and BR measures should be used in preference to doctor diagnosis where possible, particularly in middle-aged and older population samples.

Objective measures such as those obtained from spirometry testing or a bronchial provocation challenge are also impacted by errors. However, as these measures are obtained using calibrated equipment, any measurement errors are likely to be random and result in non-differential misclassification which can only attenuate the result to the null. Non-differential misclassification may result in not seeing an association when there truly is one or the association observed may be an underestimate of the true strength of the association. Using objective measures is less likely to result in associations being seen where none truly exist due to biases and confounding and should be used in research studies wherever possible.

Chapter 3 - Dietary factors as risk factors for poor lung function and lung disease

3.1 Why examine dietary factors as risk factors for poor lung function?

Previous investigations into risk factors for poor lung function and obstructive lung disease have primarily focused on asthma or COPD. Therefore, the origins of the interest in diet as a potential risk factor for poor lung function will be discussed with regards to these conditions.

In the latter half of the 20th century a marked increase in the prevalence of asthma was observed, firstly in developed countries and then in developing countries (42, 93, 94). Given such a rapid change in the prevalence of a disease cannot occur because of genetic factors alone, environmental factors are likely to have played a role. Industrialisation of the food supply occurred in the western world in the decades prior to this increase in asthma prevalence. During this time dietary intakes transitioned from a diet high in nutrient dense foods such as fruits, vegetables, wholegrains and fish, to a diet high in processed foods, which are generally high in fat, salt and sugar, and have less nutritional value (95, 96).

In the decades prior to these parallel changes in diet and asthma prevalence being recognised, the significant role of dietary factors in the development and progression of other chronic diseases such as hypertension, type 2 diabetes, cardiovascular disease and cancer was identified (97-103). Dietary advice is now a key component in the treatment and prevention of these diseases with some studies finding that changes in diet can be more effective than medication on clinical endpoints and fatalities. For example, a large RCT of 3234 non-diabetic adults, conducted by the Diabetes Prevention Program Research Group, found a lifestyle modification program, including encouragement of a healthy, low-energy, low fat diet, provided 39% greater protection against the development of diabetes than medication (metformin). The incidence of diabetes was 58% lower in the lifestyle intervention group compared to the placebo group, and 31% lower in the metformin group compared to the placebo group (98).

From the ecological evidence of the observed parallel changes in diet and asthma prevalence, and the discovery of pivotal roles of diet in the development and progression of other chronic diseases, the theory of diet as a potential risk factor for asthma was born (95). This theory was supported by the known biological functions of many dietary factors, giving rise to plausible hypotheses on the mechanisms through which diet may be involved in the development and progression of poor lung function and obstructive lung diseases.

The most commonly stated potential mechanisms are:

1. Decreased intakes of antioxidants have reduced the body's ability to neutralise cell-damaging free radicals in respiratory tissues caused by factors such as smoking, pollution, respiratory illness and ageing, leading to an increase in oxidative damage and reduced lung function (104-107);
2. Inflammation of the airways is a characteristic of obstructive lung disease and, in turn, poor lung function. Reduced intakes of oily fish and increased intakes of vegetable oils have changed the ratio of omega 6 to omega 3 PUFAs obtained from the diet. This change has led to an increase in the production of pro-inflammatory precursors and a decrease in anti-inflammatory precursors in the body thereby contributing to an increase in inflammation and a decrease in lung function (106-108).

Two more recent theories are also gaining traction:

1. Many components in the diet have been shown to be associated with inflammation. Therefore, the overall inflammatory potential of the diet, rather than solely the ratio of n-6:n-3 PUFAs, may contribute to increased inflammation of the airways leading to an increase in the risk of obstructive lung disease and a decline in lung function (109-112).
2. There has been increased interest in the role of the gut microbiota in the development and maintenance of the immune system. Gut bacteria are influenced by the food we consume and a diet high in fibre from natural sources such as fruit, vegetables and wholegrains is thought to create a healthy, diverse population of bacteria in the gut. However, changes in dietary patterns have led to a decrease in fibre intake resulting in adverse changes to the gut microbiota, impairing the immune system, reducing the body's ability to fight oxidative damage in the lungs, and consequently causing lung function impairment (113-116).

The interest in diet as a potential risk factor for COPD developed from the fact that not all cases of COPD can be explained by exposure to currently known risk factors. Smoking is considered to be by far the strongest risk factor for COPD in developed countries; however, an estimated 50% of smokers do not develop COPD, while a smaller proportion of lifelong non-smokers do (117-121). Therefore, there must be other risk factors at play, some of which are currently unknown. Diet is one such potential risk factor of interest because of the known biological mechanisms through which diet may affect lung function. The strongest and most recent mechanistic hypotheses are the same as those for asthma, described briefly above.

The identification of dietary factors that protect against lung function decline and slow the progression of obstructive lung diseases offers several benefits over medication. Firstly, if dietary recommendations originating from such research are in line with current dietary recommendations, then encouraging such a diet may assist with weight management, improve overall health and protect against a range of other chronic diseases. Diet modification generally has no serious side effects unlike some medications, and there is relatively lower risk of any contraindications than with

medications. Dietary changes may also help those who experience little relief from medication or who have trouble adhering to treatment for various reasons (e.g. difficulty with inhaler technique, forgetting to take medication, or choosing not to take medication because of side-effects or to cut down on use of medicines). These advantages provide further motivation to continue research investigating the relationship between diet, lung function and lung disease.

In this chapter I will describe the currently available methods of measuring diet, followed by the research to date on the relationship between diet and lung function. Studies of the highest quality will be discussed in detail. Studies of asthma and COPD and related endpoints will also be discussed briefly, particularly where research on the dietary factor of interest and lung function is lacking. Gaps in the current knowledge will be identified and considered.

3.2 Methods of measuring diet in epidemiological studies

The dietary measurement methods that are commonly used in research are food records, 24-hour diet recalls, dietary histories and food frequency questionnaires. Short questionnaires are sometimes used in large epidemiological studies where a particular dietary factor of interest and several other potential environmental or lifestyle risk factors are being investigated. Biological samples can also be used to assess intake of some nutrients (122, 123). All currently available methods of measuring diet have their advantages, limitations, and sources of measurement error. All of these aspects should be considered when selecting a method or tool, analysing data and interpreting results (122, 123). The most suitable method also depends on the aim of the research and the level of accuracy required. The common methods of measuring diet are described below, along with the advantages and limitations of each method (summarised in Table 3.1).

Food records – Participants are asked to record the details of all foods and beverages consumed over a specified period (i.e. brand, type e.g., full-fat, low-fat, or skim milk, etc), the cooking methods used to prepare the foods, and the quantities consumed as they are eaten. There are two types of food records, weighed and estimated. For weighed food records, participants are asked to weigh and record the quantities of the foods consumed. For estimated food records, participants estimate the quantities of the food consumed in household measures (e.g. cups, tablespoons, etc) (122-124). Weighed food records are considered the most accurate method of dietary measurement. However, they are subject to error, predominantly due to participants altering their diet to appear healthier or to make the weighing process easier, particularly when recording diet over a number of days. Food records are also expensive to carry out and are inconvenient and time consuming for participants and researchers. To estimate usual dietary intake with enough accuracy to rank individuals or use individual intakes in a regression analysis, several measurement days are required, selected and spaced to capture day-to-day and seasonal variation in the diet (122-124).

24-hour dietary recalls – Participants are asked to recall all the foods and beverages consumed in

the last 24 hours or on the previous day during an interview. Participants estimate the quantity of each food or beverage consumed in household measures with the aid of food models, images and/or photographs (122-124). The advantages of dietary recalls are lower participant burden relative to food records, particularly if only 1-2 days of recalls are conducted per participant, and participants are unlikely to modify their diet as the data is collected after the food is consumed. The disadvantages of this method include that multiple days of recalls are required to capture usual dietary intake, participants may not accurately recall everything they consumed (recall error), and this method, whilst less expensive than weighed food records, is still costly (122-124).

Dietary history – Participants complete an interview on their usual eating pattern over a specified time period during which detailed information on the foods consumed and usual portion sizes are collected. Participants then complete a food frequency questionnaire (FFQ) and the information collected from the FFQ and the interview are cross-checked to verify and clarify the data. This method requires skilled interviewers and is quite labour-intensive and, therefore, costly (122-124).

Food frequency questionnaires (FFQs) – Participants complete a questionnaire about the frequency of consumption of selected foods over a specified time period. Pre-defined response categories are given, ranging in number and time span covered depending on the tool used and the objectives of the study. Semi-quantitative FFQs also ask participants about usual portion sizes with the use of images or photographs to assist their estimation (122-124). FFQs are the most frequently used dietary measurement tool for epidemiological research because they are inexpensive, they collect data on usual intake, can be self-administered and optically scanned for automated data entry, and participant burden is relatively low. The limitations of this method include the lack of detail collected on foods, and the reduced variation in the intakes due to the use of pre-defined response options (122-124). Data from comprehensive semi-quantitative FFQs can be used to estimate food and nutrient intakes and identify dietary patterns. Depending on the tool used, this method is generally considered accurate enough to rank participants according to food and nutrient intakes (122, 123).

Short questionnaires - Short questionnaires measure the intake of particular foods, food groups or nutrients of interest. These questionnaires are quick and participant burden is low which increases response rates. Short questionnaires can be accurate enough, depending on their purpose, if they are designed appropriately. However, energy intake cannot be estimated from these questionnaires and, therefore, cannot be adjusted for during statistical analysis (122, 123).

Biological indicators – Some biomarkers have a strong direct relationship with certain nutrient intakes. The intake of these nutrients can therefore be estimated from biological samples. For example, recent intakes of protein, sodium, and potassium can be determined from 24-hour urine samples. Other biomarkers have demonstrated a correlation between biomarker concentration and intake and can therefore be used to rank individuals based on their intake of the nutrient of interest.

Examples of nutrients related to such biomarkers, and the biological sample containing the biomarker, include iodine and polyphenols from urine; fatty acids, vitamin C, carotenoids and some B vitamins from serum or plasma; selenium, fatty acids and some B vitamins from erythrocytes; selenium from hair and nails; and fatty acids from adipose tissue (122, 123). Generally, only essential fatty acids such as n-3 and n-6 polyunsaturated fatty acids can be assessed using biomarkers as these fatty acids cannot be synthesised by the body. Urine, serum and plasma nutrient levels reflect very recent intake and should not be used as an indicator of usual intake unless intake of that particular nutrient is fairly stable with little day-to-day variation (122, 123). Because of the life cycle length of erythrocytes, biomarkers in these cells reflect intake of nutrients over the past few months. Biomarkers in hair, nails and adipose tissue reflect intake over recent months or years (122, 123). These factors should be considered when selecting biological markers of nutrient intake. Although standard protocols are typically used, errors can occur during sample collection, transport, storage and laboratory analysis, which may introduce bias into the results. These potential sources of error should be considered when interpreting the results (122, 123).

Table 3.1 - Advantages and limitations of the methods of measuring diet

Dietary Measurement Method	Advantages	Limitations
Weighed food record	Most accurate method of measuring short-term dietary intake Does not rely on memory	Multiple non-consecutive days of food records needed to capture usual diet Expensive High level of participant burden Participants may change diet to appear healthier or to simplify weighing
Estimated food record	High level of accuracy Does not rely on memory	Multiple non-consecutive days of food records needed to capture usual diet Expensive High level of participant burden Participants may change diet to appear healthier Accuracy depends on participants ability to estimate quantities
24-hour diet recall	Moderate to high level of accuracy (depending on food) Low participant burden if few recalls conducted per participant Participants are unlikely to modify diet	Multiple non-consecutive days of diet recalls needed to capture usual diet Data collected retrospectively and is, therefore, subject to recall error Expensive Moderate participant burden if many recalls conducted per participant Participants' estimation of quantities is a source of error Subject to interviewer bias
Dietary history	Used to measure usual diet Data can be used to rank individuals according to intake or in correlation or regression analysis Low participant burden	Data collected retrospectively and is therefore subject to recall error Expensive Labour-intensive Participants' estimation and averaging of portion sizes is a source of error Subject to interviewer bias

Food frequency questionnaire	Used to measure usual diet Data from semi-quantitative food frequency questionnaire can be used to rank individuals according to food or nutrient intake, in correlation or regression analysis, or to analyse dietary patterns Low participant burden Can be self-administered (∴ no interviewer bias) Can be optically scanned or completed online (minimises data entry errors)	Data collected retrospectively and is therefore subject to recall error Limited response options reduce variability in the data Participants' estimation and averaging of portion sizes over a long period is a source of error Collected information limited to the foods listed
Biochemical indicators	Low participant burden Does not rely on self-reported information by participants	Can only estimate intake or rank the intake of individuals for a small number of specific nutrients Errors that can occur during sample collection, transport, storage, and laboratory analysis are a potential source of bias. Variation occurs due to differences in absorption, transport, metabolism, excretion. These factors are influenced by genetics, intake of other dietary components, lifestyle factors (e.g. smoking, exercise), and other factors.

Although weighed food records are considered to be the most accurate method of measuring diet, to accurately capture the usual diet of individuals in a sample, numerous non-consecutive days of food records would be required per participant. The actual number required depends on the food or nutrient of interest, the level of precision required, and how much an individual's diet varies which will differ depending on the study population; however, some day-to-day, weekday versus weekend, and seasonal variations are expected (122, 123). Completing so many food records would be very expensive and participant burden would be very high, potentially leading to low response and retention rates. Therefore, this method is highly impractical for capturing usual diet. The results obtained are also not likely to be an accurate measure of usual diet as participants are likely to change their diets to appear healthier or to make it easier to weigh and record, particularly as they become familiar with the process (122-124). Therefore, due to the impracticality, cost and increased error of repeated weighed food records, more practical methods are commonly used to capture usual diet in epidemiological studies. The most commonly used method is the FFQ. Although these methods are considered less accurate for capturing actual dietary intake over short periods, they could actually be more accurate at capturing usual diet, depending on the tool used and the dietary factors of interest (122, 123).

Diet is a complex exposure variable and investigating associations between dietary factors and disease is difficult. Diet can be assessed using the intakes of nutrients, foods, food groups, or various measures of dietary patterns. Intakes of some foods and nutrients are highly correlated and, therefore, examining their separate associations with the outcome is challenging (123). Including collinear variables in a multiple linear regression model should be avoided as this can give false results (125). In cases where the dietary factor of interest is highly correlated with other diet variables, using a dietary pattern or score as the exposure variable should be considered. Health attitudes and habits influence our diet and many other health measures such as physical activity, BMI, smoking and alcohol consumption. Our level of health consciousness is also influenced by socioeconomic factors such as education. This creates a complex web of inter-related factors that may be associated with diet and disease outcomes and may potentially confound any diet-disease relationships being assessed. Variables may also be highly correlated and collinear, further complicating the analyses. Confounders should therefore be carefully selected with consideration of how these factors are related to each other, which factors may lie on the casual pathway and the potential biological mechanisms underlying the associations (123, 126). It may be necessary to analyse and report the outcome of multiple regression models to examine the relationships thoroughly.

3.3 Review of the literature

There are two major limitations of the previous research investigating the relationship of diet with lung disease. Firstly, most published literature investigating diet and other risk factors for chronic

obstructive lung diseases use doctor diagnosed asthma or COPD or a questionnaire on respiratory symptoms to assess the outcome, rather than using objective lung function measures. However, doctor diagnosis or questionnaire assessments may not be appropriate for middle-aged adults in whom asthma or COPD categorization is not always clear, with elements of both diseases often present. Therefore, it is particularly important to investigate the relationship of diet and other risk factors with objective measures of lung function and BR in this population.

Secondly, amongst the studies that have used lung function or BR as the outcome, most have focussed on either asthma or COPD and excluded participants who may have the other disease. For example, some asthma studies excluded smokers because of the strong link between smoking and COPD development, and some COPD studies had a minimum age cut-off because COPD is typically diagnosed later in life. However, smokers can and do develop asthma and younger individuals can develop COPD. Because of these exclusions in previous studies, there is a lack of evidence in these population groups who may be prone to developing a particular phenotype of asthma or COPD. Further research including these population groups is needed.

Due to the limited research available, this thesis will focus on diet as a possible risk factor for increased BR and poor lung function in middle-aged and older adults. Therefore, only research conducted in adults will be reviewed here and the relevance of this research to a general middle-aged and older population (i.e. both sexes, smokers and non-smokers, etc) will be briefly discussed. Please note, this literature review is not a systematic review. I carried out the review alone using only one database to search for relevant articles, and no structured assessment of quality of the studies or meta-analyses of the data has been performed.

3.3.1 Relationships of fruit and vegetable intake, lung function and lung disease

A comprehensive search of the literature was performed using the Medline database. The search terms were “fruit*” OR “vegetable*” AND any of the following lung function related terms - “asthma”, “chronic obstructive pulmonary disease”, “pulmonary function”, “lung function”, “respiratory function”, “ventilatory function”, “forced expiratory volume”, “forced expiratory function”, “spirometry”, “forced vital capacity”, “bronchial hyper*”, “airway *responsiveness”, “bronchial provocation challenge”, “chronic bronchitis”, “emphysema”, “bronchial *reactivity”, or “airway *reactivity”. All terms were searched for as keywords and MeSH headings. The search, last updated on the 7th May 2019, identified 14 studies that assessed the relationship between fruit and vegetable intake and lung function in adults, one study that assessed fruit and vegetable intake and BHR, and one study that assessed both lung function and BR as outcomes. Of the 15 studies that examined lung function as an outcome measure, two were experimental studies (127, 128), five were prospective cohort studies (129-133), and eight were cross-sectional (134-141). Of the 2 studies that assessed BR as an outcome, one was experimental (127) and the other used a cross-sectional design (142).

Experimental studies

Randomised controlled trials (RCTs) typically provide the best evidence when investigating effects of an exposure on a disease; however, they are quite difficult to conduct when diet is the exposure of interest. There are a number of issues that can arise during dietary intervention studies which may introduce bias. The limitations of the two experimental studies are discussed in detail when interpreting the results.

Conflicting results were observed in the two experimental studies evaluating the effect of fruit and vegetable intake on lung function. The RCT, performed by Wood and colleagues, randomly assigned 137 Australian adults with stable asthma to one of three treatment arms – a high antioxidant diet (HAO) plus placebo, a low antioxidant diet (LAO) plus tomato extract, and a LAO plus placebo. The HAO included 5 serves of vegetables and 2 serves of fruit per day and the LAO included no more than 1 serve of fruit and 2 serves of vegetables per day and avoidance of other antioxidant-rich foods (127). There was no effect of the tomato extract on clinical or inflammatory markers. Therefore, the two LAO groups were combined for the diet analysis. Seventy-nine participants completed the study. This study found no difference in the change in percent predicted FEV_1, FVC and FEV_1/FVC between the HAO and LAO groups after 14 weeks on the allocated diet (p=0.49, 0.17 and 0.16 respectively). There was also no difference in the change in the dose-response slope during a bronchial provocation challenge to hypertonic saline (p=0.61) (127). This study has a number of limitations which must be considered in interpreting these results. Firstly, a large proportion of the recruited participants (42%) did not finish the study and the final sample analysed was small. Therefore, the randomisation process may not have eliminated the effects of potential confounders of the relationship between fruit and vegetable intake and lung function. The small sample size would also limit the power of the study to detect associations. The study length (14 weeks) may not have been long enough to observe an effect of fruit and vegetable intake on lung function. Lastly, current smokers, a population group which is likely to benefit most from an increase in antioxidants, were excluded.

The second experimental study was an RCT conducted by Keranis and colleagues. In this study 120 COPD patients residing in Greece (mean age (SEM) = 68.1 (1.4) years; 87.5% male) were randomly assigned to a diet high in fruit and vegetables (intervention group) or a free diet (control group). Both groups were seen at baseline and every 6 months thereafter for dietary assessment by questionnaire and lung function assessment by spirometry. Those in the intervention group were also given dietary advice to increase their fruit and vegetable consumption at these visits (128). After the 3 year follow up period, the mean annual change in FEV_1, examined using a generalised linear model, was found to be different between the two groups with those in the intervention group having better lung function than those in the control group (p=0.03). This difference increased over time ($p<0.01$). The study also found FEV_1, FVC and FEV_1/FVC measures at the end of the study were

better in the intervention group compared to the control group (128). The major limitation of this study is that the investigators excluded patients who responded to bronchodilators (15% change in FEV_1), those who had a history of asthma, and those with atopy or allergic rhinitis. Presumably these persons were excluded to ensure those studied did not have asthma. However, in doing so they may have excluded those who had elements of both diseases and those who once had asthma and then developed COPD. Therefore, it is likely the study participants represent certain phenotypes of COPD. The sample was also predominantly male, thus limiting generalisability.

Cohort studies

Cohort studies also provide good evidence for exposure-outcome relationships. This study design enables temporality to be established, that is that the exposure occurred prior to the outcome. This is a key criterion in determining if an association is causal. The results from the five cohort studies investigating the relationship between fruit and vegetable intake and lung function are mixed with some studies suggesting the effect of fruit and/or vegetable intake on lung function may exist only in certain population groups such as smokers. Some studies examined particular fruits or vegetables or categories of fruit and vegetables (e.g. stone fruit, salad vegetables), some assessed total fruit intake and/or total vegetable intake, and some assessed fruit and vegetable intake as a single variable. These differences in the study designs make it difficult to compare and draw a conclusion from the evidence available.

One such study, conducted by Garcia-Larsen and colleagues, involved a cohort of 680 adults from Germany, Norway, and the UK who were followed over a 10-year period (49.4% male; mean age 43.8±6.6 years at baseline) (129). This study found that higher total fruit intake at baseline was associated with a slower annual decline in FEV_1 and FVC over the following 10 years (β=2.99 ml/year (0.37, 5.61), p=0.025 and β=3.48 ml/year (0.04, 6.92), p=0.048 for FEV_1 and FVC respectively). There was moderate evidence that this association was modified by smoking (interaction p=0.03 and 0.04 for FEV_1 and FVC respectively), with a per tertile increase in total fruit intake being associated with a 6.41 ml/year slower decline in FEV_1 (95%CI 2.29 - 10.5, p=0.002) and 8.13 ml/year slower decline in FVC (95%CI 2.22, 14.01, p=0.007) in former smokers only (129). There was no association between total fruit intake and lung function in current or never smokers. Further, no association was observed between total vegetable intake and lung function decline. The confounders that were adjusted for in this study were age, height, sex, country, BMI, socio-economic status, physical activity, years of education, and total energy intake (129).

Similarly, Bentley and colleagues found that smoking modified the relationship between fruit and vegetable intake and lung function decline. This study involved a US cohort of 1,443 older adults (mean age (SD) = 73.5 (2.8) years) followed over 4 years. Participants were categorised as current smokers, former smokers (quit smoking prior to baseline assessment), quitters (quit smoking between baseline and follow up assessments), or never smokers (130). This study found high fruit

and vegetable intake, defined as 8 servings per day, was associated with a slower decline in FEV_1 compared to low fruit and vegetable intake, defined as 2 servings per day, in current smokers and quitters (mean difference in FEV_1 decline between high and low fruit and vegetable intakes was 25 ml/year and 41 ml/year for current smokers and quitters respectively; interaction p=0.003). There was no association in never or former smokers. No association was also observed between fruit and vegetable intake and decline in FVC or FEV_1/FVC ratio (130). Confounders adjusted for in this study included age, sex, height, race, education, family income, respiratory medication use and total energy intake. This study has a number of limitations. Firstly, fruit and vegetable intake was analysed as a single variable. Therefore, it is unclear whether the effect is from fruit only, vegetables only, or both. Those with COPD or asthma at baseline were excluded from the study. Given the age at baseline, it is likely that those included in the study are not genetically predisposed to develop asthma or COPD and may in fact have some protective biological mechanisms at play, particularly the smokers. Lastly, given the small number of smokers (n=75) and quitters (n=27) in the study, the number of confounders included in the analyses, and the inconsistency in the findings with the other lung function outcomes, these results may be chance findings and should be interpreted with caution.

Carey and colleagues also assessed the association between fruit and vegetable intake and lung function using longitudinal data; however, their analysis was slightly different. In this study, the relationship between the change in fruit and vegetable intakes and the change in lung function in a cohort of 2,171 UK adults followed over 7 years was explored (cohort age range 18-73; 43.7% male) (131). Carey and colleagues found that change in fresh fruit consumption was positively associated with change in FEV_1 which fell 107 ml (95%CI 36-178 ml) more in subjects who reduced their fruit consumption the most compared to those with no change. Average fruit intake levels were not associated with change in FEV_1 (i.e. consistently low fruit intake was not associated with more rapid lung function decline) (131). There was weak evidence of an association between change in green vegetable intake and change in FEV_1 (p=0.10). Changes in consumption of salads and fruit juice were not associated with change in FEV_1. In cross-sectional analyses of the same data, fruit intake and salad intake were positively associated with FEV_1 at baseline and follow up, fruit juice and green vegetable intakes were also positively associated with FEV_1 at baseline but not at follow up (131). This study has a number of limitations. Forty percent of the initial study cohort was lost to follow up 7 years later. This may have introduced selection bias. There was no comparison of the retained participants and those lost to follow-up. Therefore, the effect of any selection bias is unknown. There was a systematic error with the FVC results and, therefore, no analyses of FVC or FEV_1/FVC ratio were able to be performed. Subjects who reported a history of respiratory disease at either time point were excluded. This would have reduced the variation in FEV_1, reduced the power of the study to observe an association by reducing the sample size (1,406 excluded on this criterion), and introduced selection bias in the older age groups as discussed in the Bentley study above. Lastly, temporality cannot be established for the change in fruit consumption and change in lung function analysis as it is unclear which change occurred first.

The remaining two cohort studies used specific fruits as exposure variables rather than total fruit intake and/or vegetable intake. Butland and colleagues examined the relationship between the frequency of consumption of apples, citrus fruit and fruit juice and lung function in a cohort of 2,512 Welsh men aged 45-59 years at baseline (132). This study found that apple consumption was positively associated with lung function in a cross-sectional analysis of data from baseline after adjusting for confounders (≥5 apples/week vs none mean difference in FEV_1 = 138.1 ml (95% CI 58.1 to 218.1), p<0.001). In the longitudinal analysis, there was weak evidence that apple consumption was associated with slower FEV_1 decline over the 5 year follow up period (≥5 apples/week vs none mean difference in change in FEV_1 = 47.7 ml (95% CI –10.7 to 106.2), p=0.10). There were no associations between citrus fruit intake or fruit juice intake and lung function in either the cross-sectional or longitudinal analysis (132).

The final cohort study, conducted by Hanson and colleagues, investigated the relationship between 8 selected foods including banana and grapefruit, and lung function in a cohort of 2,167 adults from 12 countries (60.8% male; mean age (SD) 61.2 (8.3) years at baseline) (133). The sampling frame of this study involved recruitment of a large sample of individuals with COPD and a history of smoking and a smaller sample of controls with a mixed smoking history, all aged 40-75 years. During the 3 year follow up period, diet and lung function were assessed at 8 time points. This study found higher grapefruit intake was associated with a greater decline in post-bronchodilator percent predicted FEV_1 (β=1.00, p=0.05), but a slower decline in post-bronchodilator FEV_1/FVC ratio (β= -1.29, p=0.001) over the 3 years (133). The measure of diet in this study was crude and is a significant limitation. Participants were simply asked whether they had eaten the selected foods in the last 24 hours. The data from the 8 time points was then converted to a binary variable – yes, eaten in the previous 24 hours at any of the 8 time points, or no, not eaten in the previous 24 hours at all time points. It is unclear whether weekdays and weekend days were adequately represented or whether seasonal variation of the diet was adequately captured. Such dietary assessment is not likely to be a good measure of usual diet. It collects no information on quantity consumed, and the conversion to a binary variable eliminates almost all the variation in the information collected. This, or the numerous tests performed in this study, may explain the conflicting results obtained. Also, as the diet and lung function data used were collected at all 8 time points, temporality cannot be established as it is not clear if the dietary exposure occurred before the change in lung function.

Cross-sectional studies

Cross-sectional studies can provide good quality evidence of an association between an exposure and an outcome, in this case fruit and vegetable intake and lung function. The main downfall of cross-sectional studies is they are unable to establish the temporal relationship between diet and lung function. The results from the cross-sectional studies are suggestive of a positive association between fruit intake and lung function, although the evidence is still somewhat mixed. There is little

evidence of an association between vegetable intake and lung function.

One cross-sectional study conducted by Tabak and colleagues examined the association between fruit and vegetable intakes and lung function in 3,325 men aged 40-59 years from Finland, Italy and the Netherlands. Spirometric testing protocols differed slightly between countries with forced expired volume in 0.75 seconds ($FEV_{0.75}$) measured in Italy and Finland, and FEV_1 measured in the Netherlands (134). This study found that, in the Italian study population only, those with a fruit intake above the median (150 g) had a mean $FEV_{0.75}$ 95 ml higher (95%CI 14, 176) than those whose fruit intake was below the median, after adjusting for confounders. There was also weak evidence for an association between vegetable intake and FEV_1 in the Dutch study population (above vs below median intake β=83 ml (95%CI -4, 170)) (134). The median vegetable intake was much higher in the Dutch population (165 g) compared to the Italian and Finnish populations (42 g and 65 g respectively) which may explain why an association was observed in the Dutch group only. In this study, lung function was adjusted for age and height; and BMI, smoking, alcohol consumption, and energy intake were considered as confounders. This study was of a high quality. Response rates were greater than 80% for all groups and dietary history interviews were conducted by trained dietitians or nutritionists, limiting errors and potential bias of the results.

Tabak and colleagues then examined the association between fruit and vegetable intake and lung function in a much larger sample of Dutch adults with a wider age range (13,651 participants aged 20-59 years, 46% male) (135). For this study, fruit and vegetable intakes were divided into quartiles for the analysis. Mean fruit intakes were 73 g/day and 485 g/day for quartiles 1 and 4 respectively, and mean vegetable intakes for quartiles 1 and 4 were 69 g/day and 199 g/day respectively. After adjusting for potential confounders, this study found intakes of fruit and vegetables were positively associated with FEV_1 (mean difference between quartile 1 of intake and quartile 4 = 51 ml ($p<0.001$) and 33 ml ($p<0.01$) respectively). The fruit association remained with further adjustment for other food items (i.e. vegetables, fish, whole grains and alcohol) (Q4-Q1 = 43 ml, $p<0.001$) but the vegetable association disappeared (Q4-Q1 = 16 ml, $p\geq0.05$)(135). Unfortunately, 95% confidence intervals and exact p-values were not reported in the publication of this research. The response rate of the study was also not reported. Therefore, any potential for selection bias, and the effects of any such bias, are unknown.

Another study, conducted by Okubo and colleagues, involved 2,942 men and women, aged 58-67 years, from the UK (52.7% male) (136). This study found higher fruit and vegetable intake was associated with better FEV_1 and FVC in both sexes (Men: difference in FEV_1 per quintile increase in fruit and vegetable intake β=0.02 L (95%CI 0.00, 0.04), p=0.041; FVC β=0.02 L (95%CI -0.00, 0.05), p=0.058; Women: FEV_1 β=0.02 L (95%CI 0.01,0.04), p=0.001; FVC β=0.03 L (95%CI 0.01, 0.04), $p<0.001$). There was no association observed between fruit and vegetable intake and FEV_1/FVC ratio in men or women (136). There are a number of limitations of this study which

should be considered when interpreting the results. The response rate was low at 53%, which may have introduced selection bias. There was no comparison of those who participated in the study and those who did not. Therefore, the extent of any potential bias is unknown. Diet over the previous 3 months was assessed by FFQ during a home interview. Therefore, seasonal variation was not captured and, given the labour-intensive method used, it is unlikely all participants were assessed in the same season. Assessing short-term diet at different times of the year is a source of information bias. Fruit and vegetable intakes were combined; therefore, it is unclear if the relationships observed relate to fruit intake, vegetable intake, or a combination of the two. Okubo and colleagues also adjusted for a large number of confounders and may have over-adjusted in their analysis. For example, paracetamol intake was included as a confounder. However, based on a causal diagram, which is a widely used method of determining confounders in epidemiology, paracetamol intake is probably not a confounder of the relationship between fruit and vegetable intake and lung function. The effect of such over-adjusting on the estimates of association is unknown.

Kelly and colleagues performed another cross-sectional study, involving 6,186 Scottish residents aged 16-64 years old (44.4% male; mean age (SD) = 40.3 (13.3) years). After adjusting for confounders, this study found more frequent consumption of fruit and green vegetables was associated with higher FEV_1 (137). Those consuming fruit ≥ once/day had a mean FEV_1 132 ml higher (95%CI 77-188 ml) than those consuming fruit less than once per month and those consuming cooked green vegetables ≥ once/day had a mean FEV_1 91 ml higher (95%CI 32-150 ml) than those consuming cooked green vegetables less than once per week. Linear trends for higher FEV_1 with higher frequency of consumption of fruit and cooked green vegetables were also observed (trend p-values fruit: $p<0.001$, green vegetables: $p=0.003$). There was also a positive association observed between consumption of raw vegetables and FEV_1 after adjusting for age, sex, height, SES, smoking, and physical activity (≥ once/day vs less than once per week mean difference in FEV_1 82 ml (95%CI 32-133 ml). However, this association disappeared upon further adjustment for other food items (mean difference FEV_1 39 ml (95%CI -14-92 ml) trend p-value=0.077) (137). It is important to note that it is unclear whether the adjustment for other foods is appropriate in this study given the foods asked about (fresh fruit; cooked green, cooked root and raw vegetables; and white and non-white fish) are likely to be correlated which may have created an issue with multicollinearity in the analysis. The major limitation of this study was the measure of diet. Participants were asked only six questions about their diet, including one on the frequency of their consumption of fruit and three on the frequency of their consumption of vegetables (cooked green vegetables, cooked root vegetables, raw vegetables). Responses were collapsed for analysis to 4 categories for fruit intake and 3 categories for each of the types of vegetable intake measured, reducing the variability in the sample. For each fruit/vegetable intake variable the 1-6 times per week category represented most of the sample (54%-69%).

Strachan and colleagues investigated the relationship between fruit consumption in winter and lung

function in a sample of 1,357 current smokers and 1,502 lifelong non-smokers aged 18-69 years from the UK. This study determined predicted FEV_1 using the observed lung function of lifelong non-smokers, and age, sex and height as predictor variables. FEV_1 residuals were then calculated as the difference between the observed and predicted FEV_1 (138). A difference in mean FEV_1 residuals of 77.9 ml (95% CI: 23.9-131.9; p=0.005) between low and high intakes of fruit or juice in winter (low= never drinking pure fruit juice and eating fruit less than once a week, high= eating fruit at least once a week or drinking fruit juice at all) was seen after adjusting for region, SES and lifetime cigarette consumption. Similar differences were observed for current smokers and non-smokers. A trend for higher FEV_1 with higher frequency of fruit consumption in winter was also observed (trend p=0.03) (138). This study excluded those with current or previous respiratory issues and former, occasional, and pipe or cigar smokers, which introduces selection bias and limits the generalisability of the results to the general population. Further, those aged 70 years and over were excluded simply because of the high prevalence of a history of respiratory disease. Therefore, the relevance of these results to older populations is unclear.

Another study, performed by Garcia-Larsen and colleagues, looked at fruit and vegetable intake and lung function in a sample of 1,232 young Chilean adults aged 22-28 years (139). In this study, total fruit intake was positively associated with FVC after adjusting for confounders (highest quintile vs lowest quintile: $\beta = 0.08$ L (95%CI 0.003, 0.15), p-value for trend = 0.02); however, there was no association observed between fruit intake and FEV_1 or FEV_1/FVC. There was also no association between total vegetable intake and any of the lung function parameters (139). This study adjusted for a number of variables, some of which, according to our causal diagram, may not be confounders of the fruit/vegetable intake and lung function relationship (e.g. overcrowding, birth weight). This over-adjustment may have impacted the results and its effects are unknown. The relevance of these results to a middle-aged and older population is also unclear.

Barros and colleagues conducted a cross-sectional study in a sample of 174 Portuguese adults with asthma (81.6% female; mean age (SD) = 40 (15) years) (140). This study found neither fruit nor vegetable intake were associated with FEV_1 after adjusting for sex, age, education, rhinitis, and energy intake (fruit: β=0.011 (95%CI -0.003, 0.024); vegetables: β= -0.006 (95%CI -0.023, 0.011)). BMI, physical activity, smoking, atopy and inhaled corticosteroid use were also considered as confounders but were not associated with FEV_1 in univariate analyses and were therefore excluded in the multiple linear regression model (140). The sample size in this study was quite small, limiting the power to detect an association. The study sample was also limited to those with asthma and was predominantly female, limiting the generalisability of the results to the general population.

Another cross-sectional study, conducted by Ng and colleagues, involving 2,478 individuals aged 55 and over from Singapore also found no association between fruit and vegetable intake and pulmonary function after adjusting for relevant confounders (all p-values >0.4 for FEV_1, FVC and

FEV_1/FVC) (141). However, this study had a number of limitations and the results should be interpreted carefully. The main limitation was the assessment of diet. Participants were asked about their diet over the previous month which may not be an indicator of their usual diet and would not capture seasonal variation. Data was collected for a period of 15 months. Therefore, because of seasonal variation, the time of assessment would have introduced bias. Fruit and vegetable intake was captured as a single binary variable. Participants were asked whether they consumed at least one serving daily of fruit or vegetables, of which 91.8% responded positively. Therefore, variation in intake was not captured. This lack of variation in the data, and the grouping of fruit and vegetables together, may explain why any potential associations were not detected.

The final cross-sectional study by Woods and colleagues investigated the relationship between total fruit and vegetable intakes, and intakes of categories of fruit and vegetables, with BHR in a sample of 1,601 Australian adults aged 20-44 years (142). Complete data was available for 1,073 participants. This study found no association between total fruit intake or total vegetable intake and BHR after adjusting for age, sex, BMI, smoking status, region of birth, and family history of asthma. However, higher consumption of apples and pears was associated with a reduced odds of BHR (OR=0.88 95%CI 0.77, 1.00; p=0.05) (142). This study has a few limitations to consider when interpreting the results. Firstly, the laboratory part of the study achieved a low response rate (36%), which is a possible source of selection bias (142, 143). There were a large number of analyses performed, measuring associations between various dietary factors and five respiratory outcomes, increasing the likelihood of observing an association by chance that in reality does not exist (known as a false positive or type I error). However, apple and pear intake was also negatively associated with two other respiratory outcomes - asthma (defined as an attack of asthma or shortness of breath in the last 12 months or current use of asthma medication) and current asthma (defined as wheeze in the past 12 months and BHR). These consistent beneficial associations between apple and pear intake and lung outcomes supports the existence of a true relationship between the two. Lastly, as the age range in this study excluded middle-aged and older adults, the relevance of the findings to this population is unclear.

Summary

Table 3.2 summarises the main characteristics of the above studies and their findings. The evidence suggests a probable positive relationship between fruit intake and lung function with 8 of 11 studies finding a positive association between total fruit intake, or consumption of a particular category of fruit, and spirometry outcomes including the cohort study of longest duration. At this stage the evidence for a relationship between vegetable intake and lung function is mixed with 2 of 8 studies finding a positive association between total vegetable intake or intake of a category of vegetables and spirometry measures. However, a couple of studies found an association which disappeared when other foods were added as confounders. This may indicate that the intake of vegetables and

some other foods are too closely correlated to analyse separately, and perhaps a dietary pattern analysis might be more appropriate. Four studies combined fruit and vegetable intake (this includes the two RCTs), three of which found a positive association. Very few studies have investigated possible effect modifiers which may be important if the relationship between fruit or vegetable intake and lung function exists for specific population groups or phenotypes of obstructive disease. Therefore, further studies are needed to clarify if any true relationships exist, the magnitude of any effects, optimum levels of consumption, and population groups or disease phenotypes that should be encouraged to make dietary change for their respiratory health. Only two studies assessed the relationship between fruit and/or vegetable intake and BR, one small RCT of short duration, and one cross-sectional study in a young adult population. These studies alone cannot provide convincing evidence for or against a relationship. Again, further research is needed.

Literature search update

The literature search was repeated on 25th September 2020 to identify any research on the relationship between fruit and vegetable intake, lung function and BR published since the previous search. There was one population-based cross-sectional study of 3397 Canadian adults aged 18-79 years performed by Khanam and colleagues (144). In this study, frequency of consumption of selected foods was measured and divided into tertiles. The authors found more frequent consumption of potato was associated with a lower FEV_1 and FVC, and more frequent consumption of beans was associated with a higher FEV_1 and FVC. However, there was no clear trend across tertiles of total fruit or vegetable consumption (144). The results of this study do not change the overall summary of the literature above.

Table 3.2 - Fruit and vegetables and lung function: study characteristics and findings

Author (year)	Study design	Country	Sample size	Sample description (age/sex)	Intervention (experimental studies)	Duration (experimental/ cohort studies)	Exposure/Outcome (method of measurement)	Confounders	Findings
Wood (2012)(127)	RCT	Australia	79 asthmatics (33 HAO; 46 LAO)	HAO: age 54±14 years, 32.6% male; LAO group: age 58±15 years, 47.3% male	High antioxidant diet (HAO) vs low antioxidant diet (LAO)	14 weeks	Exposure: HAO vs LAO; Outcome: Δ % predicted FEV_1, FVC, and FEV_1/FVC, Δ DRS (%fall/ml)	Sex, age, smoking history	No difference in Δ FEV_1, FVC, FEV_1/FVC, or DRS between groups.
Keranis (2010)(128)	RCT	Greece	120 COPD patients	87.5% male; age 68.1 ± 1.4 years	High fruit + vegetable diet (IG) vs free diet (CG)	3 years	Exposure: High fruit + vegetable diet; Outcome: Δ % predicted FEV_1, FVC, and FEV_1/FVC	Sex, age, smoking, comorbid conditions, exacerbations	Mean annual Δ FEV_1 different between two groups with IG having better FEV_1 than CG. Difference ↑ over time. FVC and FEV_1/FVC also better in IG.
Garcia-Larsen (2017)(129)	Cohort	Germany, UK, and Norway	680	Age 43.8±6.6 years; 49.4% male	N/A	10 years	Exposure: total fruit intake, total vegetable intake (at baseline); Outcome: decline in FEV_1 and FVC	Age, height, country, sex, BMI, SES, physical activity, education, total energy intake	Total fruit intake at baseline ↔ slower ↓ in FEV_1 and FVC in former smokers only. No association in never or current smokers. No association between total vegetable intake and lung function decline.
Bentley (2012)(130)	Cohort	USA	1,443	Age 73.5 ±2.8 years; 49% male	N/A	4 years	Exposure: fruit + vegetable intake; Outcome: decline in FEV_1, FVC and FEV_1/FVC	Age, sex, height, race, education, family income, study site, pulmonary drug use, total energy intake	High fruit + vegetable intake ↔ slower ↓ FEV_1 in current smokers and quitters, no association in never or former smokers. No association between fruit and vegetable intake and ↓ FVC or FEV_1/FVC

Author (year)	Study design	Country	Sample size	Sample description (age/sex)	Intervention (experimental studies)	Duration (experimental/ cohort studies)	Exposure/Outcome (method of measurement)	Confounders	Findings
Carey (1998)(131)	Cohort	UK	2,171	Aged 18-73 years; 43.7% male	N/A	7 years	Exposure: mean + Δ fruit intake, Δ green vegetable intake, Δ salad intake, Δ fruit juice intake; Outcome: Δ FEV_1	Age, height, sex, region, social class, smoking	Δ fruit intake ↔ Δ FEV_1 (+); Δ green vegetable intake ↔ Δ FEV_1 (weak association); mean fruit intake, Δ salad intake, and Δ fruit juice intake not associated with Δ FEV_1
Butland (2000)(132)	Cohort	Wales	2,512	men only; age 52.1 ± 4.6 years	N/A	5 years	Exposure: consumption of apples, citrus fruit, fruit juice (frequency); Outcome: decline in FEV_1	Age, height, age squared, height squared, BMI, smoking, social class, exercise, total energy intake	Apple consumption ↔slower FEV_1 decline (weak); No association between citrus fruit or fruit juice consumption and FEV_1 decline
Hanson (2014)(133)	Cohort	International – 12 countries	2,167	60.8% male; age 61.16 ± 8.31 years	N/A	3 years	Exposure: consumption of banana and grapefruit; Outcome: Δ FEV_1 and Δ FEV_1/FVC	Age, sex, body mass index (BMI), and smoking	Grapefruit consumption ↔ faster decline in FEV_1 (% predicted) and slower decline in FEV_1/FVC over 3 years. Banana consumption not associated with decline in FEV_1 or FEV_1/FVC over 3 years.
Tabak (1999)(134)	Cross-sectional	Finland, Italy, the Netherlands	Finland (n=1248), Italy (n=1386), the Netherlands (n=691)	All men; mean age (SD) years: Finland 59.0 (5.5); Italy 54.5 (5.0); Netherlands 54.8 (5.5)	N/A	N/A	Exposure: fruit intake, vegetable intake (above vs below median); Outcome: $FEV_{0.75}$or FEV_1	Age, height, smoking, BMI, alcohol consumption, energy intake	Higher fruit intake ↔ higher $FEV_{0.75}$in Italian study samples, no association in Finnish or Dutch samples; Higher vegetable intake ↔ higher FEV_1in Dutch sample (weak), no association in Italian and Finnish samples.

Author (year)	Study design	Country	Sample size	Sample description (age/sex)	Intervention (experimental studies)	Duration (experimental/ cohort studies)	Exposure/Outcome (method of measurement)	Confounders	Findings
Tabak (2001)(135)	Cross-sectional	The Netherlands	13,651	Mean age (SD) 41.2 (10.8) years; 46% males	N/A	N/A	Exposure: fruit intake, vegetable intake; Outcome: FEV_1	Age, sex, height, smoking, BMI, energy intake. Physical activity, nationality and education did not cause a relevant change	Higher fruit intake ↔ higher FEV_1, Higher vegetable intake ↔ FEV_1, however, association disappeared after further adjustment for other foods
Okubo (2014)(136)	Cross-sectional	UK	2,942	52.7% male; mean age (SD) years: male - 65.7 (2.9), female - 66.6 (2.7)	N/A	N/A	Exposure: Fruit + vegetable intake (single variable); Outcome: FEV_1, FVC, FEV_1/FVC	Age, height, smoking (status + pack-years), home smoke exposure, education, social class, body fat mass, physical activity, dietary supplement use; inhaled or oral steroid use, paracetamol use, alcohol consumption, energy intake	Higher fruit + vegetable intake ↔ higher FEV_1 and FVC in men and women. No association between fruit + vegetable intake and FEV_1/FVC in either men or women.
Kelly (2003)(137)	Cross-sectional	Scotland	6, 186	44.4% male; mean age (SD) years: male - 40.4 (13.3), female - 40.3 (13.3)	N/A	N/A	Exposure: Intakes of Fruit, green vegetables, cooked root vegetables, and raw vegetables; Outcome: FEV_1	Age, age squared, sex, height, height squared, SES, smoking (status + average per day), work and leisure activity levels.	More frequent consumption of fruit and green vegetables ↔ higher FEV_1. Frequency of raw vegetables ↔ FEV_1, however, association disappeared after further adjustment for other foods.

Author (year)	Study design	Country	Sample size	Sample description (age/sex)	Intervention (experimental studies)	Duration (experimental/ cohort studies)	Exposure/Outcome (method of measurement)	Confounders	Findings
Strachan (1991)(138)	Cross-sectional	UK	1357 current smokers; 1502 lifelong non-smokers	aged 18-69	N/A	N/A	Exposure: low vs high fruit or juice intake, frequency of winter fruit consumption; Outcome: difference between observed and predicted FEV_1	Predicted FEV_1 calculated using sex, age and height. Analysis adjusted for pack years; region; SES.	Mean difference in FEV_1 residuals of 77.9 ml (95% CI: 23.9-131.9; p=0.005) between low and high intakes of fruit or juice in winter. Higher frequency of winter fruit consumption ↔ higher FEV_1 (trend p=0.03)
Garcia-Larsen (2015)(139)	Cross-sectional	Chile	1,232	aged 22-28 years; 45.7% male	N/A	N/A	Exposures: total fruit intake, total vegetable intake; Outcomes: FEV_1, FVC, FEV_1/FVC	Height, sex, age, current smoking, overcrowding, education, SES, birth weight, BMI, total energy intake	Higher total fruit intake ↔ higher FVC(L) (Highest quintile vs lowest quintile: β-coefficient (95%CI) = 0.08 (0.003, 0.15), trend p-value = 0.02). No association between total fruit intake and FEV_1 or FEV_1/FVC. No association between total vegetable intake and FEV_1, FVC or FEV_1/FVC.
Barros (2008)(140)	Cross-sectional	Portugal	174 Asthmatics	81.6% Female; age = 40±15years	N/A	N/A	Exposures: total fruit intake, total vegetable intake; Outcome: FEV_1	Sex, age, energy intake, BMI, education, rhinitis, atopy	Neither fruit intake nor vegetable intake were associated with FEV_1

Author (year)	Study design	Country	Sample size	Sample description (age/sex)	Intervention (experimental studies)	Duration (experimental/ cohort studies)	Exposure/Outcome (method of measurement)	Confounders	Findings
Ng (2014)(141)	Cross-sectional	Singapore	2,478	mean age (SD) = 65.9 (7.6) years, 36.9% male	N/A	N/A	Exposure: ≥ 1 serve fruit or vegetables daily over the previous month; Outcomes: FEV_1, FVC, FEV1/FVC	Sex, age, height, smoking status, housing type, past occupational exposure to dust/fumes, history of asthma and/or COPD, BMI, physical activity, and other dietary and supplement use variables	No association between daily fruit or vegetable consumption and FEV_1, FVC or FEV_1/FVC
Woods (2003)(142)	Cross-sectional	Australia	1073	Aged 20-44years	N/A	N/A	Exposure: total fruit intake, total vegetable intake, intake of apples and pears, berries, green leafy vegetables, and tomatoes; Outcome: BHR (methacholine)	Age, sex, BMI, smoking status, region of birth, family history of asthma	Higher intakes of apples and pears ↔ lower odds of BHR. No other associations were observed.

HAO=high antioxidant diet; LAO=low antioxidant diet; DRS=dose response slope

Studies examining asthma and COPD as outcomes

For reasons detailed in Chapter 2, the outcomes of interest in this thesis are lung function and BR. However, much of the relevant research to date has examined asthma and COPD as outcomes. Therefore, a brief summary of the evidence of a relationship between fruit and/or vegetable intake and asthma or COPD is provided here. However, given the limited relevance of these studies to this thesis, the quality of the studies, relevance of the findings to our population of interest (middle-aged and older adults), and the limitations of the research will not be discussed.

Studies examining asthma as an outcome

A recent systematic review and meta-analysis has been performed by Hosseini and colleagues on the relationship between fruit and vegetable intake and risk of asthma and wheeze. This study found that, of the research performed in adults, all cohort (n=4) and case-control (n=2) studies found inverse associations between fruit and/or vegetable intake, or intake of specific fruits or vegetables, and incidence or risk of asthma (145). The specific fruits and vegetables for which intake was associated with reduced asthma risk were tomatoes, carrots, leafy vegetables, apples and oranges. The findings from cross-sectional studies (n=7) were less conclusive, with three studies finding fruit and vegetable intake was negatively associated with asthma; two studies finding fruit intake was negatively associated with asthma, whilst vegetable intake had no relationship with asthma; one study finding vegetable intake was negatively associated with bronchial asthma; and one study finding no relationship between intakes of fruits and vegetables and asthma (145). Overall, these studies suggest higher consumption of fruits and vegetables is protective against asthma. The meta-analyses performed in this systematic review included studies involving adults, children and pregnant women. Several meta-analyses were performed examining associations of fruit intake and vegetable intake with risk of prevalent asthma and asthma severity, and fruit and vegetable intake combined with risk of prevalent asthma. The results of these meta-analyses were mixed, with inverse associations observed between fruit intake and asthma severity (OR=0.61 (95%CI=0.44, 0.87), p=0.005; n=2), and vegetable intake and risk of prevalent asthma (OR=0.95 (95%CI=0.92, 0.98), p=0.003; n=17) (145). There was weak evidence of an inverse association between fruit intake and risk of prevalent asthma (OR=0.98 (95%CI 0.96, 1.0) p=0.09; n=16); however, no association was observed between vegetable intake and asthma severity (OR=1.11 (95%CI=0.63, 1.94), p=0.72; n=2). The meta-analysis of studies assessing fruit and vegetable intake combined also found weak evidence of a relationship with the risk of prevalent asthma (OR=0.90 (95%CI=0.80, 1.01), p=0.07; n=6) (145). Given the heterogeneity of the included studies, the relevance of the results of these meta-analyses to middle-aged and older adult populations is unclear.

A similar systematic review and meta-analysis performed by Seyedrezazadeh and colleagues (146) was published a few years earlier than that of Hosseini and colleagues; however, these authors conducted separate meta-analyses for adults, children and pregnant women. The meta-analysis on

data from adults included 3 cohort studies, 2 case-control studies and 4 cross-sectional studies (146). This meta-analysis found negative associations between fruit intake and risk of asthma (highest vs lowest intake RR=0.77 (95%CI 0.68, 0.87)) and vegetable intake and risk of asthma (highest vs lowest intake RR=0.84 (95%CI 0.74, 0.96)) (146). These results suggest high fruit and vegetable intakes may be protective against asthma in adult populations.

Hosseini et al searched the literature up to June 2016. Since then, no further studies examining the relationship between fruit and/or vegetable intake and the risk or incidence of asthma have been published.

Studies examining COPD as an outcome

There have been no systematic reviews or meta-analyses performed on studies examining the relationship between fruit or vegetable intake and COPD; however, there have been a small number of studies conducted. I carried out a comprehensive search of the literature, identifying 14 original research articles - five longitudinal studies, four cross-sectional studies, four case-control studies, and one ecological study investigating the relationship between fruit intake and COPD (136, 147-159). Of these studies, six found an inverse relationship between fruit intake and COPD risk, incidence or mortality (148, 151, 153-156); four found no relationship between fruit intake and COPD risk (one case-control and three cross-sectional studies) (136, 149, 150, 159); and one longitudinal study found no relationship between total fruit intake and incidence of chronic non-specific lung disease (CNSLD; includes asthma, emphysema and chronic bronchitis) over 25 years (mean age at baseline was 49 years); however, a negative association was found between solid fruit intake (i.e. apples and pears) and incidence of CNSLD (152). There were two case-control studies that assessed fruit and vegetable intake as a single variable, both of which found an inverse relationship with odds of COPD (147, 158). The single ecological study also found total fruit and solid fruit consumption was inversely associated with COPD mortality 25 years later (157). Given that 10 of the 14 studies, and all of the longitudinal studies found an inverse association between fruit intake (or solid fruit intake) and COPD, it is probable that fruit intake does have a protective effect against the development and progression of COPD. However, the strength of the effect, optimum levels of fruit consumption, and population groups and COPD phenotypes that could benefit most from dietary change remain unclear.

All but one of the studies identified above examining fruit intake and COPD also investigated the relationship between vegetable intake and COPD. There were four case-controls, four cross-sectional studies, one ecological study and four cohort studies (136, 147-152, 154-159). Of these, two case-control studies, two cross-sectional studies and one longitudinal study found inverse associations between vegetable intake and COPD risk or incidence (148-151, 154); one cross-sectional study, three longitudinal studies and one ecological study found no relationship (136, 152, 155-157); and two case-control studies assessing fruit and vegetable intake as a single variable found

an inverse relationship (147, 158). There was also one cross-sectional study that found a positive association between vegetable intake and COPD risk in those aged over 65 years after adjusting for confounders; however, this association may be due to people changing their diets following diagnosis of COPD or other chronic health issues (159). This study also has a number of limitations, including using a binary variable for fruit and vegetable intakes (at least once daily vs less than once daily) and an unusually high prevalence of chronic bronchitis or emphysema in younger age groups (11% and 6% of those with chronic bronchitis or emphysema were in the 20-29 and 30-39 year age groups respectively). In summary, the research investigating the relationship between vegetable intake and COPD have provided mixed results. These inconsistent findings may be because the relationship between vegetable intake and COPD is weak, it may not be a causal relationship, the measurement error in estimating vegetable intakes may make it difficult to see associations, or it may be due to differences between the studies such as differences in vegetable intake levels or variation in vegetable intake, or differing distributions of population subgroups or COPD phenotypes.

3.3.2 Relationships of dietary patterns, lung function and lung disease

Dietary pattern analysis is a relatively new method of exploring the overall diet and its relationship with various diseases. Previously, research focused on assessing relationships between foods, food categories or groups, or nutrients and disease outcomes. However, such research ignores the fact that most foods contain many nutrients in varying quantities, and foods are not usually eaten in isolation, but in combination with other foods as part of a meal or snack (106, 123, 160). Therefore, intake of some foods or nutrients can be highly correlated. Multivariate regression analysis requires explanatory variables to be independent of each other in order to reliably estimate the regression coefficients of each explanatory variable. Highly correlated foods or nutrients in a single regression model can cause multicollinearity which makes the estimates produced unreliable and reduces the power of the model to detect a true association (161-163). Hence, it can be very difficult to determine the independent effects of a food or nutrient on a disease outcome of interest.

Dietary pattern analysis provides an alternative method of analysing diet as a risk factor for disease. There are two methods of dietary pattern analysis – the *a priori* approach and the *a posteriori* approach (106, 123, 160). Both methods assess the overall diet of an individual, however, there are some important differences. In the *a priori* approach, the investigators use the current knowledge of diet-disease relationships to define a dietary pattern. A score is then calculated for each individual according to their adherence to the pre-defined dietary pattern. This score is used as the exposure in further analysis to measure associations with disease outcomes (106, 123, 160). In the *a posteriori* approach, the dietary patterns are defined based on the correlations between the foods or nutrients in the data using a statistical method such as factor analysis or principal component analysis (PCA). With this method, more than one dietary pattern can be identified. A score is calculated for each individual for each pattern. The scores, representing adherence to each dietary pattern, are then used

as exposure measures in analysis of diet-disease relationships (106, 123, 160). Therefore, the main difference between the two methods is, in the *a priori* approach the exposure is a pre-defined dietary pattern, whereas in the *a posteriori* approach the exposure is actual dietary patterns that exist in the study population.

Dietary pattern analysis is relatively uncommon in obstructive lung disease research compared with cardiovascular disease or cancer research. I conducted a comprehensive search of the literature using the Medline database. The dietary pattern search terms were "diet* pattern*", "dietary pattern*", "Mediterranean diet", "dietary inflammatory index", "western diet", "prudent diet", and "traditional diet". The lung function related terms listed previously for the fruit and vegetable search were also used. All search terms were searched as keywords and MeSH headings. The search was last updated on 7th May 2019. From my review of the resulting list of articles, I identified 16 studies that assessed the relationship between dietary patterns and lung function in adults, one of which also assessed dietary patterns and BR. Seven of these studies used pre-defined healthy diet scores (the *a priori* method) (109, 111, 140, 164-167) and 9 studies used PCA to define dietary patterns (the *a posteriori* method) (139, 168-175). Given the significant difference between the *a priori* and *a posteriori* methods, the evidence to date will be discussed separately for each method.

3.3.2.1 *Studies using a priori methods*

Of the 7 studies using an *a priori* method to score diet, 2 were RCTs (164, 167), 4 were cross-sectional studies (111, 140, 165, 166) and one was a case-control study (109). The dietary scoring methods used represented the Mediterranean diet in 4 studies (140, 164-166), the Dietary Approaches to Stop Hypertension (DASH) diet in one study (167), two measures of a general healthy diet in one study (166), and the inflammatory potential of the diet in two studies (one study compared three scoring methods) (109, 111).

Studies using a measure of the Mediterranean diet

The Mediterranean diet is a term used to describe the traditional dietary pattern observed in populations living around the Mediterranean Sea. The diet is characterised by high intakes of fresh fruit and vegetables, pulses, nuts and olive oil; moderate amounts of fish, dairy products and alcohol; and low intakes of meat (particularly red meat) and meat products (140, 164, 165). The Mediterranean diet has been of interest to health researchers over the last 50 years following observational studies which found low incidence of, and mortality from, cardiovascular disease in Mediterranean populations. Since then further studies have extended the benefits of the Mediterranean diet to include other chronic diseases such as type 2 diabetes, obesity, and certain types of cancer (176-179). Although the mechanisms behind the benefits of the Mediterranean diet are not yet fully understood, it is likely that the pathways through which the Mediterranean diet works may also be relevant to chronic lung diseases. However, thus far the research in this area is very limited and inconclusive.

There have been 3 cross-sectional studies and 1 RCT looking at the relationship between the Mediterranean diet and lung function. The RCT, performed by Sexton and colleagues, was a small study of short duration. Thirty-eight adults with symptomatic, clinically stable asthma from Auckland, New Zealand were randomly assigned to one of three treatment arms – a high intervention (HI) group, a low intervention (LI) group and a control group (n=11, 12 and 12 for HI, LI and control groups respectively) (164). The HI group received intensive initial dietary advice encouraging a Mediterranean diet and 41 hours of support from a dietitian; the LI group received less intense advice and 2 hours with a dietitian; and the control group were offered a 1 hour session with a dietitian, recipes and free food at the end of the trial. Thirty-five participants completed the study. At the end of the 12 week follow up period the Mediterranean diet score had increased in the HI group and had not changed in the LI and control groups. However, there were no differences in mean change in pre- and post- bronchodilator FEV_1 and FVC observed between the three treatment groups (164). This study has some important limitations. The sample size was very small, and it is highly likely the randomisation process did not eliminate the effects of potential confounders of the relationship between the Mediterranean diet pattern and lung function. Comparison of baseline statistics of the three groups indicated that randomisation did not achieve even distribution of important confounding variables across the treatment groups. The small sample size also limits the power of the study to detect associations. The duration of the study (12 weeks) may not have been long enough to observe an effect of the Mediterranean diet on lung function. Participants were aware that the study was about diet and asthma, and they were aware of which treatment group they were in. This knowledge may have led to reporting bias in the follow up dietary assessments. Therefore, adherence to the Mediterranean diet in the HI group may not have been as good as the data suggests. Lastly, given the small sample size and the characteristics of the study population (e.g. inclusion of only people with stable asthma; mean (SD) age (years): 38 (4.2), 37 (4.0), and 40 (4.0) for HI, LI and control groups respectively) the relevance of these results to middle-aged and older populations, particularly those with COPD or ACO, is unclear.

The results of the 3 cross-sectional studies are more promising; however, given the limitations of cross-sectional studies, and the limited number of studies, further research is needed to confirm a positive effect of the Mediterranean diet on lung function. Barros and colleagues conducted the first cross-sectional study investigating the link between the Mediterranean diet and lung function. This study involved 174 asthma sufferers from Portugal (mean (SD) age: 40 (15) years; 82% female) (140). After adjusting for sex, age, energy intake, rhinitis and education, there was no association observed between the alternate Mediterranean diet score and percent predicted FEV_1 (140). This study has a few limitations, the main one being that the researchers only adjusted for potential confounding variables if the variable was associated with the outcome in a univariate analysis. Therefore, some important confounders were left out of the final model. This may have resulted in residual confounding. The sample size was small so confounders need to be limited, however, a confounder as critical as smoking should have been included. Those with a food allergy were also

excluded. The number of individuals excluded on this criterion is not stated, however, this may impact the phenotypes of asthma represented in the sample and the generalisability of the results to all those with asthma.

Gutierrez-Carrasquilla and colleagues performed another cross-sectional study involving 3,020 Spanish men and women of middle-age and older (men aged 45-65, women aged 50-70) (165). In this study the spirometry measures were used to create binary outcomes - abnormal FEV_1 (defined as FEV_1 <80% predicted); non-obstructive ventilatory defect (defined as FVC <80% predicted, FEV_1/FVC ratio ≥70%, and a convex-shaped flow-volume curve); and obstructive ventilatory defect (FEV_1/FVC <70%). After adjusting for age, sex, BMI, and physical activity, this study found women with a low adherence to the Mediterranean diet had greater odds of abnormal FEV_1 (OR (95%CI) 2.07 (1.06–4.06), p=0.033) and non-obstructive ventilatory defects (OR (95%CI) 2.42 (0.97–6.05) p=0.058), compared to those with high adherence to the Mediterranean diet. There was also weak evidence that low adherence to the Mediterranean diet was associated with greater odds of obstructive ventilatory defects in women (OR (95%CI) 1.99 (0.93–4.26), p=0.077)(165). In men, low adherence to the Mediterranean diet was associated with greater odds of obstructive ventilatory defects (OR (95%CI) 4.14 (1.42–12.1), p=0.009) and there was also weak evidence of an association with abnormal FEV_1 (OR (95%CI) 1.75 (0.94–3.27), p=0.078). No relationship was observed between the Mediterranean diet and non-obstructive ventilatory defects in men (165). This study has several significant limitations. Firstly, all participants were free of lung disease. This has 2 effects – 1) it limits the variability of the spirometry measures and 2) given the age of the participants, it is likely that there is a greater prevalence of genetic predisposition protecting against lung disease in the study population, particularly among the smokers. Overall, the researchers adjusted for few confounders in the analyses and they did not adjust for smoking even though smoking was associated with the Mediterranean diet score in their data. They also did not adjust for any SES measures. Therefore, there is likely to be some residual confounding. Creating binary outcomes from the spirometry measures rather than using continuous spirometry measures as outcomes has resulted in lost information and reduced the power of the analyses to detect true associations. As a screener questionnaire was used to determine the Mediterranean diet score, energy intake was not available and could not be adjusted for. P-values for the trend of associations across low, medium and high adherence to the Mediterranean diet were also not tested and would have added to the assessment of a relationship between the Mediterranean diet and lung function by indicating any possible dose-response type relationship.

The last cross-sectional study was conducted in Tehran, Iran by Yazdanpanah and colleagues. One hundred and twenty-one middle-aged and older COPD patients (mean age (SD) years: 66.1 (10.9); 85.1% men) completed spirometry testing and a questionnaire including an FFQ (166). Dietary data was used to calculate 3 diet scores – a Mediterranean diet score, the healthy eating index (HEI) 2005 and the HEI 2010. The HEI 2005 was developed from dietary guidelines and daily nutrient

requirements with high scores indicating adequate intake of fruit, vegetables (particularly dark green and orange vegetables and legumes), grains, milk, meat and beans, and fats, and limited intake of saturated fat, sodium and added sugars (166, 180). The HEI 2010 is an updated version of the HEI 2005 reflecting changes in knowledge and subsequent changes to dietary recommendations. The new score puts greater emphasis on the intake of whole grains, high protein foods (particularly seafood and plant-based proteins), and healthy fats (PUFAs and MUFAs) (166, 181). After adjusting for age, sex, smoking status, education and BMI, this study found positive associations between the Mediterranean diet score and FEV_1 and FVC (β=2.9, 95% CI (1.1, 4.8), p=0.002; β=2.8, 95% CI (0.9, 4.9), p=0.007 for FEV_1 and FVC respectively). However, there were no relationships observed between the HEI 2005 or the HEI 2010 and lung function. Further analysis indicated that vegetable intake and PUFA:SFA ratio were the components of the Mediterranean score contributing to the observed positive association with lung function (166). This study has some limitations to consider. Firstly, the scoring methods differed making it difficult to compare the results. While many of the dietary components were the same, the proportion of the score relating to each dietary component differed. Many of the components in the HEI 2005 and HEI 2010 were also energy adjusted. This is an important difference as eating becomes difficult with increasing severity of COPD and the quantity of food consumed reduces. The Mediterranean diet score has 10 dietary components - 8 related to quantity of food consumed, 6 of which score higher for intakes above the median. Therefore, the associations observed for the Mediterranean diet could be due to those in early stages of the disease with better lung function eating more food. It is also unclear from the article whether scores were analysed together, and then dietary components were analysed together, increasing the chance of multicollinearity which can greatly impact the results, or whether there were many tests conducted, thus increasing the likelihood of chance findings. Lastly, patients with both asthma and COPD were excluded, which may have excluded or reduced the representation of some COPD phenotypes.

It is important to note that all the studies investigating the Mediterranean diet and lung function used scoring methods which differed slightly, particularly in relation to certain foods such as dairy, alcohol and white meat. One study also included consumption of some discretionary foods in the scoring algorithm. The difference in scoring methods may have contributed to differences observed in the results.

Studies using the Dietary Inflammatory Index (DII)

Most of the dietary pattern scoring methods are derived from dietary guidelines which are based on research into diet-disease relationships. Most of this research focuses on CVD- or cancer-related outcomes. Diet and lung disease research is relatively limited compared to the vast amount of research into diet and CVD or cancer and it is yet to definitively demonstrate any clear relationships. Therefore, most studies looking at dietary patterns and lung function have used scoring methods

based on the research into CVD and cancer, rather than relationships between diet and lung function or lung disease.

The dietary inflammatory index (DII) is different to the other dietary scoring methods. It is designed to measure the inflammatory potential of the diet. Developed by Shivappa and colleagues, it is based on all the literature of the relationship between diet and the inflammatory biomarkers Interleukin (IL)-1β, IL-4, IL-6, IL-10, tumour necrosis factor (TNF)-α and C-reactive protein (CRP) (110). Shivappa and colleagues identified 45 dietary factors associated with these inflammatory biomarkers, some pro-inflammatory and some anti-inflammatory. They then developed a scoring algorithm to calculate the overall inflammatory potential of the diet based on associations observed and the strength of the study designs. The overall score can be negative or positive with higher scores indicating a more pro-inflammatory diet (110).

The 45 dietary factors included in the DII are listed in Table 3.3, along with the inflammatory effect score for each factor. The most pro-inflammatory dietary factors are saturated fat, total fat and *trans* fat, whilst the most anti-inflammatory factors are turmeric, fibre, flavones and isoflavones, β-carotene, green and black tea, magnesium, flavonols, ginger, vitamin D, and n-3 PUFAs (110).

Table 3.3 - Dietary factors included in the DII and their inflammatory effect scores (110)

Dietary factor	Inflammatory effect score	Dietary factor	Inflammatory effect score	Dietary factor	Inflammatory effect score
Alcohol (g)	-0.278	Magnesium (mg)	-0.484	Vitamin A (RE)	-0.401
Vitamin B_{12} (µg)	0.106	MUFAs (g)	-0.009	Vitamin C (mg)	-0.424
Vitamin B_6 (mg)	-0.365	Niacin (mg)	-0.246	Vitamin D (µg)	-0.446
β-Carotene (µg)	-0.584	n-3 PUFAs (g)	-0.436	Vitamin E (mg)	-0.419
Caffeine (g)	-0.110	n-6 PUFAs (g)	-0.159	Zinc (mg)	-0.313
Carbohydrate (g)	0.097	Onion (g)	-0.301	Green/black tea (g)	-0.536
Cholesterol (mg)	0.110	Protein (g)	0.021	Flavan-3-ol (mg)	-0.415
Energy (kcal)	0.180	PUFA (g)	-0.337	Flavones (mg)	-0.616
Eugenol (mg)	-0.140	Riboflavin (mg)	-0.068	Flavonols (mg)	-0.467
Total fat (g)	0.298	Saffron (g)	-0.140	Flavonones (mg)	-0.250
Fibre (g)	-0.663	Saturated fat (g)	0.373	Anthocyanidins (mg)	-0.131
Folic acid (µg)	-0.190	Selenium (µg)	-0.191	Isoflavones (mg)	-0.593
Garlic (g)	-0.412	Thiamin (mg)	-0.098	Pepper (g)	-0.131
Ginger (g)	-0.453	*Trans* fat (g)	0.229	Thyme/oregano (mg)	-0.102
Iron (mg)	0.032	Turmeric (mg)	-0.785	Rosemary (mg)	-0.013

MUFAs=monounsaturated fatty acids; PUFAs=polyunsaturated fatty acids; RE=retinol equivalents

As asthma and COPD are both inflammatory diseases of the airways, it is logical to assess the relationship between the inflammatory potential of the diet and lung function. However, the DII is a relatively new dietary scoring method and there have only been 2 studies to date assessing this relationship, one cross-sectional study and one case-control study (109, 111). The cross-sectional study, performed by Han and colleagues, used data from the 2007-2012 National Health and Nutrition Surveys, three large population-based cross-sectional surveys carried out in the United States. This study involved 22,294 adults aged 18-79 years (111). The DII was calculated using intakes of 27 dietary factors obtained from 24-hour dietary recalls and was energy-adjusted. After adjusting for household income, BMI, family history of asthma, serum cotinine level (an indicator of current smoking), and current asthma, higher DII (indicating a more pro-inflammatory diet) was associated with lower percent predicted FEV_1 (β (95%CI) = -0.27 (-0.47, -0.08); $p<0.01$) and percent predicted FVC (β (95%CI) = -0.36 (-0.55, -0.18; $p<0.01$). There was no association observed between DII score and percent predicted FEV_1/FVC (111). This study has some limitations including the use of a single dietary recall to assess diet of the participants. This is not an appropriate measure of usual diet in measures of association (discussed in section 3.2). Han and colleagues also used percent predicted values of FEV_1 and FVC. This is not necessary with such a large sample and it is not advised as the percent predicted values account for differences in lung function with regards to age, sex, height, and ethnicity. Therefore, these variables cannot be included as confounders in the multivariable regression analysis as the authors have induced an association between these variables and the outcome. If the sample is large enough, it is better to use the raw lung function measures and adjust for age, sex, height and ethnicity. This also makes the interpretation of the coefficients more straightforward. Lastly, serum cotinine is a measure of nicotine exposure through recent cigarette smoking; however, this does not account for history of smoking which would also be a confounder. Therefore, there is likely to be some residual confounding from smoking.

The other study investigating the relationship between the inflammatory potential of the diet and lung function was an Australian case-control study conducted by Wood and colleagues. This study involved 99 individuals with stable asthma and 61 healthy controls (109). The DII was calculated using intakes of 25 of the possible 45 dietary factors. After adjusting for age, sex, smoking (pack-years), and BMI, higher DII was found to be associated with a lower FEV_1 (β (95%CI)= −3.44 (−6.50, −0.39); p=0.040)(109). Current smokers and those with asthma who did not demonstrate BHR were excluded from the study during recruitment. Therefore, the results may not be generalisable to all asthma sufferers and the representation of some asthma phenotypes may have been affected.

Studies using other dietary scores

Two studies have assessed the relationship between dietary pattern and lung function using other

dietary pattern scores. The first is the study by Yazdanpanah and colleagues, discussed above, which found no association between the HEI 2005 and the HEI 2010 (both developed based on the dietary guidelines) and lung function (166). The other is a small RCT conducted in the USA by Ma and colleagues involving 90 adults aged 18-70 years with uncontrolled asthma (n=46 and 44 for intervention and control groups respectively; 67% female, mean (SD) age 51.8 (12.4) years) (167). The intervention in this study was a dietitian-delivered behavioural modification program encouraging the DASH diet. The DASH diet is a dietary pattern first defined in a feeding study investigating the effects of diet on blood pressure (100). It emphasises high intakes of fruits, vegetables, and low-fat dairy products; low intake of total fat, saturated fat, cholesterol, red meat, sweets, and sugar-containing beverages; and includes whole grains, poultry, fish, and nuts. The diet was later modified to include limited salt intake (100, 101). In the study by Ma and colleagues, the dietary goals of the intervention group were high intake of fruit and vegetables (7-12 serves/day), and low-fat or fat-free dairy products (2-4 serves/day), and low intake of fat (27% of calories) and salt (≤2300 mg/day) (167). The intervention was delivered in two phases – the intensive phase, involving regular one-on-one and group consultations with the dietitian, and the maintenance phase, in which phone consultations with the dietitian were conducted monthly or more frequently if needed (167). After the follow up period of 6 months, dietary assessment by 24-hour diet recalls demonstrated improved DASH scores in the intervention group and no change in the control group. However, there was no difference in FEV_1, FVC or FEV_1/FVC between the two groups (167). The main limitation of this study was the small sample size, although this was designed to be a pilot study only. The study utilised a web-based system to randomise participants into intervention or control group whilst achieving a balance between groups in age, sex, ethnicity, smoking status, DASH score and asthma control questionnaire score. However, randomisation was likely still not effective in eliminating the effects of confounding because of the small sample. The study may also have lacked the power to observe any true associations. The follow-up period may have been too short to observe the effects of the DASH diet on lung function. Lastly, those with COPD were excluded from the study. This may have affected the representation of some asthma phenotypes, although, based on the method of asthma assessment and the age of the sample, it is possible that there was some ACO in the study sample.

Summary

Table 3.4 provides a summary of the characteristics of the above studies and their findings. The dietary pattern showing the most promise for a benefit for lung function is an anti-inflammatory diet, measured by the DII. There have only been 2 studies investigating the link between the DII and lung function, both of which have shown those with a more anti-inflammatory diet have better lung function (109, 111). However, further studies are needed to confirm the relationship and gain greater insight into the magnitude of the effects, the most beneficial dietary components and their optimum levels of consumption, and population groups that will benefit the most from dietary change. To help

clarify this latter point, studies are needed looking at the relationship between DII and BR and assessing effect modifiers of the relationship between DII and lung function.

The 4 studies examining the Mediterranean diet as the exposure variable had mixed results (140, 164-166). The methods used to measure the Mediterranean diet differed between studies, and some studies had significant limitations making it difficult to compare the studies and their findings. Three of the 4 studies were quite small, and the study populations were very different. These factors may explain the inconsistent results observed. Hence, the relationship between the Mediterranean diet and lung function remains inconclusive. There have been no studies investigating the relationship between the Mediterranean diet and BR. No relationships were observed between the other dietary pattern scores examined (the DASH diet, the HEI-2005, and the HEI-2010), and lung function, however, there was only a single study on each of these scores.

Given the promising results from the studies looking at the DII, future dietary pattern studies should focus on the potential relationship between the inflammatory effect of the diet and lung function and, as mentioned above, studies also looking at BR and potential effect modifiers would provide great insight. In general, studies examining BR as an outcome are needed as BHR is potentially a clinical indicator of certain phenotypes of asthma and COPD or risk of these phenotypes developing.

Literature search update

The literature search was repeated on 25th September 2020 to identify any research on the relationship between dietary patterns, lung function and BR published since the previous search. There were four new studies identified – two cross-sectional studies on the DII and lung function (182, 183), and two cross-sectional studies on the Mediterranean diet and lung function (144, 184).

Of the two cross-sectional studies on the DII and lung function, one study, performed by Ozbey and colleagues, involved 120 Turkish adults (aged 20-65 years) diagnosed with asthma at least one year earlier. This study found a higher DII was associated with a lower FEV_1 and FVC in those with asthma (182). The other, conducted by Han et al, involved 12,687 Hispanic/Latino adults aged 18-76 years. This study found a higher DII was associated with a lower FEV_1 and FVC in those without asthma (183). There was no association in those with asthma. These studies add further evidence that there is a relationship between the inflammatory potential of the diet and lung function, however, the population groups in which this relationship exists is unclear.

Of the two cross-sectional studies of the Mediterranean diet and lung function, one study of 3397 Canadian adults (aged 18-79 years), performed by Khanam and colleagues, found greater adherence to the Mediterranean diet was associated with a lower FEV_1 and FVC (144). The other study by Papassotiriou et al, involving 2108 middle-aged and older adults (≥ 50 years), found a higher Mediterranean diet score was associated with better lung function, measured using peak expiratory

flow rate (another measure obtained by spirometry) (184). The findings from these two studies do not change the summary of the literature above. Thus, the relationship between the Mediterranean diet and lung function remains inconclusive.

Table 3.4 - Dietary pattern and lung function (*a priori* dietary pattern methods): characteristics and findings

Author (year)	Study design	Country	Sample size	Sample description (age/sex)	Intervention (experimental studies)	Duration (experimental/ cohort studies)	Exposure/Outcome (method of measurement)	Confounders	Findings
Sexton (2013)(164)	RCT	New Zealand	38 Asthmatics	HI: 18.2% male, mean (SD) age 38.0 (4.2) years; LI: 33.3% male, 37.0 (4.0) years; CG: 33.3% male, 40.2 (4.0) years	3 arms: HI - Mediterranean diet, intensive advice and support; LI - less intensive advice, little support; CG - offered one session with a dietitian.	12 weeks	Exposure: HI vs LI vs CG; Outcomes: Δ pre- and post-bronchodilator FEV_1 and FVC	Age, sex	No differences in mean change in pre- and post-BD FEV_1 or FVC observed between the 3 groups
Barros (2008)(140)	Cross-sectional	Portugal	174 Asthmatics	82% female, mean (SD) age: 40 (15) years	N/A	N/A	Exposure: Alternate Mediterranean diet score (aMDS); Outcome: % predicted FEV_1	Age, sex, energy intake, rhinitis, education	No association between aMDS and % FEV_1
Gutierrez-Carrasquilla (2019)(165)	Cross-sectional	Spain	3,020	56.0% female; men aged 45-65 years, women aged 50-70 years	N/A	N/A	Exposure: Mediterranean diet score (MDS); Outcomes: abnormal FEV_1, non-obstructive ventilatory defect, obstructive ventilatory defect	Age, sex, BMI, physical activity	Women: Low MDS ↔ ↑ abnormal FEV_1 and non-obstructive ventilatory defects. Weak evidence low MDS ↔ ↑ obstructive ventilatory defects. Men: Low MDS ↔ ↑ obstructive ventilatory defects. Weak evidence MDS ↔ ↑ abnormal FEV_1. No association between MDS and non-obstructive ventilatory defects observed.

Author (year)	Study design	Country	Sample size	Sample description (age/sex)	Intervention (experimental studies)	Duration (experimental/ cohort studies)	Exposure/Outcome (method of measurement)	Confounders	Findings
Yazdanpanah (2016)(166)	Cross-sectional	Iran	121 COPD patients	85.1% men; mean (SD) age: 66.1 (10.9) years	N/A	N/A	Exposure: MDS, HEI-2005, HEI-2010; Outcomes: FEV_1, FVC	Age, sex, smoking status, education, BMI	↑ MDS ↔ ↑ FEV_1 and ↑ FVC. No associations between HEI-2005, HEI-2010 and lung function observed.
Han (2018)(111)	Cross-sectional	USA	22,294	Age range: 18-79 years; sex distribution of whole sample not reported.	N/A	N/A	Exposure: DII score; Outcomes: % predicted FEV_1, FVC and FEV_1/FVC	Household income, BMI, family history of asthma, serum cotinine level, current asthma	↑ DII score ↔ ↓ %FEV_1 and %FVC. No association was observed between DII score and %FEV_1/FVC.
Wood (2015)(109)	Case-control	Australia	99 asthmatics, 61 controls	Cases: 40% men, mean (SD) age 57.2 (2.8) years; Controls: 41% men, mean (SD) age 46.4 (4.5) years	N/A	N/A	Exposure: DII score; Outcome: FEV_1	Age, sex, smoking (pack-years), BMI	↑ DII score ↔ ↓ FEV_1
Ma (2016)(167)	RCT	USA	90 adults with uncontrolled asthma	67% female; mean (SD) age: 51.8 (12.4) years	Dietitian delivered behavioural modification program promoting the DASH diet	6 months	Exposure: DASH dietary intervention Outcomes: FEV_1, FVC, FEV_1/FVC	Analysed using LMM. Fixed effects in model were baseline measure of the outcome, age, sex, ethnicity, smoking status, DASH score, ACQ score, treatment, time-point, treatment x time interaction	There was no difference observed in FEV_1, FVC or FEV_1/FVC between the intervention and control groups at 6 months

3.3.2.2 *Studies using a posteriori methods*

Of the nine studies using *a posteriori* methods, one was longitudinal (170), seven were cross-sectional (139, 168, 169, 171-173, 175), and one was a case-control study (174). All studies used PCA to define the dietary patterns in the study population. All examined the relationship between the dietary patterns identified and lung function. Only one study also examined the relationship of the dietary patterns with BR (172).

The study carried out by McKeever and colleagues involved cross-sectional analyses of baseline data (n=12,648; 48% male, mean (SD) age = 41.5 (11.2) years) and longitudinal analyses performed in a subgroup of the study population who were followed up 5 years later (n=2,911; 50% male, mean (SD) age = 45.0 (9.5) years at baseline) (170). Participants at baseline completed an FFQ, the data from which was used to define dietary patterns using PCA. Three dietary patterns were identified in this Dutch study population. These patterns were termed "cosmopolitan" – characterised by high intakes of fish, vegetables, wine, and rice; "traditional" – defined by high intakes of red and processed meats, potatoes and added fat, and low intakes of dairy products and breakfast cereals; and "refined foods" – comprising high intakes of french fries, high-sugar beverages, mayonaise, salty snacks, and white bread, and low intakes of whole-grain bread (170). After adjusting for smoking (smoking status and pack years), BMI, and education level, this study found greater adherence to the traditional dietary pattern was associated with a lower FEV_1 (Q5 vs Q1: β (95%CI) = -94.4 mL (-123.4, -65.5); p-trend <0.001). There were no associations observed between the other dietary patterns and FEV_1. In the longitudinal analysis, no associations were observed between the dietary patterns and FEV_1 decline over the 5 year follow up period (170). This study has a number of limitations. There was a low response rate in 2 of the 3 areas from which the sample was drawn which may be a source of selection bias. The authors also created a prediction model for FEV_1 with age, age squared, height and an age-height interaction term as explanatory variables. FEV_1 was modelled separately for each sex using data from participants who had never smoked and did not have asthma or any wheezing. These prediction models were then used to predict FEV_1 for the whole study population and residuals were calculated as the difference between actual and predicted FEV_1 for each individual. These residual values were used as the outcome in their analyses. Using residual values as the outcome makes the interpretation of results more complicated and confusing. It is simpler to use the raw data and include the explanatory variables in the statistical model. This way the co-efficients are easier to interpret.

Brigham and colleagues also conducted a large cross-sectional study investigating the link between dietary patterns and lung function. This study involved 15,256 middle-aged and older adults from the USA (55.4% male; mean (SD) age 54.2 (5.8) years) (173). Dietary data collected during an interviewer-administered semi-quantitative FFQ were grouped into 29 food categories based on nutrient content. These food groups were then analysed by PCA. Two dietary patterns were identified explaining 7% and 5% of the variability in the data respectively. The patterns identified

were a “prudent” pattern, characterised by high intake of vegetables, fruit, fish, poultry, and whole grains; and a “western” pattern, characterised by high intake of refined grains, red and processed meats, french fries, eggs, and soda (173). After adjusting for age, BMI, sex, race, education, smoking (smoking status and pack-years), total energy intake, and physical activity, higher “western” dietary pattern scores (quintile 5 vs. quintile 1) were associated with lower percent predicted FEV_1 (for each sequential increase in quintile of dietary pattern β (95%CI) = -0.74 (-1.00, -0.48); p-trend <0.001), percent predicted FVC (β (95%CI) = -0.55 (-0.78, -0.31); p-trend <0.001), and FEV_1/FVC (β (95%CI) = -0.20 (-0.32, -0.08); p-trend = 0.001). Higher “prudent” dietary pattern scores (quintile 5 vs. quintile 1) were associated with higher percent predicted FEV_1 (β (95%CI) = 0.38 (0.19, 0.58); p-trend <0.001) and FEV_1/FVC ratio (β (95%CI) = 0.22 (0.13, 0.31); p-trend <0.001) (173). This study has a number of limitations. Only a small portion of the variability in the data was explained by the dietary patterns identified. This may indicate that the food groups were not highly correlated, in which case PCA would not be an appropriate method of analysis (for further discussion refer to Chapter 7). This study also used percent predicted values of the lung function measures. As discussed previously, this is not advised in such a large sample where age, sex, height and ethnicity can simply be added to the statistical model. Lastly, the FFQ was administered by interview and, therefore, the data may be affected by interviewer bias.

Another cross-sectional study, conducted by Steinemann and colleagues, involved 2,178 Swiss adults aged 37-81 years (46.4% male, mean (SD) age 58.6 (10.6) years) (175). Intakes of food items determined from a self-administered semi-quantitative FFQ were grouped into 25 food groups based on type of food and nutrient content. These food groups were analysed by PCA. Three of the resulting components were retained representing the “prudent” diet, high in vegetables, fruits, water/tea/coffee, fish, and nuts; the "western" diet, high in meat, sausage, egg, fish, and alcohol; and the "high carbohydrate" diet, high in sweet spreads, bread, dessert, and potatoes (175). In multivariate linear regression analysis, this study found weak evidence of a positive association between the “prudent” diet and FEV_1 (mean change per one SD increase in “prudent” diet score = 22.5 ml (95%CI –2.53, 47.58); p=0.08). There were no other associations observed between any of the dietary patterns and FEV_1 or FEV_1/FVC (175). Potential confounders that were adjusted for were sex, age, height, smoking (status, pack-years, cigarettes/day), exposure to passive smoking in the last 12 months, parental smoking during childhood, education, marital status, employment status, physical activity, total energy intake, and BMI. This study has a number of limitations. The FFQ assessed diet over the previous 4 weeks which means seasonal variation in the diet was not captured and diet may have varied between participants depending on the time of year at which the FFQ was completed. It is unclear whether correlation of the food groups was investigated prior to using PCA and the proportion of variance explained by the components identified was not reported. Therefore, it is unclear if PCA was an appropriate method of analysis for the data. Lastly, the consumption of food groups was expressed as a function of body weight (g food per day/kg body weight). It is unclear why the authors did this. It is common for food consumption to be expressed as a function of

energy intake. This is done to assess the quality of the diet rather than absolute quantities of foods consumed. However, expressing food consumption as a function of body weight would not produce the same outcome and is unnecessary in this case as energy intake would have been available from the semi-quantitative FFQ. Expressing food consumption in this way has also unnecessarily complicated the interpretation of the results.

Shaheen and colleagues performed a cross-sectional analysis using data from the Hertfordshire Cohort Study conducted in the UK. This study involved 1,551 men and 1,391 women born between 1931 and 1939 (mean (SD) age 66.1 (2.8) years) (171). Foods were classified into 51 groups based on food type and nutrient content. These food groups were then analysed via PCA. Two dietary patterns were identified which together explained 13.3% of the variance in the dietary data. The patterns were the"prudent" pattern – high in fruit, vegetables, oily fish and wholemeal cereals and low consumption of white bread, added sugar, full-fat dairy products, chips and processed meat; and the "traditional" pattern - high in vegetables, fish, red meat, meat products, and puddings and low in milk drinks, reduced fat spread and breakfast cereals (171). After adjusting for potential confounders, this study found that, in females, the ‘‘prudent’’ dietary pattern was positively associated with FEV_1 (Q5 vs Q1: 0.08 L, trend p=0.008) and FVC (Q5 vs Q1: 0.07 L, p=0.007). There was no association with FEV_1/FVC. Similar results were observed in males (Q5 vs Q1: 0.18 L, p <0.001 and Q5 vs Q1: 0.07 L, p=0.044 for FEV_1 and FVC respectively); however, the “prudent” pattern was also positively associated with FEV_1/FVC (Q5 vs Q1: 0.013 L, p=0.002) (171). The main limitations of this study were poor measure of diet, low response rate, and overadjustment in the analysis. Diet was assessed during the previous 3 months only. Therefore, seasonal variation was not captured and some inter-individual variation may be due to the time of year the FFQ was completed. Weekly frequencies of consumption of food groups were used in the PCA rather than quantities of intake. This does not account for differences in serving sizes between individuals. Only 53% of those invited agreed to take part in the first part of the study (a home interview). This may be a source of response bias. Lastly, the authors adjusted for a large number of potential confounders in the multivariate analysis. The confounders were age, height, smoking (status and pack-years), second-hand smoke exposure at home, a number of SES measures (age left education, home ownership status, number of rooms for household use, number of cars for household use, social class, father’s social class at subject’s birth), body fat mass, physical activity, energy intake, alcohol consumption, dietary supplement use, birthweight, use of inhaled or oral steroids and paracetamol use. This may have resulted in overadjusting, particularly for SES.

Another study, conducted by Cho and colleagues, investigated the relationship between dietary patterns and lung function in 7,615 Korean women aged 40-90 years (mean (SD) age 56.9 (8.7) years) (169). In this study, frequency of consumption of foods was measured using an FFQ. Food items were grouped into 18 food groups which were then analysed by PCA. Two dietary patterns were identified – a “balanced” diet, high in vegetables, fish, meat, seaweeds, and mushrooms; and a

"refined" diet, high in snacks, breads, dairy products and fast foods. These diets accounted for 15.8% and 9.8% of the variance in frequency of consumption of the food groups, respectively (169). After adjusting for age, BMI, smoking (status and packs/day), secondhand smoking, exercise, and SES variables (education, income, occupation, and residence area), a "balanced" diet was positively associated with FVC (OR: 1.33 (1.09, 1.64). However, there was no association with FEV_1, predicted FEV_1, predicted FVC, and FEV_1/FVC. A "refined diet" was negatively associated with FEV_1 and predicted FEV_1 and FVC (OR 0.84 (0.68, 1.04), 0.79 (0.66, 0.93), and 0.84 (0.70, 0.99) respectively). No associations were observed between "refined diet" and FVC or FEV_1/FVC (169). The limitations of this study include the use of a food frequency questionnaire which did not consider serving size. The lung function variables were also categorised and analysed using multivariate logistic regression. This results in unneccessary loss of information. The authors could have left the variables as continuous and used linear regression instead. The interpretation of the results is also unclear due to lack of information on the categories created from the lung function measures.

Hooper and colleagues also carried out a cross-sectional study involving 1174 adults aged 29-55 years from Germany, UK and Norway (172). Participants completed an FFQ from which food and nutrient intakes were determined. The FFQs differed slightly per country. In order to combine the data, some food items were aggregated, creating 74 foods/food groups. The correlation coefficients of the 74 food groups were transformed to create a pooled correlation coefficient for the whole study population, weighted according to the sample size at each centre. These pooled correlation coefficients were analysed using PCA (172). Two dietary patterns were identified which together explained 11.2% of the variance in the dietary data. The dietary patterns were a "meat and potato" pattern, characterised by high intakes of meat and meat products, eggs, potato and chips, and in some centres, bread, butter, biscuits and cakes; and a "fish, fruit and vegetables" pattern, characterised by high intakes of fruits, vegetables, and fish (172). After adjusting for age, sex, social class, smoking status, exercise, BMI, total energy intake and the other dietary pattern, there were no associations observed between either dietary pattern and BR or FEV_1 (172). This study had some limitations. The participation rate was 51%. Although this is a good response rate for a study involving a clinic visit, it is still low and the results may be impacted by response bias. Also, the dietary patterns explained only a small portion of the variance in the data. This indicates that the data may not be highly correlated, in which case, PCA is not an appropriate method of analysis.

Another study was performed by Garcia-Larsen and colleagues, this time in a younger sample, aged 22-28 years, from Chile (n=1,187; 54.3% female) (139). Intakes of 65 foods were determined by FFQ and analysed by PCA. Two dietary patterns were identified – an "animal proteins and starchy foods" pattern, high in ribs, ham, frankfurts, eggs, cheese, potato, pasta, and bread; and a "fruits and vegetables" pattern, high in pumpkin, onion, carrot, beetroot, garlic, tomato, beans, and capsicum. These dietary patterns explained 24.7% of the variance in the original 65 food items (139). Scores

were calculated for each individual for each dietary pattern, converted to z scores, and categorised into quintiles. Multivariate linear regression analyses between each pattern and the lung function outcomes FEV_1, FVC and FEV_1/FVC showed no associations. Confounders included in the models were height, sex, age, current smoking, overcrowding, education, SES, birth weight, BMI, total energy intake (139). This was a very good quality study; however, no study is without limitations. The results may be affected by residual confounding, particularly from smoking. There are also the potential sources of bias from the use of an FFQ, as discussed in section 3.2. A cross-sectional association is not evidence of causation. This study also used a young population. Therefore, the relevance of these results to a middle-aged and older population is unclear.

The last cross-sectional study, conducted in Spain by Sorli-Aguilar and colleagues, involved 207 current smokers, aged 35-70 years, without respiratory disease (44.0% men; mean (SD) age 50.7 (9.0) years) (168). Data from a 45-item FFQ were categorised into 19 food groups based on nutrient composition and these food groups were analysed by PCA. Three dietary patterns were identified – a "Mediterranean-like" pattern, high in fruit, vegetables, legumes, potatoes, fish, eggs, dairy desserts, whole grains and refined grains; a "western" pattern, high in red and processed meats, whole and refined grains and sugary drinks, and low in fruit and vegetables; and an "alcohol-consumption" pattern, high in wine, beer, distilled drinks, and nuts and dried fruit. These patterns explained 12.8%, 9.9% and 8.3% of the variation in the food group data, respectively (168). The outcome in this study was impaired lung function defined as FEV_1 and/or FVC <80% of the predicted value and/or FEV_1/FVC <0.7. Of the 207 participants, 47 had impaired lung function (19 women and 28 men). After adjusting for age, sex, SES, height, weight, waist circumference, physical activity and cumulative tobacco use, this study found women with higher "western" pattern scores had greater risk of impaired lung function (tertile-3 (T3) vs tertile-1 (T1): OR (95%CI) 5.62 (1.17, 27.02), p=0.031; interaction p=0.011). There was no association between the "western" pattern and impaired lung function in men. The "alcohol-consumption" pattern was also positively associated with impaired lung function (T3 vs T1: OR (95%CI) 4.56 (1.58, 13.18), p = 0.005). There was no association observed between the "Mediterranean-like" pattern and impaired lung function (168). There are a number of limitations of this study. Firstly, exclusion of people with respiratory disease means the older participants are likely to be genetically protected against the development of respiratory disease, especially considering they are all smokers. There are also a number of issues with the statistical analyses. Firstly, lung function measures were categorised and then all categories indicating poor lung function were grouped. Therefore, indicators of restrictive lung disease and different types of obstructive lung disease were grouped, limiting the usefulness of the result and potentially obscuring any associations specific to the type of lung disease. As the sample size is small, the best option for the authors would have been to use the lung function measures as continuous variables in a linear regression. There were also too many confounders, particularly for the stratified analyses, which widens confidence intervals and limits the power of the analysis to detect any true associations. If all of these confounders and stratification were necessary, the

investigators should have obtained a larger study population. Lastly, p-value for the trend across tertiles of the dietary patterns were not calculated which would have been useful in determining if there was a dose-response type relationship.

The final manuscript reviewed describes a case-control study performed by Bakolis and colleagues, involving 3,206 individuals aged 15-77 years from 9 European countries (Denmark, Finland, Sweden, UK, Germany, Portugal, Belgium, Poland, and the Netherlands) (174). The study sample was drawn from the participants of the Global Asthma and Allergy Network of Excellence (GA^2 LEN) follow-up study, a population-based study conducted across 11 European countries investigating risk factors of asthma and allergy in adolescents and adults. Three groups of cases (those with asthma, those with sinusitis, and those with both asthma and sinusitis) and one group of controls (those with neither asthma nor sinusitis) were invited to take part in a follow up study. Participants completed a questionnaire, including a 239-item FFQ, and a laboratory assessment which included spirometry testing. Items consumed infrequently were excluded, leaving 196 food items. The correlation coefficients of these food items were calculated from the dietary data of the control participants in each country. These correlation coefficients were transformed and pooled correlation coefficients were determined between each food item for the whole control group, weighted according to the number of control participants in each country. The pooled correlation coefficients were then analysed by PCA, identifying two dietary patterns – an "animal proteins and carbohydrates" pattern, and a "fruit and vegetables" pattern. Together these patterns explained 16.8% of the variance in the dietary data (174). After adjusting for confounders, this study found those with higher "animal proteins and carbohydrates" pattern scores had lower log-transformed pre- and post-BD FEV_1 and FVC (all p-values <0.001)). However, there was no association with pre- or post-BD FEV_1/FVC. There was moderate evidence of a positive association between the "fruit and vegetables" pattern and pre-BD FEV_1/FVC (for each increase in quintile of F&V intake, mean FEV_1/FVC was higher by 0.02 (95%CI 0.0, 0.03; p=0.056); however no associations were observed with the other spirometry outcomes (174). Confounders considered in the analyses were age, sex, height, weight, smoking status, BMI, age at completion of full-time education, occupation, use of nutritional supplements, the other dietary pattern, and total energy intake (TEI). This study has some limitations. The FFQ used was not semi-quantitative. Food quantities were determined using a standard portion size. However, portion sizes differ between individuals, therefore, this may not provide a very accurate estimation of intake. The number of cases and controls per country was not reported. Therefore, it is unclear the amount of data from which the correlation coefficients and dietary patterns were determined. Sample sizes from half of the countries were fewer than 200 participants overall so the number of controls from these countries may have been quite small. The dietary pattern scores were classified into quintiles and this categorical variable was used as a continuous variable (i.e. Q1=1, Q2=2, etc). This is clearly not a continuous variable with an equal measurable change between quintiles. At the very least the median score for each quintile should have been calculated and these scores then used as a pseudo-continuous variable. Lastly, the dietary

patterns identified explained only a small proportion of the variance in the data. Therefore, the data may not have been highly correlated and PCA may not have been the most appropriate method of analysis.

There are a couple of other points that should be considered when interpreting and comparing the findings of the above research. Firstly, although some dietary patterns may have the same or similar names across different studies, the patterns themselves will be different as the data from which the patterns were derived is different. This makes comparison between studies difficult. The response rates of the studies were often not reported and, therefore, any effect of response bias due to low participation rates is unknown. Lastly, the majority of the studies grouped foods according to nutrient composition prior to PCA. This results in loss of information and assumes a similar relationship between each individual food in a group and the outcome, which may not be the case.

Summary

Table 3.5 provides a summary of the characteristics of the above studies and their findings. Due to the way in which dietary patterns are defined (i.e. using PCA) the patterns are different between studies. However, there are some similarities between the patterns. There are usually 2 or 3 patterns identified – one high in fruit, vegetables, fish and/or wholegrains, often termed the "prudent" pattern; one high in red and processed meats, often called the "western" or "traditional" pattern; and sometimes a third pattern is identified, high in bread, potato/fries, soft drink and sweets, often named the "refined foods" or "high carbohydrate" pattern. When this third pattern is not identified, elements of this pattern are usually part of the "western/traditional" pattern. By comparing the results of patterns with these similarities, we can see if any consistency in the findings begins to emerge.

Of the 9 studies using an *a posteriori* method, 7 had a prudent-type pattern and the remaining 2 had a fruit and vegetables pattern. The findings for these patterns were mixed, with 3 of the 7 studies with a prudent-type pattern finding moderate or stronger evidence of a positive association between this pattern and at least one lung function measure, and 1 of the 2 studies with a fruit and vegetables pattern also finding a positive association. The most convincing evidence appears to be for an inverse relationship between the western/traditional dietary pattern and lung function, with 5 of the 9 studies observing a negative association with at least one lung function measure and 3 of these 5 studies observing this relationship consistently across 3 or 4 lung function measures (169, 173, 174). This indicates that the findings are less likely to be due to chance. Neither of the studies identifying a refined foods/high-carbohydrate pattern observed a relationship between this pattern and lung function; however, only 2 studies found such a pattern. Further studies are needed to definitively conclude whether a relationship exists or not. There has only been one study assessing dietary patterns by PCA and relating these patterns to BR. This study observed no relationship between a "meat and potato" diet or a "fish, fruit and vegetables" diet and BR, but again this was only one study. Further studies are needed to clarify the association between dietary patterns and BR.

There are a few gaps in the evidence to date. Firstly, there are generally not enough good quality studies to draw a conclusion on the relationship between dietary patterns and lung function. More high-quality studies in different population groups with different levels and variabilities in intake of foods will help to determine, not only if a relationship exists, but the optimum intake levels to achieve maximum benefit and the population groups that stand to benefit most. There have not been any dietary pattern analyses by PCA using nutrients rather than foods. This is another method that can be used to provide complementary evidence to the existing dietary pattern, food/food group and nutrient data. Nutrient pattern analyses have been conducted for other diseases such as cancer and may assist in assessing the relationship between diet and lung function (185-189). Lastly, more studies assessing BR as an outcome and examining potential effect modifiers of the diet-lung function relationship are needed to help identify the disease phenotypes and/or population groups that would benefit most from dietary change. This should be the focus of future studies, the results of which will help guide future RCTs in this area by highlighting the population groups of interest.

Literature search update

The literature search was updated on 25th September 2020 to identify any recent research on the relationship between dietary patterns, lung function and BR. There were no new studies identified using *a posteriori* methods.

Table 3.5 - Dietary pattern and lung function (*a posteriori* dietary pattern methods): characteristics and findings

Author (year)	Study design	Country	Sample size	Sample description (age/sex)	Duration (cohort study only)	Exposure/Outcome (method of measurement)	Confounders	Findings
McKeever (2010) (170)	Cohort	The Netherlands	n=12,648 (cross-sectional analysis); n=2,911 (longitudinal analysis)	Cross-sectional: 48% male, mean (SD) age 41.5 (11.2) years; longitudinal: 50% male, mean (SD) age at baseline 45.0 (9.5) years	5 years	Exposures: dietary patterns derived by PCA; Outcomes: FEV_1 (cross-sectional analysis), FEV_1 decline (longitudinal analysis)	Smoking status, pack years, BMI, education level	3 dietary patterns identified: cosmopolitan, traditional, and refined foods. ↑ traditional dietary pattern score ↔ ↓ FEV_1. No association between traditional diet and FEV_1 decline. No associations between the cosmopolitan or refined foods dietary patterns and FEV_1 or FEV_1 decline.
Brigham (2018) (173)	Cross-sectional	USA	15,256	55.4% male; mean (SD) age 54.2 (5.8) years	N/A	Exposures: Dietary patterns derived by PCA; Outcomes: % predicted FEV_1 and FVC, FEV_1/FVC	Age, sex, ethnicity, BMI, energy intake, education, smoking status, pack-years, physical activity	2 dietary patterns identified: prudent and western. ↑ western dietary pattern score ↔ ↓ %FEV_1, %FVC and FEV_1/FVC. ↑ prudent dietary pattern score ↔ ↑ %FEV_1 and FEV_1/FVC. There was no association with %FVC.
Steinemann (2018) (175)	Cross-sectional	Switzerland	2,178	46.4% male; mean (SD) age 58.6 (10.6) years	N/A	Exposures: Dietary patterns derived by PCA; Outcomes: FEV_1, FEV_1/FVC	Age, sex, height, smoking status, pack-years, cigarettes/day, passive smoking in the last 12 months, parental smoking during childhood, education, marital status, employment status, BMI, physical activity, total energy intake	3 dietary patterns were identified: prudent, western and high carbohydrate. There was weak evidence of a positive association between the prudent pattern and FEV_1. No other associations were observed between any of the dietary patterns and FEV_1 or FEV_1/FVC.

Author (year)	Study design	Country	Sample size	Sample description (age/sex)	Duration (cohort study only)	Exposure/Outcome (method of measurement)	Confounders	Findings
Shaheen (2010) (171)	Cross-sectional	UK	2,942	52.7% male; mean (SD) age 66.1 (2.8) years	N/A	Exposures: Dietary patterns derived by PCA; Outcomes: FEV_1, FVC, FEV_1/FVC	Age, height, smoking status, pack-years, passive smoking at home, age left education, home ownership, number of rooms for household use, number of cars for household use, social class, father's social class at subject's birth, body fat mass, physical activity, energy intake, alcohol consumption, dietary supplement use, birthweight, use of inhaled or oral steroids, paracetamol use Analysis stratified by sex.	2 dietary patterns were identified: prudent and traditional Females: ↑ prudent pattern score ↔ ↑ FEV_1 and FVC. There was no association with FEV_1/FVC. Males: ↑ prudent pattern score ↔ ↑ FEV_1, FVC and FEV_1/FVC. There were no associations between the traditional dietary pattern and any lung function outcomes in either sex.
Cho (2014) (169)	Cross-sectional	South Korea	7,615 women	Mean (SD) age 56.9 (8.7) years	N/A	Exposures: Dietary patterns derived by PCA; Outcomes: FEV_1, FVC, % predicted FEV_1 and FVC, FEV_1/FVC	Age, smoking status, packs/day, second-hand smoking, BMI, exercise, education, income, occupation, residence area	2 dietary patterns identified: a balanced diet and a refined diet. ↑ balanced diet score ↔ ↑ FVC. There were no associations with FEV_1, %FEV_1, %FVC, or FEV_1/FVC. ↑ refined diet score ↔ ↓ FEV_1, %FEV_1, and %FVC. There was no association with FVC or FEV_1/FVC.
Hooper (2010) (172)	Cross-sectional	Germany, UK and Norway	1,174	Age range: 29-55 years. Age and sex distributions not provided.	N/A	Exposures: Dietary patterns derived by PCA; Outcomes: FEV_1, BHR slope	Age, sex, social class, smoking status, BMI, exercise, total energy intake, the other dietary pattern	2 dietary patterns were identified: "meat and potato", and "fish, fruit and vegetables". Neither dietary pattern was associated with FEV_1 or BHR slope.

Author (year)	Study design	Country	Sample size	Sample description (age/sex)	Duration (cohort study only)	Exposure/Outcome (method of measurement)	Confounders	Findings
Garcia-Larsen (2015) (139)	Cross-sectional	Chile	1,187	Aged 22-28 years (distribution not provided), 54.3% female.	N/A	Exposures: Dietary patterns derived by PCA; Outcomes: FEV_1, FVC, FEV_1/FVC	Age, sex, height, current smoking, overcrowding, education, SES birth weight, BMI, total energy intake	Two dietary patterns were identified: "animal proteins and starchy foods" and "fruits and vegetables". Neither dietary pattern was associated with FEV_1, FVC or FEV_1/FVC
Sorli-Aguilar (2016) (168)	Cross-sectional	Spain	207 current smokers	44.0% male; mean (SD) age 50.7 (9.0) years	N/A	Exposures: Dietary patterns derived by PCA; Outcomes: impaired lung function (FEV_1 and/or FVC <80% predicted and/or FEV_1/FVC <0.7)	Age, sex, height, SES, weight, waist circumference, physical activity, cumulative tobacco use	3 dietary patterns identified: "Mediterranean-like", "western" and "alcohol-consumption". ↑ western dietary pattern score ↔ ↑ risk of impaired lung function in women only. ↑ alcohol-consumption pattern score ↔ ↑ risk of impaired lung function. No association between the Mediterranean-like pattern and impaired lung function.
Bakolis (2018) (174)	Case-control	9 European countries – Denmark, Finland, Sweden, UK, Germany, Portugal, Belgium, Poland, the Netherlands	3 groups of cases (those with asthma, sinusitis, and both), control group with neither condition. total n=3,206	43.1% male; mean (SD) age 47.6 (15.1) years	N/A	Exposures: Dietary patterns derived by PCA; Outcomes: pre- and post-BD FEV_1, FVC and FEV_1/FVC	Age, sex, height, weight, smoking status, BMI, age at completion of full-time education, occupation, use of nutritional supplements, total energy intake, the other dietary pattern	Two dietary patterns identified: "animal proteins and carbohydrates" and "fruit and vegetables". ↑ "animal proteins and carbohydrates" pattern scores ↔ ↓ log-transformed pre- and post-BD FEV_1 and FVC. There was no association with pre- or post-BD FEV_1/FVC. ↑ "fruit and vegetables" pattern scores ↔ ↑ pre-BD FEV_1/FVC (p=0.056). There were no associations observed with the other spirometry outcomes.

Studies examining asthma and COPD as outcomes

A brief summary of the evidence of the relationship between dietary patterns and asthma and COPD is given below. As the outcome of interest in this thesis is lung function and BR, details relating to the quality and limitations of the studies will not be described.

Studies examining asthma as an outcome

I conducted a comprehensive search of the literature, identifying14 original research articles investigating the relationship between dietary patterns and asthma. Three of these studies used an *a priori* approach to define dietary patterns (109, 111, 190) and 11 used an *a posteriori* method (170, 172-174, 191-197). The results of studies using these two methods will be discussed separately.

Of the three studies using an *a priori* approach to define dietary patterns, two examined the inflammatory potential of the diet using the DII, and one assessed the Alternate Healthy Eating Index 2010 (AHEI 2010). The two studies using the DII had conflicting results. A case-control study found a more pro-inflammatory diet was associated with greater odds of asthma, after controlling for confounders, whilst a large cross-sectional study found no relationship (109, 111). The other study used the AHEI 2010, representing a diet high in whole grains, PUFAs (particularly omega-3s), and nuts, and low in red and processed meats, refined grains, and sugar sweetened beverages. This study was a large longitudinal study of US health professionals. No relationship was observed between the AHEI 2010 and newly diagnosed adult-onset asthma over follow up periods of 12 years and 16 years for men and women respectively (190). These studies do not provide conclusive evidence and further research is needed in this area.

Of the 11 studies using an *a posteriori* approach, 9 used PCA to define the dietary patterns, one used latent trait modelling, and the last compared the results from PCA and confirmatory factor analysis. There were 4 longitudinal studies (191, 192, 195, 197), 4 cross-sectional studies (170, 172, 173, 196), one study that performed both cross-sectional and longitudinal analyses (193), and two case-control studies (174, 194). Although the dietary patterns identified were different across the studies, there were some similarities in the patterns. The results of similar patterns are compared here to identify any consistent findings.

Eight studies identified a “prudent” dietary pattern or similar, characterised by high intakes of fish, fruit, vegetables, and/or wholegrains. None of the four longitudinal studies found any association between the “prudent” pattern and newly diagnosed adult-onset asthma (191-193, 197). The follow-up periods of these studies ranged from 10 to 16 years. Varraso and colleagues also performed a cross-sectional analysis of baseline data and found no association between the “prudent” dietary pattern and asthma prevalence (193). Of the three cross-sectional studies, two found no association with asthma prevalence and one observed an inverse relationship (172, 173, 196). The final study, a case-control design, also observed no association between a “prudent” dietary pattern and asthma (194). In summary, only one of eight studies observed an inverse association, which suggests there is

no relationship between a prudent dietary pattern and asthma. However, there are several reasons why some studies may have found no association when there truly is a relationship. These include residual confounding, studying a different population group to that within which an association exists (i.e. different age group, geographical area, disease phenotype), differences in the dietary patterns, a relationship of small magnitude that has been attenuated to the null by random errors in the data, and using PCA or a similar statistical method when the data is not highly correlated. Further studies and assessment of potential effect modifiers are needed.

Nine studies identified a "western/traditional" dietary pattern or similar, characterised by high intakes of red meat, processed meats, potato, bread, pasta, and/or sweets. Of the four longitudinal studies, one found a positive association with incidence of asthma over approximately 12 years (197) whilst the other 3 studies found no relationship (191-193). Varraso and colleagues also observed no association with asthma prevalence in cross-sectional analysis of their baseline data (193). None of the three cross-sectional studies observed a relationship between a "western/traditional" diet and asthma prevalence (170, 172, 173). The study by McKeever and colleagues identified two patterns containing elements of the "western/traditional" pattern described above. The patterns were termed "traditional" and "refined foods". The "traditional" pattern was high in red and processed meats and potato, and the "refined foods" pattern was high in fries, high sugar beverages, white bread and salty snacks. Neither pattern was associated with prevalence of asthma (170). There were also two case-control studies, one of which found a positive association between the "western/traditional" pattern and current asthma. The other study observed no association (174, 194). Therefore, 2 of the 9 studies observed a positive association. Given the differences that exist between studies and the limitations of each, this is not enough evidence to draw a definitive conclusion. Therefore, further studies are needed, ideally including an assessment of effect modifiers.

There were two case-control studies that identified a "fruit and vegetables" pattern, both of which observed no association with asthma prevalence (174, 194), and two longitudinal studies with a "nuts and wine" pattern, both of which observed no relationship with asthma incidence or prevalence (193, 197). The remaining dietary patterns from the studies using an *a posteriori* method were not similar enough across studies to compare and draw any conclusions from the results.

Two systematic reviews have been published on dietary patterns and asthma. One was a review of the western dietary pattern and adult asthma by Brigham and colleagues. However, this review included studies that were not strictly speaking dietary pattern studies, they simply asked some questions about the frequency of consumption of pre-packaged, processed and/or fast foods (198). The other review, by Lv and colleagues, included studies with outcomes of ever asthma (not asthma prevalence), asthma control, and asthma quality of life (199). Because of the issues with asthma control as an outcome measure outlined in Chapter 2, I have focussed this review on studies assessing current asthma, asthma prevalence and asthma incidence as outcomes. Hence, the findings

of these reviews have not been discussed here.

Studies examining COPD as an outcome

A comprehensive search of the literature identified 12 original studies investigating the relationship between dietary patterns and COPD. Five of these studies used an *a priori* approach (165, 190, 200-202) and 7 used an *a posteriori* method (170, 171, 173-175, 191, 192).

Of the five studies using an *a priori* approach, two examined the inflammatory potential of the diet using the DII, two explored the link between the Mediterranean diet and COPD using different Mediterranean diet scores, one assessed a measure of a general healthy diet using the Alternate Healthy Eating Index 2010 (AHEI 2010), and one looked at the DASH diet (note: one study examined two dietary pattern scores). The two studies using the DII were quite different; however, the results were consistent. One was a longitudinal study which found a more pro-inflammatory diet was associated with COPD mortality after adjusting for potential confounders (mean (SD) follow up period 20.7 ± 7.0 years) (201). The other was a cross-sectional study of current and heavy smokers which found a more pro-inflammatory diet was associated with greater odds of emphysema detected via a computed tomography (CT) scan. However, there was no association between DII and self-reported COPD/emphysema (200). The consistency of these results indicates that a true relationship may exist between the inflammatory potential of the diet and COPD prevalence and mortality, however, two studies are not enough to draw a definitive conclusion. Further studies are needed.

The findings of the two studies that assessed the Mediterranean diet were also consistent, suggesting a greater adherence to a Mediterranean diet may be protective against COPD. Both studies were cross-sectional in design. Maisonneuve and colleagues found a higher adherence to a Mediterranean diet, examined using the alternate Mediterranean diet score (aMDS), was associated with lower odds of emphysema detected by CT scan; however, there was no association with self-reported COPD/emphysema (200). Gutierrez-Carrasquilla and colleagues found a higher Mediterranean diet score was associated with lower odds of obstructive ventilatory defects in a sample of middle-aged adults free from lung disease (165). Obstructive ventilatory defects were detected by spirometry and defined as $FEV_1/FVC < 0.70$, which is a diagnostic measure of COPD. Again, although the results are consistent, no conclusions can be drawn from a couple of studies and further research is needed.

There was only one study which looked at the AHEI 2010 diet score. This longitudinal study observed an inverse association between the AHEI 2010 score and risk of newly diagnosed COPD over a period of 12 years and 16 years for men and women respectively (190). The final *a priori* study, a small case-control study, found no association between the DASH score and COPD (202). Due to the lack of studies using the AHEI 2010 and DASH scores, no conclusions on the relationship between these dietary patterns and COPD can be drawn. Again, more research is needed in a variety of population groups and exploring potential effect modifiers to fully understand the

relationships between these dietary patterns and COPD.

All 7 studies using an *a posteriori* approach defined their dietary patterns via PCA. There were 2 longitudinal studies (191, 192), 4 cross-sectional studies (170, 171, 173, 175), and one case-control study (174). Five of these studies identified a "prudent" dietary pattern. The two longitudinal studies both found higher "prudent" pattern scores were associated with a lower risk of newly diagnosed COPD over a period of 12 and 16 years (191, 192). The other three studies were cross-sectional in design. One observed a similar inverse relationship between the "prudent" pattern and COPD prevalence (173), one observed an inverse relationship in men only (171), and one observed no relationship (175). These results suggest an inverse relationship between the prudent diet and COPD incidence and prevalence, however, further studies are needed to confirm the relationship and gain greater understanding of the strength of the effect, the key components of the prudent diet that are of benefit, the optimum levels of consumption of these components, and the population groups that can benefit most from dietary change.

All 7 studies using an *a posteriori* approach found a "western/traditional" or similar dietary pattern. Both longitudinal studies found those with higher "western/traditional" diet scores had a greater incidence of COPD 12 and 16 years later respectively, after controlling for confounders (191, 192). Of the four cross-sectional studies, one observed a positive association between a "western/traditional" diet and COPD prevalence (173) and two observed no relationship (171, 175). The fourth cross-sectional study, by McKeever and colleagues, identified two patterns containing elements of the "western/traditional" pattern – the "traditional" pattern, high in red and processed meats and potato, and the "refined foods" pattern, high in fries, high sugar beverages, white bread and salty snacks. The "traditional" pattern was positively associated with the prevalence of COPD, whilst the "refined foods" pattern had no relationship (170). The final study, a case-control study, also observed no relationship between a "western/traditional" diet and COPD (174). Therefore, 4 of the 7 studies observed a positive association. These results suggest a "western/traditional" dietary pattern may contribute to the development of COPD; however, further research is needed to draw a definitive conclusion and fully understand this relationship. The different results observed in the study by McKeever and colleagues from two patterns with elements of the "western/traditional" may be highlighting the reason for the inconsistency in the findings across studies. Perhaps there truly is a relationship between diets high in red and processed meats and potato and no relationship with refined grains, sugars and processed foods and the findings of a study depend on the loadings of these foods in the dietary patterns identified. Further research exploring the relationship between dietary patterns, foods and nutrients and COPD is needed to determine if this is the case. Assessment of effect modifiers will also help clarify the population groups for which any relationships are relevant.

Only two studies observed a third dietary pattern – a "refined foods/high carbohydrate" pattern, high

in bread, potato/fries, soft drink and sweets (170, 175). Both of these studies were cross-sectional, and both found no association with the prevalence of COPD. This is, however, only two studies and more research is required to draw a definitive conclusion.

A systematic review and meta-analysis of the relationship between dietary patterns and COPD was published by Zheng and colleagues in 2016; however, this review included studies that were not strictly speaking dietary pattern studies (203). That is, they did not use a dietary pattern score or a statistical method to define dietary patterns. Therefore, the findings from this review have not been discussed here.

Overall summation

Overall, based on all the dietary pattern studies, it appears that a 'healthy diet' may assist in preserving lung function, however the results are mixed. This may be because the magnitude of the effect is small or because the errors in the data have attenuated the result to the null. A 'healthy diet' is also probably protective against the development of fixed airway obstruction (i.e. COPD), as indicated by the findings of the good quality longitudinal and cross-sectional studies. There is little evidence to suggest any benefit of a 'healthy diet' on asthma. It is important to note, however, that the dietary pattern scores used were not developed from research on lung health or disease and therefore any lack of association in these studies may simply indicate that that score does not represent the ideal diet to preserve lung function and minimise the risk of obstructive lung disease. Similarly, the lack of associations in studies using an *a posteriori* approach may be because the dietary patterns identified, or the levels at which foods are consumed, are not the ideal pattern or levels for optimum lung health.

Theoretically, the DII offers the most biologically plausible dietary pattern score for a relationship with lung function and obstructive lung disease. The limited research available indicates that a more pro-inflammatory diet is probably associated with worse lung function and/or increased risk of lung disease (5 of 6 studies observed an association), however further studies are needed to gain a greater understanding of this relationship and establish causality.

Studies using an *a posteriori* approach have shown that an 'unhealthy diet', as indicated by a "western/traditional" dietary pattern, is probably associated with poorer lung function and development of COPD. However, the results were mixed, again suggesting that the magnitude of the effect may be small or the errors in the data may have attenuated the result to the null. Further research is needed to clarify this relationship.

3.3.3 Relationships of other dietary components, lung function and lung disease

There have been several studies investigating the relationship between specific foods, food groups or nutrients and lung function or lung disease in adults. These studies are typically either exploratory studies, assessing a range of foods or nutrients as exposure variables, or studies with a particular biological mechanism in mind, for example, research examining dietary antioxidants as the exposure. The evidence for the most frequently examined dietary factors is briefly reviewed below with a focus on studies assessing lung function or bronchial responsiveness as an outcome.

3.3.3.1 *Antioxidant vitamins*

Several studies have investigated the relationship between serum concentrations or dietary intakes of the antioxidant vitamins A, C, and/or E and lung function, COPD or asthma with mixed results (105, 115, 204-207). A brief review of the studies is provided here with a focus on those assessing lung function as an outcome.

Vitamin A

The findings of observational studies examining lung function outcomes suggest higher serum and/or intake levels of vitamin A or its precursors may be associated with better lung function (205, 206). Thyagarajan and colleagues found higher serum pro-vitamin A carotenoid levels were associated with higher maximum FEV_1 and FVC, and slow decline in lung function measures in a cohort study of 2,701 U.S. adults followed up over a 20 year period (208). Similarly, Guenegou and colleagues also found higher serum β-carotene concentrations were associated with slower decline in FEV_1 in 535 French adults followed over 8 years (209). However, cohort studies using dietary intakes instead of serum levels have failed to confirm this relationship (129, 132). This may be due to the way in which these nutrients are estimated, increasing the chance of misclassification and attenuating any associations towards the null.

There are only a few supplementation RCTs which have examined the possible effect of vitamin A or its precursors on lung function. One very small study of short duration saw an improvement in lung function with vitamin A supplementation in smokers with mild COPD (n=12) (210). However, randomisation in such a small sample may not have been effective at controlling for confounding factors. Another large RCT involving 20,536 middle-aged and older adults randomised to an antioxidant supplement containing vitamins C, E, and β-carotene, or a placebo for 5 years saw no difference in lung function at follow up between the intervention and placebo groups (211). This study provides strong evidence that there is no relationship between these antioxidant vitamins and lung function. It is a very large RCT of long duration. However, it is still possible that the lack of an association may have been due to the quantities of vitamins used in the supplement or the combination of vitamins interacting with each other. Alternatively, carotenoids may be associated with intakes of other beneficial dietary factors, for example other compounds in fruit and vegetables.

This may explain the beneficial associations found in observational studies and the lack of any benefit from supplements in experimental trials.

Vitamin C

A link between vitamin C intake or status and lung function has also been suggested by several observational studies. Again, there is a lack of supplementation trials to confirm a relationship (115, 205-207). A large cohort study involving 7,106 middle-aged and older Korean adults found higher intakes of vitamin C were associated with better lung function in men and women and reduced COPD risk in men (defined by spirometry) over approximately 3 years (212). Similarly, another UK cohort study found FEV_1 decline over 9 years was slower in those with higher dietary vitamin C intake, after adjusting for confounders (213). However, a cohort study in a U.S. population found vitamin C intake was associated with slower FEV_1 decline in smokers only and some observational studies have observed no relationship or a relationship in men only, indicating the beneficial effects of vitamin C may be specific to certain population groups (130, 132, 214).

There have been few intervention studies examining the relationship between vitamin C and lung function. Those that have been performed have not confirmed a beneficial effect of vitamin C on lung function. A large RCT (discussed above) in which the intervention group received a supplement containing vitamins C, E and β-carotene observed no difference in lung function after 5 years (211). Although this is a study of high quality providing strong evidence, these results may have been due to the levels of the vitamins in the supplement or interaction between the vitamins. Another small RCT involving 35 COPD patients also observed no effect of vitamin C on lung function after 12 weeks of supplementation; however, this may have been due to confounding effects or the short duration of the study (207). Both trials used 250 mg of vitamin C daily in the intervention group which may not be adequate. The combination of nutrients in foods high in vitamin C may also be where the effect lies. Again, further studies are needed.

Vitamin E

There have been several observational studies suggesting an association between vitamin E intake or status and lung function, including a number of population-based cross-sectional studies (115, 132, 205-207, 215-218). However, most cohort studies have failed to demonstrate a link between vitamin E and lung function decline (129, 132, 213). Two cohort studies that have observed a relationship found higher vitamin E intake or status was related to slower FEV_1 decline in smokers only (130, 209). A relationship in smokers only may explain the mixed results observed across the studies. Some studies examining status in serum samples have also suggested results may depend on the isoform of vitamin E tested (206, 216).

Due to a lack of intervention studies, the effect of vitamin E on lung function cannot be conclusively

determined. One large supplementation trial (discussed previously) of long duration used a combined supplement of vitamins C, E and β-carotene and observed no difference in lung function between the intervention and placebo groups after 5 years (211). However, this may be due to the combination of vitamins or the quantities of vitamins used. Two other small RCTs of short duration also observed no improvement in lung function after supplementation with vitamin E, however this may have been due to confounding effects and/or lack of statistical power (219, 220).

There have been two other small intervention trials investigating the modification of the effect of ozone on lung function by a supplementation of antioxidant vitamins. Short term exposure to ozone has been shown to increase airway inflammation and respiratory symptoms and decrease lung function (221, 222). It is believed the effects of ozone are at least partly attributed to its oxidation capabilities and therefore, antioxidants may be able to mitigate its effects. One study was a randomised double-blind crossover study involving 47 street workers in Mexico City, an area with high ambient levels of ozone, and supplementation with vitamins C, E and β-carotene, or placebo (221). The other study was an RCT involving 38 Dutch cyclists randomly assigned to a supplement of vitamins C and E, or placebo (222). Both studies observed a protective effect of the antioxidant supplement against the damaging effects of ozone exposure on lung function parameters (221, 222). These results suggest antioxidant vitamins may protect a subgroup of the population exposed to high levels of ozone, and potentially other air pollutants and/or oxidative species, from lung damage and deficits in lung function caused by these compounds. Although these two studies showing consistent results are promising, further studies with larger sample sizes are required.

Antioxidant vitamins and BR

There is a lack of good quality evidence exploring the relationship between vitamins A, C or E and BR. The few studies examining vitamin A intake and BR have had mixed results (142, 223). Those assessing vitamin E intake and BR have found no relationship, however, these studies are very few and with mostly small sample sizes (142, 223, 224). There were several small RCTs performed in the 1970s and 1980s investigating the effects of a single dose or short course of vitamin C on BR, many of which observed a protective effect (224-228). However, the few studies that have investigated long-term vitamin C intake and BR, have had mixed results (142, 223). More high-quality studies are needed to conclusively determine if a relationship between long-term intake of antioxidant vitamins and BR exists.

3.3.3.2 ***Vitamin D***

Vitamin D is primarily obtained via synthesis in the skin with exposure to sunlight, however, some vitamin D is obtained from the diet. Studies suggest vitamin D plays an important role in the functioning of the immune system, inflammatory responses and the airway remodelling that occurs with respiratory disease (207). Hence, there has been considerable interest in the relationship between vitamin D, lung function and lung disease.

Observational studies have consistently shown a positive association between vitamin D, measured by dietary intake or serum concentration, and lung function in adults (205, 229-231). One of the largest cross-sectional assessments, performed by Black and colleagues, used data from over 14,000 adults in the NHANES, a cross-sectional survey routinely conducted in the USA. This study found the mean FEV_1 and FVC of those with serum 25-hydroxy vitamin D levels in the highest quintile was 126 ml and 172 ml higher respectively, compared to those with serum 25-hydroxy vitamin D levels in the lowest quintile, after adjusting for confounders (229). A recent meta-analysis of cross-sectional and case-control studies also found a positive relationship between serum vitamin D levels and lung function in adults with asthma. This study included 26 original studies, 6 of which were conducted in adult populations (232).

Results from cohort studies and RCTs have failed to confirm a protective effect of vitamin D on lung function decline (129, 233-236). However, Lange and colleagues found vitamin D status modifies the effect of smoking on lung function and its decline. In their study, smokers with vitamin D deficiency had lower lung function and more rapid rates of decline in FEV_1 per pack-year of smoking compared to smokers who were vitamin D sufficient (235). This suggests that vitamin D may play an important protective role against lung function decline and subsequent respiratory disease in susceptible population groups such as smokers. Two recent reviews of RCTs on the use of vitamin D supplements in the management of asthma and COPD both found no effect of vitamin D on lung function (236, 237). There was, however, a reduction in the risk of severe asthma exacerbations in asthmatics receiving vitamin D supplementation (236). Similarly, vitamin D supplementation reduced the risk of moderate to severe COPD exacerbations in COPD patients who were vitamin D deficient at baseline (237). Therefore, there is probably a protective effect of vitamin D in the prevention and/or management of obstructive lung disease. However, further studies are needed to fully understand the groups that stand to benefit from vitamin D supplementation, the mechanisms behind this protective effect, and the potential role of vitamin D in lung function and its decline.

There has been little research exploring the relationship between vitamin D and BR. Sutherland and colleagues found higher serum vitamin D levels were associated with reduced BR to methacholine in an observational study of 54 non-smoking adults with asthma (238). However, further studies are needed to confirm this association.

3.3.3.3 ***Omega-3 polyunsaturated fatty acids (n-3 PUFAs), fatty fish and fish oil***

Studies examining the relationship between the consumption of fatty fish and their anti-inflammatory fats, lung function and obstructive lung disease in adults suggest a beneficial effect of n-3 PUFAs on lung function in current and former smokers (233, 239, 240). Sharp and colleagues observed slower FEV_1 decline per pack year of smoking in smokers with high fish consumption compared to those with low fish consumption (n=2,809; mean age 54 years) (239). Similarly, Leng

and colleagues found higher consumption of the n-3 PUFAs eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) were associated with better FEV_1 and higher DPA consumption was associated with slower FEV_1 decline in current and former smokers (n=2,337; age at baseline 40-74 years) (233). In a large cross-sectional study, Shahar and colleagues also observed current and former smokers with higher n-3 PUFA intakes had better lung function and a dose-response type reduction in the odds of spirometrically detected COPD (n=8,960; age 45-64 years) (240). Garcia-Larsen and colleagues also observed a positive association between n-3 PUFA intake and lung function in a young adult study population with a high proportion of smokers (139). However, not all studies have observed a positive relationship (132, 241, 242). One very large cross-sectional study conducted by McKeever and colleagues, involving 13,820 adults aged 20-59 years found no relationship between intake of individual PUFAs and asthma, COPD or FEV_1 and no interaction between the n-3 PUFAs and smoking status (242). Conclusive evidence from RCTs is needed. There has been a small supplementation trial involving 64 former smokers with COPD randomised to receive either an n-3 PUFA supplement or an n-6 PUFA supplement. After 2 years of supplementation, no difference in FEV_1 was observed between the two groups (243). Additional supplementation trials are currently underway, the outcomes of which should provide the necessary evidence to definitively conclude if n-3 PUFAs have a beneficial effect on lung function or COPD outcomes (105, 244). Currently, there is little evidence to support a beneficial effect of n-3 PUFAs in the prevention or management of obstructive lung disease or lung function decline in never smokers (245-254).

There have been a small number of observational studies that have assessed the relationship between n-3 PUFAs and BR, the majority of which have demonstrated no association (253, 255, 256). A Cochrane review of RCTs, published in 2000, found no benefit of n-3 PUFAs on BR in adults with asthma in a meta-analysis of 3 studies (251). Notably, all 3 studies individually found no benefit of supplementation on BR (257-259). There have since been a few small intervention trials conducted (252, 260, 261), two of which observed a beneficial effect of n-3 PUFAs on BR in atopic asthmatics and elite athletes with exercise-induced bronchoconstriction, respectively (260, 261). However, these trials do not provide sufficient evidence. Larger RCTs are needed to draw a definitive conclusion.

3.3.4 Effect modifiers of the relationship between dietary factors and lung function

3.3.4.1 *Fruit and vegetable intake*

Of the 14 observational studies examining the relationship between fruit and/or vegetable intake and lung function, 9 assessed potential effect modifiers (129-132, 135-138, 141). The most common potential effect modifiers examined were sex and smoking (n=4 and n=9 respectively). Others included age, social class, activity level, and other dietary factors.

From the 4 studies examining sex as an effect modifier, two observed no interaction with fruit and/or

vegetable intake (131, 141) and the other two stratified by sex without formally testing for an interaction (136, 138). The study by Strachan and colleagues found higher fruit or juice intake was associated with greater FEV_1 in both sexes, however, a greater difference in mean FEV_1 for high vs low fruit or juice intakes was observed in women (138). This suggests possible modification of the association between fruit intake and FEV_1 by sex. The results of the other study indicate no modification by sex (136).

Seven of the 9 studies that assessed smoking as an effect modifier tested for interaction (129-132, 136, 137, 141). Two of these studies found smoking modified the relationship between fruit intake and lung function. Garcia-Larsen and colleagues found higher fruit intake was associated with slower lung function decline in ex-smokers only, with a mean decline in FEV_1 and FVC per year 6.4 ml and 8.1 ml less, respectively, for every tertile increase in fruit intake (interaction p-values 0.03 and 0.04 respectively) (129). There was no association observed in never or current smokers, however, there may not have been enough power to detect an association in current smokers after adjusting for confounders (current smokers n=109, ex-smokers n=255, never smokers n=270). Similarly, Bentley and colleagues also reported slower annual decline in FEV_1 with higher fruit and vegetable intake in smokers and quitters, but not never or former smokers (smokers n=75, quitters n=27, former smokers n=635, never smokers n=704). However, the strength of the association observed in this study was much greater than that seen by Garcia-Larsen et al (24 ml/year and 41 ml/year in smokers and quitters respectively (high vs low fruit intake), interaction p-value 0.003) (130).

Two cross-sectional studies reported a positive association between fruit intake and FEV_1 which was stronger in never smokers (135, 138). These results are inconsistent with the results from the cohort studies described above. There was no test for interaction performed. Therefore, the differences observed in the association between fruit intake and lung function in current smokers, ex-smokers and never smokers may just be some variation that would not pass a statistical test for interaction. Nevertheless, it appears there may be some interaction between fruit intake and smoking. Further studies are needed to gain a greater understanding of the relationship between fruit intake and lung function in current smokers, ex-smokers and never smokers.

Only 4 studies examined effect modifiers of the relationship between vegetable intake and lung function, none of which reported any interactions with vegetable intake (129, 131, 135, 137). It appears some studies may have tested effect modifiers only with dependent variables that were associated with lung function, in which case vegetable intake may not have been tested. Further studies examining possible effect modifiers of this relationship are needed.

3.3.4.2 ***Dietary patterns***

Of the 14 observational studies examining the relationship between dietary patterns and lung function, 6 assessed potential effect modifiers (111, 165, 168, 170, 171, 173). The most common

effect modifiers examined were sex and smoking (n=4 for each). Others possible effect modifiers assessed were BMI, physical activity, and asthma or wheeze. Four studies used PCA to define dietary patterns, one study used a Mediterranean diet score from a screener questionnaire, and one study used the DII. In order to determine if there is any consistency in the findings, the results from prudent/Mediterranean dietary patterns will be compared, and those from traditional/western dietary patterns will be compared.

Of the 4 studies that examined sex as an effect modifier of the association between the prudent diet and lung function, two found no interaction (168, 173). One study observed a greater difference in FEV_1 with increasing quintiles of the prudent pattern in men compared to women (Q5-Q1 0.18 L vs 0.08 L for men and women respectively) (171). There was also a positive association between the prudent pattern and FEV_1/FVC in men only, however no interaction p-value was reported. Similarly, in the study by Gutierrez-Carrasquilla and colleagues, there appeared to be a greater risk of obstructive ventilatory effects (defined as FEV_1/FVC <0.70) with low adherence to the Mediterranean diet in men (OR (95%CI) 4.14 (1.42–12.1), p=0.009 and 1.99 (0.93–4.26), p=0.077 for men and women respectively), however there was no test for interaction performed (165).

Of the 3 studies that investigated sex as an effect modifier of the association between the western/traditional diet and lung function, two studies observed no interaction (171, 173). The study by Sorli-Aguilar and colleagues found a positive association between the western dietary pattern and impaired lung function (defined as FEV_1 or FVC <80% predicted or FEV_1/FVC <0.70) in women only (p=0.011) (168). These results suggest that there may be a greater beneficial effect of the prudent diet on lung function in men and a greater harmful effect of the western diet on lung function in women. Alternatively, the results may be related to the association between diet and phenotypes of obstructive lung disease that are more common in men or women. Further studies are needed to clearly define the phenotypes of lung disease and their relationship with diet.

Three studies assessed the possible effect modification of the prudent diet and lung function by smoking, two of which observed no modifying effects (168, 173). The third study, by Shaheen and colleagues, found that, in men, the estimated mean differences of the positive association between quintiles of the prudent diet and FEV_1 and FEV_1/FVC were larger in current and former smokers (interaction p-values comparing current and never smokers were 0.036 and 0.002 for FEV_1 and FEV_1/FVC respectively). In current smokers, the difference in mean FEV_1 between the first and fifth quintiles after adjusting for confounders was 0.46 L (95%CI 0.08-0.83) (171). This is a very large and clinically important difference that suggests a possible beneficial effect of the prudent diet that would exceed many medications. There was no effect modification by smoking in women in this study (171). Further studies are required to confirm these results.

There was no effect modification of the western/traditional diet and lung function association by smoking in 4 studies (168, 170, 171, 173) or by BMI in 2 studies (170, 173).

There was only one study each assessing physical activity and asthma or wheeze as potential effect modifiers. Gutierrez-Carrasquilla found no effect modification of the relationship between the Mediterranean diet and odds of an abnormal FEV_1, non-obstructive ventilatory effects, or obstructive ventilatory effects by physical activity (165). Han and colleagues found a higher DII was associated with a lower % predicted FEV_1 and FVC in those without asthma or wheeze only; however, no test for effect modification was performed (111). Given the lack of research investigating physical activity and asthma or wheeze as effect modifiers, no conclusions can be drawn at this time.

Literature search update

An updated literature search was carried out on 25th September 2020. Han and colleagues have published another study of the associations between DII and lung function, this time in a large sample of Hispanic/Latino adults. Again, current asthma was examined as a potential effect modifier. The authors again found a higher DII was associated with a lower % predicted FEV_1 and FVC only in those without asthma (183). Again, no test for effect modification was performed. Although these results are consistent with those from an earlier study by the same authors, further research is still needed to confirm these findings.

3.3.5 Summary of the gaps in the literature

Poor lung function can be very debilitating and life-threatening. It is a clinical feature of lung diseases, some of which are highly prevalent. Dietary factors may be risk factors for, or alternatively may protect against, poor lung function and rapid lung function decline. These factors should be investigated thoroughly, especially in middle-aged and older populations in whom dietary change can have additional benefits such as fewer side effects compared to medication, improved weight management and reduced risk or slower progression of other chronic diseases.

There is a general lack of assessment of risk factors for poor lung function and lung function decline in middle-aged and older populations, including dietary factors. Research to date has tended to focus on either asthma or COPD, often excluding older participants and/or smokers in asthma studies and non-smokers in COPD studies due to the difficulties in distinguishing between these diseases. From my review of the literature on dietary factors and lung function in adults, the research to date suggests a probable beneficial effect of fruit on lung function, and n-3 PUFAs on lung function in smokers. There is also a possible beneficial effect of vegetable intake and vitamin D on lung function, and vitamin C on BR. Dietary pattern analysis is a relatively new method of assessing diet. The results of studies so far indicate an anti-inflammatory diet may benefit lung function, whilst a western/traditional pattern is probably harmful to lung function. A beneficial effect of the prudent and Mediterranean diets is also possible; however, further studies are needed in this area.

There have generally been very few studies examining the relationship between diet and BR and effect modifiers of the relationship between diet and lung function. Such investigations are critical at

this time given the lack of clarity surrounding the phenotypes of asthma and COPD. Examination of factors associated with BR and effect modifiers of risk factors of lung function can shed light on the phenotypes of these conditions and the population groups that can benefit from dietary and other lifestyle changes.

Therefore, in my doctoral work, I will focus on associations between dietary factors and poor lung function in middle-aged and older adults. My outcomes of interest will be spirometry measures and BR. I will examine fruit and vegetable intakes, dietary patterns defined by PCA, and the inflammatory potential of the diet, measured by the DII, as my exposure variables. I will also explore potential effect modifiers of these relationships. Prior to beginning this work, I will investigate potential methods of examining factors associated with BR and attempt to find a more suitable method than the currently used binary BHR variable or the log-transformed dose response slope.

Chapter 4 - Methods

4.1 Overview

The focus of this thesis is the relationship between dietary factors and lung function in middle-aged and older Australians. The dietary factors that will be examined are fruit and vegetable intake and dietary patterns. The outcomes of interest are spirometry measures and BR. Potential confounders will be adjusted for and possible effect modifiers of these relationships will be investigated. This chapter begins with a theoretical discussion of potential confounders, the interrelationships of which are illustrated using a directed acyclic graph (DAG), and possible effect modifiers of the relationships of interest.

The research presented in this thesis utilises data obtained in the Tasmanian Longitudinal Health Study (TAHS) 2010 follow up and the COPD Study. The TAHS is a population-based longitudinal study in which dietary information was collected for the first time as part of the 2010 follow up (262). The COPD study is a population-based cross-sectional study of middle-aged and older adults (263, 264). Both these studies recruited a large number of study participants who were of middle-age or older at the time of data collection, and included a diet questionnaire, objective lung function testing via spirometry, and a bronchial provocation challenge using methacholine. Therefore, these datasets provide an opportunity to examine the associations between various dietary factors, lung function and BR, and to assess possible effect modifiers of these relationships.

This chapter provides a detailed description of the design and data collection for these two studies as is relevant for this thesis. All analytical work presented in this thesis was performed by me, including determination of confounders using DAGs, determination of potential effect modifiers, data cleaning and exclusions as described in the sections to follow. Research questions are stated, and a general overview of the statistical methods used in the data analyses is provided. Further information on the methods employed that are specific to a particular research question are detailed in the relevant chapter.

4.2 Confounders and effect modifiers of the relationship between dietary factors and lung function

A confounder is a factor that is:

1) a risk factor of the outcome;
2) associated with the exposure; and
3) does not lie on the causal pathway between the exposure and the outcome (126).

To determine the direct effect of the risk factor of interest, the confounders must be controlled or adjusted for in epidemiological research. Not adjusting for confounders can result in incorrectly estimating the size and even direction of the association between the risk factor of interest and the

outcome. That is, the result is biased, and the effects of this bias are unpredictable.

DAGs are casual diagrams that can be useful in determining the potential confounders of an exposure-outcome relationship. They provide a visual representation of the interrelationships between factors associated with the exposure and the outcome. Below is a DAG of the theoretical relationship between diet and lung function that I developed using the DAGitty software program (Figure 4.1) (265). The variables I included in the DAG were chosen based on my own knowledge and the existing literature in the areas of diet and lung function, and factors which influence food choice. As DAGs are acyclic, no connection can have a double-headed arrow. All relationships must indicate that one factor influences the other. Therefore, I have considered the relationships between each of these variables and decided where the connections are and in which direction the arrows should go. The DAGitty program is then able to assess these relationships within the complex web of interconnections and identify the variables that meet the definition of a confounder and may bias the result. From the DAG in Figure 4.1 several potential confounders were identified including age, sex, height, BMI, physical activity, health consciousness, atopy, asthma status, and co-morbidities. Separate DAGs will be developed for each analysis based on the specific dietary factors, lung function or BR, and the covariates available for each research question. However, a general DAG is useful in considering all the potential confounders irrespective of the data and to identify potential sources of residual confounding in my analyses.

Effect modification is present when the association between the exposure and the outcome is dependent on the category or value of another variable (266). For example, in Chapter 3 I discussed the evidence for smoking status as an effect modifier of the relationship between n-3 PUFAs and lung function which suggested a probable positive association in smokers and ex-smokers and no relationship in never smokers (233, 239, 240). Examining potential effect modifiers may help identify specific population groups or disease phenotypes that can benefit from dietary change. It may also explain some of the mixed results obtained across studies.

Phenotypes of lung disease are defined based on differences in clinical, physiological and biological characteristics observed in patient groups (61, 82, 85). These differences suggest involvement of different biological processes in the manifestation of disease. Dietary factors may play a role in some of these biological pathways. Therefore, disease phenotypes are potential effect modifiers of the relationship between diet and lung function. However, there are believed to be many phenotypes of asthma and COPD and, whilst there are a few commonly recognised phenotypes of both, definitions are continually evolving in an effort to elucidate the biological mechanisms underlying the development of each phenotype and, in turn, potential treatment targets (61, 82, 85). Asthma-COPD Overlap, a newly-recognised disease entity in respiratory medicine, is also thought to be a heterogeneous condition with multiple phenotypes which are currently undefined (61). And let us

not forget that physicians currently struggle to distinguish between asthma, COPD and ACO in middle-aged adults, let alone identify the disease phenotype affecting an individual. In the absence of clearly defined phenotypes of these diseases, characteristics that will likely define, or be risk factors for, these phenotypes should be considered as potential effect modifiers of the relationship between diet and lung function.

Any known risk factors for asthma and COPD have the potential to be effect modifiers. In this thesis I will focus on characteristics with available data, a biologically plausible explanation, and sufficient prevalence in a general adult population to ensure adequate power to detect any interactions. Therefore, I will examine sex, smoking status, BMI, asthma status, and atopy. A brief explanation for the assessment of each of these variables as effect modifiers is provided below.

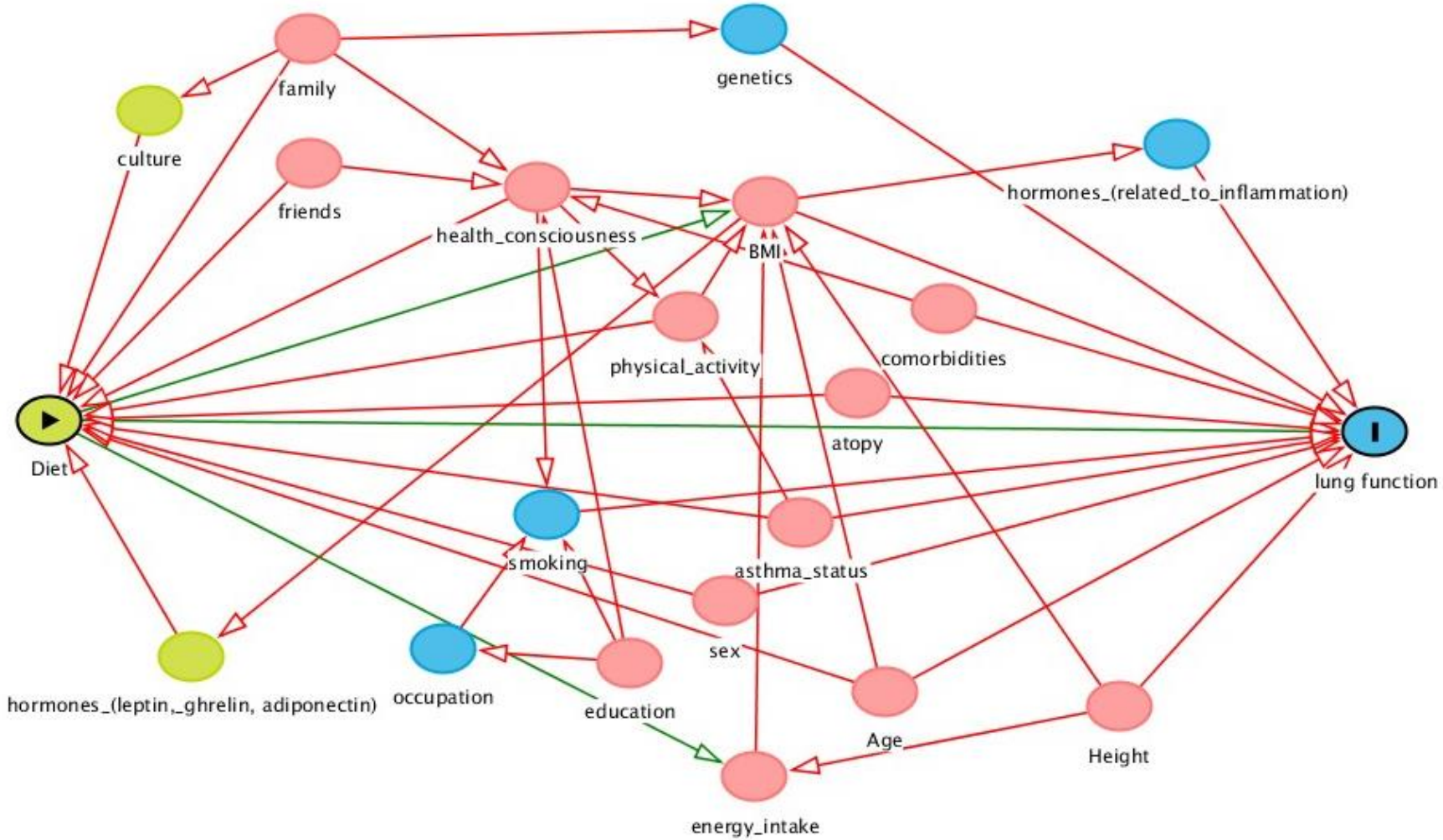


Figure 4.1 - DAG of the relationship between diet and lung function

Figure key: exposure outcome ancestor of exposure ancestor of outcome ancestor of exposure *and* outcome (i.e. a confounder)

Sex

Differences between men and women include biological differences in the levels of circulating sex hormones. These hormones are associated with the risk of asthma development. This is evident in the sex differences in asthma prevalence where asthma is more prevalent in males aged 14 years and under, and from the age of 15 onwards (around the time of puberty) into middle-age and later life, asthma is more prevalent in females (10). Research has also found sex hormone levels are associated with current asthma in adults and with lung function in children with asthma (267, 268). The increased risk of asthma in women of middle-age and older (i.e. post-menopause) relative to men may be specific to certain disease phenotypes in which hormones may play a role in disease development.

Smoking status

Smoking is a known risk factor for poor lung function and there is some evidence (although mixed) that it is also an effect modifier of the relationship between dietary factors and lung function (See Chapter 3). It is plausible that higher intake of fruit and vegetables, which are high in antioxidants, may mitigate the impact of the oxidative species in cigarette smoke on lung function. A more anti-inflammatory diet may also reduce the detrimental impact of smoking on lung function, as evidence of a positive association between n-3 PUFAs and lung function in smokers suggests (discussed in Chapter 3).

BMI

Obesity is a risk factor for poor lung function and lung function decline, it is associated with BHR, and it is already considered a characteristic that will define some asthma and COPD phenotypes (85, 269, 270). In asthma, obesity is associated with more symptoms and more frequent and severe exacerbations. Obese asthma is also less responsive to standard asthma medications (269).

There are many mechanisms through which obesity may affect lung function. For example, compression of the lungs by excess fatty tissue reduces lung volume. The fatty tissue in obese subjects is also metabolically active, releasing proinflammatory cytokines, contributing to chronic systemic inflammation. Other potential mechanisms involve changes to the gut and lung microbiome, altered adaptive and innate immune responses, and increased oxidative stress (269, 270).

Atopy

Atopy is another characteristic that may be used to define lung disease phenotypes. It is an indicator of allergic asthma phenotypes and is a risk factor for COPD development (60, 82). When the idea of asthma phenotypes first came about it was thought there were only two phenotypes – allergic and non-allergic. Allergic asthma was characterised by early onset of disease, atopy, identifiable allergic triggers, and co-existence with other allergic diseases (82). It was thought to be mediated by a Th2 immune response. The clinical biomarkers of allergic asthma included immunoglobulin E antibodies

and eosinophils (82). It is now believed that allergic asthma is a subgroup of asthma phenotypes rather than a single phenotype, however, it is likely atopy will continue to be a defining characteristic of some phenotypes of obstructive lung disease.

Asthma status

Asthma is a known risk factor for COPD. Chronic inflammation of the airways that occurs in asthma causes airway remodelling resulting in epithelial damage, thickening of the bronchial muscle and infiltration of cells by eosinophils (immune cells associated with asthma) (271, 272). This airway remodelling may contribute to further lung function decline and increase an individual's susceptibility to develop fixed airway obstruction (i.e. COPD).

4.3 The Tasmanian Longitudinal Health Study (TAHS)

4.3.1 Overview of the TAHS

The TAHS is one of the longest running population-based longitudinal studies of respiratory disease in the world. It began in 1968 with the recruitment of 8,583 school children living in Tasmania and born in 1961 (age ~7 years at baseline). These participants are referred to as 'probands'. The family members of the probands were also recruited into the study at baseline. The aim of the study was to investigate the prevalence and natural history of asthma in children (262). For the baseline study, probands and their parents and siblings were asked to complete a survey to collect basic demographic information, along with information on their respiratory health. Probands also completed spirometry testing which was conducted within schools. The response rate of the study was very high, with 98.8% of those invited completing the survey, and 93.5% completing a spirometry test (262).

Since the baseline study, the TAHS probands have been followed up in 1974, 1979, 1991, 2002, 2010 and 2012 at the approximate ages of 12, 18, 30, 43, 50 and 53 respectively. Table 4.1 summarises these studies and briefly outlines the data collected at each follow-up. Additional follow-up of the parents and siblings has also been conducted. These studies are not relevant to this thesis and will not be described.

Table 4.1 - Summary of TAHS follow up studies (262)

Study year	Sample size	Approximate age (years)	Data collected								
			Questionnaire	Anthropometry	Baseline spirometry	Post-BD spirometry	TLco (post BD)	Lung volumes and capacities	Methacholine challenge	Skin prick test	Blood sample
1968	8,583	7	✓	✓	✓						
1974	7,383	12	✓	✓	✓						
1979	846	18	✓	✓	✓						
1991	1,501	30	✓								
2002	5,729 (postal survey) 1,200 (clinical study)	43	✓ ✓	✓	✓	✓	✓	✓		✓	✓
2010	772	50	✓ (+ diet)	✓	✓				✓		✓
2012	3,609	53	✓ (+ diet)	✓	✓	✓	✓			✓	✓

BD = bronchodilator; TLco = Transfer factor of the lung for carbon monoxide; + diet = questionnaire included a diet section

The 2002 follow up study was the first follow up to attempt to contact all participants from the original 1968 study since 1974. A major effort to trace all original cohort members was undertaken and 7562 (88.1%) were successfully traced to an address. These participants were invited to take part in a postal survey with 5729 (78.4%) completing the survey. An asthma and chronic cough enriched subsample of these respondents was then invited to complete further laboratory testing. Of the 2387 participants invited, 1751 (73.4%) took part in the study with 1405 participants (58.9%) completing the full laboratory session.

The 2010 follow-up was another clinical study involving laboratory testing and the first follow-up to include questions on diet. The research presented in this thesis utilises data from this follow-up only. Participants who had completed the full laboratory study in 2002 and who had not since died or withdrawn were invited to take part in the 2010 follow-up. Of the 1375 invited, 840 participants took part in the follow up (response rate: 61%), 807 of whom attended a laboratory session between May 2010 and October 2012. During the laboratory session participants completed a questionnaire and a series of clinical tests. The questionnaire collected general demographic information as well as information on current and previous respiratory health; family history of respiratory disease; current and previous exposure to potential environmental risk factors in the home; exposure to smoking; employment history; use of medications to manage respiratory conditions; and a short questionnaire on diet (Appendices 1-2). Anthropometric measurements (height, weight, neck circumference, and waist and hip measurements) were performed by a trained respiratory scientist following a standard protocol and a blood sample was collected to measure DNA sequences and inflammatory biomarkers. A total of 696 participants completed the full testing protocol during their laboratory session (Figure 4.2).

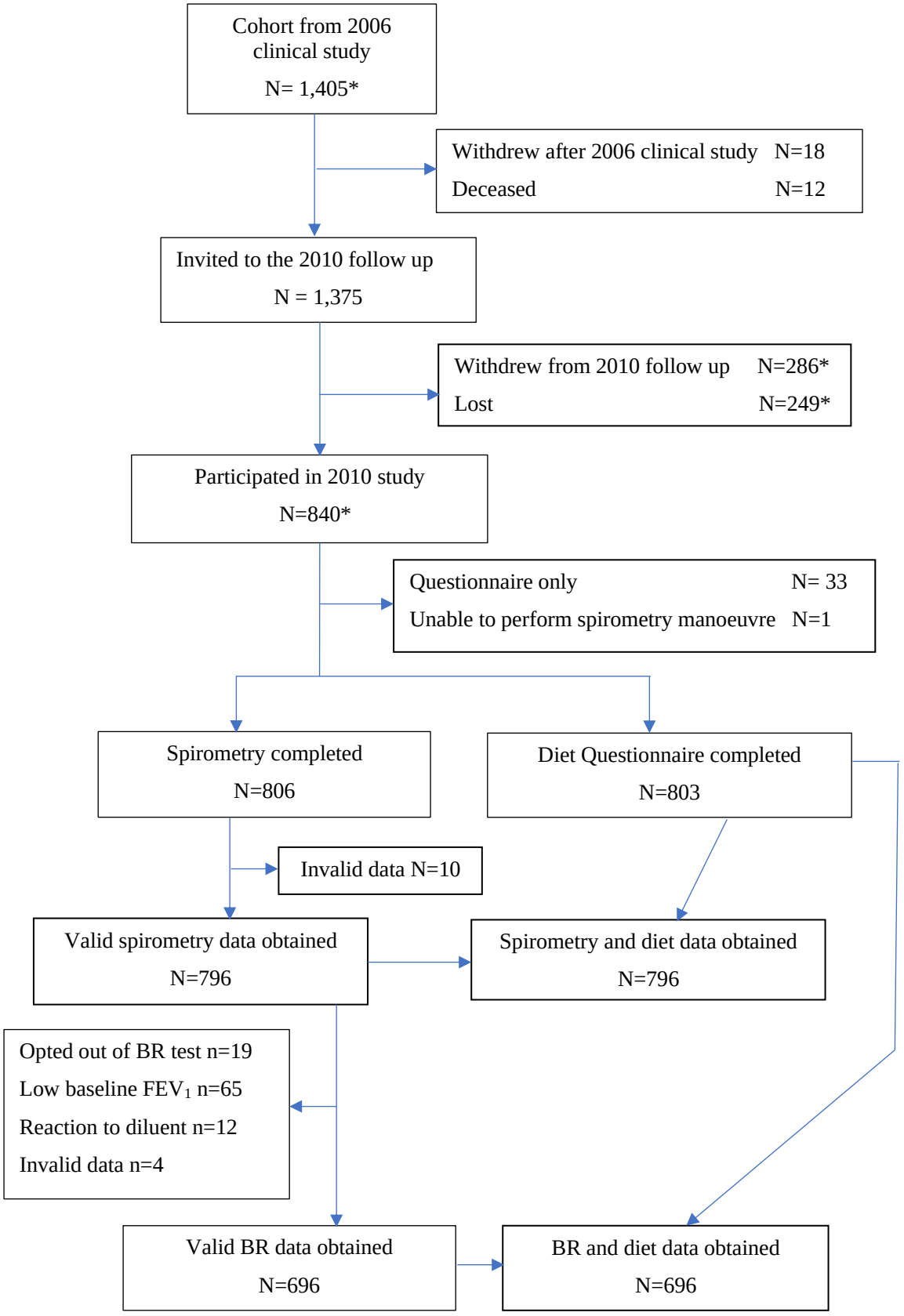


Figure 4.2 - Ascertainment of the TAHS 2010 follow up study sample included in the analyses

*Note – numbers differ slightly from a publication using the same dataset by Burgess et al (273). The numbers in the flowchart have come from the dataset provided.

4.3.2 Research questions

In this thesis I have used data from the TAHS 2010 follow up to answer the following research questions.

- Research Question 1: Is there a more suitable statistical method for examining factors associated with BR than the currently used dichotomous BHR variable or log-transformed dose response slope? If so, does this method provide more information and/or produce different results to the currently used methods?
- Research Question 2: Are fruit and vegetable intakes associated with lung function and BR in middle-age? Are any of these relationships modified by sex, smoking status, BMI or asthma status?

The methods used to collect the relevant data to answer these questions are described below.

4.3.3 Dietary questionnaire

The dietary questionnaire used in the TAHS 2010 follow up study is a modified version of the Short Fat Questionnaire (SFQ) developed by Dobson and colleagues (274). The questionnaire was designed to enable Australian adults to self-assess their dietary fat intake and food preparation behaviours and identify changes they could make to help limit their intake of dietary fats as per current dietary recommendations (274). The SFQ contains 12 questions on the frequency of consumption of a selection of discretionary foods and other foods high in saturated fat such as hot chips, cakes, burgers, chocolate, and cream (Appendix 1). Response options ranged from 'never' to '6 or more times per week'. The questionnaire also includes one question on cooking method used; one question on the amount of butter or margarine used on bread; two questions on the amount of fat on meat consumed; and one question on the type of milk consumed. The SFQ was modified for the TAHS 2010 follow up to include an additional question on the type of milk consumed (e.g. cow's or goat's milk, or soy milk) and a question each on the number of serves of fruits and vegetables consumed daily. All questions asked about the participant's usual habits but did not specify a time period.

The SFQ is designed to calculate a single score, with a low score indicating a low intake of dietary fats and a high score indicating a high intake. The possible score ranges from 0 to 63. The questionnaire has been validated in a group of 124 Australian adults against a 179-item food frequency questionnaire developed by the CSIRO (274). The CSIRO FFQ was commonly used to measure diet in studies of Australian populations at the time of the SFQ's development. The validation study demonstrated very good reproducibility of the SFQ (r=0.85, 95%CI=0.69 to 0.93) and good correlation between the score calculated from the SFQ and percentage of energy from all

fat (r=0.55, 95%CI=0.39 to 0.68) and saturated fat (r=0.67, 95%CI=0.54 to 0.77) determined from the FFQ (274). As discussed in chapter 2, FFQs are not considered to be one of the best methods of measuring diet, however, they are arguably the best method available for measuring usual diet in a large study population. The results indicate the SFQ is likely to be a satisfactory tool for use in epidemiological studies to rank study participants according to their usual dietary fat intake.

The two questions on fruit and vegetable intake that were added to the dietary questionnaire have not been validated, however, some validation studies have been conducted using similar questions (275-279). One such study was conducted by Yaroch and colleagues in the United States in 244 adults of mixed ethnicity. This study reported an overestimation of fruit intake and an underestimation of vegetable intake when the results of the fruit and vegetable questions were compared to 2 or 3 non-consecutive 24-hour diet recalls (median daily servings were 2.0 vs 0.9 and 2.0 vs 3.2 for fruit and vegetables respectively) (275). Although both items demonstrated a reasonable level of reproducibility (fruit r=0.67, vegetable r=0.65), only the fruit item correlated moderately with fruit intake calculated from diet recalls (r=0.51). No correlation was found between the vegetable item and vegetable intake (r=0.08) (275).

This validation study by Yaroch et al used 24-hour diet recalls as the reference measure of diet. Although diet recalls are considered one of the better methods to measure diet it may not be the best measure of usual diet, particularly when only 2 or 3 recalls are used. In fact, Willett suggests 3 to 4 diet recalls in each of the four seasons of the year are required to adequately capture day to day and seasonal variation and estimate long term usual dietary intake of individuals (123). Such an exercise would be costly and time consuming for both the researcher and the participant. Therefore, validated semi-quantitative food frequency questionnaires are often considered a more appropriate measure of usual intake.

Another validation study, conducted by Cook and colleagues, involved a sample of Australian adults aged 18 to 39 years. This study compared intakes determined from single questions on the number of serves of fruit and vegetables consumed (similar to the questions used in the TAHS 2010 study) to intakes calculated from a semi-quantitative FFQ developed by Cancer Council Victoria. In this study both items demonstrated moderate validity (fruit r=0.68, vegetable r=0.52) and reproducibility (fruit r=0.80, vegetable r=0.80) (276).

Coyne and colleagues also conducted a validation study on single questions measuring fruit and vegetable intake, similar to those utilised in the TAHS. This study involved a large sample of Australian adults (n=1598, aged 25 years and over) and used biochemical indicators from blood samples (serum carotenoids and red-cell folate) as the reference measures of diet (279). Using biochemical indicators to validate dietary questions is ideal as the sources of error of the two measures of diet are different, eliminating any correlation of results due to common sources of error (123). This study observed associations between intakes of fruit and vegetables, and red-cell folate

as well as 4 serum carotenoids (α-carotene, β-carotene, β-cryptoxanthin, and lutein/zeaxanthin) in regression analyses, after adjusting for confounders (all p-values <0.01) (279). There were no associations found between fruit or vegetable intake and serum lycopene levels, however, it seems fruit and vegetable intakes are poorly correlated with lycopene levels, based on the results of other validation studies using more intensive dietary assessment methods (280-282).

Based on the results of the above studies, both the fruit and vegetable items used in the TAHS 2010 questionnaire are likely to be adequate tools to rank participants based on their fruit and vegetable intakes.

4.3.4 Clinical testing methods

4.3.4.1 *Spirometry*

Baseline spirometry testing was performed under the instruction of a trained respiratory scientist. Lung function was measured using an EasyOne Pro® ultrasonic spirometer (ndd Medizintechnik, AG, Switzerland) with the subject in a seated position and using nose clips. Acceptability and repeatability of spirometry testing was assessed according to the 2005 American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines (283). The forced expired volume in one second (FEV_1) and forced vital capacity (FVC) readings from three successful manoeuvres were recorded. The highest FEV_1, FVC and FEV_1/FVC (%) values from each individual were used in the analyses. Participants were advised not to use inhalers in the 4 hours before their laboratory appointment or any oral breathing medications 8 hours prior. Participants were also requested not to smoke for 1 hour prior to their appointment.

4.3.4.2 *Methacholine Challenge*

The methacholine challenge was performed after baseline spirometry testing. Participants were excluded from the methacholine challenge if their best baseline FEV_1 was less than 1.5 litres or less than 70% of the predicted value based on their age, sex and height (n=65). FEV_1 was measured after inhalation of diluent (phosphate buffered saline) as a control measurement. A Mefar 3B dosimeter (Mefar SRL, Bovezzi, Italy) was used to administer Methacholine chloride (Provocholine®, USP Methapharm Inc. Brantford, ON, Canada) made up into solutions of varying concentrations in incremental doses (Table 4.2). Two dosing protocols were utilised during the methacholine challenge, as per ATS guidelines – a short protocol in which doses increased in quadrupling increments and a long protocol in which doses increased in doubling increments (Table 4.2) (284). The dosing protocol was selected based on the subject's history of asthma and respiratory symptoms experienced in the previous 12 months. Those performing the short protocol were changed to the long protocol if FEV_1 fell by 10% or more from the best control FEV_1. The methacholine challenge was performed until FEV_1 fell by at least 20% from the best control FEV_1 or the maximum cumulative methacholine dose of 2 mg was reached. At each measurement point two successful manoeuvres were performed and the highest FEV_1 from the two manoeuvres was used in the

analyses.

Table 4.2 - Methacholine challenge dosing protocol used in the TAHS 2010 follow up

		NUMBER OF INHALATIONS		
Concentration Methacholine	**Dose**	**SHORT Protocol**	**LONG Protocol**	**Cumulative Dose Methacholine**
0.00 mg/ml (diluent only)	Control	4	4	0.00 mg
0.39 mg/ml	1		2	0.0078 mg
	2	4	2	0.0156 mg
1.56 mg/ml	3		1	0.0312 mg
	4	3	2	0.0625 mg
6.25 mg/ml	5		1	0.125 mg
	6	3	2	0.25 mg
25.0 mg/ml	7		1	0.5 mg
	8	3	2	1.0 mg
	9	4	4	2.0 mg

Post-bronchodilator spirometry was also performed, and exhaled breath condensate samples were collected as part of the TAHS 2010 follow up study. As the results of these tests are not part of the research presented in this thesis, these tests are not described further.

4.3.4.3 *Anthropometric measures*

Height was measured to the nearest centimetre using the Leicester Height Measure Mk II (Invicta Plastics Ltd, Oadby, Leicester) with the participant shoeless. Weight was recorded in kilograms to the nearest 100 grams using the Nuweigh Personal Scale (Newcastle Weighing Services, Australia) with the participant wearing light clothing and shoeless.

Waist circumference was measured to the nearest 0.1 cm midway between the iliac crest and the costal margin (lower rib) at minimal inspiration. Hip circumference was measured to the nearest 0.1 cm at the widest circumference over the buttocks below the iliac crest.

4.3.5 Identification and assessment of covariates of interest

4.3.5.1 *Identification of covariates of interest*

Based on the theoretical DAG presented in Figure 4.1, further DAGs were developed, taking into consideration the variables available from the TAHS 2010 follow up and the relationships between these variables (Figures 4.3-4.4). In the model examining the relationship between fruit and vegetable intake and lung function (Figure 4.3), the DAGitty software identified age, BMI, sex, height, asthma status, and smoking as the minimal sufficient adjustment set. Therefore, adjustment for these variables only will minimise bias in the analyses as it closes all biasing paths. However, education and occupation were also identified as confounders. Hence, these variables were also included as confounders in the statistical analyses.

The minimal sufficient adjustment set and confounders identified in the DAG examining the relationship between fruit and vegetable intake and BR (Figure 4.3) were the same except for height which was not included in the model. Therefore, age, BMI, sex, asthma status, and smoking were the variables in the minimal sufficient adjustment set, and education and occupation were additional confounders that were included in the statistical model.

Hence, the covariates of interest in the analyses conducted are sex, height, age, BMI, asthma status, education, occupation, and smoking. The definitions used for the covariates in the statistical analyses are described below.

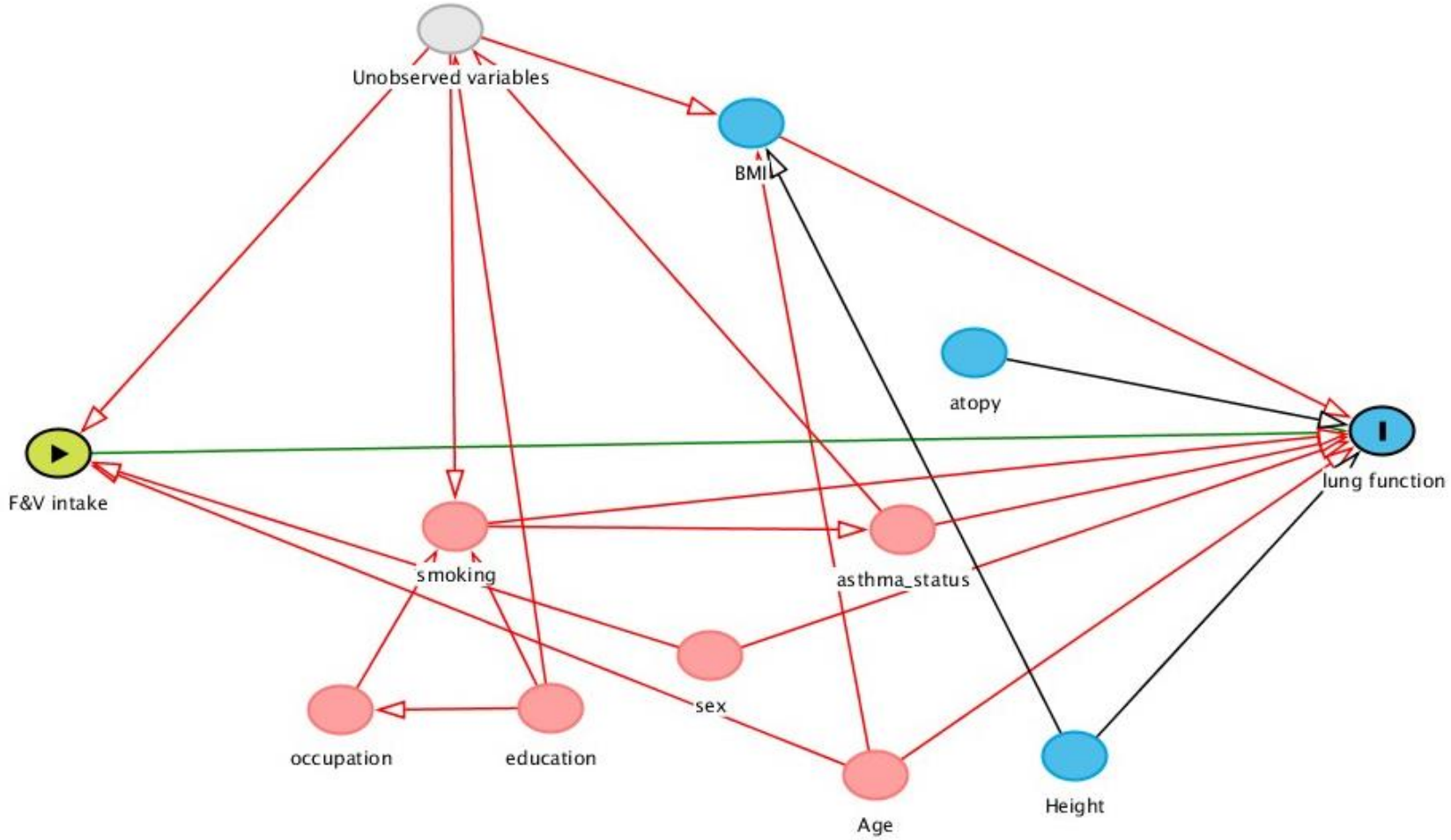


Figure 4.3 - DAG of the relationship between fruit and vegetable intake and lung function in the TAHS 2010 follow up study

Figure key: exposure outcome ancestor of outcome ancestor of exposure *and* outcome (i.e. a confounder) unobserved (latent) variable
F&V = fruit and vegetable

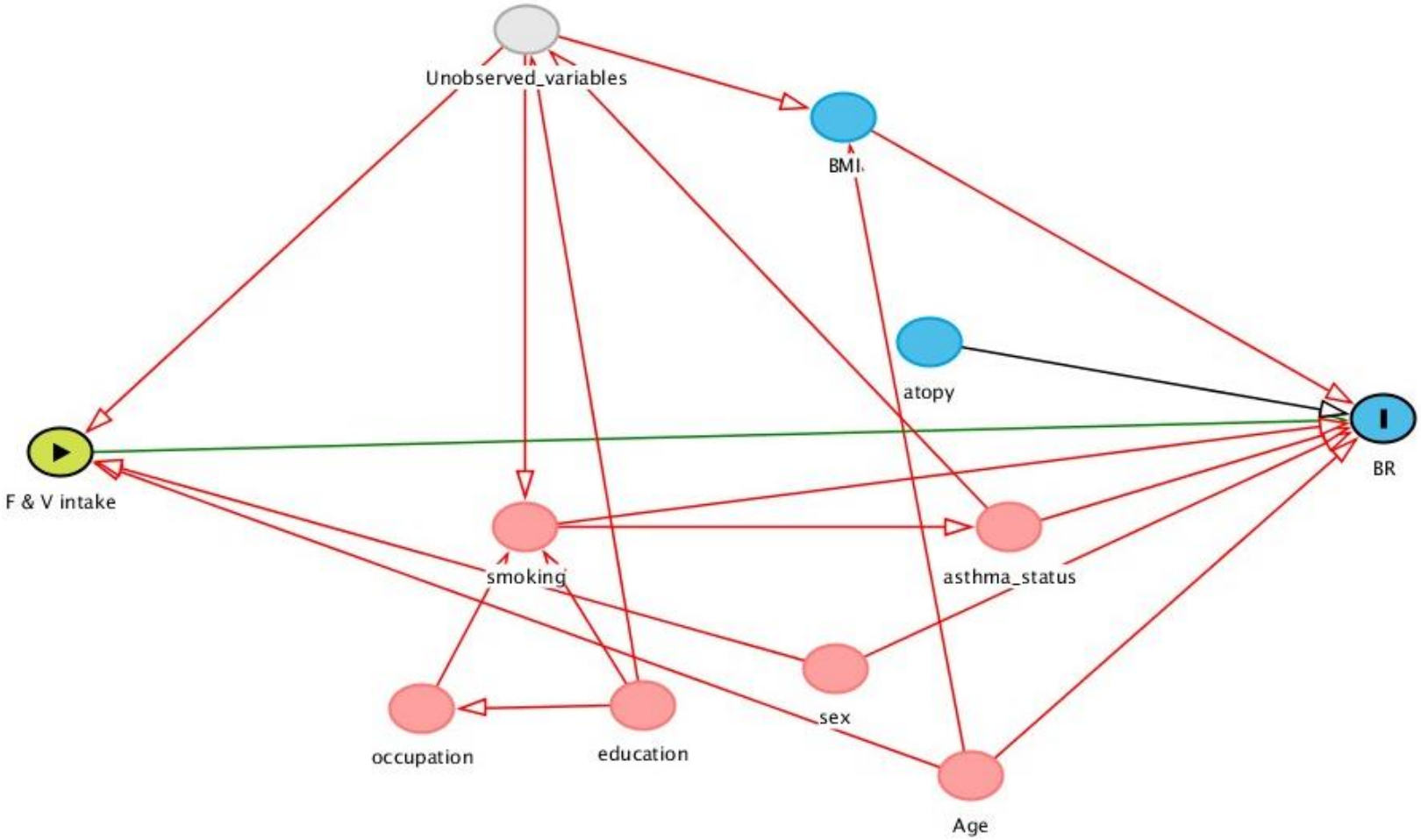


Figure 4.4 - DAG of the relationship between fruit and vegetable intake and BR in the TAHS 2010 follow up study

Figure key: exposure outcome ancestor of outcome ancestor of exposure *and* outcome (i.e. a confounder) unobserved (latent) variable
F&V = fruit and vegetable

4.3.5.2 ***Assessment of covariates of interest***

Age

The participant's age in years at the time of testing was calculated from their date of birth and their laboratory test date as follows: (laboratory appointment date – date of birth)/365.25.

BMI

BMI was calculated using the following equation and the weight and height measures obtained in the laboratory session:

$$BMI\ (kg/m^2) = \frac{\text{weight (kilograms)}}{\text{height (metres)}^2}$$

BMI was then categorised as 'healthy weight' (BMI < 25 kg/m^2), 'overweight' (25 kg/m^2 ≤ BMI <30 kg/m^2) and 'obese' (BMI ≥ 30 kg/m^2) according to the BMI cut-offs recommended by the WHO (285). The small number of underweight participants (BMI< 18.5 kg/m^2, n=3) were included in the 'healthy weight' category.

Asthma status

Asthma status was categorised into three groups, 'Never Asthma', 'Remitted Asthma' and 'Current Asthma', derived from the participant's responses to two questions - 1) 'Have you at any time in your life suffered from asthma or wheezy breathing?' 2) 'Have you had an attack of asthma or wheezy breathing in the last 12 months?' Those who responded "yes" to both questions were categorised as having current asthma. Those who answered "yes" to the asthma ever question and "no" to the "attack in the last 12 months" question were categorised as remitted asthma. Those who responded "no" to the asthma ever question were categorised as never asthma.

Smoking status

Smoking status was categorised into three groups, 'Never Smoker', 'Former Smoker' and 'Current Smoker', which was derived from the subject's responses to two questions on personal smoking habits - 1) 'In your lifetime, have you smoked at least 100 cigarettes or equal amounts of cigars, pipes or any tobacco product?' and 2) 'Do you currently smoke (within the last 4 weeks)?' Options were 'Yes' or 'No' for question 1 and 'Not at all', 'Yes, daily', 'Yes, at least weekly' and 'Yes, less than weekly'. Those who responded 'No' and 'Not at all' to the questions respectively were categorised as 'Never Smoker', those who responded 'yes' and 'Not at all' respectively were categorised as 'Former Smoker', and those who indicated they currently smoked in the second question, regardless of the frequency of their smoking, were categorised as a 'Current Smoker'.

4.3.6 Methods of statistical analysis

This section provides an overview of the statistical methods employed to analyse the data obtained from the TAHS 2010 follow up study. Further details of the statistical analyses specific to each

research question can be found in the relevant chapters.

4.3.6.1 *Data checking and cleaning*

Baseline spirometry measures FEV_1, FVC and FEV_1/FVC, and the change in FEV_1 during the methacholine challenge were the outcomes of interest. Fruit intake and vegetable intake were the exposures of interest. The covariates of interest were sex, height, age, BMI, asthma status, education, occupation, and smoking. Some data checking and cleaning had previously been performed on the 2010 follow up data by TAHS investigators. I performed the following data checking and cleaning in Stata version 13.1.

- The diet questionnaire data were in a separate datafile to the 2010 follow up laboratory and survey data. I searched for duplicate observations in the diet questionnaire data and dropped any duplicates or incomplete records (n=2). I then prepared the datafiles for merging, ensuring the merging variable (idnumber) had the same name in both files and changing variable names in the fat questionnaire file so that different variables had unique names (e.g. q1 was renamed FQq1). Labels were also added to provide a brief description of the variables for easier handling and interpretation later.
- The diet questionnaire datafile was merged into the 2010 follow up laboratory and questionnaire datafile. Observations with data in only one of the merged files were identified (n=29). Some of these were identified as dummy observations (n=3), most were participants that did not complete the diet questionnaire (n=19). The remaining observations were cross-checked with scanned electronic versions of the completed questionnaires (n=7). Data was entered manually where possible (diet questionnaire: data entered n=1, electronic data missing n=1; background questionnaire: electronic data missing n=5).
- Some participants made comments regarding their milk use at questions 15 and 16 of the diet questionnaire. Therefore, responses to these questions were cross-checked against the electronic files and amended where necessary (n=4).
- A scatterplot of height versus weight was produced and examined to identify any implausible values. There were no outliers.
- Derived variables for asthma status, smoking status, BMI and BMI categories were provided by the TAHS investigators. These variables were cross-checked against the raw data from which they were derived. The derived variables were correct and there were no missing observations except where the raw data was missing.
- There were multiple age/date of birth variables (n=4). I compared these variables and identified the variable with the least number of missing values (masterdob). I then converted this variable and the appointment date variable to numeric values, recalculated age at the time of the laboratory appointment using these variables and removed any implausible values. I compared these age values with the existing age variable. As the existing variable had fewer missing values, I updated any missing values in the existing age variable with the

newly generated age values.

- I generated the following derived variables: fruit intake (<1, 1, 2, 3, 4+ serves/day), vegetable intake (<1, 1, 2, 3, 4+ serves/day), education (categories: 1) <grade 12 or equivalent; 2) grade 12 or equivalent, trade or apprenticeship, or certificate or diploma; 3) University degree or higher), occupation (categories: 1) General manager/professional; 2) technician, tradesperson, small business owner, advanced clerical or service worker 3) Administration worker, sales, service worker), and FEV_1/FVC (%) (FEV_1/FVC x 100).
- Coding for questions 9, 17 and 18 of the SFQ were amended as per advice provided by Professor Lisa Wood, a TAHS investigator and expert in the area of diet and respiratory disease (Q9: How do you spread butter/marg on bread? – Never use butter/marg (0), thinly (2), medium (3), thickly (4); Q17: How much skin on chicken do you eat? – None of the skin/I am vegetarian (0), some of the skin (1), most or all of the skin (2); Q18: How much of the fat on meat do you eat? No fat/I am vegetarian (0), some of the fat (1), most or all of the fat (2)).
- A 'milk fat' variable was derived from questions 15 and 16 of the diet questionnaire. Values were allocated as per advice provided by Professor Lisa Wood with consideration given to the lower fat content of soy milk compared to cow or goat's milk. Responses were categorised and coded as follows: skim cow or goat's milk, skim or reduced fat soy milk, and no milk were categorised as 'skim or no milk' (coded 0); reduced fat cow or goat's milk, full cream soy milk, full cream and reduced fat soy milk, and soy milk (form not specified) were categorised as 'reduced fat' (coded 1); full cream and reduced fat cow or goat's milk was categorised as 'full cream and reduced fat' (coded 2); full cream cow or goat's milk was categorised as 'full cream' (coded 3); and condensed or evaporated cow or goat's milk was categorised as 'condensed or evaporated' (coded 4).
- A 'fat score' was calculated by summing the values given for questions 1-8, 10-14, the recoded values for questions 9, 17 and 18, and 'milk fat'.
- One participant confirmed a diagnosis of bronchiectasis in the laboratory questionnaire (ID 3124) and was excluded from further analyses.

4.3.6.2 ***Statistical methods used in analyses***

Descriptive statistics for each variable were summarized as mean (standard deviation) for normally distributed continuous variables, median (interquartile range) for non-normally distributed continuous variables and frequency (percent) for categorical variables. Univariable and multivariable linear regression methods were used to assess the associations between each diet factor of interest and the baseline spirometry measures FEV_1, FVC and FEV_1/FVC. For methods that assume a linear relationship between the outcome and exposure, the likelihood ratio test was used to check the linearity assumption. The statistical method for investigating factors associated with BR identified in research question 1 was used to examine associations between fruit and vegetable intakes and BR for research question 2. Confounders identified in the relevant DAGs were adjusted for in the models.

Effect modification was investigated in all relationships of dietary factors and lung function using the likelihood ratio test to compare models with and without interaction terms. Potential effect modifiers examined were sex, BMI, asthma status, and smoking. All analyses were conducted using Stata version 13.1 (StataCorp, College Station, TX, USA).

4.3.7 Ethics

Written informed consent was obtained from all participants during their laboratory appointment. Ethics approval for the TAHS 2010 follow up study was obtained from the Human Research Ethics Committee of the University of Melbourne (HREC Ref. number 040375).

4.4 The COPD Study

4.4.1 Overview of the COPD Study

The COPD Study is a two-stage cross-sectional epidemiological study designed to investigate the relationship between diet and other environmental and lifestyle factors and asthma and COPD in middle-aged and older adults. More specifically, the study aimed to examine occupational exposure to gases, dust and fumes, and exposure to tobacco smoke and combustion by-products in the home as possible causes of COPD and asthma in middle-age, and the possible protective effects of antioxidant vitamins and omega-3 fatty acids obtained from the diet or supplements (263, 264, 286).

A sample of 7,005 adults aged between 45 and 69 years was randomly selected from the electoral rolls of three inner south-east Melbourne electorates – Higgins, Hotham and Goldstein (263). The first stage of the study was completed in the year 2000. Subjects were sent an invitation to take part in the study via post, along with a short respiratory questionnaire. The survey was completed by 4,923 participants, achieving a response rate of 70% (263, 286, 287).

A sample of 2,900 subjects was then randomly selected from the respondents to the postal survey and invited to attend a laboratory session to complete further testing. Between December 2000 and December 2002, 1,232 participants attended the laboratory session, achieving a response rate of 42%. During the laboratory session participants completed two questionnaires and a series of clinical tests. One questionnaire was a validated semi-quantitative food frequency questionnaire to measure usual diet over the previous 12 months (Appendix 3). The other questionnaire collected basic demographic information along with information regarding current and previous respiratory health; family history of respiratory disease; work history (to ascertain possible occupational exposures); exposure to potential environmental risk factors in the home; smoking; use of medications to manage respiratory conditions; and use of fish oil or fatty acid supplements (Appendix 4). Anthropometric measurements (height and weight) were performed and recorded according to the testing protocol, and a 14 ml blood sample was collected. One thousand and fifty-four participants completed the full testing protocol during their laboratory session. Figure 4.5 displays a flow diagram of the sampling

method used in the COPD study.

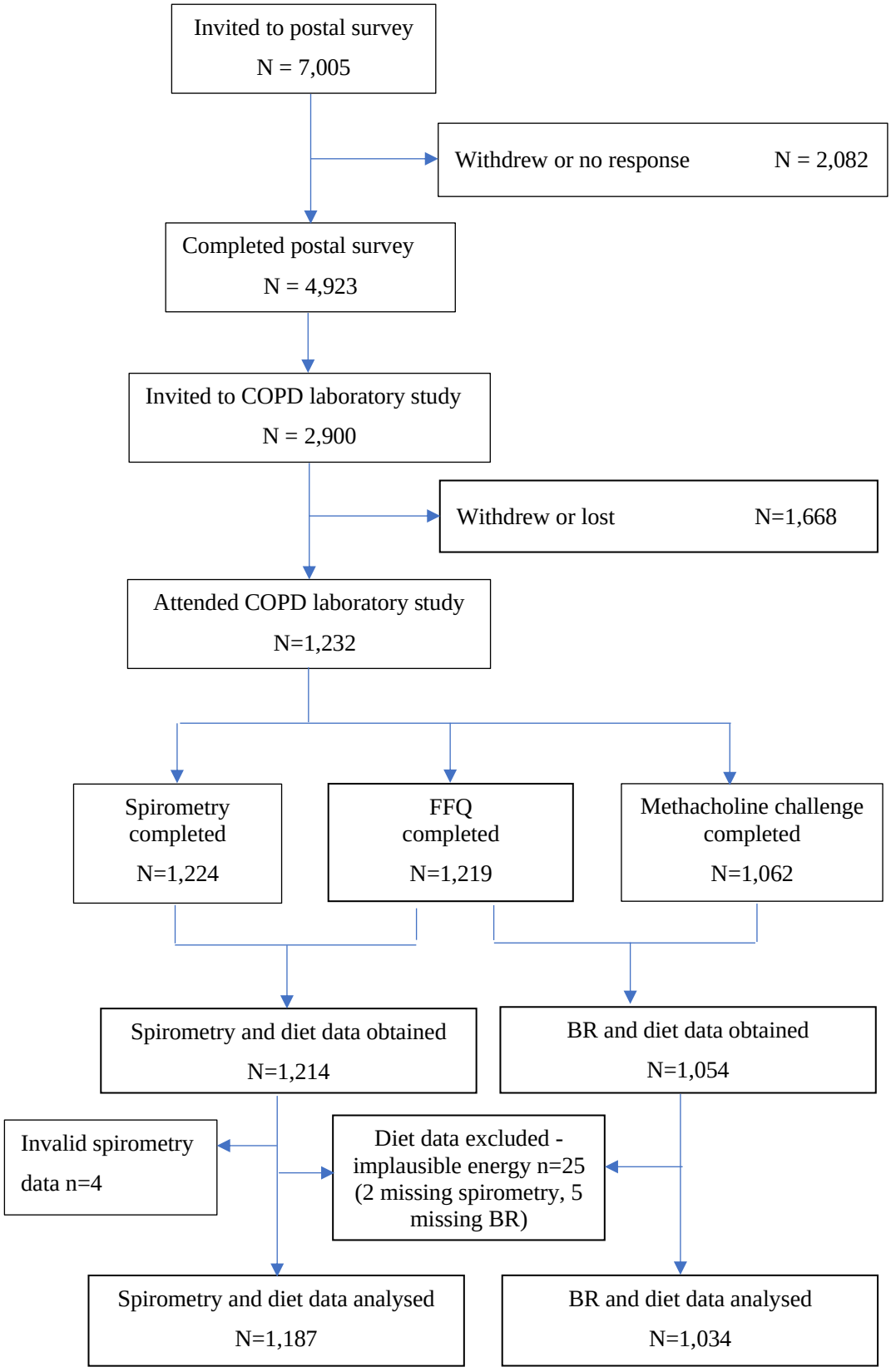


Figure 4.5 - Ascertainment of the COPD study sample included in the analyses

4.4.2 Research questions

Data from the COPD study was used to answer the following questions.

- Research Question 3: Are dietary patterns (defined using principal component analysis) associated with lung function and BR in middle-aged and older adults? Does sex, smoking status, BMI, asthma status, or atopy modify the relationships between the dietary patterns identified and lung function or BR?
- Research Question 4: Is the inflammatory potential of the diet (measured using the DII) associated with lung function and BR in middle-aged and older adults? Are any of these relationships modified by sex, smoking status, BMI, asthma status, or atopy?

The methods used to collect the relevant data to answer these questions are described below.

4.4.3 Clinical testing methods

4.4.3.1 ***Spirometry***

Baseline spirometry testing was performed under the instruction of a trained respiratory scientist following the guidelines produced by the ATS (288). Lung function was measured using a rolling seal spirometer (Sensormedics, California, USA) with the subject in a seated position and using nose clips. The FEV_1 and FVC readings from five successful manoeuvres were recorded. The highest FEV_1 and FVC values from each individual were used in the analyses and also used to calculate FEV_1/FVC (%). Participants were advised not to use inhalers in the 4 hours prior to their laboratory appointment or any oral medications for their breathing 8 hours prior. Participants were also requested not to smoke in the 6 hours before their appointment.

4.4.3.2 ***Methacholine Challenge***

Following baseline spirometry testing, a methacholine challenge was performed. FEV_1 was measured after inhalation of diluent (phosphate buffered saline) as a control measurement. Methacholine (USP Methapharm Inc. Brantford, ON, Canada) was then administered by a Mefar 3B dosimeter (Mefar, Bovezzi, Italy) in incremental doses. Two dosing protocols were used in the methacholine challenge test, as recommended by ATS guidelines – a short protocol in which doses increased in quadrupling increments and a long protocol in which doses increased in doubling increments (Table 4.3) (284). The dosing protocol was selected based on the subject's history of asthma and respiratory symptoms experienced in the previous 12 months. Those performing the short protocol were changed to the long protocol if FEV_1 fell by 10% or more from the best control FEV_1. The methacholine challenge was performed until FEV_1 fell by at least 20% from the best control FEV_1 or the maximum cumulative methacholine dose of 2 mg was reached. At each measurement point two successful manoeuvres were performed and the highest FEV_1 from the two

manoeuvres was used in the analyses.

Table 4.3 - Methacholine Challenge Dosing Protocol used in the COPD study

		NUMBER OF INHALATIONS		
Concentration Methacholine	**Dose**	**SHORT Protocol**	**LONG Protocol**	**Cumulative Dose Methacholine**
0.00 mg/ml (diluent only)	Control	4	4	0.00 mg
0.39 mg/ml	1		2	0.0078 mg
	2	4	2	0.0156 mg
1.56 mg/ml	3		1	0.0312 mg
	4	3	2	0.0625 mg
6.25 mg/ml	5		1	0.125 mg
	6	3	2	0.25 mg
12.5 mg/ml	7		2	0.5 mg
	8	6	4	1.0 mg
	9	8	8	2.0 mg

Post-bronchodilator spirometry and carbon monoxide lung transfer factor by single breath carbon monoxide were also performed as part of the COPD study. As the results of these tests are not part of this thesis these tests are not described in detail.

4.4.4 Food Frequency Questionnaire (FFQ)

The FFQ used in the COPD study is a semi-quantitative FFQ developed by the Cancer Council of Victoria (CCV) to assess an individual's usual food intake over the previous 12 months (Appendix 3). The first version of the questionnaire was developed in the 1980s and was specifically designed to measure the dietary intake of an ethnically diverse group of adults aged between 40 and 69 years living in Melbourne (289). It was updated in the 1990s to remove some items and incorporate questions on portion size to assist in estimating intakes. This updated version of the questionnaire was used in the COPD study. It contains 74 questions on the frequency of consumption of a selection of commonly eaten foods grouped into 4 categories – cereal foods, sweets and snacks; dairy products, meat and fish; fruit; and vegetables. There are 10 consumption frequency options ranging from 'never' to '3 or more times per day'. The FFQ also includes 8 questions on the consumption of

alcoholic beverages, 4 questions on portion size with photographs of different portion sizes to aid in the estimation of intakes, and 13 additional questions to further assist in calculating food and nutrient intakes, for example “What type of bread do you usually eat?”. The updated version of the questionnaire has been validated against weighed food records in a study of 63 women aged 16 to 48 years. The correlation coefficients achieved in this study for the 27 nutrients assessed ranged from 0.20 to 0.60, 0.28 to 0.78 after adjusting for energy intake (290). These correlation coefficients are comparable to those reported for other similar dietary assessment tools (291-293).

The FFQ was self-administered by the participants in the COPD study. The portion size questions were used to calculate a portion size factor for each subject. This factor and the remaining data obtained from the FFQ were then used to estimate individual intakes of 95 food items, 5 types of alcoholic beverage and 31 nutrients (Table 4.4). The intakes of 31 fatty acids, glycemic load and glycemic index were also estimated for each individual using the data obtained from the FFQ, however, these measures have not been utilised in the research contained in this thesis and will not be further detailed.

Table 4.4 - Food items, alcoholic beverages and nutrients estimated by FFQ in the COPD study

Foods (grams/day)			Nutrients (grams/day unless otherwise indicated)	Alcoholic beverages (grams/day)
Full cream milk	Cakes	Pineapple	Total energy*	Light beer
Reduced fat milk	Meat pies	Apricots	Total fat	Heavy beer
Skim milk	Pizza	Peaches	Saturated fat	Red wine
Soy milk	Hamburger	Mango	Polyunsaturated fat	White wine
Hi-fibre white bread	Chocolate	Avocado	Monounsaturated fat	Fortified spirits
White bread	Flavoured milk	Chips	Protein	Spirits
Wholemeal bread	Nuts	Potatoes	Total carbohydrates	
Rye bread	Peanut butter	Tomato sauce	Sugars	
Multigrain bread	Crisps	Tomatoes	Starch	
Margarine	Jam	Capsicum	Fibre	
Polyunsaturated margarine	Vegemite	Lettuce	Alcohol	
Monounsaturated margarine	Ice-cream	Cucumber	Calcium^	
Butter/margarine blends	Yoghurt	Celery	Cholesterol^	
Butter	Beef	Beetroot	Folate†	
Hard cheese	Veal	Carrots	Iron^	
Firm cheese	Chicken	Cabbage	Magnesium^	
Soft cheese	Lamb	Cauliflower	Niacin^	
Ricotta or cottage cheese	Pork	Broccoli	Phosphorus^	
Cream cheese	Bacon	Spinach	Potassium^	
Low fat cheese	Ham	Peas	Retinol†	
sugar	Salami	Green beans	Riboflavin^	
Eggs	Sausages	Bean sprouts	Sodium^	
All bran	Fish	Baked beans	Thiamin^	
branflakes	Fried fish	Tofu	Vitamin C^	
Weetbix	Tinned fish	Other beans	Vitamin E^	
Cornflakes	Tinned fruit	Pumpkin	Zinc^	
Porridge	Fruit juice	Onion	Alpha-carotene†	
Muesli	Oranges	Garlic	Beta-carotene†	
Rice	Apples	Mushrooms	Beta cryptoxanthin†	
Pasta	Pears	Zucchini	Lutein Zeaxanthin †	
Crackers	Bananas	Strawberries	Lycopene†	
Sweet biscuits	Melon			

* Kilojoules per day; ^ milligrams per day; †micrograms per day

4.4.5 Identification and assessment of covariates of interest

Based on the DAG presented in Figure 4.1, further DAGs were developed modelling the relationship between dietary quality (assessed by dietary patterns), lung function and BR using the data from the COPD study (Figures 4.6 and 4.7). In the model examining lung function as the outcome, the variables in the minimal sufficient adjustment set were sex, height, age, BMI, asthma status, smoking, and atopy. The variables in the minimal sufficient adjustment set in the model examining BR were the same as those for lung function except height was excluded. Therefore, the covariates of interest in the COPD study are sex, height, age, BMI, asthma status, smoking and atopy. The definitions used for these covariates are given below.

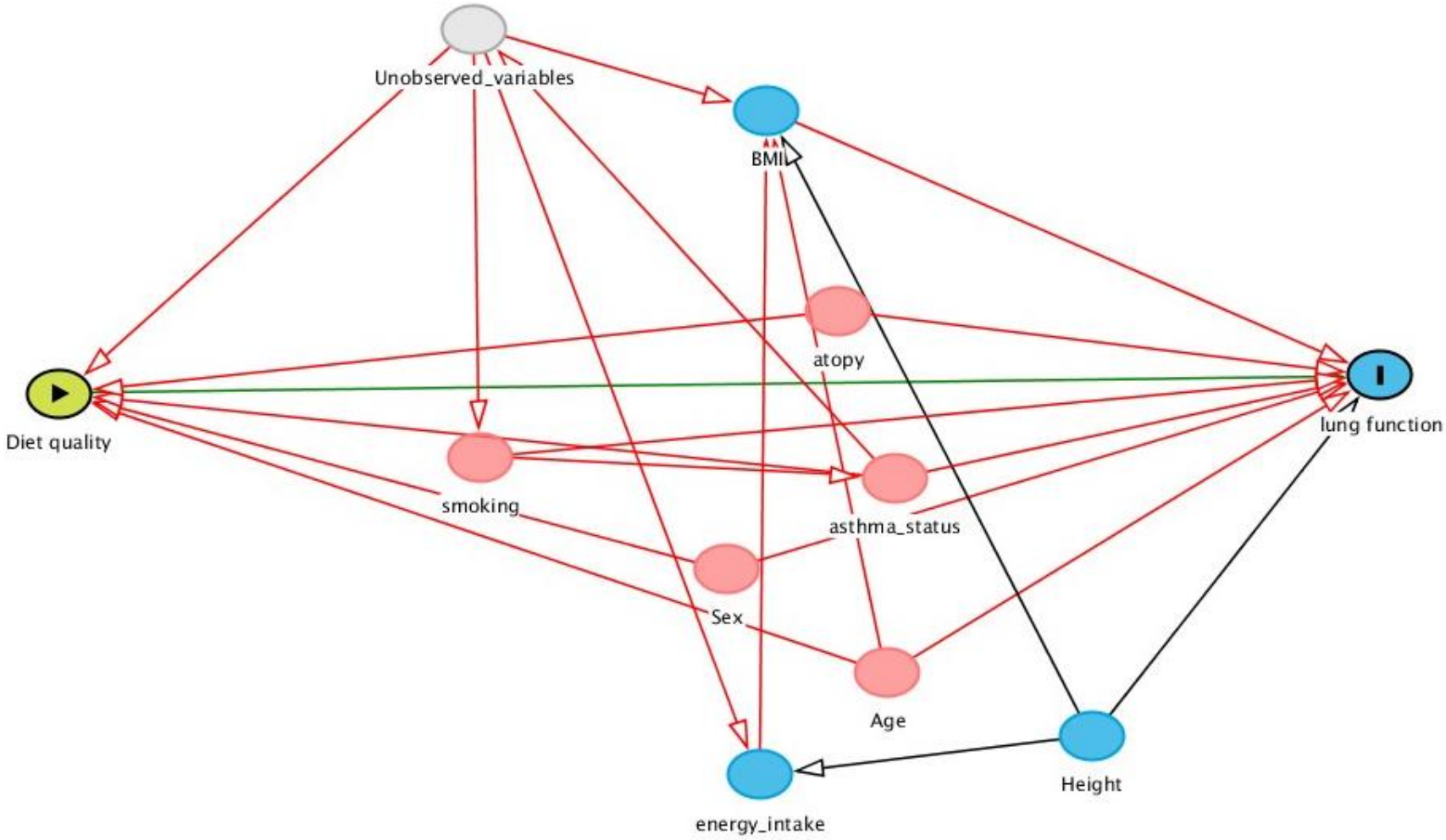


Figure 4.6 - DAG of the relationship between diet quality and lung function in the COPD study

Figure key: exposure outcome ancestor of outcome ancestor of exposure *and* outcome (i.e. a confounder) unobserved (latent) variable

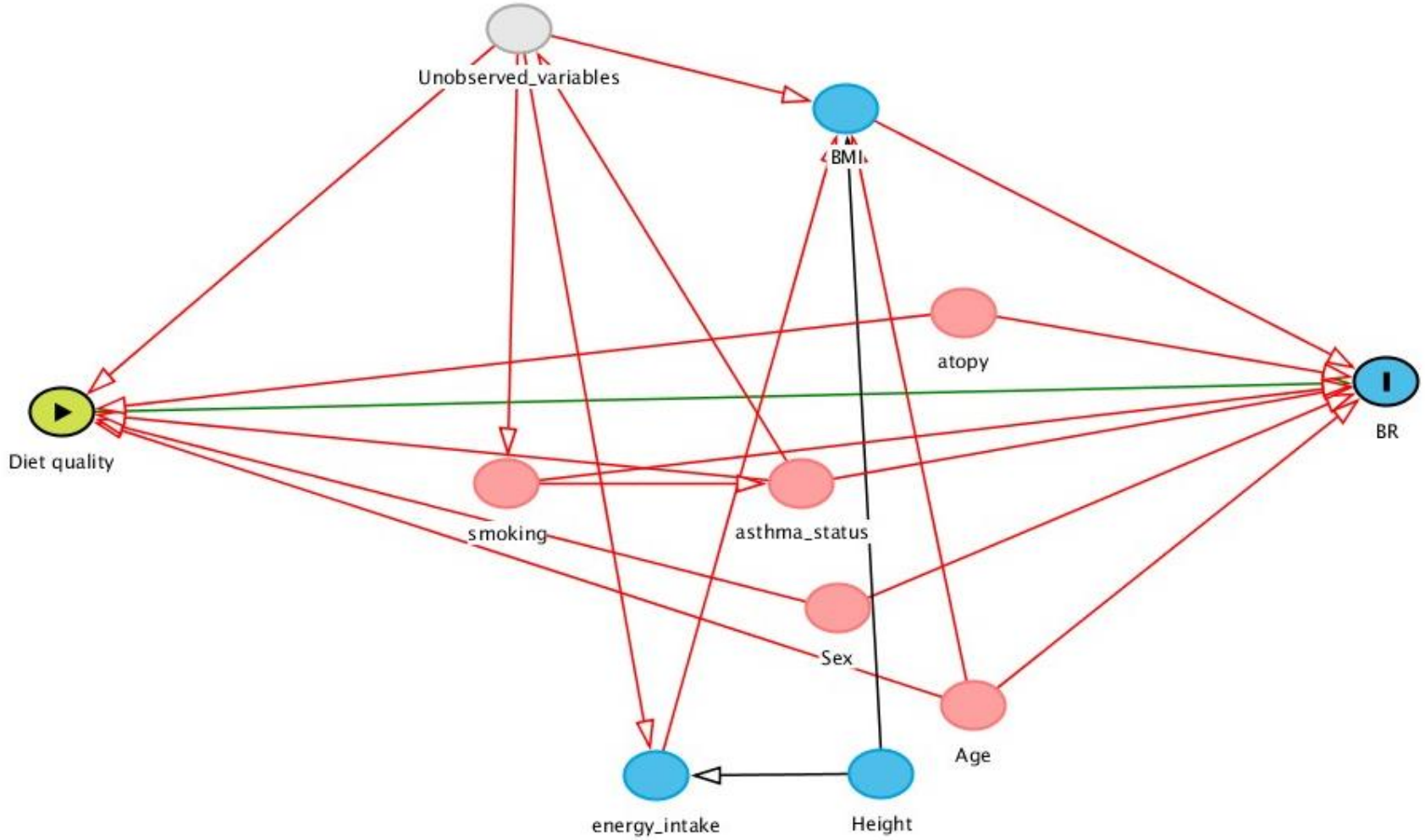


Figure 4.7 - DAG of the relationship between diet quality and BR in the COPD study

Figure key: exposure outcome ancestor of outcome ancestor of exposure *and* outcome (i.e. a confounder) unobserved (latent) variable

Age

The participant's exact age in years at the time of testing was calculated from their date of birth and their laboratory test date as follows: (laboratory test date – date of birth)/365.25.

BMI

BMI was calculated using the following equation:

$$BMI\ (kg/m^2) = \frac{\text{weight (kilograms)}}{\text{height (metres)}^2}$$

BMI was then categorised into 'healthy weight' (BMI < 25 kg/m^2), 'overweight' (25 kg/m^2 ≤ BMI <30 kg/m^2) and 'obese' (BMI ≥ 30 kg/m^2) based on the overweight and obesity BMI cut-offs recommended by the WHO (285). Due to the low number of underweight participants (BMI< 18.5 kg/m^2, n=3), these participants were included in the 'normal weight' category.

Asthma status

Asthma status was categorised into three groups, 'Never Asthma', 'Remitted Asthma' and 'Current asthma', based on the participant's responses to three questions on their respiratory health - 1) 'Have you ever had asthma?' 2) 'Was this confirmed by a doctor?' and 3) 'Have you had an attack of asthma in the last 12 months?' A 'no' response to all three questions was indicative of never suffering from asthma and was categorised as 'Never Asthma'. Those who indicated they had had asthma, but it was not confirmed by a doctor were also classified as 'Never Asthma'. Those who had been diagnosed with asthma and had not experienced an attack in the last 12 months were classed as 'Remitted Asthma' and those who responded 'yes' to all three questions were categorised as 'Current Asthma'.

Smoking status

Smoking status was categorised into three groups, 'Never Smoker', 'Former Smoker' and 'Current Smoker', based on the subject's responses to two questions on their previous and current smoking habits - 1) 'Do you currently smoke cigarettes, cigars, pipes or any other tobacco products?', the options for which were 'Not at all', 'Less often than weekly', 'At least weekly (not daily)', and 'Daily' and 2) 'In your lifetime, have you ever smoked at least 100 cigarettes or similar amount of tobacco?' Those who responded 'Not at all' and 'No' to the questions respectively were categorised as 'Never Smoker', those who responded 'Not at all' to the first question and 'yes' to the second were categorised as 'Former Smoker', and those who indicated they currently smoked in the first question, regardless of the frequency indicated by their response, were classed as 'Current Smoker'.

Atopy

Skin prick testing (SPT) was conducted to determine the allergic response to 8 common aeroallergens – Cladosporium, Alternaria, Aspergillus, ragweed, rye grass, Penicillium, house dust

mite and cat (Hollister-Stier, USA). Positive and negative controls (histamine solution and saline solution respectively) were used to establish the validity of the test. One droplet of each allergen was placed on the flexor side of the subject's forearm and the skin beneath each droplet was pricked with a lancet. The diameters of the wheals produced in response to the allergen solutions were measured and recorded in millimetres after 10-15 minutes.

The results of the SPTs were used to define atopy. Atopy was categorised as 'Yes' or 'No'. Those with a positive skin prick test (wheal size ≥ 3 mm) to at least one tested aeroallergen in the presence of a positive test result to the positive control and a negative result to the negative control (indicating a valid test) were considered atopic and classified as 'Yes'.

4.4.6 Methods of statistical analysis

This section provides an overview of the statistical methods employed to analyse the data obtained from the COPD study. Further details of the statistical analyses specific to each research question can be found in the relevant chapters.

4.4.6.1 *Data checking and cleaning*

Much of the data checking and cleaning of the COPD study laboratory data had been carried out previously by COPD investigators. However, the datafiles relating to the FFQ had been lost. The following explains my efforts to recover the data and the subsequent data checking and cleaning I performed using Microsoft Excel and Stata version 13.1.

- I visited Professor Michael Abramson (the Chief Investigator on the COPD study) at Monash University to collect the relevant electronic files for the COPD study and search their servers for the lost FFQ data with Professor Abramson's guidance. Our search was unsuccessful. Professor Abramson then requested the hardcopies of the FFQs to be retrieved from the archives which are stored offsite. Once retrieved, I collected these hardcopies from Monash University. There were 1228 hardcopy FFQs, excluding the FFQs from a repeatability study.
- The hardcopies of the FFQs had previously been sent to the Cancer Council of Victoria (CCV) for scanning and automated estimation of daily intake of foods, nutrients, and alcoholic beverages. The returned electronic files, and any cleaning that had been performed, were the files that had been lost. One of my supervisors, Associate Professor Allison Hodge, was able to locate the electronic files for the COPD study in the FFQ archives at the CCV. There were 40 electronic files in .csv excel format.
- Files relating to a repeatability study were removed. The remaining files were combined to create four files – one each for responses, estimated daily food intakes, estimated daily nutrient intakes, and estimated daily nutrient intakes from alcoholic beverages. There were 1265 observations in total and 43 occasions where the ID number occurred more than once.

- The electronic file of FFQ responses was cross-checked against the FFQ hardcopies. This cross-check identified 1219 matched records, two hard copies without electronic records, and three electronic records with no hard copy. There was also one ID number with two electronic records, neither of which matched the hard copy. Thirty-four records were rescanned due to errors in the initial scan. The initial scan for these ID numbers was, therefore, deleted. Three duplicate records and three records from the repeatability study were deleted. I also deleted the two records with the same ID number that didn't match the hard copy. Four of the electronic records for two ID numbers had no hardcopy. Therefore, it was difficult to determine if the later record was from the repeatability study or was a rescan due to reader error. Hence, all four of these records were deleted. The remaining electronic record with no hard copy was assumed correct and retained. Data from the two hard copy FFQs that were not scanned were omitted from the analysis. Deleted records were also deleted from the datafiles for daily food intakes, daily nutrient intakes, and alcohol intake.
- The final diet datasets contained 1219 observations. I prepared the diet datafiles to import them into Stata. I ensured the merging variables had the same name in all files and I changed variable names in the diet datafiles so that different variables had unique descriptive names that were compatible with Stata. I then imported the diet datafiles into Stata, reformatted the date variable in all files, and merged the files together into one diet data file (merging variables: scan_no, scan_id, scan_date). Nutrient intakes from food and alcohol were summed together to create total nutrient intakes. I then merged this diet dataset with the main COPD dataset. There were 1232 observations in the main COPD dataset, 1216 of which matched with observations from the diet datafile (unmatched: 16 from the COPD datafile, 3 from the diet datafile). All unmatched observations were dropped.
- Variables of interest that were created by COPD investigators were cross-checked with the raw laboratory data in the database. There were three variables for sex, two raw data variables and one derived. Two variables had complete data and results were consistent (one raw data and one derived variable). Therefore, I have used one of these variables. A scatterplot of height and weight identified an implausible value for height. This value was recoded as missing. Variables generated by the COPD investigators for asthma, smoking, and atopy did not match the raw laboratory data and/or the definitions for these variables were unclear. Therefore, I generated my own variables from the raw laboratory data. I also generated derived variables for age and BMI. The outcome variables FEV_1 and FVC were determined as the maximum result from five satisfactory manoeuvres. FEV_1/FVC, expressed as a percentage, was calculated as best FEV_1/best FVC x 100. Implausible values for FEV_1/FVC were identified (FEV_1/FVC >100%, n=4). The corresponding lung function values for these observations were recoded as missing. Histograms and scatterplots of various lung function variables were examined, and outliers investigated. Invalid results were identified and corrected using results from the other four replicates (FEV_1 n=1; FVC

n=3). Similar examination of the FEV_1 values from the methacholine challenge identified invalid results which were corrected using the other duplicate reading (n=2).

- To minimise the impact of implausible dietary intakes, those with energy intakes in the top and bottom 1% for each sex were excluded from the analyses (i.e. energy intakes ≤ 3054.5 kJ/day and ≥ 13955.6 kJ/day for women, and ≤ 4513.8 kJ/day and ≥ 18005.7 kJ/day for men).

4.4.6.2 *Statistical methods used in analyses*

The COPD data was analysed using the same methods as described for the TAHS data analyses. The outcomes of interest were the same; however, the exposure of interest was dietary patterns. Dietary patterns were identified using principal component analysis for research question 3 and the DII for research question 4 (110). Both methods create a score for each individual for each dietary pattern. These scores represent the individual's adherence to that dietary pattern and were used as the exposure variables in the statistical analyses. Confounders identified in the relevant DAGs were adjusted for. The same effect modifiers were also investigated with the addition of atopy. All analyses were conducted using Stata version 13.1 (StataCorp, College Station, TX, USA).

4.4.7 Ethics

Written informed consent was obtained from all participants during their laboratory appointment. Ethics approval for the COPD study was obtained from the Standing Committee on Ethics in Research on Humans at Monash University, Melbourne.

4.5 Summary

The relationships between diet, lung function and BR are complex because of the multiple ways of measuring diet, none of which are without limitations, and the complicated web of inter-relationships between diet, lung function, and the potential confounders and other factors in this relationship. Examining diet in different ways (i.e. nutrient, food and dietary pattern) and interpreting the results collectively, with consideration of the strengths and limitations of each method, may help to provide a clearer picture of these relationships. Investigating carefully selected potential effect modifiers may reveal associations that were previously hidden, identify population groups that may benefit from dietary change, and highlight biological pathways underlying the development of specific disease phenotypes. Hence, although these relationships are complex, it is important that they are investigated in a systematic and thorough manner. Every step I have taken and every decision I have made in this research has been untaken with careful consideration. One of these steps identified that the methods used in assessing risk factors for BHR had significant limitations which may produce false findings. The next chapter highlights these limitations and suggests a different methodology for analysing bronchial provocation challenge data using an established statistical method, the linear mixed model. This method will be thoroughly described in

the next chapter and then used in subsequent chapters to examine the relationship between diet and BR.

Chapter 5 - Measuring factors associated with bronchial responsiveness

5.1 Introduction

Bronchial hyperresponsiveness (BHR) is the increased sensitivity of the airways to inhaled stimuli causing the airways to narrow, making it difficult to breathe (75). BHR is a characteristic of asthma and a risk factor for COPD and ACO (38, 61, 62, 79). However, it is not a feature present in all people who suffer from these diseases, suggesting that it may be associated with certain asthma, COPD and ACO phenotypes, possibly pointing to underlying biological mechanisms of such phenotypes. Therefore, it is important to investigate the factors associated with bronchial responsiveness as this knowledge may help identify populations at risk of developing these diseases; assist in defining disease phenotypes; and aid understanding of the biological pathways involved in certain phenotypes. These biological pathways can then be targeted therapeutically.

Bronchial responsiveness is measured clinically via a bronchial provocation challenge. In this test, subjects inhale incremental doses of a provocative agent, commonly methacholine. To measure the response of the subject's airways to the provocative agent, FEV_1 is measured at baseline (no agent) and after each incremental dose. The test is complete when a pre-defined percentage fall in FEV_1 is achieved (usually 20% for methacholine) or the pre-defined maximum cumulative dose of the provocative agent has been administered (284). Therefore, at test completion, each subject has at least two FEV_1 measurements; however, usually they have more than two and those who receive the maximum cumulative dose can have up to ten FEV_1 measurements.

5.1.1 Current methods of identifying risk factors of increased BR

Factors associated with increased bronchial responsiveness are typically assessed by either 1) logistic regression of a dichotomised BHR variable in which BHR is positive if a pre-selected percentage fall in FEV_1 is achieved, and negative otherwise; or by 2) linear regression of the dose-response slope estimated from two FEV_1 measurements, usually the initial and final measurements (253, 294-299). Both these methods have several limitations. Firstly, both methods use only two FEV_1 measurements, discarding most of the data collected. This unused data may contain valuable information on an individual's response to the provocative agent and the variation in the response between individuals. Secondly, both methods calculate the fall in FEV_1 as a percentage of the initial FEV_1. The calculation is as follows

$$\%\ change\ in\ FEV_1 = \frac{FEV_{1\ (initial)} - FEV_{1\ (final)}}{FEV_{1\ (initial)}}\ x\ 100$$

Using the % change in FEV_1 to determine the outcome means that all individuals start at the same point (100%) and differences in baseline lung function between participants are not accounted for. This is an issue given baseline lung function has been shown to be associated with BR (300-308). The use of percent change in FEV_1 is also problematic as it means those with poorer lung function and lower baseline FEV_1 values are inherently more likely to be classified as having BHR. This is because they will have a greater percentage change for the same absolute change in FEV_1 as those with better lung function and higher baseline FEV_1 values.

Thirdly, a dichotomised BHR outcome may mask variability of the response to methacholine between individuals given individuals with very different responses can be grouped together. This is illustrated in Figure 5.1 which shows the dose-response curves of four TAHS 2010 follow up study participants, two classified as not having BHR as their fall in FEV_1 did not reach the 20% cut-off (persons 1 and 2 in purple and orange respectively) and two classified as having BHR (persons 3 and 4 in blue and green respectively) under a dichotomous outcome despite clear differences in their responses to methacholine.

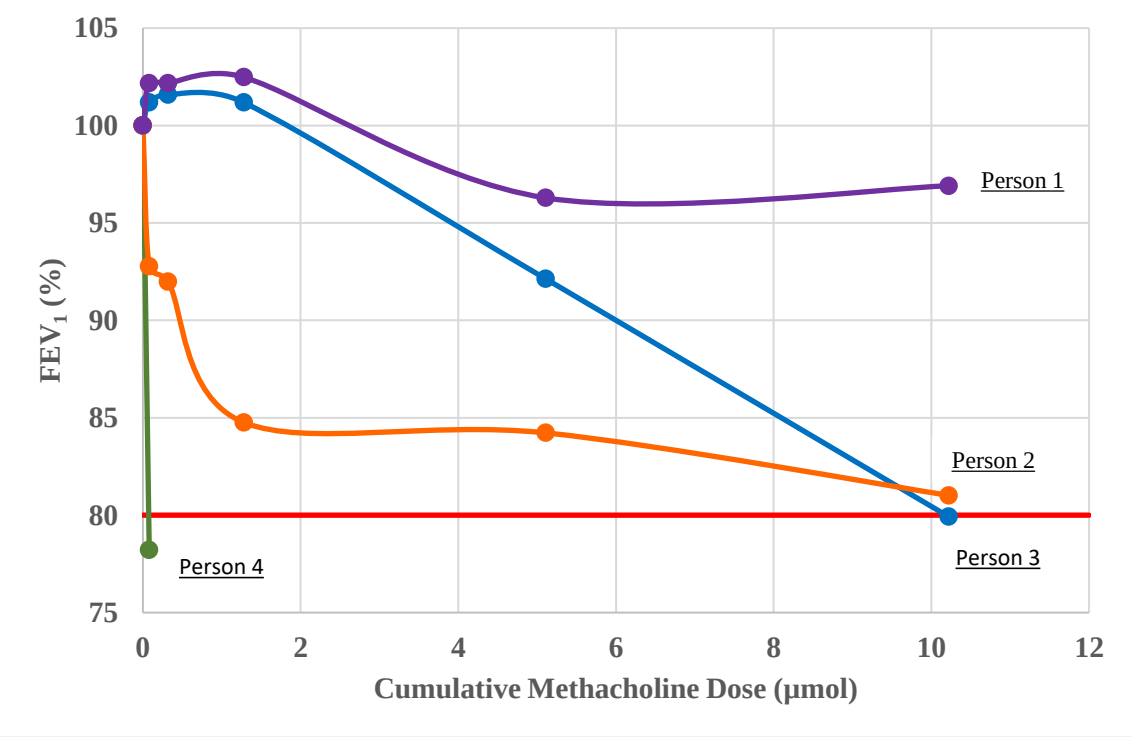


Figure 5.1 - Methacholine dose-response curves of four study participants from the TAHS 2010 follow up

Fourthly, a dichotomised BHR outcome also uses an arbitrary cut-off point (usually 20% for methacholine) which has not been specifically linked to disease risk, severity or mortality over other potential cut-off points.

Use of the dose-response slope in a linear regression has further limitations. In an effort to satisfy the

assumption of a normally distributed exposure-outcome relationship in linear regression, the dose-response slope is often log transformed. Zero and negative dose-response slopes are possible and cannot be log transformed. A zero slope can occur if the subject has no response to the provocative agent, and negative slopes can occur when the subject also has no response and becomes better at performing the spirometry manoeuvre during the test. Therefore, a small constant is added to the dose-response slope prior to transformation. This makes interpretation of the results difficult. From an extensive literature review, most (if not all) researchers do not attempt to interpret the results and simply state the direction and strength of any associations observed. I have also not come across any article reporting whether transformation successfully achieved normality, which in my experience, is rarely the case. If the normality assumption is still violated using the log-transformed dose-response slope, the results produced may not be valid. Lastly, by calculating a dose-response slope manually, the variation around the slope cannot be considered. This may lead to inappropriately small standard errors and, consequently, associations observed where none exists (also known as a type 1 error).

Other methods of assessing factors associated with bronchial responsiveness have been suggested, such as using the area under the dose-response curve (309, 310) or using the provocative dose that causes a 20% fall in FEV_1 (known as PD_{20}) in a survival analysis (311, 312). However, most of these methods still use a percent change in FEV_1 and are therefore limited as discussed above. The method currently recommended for use clinically is PD_{20} (313). This method is not suitable in studies of a general population where the majority of individuals will not achieve a 20% fall in FEV_1 during the test. Abramson et al. proposed an alternative method in which the slope is determined from a regression model with FEV_1 as the outcome and dose as the predictor. The estimated slope is then used in a separate analysis to assess factors associated with bronchial responsiveness (314). The advantages of this method include that it uses all FEV_1 measurements collected from each individual; baseline lung function is accounted for; and the estimated slope is the absolute change in FEV_1 per unit of provocative agent, thus avoiding the limitations of using a percent change in FEV_1. However, as this method is a two-step process, the variation around the slope is still not considered, increasing the likelihood of type 1 error.

5.1.2 The Linear Mixed Effects Model

Following an extensive search of the literature, consideration of the data collected during a bronchial provocation challenge, and discussion with my statistics supervisor regarding possible suitable statistical methods, I propose the use of the linear mixed effects model (LMM) for assessing factors associated with bronchial responsiveness. This well-established statistical method is ideal for analysing repeated continuous outcome measurements and overcomes the limitations of the methods currently used to examine risk factors of increased bronchial responsiveness (315, 316). The LMM will account for individual differences in baseline FEV_1 through random intercepts, and random slopes will accommodate individual differences in the rate of decline of FEV_1. In the LMM, both the estimation of the rate of decline in FEV_1 (slope) and assessment of factors associated with slope are

performed in a single step, enabling the error around each slope to be considered. The LMM also provides easy and meaningful interpretation of the results.

My aim was to demonstrate the use of the LMM for assessing factors associated with bronchial responsiveness and compare the findings to those of a regression analysis of the log dose-response slope using the same data. My findings indicate that the results can differ between these two methods. In the linear mixed effects model, sex was not associated with the change in FEV_1 with increasing methacholine; however, it was associated with the % change in FEV_1 with increasing methacholine in the regression analysis of the log dose-response slope. The difference in findings observed for sex is likely to be because females are, on average, shorter and have smaller lungs. Consequently, they have lower baseline FEV_1 values and the differences in baseline FEV_1 are accounted for in the linear mixed effects model but not in the regression analysis. Given the findings can differ, it is important that conclusions are based on the results from the most appropriate statistical method. I believe this is the LMM, as it likely has greater power, because it uses all of the FEV_1 data collected from each individual, and greater accuracy, because it accounts for differences in baseline FEV_1 and considers the error in the slope calculated for each individual. Therefore, I suggest the LMM is used for future assessment of risk factors of increased bronchial responsiveness.

The work from this chapter has been submitted for review and publication to the European Respiratory Journal. The submitted manuscript and supplementary material is available in Appendix 5.

Chapter 6 - Associations of fruit and vegetable intakes, lung function and bronchial responsiveness

6.1 Introduction

Research into the relationship between fruit and vegetable intakes and lung function has produced mixed results (see Chapter 3). These variable findings may have come about because of differences between studies such as differing distributions of population subgroups or disease phenotypes. The disease phenotypes of common chronic obstructive lung diseases are still being defined; however, one important aspect is that they may differ by the biological pathways involved. Fruit and vegetables are major sources of key nutrients in the human diet, such as antioxidants, fibre, and other vitamins and minerals, and their intakes may play a role in some of these pathways. Therefore, it is imperative that we examine effect modification of the relationship between fruit and vegetable intakes and lung function using factors that are likely to define disease phenotypes.

Bronchial hyperresponsiveness, or excessive airway sensitivity, is a factor that is likely to be part of future definitions of disease phenotypes. It is a risk factor for common chronic obstructive lung diseases and is associated with accelerated lung function decline (62, 79, 81). However, little is known about the relationship between fruit and vegetable intake and bronchial responsiveness (BR). Investigating this relationship may shed light on any relationships between fruit and vegetable intakes and lung function, and the possible biological pathways involved.

Therefore, in this chapter I aim to analyse associations between fruit and vegetable intakes, lung function and BR in a cohort of middle-aged Australian adults and examine potential effect modifiers of these relationships.

6.2 Methods

6.2.1 Study population

In this study, I utilised data collected in the 2010 follow up of the TAHS. Details of the study design and ascertainment of the study population are described in Chapter 4. Briefly, there were 796 participants available for analysis of lung function outcomes and 696 for BR, following exclusions (e.g. low baseline FEV_1 for the methacholine challenge), invalid or missing data, and those who chose not to complete testing.

6.2.2 Assessment of diet

To assess fruit and vegetable intake, participants were asked by written questionnaire “How many serves of fruit (fresh, canned, frozen) do you usually eat each day?” and “How many serves of vegetables (fresh, canned, frozen) do you usually eat each day?”. A serve was defined as “what fits

into the palm of your hand". Six response options were listed for both questions: I don't eat fruit/vegetables; less than 1 serve per day; 1 serve per day; 2 serves per day; 3 serves per day; or 4 or more servings per day. The two lowest intake options were combined, leaving 5 categories ranging from less than 1 serve per day to 4 or more serves/day.

A short questionnaire about fat intake was also completed (SFQ), as discussed in Chapter 4. The questionnaire contained 18 questions on the frequency of consumption of high fat foods and food behaviours that influence fat intake (for the full questionnaire see Appendix 1). A 'milk fat' variable was derived from two questions – 1) Q15 'What type of milk do you use on breakfast cereal or in cooking?', options were 'cow or goat's milk', 'soy milk', or 'no milk'; and 2) Q16 'What form of milk in Q15 do you consume?'; options were 'condensed or evaporated', 'full cream', 'full cream and reduced fat', 'reduced fat', or 'skim'. The derivation of the 'milk fat' variable accounted for the lower fat content of soy milk compared to cow or goat's milk and is visually presented in Figure 6.1. Further details are provided in Chapter 4. The 'milk fat' categories were scored as follows: skim or no milk = 0; reduced fat = 1; full cream and reduced fat = 2; full cream = 3; condensed or evaporated = 4.

Responses to questions 9, 17 and 18 in the SFQ were recoded for scoring purposes as per advice provided by Professor Lisa Wood, a TAHS investigator and expert in the area of diet and respiratory disease. The new coding was as follows - Q9: How do you spread butter/marg on bread? – Never use butter/marg (0), thinly (2), medium (3), thickly (4); Q17: How much skin on chicken do you eat? – None of the skin/I am vegetarian (0), some of the skin (1), most or all of the skin (2); Q18: How much of the fat on meat do you eat? No fat/I am vegetarian (0), some of the fat (1), most or all of the fat (2).

A fat score was then calculated by summing the values given for questions 1-8, 10-14, the recoded values for questions 9, 17 and 18, and 'milk fat'. The fat score can range from 0 to 64, with higher scores indicating a greater intake of fat.

The validity of the SFQ and the fruit and vegetable questions used in the TAHS are discussed in Chapter 4.

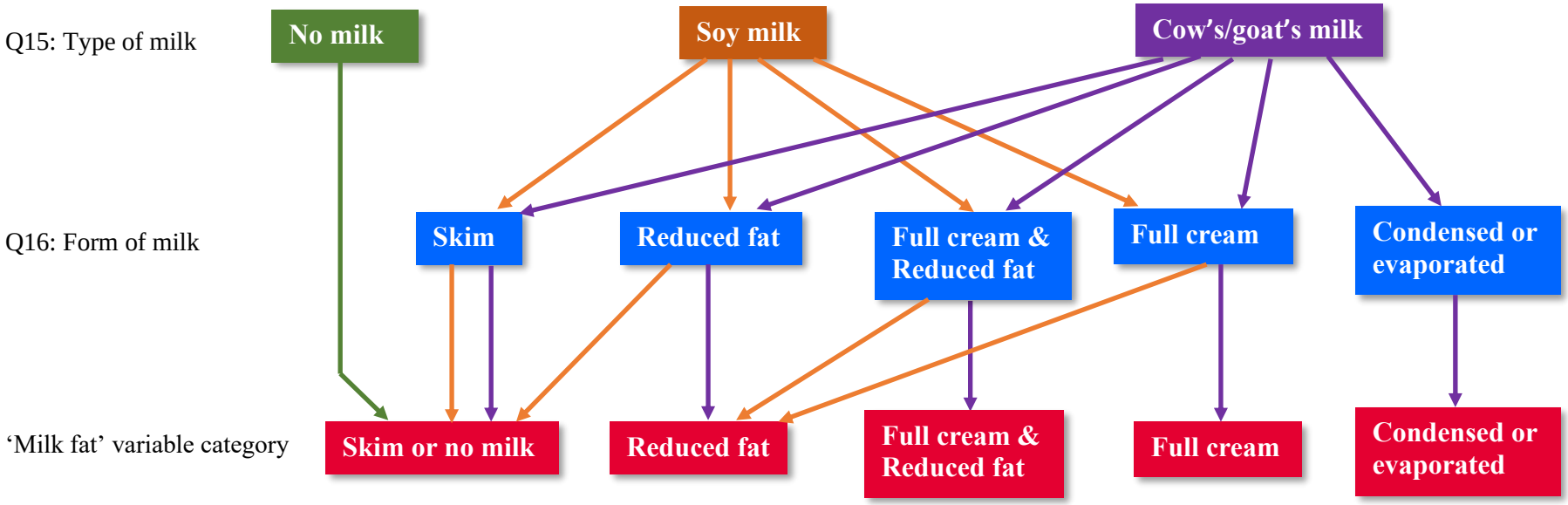


Figure 6.1 - Derivation of the 'milk fat' variable

6.2.3 Assessment of lung function and BR

The spirometry and methacholine challenge testing were performed as detailed in Chapter 4. The outcome measures of interest for this analysis are FEV_1, FVC, FEV_1/FVC, and the change in FEV_1 measurements taken during the methacholine challenge.

6.2.4 Assessment of covariates of interest

The measurement of all covariates of interest is described in Chapter 4.

6.2.5 Statistical analysis

The statistical methods used to describe and analyse the data are detailed in Chapter 4. Linear mixed effects models (detailed in Chapter 5) were used to examine the association between fruit and vegetable intakes and change in FEV_1 during the methacholine challenge. These models included all confounders of baseline FEV_1 determined from the relevant DAG (Chapter 4, Figure 4.3) and the regression analyses, as well as interactions with dose for confounders of BR as determined from the relevant DAG in Chapter 4 (Figure 4.4). Both primary exposures were modelled as categorical variables as defined above; however, models assuming a linear association between vegetable or fruit intake and the outcomes were explored. Evidence of linear trend is presented as p_{trend}. Additional adjustment for other dietary measures was conducted in separate models to determine whether these variables were confounding the relationship of interest. Acknowledging that dietary measures may be correlated, variance inflation factors (VIFs) were obtained for the regression models to assess multicollinearity (correlation) between vegetable or fruit intake and other dietary measures. A VIF greater than 10 was considered an indicator of multicollinearity (163, 317). Changes in the estimates of interest were examined to confirm if other dietary factors were confounders to be included in further statistical models. Interactions were evaluated using the likelihood ratio test comparing models with and without the interaction term. Evidence is presented in the form of a p value, $p_{interaction}$. Linearity was evaluated using the same test comparing models with the exposure variable as categorical and another as continuous and evidence is presented as $p_{linearity}$. Any relationships with moderate or stronger evidence are discussed, as well as any similar trends seen in related analyses.

6.3 Results

6.3.1 Study population characteristics

The characteristics of the study population according to their intake of fruit and vegetables are displayed in Tables 6.1 and 6.2. Those with higher intakes of fruit and vegetables were more likely to be female, to be of a healthy weight and to have never smoked. In addition, those with higher intakes of vegetables were also more likely to be university educated and have a managerial or professional occupation (see Tables 6.1 and 6.2).

Table 6.1 - Basic Characteristics of the study population by fruit intake

Characteristic	Fruit intake					Total (n=796)
	Less than 1 serve/day (n=191)	1 serve/day (n=281)	2 serves/day (n=221)	3 serves/day (n=83)	4 or more serves/day (n=20)	
Male, n (%)	105 (55.0)	168 (59.8)	85 (38.5)	30 (36.1)	7 (35.0)	395 (49.6)
Age (years)	49.6 [0.57]	49.6 [0.63]	49.6 [0.59]	49.5 [0.62]	49.5 [0.50]	49.6 [0.60]
Height (cm)	170.8 [9.4]	171.8 [8.6]	169.0 [8.0]	168.1 [8.5]	169.8 [8.4]	170.4 [8.7]
BMI category, n (%)						
Healthy weight (<25kg/m^2)	45 (23.6)	76 (27.1)	61 (27.6)	30 (36.1)	9 (45.0)	221 (27.8)
Overweight (≥25 and <30kg/m^2)	73 (38.2)	118 (42.0)	85 (38.5)	28 (33.7)	8 (40.0)	312 (39.2)
Obese (≥30kg/m^2)	73 (38.2)	87 (31.0)	75 (33.9)	25 (30.1)	3 (15.0)	263 (33.0)
Asthma, n (%)*						
Never	105 (55.0)	138 (49.1)	113 (51.4)	45 (54.2)	8 (42.1)	409 (51.5)
Remitted	49 (25.7)	70 (24.9)	50 (22.7)	21 (25.3)	4 (21.1)	194 (24.4)
Current	37 (19.4)	73 (26.0)	57 (25.9)	17 (20.5)	7 (36.8)	191 (24.1)
Smoking, n (%)						
Never smoked	73 (38.2)	127 (45.2)	105 (47.5)	39 (47.0)	14 (70.0)	358 (45.0)
Former smoker	62 (32.5)	100 (35.6)	91 (41.2)	36 (43.4)	5 (25.0)	294 (36.9)
Current smoker	56 (29.3)	54 (19.2)	25 (11.3)	8 (9.6)	1 (5.0)	144 (18.1)
Education, n (%)						
HSC not completed	70 (36.7)	62 (22.1)	50 (22.6)	22 (26.5)	7 (35.0)	211 (26.5)
HSC/trade/diploma attained	93 (48.7)	151 (53.7)	107 (48.4)	35 (42.2)	12 (60.0)	398 (50.0)
University degree or higher attained	28 (14.7)	68 (24.2)	64 (29.0)	26 (31.3)	1 (5.0)	187 (23.5)
Occupation, n (%)						
Manager/Professional	36 (18.9)	77 (27.4)	72 (32.6)	27 (32.5)	5 (25.0)	217 (27.3)
Trade/associate professional	61 (31.9)	112 (39.9)	66 (29.9)	27 (32.5)	8 (40.0)	274 (34.4)
Clerical/service worker/ labourer	94 (49.2)	92 (32.7)	83 (37.6)	29 (34.9)	7 (35.0)	305 (38.3)

All values presented are mean [standard deviation] unless otherwise stated; *n=794; HSC – Higher school certificate

Table 6.2 - Basic Characteristics of the study population by vegetable intake

Characteristic	Vegetable intake					Total (n=796)
	Less than 1 serve/day (n=46)	1 serve/day (n=240)	2 serves/day (n=193)	3 serves/day (n=218)	4 or more serves/day (n=99)	
Male, n (%)	29 (63.0)	184 (76.7)	101 (52.3)	68 (31.2)	13 (13.1)	395 (49.6)
Age (years)	49.8 [0.63]	49.6 [0.57]	49.6 [0.56]	49.6 [0.63]	49.7 [0.68]	49.6 [0.60]
Height (cm)	173.6 [8.1]	173.6 [8.6]	170.5 [8.9]	168.0 [7.8]	165.8 [7.3]	170.4 [8.7]
BMI category, n (%)						
Healthy weight (<25kg/m^2)	12 (26.1)	54 (22.5)	45 (23.3)	66 (30.3)	44 (44.4)	221 (27.8)
Overweight (≥25 and <30kg/m^2)	11 (23.9)	114 (47.5)	72 (37.3)	87 (39.9)	28 (28.3)	312 (39.2)
Obese (≥30kg/m^2)	23 (50.0)	72 (30.0)	76 (39.4)	65 (29.8)	27 (27.3)	263 (33.0)
Asthma, n (%)*						
Never	20 (43.5)	140 (58.3)	99 (51.8)	105 (48.2)	45 (45.5)	409 (51.5)
Remitted	13 (28.3)	51 (21.3)	41 (21.5)	63 (28.9)	26 (26.3)	194 (24.4)
Current	13 (28.3)	49 (20.4)	51 (26.7)	50 (22.9)	28 (28.3)	191 (24.1)
Smoking, n (%)						
Never smoked	13 (28.3)	108 (45.0)	93 (48.2)	99 (45.4)	45 (45.5)	358 (45.0)
Former smoker	17 (37.0)	81 (33.8)	65 (33.7)	86 (39.5)	45 (45.5)	294 (36.9)
Current smoker	16 (34.8)	51 (21.3)	35 (18.1)	33 (15.1)	9 (9.1)	144 (18.1)
Education, n (%)						
HSC not completed	27 (58.7)	63 (26.3)	46 (23.8)	52 (23.9)	23 (23.2)	211 (26.5)
HSC/trade/diploma attained	16 (34.8)	141 (58.8)	95 (49.2)	102 (46.8)	44 (44.4)	398 (50.0)
University degree or higher attained	3 (6.5)	36 (15.0)	52 (26.9)	64 (29.4)	32 (32.3)	187 (23.5)
Occupation, n (%)						
Manager/Professional	2 (4.4)	48 (20.0)	55 (28.5)	72 (33.0)	40 (40.4)	217 (27.3)
Trade/associate professional	20 (43.5)	89 (37.1)	70 (36.3)	70 (32.1)	25 (25.3)	274 (34.4)
Clerical/service worker/ labourer	24 (52.2)	103 (42.9)	68 (35.2)	76 (34.9)	34 (34.3)	305 (38.3)

All values presented are mean [standard deviation] unless otherwise stated; *n=794; HSC – Higher school certificate

6.3.2 Associations between fruit and vegetable intake and lung function

Vegetable intake was negatively associated with FEV_1 and FVC and positively associated with FEV_1/FVC in univariate analyses (Table 6.3, Model 1). After adjusting for age, height, sex, smoking status, asthma status, BMI category, education, and occupation, vegetable intake was positively associated with FEV_1 and FVC. Evidence of association with FEV_1/FVC became weaker, suggesting no association. Those in the highest vegetable intake category (4 or more serves/day) had a mean FEV_1 0.10 L greater than those in the lowest vegetable intake category (95%CI -0.07, 0.27; p_{trend}=0.020) and a mean FVC 0.08 L greater (95%CI -0.12, 0.28; p_{trend}=0.051). Upon further adjustment for fruit intake and fat score, the relationship between vegetable intake and FEV_1 remained; however, evidence of a relationship with FVC became weaker, indicating no association. There was no evidence of multicollinearity in these models (all VIFs <5). The additional adjustment had only a slight effect on the estimates indicating that adding fruit intake and fat score was not necessary and only reduced the power of the model. Therefore, other dietary measures were excluded as confounders in subsequent analyses.

There was no evidence of association observed between fruit intake and lung function in the unadjusted or adjusted analyses (Appendix 6, Table A1). The addition of vegetable intake and fat score in Model 3 had some effect on the estimates. Therefore, these variables were included as confounders of fruit intake and lung function in subsequent analyses.

Table 6.3 - Unadjusted and adjusted associations of vegetable intake with lung function outcomes

Lung function measure	Vegetable intake (serves/day)					p_{trend}	$p_{linearity}$
	< 1	1	2	3	4 +		
FEV_1 (L)							
Model 1	Ref	0.19 (-0.04, 0.41)	0.03 (-0.20, 0.26)	-0.12 (-0.35, 0.11)	-0.31 (-0.56, -0.62)	<0.001	0.039
Model 2	Ref	-0.005 (-0.16, 0.15)	0.08 (-0.07, 0.24)	0.11 (-0.04, 0.27)	0.10 (-0.07, 0.27)	0.020	0.58
Model 3	Ref	-0.006 (-0.16, 0.15)	0.08 (-0.08, 0.23)	0.10 (-0.06, 0.26)	0.09 (-0.09, 0.27)	0.039	0.62
FVC (L)							
Model 1	Ref	0.19 (-0.10, 0.48)	-0.10 (-0.40, 0.19)	-0.29 (-0.59, -0.00)	-0.53 (-0.85, -0.20)	<0.001	0.066
Model 2	Ref	-0.04 (-0.22, 0.14)	0.02 (-0.16, 0.20)	0.08 (-0.11, 0.26)	0.08 (-0.12, 0.28)	0.051	0.79
Model 3	Ref	-0.04 (-0.22, 0.14)	0.009 (-0.18, 0.19)	0.06 (-0.13, 0.25)	0.06 (-0.15, 0.27)	0.13	0.80
FEV_1/FVC (%)							
Model 1	Ref	1.08 (-1.00, 3.16)	2.32 (0.19, 4.44)	2.33 (0.23, 4.43)	1.79 (-0.52, 4.09)	0.031	0.24
Model 2	Ref	0.52 (-1.47, 2.50)	1.37 (-0.66, 3.40)	1.17 (-0.86, 3.21)	0.63 (-1.63, 2.89)	0.41	0.42
Model 3	Ref	0.52 (-1.48, 2.52)	1.40 (-0.66, 3.46)	1.26 (-0.82, 3.35)	0.82 (-1.51, 3.15)	0.31	0.49

β-coefficient (95%CI) presented for each vegetable category

Model 1: Unadjusted linear regression analysis

Model 2: Regression analysis adjusted for age, height, sex, smoking status, asthma status, BMI category, education, occupation

Model 3: Model 2 + fat score, fruit intake

6.3.3 Modification of the associations between fruit and vegetable intakes and lung function

The relationship between vegetable intake and FEV_1 was modified by asthma status ($p_{interaction}$=0.040; Table 6.4, Figure 6.2). In general, mean FEV_1 was lowest for those with current asthma and the never asthma group had the highest mean FEV_1. Amongst those with current asthma, people consuming four or more serves of vegetables daily had a mean FEV_1 0.27 L higher than those consuming less than one serve per day (95%CI -0.04, 0.59; p_{trend} <0.001). There was no relationship observed for the never or remitted asthma groups. A similar trend was observed for FVC; however, the evidence for an interaction was weak ($p_{interaction}$=0.087).

The relationship between vegetable intake and FEV_1 was also modified by smoking status ($p_{interaction}$=0.033; Table 6.5, Figure 6.3). In current smokers, those consuming four or more serves of vegetables daily had a mean FEV_1 0.13 L higher than those consuming less than one serve daily (95%CI -0.25, 0.52; $p\text{-}_{trend}$=0.034). A weaker positive association was also observed in former smokers; however, the evidence of a trend was weak (p_{trend}=0.098). There was no relationship observed in never smokers. A similar trend was observed for FEV_1/FVC in current smokers; however, the evidence for an interaction was weak ($p_{interaction}$=0.097). No other interactions were observed (Appendix 6, tables A6-A7).

There was no evidence of effect modification found between fruit intake and any of the potential effect modifiers tested (Appendix 6, Tables A2-A5).

Table 6.4 – Adjusted associations between vegetable intake and lung function by asthma status

Vegetable intake (serves/day)	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never asthma (n=409)	Remitted asthma (n=194)	Current asthma (n=191)	Never asthma (n=409)	Remitted asthma (n=194)	Current asthma (n=191)	Never asthma (n=409)	Remitted asthma (n=194)	Current asthma (n=191)
< 1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
1	-0.09 (-0.31, 0.13)	0.12 (-0.16, 0.41)	-0.05 (-0.34, 0.24)	-0.16 (-0.42, 0.10)	0.15 (-0.19, 0.48)	-0.04 (-0.38, 0.30)	0.48 (-2.43, 3.38)	0.62 (-3.12, 4.37)	-0.20 (-3.99, 3.58)
2	-0.06 (-0.29, 0.17)	0.11 (-0.18, 0.40)	0.27 (-0.01, 0.56)	-0.11 (-0.38, 0.16)	-0.03 (-0.38, 0.31)	0.25 (-0.09, 0.59)	0.25 (-2.73, 3.23)	2.82 (-1.02, 6.67)	1.99 (-1.79, 5.77)
3	-0.06 (-0.29, 0.17)	0.19 (-0.10, 0.47)	0.32 (0.03, 0.61)	-0.10 (-0.36, 0.17)	0.15 (-0.18, 0.48	0.29 (-0.05, 0.63)	0.22 (-2.76, 3.19)	1.20 (-2.51, 4.91)	2.83 (-0.97, 6.63)
4 +	-0.08 (-0.33, 0.17)	0.21 (-0.11 (0.52)	0.27 (-0.04, 0.59)	-0.09 (-0.39, 0.20)	0.16 (-0.21, 0.53)	0.28 (-0.09, 0.64)	-0.57 (-3.86, -2.71)	1.93 (-2.22, 6.07)	1.14 (-2.97, 5.24)
p_{trend}	1.00	0.18	<0.001	0.70	0.51	0.003	0.54	0.36	0.084
$p_{interaction}$	0.040			0.087			0.35		
$p_{linearity}$	0.45			0.38			0.53		

β-coefficient (95%CI) presented for each vegetable category

Adjusted for age, height, sex, smoking status, asthma status, BMI category, education, occupation

Table 6.5 – Adjusted associations between vegetable intake and lung function by smoking

Vegetable intake (serves/day)	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never smoker (n=358)	Former smoker (n=294)	Current smoker (n=142)	Never smoker (n=358)	Former smoker (n=294)	Current smoker (n=142)	Never smoker (n=358)	Former smoker (n=294)	Current smoker (n=142)
< 1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
1	0.08 (-0.19, 0.35)	0.01 (-0.24, 0.26)	-0.18 (-0.44, 0.09)	0.11 (-0.21, 0.43)	-0.13 (-0.42, 0.16)	-0.14 (-0.45, 0.17)	-0.05 (-3.60, 3.49)	2.51 (-0.71, 5.73)	-1.87 (-5.31, 1.58)
2	0.04 (-0.24, 0.31)	0.11 (-0.15, 0.36)	0.20 (-0.08, 0.48)	0.02 (-0.30, 0.34)	0.008 (-0.29, 0.30)	0.13 (-0.20, 0.47)	0.27 (-3.32, 3.85)	2.22 (-1.07, 5.51)	2.19 (-1.50, 5.87)
3	0.07 (-0.21, 0.35)	0.20 (-0.04, 0.45)	0.06 (-0.22, 0.34)	0.08 (-0.25, 0.40)	0.12 (-0.17, 0.41)	0.04 (-0.28, 0.37)	0.0004 (-3.61, 3.61)	2.69 (-0.52, 5.91)	0.48 (-3.18, 4.15)
4 +	0.15 (-0.14, 0.45)	0.07 (-0.20, 0.33)	0.13 (-0.25, 0.52)	0.19 (-0.16, 0.53)	0.01 (-0.30, 0.33)	0.02 (-0.43, 0.47)	0.05 (-3.78, 3.88)	0.81 (-2.68, 4.30)	3.04 (-1.96, 8.03)
p_{trend}	0.45	0.098	0.034	0.51	0.069	0.24	0.94	0.88	0.038
$p_{interaction}$	0.033			0.16			0.097		
$p_{linearity}$	0.053			0.22			0.18		

β-coefficient (95%CI) presented for each vegetable category

Adjusted for age, height, sex, smoking status, asthma status, BMI category, education, occupation

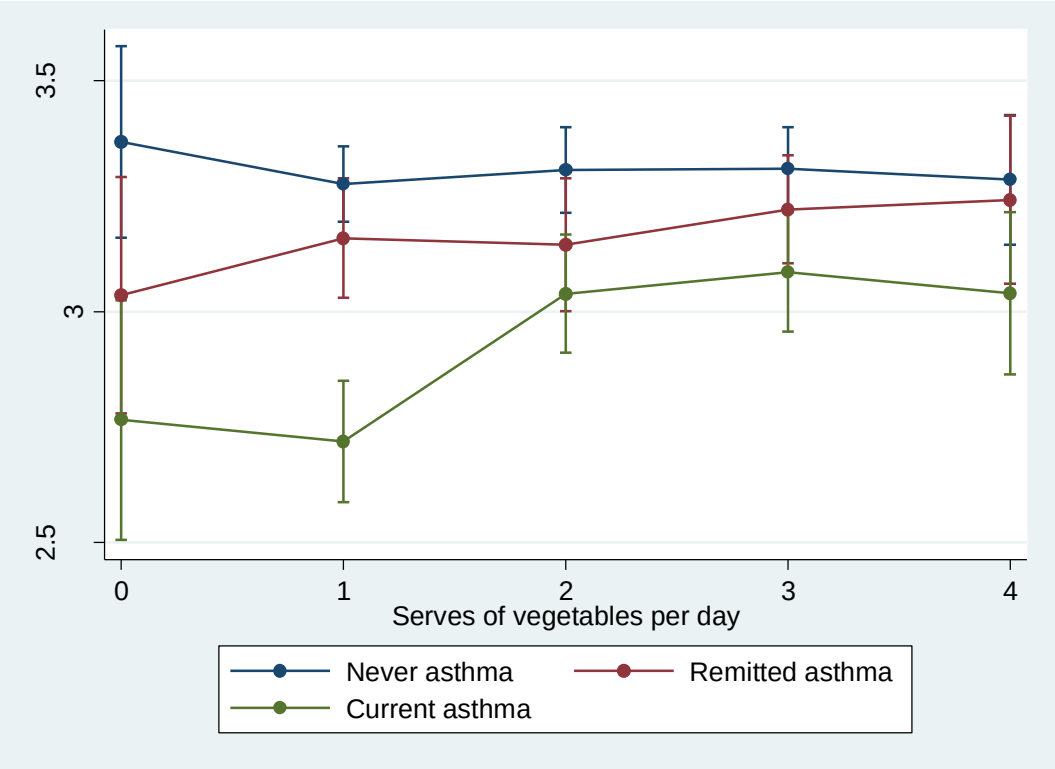


Figure 6.2 – Relationship between categories of vegetable intake and mean FEV_1 by asthma status

Adjusted for age, height, sex, smoking status, asthma status, BMI category, education, occupation; error bars represent 95%CIs

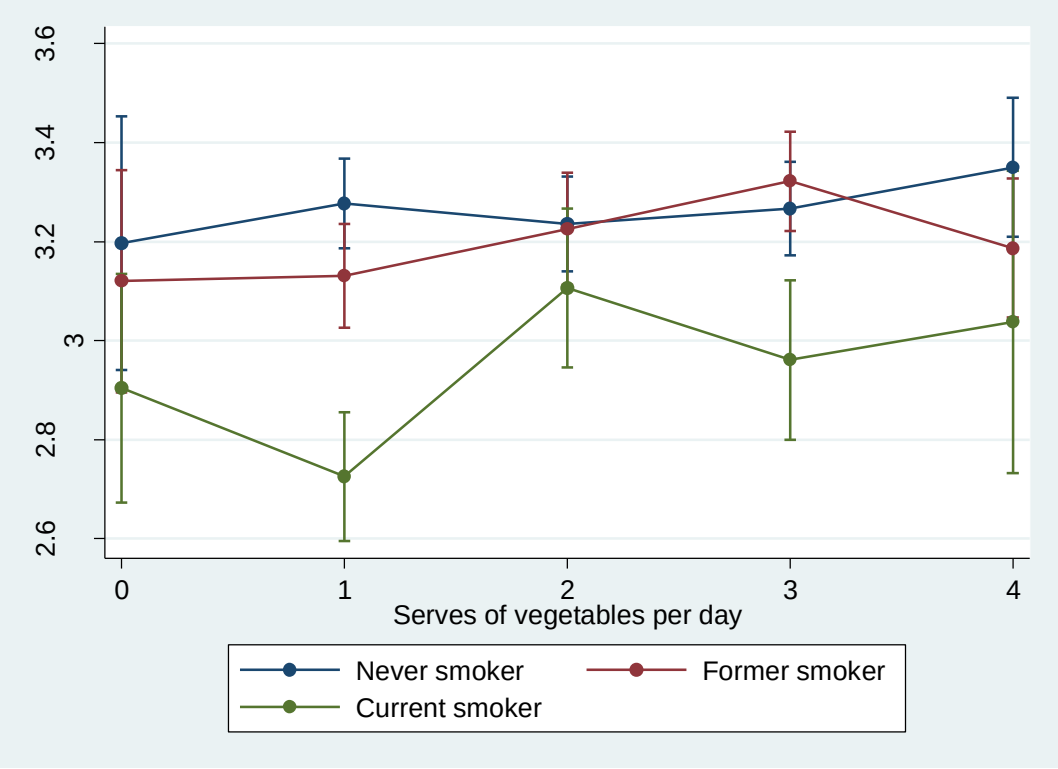


Figure 6.3 - Relationship between categories of vegetable intake and mean FEV_1 by smoking status

Adjusted for age, height, sex, smoking status, asthma status, BMI category, education, occupation; error bars represent 95%CIs

6.3.4 Associations between fruit and vegetable intake and change in FEV_1 during the methacholine challenge

In the univariate analysis, there was weak evidence of a negative association between fruit intake and the change in FEV_1 during the methacholine challenge (Table 6.6). The evidence for this relationship became stronger after adjustment for confounders, and stronger again with further adjustment for vegetable intake and fat score. The mean fall in FEV_1 per µmol methacholine was 0.02 L greater in those who consumed four or more serves of fruit per day compared with those who consumed less than one serve of fruit per day (95%CI -0.04, -0.0007; p_{trend}=0.014). Figure 6.4 visualises the rate of decline for each category of fruit intake, showing those consuming 4 serves or more had the steepest decline while those consuming <1 or 1 serve had a comparatively modest decline. The latter groups also had the highest predicted baseline FEV_1. Adjustment for vegetable intake and fat score had some effect on the estimates, indicating these variables have a confounding effect on the relationship between fruit intake and change in FEV_1. Therefore, vegetable intake and fat score were adjusted for in subsequent analyses.

There were no associations observed between vegetable intake and lung function in the unadjusted or adjusted analyses (Appendix 6, Table A8). Adjustment for the additional dietary factors had some effect on the estimates. Therefore, fruit intake and fat score were adjusted for in the effect modification analyses.

Table 6.6 - Unadjusted and adjusted associations of fruit intake with change in FEV_1 during the methacholine challenge

Diet measure	Change in FEV_1(L)/µmol methacholine		
Fruit intake (serves/day)	Model 1	Model 2	Model 3
<1	Ref	Ref	Ref
1	-0.002 (-0.01, 0.006)	-0.002 (-0.009, 0.005)	-0.004 (-0.01, 0.003)
2	-0.007 (-0.02, 0.0009)	-0.008 (-0.02, -0.0007)	-0.01 (-0.02, -0.002)
3	-0.001 (-0.01, 0.009)	-0.003 (-0.01, 0.006)	-0.006 (-0.02, 0.005)
4 +	-0.02 (-0.04, -0.0007)	-0.02 (-0.04, -0.001)	-0.02 (-0.04, -0.0007)
p_{trend}	0.086	0.035	0.014
$p_{linearity}$	0.27	0.67	0.64

β-coefficient (95%CI) presented

Model 1: Unadjusted linear mixed model

Model 2: linear mixed model adjusted for age, sex, smoking status, asthma status, BMI category, education, occupation

Model 3: Model 2 + fat score, fruit intake

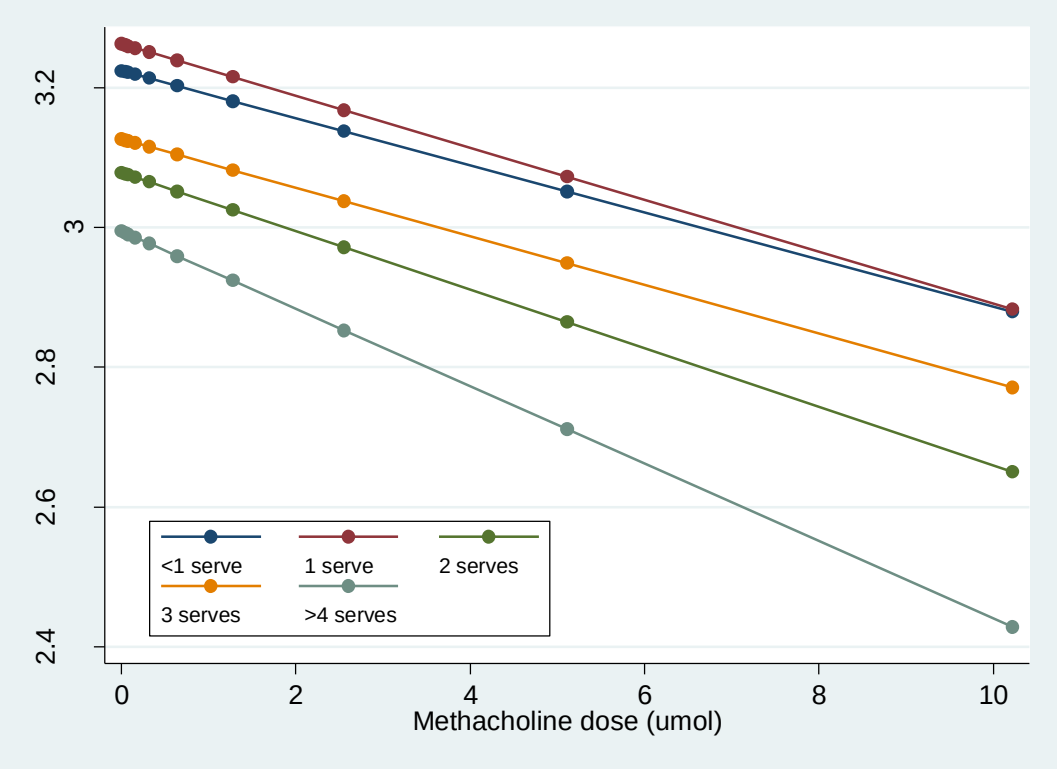


Figure 6.4 – Predicted mean FEV_1 during methacholine challenge by fruit intake (serves/day)

Adjusted for age, sex, smoking status, asthma status, BMI category, education, occupation, fat score, fruit intake

6.3.4 Modification of the associations between fruit and vegetable intakes and change in FEV_1 during the methacholine challenge

The relationship between vegetable intake and change in FEV_1 during the methacholine challenge differed by asthma status, however there was strong evidence that this relationship was non-linear ($p_{interaction}$=0.005; $p_{linearity}$=0.005; Table 6.7). Those with remitted asthma consuming less than 1 serve of vegetables daily had a mean fall in FEV_1 per µmol methacholine 0.06-0.07 L greater than any other category of intake (note: only 8 participants had remitted asthma and consumed <1 serve vegetables/day). Those with current asthma consuming one serve of vegetables per day or less also had a steeper fall in FEV_1 (mean fall in FEV_1 0.008-0.02 L greater than higher vegetable intake categories). Figure 6.5 illustrates the steeper slopes observed in the lower intake categories compared to those with higher vegetable intakes for those with remitted or current asthma, indicating greater bronchial reactivity. There were no other interactions observed with vegetable intake (Appendix 6, Table A10).

The relationship between fruit intake and the change in FEV_1 during the methacholine challenge was not modified by any of the potential effect modifiers tested (Appendix 6, Table A9).

Table 6.7 - Adjusted associations of vegetable intake with change in FEV_1 during the methacholine challenge by asthma status

Vegetable intake (serves/day)	Change in FEV_1(L)/µmol methacholine		
	Never asthma (n=371)	Remitted asthma (n=168)	Current asthma (n=155)
< 1	Ref	Ref	Ref
1	0.0006 (-0.02, 0.02)	0.06 (0.03, 0.09)	-0.002 (-0.03, 0.03)
2	0.006 (-0.01, 0.02)	0.07 (0.04, 0.10)	0.01 (-0.02, 0.04)
3	0.0007 (-0.02, 0.02)	0.06 (0.03, 0.09)	0.02 (-0.006, 0.05)
4 +	-0.0009 (-0.02, 0.02)	0.06 (0.03, 0.10)	0.008 (-0.03, 0.03)
p_{trend}	0.98	0.16	0.13
$p_{interaction}$	0.005		
$P_{linearity}$	0.005		

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, education, occupation, fruit intake, fat score

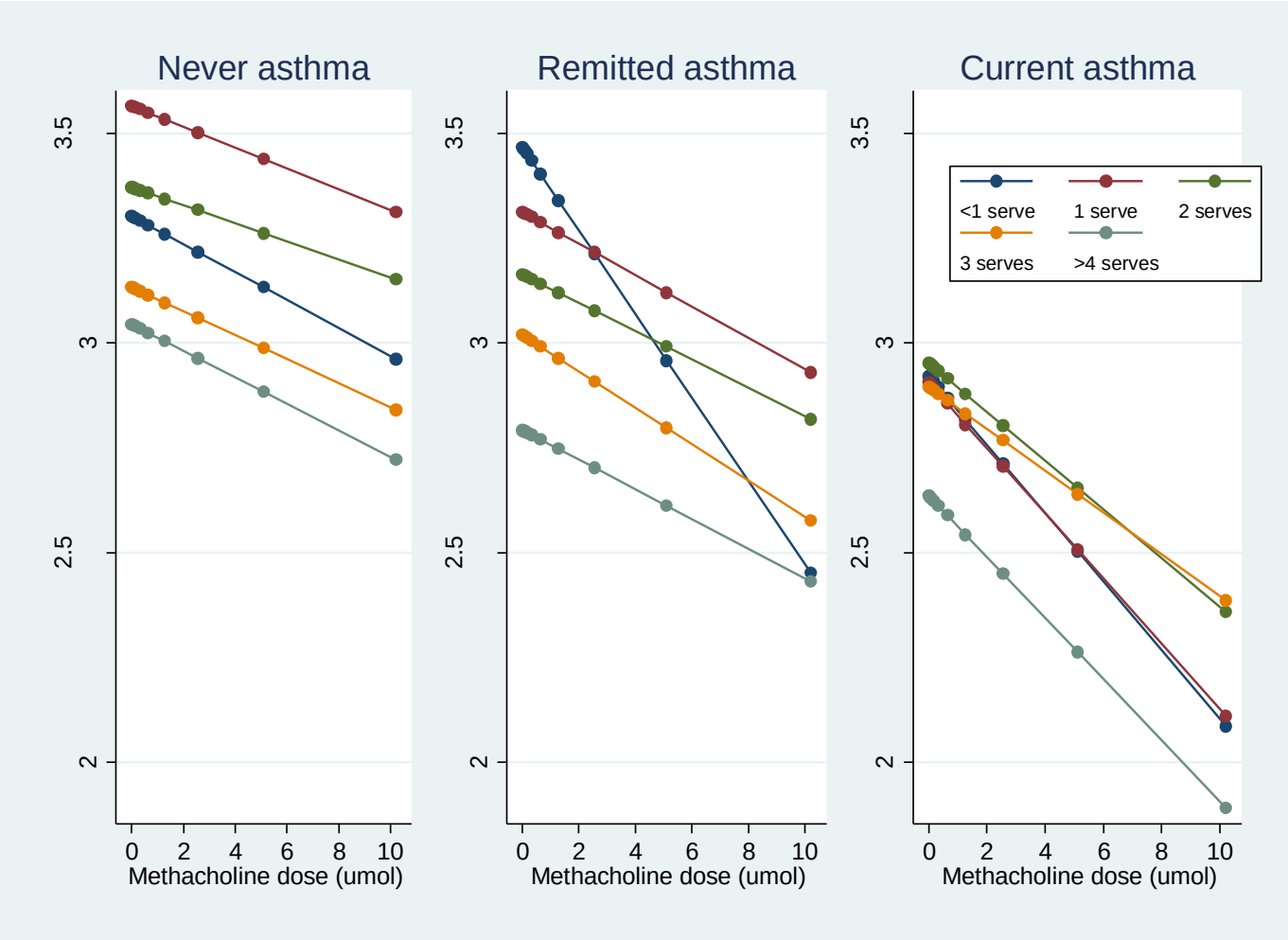


Figure 6.5 - Association between vegetable intake and change in FEV_1 during the methacholine challenge according to asthma status

Adjusted for age, sex, smoking status, asthma status, BMI category, education, occupation, fruit intake, fat score

6.4 Discussion

This study is the first that I am aware of (after an extensive search of the literature) to examine asthma status as an effect modifier of the relationships between fruit and vegetable intakes, lung function and BR. My findings suggest higher vegetable intake is associated with higher FEV_1 in those with current asthma; the mean difference in FEV_1 observed between the highest and lowest intake groups (0.27 L) is clinically important and on par with the effects of some respiratory medications (e.g. inhaled salbutamol is expected to improve FEV_1 by >200 ml in those with asthma during diagnostic testing) (61). This finding warrants confirmation and further investigation, preferably in an RCT.

This study is also the first to investigate the relationship between diet and BR using the LMM statistical method. My findings indicate that asthma status may modify the effect of vegetable intake on BR, with those having current or remitted asthma and low vegetable intakes exhibiting greater BR than their higher intake counterparts. However, these results should be interpreted with caution given the lack of a linear relationship and the small numbers in some categories. Further research is needed with larger study populations to confirm these findings.

It is biologically plausible for the relationship between vegetable intake, lung function and BR to be modified by asthma status. Vegetables are a major source of a range of nutrients and bioactive compounds including β-carotene, vitamin C, fibre, and a range of flavonoids. Vegetable intake and these specific nutrients and bioactives have been shown to be inversely associated with pro-inflammatory cytokines in observational studies and/or to reduce pro-inflammatory cytokines in RCTs (110, 318-324). Airway inflammation, a characteristic of chronic lung diseases, is thought to trigger airway remodelling which may promote airway narrowing, increased BR, further inflammation, and progressive lung function decline (325, 326). Therefore, if vegetable intake can reduce airway inflammation, this may help to maintain lung function and reduce bronchial responsiveness in people with asthma.

Smoking status also modified the association between vegetable intake and lung function, with current smokers in the highest vegetable intake group having a mean FEV_1 0.13 L higher than those in the lowest intake group. A similar trend was also observed in former smokers and for FEV_1/FVC in current smokers. No other studies have reported seeing a similar modifying effect of smoking on vegetable intake and lung function. However, few studies have examined smoking as an effect modifier of this relationship, with some studies limiting the assessment of effect modifiers to dietary factors associated with lung function in adjusted models, limiting the chances of observing an interaction (131, 135, 137, 327).

The biological mechanisms behind the modifying effect of smoking may be similar to those for asthma status. Research has demonstrated that smoking is associated with airway inflammation and triggers some airway remodelling including increased thickness and abnormalities in the airway epithelium (325, 328, 329). Further research is needed to confirm this modifying effect of smoking on the vegetable intake-lung function relationship. An RCT investigating the effect of vegetable intake on lung function in smokers

would also be of interest.

My results indicate higher fruit intake was associated with greater bronchial responsiveness. There have only been two previous studies assessing the relationship between fruit and vegetable intake and BHR or BR, one RCT of short duration and one cross-sectional study in a younger adult study population (127, 142). In contrast to my findings, both studies observed no association. It is important to note that the statistical methods used in these studies have significant limitations that the LMM overcomes. The LMM also has greater power to detect an association. However, as far as I am aware, there is no biologically plausible explanation for fruit intake to be associated with greater bronchial reactivity. As this is only one study, it is possible that these results occurred by chance or are affected by residual confounding.

This is a large cross-sectional study using objective lung function measures. Despite these strengths, there are some limitations of this research. The dietary measures may be affected by recall error and outcome measures may be affected by measurement error; however, these errors are likely to be random and attenuate the result to the null. There are some potential confounders of the relationship between fruit and vegetable intakes, lung function and BR identified in Chapter 4 that were not measured as part of the TAHS 2010 follow up (Figure 4.1). Therefore, the findings reported here may be impacted by residual confounding. There were multiple tests performed as part of this research, increasing the likelihood of a chance finding. Lastly, this is a cross-sectional analysis and cannot show a causal relationship. Reverse causation is possible; however, this is highly unlikely. Dietary change in response to disease diagnosis usually occurs around the time the diagnosis is given, often as a result of medical advice, and maintaining dietary changes overtime is very difficult. Study data indicates that the mean age of asthma diagnosis and onset of asthma symptoms in the TAHS cohort is 17 years. Therefore, diagnosis was a long time ago for most participants and dietary change as a result of diagnosis would not impact the results. Dietary change would also only be part of medical advice for asthma in overweight and obese patients and the advice would be quite general, in line with the current dietary guidelines.

In conclusion, the results of this research suggest that vegetable intake is positively associated with lung function in smokers and those with current asthma, and the difference in lung function may be clinically relevant. RCTs and large cohort studies are needed to investigate causality and confirm if increasing vegetable intake in these population groups may improve lung function or reduce lung function decline. In the meantime, recommending higher vegetable intake to these population groups can only be beneficial, particularly in this age group where vegetable intake may help prevent other chronic diseases such as cardiovascular disease and cancer.

Chapter 7 – Associations between dietary patterns, lung function and bronchial responsiveness

7.1 Introduction

Much of the research to date investigating the link between diet and lung function has focused on specific nutrients, foods or food groups (130, 132, 136, 142, 330, 331). One of the major limitations of such research is the inability to account for the inter-relationships that exist between various dietary factors. Food and nutrient intakes are often highly correlated making it difficult to measure the relationship between a food, food group or nutrient and a disease outcome of interest, independent of other dietary factors (160).

To overcome this limitation, studies of diet-disease relationships have more recently explored dietary patterns as exposure variables rather than specific foods or nutrients. Dietary patterns are defined either statistically (known as the *a posteriori* approach) or using a predefined diet score (known as the *a priori* approach). One of the methods of defining dietary patterns statistically is through the use of principal component analysis (PCA) (166, 168, 191, 332). PCA is a statistical tool for creating fewer uncorrelated components from a large number of highly correlated items (333). In a dietary pattern analysis, PCA considers all the dietary items to create a smaller number of components with each component representing a different dietary pattern. Each pattern is represented by a linear regression model in which the dietary items are explanatory variables, with the next pattern being created from the residuals of the previous regression, and so on until no remaining residuals can be regressed on. Thus, uncorrelated orthogonal patterns are created that together capture the variation in the dietary data.

Current literature investigating the relationship between dietary patterns formed using PCA and lung function is limited and findings are inconsistent (139, 168-175). This inconsistency may be because each study population produces different patterns depending on the diet of that population. Other explanations include that the majority of these studies reduced food items into fewer food groups prior to PCA and, in doing so, removed some of the variation that existed in the diet of that population. Potential effect modifiers or confounders (variously adjusted for or not adjusted for) may vary in distribution in the study populations, thus producing inconsistent findings. Other limitations of the existing studies include that they have examined very few patterns explaining only a small proportion of the variation in diet and there have been no studies conducted in an Australian population.

Bronchial responsiveness (BR) is a clinical measure of airway sensitivity, with hypersensitivity being related to an increased risk of asthma and COPD. Only one study has assessed the relationship between dietary patterns defined by PCA and BR and that study used a function of the dose response slope as the outcome, a method with many limitations as discussed in Chapter 5 (172).

Therefore, in this chapter I aim to examine associations between dietary patterns identified by PCA, lung function and BR in a population of middle-aged and older Australian adults and explore potential effect modifiers of these relationships.

7.2 Methods

The methods described here are specific to this chapter and add to the methods previously described in Chapter 4.

7.2.1 Study population

The flowchart describing the development of the study population for the COPD Study is provided in Figure 4.5 (Chapter 4). Complete FFQ data were obtained from 1,219 subjects who participated in stage 2 of the COPD study and complete spirometry data and methacholine challenge data were available for 1,210 and 1,054 of these participants, respectively.

Extreme and unrealistic dietary intakes can be reported in an FFQ. This recall error is expected to be random and, therefore, attenuate any associations between diet and the outcomes. To minimise the effect of such error, participants with energy intakes below the first or above the 99th percentile of the distribution of energy intake for each sex were excluded from the analyses. That is, males with energy intakes <4,513.78 or >18,005.67 kilojoules/day and females with energy intakes <3,054.48 or >13955.60 kilojoules/day were excluded (N=25). This exclusion criterion is one of many methods used to remove participants who report implausible dietary intakes, all of which appear to work effectively; however, this method tends to remove a smaller proportion of participants (334, 335). Those with missing values for any of the confounding variables used in the analyses were also excluded (height and BMI invalid N=1; atopy missing N=2; atopy invalid N=1) leaving 1,183 and 1,030 participants in the final analyses for lung function and BR respectively.

7.2.2 Ascertainment of Dietary Patterns – Principal Component Analysis

Individual intakes of 101 foods and beverages and 30 nutrients were calculated from the FFQ by Cancer Council Victoria (289). Responses to Questions 1 and 2 of the FFQ regarding number of pieces of fruit consumed daily and number of different vegetables consumed daily respectively, were used to scale the frequency of all fruit and vegetable consumption responses (Question 15) (289). Responses to Questions 11-14 regarding portion size were used to calculate a portion size factor for each participant. This portion size factor was then used in conjunction with the frequency data and sex-specific standard portions to estimate the quantity of each food consumed (289). Nutrient intakes were then calculated from individual food intakes using the Australian nutrient composition database NUTTAB95 (336).

Nutrient intakes are often strongly correlated with energy intake, with those with higher energy intakes also having higher intakes of most nutrients. Therefore, nutrient intakes were energy-adjusted using the residuals method. This method is considered to be the best method of adjusting for energy intake (123). In

the residuals method, regression is performed with the nutrient intake as the dependent variable and total energy intake as the independent variable and the residuals computed. These residuals represent the nutrient intake independent of energy intake and, therefore, nutrient composition or diet quality rather than quantity. Because of the way in which the residuals are calculated, by definition they are uncorrelated with energy intake.

Dietary patterns are typically defined using food intakes in a PCA, however, some studies have used nutrient intakes to obtain patterns (186-189, 337). The PCA method is designed to create fewer uncorrelated variables from many highly correlated items. Therefore, to assess the suitability of the PCA method to define dietary patterns using food intakes and/or nutrient intakes, correlation matrices of the 101 food items and the 30 energy-adjusted nutrients were examined separately. Food items were found to be weakly correlated, with only 0.7% of correlation coefficients greater than |0.3|. Nutrient intakes, however, were moderately correlated with 34.7% of correlations greater than |0.3|, and 12.6% greater than |0.5|, indicating nutrient intakes may be too correlated to be examined separately and PCA is an appropriate method to use as part of an analysis of nutrient intakes. Therefore, a PCA was performed on the 30 energy-adjusted nutrient intakes using varimax orthogonal rotation to obtain uncorrelated components for easier interpretation. The minimum number of components cumulatively explaining at least 80% of the variance in the data were retained. These components represent the dietary patterns of the study population. For each dietary pattern identified, scores were calculated for each participant using the individual nutrient intakes and the loadings of each nutrient within each component. These scores represent the individual's adherence to that dietary pattern. Each score was then divided into quintiles, creating a categorical variable for each pattern score. PCA of nutrient intakes for men and women separately identified similar nutrient patterns. Therefore males and females were analysed together.

7.2.3 Statistical analysis

The statistical methods used to describe the study population and analyse the data are outlined in Chapter 4. Linear mixed effects models were used to examine associations between quintiles of each dietary pattern score and change in FEV_1 during the methacholine challenge. These models included all confounders of diet and baseline FEV_1 identified in Chapter 4 from prior knowledge and a DAG and interactions with dose for confounders of BR, similarly identified (see Chapter 4 and DAGs in Figures 4.4 and 4.6). Models assuming a linear association were explored using pseudo-continuous variables in which subjects were allocated the quintile-specific median value. Statistical evidence of linear trend is presented as p_{trend}. Linearity was assessed using the likelihood ratio test, comparing models with each dietary pattern as categorical (i.e. quintiles) and pseudo-continuous (quintile medians). Evidence of linearity is presented as $p_{linearity}$. Where there is evidence of non-linearity, a non-linear relationship was confirmed using the likelihood ratio test, comparing models with and without the dietary pattern. Sex, BMI category, asthma status, smoking status and atopy were investigated as potential effect modifiers using the likelihood ratio test comparing, models with and without the interaction term. Evidence of an interaction is presented as $p_{interaction}$. Relationships with moderate or strong evidence are discussed, and any similar trends seen in

related analyses identified.

7.3 Results

7.3.1 Study population

The characteristics of the male and female participants included in the analyses are summarised in Table 1. The study population included 1187 participants, 51.6% of whom were male. Mean age was 58.2 ± 7.5 years. Smokers comprised 12.5% of the sample, 10.5% had current asthma, 4.2% had been diagnosed with COPD and 52.5% were atopic. The majority of the study population were overweight (45.3%) or obese (22.9%) and the mean energy intake was 8253kJs; however, this value varied considerably within the sample (standard deviation = 2696kJ).

Male participants were more likely to be overweight or obese, to be atopic and to have previously been a smoker whilst females were more likely to have never smoked, to have asthma or a history of asthma, and to have been diagnosed with COPD. As expected, males were, on average, taller and had a greater lung capacity, as indicated by their higher mean FEV_1 and FVC values compared to females. The mean FEV_1/FVC was similar in both sexes.

Table 7.1 - Summary of demographic characteristics of the study population by sex

	Male (n=610)	Female (n=573)	Total (n=1183)
Age (years)	58.4 [7.4]	57.9 [7.6]	58.2 [7.5]
Height (metres)	1.75 [0.07]	1.62 [0.06]	1.69 [0.09]
Smoking, n (%)			
- Never	227 (37.2)	315 (55.0)	542 (45.8)
- Former	301 (49.3)	194 (33.9)	495 (41.8)
- Current	82 (13.4)	64 (11.2)	146 (12.3)
Energy intake (kj)	9355 [2690]	7080 [2150]	8253 [2694]
BMI category, n (%)			
- Healthy (<25 kg/m^2)	141 (23.1)	236 (41.2)	377 (31.9)
- Overweight (≥25 and <30kg/m^2)	321 (52.6)	213 (37.2)	534 (45.1)
- Obese (≥30kg/m^2)	148 (24.3)	124 (21.6)	272 (23.0)
Asthma status, n (%)			
- Never	509 (83.4)	443 (77.3)	952 (80.5)
- Remitted	49 (8.0)	59 (10.3)	108 (9.1)
- Current	52 (8.5)	71 (12.4)	123 (10.4)
COPD, n (%)	21 (3.4)	29 (5.1)	50 (4.2)
Atopy n (%)	348 (57.1)	273 (47.6)	621 (52.5)
FEV_1 (L)	3.64 [0.74]	2.66 [0.56]	3.16 [0.82]
FVC (L)	4.88 [0.84]	3.49 [0.64]	4.21 [1.02]
FEV_1/FVC (%)	74.4 [8.0]	76.1 [7.7]	75.3 [7.9]

All values presented as mean [standard deviation] unless otherwise stated

7.3.2 Dietary patterns from analysis of nutrients

Eight principal components that in total explained 82.3% of the variance in nutrient intake were retained for further analysis. The factor loadings of nutrients in these components are presented in Table 2. The first component was characterised by higher intakes of fibre, folate, magnesium, potassium and iron and lower intakes of fat; the second by higher intakes of protein, cholesterol and zinc; the third by high intakes of polyunsaturated fat, vitamin E and starch and low intakes of alcohol; and the fourth by high intakes of sugars, vitamin C and beta-cryptoxanthin and low intakes of starch. The fifth component was high in lutein zeaxanthin and low in carbohydrates, sugars, and calcium; the sixth was high in carbohydrates, starch and lycopene and low in alcohol; the seventh was high in vitamin C and beta-cryptoxanthin and low in calcium and phosphorus; and the eighth was high in saturated fat and alpha and beta-carotene and low in lycopene. The patterns have been named according to the nutrients with the highest absolute factor loadings and are thus referred to as “high potassium & magnesium”; “high protein & zinc”; “high polyunsaturated fats (PUFAs) and vitamin E”; “high β-cryptoxanthin & vitamin C”; “low calcium and sugars”; “high starch &

lycopene"; "high vitamin C, low calcium"; and "high α-carotene, low lycopene" for patterns one to eight respectively.

Associations were observed between the dietary patterns and some demographic characteristics (Appendix 7, Tables A1-A4). Those with a higher "high potassium and magnesium" pattern score were more likely to be female, never or former smokers and currently have asthma. Those with a higher score on the second pattern were more likely to be female and to be obese. Those scoring higher on the "high PUFAs & vitamin E" pattern were more likely to be never smokers and in the healthy weight range. Those with a higher "high β-cryptoxanthin & vitamin C" dietary pattern score were more likely to be female, to have never smoked and to be a healthy weight. Those with a higher "low calcium and sugars" pattern score were more likely to be former-smokers and to be obese. Those with a higher "high starch & lycopene" dietary pattern score were more likely to be obese and those with a higher "high vitamin C, low calcium" pattern score were more likely to be male. Lastly, those with a higher "high α-carotene, low lycopene" dietary pattern score were more likely to be current smokers and to have COPD. Energy intake also differed between quintiles of a number of dietary patterns; however, this relationship appeared to be non-linear.

Table 7.2 – Factor loading matrix and explained variances for the eight components obtained by principal component analysis

Nutrient	Dietary Pattern (principal components)							
	"High potassium & magnesium"	"High protein & zinc"	"High PUFAs and vitamin E"	"High β-cryptoxanthin & vitamin C"	"Low calcium and sugars"	"High starch & lycopene"	"High vitamin C, low calcium"	"High α-carotene, low lycopene"
Proportion of variance explained (%)	29.1	12.6	11.1	8.5	6.7	5.5	4.5	4.2
Cumulative variance explained (%)	29.1	41.7	52.9	61.4	68.1	73.6	78.1	82.3
Total fat	-0.20	**0.26**	**0.25**	0.24	-	-	-	-
Saturated fat	-0.19	0.19	-	0.18	-0.21	-	0.15	**0.30**
Polyunsaturated fat	-	-	**0.39**	-	0.21	-0.23	-0.15	**-0.28**
Monounsaturated fat	-0.16	**0.25**	0.20	0.21	0.16	-	-	-
Protein	-	**0.40**	-0.20	-	-	-	-	-0.16
Carbohydrates	0.20	-0.19	0.20	-	**-0.28**	**0.33**	-	-
Sugars	0.18	-0.16	-	**0.28**	**-0.36**	-	-	-
Starch	-	-	**0.31**	**-0.29**	-	**0.38**	-	-
Alcohol	-	-0.19	**-0.31**	-0.24	0.20	**-0.34**	-	-
Fibre	**0.27**	-	0.17	-	-	-	-	-
Calcium	0.15	-	-	-	**-0.41**	-	**-0.34**	-
Cholesterol	-	**0.31**	-0.22	-	-	-	0.21	-
Folate	**0.27**	-	-	-	-	-0.23	**0.27**	-
Iron	0.24	-	-	-0.18	-	-	-	-
Magnesium	**0.29**	-	-	-	-	-0.16	-0.18	-

Niacin (equivalents)	0.23	**0.28**	-	-	-	-	0.22	-
Phosphorus	0.23	0.24	-	-	-0.16	-	**-0.28**	-0.19
Potassium	**0.29**	-	-	-	-	-	-	-
Retinol	-0.17	-	**0.27**	0.20	-0.19	-	-	-
Riboflavin	0.22	0.16	-	-	**-0.25**	**-0.25**	-	-
Sodium	-	**0.26**	0.18	-0.21	-	0.24	0.23	-
Thiamine	0.20	-	0.24	-0.17	-	-0.21	**0.25**	0.15
Vitamin C	0.18	-0.17	-	**0.33**	-	-	**0.36**	-0.16
Vitamin E	-	-	**0.34**	0.21	0.23	-0.15	-0.15	-0.17
Zinc	-	**0.37**	-0.18	-	-	-	-	-
Alpha-carotene	0.15	-	-	-	**0.25**	-	**-0.26**	**0.53**
Beta-carotene	0.19	-	-	0.24	**0.25**	0.18	-0.19	**0.34**
Beta-cryptoxanthin	0.15	-	-	**0.37**	-	-	**0.34**	-0.24
Lutein zeaxanthin	0.18	-	-	0.20	**0.27**	-	-	-
Lycopene	-	-	-	-	0.19	**0.37**	-	**-0.35**

Factor loadings between -0.15 and 0.15 are not shown for simplicity. Factor loadings ≥ |0.25| are in bold. PUFA – Polyunsaturated fatty acid

7.3.3 Associations between dietary patterns and lung function

In the univariate analysis, the "high potassium & magnesium" dietary pattern score (component 1) was negatively associated with FEV_1. There was also a non-linear relationship observed with FVC. After adjusting for age, height, sex, smoking status, asthma status, BMI category, atopy, and energy intake, this dietary pattern score was positively associated with FEV_1 and FVC, with those in quintile 5 having both a mean FEV_1 and FVC 0.12 L higher than those in quintile 1(FEV_1: 95%CI 0.02, 0.21, p_{trend}=0.003; FVC: 95%CI 0.01, 0.22, p_{trend}=0.005; Table 7.3). There was no relationship with FEV_1/FVC in either the univariate or multivariate analysis.

The unadjusted analysis of the "high protein & zinc" dietary pattern score (component 2) found negative associations with FEV_1 and FVC; however, there were no associations with any of the lung function outcomes after adjusting for confounders (Appendix 7, Table A5).

The "high PUFAs & vitamin E" dietary pattern score (component 3) was associated with FEV_1, FVC and FEV_1/FVC in the univariate analyses; however, all relationships were non-linear. After adjusting for confounders, this dietary pattern score was positively associated with FEV_1, with those in the fifth quintile having a mean FEV_1 0.12 L higher than those in quintile 1 (95%CI 0.02, 0.21; p_{trend}=0.034; Table 7.4). There was also a non-linear relationship with FVC; however, there was no obvious meaningful shape. There was no relationship between the "high PUFAs & vitamin E" dietary pattern score and FEV_1/FVC after adjusting for confounders.

Negative associations were observed between the "high β-cryptoxanthin & vitamin C" dietary pattern score (component 4) and both FEV_1 and FVC in the univariate analyses. However, these relationships disappeared upon adjustment for confounders. There was also a non-linear relationship between this dietary pattern score and FEV_1/FVC in both the unadjusted and adjusted analyses; however, there was no meaningful shape in the results (Appendix 7, Table A6).

There were no associations observed between the "low calcium & sugars" dietary pattern score (component 5) and lung function in the unadjusted or adjusted analyses (Appendix 7, Table A7).

The univariate analyses of the "high starch & lycopene" dietary pattern score (component 6) found non-linear associations with FEV_1 and FVC; however, there were no associations with any of the lung function outcomes after adjusting for confounders (Appendix 7, Table A8).

There was evidence of non-linear relationships between the "high vitamin C, low calcium" dietary pattern score (component 7) and FEV_1 and FVC in the univariate analyses. After adjusting for confounders, there was no longer a relationship with FVC; however, there was a linear negative relationship with FEV_1. Those in the highest quintile had a mean FEV_1 0.08 L less than those in the

lowest quintile (95%CI -0.18, 0.007, p_{trend}-=0.034; Table 7.5). There was no relationship with FEV_1/FVC in either the univariate or multivariate analyses.

The "high α-carotene, low lycopene" dietary pattern score (component 8) was negatively associated with FEV_1 and FEV_1/FVC in the univariate analyses. After adjustment for confounders, there were non-linear relationships with FEV_1 and FVC with no meaningful pattern. The negative association with FEV_1/FVC remained, with those in the highest quintile of score having a mean FEV_1/FVC 1.39% less than those in the lowest quintile (95%CI -2.66, -0.12, p_{trend}=0.009; Table 7.6).

Table 7.3 - Unadjusted and adjusted associations of the "high potassium & magnesium" dietary pattern score and lung function

Lung function measure	"high potassium & magnesium" dietary pattern quintiles					p_{trend}	$p_{linearity}$
	Q1	Q2	Q3	Q4	Q5		
FEV_1 (L)							
Model 1	Ref	-0.23 (-0.37, -0.08)	-0.18 (-0.33, -0.04)	-0.21 (-0.36, -0.07)	-0.19 (-0.34, -0.04)	0.028	0.060
Model 2	Ref	-0.01 (-0.10, 0.08)	-0.01 (-0.10, 0.08)	0.06 (-0.03, 0.15)	0.12 (0.02, 0.21)	0.003	0.43
FVC (L)							
Model 1	Ref	-0.32 (-0.51, -0.14)	-0.33 (-0.52, -0.15)	-0.33 (-0.51, -0.14)	-0.29 (-0.47, -0.11)	0.006	0.010
Model 2	Ref	-0.03 (-0.13, 0.08)	-0.06 (-0.16, 0.04)	0.05 (-0.06, 0.15)	0.12 (0.02, 0.22)	0.005	0.081
FEV_1/FVC (%)							
Model 1	Ref	0.26 (-1.16, 1.68)	1.43 (0.005, 2.84)	0.77 (-0.65, 2.19)	0.84 (-0.57, 2.26)	0.20	0.39
Model 2	Ref	0.09 (-1.20, 1.39)	0.57 (-0.73, 1.87)	0.60 (-0.71, 1.91)	0.83 (-0.48, 2.13)	0.15	0.97

β-coefficient (95%CI) presented for each quintile

Model 1: Unadjusted linear regression analysis

Model 2: Regression analysis adjusted for age, height, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table 7.4– Unadjusted and adjusted associations between the "High PUFAs and vitamin E" dietary pattern score and lung function

Lung function measure	"High PUFAs and vitamin E" quintiles					p_{trend}	$p_{linearity}$
	Q1	Q2	Q3	Q4	Q5		
FEV_1 (L)							
Model 1	Ref	-0.19 (-0.34, -0.04)	-0.15 (-0.30, -0.001)	-0.21 (-0.35, -0.06)	-0.005 (-0.15, 0.14)	1.00	0.002
Model 2	Ref	0.05 (-0.04, 0.14)	0.13 (0.03, 0.22)	0.03 (-0.06, 0.12)	0.12 (0.02, 0.21)	0.034	0.080
FVC (L)							
Model 1	Ref	-0.30 (-0.48, -0.12)	-0.33 (-0.51, -0.15)	-0.40 (-0.58, -0.22)	-0.06 (-0.24, 0.12)	0.44	<0.001
Model 2	Ref	0.04 (-0.06, 0.14)	0.09 (-0.01, 0.19)	-0.05 (-0.16, 0.05)	0.11 (0.01, 0.22)	0.14	0.008
FEV_1/FVC (%)							
Model 1	Ref	0.93 (-0.49, 2.34)	2.21 (0.79, 3.63)	2.20 (0.79, 3.62)	0.93 (-0.49, 2.34)	0.094	0.012
Model 2	Ref	0.45 (-0.84, 1.73)	1.27 (-0.04, 2.57)	1.56 (0.27, 2.85)	0.58 (-0.70, 1.87)	0.19	0.13

β-coefficient (95%CI) presented for each quintile; PUFA = polyunsaturated fatty acid

Model 1: Unadjusted linear regression analysis

Model 2: Regression analysis adjusted for age, height, sex, smoking status, asthma status, BMI category, atopy, and energy intake

*p-value for likelihood ratio test comparing models with and without the dietary pattern = 0.007

Table 7.5– Unadjusted and adjusted associations of the "high vitamin C, low calcium" dietary pattern score and lung function

Lung function measure	"high vitamin C, low calcium" dietary pattern quintiles					p_{trend}	$p_{linearity}$
	Q1	Q2	Q3	Q4	Q5		
FEV_1 (L)							
Model 1	Ref	-0.03 (-0.18, 0.12)	-0.12 (-0.27, 0.02)	-0.10 (-0.24, 0.05)	0.06 (-0.09, 0.21)	0.57	0.059
Model 2	Ref	-0.01 (-0.11, 0.08)	-0.06 (-0.16, 0.03)	-0.07 (-0.17, 0.02)	-0.08 (-0.18, 0.007)	0.034	0.85
FVC (L)							
Model 1	Ref	-0.07 (-0.25, 0.12)	-0.13 (-0.31, 0.06)	-0.12 (-0.31, 0.06)	0.11 (-0.08, 0.29)	0.33	0.049
Model 2	Ref	-0.03 (-0.14, 0.07)	-0.04 (-0.14, 0.07)	-0.07 (-0.17, 0.03)	-0.07 (-0.18, 0.03)	0.12	0.96
FEV_1/FVC (%)							
Model 1	Ref	0.72 (-0.70, 2.14)	-0.64 (-2.05, 0.78)	-0.09 (-1.51, 1.33)	-0.18 (-1.60, 1.24)	0.54	0.35
Model 2	Ref	0.49 (-0.79, 1.78)	-0.91 (-2.20, 0.38)	-0.63 (-1.93, 0.67)	-0.44 (-1.71, 0.84)	0.23	0.22

β-coefficient (95%CI) presented for each quintile

Model 1: Unadjusted linear regression analysis

Model 2: Regression analysis adjusted for age, height, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table 7.6– Unadjusted and adjusted associations of the "high α-carotene, low lycopene" dietary pattern score and lung function

Lung function measure	"high α-carotene, low lycopene" dietary pattern quintiles					p_{trend}	$p_{linearity}$
	Q1	Q2	Q3	Q4	Q5		
FEV_1 (L)							
Model 1	Ref	-0.09 (-0.23, 0.06)	-0.05 (-0.20, 0.10)	-0.14 (-0.29, 0.006)	-0.17 (-0.32, -0.03)	0.016	0.81
Model 2	Ref	0.008 (-0.08, 0.10)	0.09 (-0.002, 0.18)	-0.02 (-0.11, 0.07)	-0.04 (-0.13, 0.05)	0.26	0.044*
FVC (L)							
Model 1	Ref	-0.13 (-0.31, 0.05)	-0.07 (-0.25, 0.12)	-0.12 (-0.30, 0.07)	-0.13 (-0.32, 0.05)	0.21	0.71
Model 2	Ref	0.03 (-0.07, 0.13)	0.15 (0.05, 0.25)	0.05 (-0.05, 0.15)	0.006 (-0.10, 0.11)	0.89	0.013^
FEV_1/FVC (%)							
Model 1	Ref	0.43 (-0.98, 1.84)	-0.22 (-1.63, 1.19)	-1.41 (-2.83, 0.005)	-1.98 (-3.39, -0.57)	<0.001	0.34
Model 2	Ref	-0.32 (-1.60, 0.97)	-0.76 (-2.04, 0.52)	-1.48 (-2.76, -0.19)	-1.39 (-2.66, -0.12)	0.009	0.77

β-coefficient (95%CI) presented for each quintile

Model 1: Unadjusted linear regression analysis

Model 2: Regression analysis adjusted for age, height, sex, smoking status, asthma status, BMI category, atopy, and energy intake

*p-value for likelihood ratio test comparing models with and without the dietary pattern = 0.052

^p-value for likelihood ratio test comparing models with and without the dietary pattern = 0.029

7.3.4 Modification of associations between dietary patterns and lung function

7.3.4.1 *"High potassium & magnesium" dietary pattern*

Relationships between the "high potassium & magnesium" dietary pattern and FEV_1 and FVC were modified by asthma status ($p_{interaction}$=0.005 and 0.008 respectively). There was a positive association with FEV_1 in people with current asthma, with those in the highest quintile having a mean FEV_1 0.57 L greater than those in the lowest quintile (95%CI 0.30, 0.84, p_{trend}<0.001; Table 7.7, Figure 7.1). There was no relationship observed in those who never had asthma or those with remitted asthma. The relationship with FVC was non-linear; however appeared to indicate an association in those with current asthma only with a similar shape to that of FEV_1. In those with current asthma, people in the fifth quintile had a mean FVC 0.50 L higher than those in the first quintile (95%CI 0.20, 0.80, p_{trend}=0.001; Table 7.7, Figure 7.2).

There were no interactions between quintiles of the "high potassium & magnesium" dietary pattern and any of the other potential effect modifiers tested (Appendix 7, Tables A9-A12).

7.3.4.2 *"High protein & zinc" dietary pattern*

The relationship between the "high protein & zinc" dietary pattern and FVC was modified by smoking status ($p_{interaction}$=0.019); however there was strong evidence the relationship was non-linear ($p_{linearity}$=0.005; Table 7.8). There was an inverted u-shaped relationship with FVC in current smokers only (Figure 7.3). Current smokers in the third quintile of the "high protein & zinc" pattern score had a mean FVC 0.51 L higher than those in quintile 1 (95%CI 0.20, 0.81). There was no obvious shape to the results for never and former smokers.

There were no interactions observed between quintiles of the "high protein & zinc" dietary pattern and any of the other potential effect modifiers tested (Appendix 7, Tables A13-A16).

Table 7.7 - Adjusted associations between "high potassium & magnesium" dietary pattern quintiles and lung function by asthma status

Prudent pattern	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.03 (-0.13, 0.07)	0.03 (-0.26, 0.32)	0.13 (-0.17, 0.43)	-0.03 (-0.14, 0.09)	-0.06 (-0.39, 0.26)	0.03 (-0.30, 0.36)	-0.39 (-1.83, 1.06)	1.93 (-2.18, 6.03)	1.87 (-2.29, 6.03)
Q3	-0.03 (-0.13, 0.07)	-0.20 (-0.54, 0.14)	0.26 (-0.04, 0.57)	-0.08 (-0.19, 0.04)	-0.32 (-0.70, 0.06)	0.29 (-0.05, 0.63)	0.58 (-0.83, 2.00)	0.51 (-4.30, 5.32)	-0.10 (-4.41, 4.21)
Q4	0.04 (-0.06, 0.14)	0.23 (-0.10, 0.56)	0.11 (-0.17, 0.39)	0.03 (-0.08, 0.14)	0.27 (-0.09, 0.64)	0.05 (-0.26, 0.36)	0.49 (-0.96, 1.94)	1.68 (-2.92, 6.29)	0.92 (-2.98, 4.82)
Q5	0.04 (-0.06, 0.15)	0.18 (-0.14, 0.49)	0.57 (0.30, 0.84)	0.06 (-0.05, 0.17)	0.15 (-0.20, 0.51)	0.50 (0.20, 0.80)	0.10 (-1.34, 1.55)	1.50 (-2.96, 5.96)	5.23 (1.39, 9.07)
p_{trend}	0.20	0.11	<0.001	0.17	0.086	0.001	0.61	0.68	0.010
$p_{interaction}$	0.005			0.008			0.15		
$p_{linearity}$	0.14			0.018			0.61		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

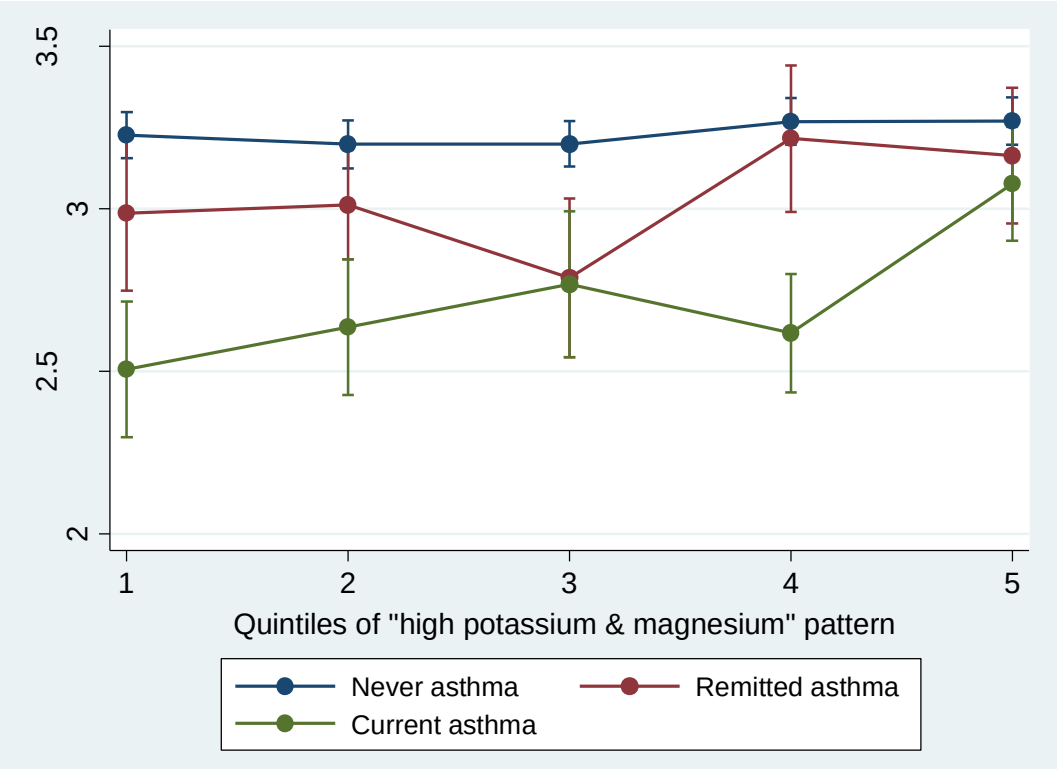


Figure 7.1 - Relationship between quintiles of the "high potassium & magnesium" dietary pattern and FEV_1 by asthma status

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy; error bars represent 95%CIs

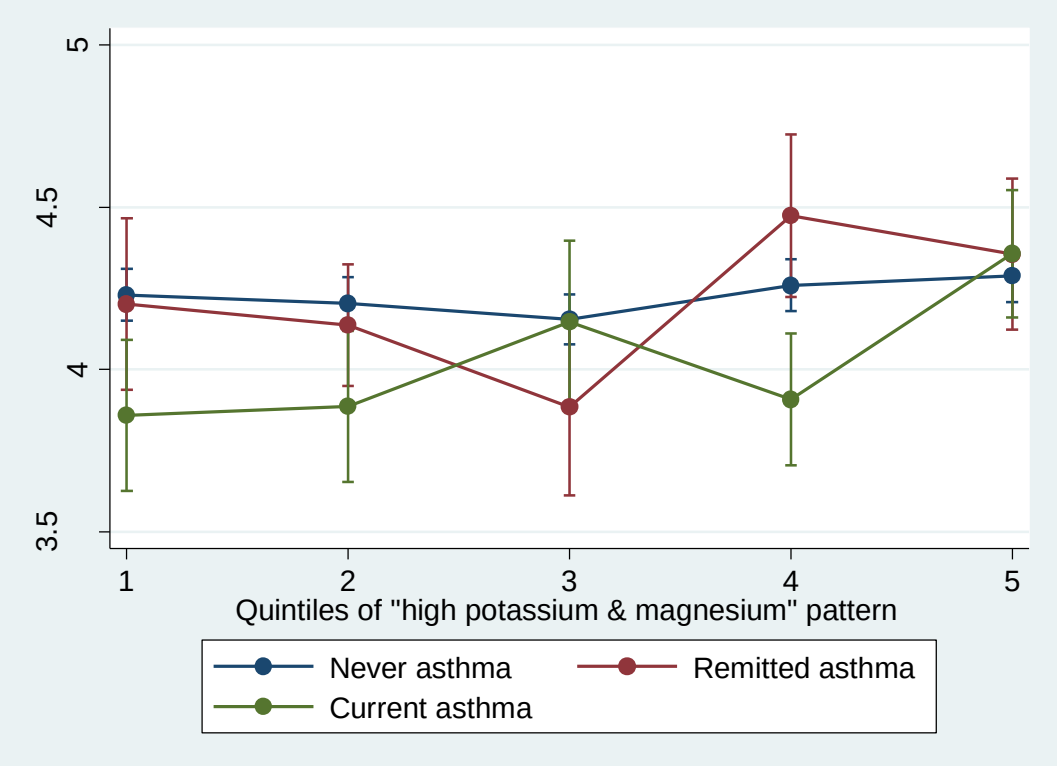


Figure 7.2 - Relationship between quintiles of the "high potassium & magnesium" dietary pattern and FVC by asthma status

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy; error bars represent 95%CIs

Table 7.8- Adjusted associations between "high protein & zinc" dietary pattern quintiles and lung function by smoking status

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.17 (0.03, 0.31)	0.01 (-0.12, 0.15)	0.10 (-0.15, 0.35)	0.12 (-0.03, 0.28)	0.10 (-0.05, 0.25)	0.24 (-0.04, 0.52)	1.84 (-0.13, 3.80)	-1.20 (-3.11, 0.70)	-1.79 (-5.34, 1.76)
Q3	0.05 (-0.09, 0.18)	0.05 (-0.08, 0.19)	0.23 (-0.05, 0.50)	0.01 (-0.14, 0.16)	0.06 (-0.09, 0.22)	0.51 (0.20, 0.81)	0.95 (-0.96, 2.85)	-0.08 (-1.99, 1.84)	-2.47 (-6.33, 1.39)
Q4	0.04 (-0.09, 0.18)	0.02 (-0.12, 0.15)	-0.05 (-0.32, 0.22)	0.004 (-0.15, 0.16)	0.13 (-0.02, 0.28)	0.09 (-0.21, 0.39)	1.43 (-0.50, 3.36)	-1.64 (-3.54, 0.27)	-2.26 (-6.06, 1.53)
Q5	0.11 (-0.03, 0.24)	-0.02 (-0.17, 0.12)	-0.02 (-0.27, 0.22)	0.11 (-0.04, 0.26)	0.01 (-0.14, 0.17)	-0.007 (-0.28, 0.27)	0.78 (-1.12, 2.68)	-0.83 (-2.83, 1.17)	-0.43 (-3.91, 3.06)
p_{trend}	0.42	0.83	0.52	0.38	0.63	0.51	0.60	0.30	0.91
$p_{interaction}$	0.37			0.019			0.23		
$p_{linearity}$	0.19			0.005			0.37		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

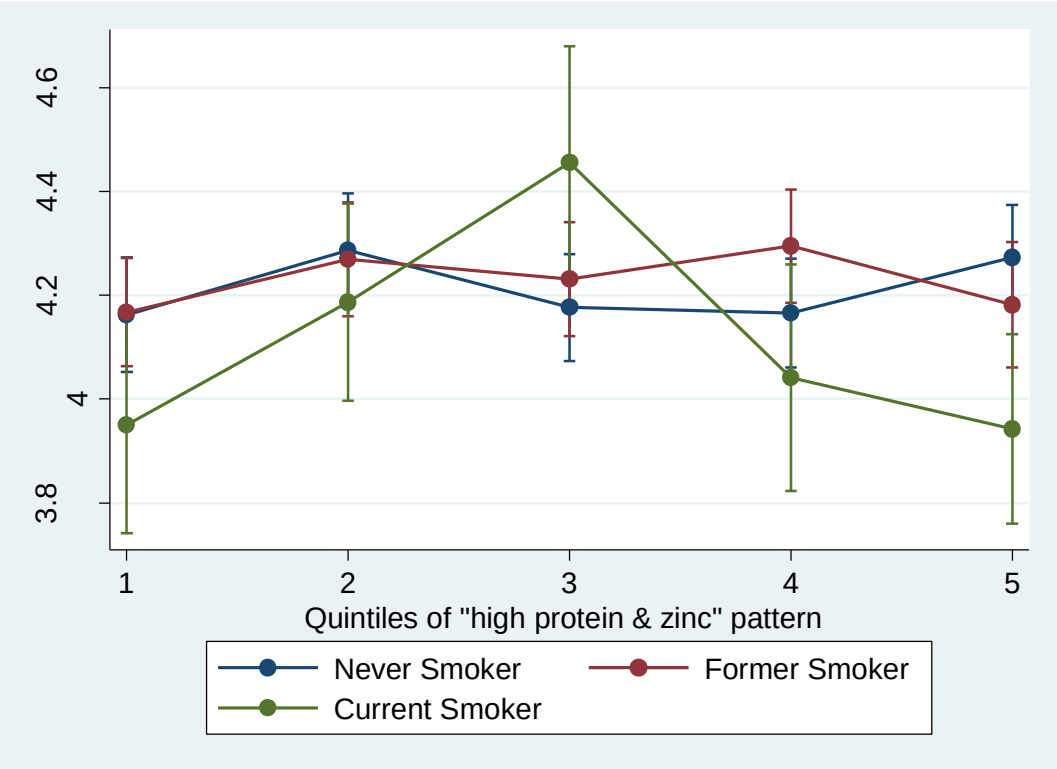


Figure 7.3 - Relationship between quintiles of the "high protein & zinc" dietary pattern and FVC by smoking status

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy; error bars represent 95%CI

7.3.4.3 *"High PUFAs and vitamin E" dietary pattern*

There was moderate evidence the relationship between the "high PUFAs & vitamin E" dietary pattern and FEV_1/FVC was modified by asthma status and moderate evidence the relationship was non-linear ($p_{interaction}$=0.047, $p_{linearity}$=0.020; Table 7.9). There was a clear non-linear relationship in the remitted asthma group; however, the shape of the results was not meaningful (Figure 7.4). There was no relationship between the "high PUFAs & vitamin E" dietary pattern and FEV_1/FVC in those with current asthma or those who have never had asthma.

The relationships between the "high PUFAs & vitamin E" dietary pattern and FEV_1 and FEV_1/FVC were modified by smoking status ($p_{interaction}$=0.018 and 0.010 respectively) and these relationships were also non-linear ($p_{linearity}$=0.014 and 0.006 respectively; Table 7.10). In current smokers, there was an inverted u-shaped pattern in the results for both lung function outcomes (Figure 7.5). The mean difference in FEV_1 between the quintile with the highest mean FEV_1 (quintile 3) and the quintile with the lowest mean FEV_1 (quintile 1) was 0.40 L (95%CI 0.15, 0.66). For FEV_1/FVC, the mean difference between the quintiles with the highest and lowest mean values (quintiles 3 and 2 respectively) was 5.19% (1.44%, 8.95%). There were no meaningful relationships between the "high PUFAs & vitamin E" dietary pattern and FEV_1 or FEV_1/FVC in former or never smokers.

There were no interactions observed between quintiles of the "high PUFAs & vitamin E" dietary pattern and any of the other potential effect modifiers tested (Appendix 7, Tables A17-A19).

Table 7.9 - Adjusted associations between "High PUFAs and vitamin E" dietary pattern quintiles and lung function by asthma status

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.04 (-0.07, 0.14)	0.02 (-0.27, 0.30)	0.19 (-0.10, 0.47)	0.06 (-0.06, 0.17)	-0.13 (-0.45, 0.19)	0.08 (-0.24, 0.40)	-0.09 (-1.52, 1.34)	2.17 (-1.84, 6.19)	3.00 (-0.98, 6.98)
Q3	0.11 (0.01, 0.22)	0.15 (-0.16, 0.47)	0.25 (-0.05, 0.54)	0.08 (-0.03, 0.20)	0.22 (-0.13, 0.57)	0.09 (-0.24, 0.42)	1.24 (-0.20, 2.67)	-0.97 (-5.36, 3.43)	3.21 (-0.94, 7.37)
Q4	0.03 (-0.07, 0.14)	0.15 (-0.15, 0.44)	-0.07 (-0.35, 0.20)	-0.03 (-0.14, 0.09)	-0.12 (-0.45, 0.21)	-0.18 (-0.49, 0.13)	1.40 (-0.05, 2.85)	5.23 (1.10, 9.36)	0.27 (-3.59, 4.14)
Q5	0.13 (0.03, 0.23)	0.06 (-0.22, 0.34)	0.06 (-0.23, 0.35)	0.12 (0.006, 0.23)	0.07 (-0.24, 0.38)	0.11 (-0.21, 0.44)	0.81 (-0.61, 2.24)	-0.009 (-3.89, 3.88)	-0.42 (-4.52, 3.68)
p_{trend}	0.022	0.55	0.69	0.14	0.65	0.99	0.11	0.73	0.42
$p_{interaction}$	0.56			0.78			0.047		
$p_{linearity}$	0.20			0.061			0.020		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

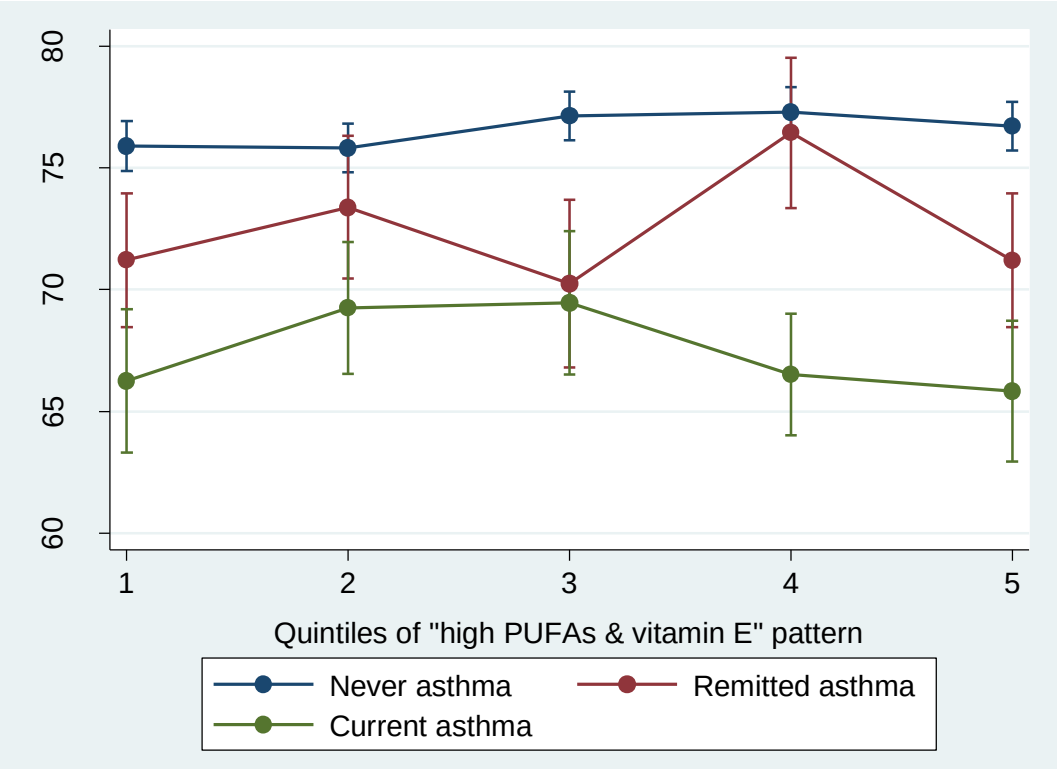


Figure 7.4 - Relationship between quintiles of the "high PUFAs & vitamin E" dietary pattern and FEV_1/FVC by asthma status

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy; error bars represent 95%CIs

Table 7.10 - Adjusted associations between quintiles of "high PUFAs &vitamin E" dietary pattern and lung function by smoking status

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.04 (-0.11, 0.19)	0.05 (-0.08, 0.18)	0.02 (-0.20, 0.24)	-0.009 (-0.18, 0.16)	0.07 (-0.08, 0.22)	0.03 (-0.22, 0.27)	1.12 (-0.99, 3.23)	0.05 (-1.82, 1.92)	-0.10 (-3.16, 2.96)
Q3	0.05 (-0.10, 0.20)	0.12 (-0.01, 0.25)	0.40 (0.15, 0.66)	0.02 (-0.15, 0.18)	0.09 (-0.06, 0.24)	0.26 (-0.03, 0.55)	0.54 (-1.53, 2.61)	1.23 (-0.65, 3.11)	5.09 (1.48, 8.70)
Q4	-0.007 (-0.15, 0.14)	-0.07 (-0.21, 0.07)	0.38 (0.14, 0.62)	-0.14 (-0.30, 0.02)	-0.07 (-0.23, 0.08)	0.22 (-0.04, 0.49)	2.41 (0.38, 4.44)	-0.61 (-2.56, 1.34)	4.96 (1.62, 8.30)
Q5	0.07 (-0.07, 0.21)	0.12 (-0.02, 0.26)	0.17 (-0.11, 0.45)	0.04 (-0.12, 0.20)	0.14 (-0.01, 0.30)	0.21 (-0.10, 0.52)	0.92 (-1.08, 2.92)	0.21 (-1.70, 2.13)	0.08 (-3.82, 3.98)
p_{trend}	0.48	0.27	0.007	0.95	0.25	0.052	0.30	0.98	0.085
$p_{interaction}$	0.018			0.44			0.010		
$p_{linearity}$	0.014			0.053			0.006		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

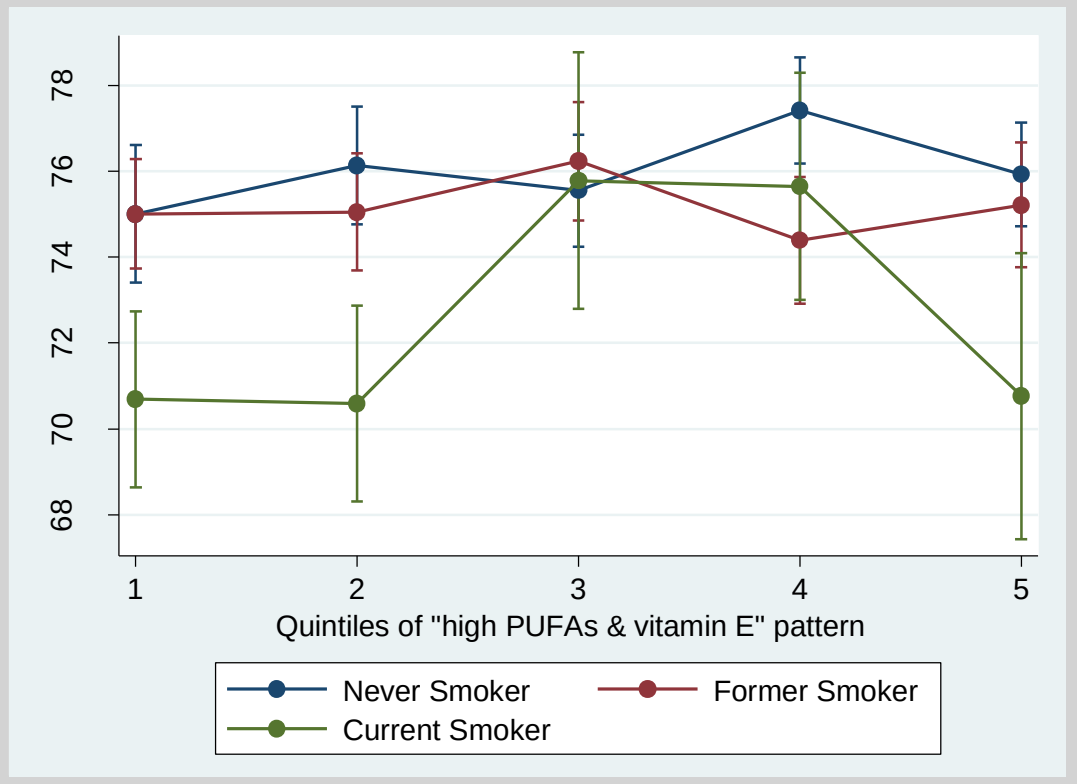


Figure 7.5 - Relationship between quintiles of the "high PUFAs & vitamin E" dietary pattern and FEV_1/FVC by smoking status

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy; error bars represent 95%CI

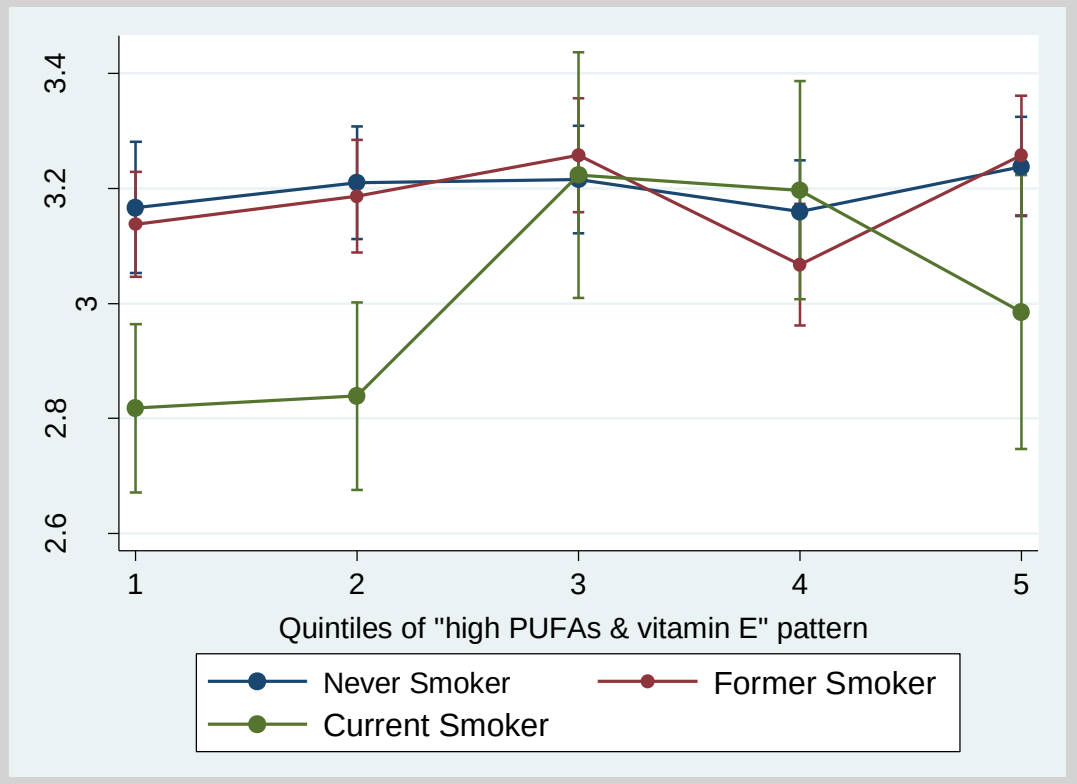


Figure 7.6 - Relationship between quintiles of the "high PUFAs & vitamin E" dietary pattern and FEV_1 by smoking status

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy; error bars represent 95%CIs

7.3.4.4 *"High β-cryptoxanthin & vitamin C" dietary pattern*

Relationships between the "high β-cryptoxanthin & vitamin C" dietary pattern and FEV_1 and FVC were modified by asthma status ($p_{interaction}$=0.020 and 0.014 respectively). There was moderate evidence the associations were non-linear ($p_{linearity}$=0.023 and 0.015 respectively); however, there were no meaningful shapes in the results (Table 7.11).

The relationship between the "high β-cryptoxanthin & vitamin C" dietary pattern and FVC was modified by atopy ($p_{interaction}$=0.020). This relationship was non-linear ($p_{linearity}$=0.034). In the non-atopic group, there was a sharp increase in mean FVC between quintiles 1 and 2, followed by a steady decline to quintile 4 and plateau between quintiles 4 and 5 (Figure 7.7). The mean difference in FVC between the highest and lowest mean FVC values (i.e. quintiles 2 and 1 respectively) was 0.24 L (95%CI 0.09, 0.39). There was no relationship observed between the "high β-cryptoxanthin & vitamin C" dietary pattern and FVC in those with atopy.

There were no interactions observed between quintiles of the "high β-cryptoxanthin & vitamin C" dietary pattern and any of the other potential effect modifiers tested (Appendix 7, Tables A20-A22).

Table 7.11 - Adjusted associations between quintiles of the "high β-cryptoxanthin & vitamin C" dietary pattern and lung function by asthma status

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.06 (-0.04, 0.16)	-0.50 (-0.82, -0.19)	-0.24 (-0.53, 0.06)	0.14 (0.03, 0.26)	-0.47 (-0.82, -0.12)	-0.22 (-0.55, 0.11)	-0.70 (-2.11, 0.70)	-3.92 (-8.33, 0.50)	-3.79 (-7.89, 0.30)
Q3	0.05 (-0.05, 0.15)	-0.18 (-0.49, 0.13)	-0.05 (-0.33, 0.24)	0.04 (-0.07, 0.16)	-0.17 (-0.52, 0.17)	-0.10 (-0.42, 0.23)	0.64 (-0.79, 2.07)	-1.05 (-5.38, 3.29)	-0.23 (-4.27, 3.80)
Q4	0.01 (-0.09, 0.11)	-0.08 (-0.40, 0.25)	-0.21 (-0.48, 0.06)	0.06 (-0.06, 0.17)	-0.06 (-0.42, 0.30)	-0.31 (-0.61, -0.009)	-0.40 (1.82, 1.03)	-1.63 (-6.19, 2.93)	-2.74 (-6.53, 1.05)
Q5	-0.01 (-0.12, 0.09)	-0.23 (-0.52, 0.06)	-0.007 (-0.29, 0.28)	0.0001 (-0.12, 0.12)	-0.26 (-0.58, 0.07)	-0.13 (-0.45, 0.18)	-0.05 (-1.50, 1.41)	-1.59 (-5.66, 2.48)	1.11 (-2.88, 5.09)
p_{trend}	0.62	0.71	0.92	0.65	0.57	0.28	0.94	0.83	0.60
$p_{interaction}$	0.020			0.014			0.55		
$p_{linearity}$	0.023			0.015			0.089		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table 7.12 - Adjusted associations between quintiles of the "high β-cryptoxanthin & vitamin C" dietary pattern and lung function by atopy

Pattern quintiles	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Non-atopic (n=562)	Atopic (n=621)	Non-atopic (n=562)	Atopic (n=621)	Non-atopic (n=562)	Atopic (n=621)
Q1	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.11 (-0.03, 0.24)	-0.12 (-0.24, 0.003)	0.24 (0.09, 0.39)	-0.09 (-0.23, 0.05)	-0.95 (-2.85, 0.94)	-1.48 (-3.21, 0.25)
Q3	0.09 (-0.04, 0.23)	-0.04 (-0.16, 0.09)	0.10 (-0.05, 0.25)	-0.05 (-0.19, 0.09)	0.69 (-1.19, 2.57)	0.22 (-1.54, 1.98)
Q4	0.001 (-0.13, 0.14)	-0.04 (-0.16, 0.09)	0.04 (-0.11, 0.19)	-0.02 (-0.16, 0.12)	-1.02 (-2.91, 0.87)	-0.50 (-2.24, 1.25)
Q5	0.04 (-0.09, 0.17)	-0.09 (-0.22, 0.04)	0.06 (-0.09, 0.21)	-0.12 (-0.26, 0.03)	0.02 (-1.83, 1.86)	-0.10 (-1.90, 1.71)
p_{trend}	0.97	0.43	0.85	0.28	0.98	0.80
$p_{interaction}$	0.11		0.020		0.93	
$p_{linearity}$	0.21		0.034		0.15	

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

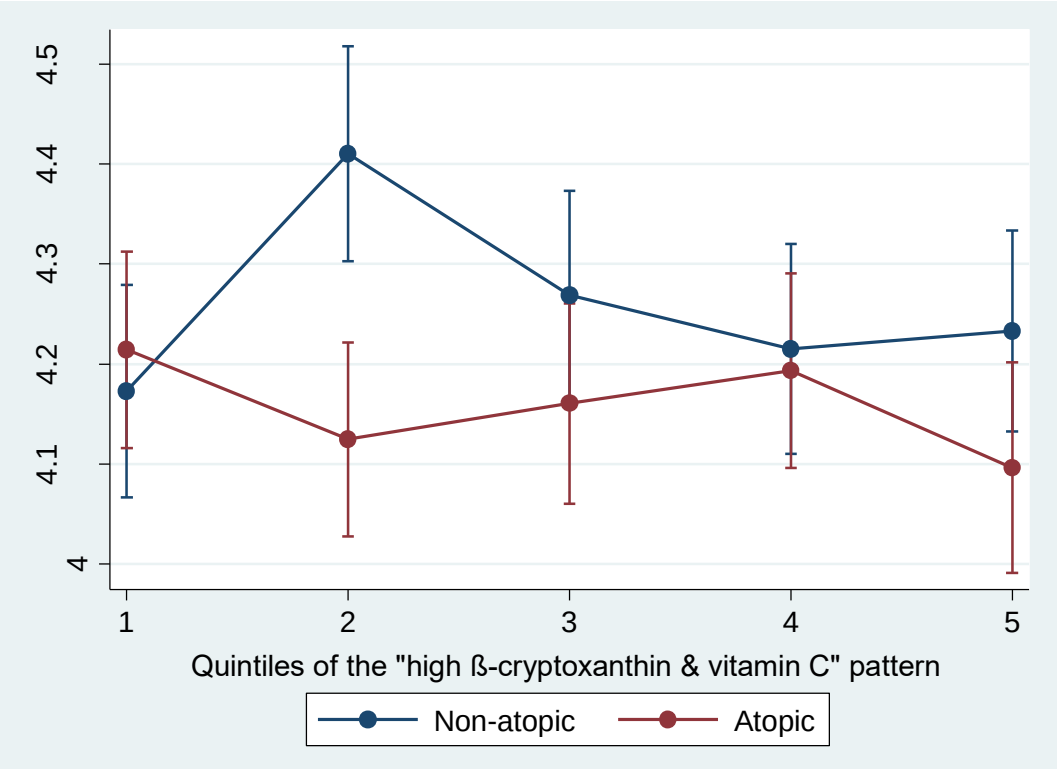


Figure 7.7 - Relationship between quintiles of the "high β-cryptoxanthin & vitamin C" dietary pattern and FVC by atopy

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy; error bars represent 95%CIs

7.3.4.5 *"Low calcium & sugars" dietary pattern*

The association between the "low calcium & sugars" dietary pattern and FEV_1 was modified by sex ($p_{interaction}$=0.012; Table 7.13), however the relationships were non-linear ($p_{linearity}$=0.016) with no meaningful shape for either sex.

The relationship between the "low calcium & sugars" dietary pattern and FVC was modified by sex ($p_{interaction}$ =0.019; Table 7.13). There was a positive association in women and a negative association in men (Figure 7.7); however, the evidence for association was weak or less for both sexes (p_{trend}=0.19 and 0.098 for men and women respectively).

Relationships between the "low calcium & sugars" dietary pattern and FEV_1 and FVC were also modified by asthma status ($p_{interaction}$=0.040 and 0.003). For FEV_1, there was a positive association in the remitted asthma group, with those in quintile 5 having a mean FEV_1 0.37 L greater than those in quintile 1 (95%CI 0.08, 0.67; Table 7.14, Figure 7.8). A similar trend was observed in the current asthma group, however evidence for a trend was weak (p_{trend}=0.084). There was no relationship between the "low calcium & sugars" dietary pattern and FEV_1 in the never asthma group. For FVC, there was moderate evidence the relationship was non-linear ($p_{linearity}$=0.023). In those with remitted asthma, there was a steady increase in mean FEV_1 between quintiles 1 to 3, followed by a sharp decrease between quintiles 3 and 4 and a plateau between quintiles 4 and 5 (Figure 7.9). Those in

quintile 3 had a mean FEV_1 0.71 L greater than those in quintile 1 (95%CI 0.36, 1.06; Table 7.14). For those with current asthma, there appeared to be a slightly curved increasing trend. The difference between the highest and lowest mean FVC values from quintiles 4 and 1 respectively was 0.36 L (95%CI 0.03, 0.68). There was no relationship between the "low calcium & sugars" dietary pattern and FVC in those who have never had asthma.

There were no interactions observed between quintiles of the "low calcium & sugars" dietary pattern and any of the other potential effect modifiers tested (Appendix 7, Tables A23-A25).

Table 7.13 - Adjusted associations between quintiles of the "low calcium & sugars" pattern and lung function outcomes by sex

Pattern quintiles	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Female (n=573)	Male (n=610)	Female (n=573)	Male (n=610)	Female (n=573)	Male (n=610)
Q1	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.14 (0.01, 0.27)	-0.05 (-0.18, 0.08)	0.13 (-0.01, 0.28)	-0.08 (-0.22, 0.07)	1.75 (-0.06, 3.56)	0.07 (-1.73, 1.86)
Q3	0.06 (-0.07, 0.19)	0.12 (-0.003, 0.25)	0.08 (-0.07, 0.22)	0.05 (-0.09, 0.19)	0.24 (-1.59, 2.07)	1.77 (-0.02, 3.56)
Q4	0.11 (-0.02, 0.25)	-0.08 (-0.21, 0.04)	0.14 (-0.01, 0.29)	-0.15 (-0.29, -0.01)	0.85 (-1.01, 2.72)	0.74 (-0.99, 2.46)
Q5	0.09 (-0.04, 0.23)	-0.01 (-0.13, 0.11)	0.11 (-0.05, 0.26)	-0.09 (-0.23, 0.04)	0.56 (-1.35, 2.48)	1.28 (-0.43, 2.98)
p_{trend}	0.28	0.70	0.19	0.098	0.86	0.10
$p_{interaction}$	0.012		0.019		0.15	
$p_{linearity}$	0.016		0.14		0.28	

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table 7.14 - Adjusted associations between quintiles of the "low calcium & sugars" dietary pattern and lung function by asthma status

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.01 (-0.09, 0.11)	0.23 (-0.06, 0.52)	0.17 (-0.13, 0.47)	-0.03 (-0.14, 0.08)	0.33 (0.002, 0.65)	0.20 (-0.13, 0.53)	0.78 (-0.63, 2.19)	1.66 (-2.41, 5.73)	2.20 (-2.00, 6.40)
Q3	0.02 (-0.08, 0.12)	0.54 (0.23, 0.85)	0.27 (-0.06, 0.59)	-0.04 (-0.15, 0.07)	0.71 (0.36, 1.06)	0.30 (-0.05, 0.66)	1.03 (-0.37, 2.43)	1.04 (-3.34, 5.41)	-0.37 (-4.87, 4.14)
Q4	-0.05 (-0.15, 0.05)	0.25 (-0.06, 0.56)	0.28 (-0.01, 0.57)	-0.08 (-0.20, 0.03)	0.19 (-0.16, 0.53)	0.36 (0.03, 0.68)	0.44 (-0.96, 1.84)	3.49 (-0.87, 7.86)	1.43 (-2.64, 5.51)
Q5	-0.03 (-0.13, 0.07)	0.37 (0.08, 0.67)	0.24 (-0.03, 0.50)	-0.06 (-0.18, 0.05)	0.26 (-0.07, 0.59)	0.25 (-0.05, 0.54)	0.08 (-1.35, 1.52)	4.80 (0.68, 8.92)	3.29 (-0.46, 7.03)
p_{trend}	0.33	0.017	0.084	0.16	0.23	0.11	0.96	0.015	0.11
$p_{interaction}$	0.040			0.003			0.19		
$p_{linearity}$	0.32			0.023			0.73		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

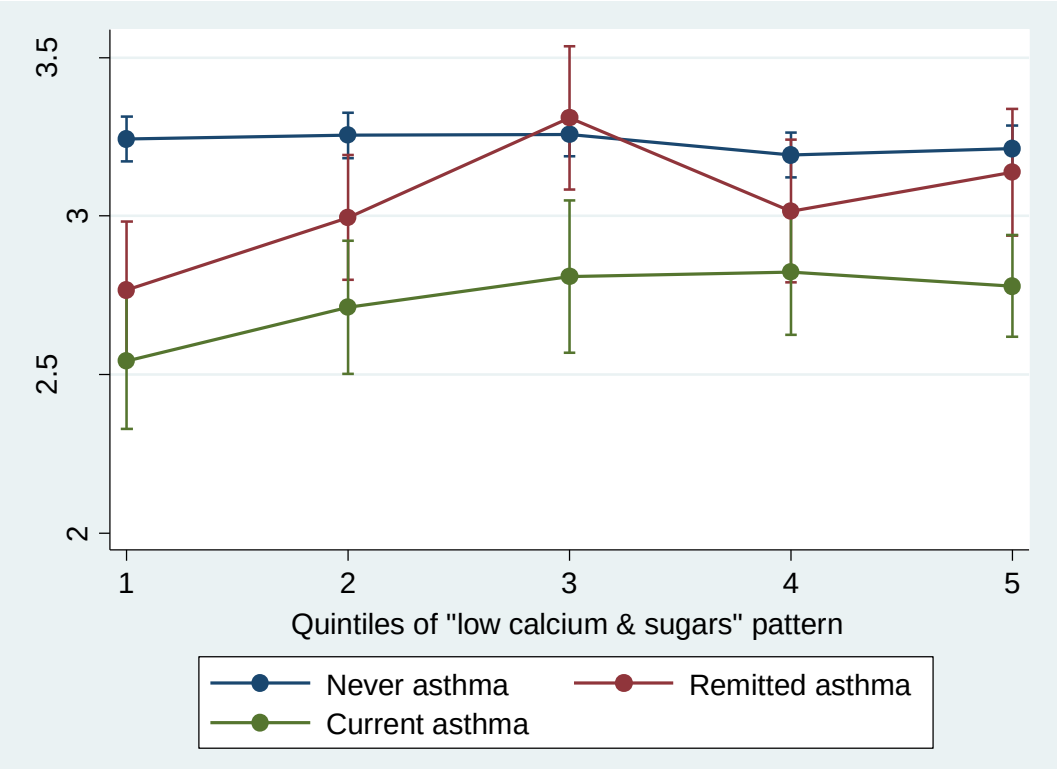


Figure 7.8 - Relationship between quintiles of the "low calcium & sugars" dietary pattern and FEV_1 by asthma status

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy; error bars represent 95%CIs

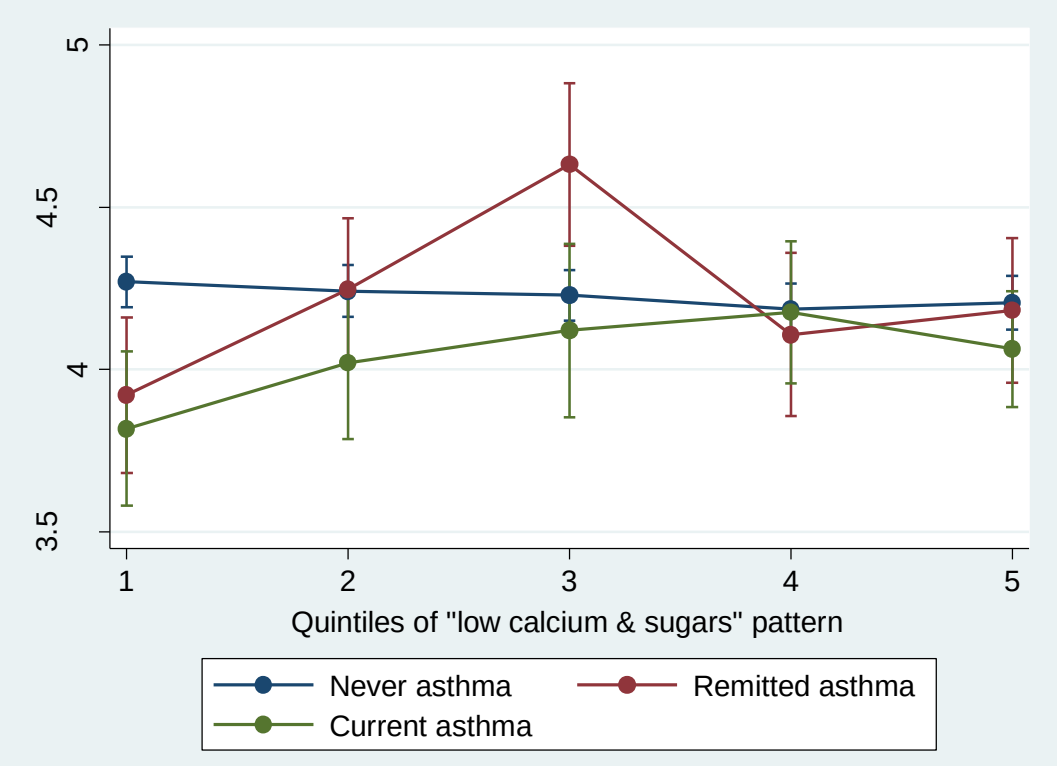


Figure 7.9 - Relationship between quintiles of the "low calcium & sugars" dietary pattern and FVC by asthma status

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy; error bars represent 95%CIs

7.3.4.6 *"High starch & lycopene" dietary pattern*

Relationships between the "high starch & lycopene" dietary pattern and FEV_1 and FVC were modified by smoking status ($p_{interaction}$=0.032 and 0.036 respectively); however, there was moderate evidence these relationships were non-linear ($p_{linearity}$=0.048 and 0.024 respectively; Table 7.15). There was a negative linear association with both outcomes in never smokers (Figures 7.10 and 7.11). Never smokers in the highest pattern score quintile had a mean FEV_1 and FVC 0.16 L and 0.15 L less than those in quintile 1 (FEV_1: 95%CI -0.30, -0.01, p_{trend}=0.019; FVC: 95%CI -0.31, 0.008, p_{trend}=0.044; Table 7.15). There was no meaningful shape in the associations for former or current smokers for either FEV_1 or FVC (Figures 7.10 and 7.11).

There were no interactions observed between quintiles of the "high starch & lycopene" dietary pattern and any of the other potential effect modifiers tested (Appendix 7, Tables A26-A29).

7.3.4.7 *"High vitamin C, low calcium" dietary pattern*

The relationship between the "high vitamin C, low calcium" dietary pattern and FVC was modified by sex ($p_{interaction}$=0.050). There was a negative association between this pattern and FVC in men only. Men in the highest pattern score quintile had a mean FVC 0.10 L less than those in the lowest quintile (95%CI -0.24, 0.03, p_{trend}=0.045; Table 7.16, Figure 7.12). There was no relationship observed between the "high vitamin C, low calcium" dietary pattern and FVC in women.

The relationship between the "high vitamin C, low calcium" dietary pattern and FEV_1/FVC was modified by asthma status ($p_{interaction}$=0.001); however, this relationship was non-linear ($p_{linearity}$=0.005) and there were no meaningful shapes in the results for any asthma category (Table 7.17, Figure 7.13).

Associations between the "high vitamin C, low calcium" dietary pattern and FEV_1 and FVC were also modified by atopy ($p_{interaction}$=0.029 and 0.041 respectively). In those without atopy, there was a negative association with FEV_1 with those in the highest quintile having a mean FEV_1 0.13 L less than those in quintile 1 (95%CI -0.26, 0.007, p_{trend}=0.048; Table 7.18). A similar trend was observed with FVC, however, the evidence for a trend across quintiles was weak (mean difference in FVC between quintile 5 and quintile 1=-0.14 L, 95%CI -0.29, 0.004, p_{trend}=0.078; Table 7.18). There was no relationship observed in those with atopy for FEV_1 or FVC.

There were no interactions observed between quintiles of the "high vitamin C, low calcium" dietary pattern and any of the other potential effect modifiers tested (Appendix 7, Tables A30-A31).

Table 7.15 - Adjusted associations between quintiles of the "high starch & lycopene" dietary pattern and lung function by smoking status

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.05 (-0.19, 0.09)	0.15 (0.02, 0.29)	0.41 (0.15, 0.66)	-0.08 (-0.24, 0.08)	0.16 (0.008, 0.31)	0.36 (0.08, 0.65)	0.43 (-1.56, 2.43)	1.02 (-0.87, 2.90)	4.56 (0.94, 8.18)
Q3	-0.06 (-0.20, 0.08)	0.06 (-0.08, 0.20)	0.14 (-0.13, 0.40)	-0.04 (-0.19, 0.12)	-0.02 (-0.17, 0.14)	-0.03 (-0.32, 0.26)	-0.47 (-2.43, 1.48)	1.31 (-0.66, 3.29)	4.20 (0.50, 7.90)
Q4	-0.12 (-0.26, 0.02)	0.02 (-0.11, 0.16)	0.37 (0.11, 0.64)	-0.14 (-0.30, 0.01)	-0.06 (-0.21, 0.09)	0.25 (-0.05, 0.54)	-0.12 (-2.10, 1.87)	1.20 (-0.71, 3.10)	5.45 (1.74, 9.17)
Q5	-0.16 (-0.30, -0.01)	0.05 (-0.08, 0.19)	0.20 (-0.06, 0.47)	-0.15 (-0.31, 0.008)	0.0006 (-0.15, 0.15)	0.18 (-0.12, 0.47)	-0.63 (-2.65, 1.39)	0.89 (-0.99, 2.77)	2.63 (-1.08, 6.34)
p_{trend}	0.019	0.81	0.23	0.044	0.43	0.45	0.44	0.31	0.18
$p_{interaction}$	0.032			0.036			0.33		
$p_{linearity}$	0.048			0.024			0.30		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

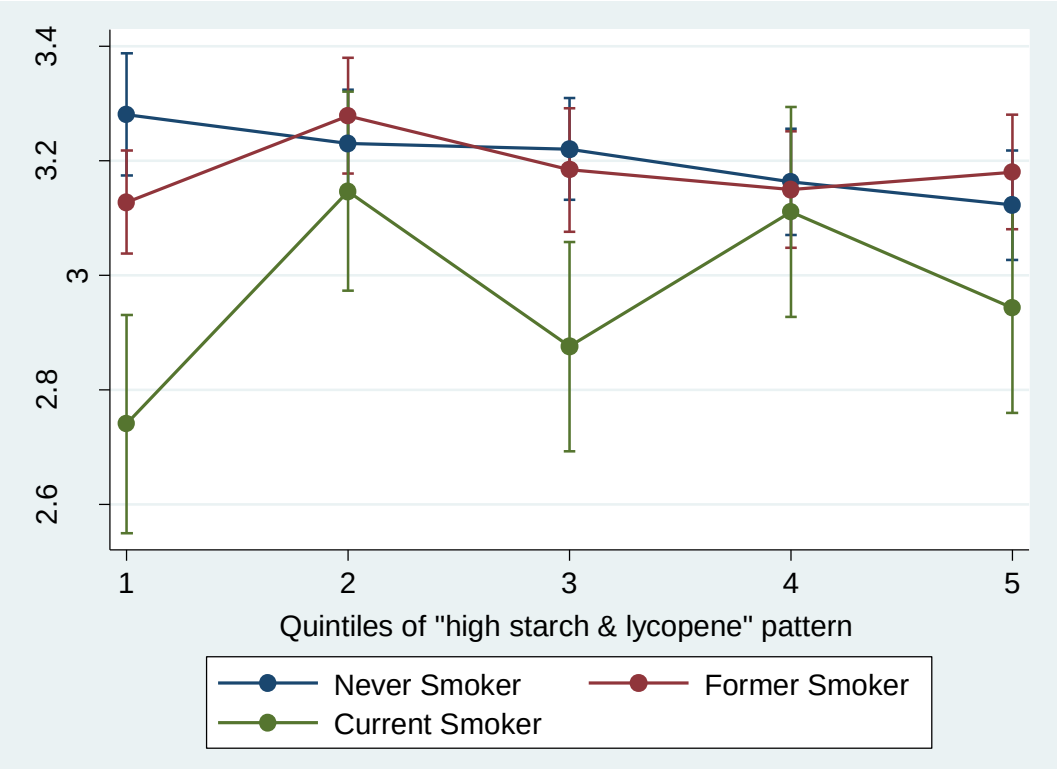


Figure 7.10 - Relationship between quintiles of the "high starch & lycopene" dietary pattern and FEV_1 by smoking status

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy; error bars represent 95%CIs

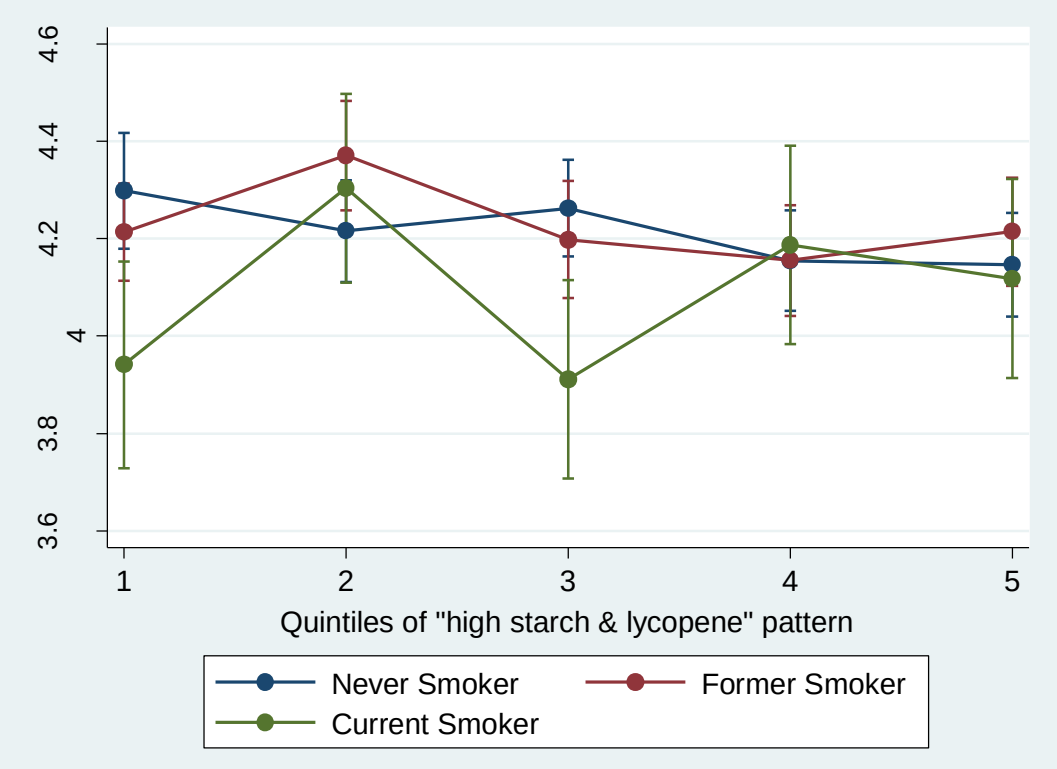


Figure 7.11 - Relationship between quintiles of the "high starch & lycopene" dietary pattern and FVC by smoking status

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy; error bars represent 95%CIs

Table 7.16 - Adjusted associations between quintiles of the "high vitamin C, low calcium" dietary pattern and lung function by sex

Pattern quintiles	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Female (n=573)	Male (n=610)	Female (n=573)	Male (n=610)	Female (n=573)	Male (n=610)
Q1	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.02 (-0.15, 0.11)	-0.01 (-0.14, 0.12)	-0.06 (-0.20, 0.08)	-0.01 (-0.16, 0.14)	1.01 (-0.75, 2.78)	-0.09 (-1.94, 1.76)
Q3	0.004 (-0.12, 0.13)	-0.14 (-0.28, -0.009)	0.02 (-0.12, 0.16)	-0.10 (-0.25, 0.05)	-0.11 (-1.86, 1.65)	-1.81 (-3.67, 0.05)
Q4	0.02 (-0.11, 0.15)	-0.17 (-0.30, -0.04)	0.05 (-0.09, 0.19)	-0.19 (-0.33, -0.04)	-0.31 (-2.11, 1.49)	-1.02 (-2.85, 0.82)
Q5	-0.06 (-0.20, 0.08)	-0.12 (-0.24, 0.005)	-0.06 (-0.21, 0.10)	-0.10 (-0.24, 0.03)	0.02 (-1.89, 1.94)	-0.94 (-2.67, 0.80)
p_{trend}	0.60	0.017	0.92	0.045	0.66	0.23
$p_{interaction}$	0.13		0.050		0.76	
$p_{linearity}$	0.37		0.23		0.42	

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

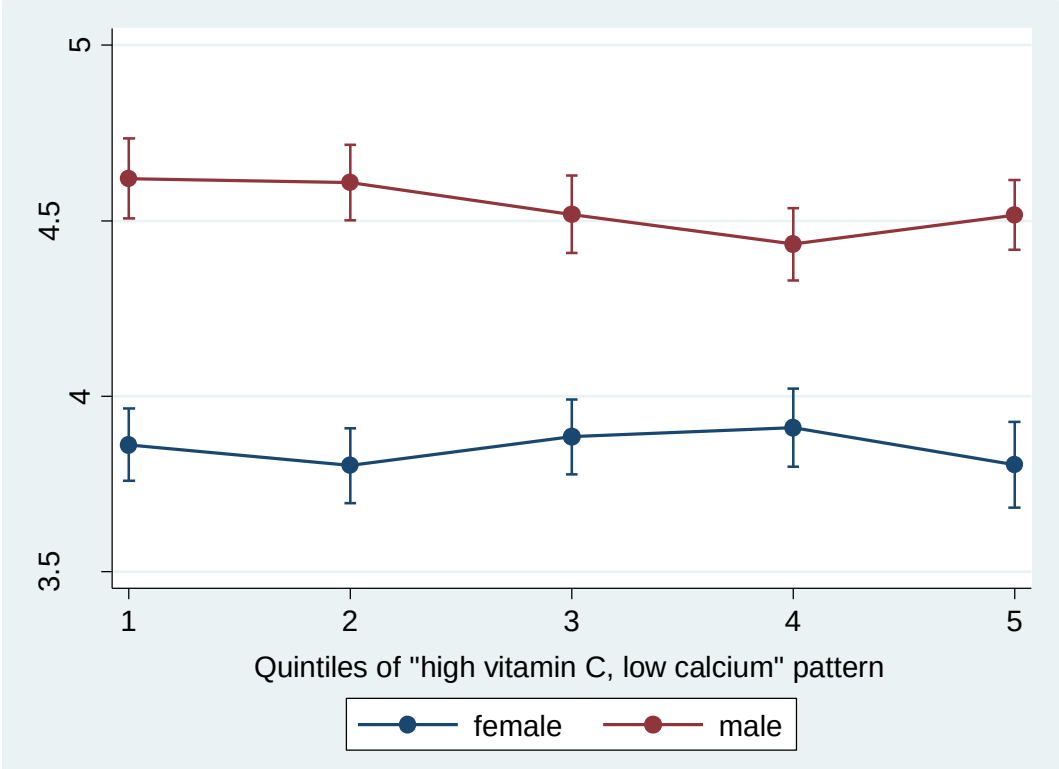


Figure 7.12 - Relationship between quintiles of the "high vitamin C, low calcium" dietary pattern and FVC by sex

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy; error bars represent 95%CIs

Table 7.17 - Adjusted associations between quintiles of the "high vitamin C, low calcium" dietary pattern and lung function by asthma status

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.02 (-0.12, 0.08)	0.04 (-0.28, 0.35)	-0.03 (-0.31, 0.25)	-0.04 (-0.15, 0.08)	0.01 (-0.34, 0.36)	-0.08 (-0.40, 0.23)	0.41 (-1.00, 1.83)	1.34 (-3.01, 5.69)	0.84 (-3.07, 4.74)
Q3	-0.07 (-0.17, 0.03)	0.10 (-0.21, 0.41)	-0.17 (-0.43, 0.10)	-0.05 (-0.16, 0.07)	0.14 (-0.20, 0.49)	-0.08 (-0.38, 0.21)	-0.61 (-2.04, 0.81)	-0.47 (-4.75, 3.81)	-3.41 (-7.08, 0.25)
Q4	-0.04 (-0.14, 0.07)	-0.007 (-0.29, 0.28)	-0.45 (-0.73, -0.17)	-0.05 (-0.16, 0.07)	-0.06 (-0.38, 0.25)	-0.24 (-0.56, 0.07)	0.21 (-1.23, 1.66)	0.93 (-2.99, 4.86)	-8.89 (-12.83, -4.95)
Q5	-0.10 (-0.20, 0.0003)	0.19 (-0.11, 0.48)	-0.18 (-0.47, 0.10)	-0.11 (-0.23, 0.0009)	0.25 (-0.08, 0.58)	-0.06 (-0.38, 0.25)	-0.28 (-1.69, 1.13)	0.81 (-3.32, 4.93)	-2.49 (-6.42, 1.44)
p_{trend}	0.048	0.29	0.034	0.056	0.22	0.49	0.62	0.74	0.007
$p_{interaction}$	0.083			0.39			0.001		
$p_{linearity}$	0.38			0.80			0.005		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

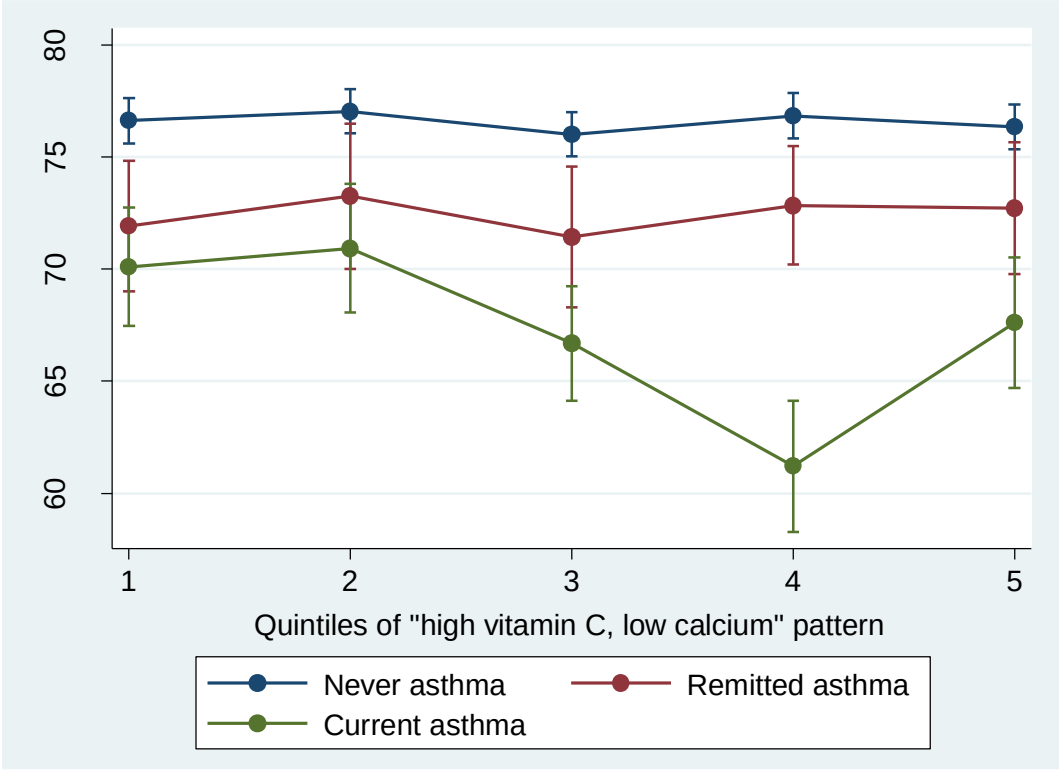


Figure 7.13 - Relationship between quintiles of the "high vitamin C, low calcium" dietary pattern and FEV_1/FVC by asthma status

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy; error bars represent 95%CIs

Table 7.18 - Adjusted associations between quintiles of the "high vitamin C, low calcium" dietary pattern and lung function by atopy

Pattern quintiles	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Non-atopic (n=562)	Atopic (n=621)	Non-atopic (n=562)	Atopic (n=621)	Non-atopic (n=562)	Atopic (n=621)
Q1	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.07 (-0.06, 0.19)	-0.10 (-0.23, 0.03)	0.02 (-0.12, 0.17)	-0.10 (-0.24, 0.05)	1.23 (-0.56, 3.02)	-0.27 (-2.08, 1.55)
Q3	-0.06 (-0.19, 0.07)	-0.07 (-0.20, 0.06)	-0.04 (-0.18, 0.11)	-0.04 (-0.18, 0.10)	-1.26 (-3.08, 0.57)	-0.64 (-2.43, 1.15)
Q4	0.02 (-0.11, 0.15)	-0.16 (-0.29, -0.03)	0.03 (-0.12, 0.17)	-0.15 (-0.30, -0.01)	0.04 (-1.81, 1.88)	-1.24 (-3.04, 0.55)
Q5	-0.13 (-0.26, 0.007)	-0.06 (-0.19, 0.06)	-0.14 (-0.29, 0.004)	-0.03 (-0.17, 0.11)	-0.37 (-2.23, 1.49)	-0.56 (-2.31, 1.18)
p_{trend}	0.048	0.29	0.078	0.63	0.41	0.38
$p_{interaction}$	0.029		0.041		0.42	
$p_{linearity}$	0.086		0.16		0.22	

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

7.3.4.8 *"High alpha-carotene, low lycopene" dietary pattern*

The association between the "high alpha-carotene, low lycopene" dietary pattern and FEV_1 was modified by BMI category ($p_{interaction}$=0.032; Table 7.19), however this relationship was non-linear ($p_{linearity}$=0.015). Those in quintile 3 of the score had the highest mean FEV_1 in both the healthy weight and obese BMI categories (Figure 7.14). In those of a healthy weight, the difference in mean FEV_1 between the quintiles with the highest and lowest mean FEV_1 values (quintiles 3 and 5 respectively) was 0.29 L (95%CI 0.13, 0.45). In obese subjects, the mean difference between the quintiles with the highest and lowest mean FEV_1 values (quintiles 3 and 4 respectively) was 0.25 L (95%CI 0.05, 0.45). A similar trend was observed for FVC; however, evidence for an interaction was weak ($p_{interaction}$=0.084). The relationship between the "high alpha-carotene, low lycopene" dietary pattern and FEV_1/FVC was also modified by BMI category ($p_{interaction}$=0.001; Table 7.19) with evidence of non-linear relationships ($p_{linearity}$=0.007); however, there were no meaningful shapes in the results for any BMI category (Figure 7.15).

There were no interactions observed between quintiles of the "high vitamin C, low calcium" dietary pattern and any of the other potential effect modifiers tested (Appendix 7, Tables A30-A31).

Table 7.19 - Adjusted associations between quintiles of the "high alpha-carotene, low lycopene" dietary pattern and lung function by BMI category

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.05 (-0.21, 0.12)	0.10 (-0.03, 0.23)	-0.11 (-0.29, 0.08)	-0.006 (-0.19, 0.18)	0.16 (0.02, 0.31)	-0.17 (-0.38, 0.03)	-0.68 (-3.00, 1.63)	-0.63 (-2.49, 1.22)	0.68 (-1.91, 3.26)
Q3	0.16 (-0.006, 0.32)	0.08 (-0.05, 0.21)	0.03 (-0.16, 0.22)	0.15 (-0.04, 0.33)	0.19 (0.04, 0.34)	0.09 (-0.12, 0.30)	0.68 (-1.61, 2.98)	-1.70 (-3.56, 0.16)	-0.80 (-3.39, 1.79)
Q4	-0.08 (-0.24, 0.08)	0.11 (-0.02, 0.24)	-0.22 (-0.41, -0.03)	-0.10 (-0.28, 0.09)	0.21 (0.06, 0.36)	-0.07 (-0.28, 0.15)	-0.55 (-2.80, 1.71)	-1.09 (-2.95, 0.76)	-4.06 (-6.74, -1.38)
Q5	-0.13 (-0.29, 0.03)	0.06 (-0.08, 0.20)	-0.09 (-0.27, 0.09)	-0.02 (-0.20, 0.15)	0.08 (-0.07, 0.24)	-0.10 (-0.31, 0.10)	-3.33 (-5.52, -1.14)	-0.17 (-2.13, 1.79)	-0.45 (-2.98, 2.07)
p_{trend}	0.063	0.40	0.22	0.53	0.22	0.54	0.003	0.72	0.17
$p_{interaction}$	0.032			0.084			0.001		
$p_{linearity}$	0.015			0.008			0.007		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

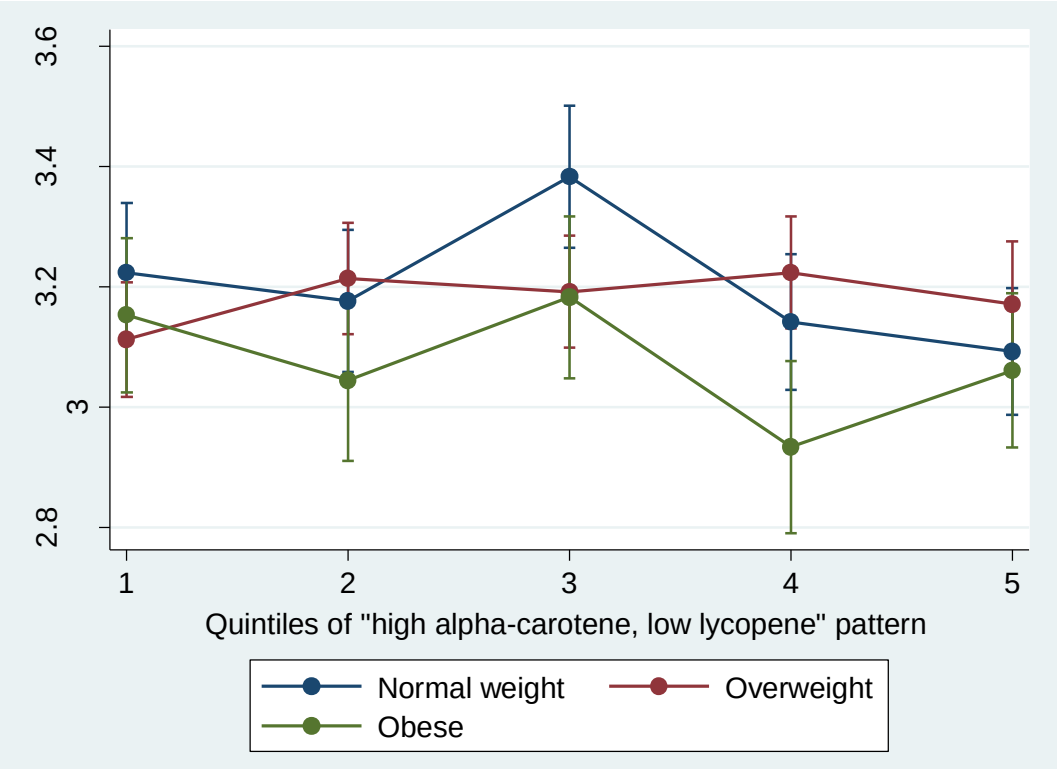


Figure 7.14 - Relationship between quintiles of the "high alpha-carotene, low lycopene" dietary pattern and FEV_1/FVC by BMI category

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy; error bars represent 95%CIs

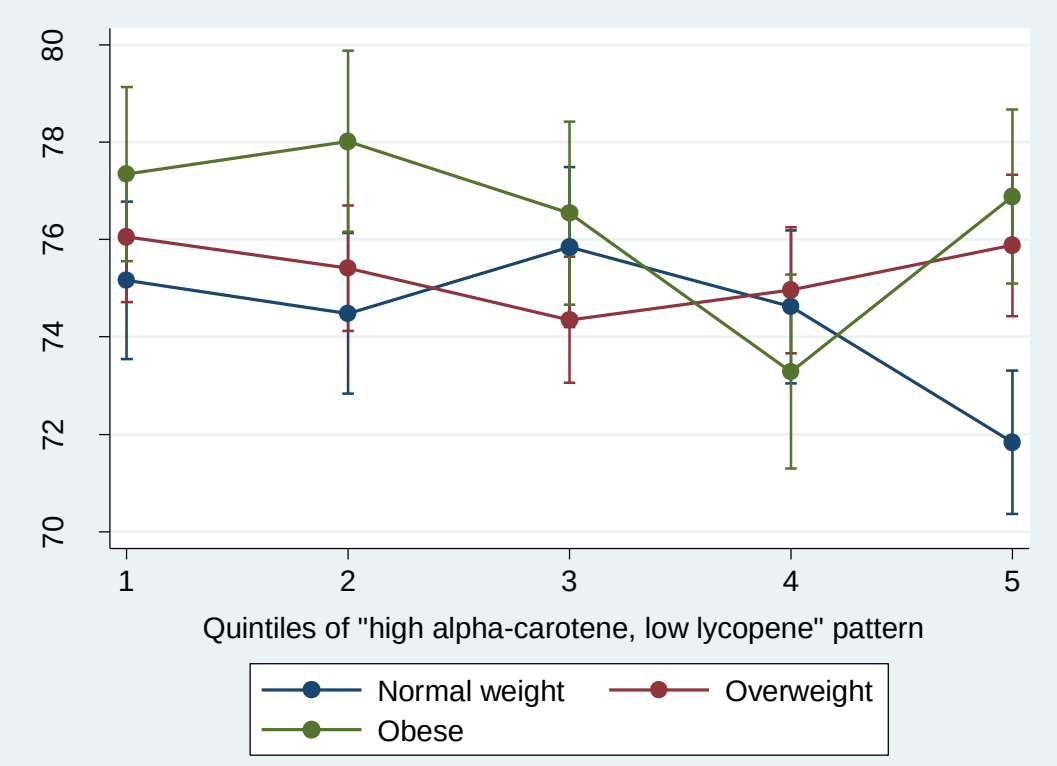


Figure 7.15 - Relationship between quintiles of the "high alpha-carotene, low lycopene" dietary pattern and FEV_1/FVC by BMI category

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy; error bars represent 95%CIs

7.3.5 Associations between dietary patterns and change in FEV_1 during the methacholine challenge

In the univariate analyses, there were no relationships observed between any of the dietary patterns and change in FEV_1 during the methacholine challenge (Table 7.20 and Appendix 7, Table A36). After adjusting for confounders, the relationship between quintiles of the "low calcium and sugars" dietary pattern and change in FEV_1 formed a u-shaped curve ($p_{linearity}$=0.003). During the challenge, those in quintile 3 had a mean fall in FEV_1 0.03 L greater per mg methacholine compared to those in quintile 5 (95%CI 0.02, 0.06) or those in quintile 1 (95%CI 0.05, 0.008, Table 7.20). No associations were observed between the other dietary patterns and change in FEV_1 during the methacholine challenge (Table 7.20 and Appendix 7, Table A36).

Table 7.20 - Unadjusted and adjusted associations between quintiles for patterns 5-8 and change in FEV_1 during methacholine challenge

Pattern quintiles	Change in FEV_1 (L) per mg Methacholine for each dietary pattern							
	Low calcium and sugars pattern		High starch and lycopene pattern		High vitamin C, low calcium pattern		High alpha-carotene, low lycopene pattern	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.004 (-0.03, 0.02)	-0.003 (-0.03, 0.02)	0.007 (-0.02, 0.03)	0.02 (-0.006, 0.04)	-0.01 (-0.03, 0.01)	-0.01, (-0.04, 0.009)	-0.02 (-0.04, 0.006)	-0.01 (-0.04, 0.008)
Q3	-0.03 (-0.05, -0.006)	-0.03 (-0.05, -0.008)	-0.006 (-0.03, 0.02)	-0.003 (-0.03, 0.02)	-0.02 (-0.04, 0.008)	-0.02 (-0.04, 0.007)	-0.01 (-0.03, 0.01)	-0.01 (-0.03, 0.01)
Q4	-0.01 (-0.04, 0.01)	-0.009 (-0.03, 0.01)	-0.007 (-0.03, 0.02)	-0.005 (-0.03, 0.02)	-0.005 (-0.03, 0.02)	-0.01 (-0.03, 0.01)	0.002 (-0.02, 0.02)	0.005 (-0.02, 0.03)
Q5	-0.003 (-0.03, 0.02)	0.003 (-0.02, 0.03)	0.004 (-0.02, 0.03)	0.0002 (-0.02, 0.02)	-0.01 (-0.03, 0.01)	-0.01 (-0.04, 0.009)	0.0001 (-0.02, 0.02)	0.006 (-0.02, 0.03)
p_{trend}	0.59	0.95	0.88	0.52	0.52	0.34	0.54	0.26
$p_{linearity}$	0.019*	0.003^	0.056	0.25	0.56	0.94	0.47	0.21

β-coefficient (95%CI) presented for each quintile

Model 1: Unadjusted analysis

Model 2: Adjusted for sex, age, BMI category, asthma status, smoking status, atopy and energy intake

*p-value for likelihood ratio test comparing models with and without the dietary pattern as a predictor of change in FEV_1= 0.084

^p-value for likelihood ratio test comparing models with and without the dietary pattern as a predictor of change in FEV_1 = 0.025

7.3.6 Modification of the associations between dietary patterns and change in FEV_1 during the methacholine challenge

7.3.6.1 *"High potassium &magnesium" dietary pattern*

There were no interactions observed between quintiles of the "high potassium & magnesium" dietary pattern and any of the potential effect modifiers tested (Appendix 7, Tables A37-A38).

7.3.6.2 *"High protein & zinc" dietary pattern*

The relationship between the "high protein & zinc" dietary pattern and change in FEV_1 during the methacholine challenge was modified by sex ($p_{interaction}$=0.008) and there was evidence the relationship was non-linear ($p_{linearity}$=0.002; Appendix 7, Table A39). However, there was no meaningful shape in the results for either sex.

The relationship between the "high protein & zinc" dietary pattern and change in FEV_1 during the methacholine challenge was modified by asthma status ($p_{interaction}$=0.021) with evidence the relationship was non-linear. In those with current asthma, there was a plateau between quintiles 1 and 2, then an increase between quintiles 2 and 3, followed by another plateau between quintiles 3 and 5. That is, in those with current asthma, the mean fall in FEV_1 for those in quintiles 3 to 5 was less than those in quintiles 1 and 2 by up to 0.16 L per mg methacholine (95%CI 0.04, 0.28; Table 7.21, Figure 7.16). There were no meaningful shapes in the results for the remitted asthma and never asthma groups.

There were no interactions observed between quintiles of the "high protein & zinc" dietary pattern and the other potential effect modifiers tested (Appendix 7, Tables A39-A40).

Table 7.21 - Adjusted associations of quintiles of the "high protein & zinc" dietary pattern with change in FEV_1 during the methacholine challenge by asthma status

Pattern quintiles	Asthma status		
	Never asthma (n=846)	Remitted asthma (n=90)	Current asthma (n=94)
Q1	Ref	Ref	Ref
Q2	-0.02 (-0.04, 0.007)	0.07 (-0.03, 0.16)	0.01 (-0.12, 0.14)
Q3	-0.001 (-0.03, 0.02)	0.03 (-0.06, 0.12)	0.15 (0.02, 0.28)
Q4	-0.009 (-0.03, 0.02)	0.04 (-0.05, 0.13)	0.11 (-0.02, 0.24)
Q5	0.007 (-0.02, 0.03)	0.01 (-0.08, 0.10)	0.16 (0.04, 0.28)
p_{trend}	0.51	0.82	0.001
$p_{interaction}$	0.021		
$p_{linearity}$	0.030		

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

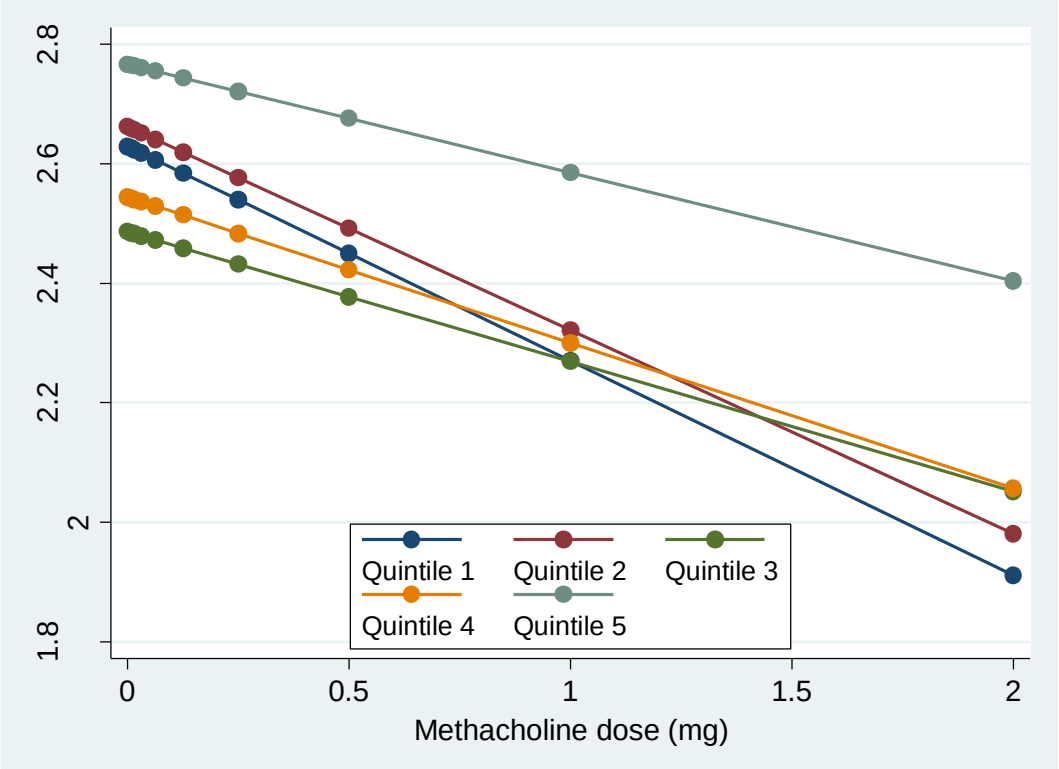


Figure 7.16 - Relationship between quintiles of the "high protein & zinc" dietary pattern and change in mean FEV_1 during the methacholine challenge in those with current asthma

Adjusted for age, sex, smoking status, energy intake, BMI category, asthma status and atopy

7.3.6.3 *"High PUFAs & vitamin E" dietary pattern*

The relationship between the "high PUFAs & vitamin E" dietary pattern and change in FEV_1 during the methacholine challenge was modified by asthma status ($p_{interaction}$=0.032). In those with current asthma, there was a positive association between quintiles of the "high PUFAs & vitamin E" dietary pattern and change in FEV_1 with those in the highest quintile having a mean fall in FEV_1 0.16 L less per mg methacholine compared to those in the lowest quintile (95%CI 0.04, 0.27, p_{trend}=0.001; Table 7.22, Figure 7.17). There was no association observed in the remitted asthma and never asthma groups.

There were no interactions observed between quintiles of the "high PUFAs & vitamin E" dietary pattern and the other potential effect modifiers tested (Appendix 7, Tables A41-A42).

7.3.6.4 *"High β-cryptoxanthin & vitamin C" dietary pattern*

There were no interactions observed between quintiles of the "high β-cryptoxanthin & vitamin C" dietary pattern and any of the potential effect modifiers tested (Appendix 7, Tables A43-A44).

7.3.6.5 *"Low calcium and sugars" dietary pattern*

The association between the "low calcium & sugars" dietary pattern and change in FEV_1 during the methacholine challenge was modified by asthma status ($p_{interaction}$=0.043) and the relationship is non-linear ($p_{linearity}$=0.014; Table 7.23). In the current asthma group there was a sharp increase in mean change in FEV_1 between quintiles 1 and 2, a plateau between quintiles 2 and 3, another smaller increase between quintiles 3 and 4, followed by another plateau between quintiles 4 and 5. In those with current asthma, the mean fall in FEV_1 in those in quintile 4 of the "low calcium & sugars" pattern score was 0.19 L less per mg methacholine compared to those in quintile 1 (95%CI 0.08, 0.31; Table 7.23, Figure 7.18). There was also a u-shaped relationship between the "low calcium & sugars" dietary pattern and change in FEV_1 in the never asthma group, with those in quintile 3 of score having a mean change in FEV_1 0.03 L less per mg methacholine compared to those in quintile 1 (Table 7.23). There was no relationship observed in the remitted asthma group.

There were no interactions observed between quintiles of the "low calcium & sugars" dietary pattern and the other potential effect modifiers tested (Appendix 7, Tables A45-A46).

Table 7.22 - Adjusted associations of quintiles of the "high PUFAs & vitamin E" dietary pattern with change in FEV_1 during the methacholine challenge by asthma status

Pattern quintiles	Asthma status		
	Never asthma (n=846)	Remitted asthma (n=90)	Current asthma (n=94)
Q1	Ref	Ref	Ref
Q2	-0.004 (-0.03, 0.02)	0.01 (-0.06, 0.09)	0.09 (-0.02, 0.20)
Q3	0.005 (-0.02, 0.03)	-0.005 (-0.10, 0.09)	0.05 (-0.05, 0.16)
Q4	-0.002 (-0.03, 0.02)	0.03 (-0.04, 0.11)	0.18 (0.08, 0.28)
Q5	0.001 (-0.02, 0.03)	0.02 (-0.06, 0.09)	0.16 (0.04, 0.27)
p_{trend}	0.86	0.55	0.001
$p_{interaction}$	0.032		
$p_{linearity}$	0.31		

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

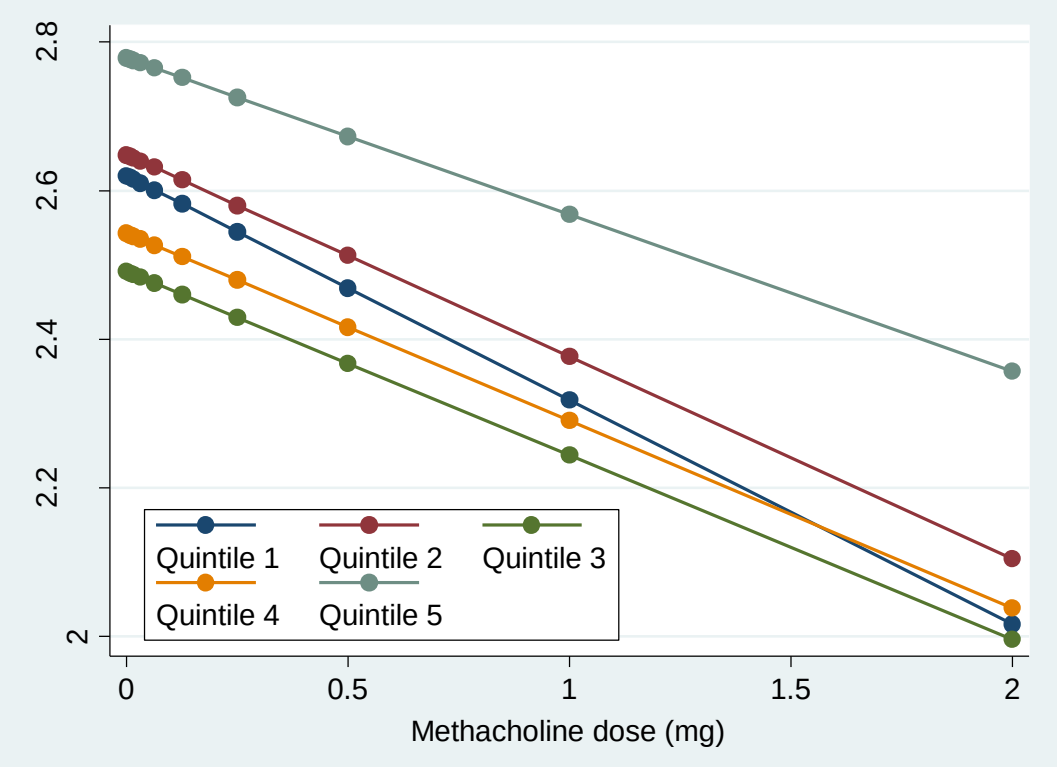


Figure 7.17 – Relationship between quintiles of the "high PUFAs & vitamin E" dietary pattern and change in mean FEV_1 during the methacholine challenge in those with current asthma

Adjusted for age, sex, smoking status, energy intake, BMI category, asthma status and atopy

Table 7.23 - Adjusted associations of quintiles of the "low calcium & sugars" dietary pattern with change in FEV_1 during the methacholine challenge by asthma status

Pattern quintiles	Asthma status		
	Never asthma (n=846)	Remitted asthma (n=90)	Current asthma (n=94)
Q1	Ref	Ref	Ref
Q2	-0.006 (-0.03, 0.02)	-0.02 (-0.10, 0.06)	0.13 (0.009, 0.24)
Q3	-0.03 (-0.06, -0.009)	-0.08 (-0.16, 0.008)	0.12 (-0.006, 0.24)
Q4	-0.02 (-0.04, 0.008)	-0.03 (-0.12, 0.05)	0.19 (0.08, 0.31)
Q5	-0.006 (-0.03, 0.02)	0.006 (-0.07, 0.08)	0.18 (0.07, 0.29)
p_{trend}	0.39	0.87	0.002
$p_{interaction}$	0.043		
$p_{linearity}$	0.014		

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

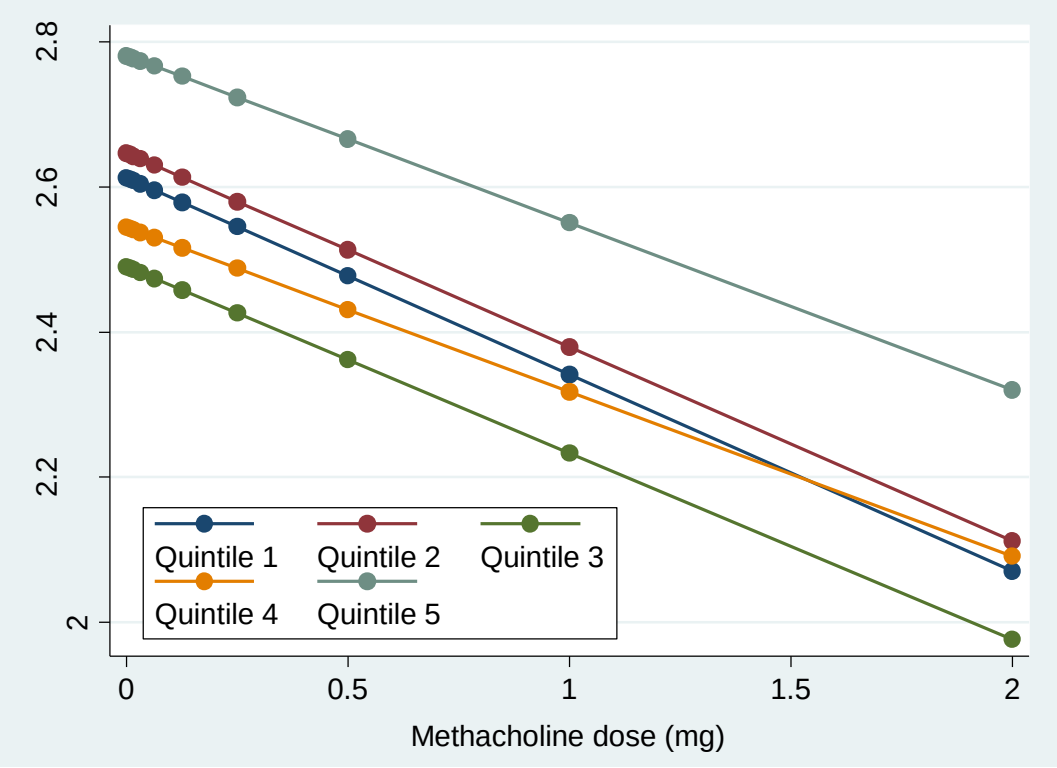


Figure 7.18 – Relationship between quintiles of the "low calcium & sugars" dietary pattern and change in mean FEV_1 during the methacholine challenge in those with current asthma

Adjusted for age, sex, smoking status, energy intake, BMI category, asthma status and atopy

7.3.6.6 *"High starch & lycopene" dietary pattern*

There were no interactions observed between quintiles of the "high starch & lycopene" dietary pattern and any of the potential effect modifiers tested (Appendix 7, Tables A47-A48).

7.3.6.7 *"High vitamin C, low calcium" dietary pattern*

There was evidence the relationship between the "high vitamin C, low calcium" dietary pattern and change in FEV_1 during the methacholine challenge was modified by atopy ($p_{interaction}$=0.021); however, there was little evidence for a trend in either of the atopy groups (p_{trend}=0.77 and 0.10 for non-atopic and atopic groups respectively; Appendix 7, Table A50).

There were no interactions observed between quintiles of the "high vitamin C, low calcium" dietary pattern and any of the other potential effect modifiers tested (Appendix 7, Tables A49-A50).

7.3.6.8 *"High α-carotene, low lycopene" dietary pattern*

The relationship between the "high α-carotene, low lycopene" dietary pattern and change in FEV_1 during the methacholine challenge was modified by asthma status ($p_{interaction}$=0.015) with a positive association observed in the current asthma group (p_{trend}=0.001). Those with current asthma in quintile 5 of this dietary pattern score had a mean fall in FEV_1 0.13 L less per mg methacholine compared to those in quintile 1 (95%CI 0.03, 0.22; Table 7.24, Figure 7.19). There was no relationship observed in the remitted and never asthma groups.

There were no interactions observed between quintiles of the "high α-carotene, low lycopene" dietary pattern and any of the other potential effect modifiers tested (Appendix 7, Tables A51-A52).

Table 7.24- Adjusted associations of quintiles of the "high α-carotene, low lycopene" dietary pattern with change in FEV_1 during the methacholine challenge by asthma status

Pattern quintiles	Asthma status		
	Never asthma (n=846)	Remitted asthma (n=90)	Current asthma (n=94)
Q1	Ref	Ref	Ref
Q2	-0.02 (-0.04, 0.005)	0.008 (-0.07, 0.09)	0.0002 (-0.10, 0.10)
Q3	-0.02 (-0.04, 0.007)	0.03 (-0.05, 0.11)	0.05 (-0.04, 0.14)
Q4	0.002 (-0.02, 0.03)	-0.06 (-0.15, 0.03)	0.13 (0.03, 0.22)
Q5	-0.004 (-0.03, 0.02)	0.04 (-0.04, 0.12)	0.13 (0.03, 0.22)
p_{trend}	0.79	0.59	0.001
$p_{interaction}$	0.015		
$p_{linearity}$	0.14		

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

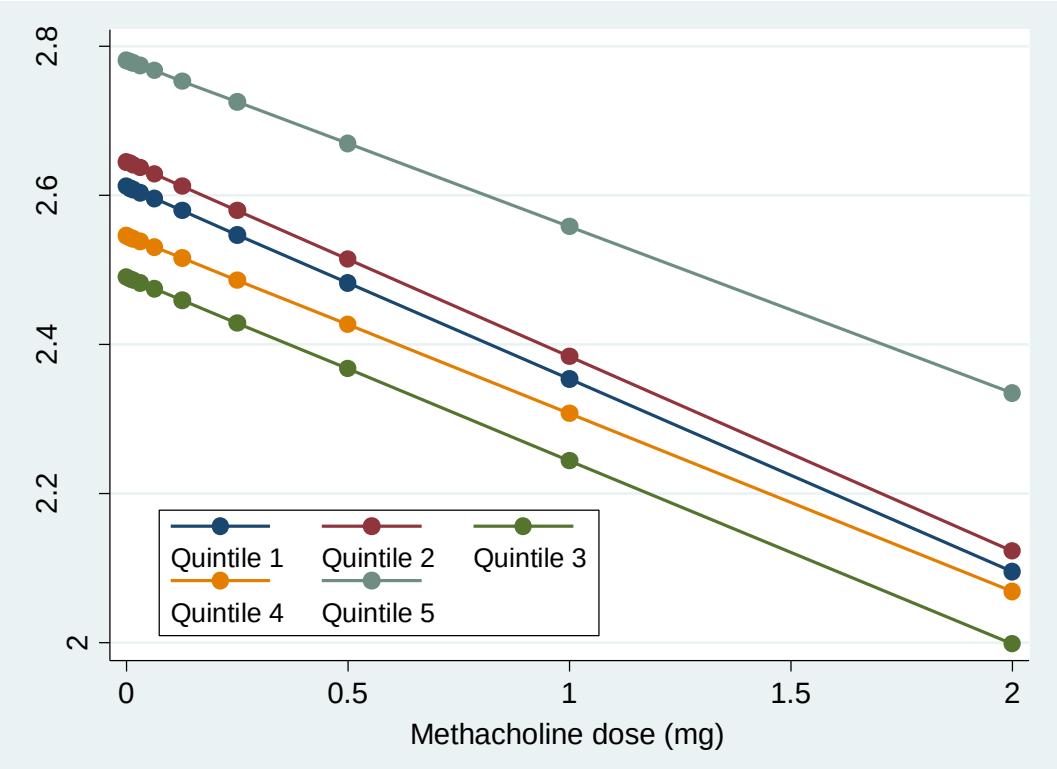


Figure 7.19 - Relationship between quintiles of the "high α-carotene, low lycopene" dietary pattern and change in mean FEV_1 during the methacholine challenge in those with current asthma

Adjusted for age, sex, smoking status, energy intake, BMI category, asthma status and atopy

7.4 Discussion

This is the first study that I am aware of (following an extensive literature search) to assess asthma status as an effect modifier of relationships between dietary patterns defined using PCA, lung function and BR. I found several relationships existed in those with asthma or a history of asthma only. My findings suggest, in those with asthma or a history of asthma, higher intakes of the "high potassium & magnesium" and "low calcium & sugars" patterns are associated with better lung function; and higher intakes of the "high protein & zinc", "high PUFAs & vitamin E", "low calcium & sugars" and "high α-carotene, low lycopene" patterns are associated with reduced BR. Some of the mean differences observed here indicate clinically important differences, with effects potentially on par with medication used to treat asthma and COPD. Should these relationships be proven to be causal, a diet modification program should be added as a treatment option, particularly for patients with co-morbidities, those who experience side-effects from medication, or those who prefer a "natural" alternative to medication. Further research is needed to demonstrate causality, ideally with an RCT study design.

Higher intakes of the "high potassium & magnesium" pattern were associated with better FEV_1 in current asthmatics and there was a similar trend observed for FVC. This pattern was characterised by higher intakes of fibre, folate, magnesium, potassium and iron, and lower intakes of fat. This is indicative of a diet high in fruits, vegetables and wholegrains, typically referred to as a prudent diet. Such a diet is rich in antioxidants and promotes a healthy, diverse gut microbiota, both of which support the immune system. A well-functioning immune system may reduce frequency and severity of respiratory infections that can trigger asthma exacerbations and, in turn, lung function decline. Previous studies of dietary patterns and lung function have found mixed results with prudent or similar dietary patterns (discussed in Chapter 3). This inconsistency in findings may be at least partly explained by the modifying effect of asthma which has not been assessed previously. Assessing the study population as a whole when there is only an association in a sub-population will dilute the effect.

There was a positive relationship between the "low calcium & sugars" pattern and FEV_1 in those with remitted asthma. Similar trends were also observed for FEV_1 and FVC in the current asthma group. Higher intakes of this pattern were also associated with reduced BR in those with current asthma. The "low calcium & sugars" pattern was characterised by higher intakes of lutein zeaxanthin and lower intakes of carbohydrates, sugars and calcium. This is indicative of a diet rich in vegetables and low in sugar and dairy products. A diet high in vegetables is rich in antioxidants which support the immune system (lutein and zeaxanthin are antioxidants). Research also suggests higher intakes of sugar are associated with increased inflammation (318, 338-340). Asthma and COPD are both associated with increased airway inflammation. Therefore, low sugar intake may reduce airway inflammation, particularly in those with asthma or a history of asthma who are prone to developing

such inflammation. The prudent dietary pattern is often characterised by lower intakes of sugar, and its relationship with lung function is inconclusive. Two studies have examined a "high carbohydrate/refined foods" dietary pattern and lung function. Steinmann and colleagues observed no relationship between a "high carbohydrate" pattern, high in sweet spreads, bread, dessert and potatoes, and FEV_1 and FEV_1/FVC in a study population of Swiss adults aged 37-81 years (175). However, McKeever et al. found a trend for a positive relationship between a "refined foods" pattern, high in sugar-sweetened beverages, white bread and other processed foods, and lung function decline over 5 years (p_{trend}=0.11) in a longitudinal study of 2,911 Dutch adults (mean (SD) age=45.0 ± 9.5years) (170). Neither of these studies assessed asthma status as an effect modifier. In my study, there was no relationship observed between the "low calcium & sugars" pattern and lung function or BR in the overall population, which is similar to the results in these studies.

The finding that higher intakes of the "high protein & zinc" pattern was associated with reduced BR in those with current asthma was unexpected. This dietary pattern was characterised by higher intakes of protein, cholesterol and zinc which is indicative of a diet high in animal products such as meat and eggs. There is some biological plausibility for this result; zinc is important for the functioning of the immune system. Therefore, inadequate zinc intake may increase the occurrence of respiratory infections, increasing exacerbations and airway inflammation. There has only been one previous study on dietary patterns and BR. This cross-sectional study of German adults found no association between a "meat and potato" dietary pattern and BR (172). Whilst evidence has been mixed, overall research suggests a "traditional" or "western" diet high in red and processed meats may be associated with poorer lung function (discussed in chapter 3). The contrast of my results with these studies may be due to differences in the dietary patterns obtained or differences in amounts and types of animal products consumed between study populations (e.g. chicken, eggs or red meat). Alternatively, this may be a chance finding.

High intake of the "high PUFAs & vitamin E" dietary pattern was associated with lower BR in those with current asthma. This association may be due to the anti-inflammatory effects of n-3 PUFAs or the antioxidant properties of vitamin E. My results are consistent with those from a couple of small intervention studies that observed a beneficial effect of n-3 PUFAs on BR in those with allergic asthma and elite athletes with exercise-induced asthma (260, 261). Adams et al also found higher total n-3 PUFAs and the n-3 PUFAs eicosapentaenoic acid and docosapentaenoic acid measured in serum phospholipids were associated with a reduced risk of BHR in a cross-sectional study of 642 South African adults living in a coastal fishing village (255). However, most observational and intervention studies have observed no relationship (251-253, 256). The contrasting findings may be due to differences in study populations, lack of assessment of asthma status as an effect modifier in population-based studies, and the use of the LMM here which should produce more accurate and reliable results. There is a lack of good quality evidence on the relationship between vitamin E and BR (discussed in Chapter 3). Further research is needed to determine if a relationship exists and, if

so, the nature of this relationship.

High intakes of the "high α-carotene, low lycopene" dietary pattern was also associated with lower BR in those with current asthma. This diet was high in saturated fat, α-carotene and β-carotene and low in lycopene and PUFAs, reflective of a diet high in animal products and vegetables but low in tomatoes, tomato products and dietary sources of PUFAs such as fish and margarine. No other studies have assessed the relationship between a similar dietary pattern and BR; however, α- and β-carotenes may provide protection against increased BR through their antioxidant properties. Again, further research is needed to confirm this result and the potential biological mechanism.

Overall, I observed numerous relationships between diet, lung function and BR in those with asthma or a history of asthma. There is a biological explanation why these relationships might exist in this population group only. Exacerbations or asthma attacks trigger structural changes in the airways known as airway remodelling. These changes cause further narrowing of the airways, increased airway inflammation and BR, and lung function decline (325, 326). Therefore, any dietary factors that can reduce the frequency or severity of exacerbations, minimise these structural changes or reduce airway inflammation are likely to have a greater effect in those with asthma or a history of asthma. As this group is at greater risk of poor lung function, lung function decline and increased BR, it is critical that further research is performed to conclusively determine and better understand these relationships.

Several of the patterns associated with lung function and BR in those with asthma or a history of asthma in this study suggest higher intakes of vegetables may be beneficial for lung health in middle-age. These results are consistent with my findings in Chapter 6 in which higher vegetable intake was associated with better FEV_1 and FVC in those with asthma. This research was performed in a separate study sample of middle-aged Australian adults which adds strength that these findings may reflect the true relationship between vegetable intake and lung health in asthma.

There were also other relationships observed in this study. In the overall study population, higher intakes of the "high PUFAs & vitamin E" dietary pattern were associated with a high FEV_1, potentially due to the anti-inflammatory and antioxidant effects of n-3 PUFAs and vitamin E respectively. Higher intakes of the "high α-carotene, low lycopene" dietary pattern were also associated with a lower FEV_1/FVC. However, the magnitude of the effect was small and not clinically important. In never smokers, higher intake of the "high starch & lycopene" dietary pattern was associated with a lower FEV_1 and FVC. Lastly, higher intakes of the "high vitamin C, low calcium" pattern were associated with lower FVC in men only and lower FEV_1 in those without atopy. Given the inconsistency in some of these findings across lung function outcomes and the lack of potential biologically plausible mechanisms, some of these results may be chance findings.

There were several non-linear relationships observed in this study. These results are difficult to

interpret given the exposure variables are dietary patterns comprised of many nutrient intakes. Therefore, a u-shaped relationship, for example, may result from 1 or more nutrients having a u-shaped relationship with the outcome, 1 or more nutrients having opposing relationships with the outcome, or a combination of different linear and non-linear relationships across the relevant nutrients. It is also dependent on the mean and range of the nutrient intakes in this sample. Therefore, the non-linear results that are difficult to interpret have not been discussed here.

This study has a number of strengths. It is the first study to assess the relationship between dietary patterns and BR using the more reliable and accurate LMM. It is also the first to assess effect modifiers of these relationships and the first to examine asthma status as an effect modifier of the relationship between dietary patterns and lung function. This is also the first study to investigate the relationship between dietary patterns and lung function using dietary patterns derived from nutrient intakes. Defining dietary patterns in this way has enabled the identification of eight dietary patterns explaining a very high proportion of the variance in nutrient intakes (82%) in this study population. I also used data from a validated semi-quantitative FFQ to assess diet and objective clinical tests to measure lung function and BR. However, there are also a few limitations that should be considered when interpreting the results. There have been multiple analyses performed in this study, increasing the likelihood of chance findings (i.e. observing by chance an association that does not exist). There may be a lack of power for some modification analyses where the sample size of particular sub-groups is small (ie. current and remitted asthma groups and current smokers). The results may be affected by random errors in the data (recall error in the dietary measures, measurement error in the outcome measures) which attenuate the result to the null, and residual confounding, the effects of which are difficult to predict. The study sample was drawn from areas of high socio-economic status in Melbourne and, therefore, the study sample and the dietary patterns deduced from the sample may not be representative of the general population. The low response rate for the clinic study may also be a source of selection bias. Unfortunately, the data was unavailable to compare baseline characteristics of those who completed the study and those who did not. However, this has been examined in a previously published article from this study population which found a significantly higher proportion of current smokers and women in the non-responders group (264). This group was also slightly older than those that attended the laboratory clinic. This confirms the possibility of selection bias due to the response rate. Lastly, this is a cross-sectional study and causal relationships cannot be determined.

In conclusion, the findings of this study strongly suggest a relationship between diet, lung function and BR in middle-aged and older adults with asthma. If these findings are confirmed, appropriate dietary changes could make a considerable difference to the lung health of this large population group at high-risk of further lung function decline and development of COPD.

Chapter 8 – Associations between the inflammatory potential of the diet, lung function and bronchial responsiveness

8.1 Introduction

In recent years, research investigating the relationship between diet and disease has evolved to assess the diet as a whole, rather than individual food or nutrient components of the diet. There are two main ways of assessing the overall diet – 1) using the *a posteriori* approach in which dietary patterns are determined statistically (as I have done in Chapter 7), or 2) using the *a priori* approach in which a diet quality index or diet score is employed, which measures how similar an individual's diet is to a specific, well-defined diet (see Chapter 3 for further discussion of these methods) (341). There are a number of different diet scores that have been developed, most of which assess how similar an individual's diet is to a healthy diet as described by the dietary guidelines (100, 180, 181, 342).

The Dietary Inflammatory Index (DII) is one such score, however, it is theoretically different to most other diet scores. The DII was developed by Shivappa and colleagues who reviewed all the literature published between 1950 and 2010 examining the relationship between diet and six inflammatory biomarkers – IL-1β, IL-4, IL-6, IL-10, TNF-α and CRP (110). From this review, forty-five dietary factors were identified as being associated with the concentrations of these biomarkers. A scoring algorithm measuring the overall inflammatory potential of the diet was then established, with consideration given to the strength of the evidence provided by different study designs (110).

The relationship between the DII and chronic respiratory disease is of particular interest because asthma and COPD, the most prevalent chronic lung diseases, are characterised by airway inflammation (38, 61). Studies have also found that some of the inflammatory biomarkers that were assessed in the development of the DII are associated with poorer lung function and/or accelerated lung function decline in adults (343, 344). Therefore, inflammation is a potential mechanism through which diet may affect lung function.

The DII is a newly developed score and as such there have only been a few studies investigating its relationship with lung function. Wood and colleagues conducted a small case-control study in Australian adults (109), and Han and colleagues performed two large cross-sectional studies, one in a general adult US population and one in Hispanic/Latino American adults (111, 345). All these studies found a more pro-inflammatory diet was associated with poorer lung function, although the results suggest the difference in lung function is small and may not be clinically important. However, these studies were limited by being small or being conducted across a broad and potentially heterogeneous study population. A stronger relationship of greater magnitude may exist in specific population groups or within particular disease phenotypes.

Bronchial hyperresponsiveness (BHR) is an important characteristic of asthma and risk factor for COPD and Asthma-COPD Overlap that will likely be a defining factor of phenotypes of these diseases in the future, as definitions of these phenotypes evolve. Bronchial responsiveness (BR), has not yet been studied with respect to the DII. Such investigations will build on prior research into the DII and lung function and may provide insight into the relationship between diet and disease phenotypes associated with BHR, and the potential underlying biological mechanisms of such relationships.

Therefore, the aim of this study is to investigate the relationship between the inflammatory potential of the diet, lung function and BR in a population of middle-aged and older adults, an age group in which chronic systemic inflammation is more prevalent (346). I will also assess potential effect modifiers to further investigate this relationship in different population groups and phenotypes of chronic lung disease.

8.2 Methods

8.2.1 Study population

The design of the COPD study and ascertainment of the study population are detailed in Chapter 4. Briefly, there were 1,214 participants who completed the FFQ and spirometry testing, 1,054 of whom also completed the methacholine challenge. Following exclusions due to invalid spirometry data (n=4), missing or invalid data for covariates (height and BMI invalid n=1; atopy missing n=2; atopy invalid n=1), and extreme energy intakes (n=25; detailed in Chapter 7), there were 1,183 available for analysis of lung function outcomes and 1,030 for BR.

8.2.2 Ascertainment of the DII

Usual diet over the past 12 months was assessed by a self-administered semi-quantitative FFQ developed by Cancer Council Victoria (see chapter 4 for a detailed description of the FFQ). Individual intakes of 95 food items and 31 nutrients were determined from the FFQ, 25 of which were used in the calculation of the DII. These foods/nutrients were (in g/day unless otherwise stated) onion, garlic, total fat, saturated fat, polyunsaturated fat, monounsaturated fat, fibre, cholesterol (mg/day), retinol (µg/day), thiamin (mg/day), vitamin E (mg/day), zinc (mg/day), beta-carotene (µg/day), alcohol, total energy (kJ/day), protein, carbohydrate, folate (µg/day), iron (mg/day), magnesium (µg/day), niacin (µg/day), riboflavin (µg/day), vitamin C, omega-3 fatty acids, and omega-6 fatty acids. I prepared the datafile with the relevant diet data and sent it to Dr Nitin Shivappa and colleagues. Dr Shivappa and his team linked this data to a regionally representative world database containing estimated means and standard deviations for each DII food parameter from 11 countries (USA, Mexico, England, Denmark, India, Australia, New Zealand, Bahrain, Scotland, South Korea and Japan). Using this database, z-scores were calculated for each food parameter for each individual. The z-scores were converted to centred proportions and then

multiplied by the inflammation effect score specific to each food parameter. The overall DII for each individual was determined as the sum of these food parameter scores. The DII was then energy-adjusted (creating the E-DII^{TM}) using the energy density approach (i.e. converted to per 1000 calories consumed). The E-DII for each individual was then sent back to me to perform the association analyses. The final E-DII ranged from -4.30 to 4.47 with lower, more negative values indicating a more anti-inflammatory diet and higher, more positive values indicating a more pro-inflammatory diet.

8.2.3 Assessment of lung function and BR

Spirometry and methacholine challenge tests were conducted as described in Chapter 4. The outcome measures for this analysis are FEV_1, FVC, and FEV_1/FVC, and change in FEV_1 during the methacholine challenge.

8.2.4 Assessment of covariates

The measurement and definition of all covariates of interest are detailed in Chapter 4.

8.2.5 Statistical analysis

The statistical methods used to describe and analyse the data are detailed in Chapter 4. Correlations between the E-DII and the food parameters used to calculate the E-DII were assessed. Linear mixed effects models (detailed in Chapter 5) were used to examine the association between the E-DII and change in FEV_1 during the methacholine challenge. These models included all confounders of diet and baseline FEV_1 as determined from the diet quality-lung function DAG in Chapter 4 (Figure 4.6) and interactions with dose for confounders of BR as determined from the diet quality-BR DAG, (Chapter 4, Figure 4.4). E-DII was categorised into quintiles and modelled as a categorical variable. Models assuming a linear association between E-DII and the outcome variables were explored using a pseudo-continuous form of the E-DII in which subjects were allocated the quintile-specific median value. Statistical evidence of linear trend is presented as p_{trend}. Linearity was assessed using the same test comparing models with the E-DII as categorical (i.e. quintiles) and pseudo-continuous (quintile medians). Statistical evidence of linearity is presented as $p_{linearity}$. Where there is evidence of a non-linear relationship, such a relationship was confirmed using the same test comparing models with and without the dietary pattern (in categorical form). Interactions were investigated using the likelihood ratio test comparing models with and without the interaction term, with evidence presented as $p_{interaction}$. Relationships with moderate or strong evidence are discussed, as well as any similar trends seen in related analyses.

8.3 Results

8.3.1 Study population characteristics

The characteristics of the study population according to E-DII quintile are displayed in Table 8.1.

Those with a higher E-DII were more likely to be male and current smokers, and were, on average, slightly younger, slightly taller and had a higher energy intake compared to those with a lower E-DII.

Table 8.1 - Characteristics of the study population by E-DII quintile

Characteristic	E-DII quintiles					Total
	Q1 (n=238)	Q2 (n=239)	Q3 (n=239)	Q4 (n=238)	Q5 (n=233)	(n=1187)
Male, n (%)	82 (34.5)	111 (46.4)	122 (51.1)	133 (55.9)	164 (70.4)	612 (51.6)
Age (years)	59.7 [7.4]	58.3 [7.6]	57.9 [7.4]	57.8 [7.4]	57.1 [7.5]	58.2 [7.5]
Height (metres)	1.66 [0.09]	1.68 [0.09]	1.69 [0.09]	1.69 [0.09]	1.71 [0.09]	1.69 [0.09]
BMI category, n (%)						
Healthy weight (<25kg/m^2)	83 (34.9)	78 (32.6)	75 (31.4)	71 (29.8)	70 (30.2)	377 (31.8)
Overweight (≥25 and <30kg/m^2)	108 (45.4)	108 (45.2)	108 (45.2)	113 (47.5)	100 (43.1)	537 (45.3)
Obese (≥30kg/m^2)	47 (19.8)	53 (22.2)	56 (23.4)	54 (22.7)	62 (26.7)	272 (22.9)
Asthma, n (%)						
Never	179 (75.2)	201 (84.1)	191 (79.9)	188 (79.0)	195 (83.7)	954 (80.4)
Remitted	26 (10.9)	18 (7.5)	20 (8.4)	22 (9.2)	23 (9.9)	109 (9.2)
Current	33 (13.9)	20 (8.4)	28 (11.7)	28 (11.8)	15 (6.4)	124 (10.5)
Smoking, n (%)						
Never smoked	115 (48.3)	120 (50.2)	110 (46.0)	112 (47.1)	86 (36.9)	543 (45.8)
Former smoker	112 (47.1)	98 (41.0)	106 (44.4)	91 (38.2)	89 (38.2)	496 (41.8)
Current smoker	11 (4.6)	21 (8.8)	23 (9.6)	35 (14.7)	58 (24.9)	148 (12.5)
Atopy, n (%)	121 (50.8)	130 (54.6)	129 (54.0)	110 (46.4)	132 (56.9)	622 (52.5)
Energy intake (kilojoules/day)	7315 [2339]	7752 [2489]	7956 [2438]	8796 [2865]	9474 [2770]	8253 [2696]

All values presented are mean [standard deviation] unless otherwise stated; E-DII – Energy-adjusted dietary inflammatory index

8.3.2 Correlation between the E-DII and its dietary components

There were nine food parameters that were correlated with the E-DII (Table 8.2). These were fat, saturated fat, monounsaturated fat, fibre, cholesterol, retinol, beta-carotene, vitamin C and energy intake.

Table 8.2 - Correlation between E-DII and its food parameters

Food parameter	Correlation with E-DII*	Food parameter	Correlation with E-DII*	Food parameter	Correlation with E-DII*
Onion	-	Thiamin	-	Folate	-
Garlic	-	Vitamin E	-	Iron	-
Fat	0.42	Zinc	-	Magnesium	-
Saturated fat	0.58	Beta-carotene	-0.41	Niacin	-
Poly-unsaturated fat	-	Alcohol	-	Riboflavin	-
Mono-unsaturated fat	0.36	Energy intake	0.28	Vitamin C	-0.26
Fibre	-0.30	Protein	-	Omega-3 fatty acids	-
Cholesterol	0.45	Carbohydrates	-	Omega-6 fatty acids	-
Retinol	0.44				

*Only correlations ≥ 0.25 or ≤ -0.25 are displayed

8.3.3 Associations between E-DII and lung function

In the univariate analyses, E-DII was positively associated with FVC and there were non-linear relationships with FEV_1 and FEV_1/FVC (Table 8.3, Model 1). After adjusting for age, height, sex, smoking status, asthma status, BMI category, atopy, and energy intake, E-DII was associated with FEV_1 and there was moderate evidence that this association was non-linear. Results for each quintile show a similar mean FEV_1 for quintiles 1-3, after which there is a substantial drop and similar results for quintiles 4 and 5. Those in quintiles 4 and 5 had a mean FEV_1 0.19 L and 0.14 L less, respectively, compared to those in quintile 1 (95%CIs Q4: -0.28, -0.10; Q5: -0.23, -0.04).

After adjusting for confounders, there was a negative association between E-DII and FVC. Those in the highest E-DII quintile had a mean FVC 0.14 L less than those in the lowest quintile (95%CI -0.25, -0.03; $p_{trend}<0.001$).

E-DII was also associated with FEV_1/FVC after adjusting for confounders; however, there was moderate evidence the relationship was non-linear and no obvious meaningful pattern in the results was observed.

Table 8.3 - Unadjusted and adjusted associations of E-DII with lung function

Lung function measure	E-DII (quintiles)					p_{trend}	$p_{linearity}$
	Q1	Q2	Q3	Q4	Q5		
FEV_1 (L)							
Model 1	Ref	0.21 (0.06, 0.36)	0.23 (0.09, 0.38)	0.13 (-0.01, 0.28)	0.34 (0.19, 0.49)	< 0.001	0.033
Model 2	Ref	-0.01 (-0.10, 0.08)	-0.03 (-0.12, 0.06)	-0.19 (-0.28, -0.10)	-0.14 (-0.23, -0.04)	<0.001	0.031*
FVC (L)							
Model 1	Ref	0.25 (0.07, 0.43)	0.26 (0.08, 0.44)	0.25 (0.07, 0.43)	0.49 (0.31, 0.67)	<0.001	0.21
Model 2	Ref	-0.02 (-0.11, 0.08)	-0.08 (-0.18, 0.02)	-0.18 (-0.28, -0.08)	-0.14 (-0.25, -0.03)	<0.001	0.19
FEV_1/FVC (%)							
Model 1	Ref	0.28 (-1.11, 1.68)	0.77 (-0.63, 2.16)	-1.47 (-2.87, -0.07)	-0.89 (-2.29, 0.52)	0.033	0.040
Model 2	Ref	-0.32 (-1.57, 0.93)	0.50 (-0.74, 1.75)	-1.51 (-2.78, -0.23)	-1.01 (-2.34, 0.33)	0.043	0.047^

β-coefficient (95%CI) presented for each E-DII quintile

Model 1: Unadjusted linear regression analysis

Model 2: Regression analysis adjusted for age, height, sex, smoking status, asthma status, BMI category, atopy, and energy intake

* p-value for likelihood ratio test comparing models with and without E-DII <0.001

^ p-value for likelihood ratio test comparing models with and without E-DII <0.001

8.3.4 Modification of the associations between E-DII and lung function

Relationships between E-DII and FEV_1 and between E-DII and FVC were modified by asthma status ($p_{interaction}$=0.001 and <0.001 respectively); however, both relationships were non-linear ($p_{linearity}$=0.009 and 0.014 respectively; Table 8.4). In general, mean FEV_1 was lowest for those with current asthma and highest in the never asthma group. There was a substantial drop in mean FEV_1 between quintiles 3 and 4 of E-DII in those with remitted and current asthma (Figure 8.1). In the remitted asthma group, those in quintiles 4 and 5 had a mean FEV_1 0.45 L and 0.36 L less, respectively, compared to those in quintile 1 (95%CIs Q4: -0.74, -0.17; Q5: -0.64, -0.08; Table 8.4). In the current asthma group, mean FEV_1 for quintiles 4 and 5 were 0.41 L and 0.51 L less than quintile 1, respectively (95%CIs Q4: -0.66, -0.16; Q5: -0.82, -0.21). There was also a relationship of smaller magnitude in those who had never had asthma, with those in quintile 4 of the E-DII having the lowest mean FEV_1 (0.12 L less than quintile 1, 95%CI -0.21, -0.01; Table 8.3).

For FVC, in the current and remitted asthma groups there was a substantial drop between quintiles 3 and 4 of E-DII, with mean FVC lower in quintiles 4 and 5 compared to quintiles 1-3 (Figure 8.2). In the remitted asthma group, mean FVC for quintiles 4 and 5 was 0.63 L and 0.40 L less, respectively, compared to quintile 1 (95%CIs Q4: -0.94, -0.32; Q5: -0.71, -0.09) and in the current asthma group, mean FVC for quintiles 4 and 5 was 0.38 L and 0.59 L less (95%CIs Q4: -0.66, -0.11; Q5: -0.93, -0.26). There was no relationship between E-DII and FVC in the never asthma group.

There was moderate to weak evidence that the relationships between E-DII and both FEV_1 and FEV_1/FVC were modified by smoking status ($p_{interaction}$=0.067 and 0.090 respectively; Appendix 8, Table A3). These results will not be discussed in detail as the evidence was not strong enough. However, it is worth noting the effect sizes were considerable in the current smoking group and further investigation with a larger sample of current smokers is recommended.

The relationship between E-DII and lung function was not modified by any of the other potential effect modifiers tested (Appendix 8, Tables A1, A2 and A4).

Table 8.4 - Adjusted associations between E-DII quintiles and lung function by asthma status

E-DII quintile	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.02 (-0.08, 0.12)	-0.11 (-0.41, 0.18)	-0.10 (-0.38, 0.17)	0.05 (-0.06, 0.16)	-0.20 (-0.53, 0.12)	-0.26 (-0.56, 0.04)	-0.76 (-2.16, 0.63)	0.81 (-3.34, 4.97)	2.31 (-1.51, 6.13)
Q3	-0.05 (-0.15, 0.06)	-0.06 (-0.35, 0.23)	0.14 (-0.11, 0.38)	-0.07 (-0.18, 0.04)	-0.26 (-0.58, 0.05)	0.06 (-0.21, 0.33)	-0.09 (-1.50, 1.33)	2.58 (-1.43, 6.58)	2.72 (-0.74, 6.18)
Q4	-0.12 (-0.22, -0.01)	-0.45 (-0.74, -0.17)	-0.41 (-0.66, -0.16)	-0.08 (-0.20, 0.03)	-0.63 (-0.94, -0.32)	-0.38 (-0.66, -0.11)	-1.46 (-2.91, -0.02)	-0.13 (-4.04, 3.79)	-3.21 (-6.72, 0.30)
Q5	-0.06 (-0.17, 0.04)	-0.36 (-0.64, -0.08)	-0.51 (-0.82, -0.21)	-0.05 (-0.17, 0.07)	-0.40 (-0.71, -0.09)	-0.59 (-0.93, -0.26)	-0.93 (-2.41, 0.56)	-1.58 (-5.50, 2.34)	-2.14 (-6.36, 2.07)
p_{trend}	0.047	0.002	<0.001	0.100	0.001	<0.001	0.17	0.37	0.071
$P_{interaction}$	0.001			<0.001			0.19		
$p_{linearity}$	0.009			0.014			0.040		

β-coefficient (95%CI) presented for each E-DII quintile

Adjusted for age, height, sex, smoking status, asthma status, BMI category, atopy, and energy intake

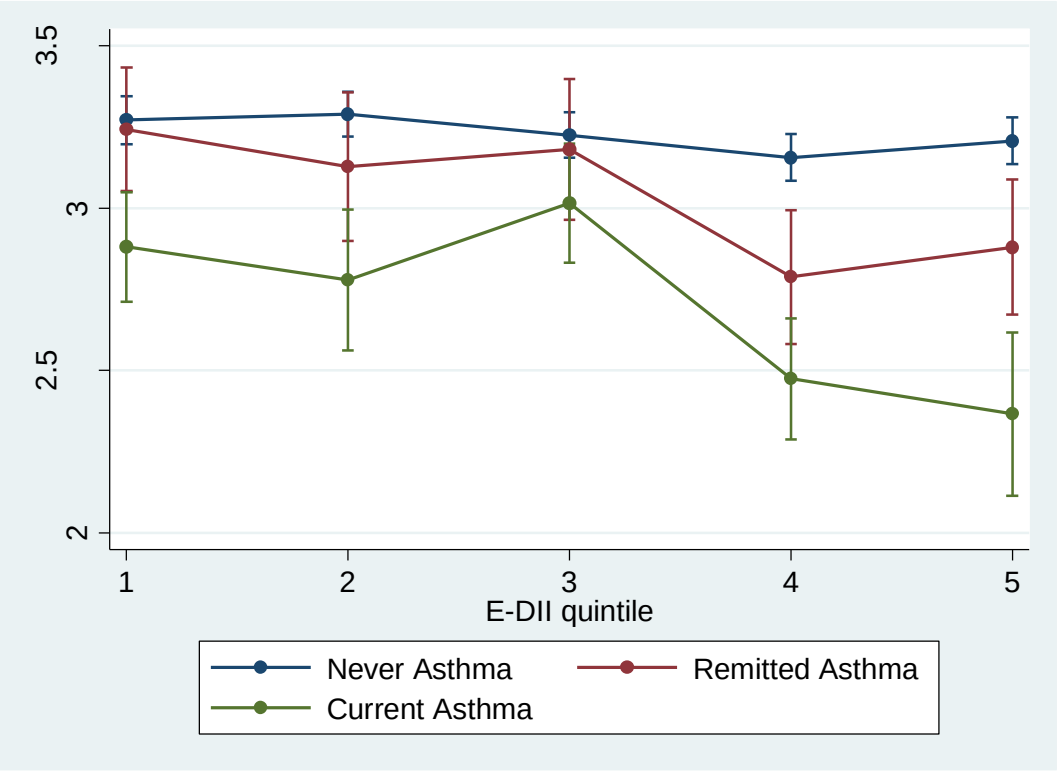


Figure 8.1 - Relationship between quintiles of E-DII and mean FEV_1 by asthma status

Adjusted for age, height, sex, smoking status, asthma status, BMI category, atopy, and energy intake; error bars represent 95%CIs

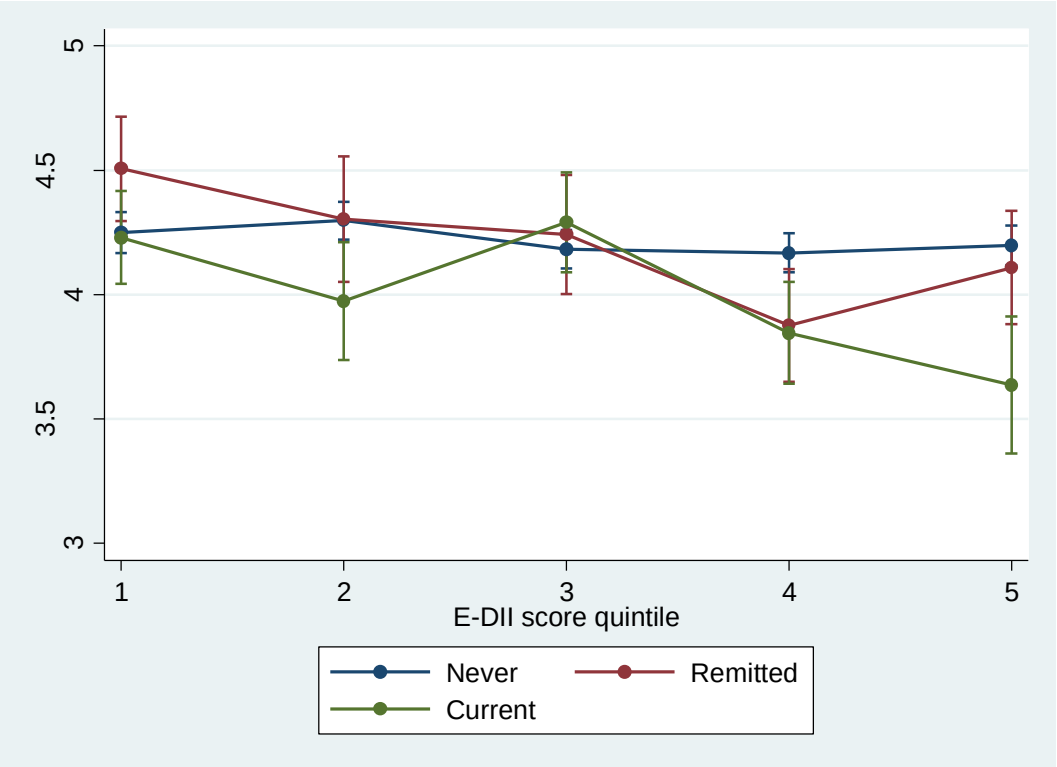


Figure 8.2 - Relationship between quintiles of E-DII and mean FVC by asthma status

Adjusted for age, height, sex, smoking status, asthma status, BMI category, atopy, and energy intake; error bars represent 95%CIs

8.3.5 Association between E-DII and change in FEV_1 during the methacholine challenge

There was no association between E-DII and change in FEV_1 during the methacholine challenge in either the univariate or multivariate analyses (Appendix 8, Table A5).

8.3.6 Modification of the association between E-DII and change in FEV_1 during the methacholine challenge

There was moderate evidence that the relationship between E-DII and change in FEV_1 during the methacholine challenge was modified by sex ($p_{interaction}$=0.051); however the relationship was non-linear in both males and females ($p_{linearity}$=0.006; Table 8.4). There was no apparent pattern in the results for females. After adjusting for confounders, males in quintile 1 of E-DII had the fastest mean fall in FEV_1 during the methacholine challenge, whilst males in quintile 2 had the slowest, with a mean fall in FEV_1 46 ml less per mg methacholine compared to quintile 1 (95%CI 0.01, 0.08; Table 8.5). After quintile 2, mean fall in FEV_1 increased as E-DII increased (Figure 8.3).

The relationship between E-DII and the change in FEV_1 during the methacholine challenge was not modified by any of the other potential effect modifiers tested (Appendix 8, Table A6).

Table 8.5 - Adjusted associations of E-DII with change in FEV_1 during the methacholine challenge by sex

E-DII quintile	Change in FEV_1(L)/mg methacholine	
	Female (n=498)	Male (n=532)
Q1	Ref	Ref
Q2	0.002 (-0.03, 0.03)	0.05 (0.01, 0.08)
Q3	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.06)
Q4	-0.01 (-0.05, 0.02)	0.03 (-0.007, 0.06)
Q5	0.02 (-0.02, 0.05)	0.005 (-0.03, 0.04)
p_{trend}	0.62	0.30
$p_{interaction}$	0.051	
$P_{linearity}$	0.006	

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, education, occupation, fruit intake, fat score

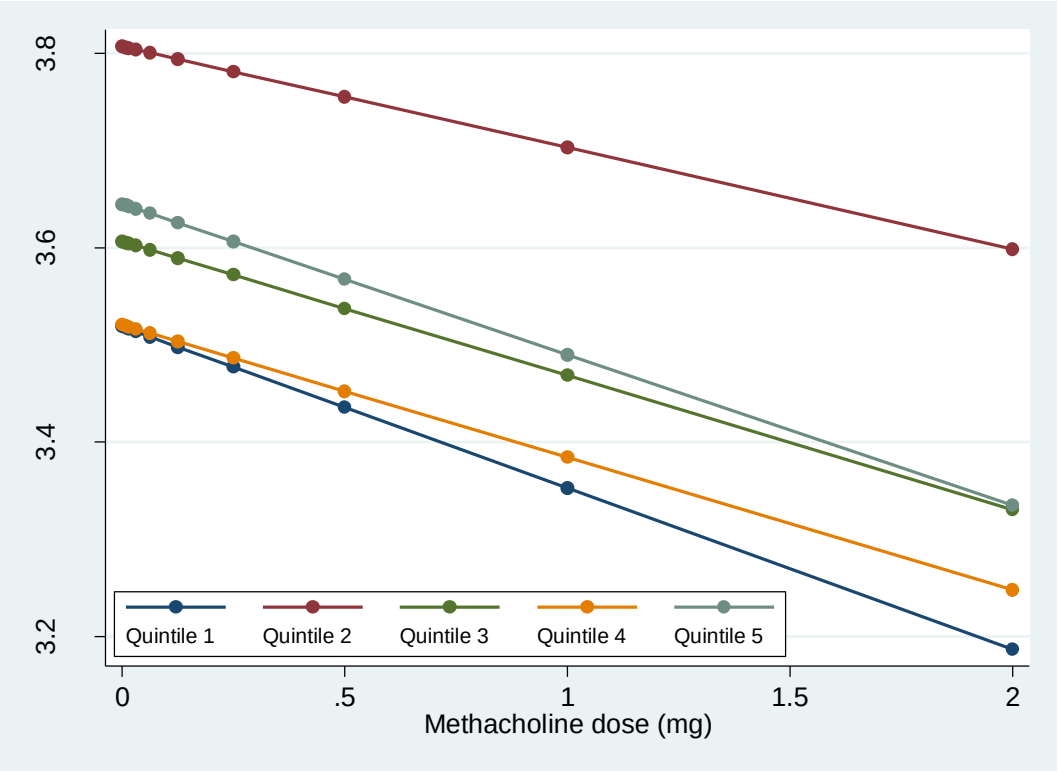


Figure 8.3 - Relationship between quintiles of E-DII and change in mean FEV_1 during the methacholine challenge in males

Adjusted for age, smoking status, asthma status, BMI category, atopy, and energy intake

8.4 Discussion

The findings of this study suggest a pro-inflammatory diet is associated with lower FEV_1 and lower FVC, particularly in those with current asthma or a history of asthma. They also indicate that there may be a threshold beyond which lower lung function is observed. In the current asthma group, those with a pro-inflammatory diet had a mean FEV_1 and FVC up to 0.65 L less than those with a neutral or anti-inflammatory diet. In those with remitted asthma, the mean differences were up to 0.45 L and 0.63 L for FEV_1 and FVC, respectively. The differences observed in this study are clinically important. Further research is needed to confirm these results and demonstrate a causal relationship; however, if these associations are found to be causal, these results suggest dietary change may be more effective at improving lung function and/or reducing lung function decline than medication (see Chapter 9 for a detailed discussion) (347-349).

There have been two other studies that have assessed asthma status as an effect modifier of the relationship between DII and lung function. Both these studies, by Han and colleagues, found a negative association between E-DII and percent predicted FEV_1 and FVC of small magnitude in adults without asthma (111, 345). This is similar to the results I have observed in those who have never had asthma, although stronger associations are implied by my data. In contrast to my findings, neither study found any association in adults with asthma (111, 345). These conflicting findings may be due to differences in the study populations and statistical methods. Both these studies by Han et al involved adult populations aged 18-74+ years, whereas the COPD study focused on a middle-aged and older population group (aged 45-69 years). Chronic systemic inflammation is more common in older age groups (346). Therefore, diet may have more of an impact on inflammation in this group. Both studies used percent predicted values of lung function which standardises lung function measures according to age, sex, height and ethnicity. In my study, I used raw lung function values. The two methods imply different relationships between raw lung function and age, sex, height and ethnicity. The effect of using percent predicted lung function values instead of using raw values and adjusting for age, sex, height and ethnicity is unknown. Both studies used E-DII as a continuous variable, preventing a non-linear relationship from being detected. Both studies also assessed diet using one or two 24-hour dietary recalls per participant which is not an appropriate measure of usual diet to examine associations between diet and health outcomes. One of the studies excluded people with heart disease, a condition that is associated with inflammation, asthma and COPD (111, 350-352). This may have led to an inadvertent exclusion of older adults with chronic inflammation and poor lung function which may have biased the results. The other study was conducted in a Hispanic/Latino American population (345). Therefore, the relevance of these results to a Caucasian population such as that in the COPD study is unclear.

It is biologically plausible for the relationship between the inflammatory potential of the diet and lung function to differ by asthma status. In asthma, structural changes of the airways occur (known

as airway remodelling) resulting in narrowing of the airways, increased BR, increased airway inflammation and further declines in lung function (325, 326). Airway inflammation may also play a role in initiating airway remodelling, leading to a cycle of continued airway inflammation and worsening lung function (325, 326). It is currently unclear whether airway remodelling is permanent or reversible (353, 354). Therefore, these structural changes may be present in those with current asthma and those with a history of asthma. An anti-inflammatory diet may reduce airway inflammation and, in turn, mitigate airway remodelling and lung function decline.

To my knowledge, following an extensive review of the literature, this study is the first study to examine the relationship between the inflammatory potential of the diet and bronchial responsiveness. I found men with the most anti-inflammatory diet were most responsive to methacholine and those in the second most anti-inflammatory category were the least responsive, followed by greater responsiveness with further increases in the inflammatory potential of the diet. I expected that, if there was any relationship, responsiveness would be higher as the diet became more pro-inflammatory. This expectation was based on the contribution of airway inflammation to airway remodelling and the relation between these structural changes and BR (325, 326, 355). The finding that men with the most anti-inflammatory diet had the greatest bronchial responsiveness was unexpected. This may be a chance result; however, there is a possible biologically plausible explanation. The correlations between DII and its food parameters indicate that those with a low DII are consuming less fat and less animal products (i.e. meat and dairy), which are high in saturated fat, cholesterol and retinol. It is possible those with the most anti-inflammatory diet are not consuming enough fat to support normal biological functions. One such function that may be impaired is the absorption, transport and storage of fat-soluble vitamins (i.e. vitamins A, D, E and K). Vitamins A and E are antioxidant vitamins, which are important for immune function. Vitamin D is also thought to play an important role in immune system function and airway remodelling (207). Higher serum vitamin D levels have also been shown to be associated with reduced BHR (238). There are no recommendations for absolute fat intake; however, the recommended intake of fat as a percentage of total energy intake is 20-35% (356). Although mean percentage of energy from fat was higher with higher E-DII in our study population, only a small number of men had fat intakes less than 20% of energy and the distribution of these men across quintiles of E-DII was fairly even. However, these recommendations are based on research related to risk of heart disease, obesity and diseases for which obesity is a risk factor (356). The fat intake required for optimal lung health is unknown and may be different to these recommendations. Mean absolute fat intake was also higher with higher E-DII and an absolute minimum cut-off may be more relevant for lung health.

Those with a lower E-DII are also consuming fewer animal products. This may be affecting their levels of vitamin B12, a nutrient that can only be sourced from meat, animal products and fortified foods. Vitamin B12 plays important roles in the formation of red blood cells, DNA synthesis and nervous system function, and deficiency is common in older populations as absorption decreases

with age. Its relationship with lung function is unclear; however, lower serum vitamin B12 levels have been associated with more frequent exacerbations requiring hospitalisation in COPD patients (357). Vitamin B12 supplementation has also been shown to benefit exercise tolerance in advanced COPD (358). Despite the potential biological plausibility of this result, further research is needed to confirm these findings.

This study is a large cross-sectional study utilising a validated semi-quantitative FFQ and objective lung function measures. However, there are some limitations. Recall error and measurement error may impact the diet and lung function measures, respectively. However, lung function measurement errors are likely to be random and dietary recall errors are not expected to occur differently in those with poor lung function and those with good lung function, particularly in a study such as this, where many potential risk factors are measured. Therefore, these errors are likely to be non-differential and would be expected to attenuate the true association to the null. There are some potential confounders that were not measured as part of the COPD study (e.g. physical activity, comorbidities, SES measures; see Chapter 4 Figure 4.1). Therefore, there may be some residual confounding affecting the results. This is particularly relevant for the analyses of bronchial responsiveness as the known risk factors of BHR have been determined using statistical methods that can produce erroneous results, as shown in Chapter 5. There may be a lack of power for some analyses assessing effect modification, reducing the chance of detecting an association if one exists. This has likely impacted the assessment of smoking as an effect modifier of the relationship between the inflammatory potential of the diet and lung function. This possible interaction requires further investigation. Only 25 of the possible 45 food parameters were used in the calculation of the E-DII and some of the missing parameters are those with the greatest effects on inflammation (e.g. turmeric, green and black tea, and isoflavones) (110). Whether the 25 food parameters used adequately capture the variation in the inflammatory potential of the diet in the study population is unknown. Interleukin-4 is considered anti-inflammatory in the calculation of the E-DII, however, it has been shown to have a pro-inflammatory effect with regards to asthma (110, 359, 360). The impact of this systematic error on the results is unclear. Lastly, this study is cross-sectional in design and the associations produced do not reflect temporal relationships. However, validation studies of the DII have demonstrated its association with inflammatory biomarkers (361, 362). Therefore, given the evidence supporting the proposed biological mechanism, and the low likelihood that asthmatics with better lung function would change their diet, reverse causation is highly unlikely.

In conclusion, the findings from this research suggest that a more pro-inflammatory diet is associated with poorer lung function, particularly in those with current asthma or a history of asthma, and the difference in lung function in these groups may be clinically important. My findings also suggest a more pro-inflammatory diet is associated with greater bronchial reactivity, particularly in men. Further studies in the form of RCTs and large cohort studies are needed to confirm these results and investigate temporal relationships. In the meantime, recommending an anti-inflammatory

diet, high in fruit and vegetables, low in saturated fat and with adequate healthy fats (from fatty fish, avocado, and nuts and seeds) is advised.

Chapter 9 – Overall discussion and conclusions

9.1 Introduction

The aims of my doctoral work were to investigate relationships between dietary factors, lung function and BR in middle-aged and older Australians, and examine whether these relationships are modified by other factors. Based on a review of the literature, the dietary factors of interest were fruit and vegetable intakes, dietary patterns defined by PCA, and the inflammatory potential of the diet, measured using the DII. The potential effect modifiers explored were sex, smoking, asthma status, BMI, and atopy, based on possible biological plausibility.

Current statistical methods used to examine factors associated with BR have significant limitations. Therefore, a secondary aim of this research was to identify a more suitable statistical approach to analyse data collected during a bronchial provocation challenge with the purpose of assessing factors associated with BR; to demonstrate its application; and to compare its findings to those from one of the most commonly used statistical methods.

In this chapter, I briefly summarise the key findings from my doctoral work and discuss the interpretation of these findings with consideration given to results from previous studies and the strengths and limitations of my research. I will also discuss the generalisability of my results and the implications of my work for public health, clinical practice, and future research.

9.2 Summary of results

9.2.1 Measurement of factors associated with bronchial responsiveness using a linear mixed model

I propose the use of the LMM for investigating factors associated with BR. The LMM is a well-established statistical technique that can be used to analyse repeated continuous outcome measurements (in this case FEV_1). This method overcomes many of the limitations of the existing methods by 1) modelling all FEV_1 values collected; 2) allowing for differences in baseline FEV_1 between individuals, by including random intercepts; 3) allowing for differences in the change in FEV_1 with increasing methacholine dose between individuals, by including random slopes; and 4) considering error around the slope in the association analyses by calculating the slope and assessing factors associated with the slope in a single step. The LMM has the additional benefit of direct and meaningful interpretation of its results.

In chapter 5, I demonstrated the application of the LMM to data from a bronchial provocation challenge and explained the interpretation of the results produced. I also showed that conclusions drawn from an LMM can differ from those of a regression of the log-transformed dose-response slope. Hence the importance of using the most suitable method that will produce the most accurate,

reliable results.

9.2.2 Associations between dietary factors, lung function and BR

9.2.2.1 *Fruit and Vegetable intake*

In chapter 6, I investigated associations between fruit and vegetable intakes, lung function and BR using data from the TAHS 2010 follow up. I found the relationship between vegetable intake and lung function was modified by asthma status, with a higher vegetable intake being associated with a higher FEV_1 in those with current asthma only. The mean difference in FEV_1 between the highest and lowest intake categories (0.27 L) for people with asthma is clinically important. There was also a similar trend for FVC, however the evidence for interaction was weak.

A higher fruit intake was also associated with greater BR, with those consuming 4 or more serves of fruit daily having a mean fall in FEV_1 0.11 L greater per mg methacholine compared to those consuming less than one serve of fruit per day. Note this result has been converted from L/µmol methacholine for easy comparison with other findings (µmol = mg x 5.11).

9.2.2.2 *Dietary patterns (defined by PCA)*

In chapter 7, I investigated associations between dietary patterns defined by principal component analysis (PCA), lung function and BR using data from the COPD study.

Lung function findings

I found relationships between the "high potassium & magnesium" pattern and FEV_1 and FVC were modified by asthma status, with a higher pattern score being associated with a higher FEV_1 and FVC in those with current asthma only. The mean differences between the highest and lowest quintiles of this pattern (0.57 L and 0.50 L for FEV_1 and FVC respectively) would be considered clinically important.

Relationships between the "low calcium & sugars" dietary pattern and FEV_1 and FVC were modified by asthma status. A higher dietary pattern score was associated with a higher FEV_1 in those with remitted asthma (Q5-Q1 mean difference = 0.37 L), with a similar trend observed in the current asthma group. Similarly, for FVC, in those with current asthma, there was an almost linear positive association with a slight curve (Q4-Q1 mean difference = 0.36 L).

BR findings

Several relationships between dietary patterns and BR were modified by asthma status, with associations existing in those with current asthma only. Although some of these relationships were non-linear, the findings suggest greater adherence to these dietary patterns was associated with a more gradual fall in FEV_1 during a methacholine challenge and, therefore, less BR. These dietary patterns were the "high protein & zinc" pattern (Q5-Q1 mean difference=0.16 L/mg methacholine); the "high PUFAs & vitamin E pattern (Q5-Q1 mean difference = 0.16 L/mg methacholine); the "low

calcium & sugars" pattern (Q4-Q1 mean difference=0.19 L/mg methacholine); and the "high α-carotene, low lycopene" pattern (Q5-Q1 mean difference = 0.13 L/mg methacholine).

9.2.2.3 *The inflammatory potential of the diet*

In chapter 8, I investigated associations between the inflammatory potential of the diet, measured using the E-DII, lung function and BR using data from the COPD study. I found relationships between the E-DII and FEV_1 and FVC were modified by asthma status. Although these relationships were non-linear, the findings indicate that, in those with remitted and current asthma, a more proinflammatory diet was associated with a lower mean FEV_1 and FVC (remitted asthma: Q4-Q1 mean difference in FEV_1 and FVC was 0.45 L and 0.63 L respectively; current asthma: Q5-Q3 mean difference in FEV_1 and FVC was 0.65 L for both). There was also a relationship of smaller magnitude with FEV_1 in those who have never had asthma (Q4-Q1 mean difference 0.12 L).

9.3 Overall interpretation of results

9.3.1 Asthma status modifies relationships between diet, lung function and BR

9.3.1.1 *Interpretation of lung function results*

Asthma status modified several relationships between dietary factors, lung function and BR, and generally indicated stronger associations of greater magnitude in those with current asthma. The findings with the greatest clinical importance were from the dietary pattern and E-DII studies. The E-DII study found in those with current asthma, consuming a pro-inflammatory diet (representing a diet high in animal products and low in fruits and vegetables in this study population) was associated with a mean FEV_1 and FVC up to 0.65 L less than those consuming a neutral or anti-inflammatory diet. The findings were similar in those with remitted asthma, with differences in mean FEV_1 and FVC of 0.45 L and 0.63 L respectively. In the dietary pattern study, for those with current asthma, consuming a "high potassium & magnesium" pattern (indicative of a diet high in fruits, vegetables and wholegrains) was associated with a mean FEV_1 and FVC 0.57 L and 0.50 L higher than those with a poor adherence to this pattern. Greater adherence to a "low calcium & sugars" dietary pattern (indicative of a diet high in vegetables and low in sugar and dairy products) was associated with a higher FEV_1 in those with remitted asthma (Q5 v Q1 mean difference = 0.37 L), with a similar trend in the current asthma group, and a higher FVC in those with current asthma, with a mean difference between quintile 4 and quintile 1 of the dietary pattern score of 0.36 L. These results are all consistent with findings from the fruit and vegetable intake study, in which a higher vegetable intake was associated with a higher FEV_1 in those with current asthma, with a mean difference of 0.27 L observed between the highest and lowest intake categories.

These findings together suggest a diet low in animal products, and high in fruits, vegetables, and wholegrains may improve lung function or reduce lung function decline. The consistency across these results strengthen the evidence for there being a true relationship between diet and lung

function. The effect sizes may be impacted by residual confounding or recall or measurement error; however, recall or measurement error would result in an underestimation of the effect size. The large effect estimates strengthen the evidence that, even with residual confounding, a true association exists.

Comparison of observed effect sizes with the efficacy of medication

The effect sizes observed here are not just clinically important but are on par with the effects of medications used to treat asthma and COPD. According to the Global Initiative for Asthma, a clinically important difference in FEV_1 is any difference equal to or greater than 10%, which is an amount perceptible to the patient (61). Using the mean FEV_1 values of those in the TAHS and COPD studies (3.27 L and 3.16 L respectively), the mean differences in FEV_1 observed in my research (e.g. 0.57 L for the "high potassium and magnesium" dietary pattern and 0.65 L for the E-DII) represent differences of up to 21%, which is clinically important. Salbutamol (commonly known by its brand name, Ventolin) is the typical treatment for immediate relief of airway narrowing during an asthma attack or exacerbation. An improvement in FEV_1 of greater than 12% or 200 ml upon administration of salbutamol confirms reversible airflow obstruction and a diagnosis of asthma (61). Salbutamol and other short-acting β-agonist drugs can improve lung function well above this diagnostic cut-off, with one small study of adults with asthma demonstrating a mean increase in FEV_1 of 0.5 L 20 minutes after administration (347). However, short-acting β-agonists also have detrimental health effects with regular, prolonged use, including increased lung function decline, and an increased risk of exacerbations, hospitalisation and death (61, 363-366). Hence, asthma treatment usually consists of a corticosteroid inhaler with or without a long-acting β-agonist, and the use of salbutamol or an equivalent on an "as-needed" basis (61). Additional medications may be considered for uncontrolled or severe asthma.

Studies suggest a more modest improvement in FEV_1 with long-term use of inhaled corticosteroids and long-acting beta-agonists compared to the potential improvements from dietary change, as indicated by my findings. A Cochrane review of randomised controlled trials assessing the efficacy of a commonly used corticosteroid inhaler, beclomethasone dipropionate, in the treatment of chronic asthma found daily use for a minimum of four weeks improved mean FEV_1 by 0.36 L (95%CI 0.26, 0.46) (348). A randomised controlled trial of 408 people with asthma found 24 weeks of treatment with a common long-acting beta-agonist, salmeterol, lead to an improvement in mean FEV_1 of 0.26 L (349). Therefore, based on my results and the findings from these studies, dietary change may be at least as effective in maintaining lung function as current medications. However, further research is needed to establish causality.

Comparison of my findings with other studies

Only two other studies have been published in which asthma status was assessed as an effect modifier of the relationships between dietary factors and lung function (111, 183). Both studies were

large cross-sectional studies, one in a general US population, and one in a US Hispanic/Latino population. Both studies observed a higher E-DII was associated with a modestly lower percent predicted FEV_1 and FVC in those without asthma. This is similar to my finding of a negative relationship between E-DII and FEV_1 in those who have never had asthma. However, in contrast to my findings, neither study found a relationship between E-DII and lung function in those with asthma. This may be because the age range of the study populations in these studies (18 - 74 years and 18 – 79 years) differs from my studies in middle-aged and older adults. By selecting middle-aged and older populations, there is likely to be a higher proportion of phenotypes that affect older age-groups (e.g. adult-onset asthma and obesity-related asthma), or phenotypes that are more likely to develop in women, given women are at a greater risk of developing asthma in adulthood. There is also more likely to be more systemic inflammation.

Other research examining fruit and vegetable intakes, or "healthy" dietary patterns and lung function has shown mixed results (see literature review in Chapter 3). This may be because of the modifying effect of asthma and differences between study populations (e.g. age, ethnicity, obesity, geographical location, etc). Not assessing asthma status as an effect modifier and looking at the relationship in the overall population only, may miss associations within specific subgroups.

Biological plausibility

A diet low in animal products, and high in fruits, vegetables, and wholegrains is low in saturated fat and high in fibre and antioxidants such as vitamins A and C and flavonoids. It is biologically plausible that such a diet would not only be beneficial for lung function but would have a greater benefit in those with asthma or a history of asthma. This type of diet is associated with less systemic inflammation and, in turn, less airway inflammation; it promotes a healthy body weight, reducing the likelihood of obesity and related reductions in lung function; it promotes a healthy, diverse gut microbiota, which aids immune function; and it is high in antioxidants, which also play an important role in the functioning of the immune system (109, 110, 115, 122, 319, 320, 343, 344). Asthma attacks or exacerbations initiate structural changes in the airways (known as airway remodelling). The airways become narrow and more sensitive to inhaled stimuli (i.e. greater BR), inflammation in the airways increases and lung function worsens (325, 326). Airway inflammation is also thought to play a role in triggering airway remodelling, leading to a cycle of continued airway inflammation and further lung function decline (325, 326). Asthma exacerbations are often triggered by acute respiratory illnesses. Therefore, supporting the immune system through a diet high in antioxidants that promotes a healthy gut microbiota may reduce the frequency and severity of respiratory illnesses in the general population, and asthma exacerbations and airway remodelling in those with asthma. Reducing airway inflammation through an anti-inflammatory diet may also reduce airway remodelling with beneficial effects on lung function, BR, and severity and frequency of asthma exacerbations.

Generalisability

My findings were consistent in two separate population-based cross-sectional studies of middle-aged and older adults. Both study populations are predominantly Caucasian. Therefore, these results can be extended to a general Caucasian middle-aged and older adult population. However, these findings are yet to be replicated in studies of other age groups or ethnicities. Therefore, at this stage, it is not possible to determine whether these findings are generalisable beyond Caucasian adults of middle-age and older.

9.3.1.2 *Interpretation of bronchial responsiveness results*

Several dietary factors were associated with reduced BR in people with current asthma. These were 1) a "high protein & zinc" dietary pattern, indicative of a diet high in meat and animal products (mean difference of up to 0.16 L/mg methacholine); 2) a "high PUFAs & vitamin E" pattern, indicative of a diet high in oily fish, complex carbohydrates and vegetable oils (mean difference of 0.16 L/mg methacholine); 3) a "low calcium & sugars" pattern, indicative of a diet high in vegetables and low in sugar and dairy products (mean difference of up to 0.19 L/mg methacholine); and 4) a "high α-carotene, low lycopene" pattern, indicative of a diet high in animal products and vegetables and low in tomatoes and dietary sources of PUFAs (e.g. fish and margarine) (mean difference of 0.13 L/mg methacholine). Those who reach the end of the provocation challenge will have been administered a cumulative dose of two milligrams of methacholine. Therefore, if the associations I observed are found to be causal, my estimated effect sizes suggest dietary change could reduce an individual's fall in FEV_1 during the methacholine challenge by up to 0.26-0.38 L, depending on the dietary pattern. This change may contribute significantly to an individual's response to methacholine and could prevent someone crossing the 20% threshold for a clinically positive BHR result.

Comparison of my findings with other studies

To my knowledge, this is the first time (i) asthma status has been assessed as a potential effect modifier of BR, (ii) a linear mixed model has been used to examine factors associated with BR, and (iii) many of these dietary patterns have been identified in an analysis of dietary patterns and BR. There has only been one previous study investigating the relationship between dietary patterns defined by PCA and BR slope. This study, by Hooper and colleagues, found no relationships between the two dietary patterns identified, a "meat and potato" pattern and a "fish, fruit and vegetables" pattern, and BR slope (172). However, the interaction with asthma status was not explored. My results suggest there is a relationship of clinical importance between diet and BR in those with asthma. However, due to the lack of previous research in this area, further studies are needed to clarify this relationship.

9.3.2 Higher fruit intake may be associated with increased BR

In chapter 6 I reported that those with a higher fruit intake had greater BR. This result contrasts with

findings from a previous study by Woods et al. in which higher intakes of apples and pears were associated with lower odds of BHR. However, this study was conducted in younger adults aged 20-44 years (142). No other studies have examined the relationship between fruit intake and BR. It is possible that excess fruit intake contributes to a high sugar intake which is associated with increased inflammation (318, 338-340). Such an effect may be heightened in a middle-aged population where systemic inflammation and related diseases are more prevalent (346). There is generally a lack of research of the relationship between diet and BR and more work is needed to conclusively identify and understand any relationships that may exist.

9.4 Strengths and limitations of this research

The major strength of this body of work is the consistency in the findings from two separate study populations of middle-aged adults. This adds strength that these findings reflect true relationships between dietary factors and lung function and are not chance observations. Other major strengths include the use of a more suitable statistical method (the LMM) to assess relationships between diet and BR, and identification of dietary patterns explaining a very large proportion of variation in diet compared to other studies. This research also used objective measures of lung function and BR, which minimises bias, and the COPD study used a validated semi-quantitative FFQ, which is the best method of assessing usual diet in population-based studies.

The major limitation of this work is that both the TAHS 2010 follow up and the COPD studies were cross-sectional in design. Therefore, associations have been observed and further work is needed to identify causal relationships and their direction. My hypothesis is that a healthy diet helps to maintain lung function, reduces lung function decline and reduces BR. Reverse causality is unlikely. That is, it is unlikely those with better lung function have changed to a healthier diet, and it is unlikely those with worse lung function have changed to an unhealthy diet. As discussed in chapter 6, the majority of participants with asthma in these studies were diagnosed many years ago. Therefore, diagnosis is unlikely to affect their dietary habits now. It is possible those with COPD and very poor lung function may consume more energy dense foods to ensure they consume enough energy before they become too tired to continue eating. However, only 4.2% of participants in the COPD study had been diagnosed with COPD and the TAHS study population was, on average, younger. Therefore, the proportion of participants with advanced COPD would be very small in both studies.

It is possible that those with asthma who adhere to treatment recommendations may be more health conscious and, therefore, may also be more likely to have a healthier diet. It is known that asthma treatment reduces exacerbations which, in turn, reduces airway remodelling and lung function decline. Therefore, the results observed could be due to greater treatment adherence in those with a healthier diet (i.e. diet is acting as a proxy measure of treatment adherence). However, there was no difference in the distribution of the use or regular use of corticosteroid inhalers (the first line of

preventative asthma treatment) across dietary pattern or DII quintiles in the COPD study or across vegetable intake categories in the TAHS 2010 follow up. Therefore, my results are not caused by differences in treatment adherence across quintiles or categories of the relevant dietary factors.

Another limitation of this work is that multiple testing was performed, increasing the likelihood of observing associations by chance. There are methods available to correct p-values for multiple testing; however, it would be difficult to use such methods in this thesis due to the use of different statistical methods, outcomes and datasets. Sample sizes may have not been adequate to detect associations in some of the interaction models (e.g. interactions with smoking and asthma). Hence, effect sizes in these models should be considered for directions of future research. The dietary assessments may be affected by recall error and the lung function measures may be affected by measurement error; however, these errors are expected to be random, attenuating the result to the null. The results may also be affected by residual confounding, the effects of which are unknown.

9.5 Implications of this research

9.5.1 Public health implications

Asthma is a highly prevalent chronic lung disease affecting 339 million people globally and 11% of Australians (6, 9). It is associated with significant disease burden, impacting an individual's physical, mental and social wellbeing, and the healthcare system. It is also associated with an increased risk of developing COPD, which is the 3rd leading cause of death globally and the 5th leading cause of death in Australia, despite the decline in smoking rates in recent decades (6, 7).

Currently there are no dietary recommendations for the treatment of asthma or the prevention of COPD other than to maintain a healthy body weight. My findings suggest a diet low in animal products, and high in vegetables, and wholegrains may be more effective at maintaining lung function than current medications. Such a diet is in line with current dietary recommendations. Should these relationships be proven to be causal, large-scale population-based dietary modification programs could significantly reduce the burden of asthma on individuals and society and reduce COPD prevalence and mortality rates.

9.5.2 Clinical implications

Further research is needed to confirm the findings from my research and establish a causal relationship, ideally using an RCT study design. However, my research suggests significant potential benefits of a diet low in animal products and high in vegetables and wholegrains to lung function in middle-aged and older adults with asthma. These dietary changes are also in line with the current Australian dietary guidelines. Therefore, I encourage general practitioners and respiratory physicians to recommend a dietary modification program to their middle-aged and older asthma patients. This program should encourage patients to 1) limit intake of animal products; 2) eat a diet rich in a variety

of vegetables; 3) choose wholegrain foods; and 4) limit fruit intake to the recommended 2 serves per day. Given my finding that higher fruit intake is associated with increased BR, I would advise patients limit their fruit intake to the currently recommended two serves daily until further research conclusively determines the relationship between fruit intake and BR.

The recommended dietary modification has several benefits in addition to the potential benefits to lung function. Firstly, it will have few side-effects, none of which are likely to worsen asthma symptoms or increase the risk of death. In contrast, medications used to treat asthma and COPD all have potential side-effects. As mentioned previously, regular, long-term use of salbutamol and similar medications can cause serious asthma exacerbations resulting in hospitalisation and death (61, 363-366). There have also been some concerns regarding the safety of long-term use of long-acting beta-agonists, with some studies suggesting they may also increase the risk of serious exacerbations, hospitalisation and death in some population groups (367, 368). Dietary change is highly unlikely to have such serious side-effects.

There are some phenotypes of asthma and COPD that respond poorly to current medication. Dietary change provides an alternative to medication that may improve the condition of these patients. In middle-aged and older adults, such dietary change would also reduce the risk or progression of other chronic diseases including some types of cancer and cardiovascular disease.

Hence, this dietary modification program should be recommended to all middle-aged and older adults with asthma, in particular targeting those who experience unpleasant side-effects from asthma medication; those at an increased risk of serious side-effects from asthma medicines; those whose asthma is not responsive to medication; and those with lifestyle-related co-morbidities such as type 2 diabetes, hypertension, high cholesterol, obesity, or a genetic predisposition to cardiovascular disease. For patients who prefer a more “natural” approach to their treatment, dietary modification may be an appealing treatment option.

9.5.3 Future research implications

My research suggests relationships between diet, lung function and BR in middle-aged and older Caucasian adults are modified by asthma status, with some large effect sizes observed for lung function in those with current asthma. This research is among the first to examine asthma status as an effect modifier. Further studies are urgently needed to confirm these relationships and establish causality, ideally via a large RCT of middle-aged and older adults with asthma. Future research should also investigate whether asthma status modifies diet-lung function relationships in other age groups and ethnicities.

A number of associations were found between dietary factors and BR in my research; however, there were very few studies to compare these results to. Increased BR (or BHR) is a clinical characteristic of asthma; it is associated with ACO, COPD, accelerated lung function decline and future

development of obstructive lung disease in asymptomatic individuals; and it may be associated with specific asthma and COPD phenotypes (62, 78-81). It is important that we understand the causes of increased BR to adequately treat individuals with asthma and COPD, and possibly prevent asthma and COPD in asymptomatic individuals with BHR. My research suggests there are relationships between dietary factors and BR. Further research is needed to conclusively determine the nature of these relationships. The LMM is a more suitable statistical method for analysing data from a bronchial provocation challenge than methods typically used for this purpose. Therefore, I suggest this method be used for future investigations of factors associated with BR. It is important to note the LMM assumes a linear change in FEV_1 with increasing methacholine dose. This assumption should be checked prior to its use.

A number of previous studies investigating relationships between fruit and vegetable intakes, lung function and BR have combined fruit intake and vegetable intake into a single variable (130, 136, 141). Given my finding of higher fruit intake being associated with increased BR, I would encourage future studies to examine fruit intake and vegetable intake separately, particularly in middle-aged and older population groups.

It should be noted that some interaction models may not have been adequately powered. My results suggest the relationship between the inflammatory potential of the diet and lung function may be modified by smoking status with potentially large effects in current smokers. Further investigations with a larger sample of current smokers is recommended.

9.6 Conclusion

Previous studies of relationships between dietary factors and lung function have produced mixed results. This inconsistency may be due to other factors modifying the relationships, however few studies have investigated potential effect modifiers. My research strongly suggests asthma status modifies these relationships in middle-aged and older adults. A diet low in animal products, high in vegetables and wholegrains, and with limited fruit may improve lung function or slow lung function decline. Further research is urgently needed to establish causality; however, following these recommendations now would not be harmful and may be particularly beneficial for certain population groups.

Few studies have investigated relationships between dietary factors and BR. I found several relationships in my doctoral work. Further research is needed to confirm these findings and determine any causal links. Such work, and any other work examining potential risk factors for increased BR, should use the LMM statistical method to analyse data from a bronchial provocation challenge.

References

1. Lumen Learning. Anatomy and normal microbiota of the respiratory tract: Lumen Learning; [cited 2019 23 July]. Available from: https://courses.lumenlearning.com/suny-microbiology/chapter/anatomy-and-normal-microbiota-of-the-respiratory-tract/.

2. Johns DP, Pierce R. Spirometry: The measurement and interpretation of ventilatory function in clinical practice. The Thoracic Society of Australia and New Zealand; 2008.

3. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J. 2012;40(6):1324-43.

4. Australian Institute of Health and Welfare. COPD (chronic obstructive pulmonary disease) snapshot: AIHW; 2018 [cited 2019 25 January]. Available from: https://www.aihw.gov.au/reports/chronic-respiratory-conditions/copd/contents/copd.

5. Toelle BG, Xuan W, Bird TE, Abramson MJ, Atkinson DN, Burton DL, et al. Respiratory symptoms and illness in older Australians: the Burden of Obstructive Lung Disease (BOLD) study. Med J Aust. 2013;198(3):144-8.

6. Australian Institute of Health and Welfare. Australia's health 2018. Canberra: AIHW; 2018.

7. World Health Organisation. The top 10 causes of death: World Health Organisation; 2018 [cited 2019 11 February]. Available from: https://www.who.int/en/news-room/fact-sheets/detail/the-top-10-causes-of-death.

8. Australian Institute of Health and Welfare. Asthma snapshot: AIHW; 2018 [cited 2019 25 January]. Available from: https://www.aihw.gov.au/reports/chronic-respiratory-conditions/asthma/contents/asthma.

9. World Health Organisation. Asthma: World Health Organisation; 2017 [cited 2019 25 January]. Available from: https://www.who.int/en/news-room/fact-sheets/detail/asthma.

10. Australian Bureau of Statistics. National Health Survey: First Results, 2017-18. Canberra, ABS; 2018 25 January 2019. Report No.: 4364.0.55.001

11. National Asthma Council Australia. Australian asthma handbook: diagnosing asthma in adults: National Asthma Council Australia; 2019 [cited 2019 24 July]. Available from: https://www.asthmahandbook.org.au/diagnosis/adults.

12. Global Asthma Network. The Global Asthma Report 2018. Auckland, New Zealand: Global Asthma Network; 2018.

13. Lung Foundation Australia. Overview: Bronchiectasis: Lung Foundation Australia; 2019 [cited 2019 28 January]. Available from: https://lungfoundation.com.au/patients-carers/living-with-a-lung-disease/bronchiectasis/overview/.

14. World Health Organisation. Chronic respiratory diseases: Bronchiectasis: WHO; 2019 [cited 2019 25 January]. Available from: https://www.who.int/respiratory/other/bronchiectasis/en/.

15. Chang AB, Bell SC, Byrnes CA, Grimwood K, Holmes PW, King PT, et al. Chronic suppurative lung disease and bronchiectasis in children and adults in Australia and New Zealand. Med J Aust. 2010;193(6):356-65.

16. Weycker D, Edelsberg J, Oster G, Tino G. Prevalence and Economic Burden of Bronchiectasis. Clin Pulm Med. 2005;12(4):205-9.

17. Quint JK, Millett ER, Joshi M, Navaratnam V, Thomas SL, Hurst JR, et al. Changes in the incidence, prevalence and mortality of bronchiectasis in the UK from 2004 to 2013: a population-based cohort study. Eur Respir J. 2016;47(1):186-93.

18. Chang AB, Masel JP, Boyce NC, Wheaton G, Torzillo PJ. Non-CF bronchiectasis: clinical and HRCT evaluation. Pediatr Pulmonol. 2003;35(6):477-83.

19. National Heart L, and Blood Institute; National Institutes of Health; U.S. Department of Health and Human Services,. Cystic Fibrosis: NHLBI, NIH; [cited 2019 28 January]. Available from: https://www.nhlbi.nih.gov/health-topics/cystic-fibrosis.

20. Cystic Fibrosis Australia. What is CF: Cystic Fibrosis Australia; 2017 [cited 2019 28 January]. Available from: https://www.cysticfibrosis.org.au/about-cf/what-is-cf.

21. Cystic Fibrosis Foundation. About cystic fibrosis: Cystic Fibrosis Foundation; [cited 2019 28 January]. Available from: https://www.cff.org/What-is-CF/About-Cystic-Fibrosis/.

22. Rasa Ruseckaite SA, Tom Ranger, Mark Tacey, Joanna Dean, Madeleine Gardam, Scott Bell, Nettie Burke on behalf of the Australian Cystic Fibrosis Data Registry,. The Australian Cystic Fibrosis Data Registry Annual Report, 2016. Monash University, Department of Epidemiology and Preventive Medicine; 2018. Report No.: 19.

23. Troy L, Corte T. Interstitial lung disease in 2015: where are we now? Aust Fam Physician. 2015;44(8):546-52.

24. Australian Institute of Health and Welfare. The burden of chronic respiratory conditions in Australia: a detailed analysis of the Australian Burden of Disease Study 2011. Canberra: AIHW; 2017. Contract No.: BOD 15.

25. American Thoracic Society. Chapter 10 Interstitial Lung Disease. 2010. In: Breathing in America: Diseases, Progress, and Hope [Internet]. American Thoracic Society. Available from: https://www.thoracic.org/patients/patient-resources/breathing-in-america/resources/chapter-10-interstitial-lung-disease.pdf.

26. Raghu G, Nyberg F, Morgan G. The epidemiology of interstitial lung disease and its association with lung cancer. Br J Cancer. 2004;91 Suppl 2:S3-10.

27. Ward J, McDonald C. Interstitial Lung Disease: An Approach to Diagnosis and Management. Aust Fam Physician. 2010(3):106.

28. Coultas DB, Zumwalt RE, Black WC, Sobonya RE. The epidemiology of interstitial lung diseases. Am J Respir Crit Care Med. 1994;150(4):967-72.

29. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med. 2011;183(6):788-824.

30. Australian Institute of Health and Welfare. Chronic respiratory diseases in Australia: their prevalence, consequences and prevention. Canberra: AIHW; 2005. Contract No.: AIHW Cat. No. PHE 63.

31. Lung Foundation Australia. Factsheet: Sarcoidosis: Lung Foundation Australia; 2018 [cited 2019 30 January]. Available from: https://lungfoundation.com.au/wp-content/uploads/2018/09/Factsheet-Sarcoidosis-Jul2016.pdf.

32. Patterson KC, Strek ME. Pulmonary fibrosis in sarcoidosis. Clinical features and outcomes. Ann Am Thorac Soc. 2013;10(4):362-70.

33. Wells AU, Hirani N. Interstitial lung disease guideline. Thorax. 2008;63(Suppl 5):v1-v58.

34. Statement on Sarcoidosis. Am J Respir Crit Care Med. 1999;160(2):736-55.

35. American Thoracic Society. Chapter 21 Sarcoidosis. 2010. In: Breathing in America: Diseases, Progress, and Hope [Internet]. American Thoracic Society. Available from: https://www.thoracic.org/patients/patient-resources/breathing-in-america/resources/chapter-21-sarcoidosis.pdf.

36. World Health Organisation. Chronic respiratory diseases: Chronic obstructive pulmonary disease (COPD): WHO; 2019 [cited 2019 25 January]. Available from: http://www.who.int/respiratory/copd/en/.

37. World Health Organisation. Global surveillance, prevention and control of chronic respiratory diseases: A comprehensive approach. World Health Organisation; 2007.

38. Global Initiative for Chronic Obstructive Lung Disease. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. 2018.

39. World Health Organisation. Chronic obstructive pulmonary disease (COPD): World Health Organisation; 2017 [cited 2019 11 February]. Available from: https://www.who.int/en/news-room/fact-sheets/detail/chronic-obstructive-pulmonary-disease-(copd).

40. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med. 2006;3(11):e442.

41. Queensland Health Statewide Respiratory Clinical Network and Lung Foundation Australia. Better Living with Chronic Obstructive Pulmonary Disease: A Patient Guide Queensland Health and Lung Foundation Australia; 2016 [cited 2019 12 February]. Available from: https://lungfoundation.com.au/wp-content/uploads/2018/09/Book-Better-Living-with-COPD-Dec2016.pdf.

42. Australian Centre for Asthma Monitoring. Asthma in Australia 2011. Canberra: AIHW; 2011.

43. Australian Institute of Health and Welfare. Asthma, chronic obstructive pulmonary disease and other respiratory diseases in Australia. Canberra: AIHW; 2010. Report No.: ACM 20.

44. Mannino DM, Buist AS. Global burden of COPD: risk factors, prevalence, and future trends. Lancet. 2007;370(9589):765-73.

45. Holguin F, Folch E, Redd SC, Mannino DM. Comorbidity and mortality in COPD-related hospitalizations in the United States, 1979 to 2001. Chest. 2005;128(4):2005-11.

46. Australian Institute of Health and Welfare. COPD, associated comorbidities and risk factors: AIHW; 2016 [cited 2019 13 February]. Available from: https://www.aihw.gov.au/reports/chronic-respiratory-conditions/copd-associated-comorbidities-risk-factors/contents/about-copd-and-associated-comorbidities.

47. Australian Bureau of Statistics. Australian demographic statistics. Canberra, ABS; 2018 13 February 2019. Report No.: 3101.0.

48. World Health Organisation. Chronic respiratory diseases: causes of COPD: WHO; 2019 [cited 2019 13 February]. Available from: https://www.who.int/respiratory/copd/causes/en/.

49. Asthma Australia. Asthma Basic Facts: Staying safe and well with asthma. For people with asthma and their carers: Asthma Australia; 2018 [24th February 2019]. Available from: https://www.asthmaaustralia.org.au/national/about-asthma/resources/brochures.

50. National Asthma Council Australia. Australian asthma handbook: Assessing lung function to investigate asthma-like symptoms in adults: National Asthma Council Australia; 2019 [cited 2019 25th February]. Available from: http://www.asthmahandbook.org.au/diagnosis/adults/initial-

investigations/lung-function.

51. Australian Institute of Health and Welfare. Asthma among older people in Australia. Canberra: AIHW; 2010. Report No.: ACM 19.

52. Lavoie KL, Bacon SL, Barone S, Cartier A, Ditto B, Labrecque M. What is worse for asthma control and quality of life: depressive disorders, anxiety disorders, or both? Chest. 2006;130(4):1039-47.

53. Oraka E, King ME, Callahan DB. Asthma and serious psychological distress: prevalence and risk factors among US adults, 2001-2007. Chest. 2010;137(3):609-16.

54. Scott KM, Von Korff M, Ormel J, Zhang MY, Bruffaerts R, Alonso J, et al. Mental disorders among adults with asthma: results from the World Mental Health Survey. Gen Hosp Psychiatry. 2007;29(2):123-33.

55. Australian Institute of Health and Welfare. Asthma hospitalisations in Australia 2010-11. Canberra: AIHW; 2013. Report No.: ACM 27.

56. Asthma Australia. Statistics: Asthma Australia; 2019 [26 March 2019]. Available from: https://www.asthmaaustralia.org.au/national/about-asthma/what-is-asthma/statistics.

57. Reddel HK, Sawyer SM, Everett PW, Flood PV, Peters MJ. Asthma control in Australia: a cross-sectional web-based survey in a nationally representative population. Med J Aust. 2015;202(9):492-7.

58. Britt H MG, Henderson J, Bayram C, Harrison C, Valenti L, Pan Y, Charles J, Pollack AJ, Wong C, Gordon J. General practice activity in Australia 2015–16. Sydney: Sydney University Press; 2016. Report No.: General practice series no. 40.

59. Australian Bureau of Statistics. Australian Aboriginal and Torres Strait Islander Health Survey: First Results, Australia, 2012─13. Canberra: Australian Bureau of Statistics; 2013. Report No.: 4727.0.55.001

60. Postma DS, Rabe KF. The Asthma-COPD Overlap Syndrome. N Engl J Med. 2015;373(13):1241-9.

61. Global Initiative for Asthma. Global strategy for asthma management and prevention. 2018.

62. Gibson PG, Simpson JL. The overlap syndrome of asthma and COPD: what are its features and how important is it? Thorax. 2009;64(8):728-35.

63. Kauppi P, Kupiainen H, Lindqvist A, Tammilehto L, Kilpelainen M, Kinnula VL, et al. Overlap syndrome of asthma and COPD predicts low quality of life. J Asthma. 2011;48(3):279-85.

64. Marsh SE, Travers J, Weatherall M, Williams MV, Aldington S, Shirtcliffe PM, et al. Proportional classifications of COPD phenotypes. Thorax. 2008;63(9):761-7.

65. Alshabanat A, Zafari Z, Albanyan O, Dairi M, FitzGerald JM. Asthma and COPD overlap syndrome (ACOS): A systematic review and meta analysis. PLoS One. 2015;10(9):e0136065.

66. Jenkins CR, Jones PW, Calverley PM, Celli B, Anderson JA, Ferguson GT, et al. Efficacy of salmeterol/fluticasone propionate by GOLD stage of chronic obstructive pulmonary disease: analysis from the randomised, placebo-controlled TORCH study. Respir Res. 2009;10:59.

67. Hurst JR, Vestbo J, Anzueto A, Locantore N, Mullerova H, Tal-Singer R, et al. Susceptibility to exacerbation in chronic obstructive pulmonary disease. N Engl J Med. 2010;363(12):1128-38.

68. Mannino DM, Doherty DE, Sonia Buist A. Global Initiative on Obstructive Lung Disease

(GOLD) classification of lung disease and mortality: findings from the Atherosclerosis Risk in Communities (ARIC) study. Respir Med. 2006;100(1):115-22.

69. Vestbo J, Anderson W, Coxson HO, Crim C, Dawber F, Edwards L, et al. Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE). Eur Respir J. 2008;31(4):869-73.

70. Osborne ML, Pedula KL, O'Hollaren M, Ettinger KM, Stibolt T, Buist AS, et al. Assessing future need for acute care in adult asthmatics: the Profile of Asthma Risk Study: a prospective health maintenance organization-based study. Chest. 2007;132(4):1151-61.

71. Hole DJ, Watt GC, Davey-Smith G, Hart CL, Gillis CR, Hawthorne VM. Impaired lung function and mortality risk in men and women: findings from the Renfrew and Paisley prospective population study. BMJ. 1996;313(7059):711-5; discussion 5-6.

72. Vonk JM, Jongepier H, Panhuysen CI, Schouten JP, Bleecker ER, Postma DS. Risk factors associated with the presence of irreversible airflow limitation and reduced transfer coefficient in patients with asthma after 26 years of follow up. Thorax. 2003;58(4):322-7.

73. Hansen EF, Phanareth K, Laursen LC, Kok-Jensen A, Dirksen A. Reversible and Irreversible Airflow Obstruction as Predictor of Overall Mortality in Asthma and Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med. 1999;159(4):1267-71.

74. Lange P, Nyboe J, Appleyard M, Jensen G, Schnohr P. Relation of ventilatory impairment and of chronic mucus hypersecretion to mortality from obstructive lung disease and from all causes. Thorax. 1990;45(8):579-85.

75. Sterk PJ, Bel EH. Bronchial hyperresponsiveness: the need for a distinction between hypersensitivity and excessive airway narrowing. Eur Respir J. 1989;2(3):267-74.

76. Cockcroft DW. Direct challenge tests: Airway hyperresponsiveness in asthma: its measurement and clinical significance. Chest. 2010;138(2 Suppl):18S-24S.

77. National Asthma Council Australia. Australian asthma handbook: Airway hyperresponsiveness tests for diagnosis in adults: National Asthma Council of Australia,; 2016 [cited 2016 23rd February]. Available from: http://www.asthmahandbook.org.au/diagnosis/adults/further-investigations/airway-hyperresponsiveness-tests.

78. Ramsdale EH, Morris MM, Roberts RS, Hargreave FE. Bronchial responsiveness to methacholine in chronic bronchitis: relationship to airflow obstruction and cold air responsiveness. Thorax. 1984;39(12):912-8.

79. Brutsche MH, Downs SH, Schindler C, Gerbase MW, Schwartz J, Frey M, et al. Bronchial hyperresponsiveness and the development of asthma and COPD in asymptomatic individuals: SAPALDIA cohort study. Thorax. 2006;61(8):671-7.

80. Peat JK, Woolcock AJ, Cullen K. Rate of decline of lung function in subjects with asthma. Eur J Respir Dis. 1987;70(3):171-9.

81. Van Schayck CP, Dompeling E, Van Herwaarden CL, Wever AM, Van Weel C. Interacting effects of atopy and bronchial hyperresponsiveness on the annual decline in lung function and the exacerbation rate in asthma. Am Rev Respir Dis. 1991;144(6):1297-301.

82. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med. 2012;18(5):716-25.

83. Kirby JG, Hargreave FE, Gleich GJ, O'Byrne PM. Bronchoalveolar cell profiles of asthmatic and nonasthmatic subjects. Am Rev Respir Dis. 1987;136(2):379-83.

84. Wardlaw AJ, Dunnette S, Gleich GJ, Collins JV, Kay AB. Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. Relationship to bronchial hyperreactivity. Am Rev Respir Dis. 1988;137(1):62-9.

85. Carolan BJ, Sutherland ER. Clinical phenotypes of chronic obstructive pulmonary disease and asthma: recent advances. J Allergy Clin Immunol. 2013;131(3):627-34; quiz 35.

86. Pembrey L, Barreto ML, Douwes J, Cooper P, Henderson J, Mpairwe H, et al. Understanding asthma phenotypes: the World Asthma Phenotypes (WASP) international collaboration. ERJ Open Res. 2018;4(3).

87. Bateman ED, Reddel HK, van Zyl-Smit RN, Agusti A. The asthma-COPD overlap syndrome: towards a revised taxonomy of chronic airways diseases? Lancet Respir Med. 2015;3(9):719-28.

88. Han MK, Agusti A, Calverley PM, Celli BR, Criner G, Curtis JL, et al. Chronic obstructive pulmonary disease phenotypes: the future of COPD. Am J Respir Crit Care Med. 2010;182(5):598-604.

89. Cockcroft DW, Swystun VA. Asthma control versus asthma severity. J Allergy Clin Immunol. 1996;98(6 Pt 1):1016-8.

90. Teeter JG, Bleecker ER. Relationship between airway obstruction and respiratory symptoms in adult asthmatics. Chest. 1998;113(2):272-7.

91. Kendrick AH, Higgs CM, Whitfield MJ, Laszlo G. Accuracy of perception of severity of asthma: patients treated in general practice. BMJ. 1993;307(6901):422-4.

92. Osborne ML, Vollmer WM, Pedula KL, Wilkins J, Buist AS, O'Hollaren M. Lack of correlation of symptoms with specialist-assessed long-term asthma severity. Chest. 1999;115(1):85-91.

93. Devereux G. The increase in the prevalence of asthma and allergy: food for thought. Nature Reviews Immunology. 2006;6(11):869-74.

94. Devenny A, Wassall H, Ninan T, Omran M, Khan SD, Russell G. Respiratory symptoms and atopy in children in Aberdeen: questionnaire studies of a defined school population repeated over 35 years. BMJ. 2004;329(7464):489-90.

95. Seaton A, Godden DJ, Brown K. Increase in asthma: a more toxic environment or a more susceptible population? Thorax. 1994;49(2):171-4.

96. Torres-Borrego J, Moreno-Solis G, Molina-Teran AB. Diet for the prevention of asthma and allergies in early childhood: much ado about something? Allergol Immunopathol (Madr). 2012;40(4):244-52.

97. Cross AJ, Leitzmann MF, Gail MH, Hollenbeck AR, Schatzkin A, Sinha R. A prospective study of red and processed meat intake in relation to cancer risk. PLoS Med. 2007;4(12):e325.

98. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002;346(6):393-403.

99. Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med. 2001;344(18):1343-50.

100. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, et al. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. N Engl J Med. 1997;336(16):1117-24.

101. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, et al. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. N Engl J Med. 2001;344(1):3-10.

102. Investigators G-P. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. . Lancet. 1999;354(9177):447-55.

103. Burr ML, Fehily AM, Gilbert JF, Rogers S, Holliday RM, Sweetnam PM, et al. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). Lancet. 1989;2(8666):757-61.

104. Eisner MD, Anthonisen N, Coultas D, Kuenzli N, Perez-Padilla R, Postma D, et al. An official American Thoracic Society public policy statement: Novel risk factors and the global burden of chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2010;182(5):693-718.

105. Berthon BS, Wood LG. Nutrition and respiratory health--feature review. NUTRIENTS. 2015;7(3):1618-43.

106. Varraso R. Nutrition and asthma. Curr Allergy Asthma Rep. 2012;12(3):201-10.

107. Allan K, Devereux G. Diet and asthma: nutrition implications from prevention to treatment. J Am Diet Assoc. 2011;111(2):258-68.

108. Black PN, Sharpe S. Dietary fat and asthma: is there a connection? Eur Respir J. 1997;10(1):6-12.

109. Wood LG, Shivappa N, Berthon BS, Gibson PG, Hebert JR. Dietary inflammatory index is related to asthma risk, lung function and systemic inflammation in asthma. Clin Exp Allergy. 2015;45(1):177-83.

110. Shivappa N, Steck SE, Hurley TG, Hussey JR, Hebert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. Public Health Nutr. 2014;17(8):1689-96.

111. Han YY, Forno E, Shivappa N, Wirth MD, Hebert JR, Celedon JC. The Dietary Inflammatory Index and Current Wheeze Among Children and Adults in the United States. J Allergy Clin Immunol Pract. 2018;6(3):834-41 e2.

112. de Boer A, van de Worp W, Hageman GJ, Bast A. The effect of dietary components on inflammatory lung diseases - a literature review. Int J Food Sci Nutr. 2017;68(7):771-87.

113. Thorburn AN, Macia L, Mackay CR. Diet, metabolites, and "western-lifestyle" inflammatory diseases. Immunity. 2014;40(6):833-42.

114. West CE, Renz H, Jenmalm MC, Kozyrskyj AL, Allen KJ, Vuillermin P, et al. The gut microbiota and inflammatory noncommunicable diseases: associations and potentials for gut microbiota therapies. J Allergy Clin Immunol. 2015;135(1):3-13; quiz 4.

115. Guilleminault L, Williams EJ, Scott HA, Berthon BS, Jensen M, Wood LG. Diet and asthma: Is it time to adapt our message? NUTRIENTS. 2017;9(11):08.

116. Richards JL, Yap YA, McLeod KH, Mackay CR, Marino E. Dietary metabolites and the gut microbiota: an alternative approach to control inflammatory and autoimmune diseases. Clinical & Translational Immunology. 2016;5(5):e82.

117. Rennard SI, Vestbo J. Natural histories of chronic obstructive pulmonary disease. Proc Am Thorac Soc. 2008;5(9):878-83.

118. Mannino DM, Buist AS, Petty TL, Enright PL, Redd SC. Lung function and mortality in the United States: data from the First National Health and Nutrition Examination Survey follow up

study. Thorax. 2003;58(5):388-93.

119. Mannino DM, Gagnon RC, Petty TL, Lydick E. Obstructive lung disease and low lung function in adults in the United States: data from the National Health and Nutrition Examination Survey, 1988-1994. Arch Intern Med. 2000;160(11):1683-9.

120. Rennard SI, Vestbo J. COPD: the dangerous underestimate of 15%. Lancet. 2006;367(9518):1216-9.

121. Varraso R, Shaheen SO. Could a healthy diet attenuate COPD risk in smokers? 2017;1(6):491-2.

122. Gibson RS. Principles of Nutritional Assessment. 2nd ed. New York, NY: Oxford University Press; 2005.

123. Willett W. Nutritional Epidemiology. 3rd ed. New York, NY: Oxford University Press; 2013.

124. Rutishauser IH. Dietary intake measurements. Public Health Nutr. 2005;8(7A):1100-7.

125. Kirkwood B, Sterne J. Essential Medical Statistics. 2nd ed. Massachusetts, USA: Blackwell Science; 2003.

126. Webb P, Bain, C. Essential Epidemiology: An Introduction for Students and Health Professionals. 2nd ed. Cambridge, UK: Cambridge University Press; 2011.

127. Wood LG, Garg ML, Smart JM, Scott HA, Barker D, Gibson PG. Manipulating antioxidant intake in asthma: a randomized controlled trial. Am J Clin Nutr. 2012;96(3):534-43.

128. Keranis E, Makris D, Rodopoulou P, Martinou H, Papamakarios G, Daniil Z, et al. Impact of dietary shift to higher-antioxidant foods in COPD: a randomised trial. The European Respiratory Journal. 2010;36(4):774-80.

129. Garcia-Larsen V, Potts JF, Omenaas E, Heinrich J, Svanes C, Garcia-Aymerich J, et al. Dietary antioxidants and 10-year lung function decline in adults from the ECRHS survey. Eur Respir J. 2017;50(6):12.

130. Bentley AR, Kritchevsky SB, Harris TB, Holvoet P, Jensen RL, Newman AB, et al. Dietary antioxidants and forced expiratory volume in 1 s decline: the Health, Aging and Body Composition study. Eur Respir J. 2012;39(4):979-84.

131. Carey IM, Strachan DP, Cook DG. Effects of changes in fresh fruit consumption on ventilatory function in healthy British adults. Am J Respir Crit Care Med. 1998;158(3):728-33.

132. Butland BK, Fehily AM, Elwood PC. Diet, lung function, and lung function decline in a cohort of 2512 middle aged men. Thorax. 2000;55(2):102-8.

133. Hanson C, Sayles H, Rutten E, Wouters EFM, MacNee W, Calverley P, et al. The Association Between Dietary Intake and Phenotypical Characteristics of COPD in the ECLIPSE Cohort. Chronic Obstructive Pulmonary Diseases. 2014;1(1):115-24.

134. Tabak C, Smit HA, Rasanen L, Fidanza F, Menotti A, Nissinen A, et al. Dietary factors and pulmonary function: a cross sectional study in middle aged men from three European countries. Thorax. 1999;54(11):1021-6.

135. Tabak C, Smit HA, Heederik D, Ocke MC, Kromhout D. Diet and chronic obstructive pulmonary disease: independent beneficial effects of fruits, whole grains, and alcohol (the MORGEN study). Clin Exp Allergy. 2001;31(5):747-55.

136. Okubo H, Shaheen SO, Ntani G, Jameson KA, Syddall HE, Sayer AA, et al. Processed meat consumption and lung function: modification by antioxidants and smoking. Eur Respir J.

2014;43(4):972-82.

137. Kelly Y, Sacker A, Marmot M. Nutrition and respiratory health in adults: findings from the health survey for Scotland. Eur Respir J. 2003;21(4):664-71.

138. Strachan DP, Cox BD, Erzinclioglu SW, Walters DE, Whichelow MJ. Ventilatory function and winter fresh fruit consumption in a random sample of British adults. Thorax. 1991;46(9):624-9.

139. Garcia-Larsen V, Amigo H, Bustos P, Bakolis I, Rona RJ. Ventilatory function in young adults and dietary antioxidant intake. NUTRIENTS. 2015;7(4):2879-96.

140. Barros R, Moreira A, Fonseca J, de Oliveira JF, Delgado L, Castel-Branco MG, et al. Adherence to the Mediterranean diet and fresh fruit intake are associated with improved asthma control. Allergy. 2008;63(7):917-23.

141. Ng TP, Niti M, Yap KB, Tan WC. Dietary and supplemental antioxidant and anti-inflammatory nutrient intakes and pulmonary function. Public Health Nutr. 2014;17(9):2081-6.

142. Woods RK, Walters EH, Raven JM, Wolfe R, Ireland PD, Thien FC, et al. Food and nutrient intakes and asthma risk in young adults. Am J Clin Nutr. 2003;78(3):414-21.

143. Newman G, Kopras, A. Socioeconomic Indexes for Electoral Divisions. Canberra: Department of the Parliamentary Library; 1998.

144. Khanam UA, Rennie DC, Davis K, Lawson JA. Are Dietary Factors Associated with Lung Function in Canadian Adults? Canadian Journal of Dietetic Practice & Research. 2020;81(1):28-36.

145. Hosseini B, Berthon BS, Wark P, Wood LG. Effects of Fruit and Vegetable Consumption on Risk of Asthma, Wheezing and Immune Responses: A Systematic Review and Meta-Analysis. NUTRIENTS. 2017;9(4):29.

146. Seyedrezazadeh E, Moghaddam MP, Ansarin K, Vafa MR, Sharma S, Kolahdooz F. Fruit and vegetable intake and risk of wheezing and asthma: a systematic review and meta-analysis. Nutr Rev. 2014;72(7):411-28.

147. Lin YC, Wu TC, Chen PY, Hsieh LY, Yeh SL. Comparison of plasma and intake levels of antioxidant nutrients in patients with chronic obstructive pulmonary disease and healthy people in Taiwan: a case-control study. Asia Pac J Clin Nutr. 2010;19(3):393-401.

148. Watson L, Margetts B, Howarth P, Dorward M, Thompson R, Little P. The association between diet and chronic obstructive pulmonary disease in subjects selected from general practice. Eur Respir J. 2002;20(2):313-8.

149. Hirayama F, Lee AH, Binns CW, Zhao Y, Hiramatsu T, Tanikawa Y, et al. Do vegetables and fruits reduce the risk of chronic obstructive pulmonary disease? A case-control study in Japan. Prev Med. 2009;49(2-3):184-9.

150. La Vecchia C, Decarli A, Pagano R. Vegetable consumption and risk of chronic disease. Epidemiology. 1998;9(2):208-10.

151. Kaluza J, Larsson SC, Orsini N, Linden A, Wolk A. Fruit and vegetable consumption and risk of COPD: a prospective cohort study of men. Thorax. 2017;72(6):500-9.

152. Miedema I, Feskens EJ, Heederik D, Kromhout D. Dietary determinants of long-term incidence of chronic nonspecific lung diseases. The Zutphen Study. Am J Epidemiol. 1993;138(1):37-45.

153. Du H, Li L, Bennett D, Yang L, Guo Y, Key TJ, et al. Fresh fruit consumption and all-cause and cause-specific mortality: findings from the China Kadoorie Biobank. Int J Epidemiol.

2017;46(5):1444-55.

154. Meteran H, Thomsen SF, Miller MR, Hjelmborg J, Sigsgaard T, Backer V. Self-reported intake of fruit and vegetables and risk of chronic obstructive pulmonary disease: A nation-wide twin study. Respir Med. 2018;144:16-21.

155. Kaluza J, Harris HR, Linden A, Wolk A. Long-term consumption of fruits and vegetables and risk of chronic obstructive pulmonary disease: a prospective cohort study of women. Int J Epidemiol. 2018;47(6):1897-909.

156. Walda IC, Tabak C, Smit HA, Rasanen L, Fidanza F, Menotti A, et al. Diet and 20-year chronic obstructive pulmonary disease mortality in middle-aged men from three European countries. Eur J Clin Nutr. 2002;56(7):638-43.

157. Tabak C, Feskens EJ, Heederik D, Kromhout D, Menotti A, Blackburn HW. Fruit and fish consumption: a possible explanation for population differences in COPD mortality (The Seven Countries Study). Eur J Clin Nutr. 1998;52(11):819-25.

158. Celik F, Topcu F. Nutritional risk factors for the development of chronic obstructive pulmonary disease (COPD) in male smokers. Clin Nutr. 2006;25(6):955-61.

159. Vukovic DS, Nagorni-Obradovic LM, Vukovic GM. Lifestyle and perceived health in subjects with chronic bronchitis or emphysema: a cross-sectional study. BMC Public Health. 2010;10:546.

160. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. Curr Opin Lipidol. 2002;13(1):3-9.

161. Chatterjee S, Simonoff JS. Handbook of regression analysis: Wiley; 2013.

162. Freund RJ, Wilson WJ, Sa P. Regression analysis : statistical modeling of a response variable. 2nd ed: Elsevier Academic Press; 2006.

163. UCLA institute for Digital Research and Education. Regression with stata chapter 2 – regression diagnostics: UCLA [cited 2019 28 September]. Available from: https://stats.idre.ucla.edu/stata/webbooks/reg/chapter2/stata-webbooksregressionwith-statachapter-2-regression-diagnostics/.

164. Sexton P, Black P, Metcalf P, Wall CR, Ley S, Wu L, et al. Influence of mediterranean diet on asthma symptoms, lung function, and systemic inflammation: a randomized controlled trial. J Asthma. 2013;50(1):75-81.

165. Gutierrez-Carrasquilla L, Sanchez E, Hernandez M, Polanco D, Salas-Salvado J, Betriu A, et al. Effects of Mediterranean Diet and Physical Activity on Pulmonary Function: A Cross-Sectional Analysis in the ILERVAS Project. NUTRIENTS. 2019;11(2):03.

166. Yazdanpanah L, Paknahad Z, Moosavi AJ, Maracy MR, Zaker MM. The relationship between different diet quality indices and severity of airflow obstruction among COPD patients. Med J Islam Repub Iran. 2016;30:380.

167. Ma J, Strub P, Lv N, Xiao L, Camargo CA, Jr., Buist AS, et al. Pilot randomised trial of a healthy eating behavioural intervention in uncontrolled asthma. Eur Respir J. 2016;47(1):122-32.

168. Sorli-Aguilar M, Martin-Lujan F, Flores-Mateo G, Arija-Val V, Basora-Gallisa J, Sola-Alberich R, et al. Dietary patterns are associated with lung function among Spanish smokers without respiratory disease. BMC Pulm Med. 2016;16(1):162.

169. Cho Y, Chung HK, Kim SS, Shin MJ. Dietary patterns and pulmonary function in Korean women: findings from the Korea National Health and Nutrition Examination Survey 2007-2011. Food Chem Toxicol. 2014;74:177-83.

170. McKeever TM, Lewis SA, Cassano PA, Ocke M, Burney P, Britton J, et al. Patterns of dietary intake and relation to respiratory disease, forced expiratory volume in 1 s, and decline in 5-y forced expiratory volume. Am J Clin Nutr. 2010;92(2):408-15.

171. Shaheen SO, Jameson KA, Syddall HE, Aihie Sayer A, Dennison EM, Cooper C, et al. The relationship of dietary patterns with adult lung function and COPD. Eur Respir J. 2010;36(2):277-84.

172. Hooper R, Heinrich J, Omenaas E, Sausenthaler S, Garcia-Larsen V, Bakolis I, et al. Dietary patterns and risk of asthma: results from three countries in European Community Respiratory Health Survey-II. Br J Nutr. 2010;103(9):1354-65.

173. Brigham EP, Steffen LM, London SJ, Boyce D, Diette GB, Hansel NN, et al. Diet Pattern and Respiratory Morbidity in the Atherosclerosis Risk in Communities Study. Annals of the American Thoracic Society. 2018;15(6):675-82.

174. Bakolis I, Hooper R, Bachert C, Lange B, Haahtela T, Keil T, et al. Dietary patterns and respiratory health in adults from nine European countries-Evidence from the GA²LEN study. Clin Exp Allergy. 2018;48(11):1474-82.

175. Steinemann N, Grize L, Pons M, Rothe T, Stolz D, Turk A, et al. Associations between Dietary Patterns and Post-Bronchodilation Lung Function in the SAPALDIA Cohort. Respiration. 2018;95(6):454-63.

176. Keys A. Coronary heart disease in seven countries. Circulation. 1970;41(1):1-211.

177. Godos J, Zappala G, Bernardini S, Giambini I, Bes-Rastrollo M, Martinez-Gonzalez M. Adherence to the Mediterranean diet is inversely associated with metabolic syndrome occurrence: a meta-analysis of observational studies. Int J Food Sci Nutr. 2017;68(2):138-48.

178. Esposito K, Maiorino MI, Ciotola M, Di Palo C, Scognamiglio P, Gicchino M, et al. Effects of a Mediterranean-style diet on the need for antihyperglycemic drug therapy in patients with newly diagnosed type 2 diabetes: a randomized trial. Ann Intern Med. 2009;151(5):306-14.

179. Schwingshackl L, Schwedhelm C, Galbete C, Hoffmann G. Adherence to Mediterranean Diet and Risk of Cancer: An Updated Systematic Review and Meta-Analysis. NUTRIENTS. 2017;9(10):1063.

180. Guenther PM, Reedy J, Krebs-Smith SM. Development of the Healthy Eating Index-2005. J Am Diet Assoc. 2008;108(11):1896-901.

181. Guenther PM, Casavale KO, Reedy J, Kirkpatrick SI, Hiza HA, Kuczynski KJ, et al. Update of the Healthy Eating Index: HEI-2010. J Acad Nutr Diet. 2013;113(4):569-80.

182. Ozbey U, Ucar A, Shivappa N, Hebert JR. The Relationship between Dietary Inflammatory Index, Pulmonary Functions and Asthma Control in Asthmatics. Iranian Journal of Allergy Asthma & Immunology. 2019;18(6):605-14.

183. Han YY, Jerschow E, Forno E, Hua S, Mossavar-Rahmani Y, Perreira KM, et al. Dietary Patterns, Asthma, and Lung Function in the Hispanic Community Health Study/Study of Latinos. Annals of the American Thoracic Society. 2020;17(3):293-301.

184. Papassotiriou I, Shariful Islam SM. Adherence to Mediterranean Diet Is Associated With Lung Function in Older Adults: Data From the Health and Retirement Study. J Am Coll Nutr. 2020:1-6.

185. Bosetti C, Bravi F, Turati F, Edefonti V, Polesel J, Decarli A, et al. Nutrient-based dietary patterns and pancreatic cancer risk. Ann Epidemiol. 2013;23(3):124-8.

186. Deneo-Pellegrini H, Boffetta P, De Stefani E, Correa P, Ronco AL, Acosta G, et al. Nutrient-

based dietary patterns of head and neck squamous cell cancer: a factor analysis in Uruguay. Cancer Causes Control. 2013;24(6):1167-74.

187. Edefonti V, Bravi F, Garavello W, La Vecchia C, Parpinel M, Franceschi S, et al. Nutrient-based dietary patterns and laryngeal cancer: evidence from an exploratory factor analysis. Cancer Epidemiol Biomarkers Prev. 2010;19(1):18-27.

188. Edefonti V, La Vecchia C, Di Maso M, Crispo A, Polesel J, Libra M, et al. Association between nutrient-based dietary patterns and bladder cancer in Italy. NUTRIENTS. 2020;12(6).

189. Prinelli F, Fratiglioni L, Musicco M, Johansson I, Adorni F, Shakersain B, et al. The impact of nutrient-based dietary patterns on cognitive decline in older adults. Clin Nutr. 2019;38(6):2813-20.

190. Varraso R, Chiuve SE, Fung TT, Barr RG, Hu FB, Willett WC, et al. Alternate Healthy Eating Index 2010 and risk of chronic obstructive pulmonary disease among US women and men: prospective study. BMJ. 2015;350:h286.

191. Varraso R, Fung TT, Barr RG, Hu FB, Willett W, Camargo CA, Jr. Prospective study of dietary patterns and chronic obstructive pulmonary disease among US women. Am J Clin Nutr. 2007;86(2):488-95.

192. Varraso R, Fung TT, Hu FB, Willett W, Camargo CA. Prospective study of dietary patterns and chronic obstructive pulmonary disease among US men. Thorax. 2007;62(9):786-91.

193. Varraso R, Kauffmann F, Leynaert B, Le Moual N, Boutron-Ruault MC, Clavel-Chapelon F, et al. Dietary patterns and asthma in the E3N study. Eur Respir J. 2009;33(1):33-41.

194. Bakolis I, Hooper R, Thompson RL, Shaheen SO. Dietary patterns and adult asthma: population-based case-control study. Allergy. 2010;65(5):606-15.

195. Butler LM, Koh WP, Lee HP, Tseng M, Yu MC, London SJ, et al. Prospective study of dietary patterns and persistent cough with phlegm among Chinese Singaporeans. Am J Respir Crit Care Med. 2006;173(3):264-70.

196. Barros R, Moreira A, Padrao P, Teixeira VH, Carvalho P, Delgado L, et al. Dietary patterns and asthma prevalence, incidence and control. Clin Exp Allergy. 2015;45(11):1673-80.

197. Bedard A, Garcia-Aymerich J, Sanchez M, Le Moual N, Clavel-Chapelon F, Boutron-Ruault MC, et al. Confirmatory Factor Analysis Compared with Principal Component Analysis to Derive Dietary Patterns: A Longitudinal Study in Adult Women. J Nutr. 2015;145(7):1559-68.

198. Brigham EP, Kolahdooz F, Hansel N, Breysse PN, Davis M, Sharma S, et al. Association between Western diet pattern and adult asthma: a focused review. Ann Allergy Asthma Immunol. 2015;114(4):273-80.

199. Lv N, Xiao L, Ma J. Dietary pattern and asthma: a systematic review and meta-analysis. J Asthma Allergy. 2014;7:105-21.

200. Maisonneuve P, Shivappa N, Hebert JR, Bellomi M, Rampinelli C, Bertolotti R, et al. Dietary inflammatory index and risk of lung cancer and other respiratory conditions among heavy smokers in the COSMOS screening study. Eur J Nutr. 2016;55(3):1069-79.

201. Shivappa N, Blair CK, Prizment AE, Jacobs DR, Jr., Steck SE, Hebert JR. Association between inflammatory potential of diet and mortality in the Iowa Women's Health study. Eur J Nutr. 2016;55(4):1491-502.

202. Ardestani ME, Onvani S, Esmailzadeh A, Feizi A, Azadbakht L. Adherence to Dietary Approaches to Stop Hypertension (DASH) Dietary Pattern in Relation to Chronic Obstructive

Pulmonary Disease (COPD): A Case-Control Study. J Am Coll Nutr. 2017;36(7):549-55.

203. Zheng PF, Shu L, Si CJ, Zhang XY, Yu XL, Gao W. Dietary Patterns and Chronic Obstructive Pulmonary Disease: A Meta-analysis. Copd: Journal of Chronic Obstructive Pulmonary Disease. 2016;13(4):515-22.

204. Allen S, Britton JR, Leonardi-Bee JA. Association between antioxidant vitamins and asthma outcome measures: systematic review and meta-analysis. Thorax. 2009;64(7):610-9.

205. Hanson C, Rutten EP, Wouters EF, Rennard S. Diet and vitamin D as risk factors for lung impairment and COPD. Transl Res. 2013;162(4):219-36.

206. Zhai T, Li S, Hu W, Li D, Leng S. Potential Micronutrients and Phytochemicals against the Pathogenesis of Chronic Obstructive Pulmonary Disease and Lung Cancer. NUTRIENTS. 2018;10(7):25.

207. Tsiligianni IG, van der Molen T. A systematic review of the role of vitamin insufficiencies and supplementation in COPD. Respir Res. 2010;11:171-8.

208. Thyagarajan B, K AM, Smith LJ, Beckett WS, Williams OD, Gross MD, et al. Serum carotenoid concentrations predict lung function evolution in young adults: the Coronary Artery Risk Development in Young Adults (CARDIA) study. Am J Clin Nutr. 2011;94(5):1211-8.

209. Guenegou A, Leynaert B, Pin I, Le Moel G, Zureik M, Neukirch F. Serum carotenoids, vitamins A and E, and 8 year lung function decline in a general population. Thorax. 2006;61(4):320-6.

210. Paiva SAR, Godoy I, Vannucchi H, Favaro RMD, Geraldo RRC, Campana AO. Assessment of vitamin A status in chronic obstructive pulmonary disease patients and healthy smokers. Am J Clin Nutr. 1996;64(6):928-34.

211. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet. 2002;360 North American Edition(9326):23-33.

212. Joshi P, Kim WJ, Lee SA. The effect of dietary antioxidant on the COPD risk: the community-based KoGES (Ansan–Anseong) cohort. International Journal of COPD. 2015(Issue 1):2159.

213. McKeever TM, Scrivener S, Broadfield E, Jones Z, Britton J, Lewis SA. Prospective Study of Diet and Decline in Lung Function in a General Population. Am J Respir Crit Care Med. 2002;165(9):1299-303.

214. Chen R, Tunstall-Pedoe H, Bolton-Smith C, Hannah MK, Morrison C. Association of Dietary Antioxidants and Waist Circumference with Pulmonary Function and Airway Obstruction. Am J Epidemiol. 2001;153(2):157-63.

215. McKeever TM, Lewis SA, Smit HA, Burney P, Cassano PA, Britton J. A multivariate analysis of serum nutrient levels and lung function. Respir Res. 2008;9:67.

216. Hanson C, Lyden E, Furtado J, Campos H, Sparrow D, Vokonas P, et al. Serum tocopherol levels and vitamin E intake are associated with lung function in the normative aging study. Clin Nutr. 2016;35(1):169-74.

217. Schunemann HJ, McCann S, Grant BJ, Trevisan M, Muti P, Freudenheim JL. Lung function in relation to intake of carotenoids and other antioxidant vitamins in a population-based study. Am J Epidemiol. 2002;155(5):463-71.

218. Britton JR, Pavord ID, Richards KA, Knox AJ, Wisniewski AF, Lewis SA, et al. Dietary antioxidant vitamin intake and lung function in the general population. Am J Respir Crit Care Med. 1995;151(5):1383-7.

219. Nadeem A, Raj HG, Chhabra SK. Effect of vitamin E supplementation with standard treatment on oxidant-antioxidant status in chronic obstructive pulmonary disease. Indian J Med Res. 2008;128(6):705-11.

220. Daga MK, Chhabra R, Sharma B, Mishra TK. Effects of exogenous vitamin E supplementation on the levels of oxidants and antioxidants in chronic obstructive pulmonary disease. J Biosci. 2003;28(1):7-11.

221. Romieu I, Meneses F, Ramirez M, Ruiz S, Padilla RP, Sienra JJ, et al. Antioxidant Supplementation and Respiratory Functions among Workers Exposed to High Levels of Ozone. Am J Respir Crit Care Med. 1998;158(1):226-32.

222. Grievink L, Zijlstra AG, Ke X, Brunekreef B. Double-blind intervention trial on modulation of ozone effects on pulmonary function by antioxidant supplements. Am J Epidemiol. 1999;149(4):306-14.

223. Soutar A, Seaton A, Brown K. Bronchial reactivity and dietary antioxidants. Thorax. 1997;52(2):166-70.

224. Chatham MD, Eppler JH, Jr., Sauder LR, Green D, Kulle TJ. Evaluation of the effects of vitamin C on ozone-induced bronchoconstriction in normal subjects. Ann N Y Acad Sci. 1987;498:269-79.

225. Schachter EN, Schlesinger A. The attenuation of exercise-induced bronchospasm by ascorbic acid. Ann Allergy. 1982;49(3):146-51.

226. Zuskin E, Lewis AJ, Bouhuys A. Inhibition of histamine-induced airway constriction by ascorbic acid. J Allergy Clin Immunol. 1973;51(4):218-26.

227. Malo JL, Cartier A, Pineau L, L'Archeveque J, Ghezzo H, Martin RR. Lack of acute effects of ascorbic acid on spirometry and airway responsiveness to histamine in subjects with asthma. J Allergy Clin Immunol. 1986;78(6):1153-8.

228. Mohsenin V, Dubois AB, Douglas JS. Effect of ascorbic acid on response to methacholine challenge in asthmatic subjects. Am Rev Respir Dis. 1983;127(2):143-7.

229. Black PN, Scragg R. Relationship between serum 25-hydroxyvitamin d and pulmonary function in the third national health and nutrition examination survey. Chest. 2005;128(6):3792-8.

230. Semba RD, Chang SS, Sun K, Cappola AR, Ferrucci L, Fried LP. Serum 25-hydroxyvitamin D and pulmonary function in older disabled community-dwelling women. J Gerontol A Biol Sci Med Sci. 2012;67(6):683-9.

231. Shaheen SO, Jameson KA, Robinson SM, Boucher BJ, Syddall HE, Sayer AA, et al. Relationship of vitamin D status to adult lung function and COPD. Thorax. 2011;66(8):692-8.

232. Liu J, Dong YQ, Yin J, Yao J, Shen J, Sheng GJ, et al. Meta-analysis of vitamin D and lung function in patients with asthma. Respir Res. 2019;20(1):161.

233. Leng S, Picchi MA, Tesfaigzi Y, Wu G, Gauderman WJ, Xu F, et al. Dietary nutrients associated with preservation of lung function in Hispanic and non-Hispanic white smokers from New Mexico. International Journal of COPD. 2017:3171.

234. Kunisaki KM, Niewoehner DE, Singh RJ, Connett JE. Vitamin D status and longitudinal lung function decline in the Lung Health Study. Eur Respir J. 2011;37(2):238-43.

235. Lange NE, Sparrow D, Vokonas P, Litonjua AA. Vitamin D deficiency, smoking, and lung function in the Normative Aging Study. Am J Respir Crit Care Med. 2012;186(7):616-21.

236. Whitehead L. Vitamin D for the management of asthma: A Cochrane Review Summary. Int J Nurs Stud. 2017;73:105-6.

237. Jolliffe DA, Greenberg L, Hooper RL, Mathyssen C, Rafiq R, de Jongh RT, et al. Vitamin D to prevent exacerbations of COPD: systematic review and meta-analysis of individual participant data from randomised controlled trials. Thorax. 2019;74(4):337-45.

238. Sutherland ER, Goleva E, Jackson LP, Stevens AD, Leung DY. Vitamin D levels, lung function, and steroid response in adult asthma. Am J Respir Crit Care Med. 2010;181(7):699-704.

239. Sharp DS, Rodriguez BL, Shahar E, Hwang LJ, Burchfiel CM. Fish consumption may limit the damage of smoking on the lung. 1994.

240. Shahar E, Folsom AR, Melnick SL, Tockman MS, Comstock GW, Gennaro V, et al. Dietary n-3 polyunsaturated fatty acids and smoking-related chronic obstructive pulmonary disease. Atherosclerosis Risk in Communities Study Investigators.[Reprint of Am J Epidemiol. 2008 Oct 1;168(7):796-801; PMID: 18820272]. N Engl J Med. 1994;331(4):228-33.

241. Wood LG, Attia J, McElduff P, McEvoy M, Gibson PG. Assessment of dietary fat intake and innate immune activation as risk factors for impaired lung function. Eur J Clin Nutr. 2010;64(8):818-25.

242. McKeever TM, Lewis SA, Cassano PA, Ocke M, Burney P, Britton J, et al. The relation between dietary intake of individual fatty acids, FEV1 and respiratory disease in Dutch adults. Thorax. 2008;63(3):208-14.

243. Matsuyama W, Mitsuyama H, Watanabe M, Oonakahara K, Higashimoto I, Osame M, et al. Effects of omega-3 polyunsaturated fatty acids on inflammatory markers in COPD. Chest. 2005;128(6):3817-27.

244. Fulton AS, Hill AM, Williams MT, Howe PR, Frith PA, Wood LG, et al. Feasibility of omega-3 fatty acid supplementation as an adjunct therapy for people with chronic obstructive pulmonary disease: study protocol for a randomized controlled trial. Trials [Electronic Resource]. 2013;14:107.

245. Li J, Xun P, Zamora D, Sood A, Liu K, Daviglus M, et al. Intakes of long-chain omega-3 (n-3) PUFAs and fish in relation to incidence of asthma among American young adults: the CARDIA study. Am J Clin Nutr. 2013;97(1):173-8.

246. Nagel G, Linseisen J. Dietary intake of fatty acids, antioxidants and selected food groups and asthma in adults. Eur J Clin Nutr. 2005;59(1):8-15.

247. Yang H, Xun P, He K. Fish and fish oil intake in relation to risk of asthma: a systematic review and meta-analysis. PLoS ONE [Electronic Resource]. 2013;8(11):e80048.

248. de Luis DA, Armentia A, Aller R, Asensio A, Sedano E, Izaola O, et al. Dietary intake in patients with asthma: a case control study. Nutrition. 2005;21(3):320-4.

249. Troisi RJ, Willett WC, Weiss ST, Trichopoulos D, Rosner B, Speizer FE. A prospective study of diet and adult-onset asthma. Am J Respir Crit Care Med. 1995;151(5):1401-8.

250. Shahar E, Boland LL, Folsom AR, Tockman MS, McGovern PG, Eckfeldt JH. Docosahexaenoic acid and smoking-related chronic obstructive pulmonary disease. The Atherosclerosis Risk in Communities Study Investigators. Am J Respir Crit Care Med. 1999;159(6):1780-5.

251. Woods RK, Thien FC, Abramson MJ. Dietary marine fatty acids (fish oil) for asthma. Cochrane Database Syst Rev. 2000(4):CD001283.

252. Brannan JD, Bood J, Alkhabaz A, Balgoma D, Otis J, Delin I, et al. The effect of omega-3 fatty

acids on bronchial hyperresponsiveness, sputum eosinophilia, and mast cell mediators in asthma. Chest. 2015;147(2):397-405.

253. Kompauer I, Demmelmair H, Koletzko B, Bolte G, Linseisen J, Heinrich J. Association of fatty acids in serum phospholipids with lung function and bronchial hyperresponsiveness in adults. Eur J Epidemiol. 2008;23(3):175-90.

254. Okamoto M, Mitsunobu F, Ashida K, Mifune T, Hosaki Y, Tsugeno H, et al. Effects of dietary supplementation with n-3 fatty acids compared with n-6 fatty acids on bronchial asthma. Intern Med. 2000;39(2):107-11.

255. Adams S, Lopata AL, Smuts CM, Baatjies R, Jeebhay MF. Relationship between serum omega-3 fatty acid and asthma endpoints. International Journal of Environmental Research & Public Health [Electronic Resource]. 2018;16(1):25.

256. Kitz R, Rose MA, Schubert R, Beermann C, Kaufmann A, Bohles HJ, et al. Omega-3 polyunsaturated fatty acids and bronchial inflammation in grass pollen allergy after allergen challenge. Respir Med. 2010;104(12):1793-8.

257. Arm JP, Horton CE, Mencia-Huerta JM, House F, Eiser NM, Clark TJ, et al. Effect of dietary supplementation with fish oil lipids on mild asthma. Thorax. 1988;43(2):84-92.

258. Arm JP, Horton CE, Spur BW, Mencia-Huerta JM, Lee TH. The effects of dietary supplementation with fish oil lipids on the airways response to inhaled allergen in bronchial asthma. Am Rev Respir Dis. 1989;139(6):1395-400.

259. Thien FC, Mencia-Huerta JM, Lee TH. Dietary fish oil effects on seasonal hay fever and asthma in pollen-sensitive subjects. Am Rev Respir Dis. 1993;147(5):1138-43.

260. Villani F, Comazzi R, De Maria P, Galimberti M. Effect of dietary supplementation with polyunsaturated fatty acids on bronchial hyperreactivity in subjects with seasonal asthma. Respiration. 1998;65(4):265-9.

261. Mickleborough TD, Murray RL, Ionescu AA, Lindley MR. Fish oil supplementation reduces severity of exercise-induced bronchoconstriction in elite athletes. Am J Respir Crit Care Med. 2003;168(10):1181-9.

262. Matheson MC, Abramson MJ, Allen K, Benke G, Burgess JA, Dowty JG, et al. Cohort Profile: The Tasmanian Longitudinal Health Study (TAHS). Int J Epidemiol. 2016.

263. Abramson M, Matheson M, Wharton C, Sim M, Walters EH. Prevalence of respiratory symptoms related to chronic obstructive pulmonary disease and asthma among middle aged and older adults. Respirology. 2002;7(4):325-31.

264. Matheson MC, Abeysena C, Raven JM, Skoric B, Johns DP, Abramson MJ, et al. How have we been managing chronic obstructive pulmonary disease in Australia? Intern Med J. 2006;36(2):92-9.

265. Textor J. Drawing and analyzing causal DAGs with DAGitty 2015 [cited 2018 1 May]. Available from: http://www.dagitty.net/manual-2.x.pdf.

266. Webb P, Bain C. Essential epidemiology : an introduction for students and health professionals. 2nd ed. ed: Cambridge University Press; 2011.

267. Han YY, Forno E, Celedon JC. Sex Steroid Hormones and Asthma in a Nationwide Study of U.S. Adults. Am J Respir Crit Care Med. 2020;201(2):158-66.

268. DeBoer MD, Phillips BR, Mauger DT, Zein J, Erzurum SC, Fitzpatrick AM, et al. Effects of endogenous sex hormones on lung function and symptom control in adolescents with asthma.

BMC Pulm Med. 2018;18(1):58.

269. Peters U, Dixon AE, Forno E. Obesity and asthma. J Allergy Clin Immunol. 2018;141(4):1169-79.

270. Hanson C, Rutten EP, Wouters EF, Rennard S. Influence of diet and obesity on COPD development and outcomes. International Journal of Copd. 2014;9:723-33.

271. Ulrik CS. Outcome of asthma: longitudinal changes in lung function. Eur Respir J. 1999;13(4):904-18.

272. Boulet LP. Airway remodeling in asthma: update on mechanisms and therapeutic approaches. Curr Opin Pulm Med. 2018;24(1):56-62.

273. Burgess JA, Matheson MC, Diao F, Johns DP, Erbas B, Lowe AJ, et al. Bronchial hyperresponsiveness and obesity in middle age: insights from an Australian cohort. Eur Respir J. 2017;50(3).

274. Dobson AJ, Blijlevens R, Alexander HM, Croce N, Heller RF, Higginbotham N, et al. Short fat questionnaire: a self-administered measure of fat-intake behaviour. Aust J Public Health. 1993;17(2):144-9.

275. Yaroch AL, Tooze J, Thompson FE, Blanck HM, Thompson OM, Colón-Ramos U, et al. Evaluation of Three Short Dietary Instruments to Assess Fruit and Vegetable Intake: The National Cancer Institute's Food Attitudes and Behaviors Survey. J Acad Nutr Diet. 2012;112(10):1570-7.

276. Cook A, Roberts K, O'Leary F, Allman-Farinelli MA. Comparison of single questions and brief questionnaire with longer validated food frequency questionnaire to assess adequate fruit and vegetable intake. Nutrition. 2015;31(7-8):941-7.

277. Rutishauser IHE. Evaluation of short dietary questions from the 1995 national nutrition survey. Canberra: Health and Aged Care; 2001.

278. Resnicow K, Odom E, Wang T, Dudley WN, Mitchell D, Vaughan R, et al. Validation of Three Food Frequency Questionnaires and 24-Hour Recalls with Serum Carotenoid Levels in a Sample of African-American Adults. Am J Epidemiol. 2000;152(11):1072-80.

279. Coyne T, Ibiebele TI, McNaughton S, Rutishauser IH, O'Dea K, Hodge AM, et al. Evaluation of brief dietary questions to estimate vegetable and fruit consumption - using serum carotenoids and red-cell folate. Public Health Nutr. 2005;8(3):298-308.

280. Campbell DR, Gross MD, Martini MC, Grandits GA, Slavin JL, Potter JD. Plasma carotenoids as biomarkers of vegetable and fruit intake. Cancer Epidemiology Biomarkers & Prevention. 1994;3(6):493.

281. Polsinelli ML, Rock CL, Henderson SA, Drewnowski A. Plasma Carotenoids as Biomarkers of Fruit and Vegetable Servings in Women. J Am Diet Assoc. 1998;98(2):194-6.

282. Bogers RP, Dagnelie PC, Westerterp KR, Kester ADM, van Klaveren JD, Bast A, et al. Using a Correction Factor to Correct for Overreporting in a Food-Frequency Questionnaire Does Not Improve Biomarker-Assessed Validity of Estimates for Fruit and Vegetable Consumption. The Journal of Nutrition. 2003;133(4):1213-9.

283. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26(2):319-38.

284. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, et al. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. Am J Respir Crit Care

Med. 2000;161(1):309-29.

285. World Health Organisation. Factsheet: Obesity and overweight 2016 [cited 2017 18 September]. Available from: http://www.who.int/mediacentre/factsheets/fs311/en/.

286. Matheson MC, Benke G, Raven J, Sim MR, Kromhout H, Vermeulen R, et al. Biological dust exposure in the workplace is a risk factor for chronic obstructive pulmonary disease. Thorax. 2005;60(8):645-51.

287. Matheson MC, Ellis JA, Raven J, Johns DP, Walters EH, Abramson MJ. Beta2-adrenergic receptor polymorphisms are associated with asthma and COPD in adults. J Hum Genet. 2006;51(11):943-51.

288. American Thoracic Society. Standardization of Spirometry, 1994 Update. American Thoracic Society. Am J Respir Crit Care Med. 1995;152(3):1107-36.

289. Giles GG, Ireland PD. Dietary Questionnaire for Epidemiological Studies (Version 2). Melbourne: Cancer Council Victoria; 1996.

290. Hodge A, Patterson AJ, Brown WJ, Ireland P, Giles G. The Anti Cancer Council of Victoria FFQ: relative validity of nutrient intakes compared with weighed food records in young to middle-aged women in a study of iron supplementation.[Erratum appears in Aust N Z J Public Health. 2003 Aug;27(4):468]. Aust N Z J Public Health. 2000;24(6):576-83.

291. Steinemann N, Grize L, Ziesemer K, Kauf P, Probst-Hensch N, Brombach C. Relative validation of a food frequency questionnaire to estimate food intake in an adult population. Food Nutr Res. 2017;61(1):1305193.

292. Subar AF, Thompson FE, Kipnis V, Midthune D, Hurwitz P, McNutt S, et al. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires : the Eating at America's Table Study. Am J Epidemiol. 2001;154(12):1089-99.

293. Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol. 1992;135(10):1114-26; discussion 27-36.

294. Peat JK, Salome CM, Woolcock AJ. Factors associated with bronchial hyperresponsiveness in Australian adults and children. Eur Respir J. 1992;5(8):921-9.

295. Arshad SH, Kurukulaaratchy RJ, Fenn M, Matthews S. Early life risk factors for current wheeze, asthma, and bronchial hyperresponsiveness at 10 years of age. Chest. 2005;127(2):502-8.

296. Leynaert B, Bousquet J, Henry C, Liard R, Neukirch F. Is bronchial hyperresponsiveness more frequent in women than in men? A population-based study. Am J Respir Crit Care Med. 1997;156(5):1413-20.

297. Litonjua AA, Sparrow D, Weiss ST. The FEF25-75/FVC ratio is associated with methacholine airway responsiveness. The normative aging study. Am J Respir Crit Care Med. 1999;159(5 Pt 1):1574-9.

298. Lau S, Illi S, Sommerfeld C, Niggemann B, Bergmann R, von Mutius E, et al. Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. Lancet. 2000;356(9239):1392.

299. O'Connor G, Sparrow D, Taylor D, Segal M, Weiss S. Analysis of dose-response curves to methacholine. An approach suitable for population studies. Am Rev Respir Dis. 1987;136(6):1412-7.

300. Britton J, Pavord I, Richards K, Knox A, Wisniewski A, Wahedna I, et al. Factors influencing the

occurrence of airway hyperreactivity in the general population: the importance of atopy and airway calibre. Eur Respir J. 1994;7(5):881-7.

301. Rijcken B, Schouten JP, Weiss ST, Speizer FE, van der Lende R. The relationship between airway responsiveness to histamine and pulmonary function level in a random population sample. Am Rev Respir Dis. 1988;137(4):826-32.

302. Trigg CJ, Bennett JB, Tooley M, Sibbald B, D'Souza MF, Davies RJ. A general practice based survey of bronchial hyperresponsiveness and its relation to symptoms, sex, age, atopy, and smoking. Thorax. 1990;45(11):866-72.

303. Bakke PS, Baste V, Gulsvik A. Bronchial responsiveness in a Norwegian community. Am Rev Respir Dis. 1991;143(2):317-22.

304. Paoletti P, Carrozzi L, Viegi G, Modena P, Ballerin L, Di Pede F, et al. Distribution of bronchial responsiveness in a general population: effect of sex, age, smoking, and level of pulmonary function. Am J Respir Crit Care Med. 1995;151(6):1770-7.

305. Sparrow D, O'Connor G, Colton T, Barry CL, Weiss ST. The relationship of nonspecific bronchial responsiveness to the occurrence of respiratory symptoms and decreased levels of pulmonary function. The Normative Aging Study. Am Rev Respir Dis. 1987;135(6):1255-60.

306. Schwartz J, Schindler C, Zemp E, Perruchoud AP, Zellweger JP, Wuthrich B, et al. Predictors of methacholine responsiveness in a general population. Chest. 2002;122(3):812-20.

307. Cockcroft DW, Killian DN, Mellon JJ, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. Clin Allergy. 1977;7(3):235-43.

308. Wassmer G, Jörres RA, Heinrich J, Wjst M, Reitmeir P, Wichmann HE. The association between baseline lung function and bronchial responsiveness to methacholine. Eur J Med Res. 1997;2(2):47-54.

309. Hopp RJ, Weiss SJ, Nair NM, Bewtra AK, Townley RG. Interpretation of the results of methacholine inhalation challenge tests. J Allergy Clin Immunol. 1987;80(6):821-30.

310. Aerts JG, Bogaard JM, Overbeek SE, Verbraak AF, Thio P. Extrapolation of methacholine log-dose response curves with a Cumulative Gaussian Distribution function. Eur Respir J. 1994;7(5):895-900.

311. de Marco R, Bugiani M, Zanolin E, Verlato G, Rijcken B. Doses and models in risk assessment analysis for bronchial hyperresponsiveness. J Clin Epidemiol. 1998;51(1):19-28.

312. Gurrin LC, Scurrah KJ, Hazelton ML. Tutorial in biostatistics: spline smoothing with linear mixed models. Stat Med. 2005;24(21):3361-81.

313. Coates AL, Wanger J, Cockcroft DW, Culver BH, Bronchoprovocation Testing Task Force: Kai-Hakon C, Diamant Z, et al. ERS technical standard on bronchial challenge testing: general considerations and performance of methacholine challenge tests. Eur Respir J. 2017;49(5):05.

314. Abramson MJ, Saunders NA, Hensley MJ. Analysis of bronchial reactivity in epidemiological studies. Thorax. 1990;45(12):924-9.

315. Verbeke G, Molenberghs G. Linear mixed models for longitudinal data: Springer; 2009.

316. De Livera AM, Zaloumis S, Simpson JA. Models for the analysis of repeated continuous outcome measures in clinical trials. Respirology. 2014;19(2):155-61.

317. Kutner MH, Nachtsheim C, Neter J. Applied linear statistical models. 5th ed. Boston: McGraw-Hill Irwin; 2005.

318. Giugliano D, Ceriello A, Esposito K. The effects of diet on inflammation: Emphasis on the metabolic syndrome. J Am Coll Cardiol. 2006;48(4):677-85.

319. King DE, Egan BM, Geesey ME. Relation of dietary fat and fiber to elevation of C-reactive protein. The American Journal of Cardiology. 2003;92(11):1335-9.

320. Gao X, Bermudez OI, Tucker KL. Plasma C-reactive protein and homocysteine concentrations are related to frequent fruit and vegetable intake in Hispanic and non-Hispanic white elders. J Nutr. 2004;134(4):913-8.

321. Brighenti F, Valtueña S, Pellegrini N, Ardigò D, Del Rio D, Salvatore S, et al. Total antioxidant capacity of the diet is inversely and independently related to plasma concentration of high-sensitivity C-reactive protein in adult Italian subjects. Br J Nutr. 2005;93(5):619-25.

322. van Herpen-Broekmans WM, Klopping-Ketelaars IA, Bots ML, Kluft C, Princen H, Hendriks HF, et al. Serum carotenoids and vitamins in relation to markers of endothelial function and inflammation. Eur J Epidemiol. 2004;19(10):915-21.

323. Ajani UA, Ford ES, Mokdad AH. Dietary fiber and C-reactive protein: findings from national health and nutrition examination survey data. J Nutr. 2004;134(5):1181-5.

324. Watzl B, Kulling SE, Moseneder J, Barth SW, Bub A. A 4-wk intervention with high intake of carotenoid-rich vegetables and fruit reduces plasma C-reactive protein in healthy, nonsmoking men. Am J Clin Nutr. 2005;82(5):1052-8.

325. James AL, Wenzel S. Clinical relevance of airway remodelling in airway diseases. Eur Respir J. 2007;30(1):134-55.

326. Fixman ED, Stewart A, Martin JG. Basic mechanisms of development of airway structural changes in asthma. Eur Respir J. 2007;29(2):379-89.

327. Garcia-Larsen V, Arthur R, Potts JF, Howarth PH, Ahlstrom M, Haahtela T, et al. Is fruit and vegetable intake associated with asthma or chronic rhino-sinusitis in European adults? Results from the Global Allergy and Asthma Network of Excellence (GA²LEN) Survey. Clinical and Translational Allergy. 2017;7:3.

328. St-Laurent J, Bergeron C, Page N, Couture C, Laviolette M, Boulet LP. Influence of smoking on airway inflammation and remodelling in asthma. Clin Exp Allergy. 2008;38(10):1582-9.

329. Amin K, Ekberg-Jansson A, Lofdahl CG, Venge P. Relationship between inflammatory cells and structural changes in the lungs of asymptomatic and never smokers: a biopsy study. Thorax. 2003;58(2):135-42.

330. Kan H, Stevens J, Heiss G, Rose KM, London SJ. Dietary fiber, lung function, and chronic obstructive pulmonary disease in the atherosclerosis risk in communities study. Am J Epidemiol. 2008;167(5):570-8.

331. Jiang R, Jacobs DR, He K, Hoffman E, Hankinson J, Nettleton JA, et al. Associations of dairy intake with CT lung density and lung function. J Am Coll Nutr. 2010;29(5):494-502.

332. Rice JL, Romero KM, Galvez Davila RM, Meza CT, Bilderback A, Williams DL, et al. Association Between Adherence to the Mediterranean Diet and Asthma in Peruvian Children. Lung. 2015;193(6):893-9.

333. Jolliffe IT. Principal Component Analysis. [electronic resource]. New York, NY: Springer; 1986.

334. Rhee JJ, Sampson L, Cho E, Hughes MD, Hu FB, Willett WC. Comparison of methods to account for implausible reporting of energy intake in epidemiologic studies. Am J Epidemiol. 2015;181(4):225-33.

335. Hodge AM, English DR, O'Dea K, Giles GG. Glycemic index and dietary fiber and the risk of type 2 diabetes. Diabetes Care. 2004;27(11):2701-6.

336. Food Standards Australia and New Zealand. NUTTAB95 Nutrient Data Table for Use in Australia. Canberra1995.

337. Rosato V, Edefonti V, Bravi F, Bosetti C, Bertuccio P, Talamini R, et al. Nutrient-based dietary patterns and prostate cancer risk: a case-control study from Italy. Cancer Causes Control. 2014;25(4):525-32.

338. de Koning L, Malik VS, Kellogg MD, Rimm EB, Willett WC, Hu FB. Sweetened beverage consumption, incident coronary heart disease, and biomarkers of risk in men. Circulation. 2012;125(14):1735-41, S1.

339. Hert KA, Fisk PS, 2nd, Rhee YS, Brunt AR. Decreased consumption of sugar-sweetened beverages improved selected biomarkers of chronic disease risk among US adults: 1999 to 2010. Nutr Res. 2014;34(1):58-65.

340. Schulze MB, Hoffmann K, Manson JE, Willett WC, Meigs JB, Weikert C, et al. Dietary pattern, inflammation, and incidence of type 2 diabetes in women. Am J Clin Nutr. 2005;82(3):675-84.

341. Willett W. Nutritional epidemiology: New York : Oxford University Press, c2013.

3rd ed.; 2013.

342. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med. 2003;348(26):2599-608.

343. Kalhan R, Tran BT, Colangelo LA, Rosenberg SR, Liu K, Thyagarajan B, et al. Systemic Inflammation in Young Adults Is Associated with Abnormal Lung Function in Middle Age. PLoS One. 2010;5(7):1-8.

344. Thorleifsson SJ, Margretardottir OB, Gudmundsson G, Olafsson I, Benediktsdottir B, Janson C, et al. Chronic airflow obstruction and markers of systemic inflammation: Results from the BOLD study in Iceland. Respir Med. 2009;103(10):1548-53.

345. Han YY, Jerschow E, Forno E, Hua S, Mossavar-Rahmani Y, Perreira K, et al. Dietary Patterns, Asthma, and Lung Function in the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). Annals of the American Thoracic Society. 2019;05:05.

346. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A Biol Sci Med Sci. 2014;69 Suppl 1:S4-9.

347. Ullman A, Svedmyr N. Salmeterol, a new long acting inhaled beta 2 adrenoceptor agonist: comparison with salbutamol in adult asthmatic patients. Thorax. 1988;43(9):674-8.

348. Adams NP, Bestall JB, Malouf R, Lasserson TJ, Jones PW. Inhaled beclomethasone versus placebo for chronic asthma. Cochrane Database Syst Rev. 2005(1):CD002738.

349. Rosenthal RR, Busse WW, Kemp JP, Baker JW, Kalberg C, Emmett A, et al. Effect of Long-term Salmeterol Therapy Compared With As-Needed Albuterol Use on Airway Hyperresponsiveness*. Chest. 1999;116(3):595-602.

350. Geovanini GR, Libby P. Atherosclerosis and inflammation: overview and updates. Clin Sci (Lond). 2018;132(12):1243-52.

351. Liu CL, Wang Y, Liao M, Santos MM, Fernandes C, Sukhova GK, et al. Allergic lung inflammation promotes atherosclerosis in apolipoprotein E-deficient mice. Transl Res. 2016;171:1-16.

352. Decramer M, Janssens W. Chronic obstructive pulmonary disease and comorbidities. The Lancet Respiratory Medicine. 2013;1(1):73-83.

353. Boulet LP, Sterk PJ. Airway remodelling: the future. Eur Respir J. 2007;30(5):831-4.

354. Elias JA, Zhu Z, Chupp G, Homer RJ. Airway remodeling in asthma. J Clin Invest. 1999;104(8):1001-6.

355. Busse WW. The relationship of airway hyperresponsiveness and airway inflammation: Airway hyperresponsiveness in asthma: its measurement and clinical significance. Chest. 2010;138(2 Suppl):4S-10S.

356. Institute of Medicine. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. [electronic resource]. Washington, DC: National Academy Press; 2002.

357. Horadagoda C, Dinihan T, Roberts M, Kairaitis K. Body composition and micronutrient deficiencies in patients with an acute exacerbation of chronic obstructive pulmonary disease. Intern Med J. 2017;47(9):1057-63.

358. Paulin FV, Zagatto AM, Chiappa GR, Muller PT. Addition of vitamin B12 to exercise training improves cycle ergometer endurance in advanced COPD patients: A randomized and controlled study. Respir Med. 2017;122:23-9.

359. Steinke JW, Borish L. Th2 cytokines and asthma. Interleukin-4: its role in the pathogenesis of asthma, and targeting it for asthma treatment with interleukin-4 receptor antagonists. Respir Res. 2001;2(2):66-70.

360. Riffo-Vasquez Y, Spina D. Role of cytokines and chemokines in bronchial hyperresponsiveness and airway inflammation. Pharmacol Ther. 2002;94(3):185-211.

361. Shivappa N, Steck SE, Hurley TG, Hussey JR, Ma Y, Ockene IS, et al. A population-based dietary inflammatory index predicts levels of C-reactive protein in the Seasonal Variation of Blood Cholesterol Study (SEASONS). Public Health Nutr. 2014;17(8):1825-33.

362. Cavicchia PP, Steck SE, Hurley TG, Hussey JR, Ma Y, Ockene IS, et al. A new dietary inflammatory index predicts interval changes in serum high-sensitivity C-reactive protein. J Nutr. 2009;139(12):2365-72.

363. Sears MR, Taylor DR, Print CG, Lake DC, Li Q, Flannery EM, et al. Regular inhaled beta-agonist treatment in bronchial asthma. The Lancet. 1990(8728):1391.

364. Crane J, Flatt A, Jackson R, Ball M, Pearce N, Burgess C, et al. Prescribed fenoterol and death from asthma in New Zealand, 1981-83; case-control study. The Lancet. 1989;333(8644):917-22.

365. Walter O S, Samy S, Pierre E, Ralph I H, Brian H, Donald C, et al. The Use of β-Agonists and the Risk of Death and near Death from Asthma. The New England Journal of Medicine. 1992;326(8):501-6.

366. Taylor DR, Sears MR, Herbison GP, Flannery EM, Print CG, Lake DC, et al. Regular inhaled beta agonist in asthma: effects on exacerbations and lung function. Thorax. 1993;48(2):134-8.

367. Martinez FD. Safety of long-acting beta-agonists--an urgent need to clear the air. N Engl J Med. 2005;353(25):2637-9.

368. Khianey R, Oppenheimer J. Controversies regarding long-acting [beta]2-agonists. Curr Opin Allergy Clin Immunol. 2011;11(4):345-54.

Appendices

Appendix 1: TAHS diet questionnaire

IDnumber :	Initials:
DOB:	Family ID:

Short Fat Questionnaire

Tick the box that best describes your usual eating habits

1. How often do you eat fried food with batter or breadcrumb coating?

❒ 6 or more times per week
❒ 3-5 times per week
❒ 1-2 times per week
❒ Less than once a week
❒ Never

2. How often do you eat gravy, cream sauces or cheese sauces?

❒ 6 or more times per week
❒ 3-5 times per week
❒ 1-2 times per week
❒ Less than once a week
❒ Never

3. How often do you add butter, margarine, oil or sour cream to vegetables, rice, spaghetti?

❒ 6 or more times per week
❒ 3-5 times per week
❒ 1-2 times per week
❒ Less than once a week
❒ Never

4. How often do you eat vegetables that are fried or roasted with fat or oil?

❒ 6 or more times per week
❒ 3-5 times per week
❒ 1-2 times per week
❒ Less than once a week
❒ Never

5. How many times a week do you eat meat pies, sausages, salami, burgers or bacon?
❒ 6 or more times per week
❒ 3-5 times per week
❒ 1-2 times per week
❒ Less than once a week
❒ Never

6. How often do you eat pastries, cakes, sweet biscuits or croissants?

❒ 6 or more times per week
❒ 3-5 times per week
❒ 1-2 times per week
❒ Less than once a week
❒ Never

7. How many times a week do you eat hot chips?

❒ 6 or more times per week
❒ 3-5 times per week
❒ 1-2 times per week
❒ Less than once a week
❒ Never

8. How is your meat usually cooked?

❒ Fried
❒ Stewed
❒ Grilled/roasted with added fat/oil
❒ Grilled/roasted without fat/oil
❒ Eat meat occasionally or never

9. How do you spread butter/marg on bread?

- ❒ Thickly
- ❒ Medium
- ❒ Thinly
- ❒ Never use butter/marg

10. How many times a week do you eat chocolate or sweet snack bars?

- ❒ 6 or more times per week
- ❒ 3-5 times per week
- ❒ 1-2 times per week
- ❒ Less than once a week
- ❒ Never

11. How many times a week do you eat crisps, corn chips or nuts?

- ❒ 6 or more times per week
- ❒ 3-5 times per week
- ❒ 1-2 times per week
- ❒ Less than once a week
- ❒ Never

12. How often do you eat cream?

- ❒ 6 or more times per week
- ❒ 3-5 times per week
- ❒ 1-2 times per week
- ❒ Less than once a week
- ❒ Never

13. How often do you eat more than a small serve of ice cream?

- ❒ 6 or more times per week
- ❒ 3-5 times per week
- ❒ 1-2 times per week
- ❒ Less than once a week
- ❒ Never

14. How often do you eat more than a small piece of cheese (exclude low fat cheese)?

- ❒ 6 or more times per week
- ❒ 3-5 times per week
- ❒ 1-2 times per week
- ❒ Less than once a week
- ❒ Never

15. What type of milk do you use on breakfast cereal or in cooking?

- ❒ Cow or goat's milk
- ❒ Soy milk

16. What form of milk in Q15 do you consume?

- ❒ Condensed or evaporated
- ❒ Full cream
- ❒ Full cream and reduced fat
- ❒ Reduced fat
- ❒ Skim

17. How much skin on chicken do you eat?

- ❒ Most or all of the skin
- ❒ Some of the skin
- ❒ None of the skin /I am vegetarian

18. How much of the fat on meat do you eat?

- ❒ Most or all of the fat
- ❒ Some of the fat
- ❒ No fat /I am vegetarian

19. How many serves of fruit (fresh, canned, frozen) do you usually eat each day? (a serve is what fits into the palm of your hand)

- ❒ I don't eat fruit
- ❒ Less than I serving per day
- ❒ 1 serving per day
- ❒ 2 servings per day
- ❒ 3 servings per day
- ❒ 4 or more servings per day

20. How many serves of vegetables (fresh, canned, frozen) do you usually eat each day? (a serve is what fits into the palm of your hand)

- ❒ I don't eat vegetables
- ❒ Less than I serving per day
- ❒ 1 serving per day
- ❒ 2 servings per day
- ❒ 3 servings per day
- ❒ 4 or more servings per day

Thank you for your assistance with this Questionnaire

Appendix 2: Other TAHS 2010 follow up questionnaires

The Tasmanian Longitudinal Health Study

What are the causes of increased airway sensitivity in middle-age?

Thank you for your help with this medical research.
All information you provide is kept strictly confidential.
If you have any questions please call **1800 110 711** (free call in Australia)

Current Contact Details:

Current name:	
DOB:	
IDnumber:	FamilyID:
Current address:	

Please provide your phone numbers:

Home: ( )

Work: ( )

Mobile:

Today's date:

1

For questions where the subject has to choose one answer, read out the question and all the categories before expecting them to respond. Definitions of ambiguous terms are given in text boxes. To standardise the questionnaire administration across centres, avoid providing any further explanation other than what is given in the questionnaire

GENERAL DEMOGRAPHIC INFORMATION

A. What is your height without shoes?
(Answer either in feet & inches **OR** centimeters.)

☐☐ *feet and* ☐ *inches*

OR

☐☐☐ *Centimeters*

B. What is your weight?
(Answer either in stone & pounds **OR** in kilograms.)

☐☐ *stone and* ☐☐ *pounds*

OR

☐☐☐ *kilograms*

C. What is the highest educational or vocational qualification that you have completed? ***(tick one only)***

- ☐ Grade 1 to 6
- ☐ Grade 7 to 9
- ☐ Grade 10 or 11
- ☐ Grade 12 or equivalent (eg. Higher School Certificate)
- ☐ Trade/ Apprenticeship (eg. Hairdresser, electrician, plumber)
- ☐ Certificate or Diploma (eg. Child care, technician etc)
- ☐ University degree (eg. Bachelor)
- ☐ Higher University degree (eg. Graduate diploma, masters, PhD)

D. Are you currently employed (including self-employed)?
(tick one only)

- ☐ Yes
- ☐ No
- ☐ No, studying
- ☐ No, retired

E. What is/was your main occupation? ***(tick one only)***

Manager or administrator ..... ☐
(eg. Magistrate, general manager, school principal, director of nursing)

Professional ..... ☐
(eg. Scientist, nurse, allied health professional, teacher, artist)

Associate professional ..... ☐
(eg. Technician, manager, police officer, small business owner)

Tradesperson or related worker ..... ☐
(eg. Hairdresser, gardener, florist)

Advanced clerical or service worker ..... ☐
(eg. Secretary, flight attendant, law clerk, personal assistant)

Intermediate clerical, sales, service worker ..... ☐
(eg. Administration worker, child care worker, nursing assistant, hospitality worker)

Intermediate production or transport worker ..... ☐
(eg. Machine operator, bus driver, sewing machinist)

Elementary clerical, sales or service worker ..... ☐
(eg. Filing/mail clerk, parking inspector, sales assistant, housekeeper)

Labourer or related worker ..... ☐
(eg. Cleaner, factory worker, farm hand, kitchen hand)

House person..... ☐

F. What is your martial status? *(tick one only)*

☐ Never married = 1
☐ Widowed = 2
☐ Divorced = 3
☐ Separated but not divorced = 4
☐ De facto = 5
☐ Married = 6

HOME ENVIRONMENT

First I am going to ask few questions about your home and the area where you live.

1a. What is your current postcode? ☐☐☐☐

1. Have you changed residence from when we last interviewed you (interviews were between 2004 and 2008)? ❒ No ❒ Yes

If no, go to Q2

If Yes☞ **1.1** For how many years have you lived in your present home? *(<1year code as 1)* ☐☐

1.2 In which decade was your present home built? *Tick one* ☑

- ❒ Before 1940
- ❒ 1941-1960
- ❒ 1961-1970
- ❒ 1971-1980
- ❒ 1981-1990
- ❒ 1990- Present
- ❒ Don't know

1.3 What is the base structure of your home? *Tick one* ☑

- ❒ Concrete slab
- ❒ Stumps and wooden flooring
- ❒ Combination of above
- ❒ Don't know

2. How old is the mattress in your bed? *Tick one* ☑

- ❒ Less than 12 months old
- ❒ 1-5 years old
- ❒ More than 5 years old
- ❒ Don't know
- ❒ Not relevant (e.g. waterbed)

3. Is there fitted carpet in the bedroom? ❒ No ❒ Yes

If Yes☞ 3.1 What is the age of the carpet? *Tick one*

- ❒ Less than 12 months old
- ❒ 1 - 5 years old
- ❒ More than 5 years old
- ❒ Don't know

4. On average, how often is your bedroom vacuumed, or if it has a hard floor, how often is it swept or mopped? (*Tick one)*

- ❒ 5 or more times each week
- ❒ 2-4 times each week
- ❒ Once a week
- ❒ Less than once a week but more than once a month
- ❒ Once a month or less

5. On average, how often is your bedroom aired by opening windows for at least for 1-hour? (*Tick one)*

- ❒ 5 or more times each week
- ❒ 2-4 times each week
- ❒ Once a week
- ❒ Less than once a week but more than once a month
- ❒ Once a month or less

6. Which types of heating do you use at home? *(Tick all that apply)*

- ❒ Gas ducted central heating
- ❒ Coal or wood fire
- ❒ Gas room heater
- ❒ Electric heater (eg: radiator, fan or Dimplex type)
- ❒ Other central heating (eg: electric hydronic, slab floor)
- ❒ Reverse cycle air-conditioning
- ❒ Other? Specify _____

7. What kind of stove do you mostly use for cooking?

- ❒ Gas
- ❒ Electric
- ❒ Coal, coke or wood
- ❒ Other? Specify _____

7.1 Do you have an exhaust fan over the stove? ❒ No ❒ Yes

If no, go to Q8

If yes ☞ 7.2 When cooking how often do you use the fan?

- ❒ All of the time
- ❒ Some of the time
- ❒ None of the time

7.3 Does the fan take the fumes outside the house? ❒ No ❒ Yes

8. Has there ever been mould or mildew on any surface, other than food, in your home? ❒ No ❒ Yes

If no, go to Q9

If yes ☞ 8.1 Which rooms have been affected?

- ❒ Bathrooms
- ❒ Living rooms
- ❒ Your bedroom
- ❒ Kitchen
- ❒ Other bedrooms
- ❒ Any other area/s

8.2 Has there ever been mould or mildew on any surface, other than food, in your home in the last 12 months? ❒ No ❒ Yes

9. Do you keep or own any cats? ❒ No ❒ Yes

If no, go to Q10

If yes ☞ 9.1 How many? Number ☐☐

9.2 Are the cats **allowed** indoors? ❒ No ❒ Yes

If no, go to Q10

If yes ☞ 9.2.1 Are the cats **allowed** in the bedroom? ❒ No ❒ Yes

10. Has there been a cat in the house in the last 12 months? ❒ No ❒ Yes ❒ DK

11. Do you keep or own any dogs? ❒ No ❒ Yes

If no, go to Q12

If yes ☞ 11.1 How many? Number ☐☐

11.2 Are the dogs **allowed** indoors? ❒ No ❒ Yes

If no, go to Q12

If yes ☞ 11.2.1 Are the dogs **allowed** in the bedroom? ❒ No ❒ Yes

12. Has there been a dog in the house in the last 12 months? ❒ No ❒ Yes ❒ DK

CHILDHOOD ENVIRONMENT

13. What term best describes the place you lived most of the time when you were under the age of five years? *Tick one*

- ❒ Farm
- ❒ Country town
- ❒ Suburb of a city
- ❒ Inner city
- ❒ Don't know

14. How many of your brothers, sisters or other children regularly slept in your bedroom before you were five years old, not including yourself?

☐☐ (number of sibs)

15. Did you have a serious respiratory infection before the age of five years?

❒ No ❒ Yes

16. Did you go to school, pre-school, kindergarten, or a day care centre before the age of five years? ❒ No ❒ Yes

17. At what age did you first attend a school, pre-school, kindergarten, or day care?

☐☐ (age in years)

18. Did your father smoke:

During the first year of your life?	❒ No	❒ Yes	❒ DK
When you were aged 1-4 years?	❒ No	❒ Yes	❒ DK
When you were aged 5-15 years?	❒ No	❒ Yes	❒ DK

19. Did your mother smoke:

During the first year of your life?	❒ No	❒ Yes	❒ DK
When you were aged 1-4 years?	❒ No	❒ Yes	❒ DK
When you were aged 5-15 years?	❒ No	❒ Yes	❒ DK

20. Was there a cat in your home:

During the first year of your life?	❒ No	❒ Yes	❒ DK
When you were aged 1-4 years?	❒ No	❒ Yes	❒ DK
When you were aged 5-15 years?	❒ No	❒ Yes	❒ DK

21. Did you have carpet (or a rug) covering the floor in your bedroom:

During the first year of your life?	❒ No	❒ Yes	❒ DK
When you were aged 1-4 years?	❒ No	❒ Yes	❒ DK
When you were aged 5-15 years?	❒ No	❒ Yes	❒ DK

22. What was the main type of heating your home had when you were under the age of five years? (tick all that apply)

- ❒ Gas ducted central heating
- ❒ Coal or wood fire
- ❒ Gas room heater
- ❒ Electric heater (eg. Radiator, fan or dimplex-type)
- ❒ Other central heating (eg. Electric, hydronic, slab floor)
- ❒ Reverse cycle air-conditioning
- ❒ Other
- ❒ No heating

SMOKING

23. In your lifetime, have you smoked at least 100 cigarettes or equal amounts of cigars, pipes or any tobacco product?

❒ No ❒ Yes

If no, go to Q24

If yes ☞ 23.1 How old were you when you started smoking? ☐☐ (Age in years)

23.2 Do you currently smoke (within the last 4 weeks)?

- ❒ Not at all ☞ Go to Q23.4
- ❒ Yes, daily ☞ Go to Q 23.3
- ❒ Yes, at least weekly ☞ Go to Q 23.3
- ❒ Yes, less than weekly ☞ Go to Q 23.3

23.3 On average, how much do you currently smoke (total number of cigarettes or equivalent product)? *Provide the average number per day or per week or per month*

☐☐ per day ☞ Go to Q24
or
☐☐ per week ☞ Go to Q24
or
☐☐ per month ☞ Go to Q24

23.4 How old were you when you stopped smoking? ☐☐ (Age in years)

23.5 On average, during periods when you smoked, how much did you smoke (total number of cigarettes or equivalent product)? *Provide the average number per day or per week or per month*

☐☐ per day

or

☐☐ per week
☐☐ or
per month

24. Not counting yourself, how many people in your household currently smoke regularly (most days of the week) inside the house? (number) ☐☐

25. On average, how many hours per day are you exposed to other people's tobacco smoke (work and home)? (hours per day) ☐☐

RESPIRATORY AND ALLERGY SYMPTOMS

ECZEMA

26. Have you ever had eczema or any kind of skin allergy? ❒ No ❒ Yes

27. Have you ever had an itchy rash that was coming and going for at least 6 months? ❒ No ❒ Yes

If yes ☞ 27.1 How old were you when you first had this itchy rash? ☐☐ (Age in years)

27.2 Have you had this itchy rash in the last 12 months? ❒ No ❒ Yes

27.3 Has this rash at any time affected any of the following places: *(tick all that apply)*

- ❒ Folds of the elbows
- ❒ Behind the knees
- ❒ In front of the ankles
- ❒ Under the buttocks
- ❒ Around the neck, ears or eyes
- ❒ None of the above

HAY FEVER

28. Have you ever had hay fever or nasal allergies (that is sneezing, running or blocked nose when you do not have a cold or the flu)? ❒ No ❒ Yes

If yes ☞ 28.1 Have you had this problem in the last 12 months? ❒ No ❒ Yes

28.2 Was this problem accompanied by itchy or watery eyes?

❒ No ❒ Yes

28.3 How old were you when you first had hayfever or nasal allergies?

☐☐ (Age in years)

29. When you are near animals, such as cats, dogs, or horses; near feathers, including pillows, quilts or doonas; or in a dusty part of the house, do you ever: (*tick all that apply*)

- ❒ Start to cough?
- ❒ Start to wheeze?
- ❒ Get a feeling of tightness in the chest?
- ❒ Start to feel short of breath?
- ❒ Get a runny or stuffy nose or start to sneeze?
- ❒ Get itchy or watery eyes?

30. When you are near trees, grass or flowers, or when there is a lot of pollen about, do you ever:

- ❒ Start to cough?
- ❒ Start to wheeze?
- ❒ Get a feeling of tightness in the chest?
- ❒ Start to feel short of breath?
- ❒ Get a runny or stuffy nose or start to sneeze?
- ❒ Get itchy or watery eyes?

If yes to any of the above☞ 30.1 At which time of the year does this happen?

- ❒ Winter
- ❒ Spring
- ❒ Summer
- ❒ Autumn

FOOD ALLERGY

31. Have you ever had any food allergies? ❒ No ❒ Yes

If yes ☞

	Peanut	Tree nut	Shellfish	Fish	Cow's milk	Egg	Wheat*	Sesame	Other
What food?									
Was it confirmed by a doctor?	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
What age did you develop it? (years)	____	____	____	____	____	____	____	____	____
Are you still allergic to it?	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Have you been prescribed an Epipen?	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Specify the name/s of Tree Nut or other food if relevant		____							____

*include Coeliac disease

RESPIRATORY AND SLEEP CONDITIONS

32. Have you, at anytime in your life, suffered from attacks of asthma of wheezy breathing? (Regard asthma and wheezy breathing as being much the same thing for this question.)

❒ No ❒ Yes

If yes ☞ 32.1 How old were you when you had your first attack of asthma or wheezy breathing? ☐☐ *(age in years)*

32.2 How old were you when you had your most recent attack of asthma or wheezy breathing? ☐☐ *(age in years)*

32.3 Have you had an attack of asthma or wheezy breathing in the last 12 months? ❒ No ❒ Yes

32.4 Have you taken any medicines including inhalers or tablets for asthma or wheezy breathing in the last 12 months? ❒ No ❒ Yes

33. Have you had wheezing or whistling in your chest in the last 12 months?
(Wheezing means a whistling sound, however high or low pitched and however faint.)

❒ No ❒ Yes

If yes ☞ 33.1 Have you been at all breathless when the wheezing noise was present? ❒ No ❒ Yes

33.2 Have you had this wheezing or whistling when you did not have a cold? ❒ No ❒ Yes

34. Have you, at any time in the last 12 months, had an attack of shortness of breath at rest? ❒ No ❒ Yes

35. Have you, at any time in the last 12 months, had an attack of shortness of breath after exercise? ❒ No ❒ Yes

36. Have you, at any time in the last 12 months, woken due to a feeling of tightness in your chest? ❒ No ❒ Yes

36.1 During the last month, do you or have you been told you snore loudly in sleep? ❒ No ❒ Yes ❒ Don't know

If yes ☞ 36.1.1 On average, how often? *Tick one*☑

- ❒ Rarely, less than once a week
- ❒ 1 – 2 times per week
- ❒ 3 – 4 times per week
- ❒ 5 – 7 times per week
- ❒ Don't know

36.2 During the last month, do you or have you been told you snort or gasp in sleep? ❒ No ❒ Yes ❒ Don't know

If yes ☞ 36.2.1 On average, how often? *Tick one*☑

- ❒ Rarely, less than once a week
- ❒ 1 – 2 times per week
- ❒ 3 – 4 times per week
- ❒ 5 – 7 times per week
- ❒ Don't know

36.3 During the last month, do you or have you been told you choke or stop breathing in sleep? ❒ No ❒ Yes ❒ Don't know

If yes ☞ 36.3.1 On average, how often? *Tick one*☑

- ❒ Rarely, less than once a week
- ❒ 1 – 2 times per week
- ❒ 3 – 4 times per week
- ❒ 5 – 7 times per week
- ❒ Don't know

36.4 During the last month, have you had excessive sleepiness during the day? ❒ No ❒ Yes

37. Have you, at any time in the last 12 months, been woken at night by an attack of shortness of breath? ❒ No ❒ Yes

38. Do you usually cough when you do not have a cold? ❒ No ❒ Yes

If yes ☞ 38.1 Are there months in which you cough on most days? ❒ No ❒ Yes

If yes ☞ 38.1.1 Do you cough on most days for at least three months of each year? ❒ No ❒ Yes

38.1.2 For how many years have you had this cough?
- ❒ Less than 2 years
- ❒ 2 – 5 years
- ❒ More than 5 years

39. Do you usually have phlegm in your chest when you do not have a cold? ❒ No ❒ Yes

If yes ☞ 39.1 Are there months in which you have phlegm in your chest on most days? ❒ No ❒ Yes

If yes ☞ 39.1.1 Do you bring up this phlegm on most days for at least three months of each year? ❒ No ❒ Yes

39.1.2 For how many years have you had this phlegm?
- ❒ Less than 2 years
- ❒ 2 – 5 years
- ❒ More than 5 years

40. Have you, at anytime in your life, suffered from cough with phlegm in the chest (with or without a cold)? ❒ No ❒ Yes

If yes ☞ 40.1 Have you had this cough with phlegm on most days for at least three months and for two years in a row? ❒ No ❒ Yes

41. Have you at any time in your life suffered from attacks of bronchitis or attacks of cough with sputum (phlegm) in the chest ("loose" or "rattly" cough)? ❒ No ❒ Yes

If yes ☞ 41.1 How long is it since the last attack
- ❒<=6months
- ❒<=1 year but > 6 months
- ❒<=2 years but > 1 year
- ❒> 2 years

41.2 At what age did these attacks begin? ☐☐ *(age in years)*

42. Has your doctor ever told you that you have or had chronic bronchitis?

❒ No ❒ Yes

If yes ☞ 42.1 How old were you when you were told you had chronic bronchitis? ☐☐ *(age in years)*

42.2 Have you taken any medicine (including inhalers or tablets) for chronic bronchitis in the last three months?

❒ No ❒ Yes

43. Has your doctor ever told you that you have or had emphysema?

❒ No ❒ Yes

If yes ☞ 43.1 How old were you when you were told you had emphysema? ☐☐ *(age in years)*

43.2 Have you taken any medicine (including inhalers or tablets) for emphysema in the last three months?

❒ No ❒ Yes

44. Has your doctor ever told you that you have or had chronic obstructive pulmonary disease (COPD) or chronic obstructive airways disease (COAD)?

❒ No ❒ Yes

If yes ☞ 44.1 How old were you when you were told you had chronic obstructive pulmonary disease? ☐☐ *(age in years)*

44.2 Have you taken any medicine (including inhalers or tablets) for chronic obstructive pulmonary disease in the last three months?

❒ No ❒ Yes

45. Has your doctor ever told you that you have or had obstructive sleep apnoea?

❒ No ❒ Yes

If yes ☞ 45.1 How old were you when you were told you had obstructive sleep apnoea? ☐☐ *(age in years)*

45.2 Are you currently being treated for obstructive sleep apnoea with CPAP, surgery, adequate weight loss or other device?

❒ No ❒ Yes

CHEST COLDS AND CHEST ILLNESSES

46. If you get a cold, does it usually go to your chest? (Usually means more than half of the time)

❒ No ❒ Yes ❒ Do not get colds

47. During the past 3 years, have you had any chest illnesses that have kept you off work, indoors at home, or in bed?

❒ No ❒ Yes

If yes ☞ 47.1 Did you produce phlegm with any of the chest illnesses?

❒ No ❒ Yes

47.2. In the last 3 years, how many such illnesses, with increased phlegm, did you have which lasted a week or more? (if none enter zero)

Number of illnesses ☐☐

48. Have you ever had Pneumonia?

❒ No ❒ Yes

If yes ☞ 48.1 Was this confirmed by a doctor?

❒ No ❒ Yes

48.2 At what age did you first have it? ☐☐ Age in *years*

48.3 How many times have you had it? ☐☐ number

48.4 Have you ever been hospitalised for Pneumonia?

❒ No ❒ Yes

49. Have you ever been hospitalised for any other chest illness?

❒ No ❒ Yes

If yes ☞ please specify

	Diagnosis	Age at first occurrence	No. occurrences
1			
2			
3			

ASTHMA

Now I am going to ask few detailed questions about asthma that you may or may not have.

50. Have you ever had asthma ? ❒ No ❒ Yes

If no, go to Q51

If yes continue

50.1 How old were you when you had your first symptoms of asthma? (Age in years) ☐☐

50.2 Was this confirmed by a doctor? ❒ No ❒ Yes

If yes ☞ 50.2 How old were you when this was confirmed? ☐☐ *years old*

50.3 How old were you when you had your most recent symptoms of asthma? *years old* ☐☐

Symptom Severity

50.4. Have you been woken from your sleep by your asthma?

last 12 months		*last 1 month*	
❒ No	❒ Yes	❒ No	❒ Yes

If Yes to last month:
49.4.1 How many nights were you woken from sleep by your asthma the last week?

☐☐ *No. of nights*

50.5. Have you had asthma symptoms when you wake in the morning?

last 12 months		*last 1 month*	
❒ No	❒ Yes	❒ No	❒ Yes

If Yes to last month:
50.5.1 How many mornings in the last week? ☐☐ *No. of mornings*

50.6 Have you been limited in any of the following activities because of asthma?

Tick the appropriate boxes in each category:

	last 12 months		*last 1 month*	
50.6.1 All activities	❒ No	❒ Yes	❒ No	❒ Yes
50.6.2 When dressing	❒ No	❒ Yes	❒ No	❒ Yes
50.6.3 Walking on level ground	❒ No	❒ Yes	❒ No	❒ Yes
50.6.4 Hurrying on level ground	❒ No	❒ Yes	❒ No	❒ Yes
50.6.5 Walking up stairs or up hills	❒ No	❒ Yes	❒ No	❒ Yes
50.6.6 Active sports	❒ No	❒ Yes	❒ No	❒ Yes
50.6.7 Other (Specify) _____	❒ No	❒ Yes	❒ No	❒ Yes

☞ *If Yes to any of* 50*.6.1 to* 50*.6.7, go to* 50*.7.*
If all were No, answer 50*.6.8 first.*

50.6.8 Would you agree or disagree with the following statement:

"My asthma has not limited any of my activities."

last 12 months		*last month*	
❒ agree	❒ disagree	❒ agree	❒ disagree

50.7. How frequent have your asthma symptoms (of any severity) been?

Tick one

- ❒ No asthma in the past 12 months
- ❒ Asthma symptoms in the past 12 months but not in the last month
- ❒ Asthma symptoms in the last month, but not frequent (less than once per week)
- ❒ Frequent (once per week or more but not daily) in the last 1 month
- ❒ Persistent (daily)

☞ *If 'No asthma in the past 12 months', go to* 50*.14. If any asthma in last 12 months continue*

50.8. How frequent have your asthma attacks/flare ups been over the past 12 months?

An "attack" or "flare up" of asthma is a period of time when asthma symptoms are worse or more frequent than usual. One could have asthma symptoms regularly or intermittently without getting attacks or flare-ups.

Tick one

- ❒ None in the past 12 months
- ❒ 3 or less in the past 12 months
- ❒ 4 or more but less than monthly
- ❒ More than monthly in the last 12 months
- ❒ More than weekly or persistent
- ❒ Not sure

50.9. Have you had an episode of asthma which has made you unable to speak or severe enough to limit your speech to only 1 or 2 words between breaths?

last 12 months		*last 1 month*	
❒	❒	❒	❒
No	Yes	No	Yes

50.10. On average, how would **you** rate the severity of your asthma?

	last 12 months *tick one*	*last 1 month* *tick one*
Not severe at all	❒	❒
Mild	❒	❒
Moderate	❒	❒
Severe	❒	❒
Not sure	❒	❒

Events

50.11. Have you lost any days from work, school or usual activities because of your asthma?

last 12 months		*last 1 month*	
❒	❒	❒	❒
No	Yes	No	Yes

If yes ☞: 50.11.1. How many? ☐☐ *last 12 months* ☐☐ *last 1 month*

50.12. Have you had an attack or symptoms of asthma that was so bad, you needed to call your general practitioner, ambulance, emergency locum or 24 hour clinic?

last 12 months		*last 1 month*	
❒	❒	❒	❒
No	Yes	No	Yes

If yes ☞ 50.12.1. How many? ☐☐ *last 12 months* ☐☐ *last 1 month*

50.13. Have you had an attack or symptoms of asthma that was so bad you had to go to a hospital emergency or casualty department?

	last 12 months		*last 1 month*	
	❒ No	❒ Yes	❒ No	❒ Yes
If yes ☞ 50.13.1. How many?	☐☐ *last 12 months*		☐☐ *last 1 month*	

50.14. Have you ever been admitted to a hospital because of your asthma? ❒ No ❒ Yes

If no, go to Q50.15

If yes ☞ 50.14.1 In the past 12 months? ❒ No ❒ Yes

If yes ☞ 50.14.1.1 How many times in the last 12 months? ☐☐ *Number*

50.14.1.2 How many times in the last 1 month? ☐☐ *Number*

50.15. Have you ever had an attack or symptoms of asthma that resulted in an admission to a hospital intensive care unit? ❒ No ❒ Yes

50.15a How frequently have you seen the following health professionals for your asthma in the last 12 months? (enter zero if not at all)

1) General Practitioner (No. of times last 12 months) ☐☐ *Number*

2) Respiratory specialist (No. of times last 12 months) ☐☐ *Number*

3) Nurse/Asthma educator (No. of times last 12 months) ☐☐ *Number*

4) Pharmacist (No. of times last 12 months) ☐☐ *Number*

50.16 Have you ever been given a demonstration on the correct use of your metered dose inhaler? ❒ No ❒ Yes

If yes 50.16.1 In the last 12 months? ..... ❒ No ❒ Yes

50.17 Has your doctor ever checked your inhaler technique? ❒ No ❒ Yes

If yes 50.17.1 In the last 12 months? ..... ❒ No ❒ Yes

50.18 Do you have written instructions from your doctor on how to manage your asthma if it gets worse or if you have an attack?
..... ❒ No ❒ Yes

If yes 50.18.1 In the last 12 months? ..... ❒ No ❒ Yes

50.19 Has your doctor given you a verbal plan telling you how to manage your asthma if it gets worse or if you have an attack?
..... ❒ No ❒ Yes

If yes 50.19.1 In the last 12 months? ..... ❒ No ❒ Yes

50.20 Do you have a peak flow meter of your own? ..... ❒ No ❒ Yes

If yes 50.20.1 How often have you used it in the last 3 months?

A) Never ❒
B) Some days ❒
C) Most days ❒

50.21 Has your doctor ever measured your breathing in his/her surgery (including peak flows/spirometry/bronchodilator response)? ..... ❒ No ❒ Yes

If yes 50.21.1 In the last 12 months? ..... ❒ No ❒ Yes

FAMILY PREDISPOSITION

I am now going to ask about the respiratory conditions of your family members. It is possible that you are not aware of these details but respond according to what you know about your family.

51. Has your biological mother ever had self reported or doctor diagnosed:

51.1 Asthma? ❒ No ❒ Yes ❒ Don't know

51.2 COPD, COAD, chronic bronchitis or emphysema? ❒ ❒ ❒

No Yes Don't know

52. Has your biological father ever had self reported or doctor diagnosed:

52.1 Asthma? ❒ No ❒ Yes ❒ Don't know

52.2 COPD, COAD, chronic bronchitis or emphysema? ❒ No ❒ Yes ❒ Don't know

53. Do you, or did you, have any biological brothers or sisters? This includes half-brothers and half-sisters, but not step-brothers or step-sisters. ❒ No ❒ Yes ❒ Don't know

If No, go to Q54

If yes ☞ 53.1 How many? ☐☐ Number ❒ Don't know

53.2 How many of your biological brothers or sisters have ever had self reported or doctor diagnosed:

53.2.1 Asthma? ☐☐ Number ❒ Don't know

53.2.2 COPD, COAD, chronic bronchitis, emphysema? ☐☐ Number ❒ Don't know

54. Do you, or did you, have any biological children? ❒ No ❒ Yes

If No, go to Q54 *If Yes continue*

53.1 How many? ☐☐ Number

53.2. How many of them have ever had self reported or doctor diagnosed asthma? ☐☐ Number ❒ Don't know

55. Has any member of your family or close relatives died from asthma? By family and close relatives I mean children, parents, siblings, nephews, nieces, grand parents, first cousins, uncles and aunts ❒ No ❒ Yes ❒ Don't know

If Yes: 55.1 How many? ☐☐ Number

56. Has any member of your family or close relatives died from COPD/COAD/Chronic Bronchitis/Emphysema?

❒ No ❒ Yes ❒ Don't know

If Yes: 56.1 How many? ☐☐ Number

When administering Q57 and Q58:
First ask the main question as given below i.e. "Have you used any inhaled medicines to help your breathing in the last 12 months?"
If the response is yes, get the participant to tell what medication/s he/she has used and find what group of drugs the participant's drug belongs to from the medication list provided with this questionnaire.
Then administer the sub questions under each medication (i.e. if a ***Short Acting beta-2-agonist inhaler is used complete 57.1 to 57.1.2)***

MEDICINES AND INHALERS

57. Have you used any inhaled medicines to help your breathing in the last 12 months? ❒ No ❒ Yes

If No, go to Q58. If Yes, continue - **Which have been used in the last 12 months?**

	Medication 1	Medication 2	Medication 3	Medication 4
57.1 Which one?				
57.2 Which type of inhaler device?				
57.3 Strength/dose per puff (mcg)?				
57.4Are you currently using this medication?	□ Yes □ No	□ Yes □ No	□ Yes □ No	□ Yes □ No
57.5 how long have you been using this med?	_ _ _ _ Days/Mths/Yrs	_ _ _ _ Days/Mths/Yrs	_ _ _ _ Days/Mths/Yrs	_ _ _ _ Days/Mths/Yrs
57.6 Last 12mths, how have you used them:				
57.6a) when needed - Average number of puffs per month	_ _ _ _ (puff/mth)	_ _ _ _ (puff/mth)	_ _ _ _ (puff/mth)	_ _ _ _ (puff/mth)
57.6b) in short courses -Number of courses in last 12 months -Average number of puffs per day during flare-up -Average number of days of flare-up	_ _ _ _ (number) _ _ _ _ (puff/day) _ _ _ _ (days)	_ _ _ _ (number) _ _ _ _ (puff/day) _ _ _ _ (days)	_ _ _ _ (number) _ _ _ _ (puff/day) _ _ _ _ (days)	_ _ _ _ (number) _ _ _ _ (puff/day) _ _ _ _ (days)
57.6c) continuously - Average number of puffs per day	_ _ _ _ (puffs)	_ _ _ _ (puffs)	_ _ _ _ (puffs)	_ _ _ _ (puffs)
57.6d) not at all	□	□	□	□
57.7 Last 1mth, how have you used them:				
57.7a) when needed - Average number of puffs per month	_ _ _ _ (puff/mth)	_ _ _ _ (puff/mth)	_ _ _ _ (puff/mth)	_ _ _ _ (puff/mth)
57.7b) in short courses -Number of courses in last 12 months -Average number of puffs per day during flare-up -Average number of days of flare-up	_ _ _ _ (number) _ _ _ _ (puff/day) _ _ _ _ (days)	_ _ _ _ (number) _ _ _ _ (puff/day) _ _ _ _ (days)	_ _ _ _ (number) _ _ _ _ (puff/day) _ _ _ _ (days)	_ _ _ _ (number) _ _ _ _ (puff/day) _ _ _ _ (days)
57.7c) continuously - Average number of puffs per day	_ _ _ _ (puffs)	_ _ _ _ (puffs)	_ _ _ _ (puffs)	_ _ _ _ (puffs)
57.7d) not at all	□	□	□	□
57.8) Is this medication the same type as prescribed by your doctor?	□ Yes □ No □ DK	□ Yes □ No □ DK	□ Yes □ No □ DK	□ Yes □ No □ DK
57.9) Are you taking this medication at the same dose as prescribed by your doctor?	□ Yes □ No □ DK	□ Yes □ No □ DK	□ Yes □ No □ DK	□ Yes □ No □ DK
57.9a) If no to Q57.8 or Q57.9 why not the same: (tick all that apply)	□ Cost □Side effects □ Symptoms resolved □ forget to take □ Other?Specify _____	□ Cost □Side effects □ Symptoms resolved □ forget to take □ Other?Specify _____	□ Cost □Side effects □ Symptoms resolved □ forget to take □ Other?Specify _____	□ Cost □Side effects □ Symptoms resolved □ forget to take □ Other?Specify _____

58. Have you used any pills, capsules, tablets or medicines, other than inhaled medicines to help your breathing at any time in the last 12 months? ❒ No ❒ Yes

If No go to Q 59, if Yes continue - **Which have been used in the last 12 months?**

	Medication 1	Medication 2	Medication 3	Medication 4
58.1 Which one?				
58.2 Strength/dose per tablet (mg)?				
58.3 Are you currently using this medication?	□ Yes □ No	□ Yes □ No	□ Yes □ No	□ Yes □ No
58.4 how long have you been using this med?	_ _ _ _ Days/Mths/Yrs	_ _ _ _ Days/Mths/Yrs	_ _ _ _ Days/Mths/Yrs	_ _ _ _ Days/Mths/Yrs
58.5 Last 12mths, how have you used them:				
58.5a) when needed - Average number of tablets per month	_ _ _ _ (puff/mth)	_ _ _ _ (puff/mth)	_ _ _ _ (puff/mth)	_ _ _ _ (puff/mth)
58.5b) in short courses - Number of courses in last 12 months - Number of tablets per day during flare-up - Average number of days of flare-up	_ _ _ _ (number) _ _ _ _ (tablets/day) _ _ _ _ (days)	_ _ _ _ (number) _ _ _ _ (tablets/day) _ _ _ _ (days)	_ _ _ _ (number) _ _ _ _ (tablets/day) _ _ _ _ (days)	_ _ _ _ (number) _ _ _ _ (tablets/day) _ _ _ _ (days)
58.5c) continuously - Average number of puffs per day	_ _ _ _ (puffs)	_ _ _ _ (puffs)	_ _ _ _ (puffs)	_ _ _ _ (puffs)
58.5d) not at all	□	□	□	□
58.6 Last 1mth, how have you used them:				
58.6a) when needed - Average number of puffs per month	_ _ _ _ (puff/mth)	_ _ _ _ (puff/mth)	_ _ _ _ (puff/mth)	_ _ _ _ (puff/mth)
58.6b) in short courses - Number of courses in last 12 months - Average number of tablets per day during flare-up - Average number of days of flare-up	_ _ _ _ (number) _ _ _ _ (tablets/day) _ _ _ _ (days)	_ _ _ _ (number) _ _ _ _ (tablets/day) _ _ _ _ (days)	_ _ _ _ (number) _ _ _ _ (tablets/day) _ _ _ _ (days)	_ _ _ _ (number) _ _ _ _ (tablets/day) _ _ _ _ (days)
58.6c) continuously - Average number of puffs per day	_ _ _ _ (puffs)	_ _ _ _ (puffs)	_ _ _ _ (puffs)	_ _ _ _ (puffs)
58.6d) not at all	□	□	□	□
58.7) Is this medication the same type as prescribed by your doctor?	□ Yes □ No □ DK	□ Yes □ No □ DK	□ Yes □ No □ DK	□ Yes □ No □ DK
58.8) Are you taking this medication at the same dose as prescribed by your doctor?	□ Yes □ No □ DK	□ Yes □ No □ DK	□ Yes □ No □ DK	□ Yes □ No □ DK
58.8a) If no to Q58.7 or Q58.8 why not the same: (tick all that apply)	□ Cost □Side effects □ Symptoms resolved □ forget to take □ Other?Specify _____	□ Cost □Side effects □ Symptoms resolved □ forget to take □ Other?Specify _____	□ Cost □Side effects □ Symptoms resolved □ forget to take □ Other?Specify _____	□ Cost □Side effects □ Symptoms resolved □ forget to take □ Other?Specify _____

59. Have you ever used inhaled steroids to help your breathing? ❒ No ❒ Yes

If No go to Q 59, if Yes continue

59.1 At what age did you start using inhaled steroids? ☐☐ years

59.2 In the past 5 years, how many months would you have used inhaled steroids on most days? ☐☐ months

60. Have you ever been prescribed home oxygen therapy? ❒ No ❒ Yes

If no go to Q60. If Yes continue

60.1 Are you currently using oxygen therapy at home? ❒ No ❒ Yes

60.2 For how many years have you been using oxygen therapy at home? ☐☐ Years

60.3 How have you used oxygen therapy during the last month?

60.3.1 For relief of symptoms or when needed ❒ No ❒ Yes

60.3.2 For flare-ups or attacks ❒ No ❒ Yes

60.3.3 Regularly, on a daily basis ❒ No ❒ Yes

61. Have you ever had an influenza vaccination? ❒ No ❒ Yes

If yes: 61.1 Have you been vaccinated for influenza in the last 12months? ❒ No ❒ Yes

62. Have you ever had a pneumonia vaccination? ❒ No ❒ Yes

If yes: 62.1 Have you been vaccinated for pneumonia in the last 5 years? ❒ No ❒ Yes

63. Have you ever been vaccinated or desensitised for allergy? ❒ No ❒ Yes

If yes: 63.1 Have you been vaccinated for allergy in the last 12 months? ❒ No ❒ Yes

64. Have you had any other injections to help your breathing at any time in the last 12 months? ❒ No ❒ Yes

If yes: 64.1 what injections? _____

OTHER CONDITIONS

65. Has a doctor ever told you that you have/had any of the following conditions?

- ❒ Angina, heart attack or myocardial infarction
- ❒ Transient ischaemic attack (TIA) or a stroke
- ❒ High blood pressure or Hypertension
- ❒ High levels of cholesterol/ triglycerides
- ❒ Diabetes or high sugar levels in the blood or urine
- ❒ Cancer
- ❒ Rheumatoid arthritis
- ❒ Psychiatric/ mental health problem
- ❒ Multiple Sclerosis
- ❒ Thyroid Problems
- ❒ Lupus/ Systemic Lupus Erythematosus

If FEMALE please continue ……
If MALE thank you for your assistance with this Questionnaire

FEMALES ONLY

66. Have you ever had a menstrual period? ❒ No ❒ Yes

If yes: 66.1 What was your age when you had your first period? ☐☐ (age in years)

66.2 Have you had a menstrual period in the last 12 months?
- ❒ No ☞ Go to 66.3
- ❒ Yes ☞ Go to 67
- ❒ Don't know ☞ Go to 67

66.3 Have your menstrual periods stopped permanently or only temporarily due to pregnancy, breast feeding or other condition?
- ❒ Stopped permanently ☞ continue
- ❒ Stopped temporarily ☞ Go to 67

66.4 How old were you when your periods stopped permanently? ☐☐ *(age in years)*

67. Have you ever used birth control pills or other hormonal contraceptives (implants or injections)? ❒ No ❒ Yes

If yes: 67.1 At what age did you first use birth control pills or other hormonal contraceptives? (age in years) ☐☐

67.2 Are you currently taking birth control pills or other hormonal contraceptives? ❒ No ☐ Yes

67.3 Over your whole lifetime, in total how many months or years have you taken birth control pills or other hormonal contraceptives?

☐☐ Months OR ☐☐ Years

68. Are you currently pregnant? ❒ No ❒ Yes

69. Have you ever been pregnant in the past? ❒ No ❒ Yes

70. How many live births have you had? ☐☐

71. How many miscarriages or abortions have you had? ☐☐

72. Have you ever had a mammogram? ❒ No ❒ Yes

73. Have you ever taken oestrogen, progesterone or other female hormones for menopause (that is, prescription hormone replacement therapy or HRT)?
The preparation may be pills, injections or skin patches. This question does not include birth control pill or hormonal contraceptives. ❒ No ❒ Yes

If yes, complete HRT section

Thank you for your assistance with this Questionnaire

MONASH UNIVERSITY

UNIVERSITY OF TASMANIA

THE UNIVERSITY OF MELBOURNE

TASMANIAN LONGITUDINAL HEALTH STUDY (TAHS):

What are the causes of increased airway reactivity in middle-age?

Tasmanian Longitudinal Health Study

LAB BOOKLET

Tasmanian Longitudinal Health Study

- Associate Professor Shyamali Dharmage, The University of Melbourne
- Professor E. Haydn Walters, The University of Tasmania
- Professor Michael Abramson, Monash University
- Associate Professor Paul Thomas, The University of New South Wales
- Doctor Bircan Erbas, La Trobe University
- Doctor Melanie Matheson, The University of Melbourne
- Associate Professor David Johns, The University of Tasmania
- Doctor Jim Markos, Launceston General Hospital

Associate Investigators:

- Doctor Stephen Morrison, Royal Brisbane and Women's Hospital
- Associate Professor Richard Wood-Baker, Royal Hobart Hospital
- Doctor Greg Haugh, Launceston General Hospital
- Doctor Ian Feather, Gold Coast Hospital
- Doctor Geza Benke, Monash University
- Associate Professor Justin Walls, The University of Tasmania
- Doctor John Marrone, The University of Tasmania

Participant ID: | | | | | | **Family ID:** | | | | | | |

Participant Initials : | | |

Participant DOB : | | / | | / | | | | |

Appointment Date : | | / | | / | | | | |

Scientist initials | | | **Centre Number** |

(Centre number: Menzies Research Institute = 1, Launceston General Hospital= 2, Burnie (North West Regional) Hospital= 3, Alfred Hospital= 4, Royal Brisbane and Women's Hospital= 5, Gold Coast Hospital= 6, Prince of Wales Hospital= 7)

Participant ID: | | | | | | |

PARTICIPANT CONTACT DETAILS

Please record your full name and contact details below:

Full name: _____

Street address: _____

Suburb: _____ State: _____ Postcode: _____

Home phone: _____

Work phone: _____

Mobile phone: _____

Email address: _____

A lot of money and staff time goes into just trying to locate participants for follow-up research studies. It would be appreciated if you could provide the name and contact details of one person who is not part of your immediate family and who will know your whereabouts in future years (and lives at a different address to you). We would only contact this person if another follow-up study is done in future and we can't locate you through other means. These details will be kept confidential.

Person's name: _____

Relationship: _____

Street address: _____

Suburb: _____ State: _____ Postcode: _____

Home phone: _____

Work phone: _____

Mobile phone:

Email address:

Participant ID: | | | | | | |

LUNG FUNCTION QUESTIONNAIRE

1. Have you had a cigarette (or any other tobacco product) in the last 24 hours? ☐ No ☐ Yes

If yes: How many hours ago was your last smoke? ☐☐ Hours

(NB: if under one hour delay test until one hour – at least – has elapsed)

2. Have you used a puffer or inhaler in the last 24 hours? ☐ No ☐ Yes

If yes: What inhaler(s) did you use and how many hours ago was the last dose taken?

_____ ☐☐ Hours

_____ ☐☐ Hours

(NB: if taken under one hour delay test until one hour – at least – has elapsed)

3. Have you had a respiratory infection in the last 6 weeks? ☐ No ☐ Yes

If yes: How many days ago did it end? ☐☐ Days

(NB: if currently infected delay test until participant is well)

4. Have you taken any medication for breathing (other than inhalers) in the last 24 hours? ☐ No ☐ Yes

If yes: Which medication(s) did you take and how many hours ago was the last dose taken?

_____ ☐☐ Hours

_____ ☐☐ Hours

5. Have you taken an antihistamine (any medication for allergy including hay fever) or cough medicine in the last 72 hours? ☐ No ☐ Yes

If yes: What medicine(s) did you use and how many hours ago was the last dose taken?

_____ ☐☐ Hours

_____ ☐☐ Hours

6. Have you taken any anti-depressants in the last month or hours? ☐ No ☐ Yes

If yes: What medicine(s) did you use and how many hours/day ago was the last dose taken?

_____ ☐☐ Hrs/Days

_____ ☐☐ Hrs/Days

7. How many hours ago did you have your last meal? ☐☐ Hours

Participant ID: | | | | | | |

******NB: IF "YES" TO ANY OF QUESTIONS 8 through to 10**
DO NOT CARRY OUT METHACHOLINE CHALLENGE TESTING****

8. Have you had a heart attack or stroke in the last 3 months? ☐ No ☐ Yes
(If Yes, defer spirometry also for at least 3 months)

8a. Have you been diagnosed as having bronchiectasis
(abnormal or permanent widening of the airways)? ☐ No ☐ Yes

9. Have you taken any medication for high blood pressure, ☐ No ☐ Yes
a heart condition, epilepsy or used eye drops to treat glaucoma
in the last 72 hours?

If yes: Which medication(s) did you take and how many hours ago was the last dose taken?

_____ ☐☐ Hours
_____ ☐☐ Hours
_____ ☐☐ Hours
_____ ☐☐ Hours

NB: If any of the above medications are beta-blockers use Atrovent instead of Ventolin for BD challenge only (you must wait 30mins after administering Atrovent for post PD spirometry).

9a. Do you *s*nore loudly (louder than talking or can be heard through closed doors)? ❒ No ❒ Yes

9b. Do you often feel tired, fatigued, or sleepy during daytime? ❒ No ❒ Yes

9c. Has anyone observed you stop breathing during your sleep? ❒ No ❒ Yes

9d. Do you have or are you being treated for high blood *p*ressure? ❒ No ❒ Yes

IF FEMALE:

10. Are you pregnant or breastfeeding? ☐ No ☐ Yes
(If Yes, still able to carry out BD challenge only using Ventolin)

11. Have you had a menstrual period in the last 12 months? ☐ No ☐ Yes

IF Yes ☞ **11.1** What was the date of your last menstrual period
(or if currently experiencing a period, the first day it began)? Date……

Participant ID: | | | | | | |

ANTHROPOMETRIC DATA

12. Height (cm) Range 00-260cm **13.** Weight (kg) • Range 00-200.0 kg

13a. Neck circumference (cm) : •

14. Waist measurements (cm) : • •

Range 00-260.0 cm (both must be within 2cm otherwise re-measure)

15. Hip measurements (cm) : • •

Range 00-260.0 cm (both must be within 2cm otherwise re-measure)

SPIROMETRY - BASELINE TESTING

16. Baseline FVC, FEV_1 and PEF: (The maximum total number of attempts allowed is nine to record 3 technically satisfactory manoeuvres within 150mls)

	Best attempt 1	Best attempt 2	Best attempt 3
FVC(L)	•	•	•
FEV_1(L)	•	•	•
PEF(L/s)	•	•	•

16a. Best **Baseline FEV_1** (from recorded blows) •

17. Number of rejected attempts

18. Predicted FEV_1 •

19. Best baseline FEV_1 as % of predicted FEV_1 • %

19a. Is best baseline FEV_1 : a) less than 70% predicted (Q19) ? ❒ No ❒ Yes
b) less than 1.5 litres (Q16a) ? ❒ No ❒ Yes

IF YES to (a) or (b) ☞ SKIP METHACHOLINE & GO TO BRONCHODILATOR CHALLENGE (Page 9)

OTHERWISE CONTINUE TO METHOCHOLINE CHALLENGE

Participant ID: | | | | | | |

METHACHOLINE CHALLENGE

20. CONTROL FEV_1 following inhalation of diluent:

20.1 Record 2 technically satisfactory manoeuvres within 150mls

Blow 1	Blow 2
•	•

20.2 Number of rejected attempts

21. Best **CONTROL FEV_1** as a % of **Baseline FEV_1** • %

([Control FEV_1] / [Baseline FEV_1] x 100)

21.1 Is this value less than 90% ? ❒ No ❒ Yes

IF YES ☞ STOP CHALLENGE & GO TO REVERSAL OF BRONCHOCONSTRICTION (PAGE 8)

OTHERWISE CONTINUE WITH METHOCHOLINE CHALLENGE

22. Does the participant have any of the following symptoms (Tick all that apply) :

- ❒ woken with tightness of chest in the last 12 months
- ❒ had an attack of shortness of breath during the day while at rest in the last 12 months
- ❒ been woken by an attack of shortness of breath in the last 12 months
- ❒ trouble with their breathing
- ❒ ever had asthma

IF YES to any of the above questions ☞ Long protocol

22a. Will the long or short protocol be followed? long ❒ short ❒

After each dose, calculate the Post Mch FEV_1 as a % of the CONTROL FEV_1
[Post Mch FEV_1]/ [Control FEV_1] x 100

During Short Protocol:

- ☞ **Change to Long Protocol** if Post Mch FEV_1 falls to 90% of Control FEV_1
- ☞ **Stop Methacholine Challenge** if Post Mch FEV_1 falls to ≤ 80% Control FEV_1

During Long Protocol:

- ☞ **Stop Methacholine Challenge** if Post Mch FEV_1 falls to ≤ 80% Control FEV_1

NOW RECORD **90% of control FEV_1** •

80% of control FEV_1 •

Participant ID: | | | | | | |

METHACHOLINE CHALLENGE - continued

23. METHACHOLINE BATCH NUMBER
(Refer to supplied pharmacy paperwork)

SESSION NUMBER
(Maximum of 6 sessions per batch of methacholine)

ORDER IN SESSION (1 to 6)

Dose Level	**Cumulative dose** (mg)	**Inhalations**		FEV_1	FEV_1	No. rejected attempts
		SHORT	**LONG**			
1	**0.0078**	**-**	**2**	.	.	
2	**0.0156**	**4**	**2**	.	.	
3	**0.0312**	**-**	**1**	.	.	
4	**0.0625**	**3**	**2**	.	.	
5	**0.125**	**-**	**1**	.	.	
6	**0.25**	**3**	**2**	.	.	
7	**0.5**	**-**	**1**	.	.	
8	**1.0**	**3**	**2**	.	.	
9	**2.0**	**4**	**4**	.	.	

Participant ID: | | | | | | |

METHACHOLINE CHALLENGE - continued

24. Why was methacholine challenge stopped? TICK BOX

a) end of test reached (cumulative dose of 2mgs inhaled) 1

b) 20% fall in FEV_1 achieved 2

c) participant's technique not satisfactory 3

d) participant asked to stop 4

e) other (specify) _____ 5

REVERSAL OF BRONCHOCONSTRICTION

25. Record the first 2 technically satisfactory maneuvers

	Best attempt 1	Best attempt 2
FVC(L)	.	.
FEV_1(L)	.	.
PEF(L/s)	.	.

25.1 Number of rejected attempts

26. Best POST-BRONCHODILATOR FEV_1 as % of BASELINE FEV1 . %

NO YES

27. Has the subject's FEV_1 returned to within 10% of baseline measurement?

Ensure subject's lung function is within 10% of baseline measurement before he/she leaves the testing centre

Participant ID: | | | | | | |

BRONCHODILATOR CHALLENGE

28. Record the first 2 technically satisfactory maneuvers

	Best attempt 1	Best attempt 2
FVC(L)	•	•
FEV_1(L)	•	•
PEF(L/s)	•	•

28.1 Number of rejected attempts

CHECK LIST

29.1 Consent ❒ No ❒ Yes

29.2 Dietary fat questionnaire collected ❒ No ❒ Yes

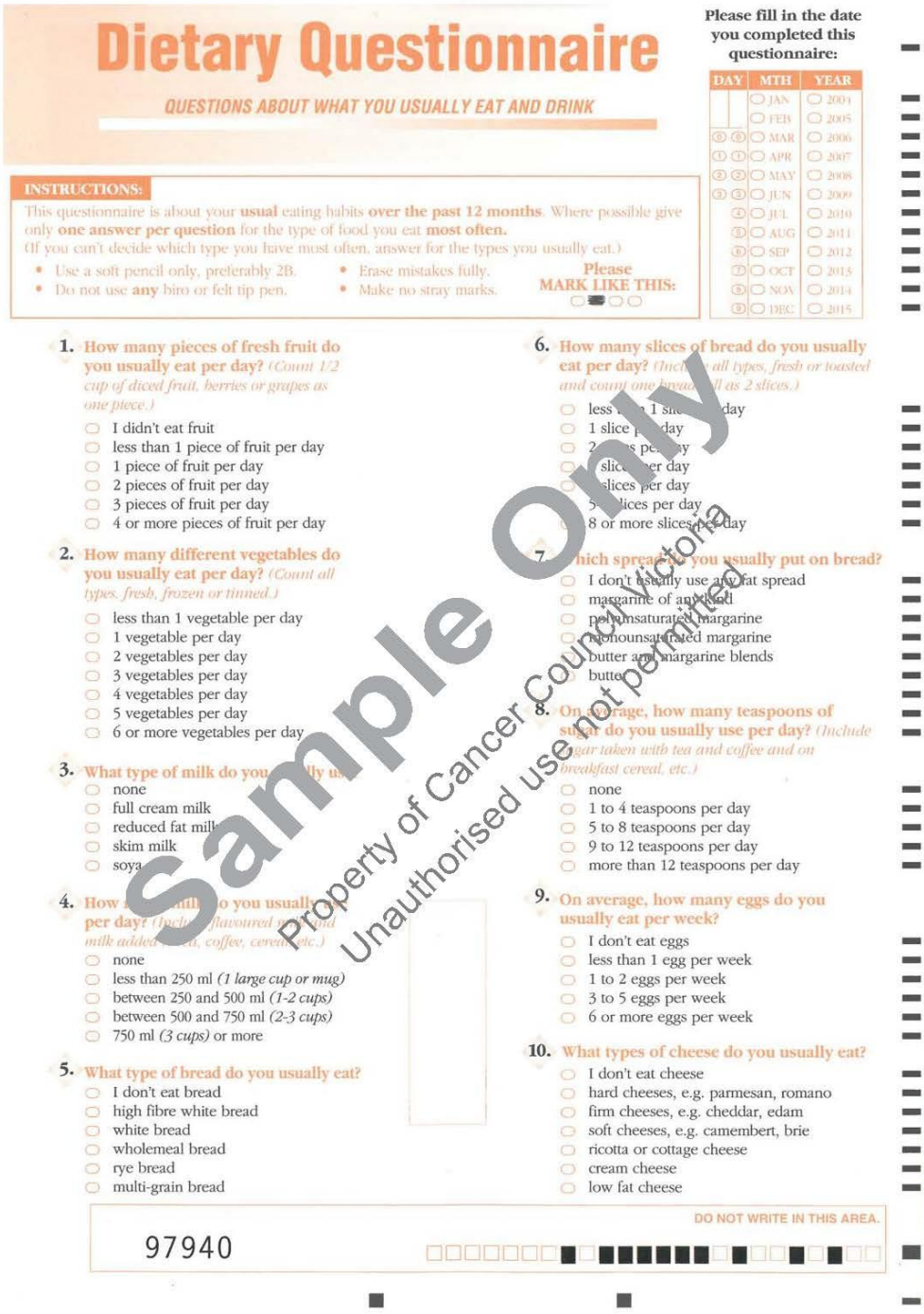
29.3 Lung Function measures collected :
- EBC measurement ❒
- VD measurements ❒
- Spirometry ❒
- Methacholine Challenge ❒
- Bronchodilator Challenge ❒

29.4 Biological specimens collected :
- Full Bloods ❒
- Part Bloods ❒
- Saliva ❒
- No sample ❒

29.5 Information about TAHS Breast Density Study given to participant ❒ No ❒ Yes ❒ N/A

29.6 Sleep questionnaire collected ❒ No ❒ Yes

Appendix 3: COPD Study food frequency questionnaire



Dietary Questionnaire

QUESTIONS ABOUT WHAT YOU USUALLY EAT AND DRINK

Please fill in the date you completed this questionnaire:

DAY	MTH	YEAR
	○ JAN	○ 2004
	○ FEB	○ 2005
⓪ ⓪	○ MAR	○ 2006
① ①	○ APR	○ 2007
② ②	○ MAY	○ 2008
③ ③	○ JUN	○ 2009
	④ ○ JUL	○ 2010
	⑤ ○ AUG	○ 2011
	⑥ ○ SEP	○ 2012
	⑦ ○ OCT	○ 2013
	⑧ ○ NOV	○ 2014
	⑨ ○ DEC	○ 2015

INSTRUCTIONS:

This questionnaire is about your **usual** eating habits **over the past 12 months**. Where possible give only **one answer per question** for the type of food you eat **most often.**
(If you can't decide which type you have most often, answer for the types you usually eat.)

- Use a soft pencil only, preferably 2B.
- Do not use **any** biro or felt tip pen.
- Erase mistakes fully.
- Make no stray marks.

Please MARK LIKE THIS: ○●○○

1. How many pieces of fresh fruit do you usually eat per day? *(Count 1/2 cup of diced fruit, berries or grapes as one piece.)*

- ○ I didn't eat fruit
- ○ less than 1 piece of fruit per day
- ○ 1 piece of fruit per day
- ○ 2 pieces of fruit per day
- ○ 3 pieces of fruit per day
- ○ 4 or more pieces of fruit per day

2. How many different vegetables do you usually eat per day? *(Count all types, fresh, frozen or tinned.)*

- ○ less than 1 vegetable per day
- ○ 1 vegetable per day
- ○ 2 vegetables per day
- ○ 3 vegetables per day
- ○ 4 vegetables per day
- ○ 5 vegetables per day
- ○ 6 or more vegetables per day

3. What type of milk do you ... ly u...

- ○ none
- ○ full cream milk
- ○ reduced fat mil...
- ○ skim milk
- ○ soya

4. How ... milk do you usually ... per day? *(Inclu... flavoured ... and milk added ... tea, coffee, cereal, etc.)*

- ○ none
- ○ less than 250 ml *(1 large cup or mug)*
- ○ between 250 and 500 ml *(1-2 cups)*
- ○ between 500 and 750 ml *(2-3 cups)*
- ○ 750 ml *(3 cups)* or more

5. What type of bread do you usually eat?

- ○ I don't eat bread
- ○ high fibre white bread
- ○ white bread
- ○ wholemeal bread
- ○ rye bread
- ○ multi-grain bread

6. How many slices of bread do you usually eat per day? *(Inc... all types, fresh or toasted and count one bread ... as 2 slices.)*

- ○ less ... 1 sli... day
- ○ 1 slice ... day
- ○ 2 ... s pe... ay
- ○ slic... er day
- ○ ...slices per day
- ○ 5 ...ices per day
- ○ 8 or more slices per day

7. ...hich spread ... you usually put on bread?

- ○ I don't usually use any fat spread
- ○ margarine of any kind
- ○ polyunsaturated margarine
- ○ monounsaturated margarine
- ○ butter and margarine blends
- ○ butter

8. On ...erage, how many teaspoons of sug... do you usually use per day? *(Include sugar taken with tea and coffee and on breakfast cereal, etc.)*

- ○ none
- ○ 1 to 4 teaspoons per day
- ○ 5 to 8 teaspoons per day
- ○ 9 to 12 teaspoons per day
- ○ more than 12 teaspoons per day

9. On average, how many eggs do you usually eat per week?

- ○ I don't eat eggs
- ○ less than 1 egg per week
- ○ 1 to 2 eggs per week
- ○ 3 to 5 eggs per week
- ○ 6 or more eggs per week

10. What types of cheese do you usually eat?

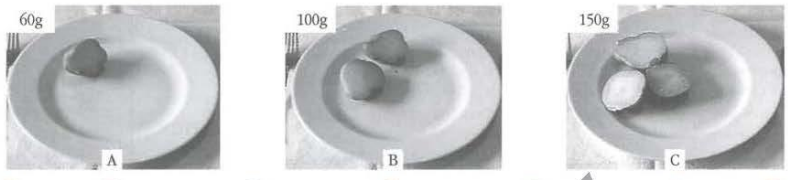
- ○ I don't eat cheese
- ○ hard cheeses, e.g. parmesan, romano
- ○ firm cheeses, e.g. cheddar, edam
- ○ soft cheeses, e.g. camembert, brie
- ○ ricotta or cottage cheese
- ○ cream cheese
- ○ low fat cheese

DO NOT WRITE IN THIS AREA.

97940

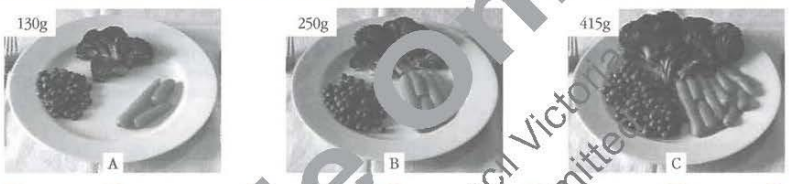
For each food shown on this page, indicate ***how much on average you would usually have eaten at main meals during the past 12 months.*** *When answering each question, think of the* ***amount*** *of that food you usually ate, even though you may rarely have eaten the food on its own.*
If you usually ate more than one helping, fill in the oval for the serving size closest to the ***total amount*** *you ate.*

11. When you ate potato, did you usually eat: ○ I never ate potato



○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

12. When you ate vegetables, did you usually eat: ○ I never ate vegetables

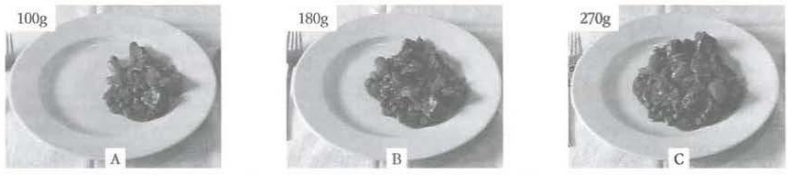


○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

13. When you ate steak, did you usually eat: ○ I never ate steak

○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

14. When you ate meat *or* vegetable casserole, did you usually eat: ○ I never ate casserole



○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

15. **Over the last 12 months, on average, *how often* did you eat the following foods?** *Please completely fill one oval in every line.* Please MARK LIKE THIS: ○●○○ NOT LIKE THIS: ✓ ✗ —○

Times You Have Eaten	NEVER	less than once per month	1 to 3 times per month	1 time per week	2 times per week	3 to 4 times per week	5 to 6 times per week	1 time per day	2 times per day	3 or more times per day
CEREAL FOODS, SWEETS & SNACKS										
All Bran™ A1	○	○	○	○	○	○	○	○	○	○
Sultana Bran™, FibrePlus™, Branflakes™ A2	○	○	○	○	○	○	○	○	○	○
Weet Bix™, Vita Brits™, Weeties™ A3	○	○	○	○	○	○	○	○	○	○
Cornflakes, Nutrigrain™, Special K™ A4	○	○	○	○	○	○	○	○	○	○
Porridge A5	○	○	○	○	○	○	○	○	○	○
Muesli A6	○	○	○	○	○	○	○	○	○	○
Rice A7	○	○	○	○	○	○	○	○	○	○
Pasta or noodles (include lasagne) A8	○	○	○	○	○	○	○	○	○	○
Crackers, crispbreads, dry biscuits A9	○	○	○	○	○	○	○	○	○	○
Sweet biscuits A10	○	○	○	○	○	○	○	○	○	○
Cakes, sweet pies, tarts and other sweet pastries A11	○	○	○	○	○	○	○	○	○	○
Meat pies, pasties, quiche and other savoury pastries A12	○	○	○	○	○	○	○	○	○	○
Pizza A13	○	○	○	○	○	○	○	○	○	○
Hamburger with a bun A14	○	○	○	○	○	○	○	○	○	○
Chocolate A15	○	○	○	○	○	○	○	○	○	○
Flavoured milk drink (cocoa, Milo™, etc.) A16	○	○	○	○	○	○	○	○	○	○
Nuts A17	○	○	○	○	○	○	○	○	○	○
Peanut butter or peanut paste A18	○	○	○	○	○	○	○	○	○	○
Corn chips, potato crisps, Twisties™, etc. A19	○	○	○	○	○	○	○	○	○	○
Jam, marmalade, honey or syrups A20	○	○	○	○	○	○	○	○	○	○
Vegemite™, Marmite™ or Promite™ A21	○	○	○	○	○	○	○	○	○	○
DAIRY PRODUCTS, MEAT & FISH										
[illegible] B1	○	○	○	○	○	○	○	○	○	○
Ice-[illegible]m B2	○	○	○	○	○	○	○	○	○	○
Yoghurt B3	○	○	○	○	○	○	○	○	○	○
Beef B4	○	○	○	○	○	○	○	○	○	○
Veal B5	○	○	○	○	○	○	○	○	○	○
Chicken B6	○	○	○	○	○	○	○	○	○	○
Lamb B7	○	○	○	○	○	○	○	○	○	○
Pork B8	○	○	○	○	○	○	○	○	○	○
Bacon B9	○	○	○	○	○	○	○	○	○	○
Ham B10	○	○	○	○	○	○	○	○	○	○
Canned meat, luncheon meat or salami B11	○	○	○	○	○	○	○	○	○	○
Sausages or frankfurters B12	○	○	○	○	○	○	○	○	○	○
Fish, steamed, grilled or baked B13	○	○	○	○	○	○	○	○	○	○
Fish, fried (include take-away) B14	○	○	○	○	○	○	○	○	○	○
Fish, tinned (salmon, tuna, sardines, etc.) B15	○	○	○	○	○	○	○	○	○	○
FRUIT										
Tinned or frozen fruit (any kind) C1	○	○	○	○	○	○	○	○	○	○
Fruit juice C2	○	○	○	○	○	○	○	○	○	○
Oranges or other citrus fruit C3	○	○	○	○	○	○	○	○	○	○
Apples C4	○	○	○	○	○	○	○	○	○	○
Pears C5	○	○	○	○	○	○	○	○	○	○
Bananas C6	○	○	○	○	○	○	○	○	○	○
Watermelon, rockmelon (cantaloupe), honeydew, etc. C7	○	○	○	○	○	○	○	○	○	○
Pineapple C8	○	○	○	○	○	○	○	○	○	○
Strawberries C9	○	○	○	○	○	○	○	○	○	○
Apricots C10	○	○	○	○	○	○	○	○	○	○
Peaches or nectarines C11	○	○	○	○	○	○	○	○	○	○
Mango or paw paw C12	○	○	○	○	○	○	○	○	○	○
Avocado C13	○	○	○	○	○	○	○	○	○	○

Times You Have Eaten
CONTINUED

		NEVER	less than once per month	1 to 3 times per month	1 time per week	2 times per week	3 to 4 times per week	5 to 6 times per week	1 time per day	2 times per day	3 or more times per day
VEGETABLES (INCLUDING FRESH, FROZEN AND TINNED)											
Potatoes, roasted or fried (include hot chips)	D1	○	○	○	○	○	○	○	○	○	○
Potatoes cooked without fat	D2	○	○	○	○	○	○	○	○	○	○
Tomato sauce, tomato paste or dried tomatoes	D3	○	○	○	○	○	○	○	○	○	○
Fresh or tinned tomatoes	D4	○	○	○	○	○	○	○	○	○	○
Peppers (capsicum)	D5	○	○	○	○	○	○	○	○	○	○
Lettuce, endive, or other salad greens	D6	○	○	○	○	○	○	○	○	○	○
Cucumber	D7	○	○	○	○	○	○	○	○	○	○
Celery	D8	○	○	○	○	○	○	○	○	○	○
Beetroot	D9	○	○	○	○	○	○	○	○	○	○
Carrots	D10	○	○	○	○	○	○	○	○	○	○
Cabbage or Brussels sprouts	D11	○	○	○	○	○	○	○	○	○	○
Cauliflower	D12	○	○	○	○	○	○	○	○	○	○
Broccoli	D13	○	○	○	○	○	○	○	○	○	○
Silverbeet or spinach	D14	○	○	○	○	○	○	○	○	○	○
Peas	D15	○	○	○	○	○	○	○	○	○	○
Green beans	D16	○	○	○	○	○	○	○	○	○	○
Bean sprouts or alfalfa sprouts	D17	○	○	○	○	○	○	○	○	○	○
Baked beans		○	○	○	○	○	○	○	○	○	○
Soy beans, soy bean curd or tofu	D19	○	○	○	○	○	○	○	○	○	○
Other beans (include chick peas, lentils, etc.)		○	○	○	○	○	○	○	○	○	○
Pumpkin		○	○	○	○	○	○	○	○	○	○
Onion or leeks	D22	○	○	○	○	○	○	○	○	○	○
Garlic (not garlic tablets)	D23	○	○	○	○	○	○	○	○	○	○
Mushrooms	D24	○	○	○	○	○	○	○	○	○	○
[illegible]	D25	○	○	○	○	○	○	○	○	○	○

16. Over the last 12 months, how often did you drink beer, wine and/or spirits?

Times That You Drank

		NEVER	less than once a month	1-3 days per month	1 day per week	2 days per week	3 days per week	4 days per week	5 days per week	6 days per week	every day
Beer (low alcohol)		○	○	○	○	○	○	○	○	○	○
Beer (full strength)	4	○	○	○	○	○	○	○	○	○	○
Red wine		○	○	○	○	○	○	○	○	○	○
White wine (include sparkling wines)		○	○	○	○	○	○	○	○	○	○
Fortified wines (port, sherry etc.)		○	○	○	○	○	○	○	○	○	○
Spirits, liqueurs etc.		○	○	○	○	○	○	○	○	○	○

To answer the next two questions, please convert the amounts you drank into glasses using the examples given below. For spirits, liqueurs and mixed drinks containing spirits, please count each nip (30 ml) as one glass.

1 can or stubby of beer = 2 glasses
1 large bottle beer (750 ml) = 4 glasses
1 bottle wine (750 ml) = 6 glasses
1 bottle of port or sherry (750 ml) = 12 glasses

17. Over the last 12 months, on days when you were drinking, how many glasses of beer, wine and/or spirits altogether did you *usually* drink?

TOTAL NUMBER OF GLASSES PER DAY	1	2	3	4	5	6	7	8	9	10 or more
	○	○	○	○	○	○	○	○	○	○

18. Over the last 12 months, what was the *maximum* number of glasses of beer, wine and/or spirits that you drank in 24 hours?

MAXIMUM NUMBER OF GLASSES PER 24 HOURS	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19 or more
	○	○	○	○	○	○	○	○	○	○

Thank You for completing this questionnaire

DO NOT WRITE IN THIS AREA.

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Appendix 4: Other COPD Study questionnaires

COPD LABORATORY QUESTIONNAIRE 266

Identification No: ☐☐☐☐

Initials: ☐☐☐

Sample *(2001=201)*: ☐☐☐

Date: ☐☐ ☐☐ ☐☐☐☐

Fieldworker No: ☐

Interview Type: ☐

I am going to ask you some questions. At first these will be about your breathing.

Wherever possible, please give a straight yes or no answer.

Wheeze and Tightness in the Chest *No* *Yes*

1. Have you had wheezing or whistling in your chest at any time in the last 12 months? ☐ ☐

If yes: 1.1 Have you been at all breathless when the wheezing noise was present? ☐ ☐

1.2 Have you had this wheezing or whistling when you did not have a cold? ☐ ☐

No *Yes*

2. Have you woken up with a feeling of tightness in your chest at any time in the last 12 mths? ☐ ☐

Shortness of Breath

3. Have you had an attack of shortness of breath that came on during the day when you were at rest at anytime in the last 12 months? ☐ ☐

4. Have you had an attack of shortness of breath that came on following strenuous activity at any time in the last 12 months? ☐ ☐

No *Yes*

5. Have you been woken by an attack of shortness of breath at any time in the last 12 months? ☐ ☐

If yes: 5.1 Have you been woken by an attack of shortness of breath in the past 3 mths? ☐ ☐

If yes: 5.1.1 Have you been woken by an attack of shortness of breath at least once a week in the last 3 months? ☐ ☐

Times

*If yes: 5.*1.1.1 How many times a week on average are you woken by an attack of shortness of breath? ☐☐

Cough and Phlegm from the Chest *No* *Yes*

6. Have you been woken by an attack of coughing at any time in the last 12 months? ☐ ☐

7. Do you **usually** cough first thing in the morning in the winter? ☐ ☐

8. Do you **usually** cough during the day, or at night, in the winter? ☐ ☐

If yes: 8.1 Do you cough like this on most days for as much as 3 months each year? ☐ ☐

9. Do you **ever cough up** phlegm or sputum **from your chest**? *No* *Yes*
If no go to Q13
If yes: 9.1 Do you **usually** bring up phlegm from your chest first thing in the morning in the winter?
9.2 Do you **usually** bring up phlegm from your chest during the day, or at night in the winter?
If yes: 9.2.1. Have you brought up phlegm on most days for as much as 3 months of a year for at least 2 successive years?

10. Do you bring up phlegm regularly (4 or more days per week) from your chest in any other seasons? *No* *Yes*
If yes: 10.1 Summer
10.2 Spring
10.3 Autumn

11. In the last 12 months have you brought up phlegm from your chest: *No* *Yes*
11.1 Regularly (4 or more days per week for more than 3 months)
If yes: 11.1.1 For how many years has this occurred? *Years*
OR
11.2 Only during exacerbations, attacks or infections *No* *Yes*
If yes: 11.2.1 How many exacerbations in the last 12 months? *Number*
11.3 How would you describe the usual colour of your sputum? *Tick one*
11.3.1 Clear or white
11.3.2 Creamy or yellow
11.3.3 Green
11.3.4 Other (specify) _____

An exacerbation to be identified by any 2 of the following criteria for more than 2 days:
worsening cough
increased shortness of breath
increased sputum volume
change of colour of sputum

12. Do you ever have exacaberations or flare-ups of your respiratory symptoms? *No* *Yes*
If yes: Do you experience:
12.1 worsening cough
12.2 increased shortness of breath
12.3 increased sputum volume
12.4 change of colour of sputum

Breathing

	No	Yes
13. Do you ever have trouble with your breathing?	☐	☐
If yes: 13.1 Do you have this trouble?	*Tick one*	
a) Continuously so that your breathing is never quite right?	☐	
b) Repeatedly, but it always gets completely better?	☐	
c) Only rarely?	☐	
14. Are you disabled from walking by a condition other than heart or lung disease?	☐	☐
If yes: 14.1 What condition? _____		
15. Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill?	☐	☐
If yes: 15.1 Do you get short of breath walking with other people of your own age on level ground?	☐	☐
15.2 Do you have to stop for breath when walking at your own pace on level ground?	☐	☐

Asthma

	No	Yes
16. Have you ever had asthma?	☐	☐
If yes: 16.1 Was this confirmed by a doctor?	☐	☐
	Years	
16.2 How old were you when you had your first attack of asthma?	☐☐	
16.3 How old were you when you had your most recent attack of asthma?	☐☐	
	No	Yes
16.4 Do you suffer asthma symptoms seasonally?	☐	☐
If yes: Which seasons: 16.4.1 Winter	☐	☐
16.4.2 Spring	☐	☐
16.4.3 Summer	☐	☐
16.4.4 Autumn	☐	☐
	No	Yes
16.5 Have you had an attack of asthma in the last 12 months?	☐	☐
	Number	
If yes: 16.5.1 How many attacks of asthma have you had in the last 12 months?	☐☐	
16.5.2 How many attacks of asthma have you had in the last 3 months?	☐☐	

If any attacks: 16.5.2.1 How many times have you woken up because of your asthma in the last 3 months?

Tick one

a) Almost every night ☐
b) More than once a week ☐
c) More than twice a month ☐
d) Equal or less than once a month ☐
e) Not at all ☐

16.5.2.2 How often have you had trouble with your breathing because of your asthma in the last 3 mths?

Tick one

a) Continuously ☐
b) Once a day ☐
c) More than twice a week ☐
d) Equal or less than twice a week ☐
e) Once a week ☐
f) Less than once a week ☐

Other Conditions

	No	*Yes*
17. Have you ever had emphysema or chronic obstructive lung disease?	☐	☐
If yes: 17.1 Was this confirmed by a doctor?	☐	☐

17.2 At what age was it first diagnosed? *Years* ☐☐

	No	*Yes*
18. Have you ever had any nasal allergies including "hayfever"?	☐	☐
If yes: 18.1 Have you had nasal allergy or hayfever in the last 12 months?	☐	☐

More about Yourself and Your family

19. What is your date of birth? *Day* ☐☐ *Month* ☐☐ *Year* ☐☐☐☐

20. In what country were you born? _____

	Male	*Female*
21. Gender: Male / Female	☐	☐

No Yes

22. Has your mother ever had asthma? ☐☐
23. Has your mother ever had chronic bronchitis or emphysema? ☐☐

24. Has your father ever had asthma? ☐☐
25. Has your father ever had chronic bronchitis or emphysema? ☐☐

26. Did you have a serious respiratory infection before the age of 5 years? (include: croup requiring hospitalisation, bronchiolitis, wheezy bronchitis, pneumonia, diphtheria, tuberculosis, whooping cough ☐☐
If yes: 26.1 Which one? _____

No Yes

27. As a child did you have regular contact with livestock (cows, horses, sheep, pigs and goats) or poultry? ☐☐

Occupation

Tick one

28. Are you currently?
    a) Employed or self employed ☐
    b) Not employed ☐
    c) Houseperson ☐
    d) Retired ☐

No Yes

29. Have you **ever** been employed in any job for six continuous months or longer? ☐☐
    If yes: . **Complete work history calendar**

Home Environment

Years

30. For how many years have you lived in your present home? ☐☐

No Yes

30.1 Do you live in the same home as at the time you completed the postal survey? ☐☐
(ie. June – October, 2000)

Times

If no: 30.1.1 How many times have you moved since completing the postal survey? ☐☐

30.1.2 Where do you currently live?

Tick one

a) A different home in sampling area ☐
b) Outside sampling area, but still in Melbourne metropolitan area ☐
c) Country Victoria ☐
d) Elsewhere ☐

Years

31. For how many years have you lived in the Melbourne suburbs? ☐☐

Tick one

32. In which decade was your present home built?

- a) Before 1940 ☐
- b) 1941-1960 ☐
- c) 1961-1970 ☐
- d) 1971-1980 ☐
- e) 1981-1990 ☐
- f) Later than 1990 ☐
- g) Don't know ☐

Tick one

33. Which best describes your home?

- a) Freestanding home ☐
- b) A semi detached house / terrace ☐
- c) A unit ☐
- d) An apartment / flat ☐
- e) Other (specify) _____ ☐

34. Does your home have any of the following?

	No	*Yes*
34.1. Ducted air heating	☐	☐
34.2 other central heating (hydronic, slab floor heating)	☐	☐
34.3 air conditioning / evaporative cooling	☐	☐
34.4 reverse cycle air conditioning	☐	☐

35. Which of the following do you use for **heating**?

	No	*Yes*
35.1 gas-fired furnace (ducted central heating)	☐	☐
35.2 coal or wood fire	☐	☐
35.3 gas heater	☐	☐
35.4 electric heater (eg. radiator, fan or Dimplex-type)	☐	☐
35.5 other central heating (hydronic, slab floor heating)	☐	☐
35.6 reverse cycle air conditioning	☐	☐
35.7 other _____	☐	☐

36. Which of the following do you use for <u>hot water</u>?

		No	Yes
36.1 gas		☐	☐
If yes: 36.1.1 Is the unit	a) internal	☐	☐
	b) external	☐	☐
		No	*Yes*
36.2 electric		☐	☐
If yes: 36.2.1 Is the unit	a) internal	☐	☐
	b) external	☐	☐
		No	*Yes*
36.3 solar		☐	☐
36.4 other _____		☐	☐

		Tick one
37. What kind of stove do you **mostly** use for <u>cooking?</u>	37.1 gas	☐
	37.2 electric	☐
	37.3 microwave	☐
	37.4 coal, coke or wood solid fuel	☐
	37.5 other	☐

		No	Yes
38. Do you have an extractor fan over the cooker?		☐	☐
If yes: 38.1 When cooking do you use the fan?		*Tick one*	
	a) All of the time?	☐	
	b) Some of the time?	☐	
	c) None of the time?	☐	
		No	*Yes*
38.2 Does the fan take the fumes outside the house?		☐	☐

	No	Yes
39. Has there **<u>ever</u>** been evidence of water damage to the home (eg broken pipes/leaks/floods)	☐	☐
If yes: 39.1 Has there been water damage in the last 12 months?	☐	☐

	No	Yes
40. Has there **<u>ever</u>** been visible damp or condensation on windows inside the house?	☐	☐

		No	Yes
If yes: 40.1 Which rooms have been affected?	40.1.1 bathrooms	☐	☐
	40.1.2 your bedroom	☐	☐
	40.1.3 other bedrooms	☐	☐
	40.1.4 living areas	☐	☐
	40.1.5 kitchen	☐	☐
	40.1.6 other _____	☐	☐

	No	Yes
40.2 Has there been visible damp or condensation in the last 12mths?	☐	☐

	No	Yes
41. Has there **ever** been mould or mildew on any surfaces, other than food, inside the home?	☐	☐
If yes: 41.1 Which rooms have been affected?		
41.1.1 bathrooms	☐	☐
41.1.2 your bedroom	☐	☐
41.1.3 other bedrooms	☐	☐
41.1.4 living areas	☐	☐
41.1.5 kitchen	☐	☐
41.1.6 other _____	☐	☐
41.2 Has there been mould or mildew on any surface, other than food, inside the home in the last 12 months?	☐	☐
42. Have you used a humidifier, including any humidifier system built into your heating system or an evaporative cooler in the last 12 months?	☐	☐
If yes: 42.1 What type of humidifier have you used?		
a) Humidifier built into the heating system	☐	☐
b) Portable hot / cold mist vaporiser	☐	☐
c) Evaporative cooler	☐	☐
d) Other _____	☐	☐
42.2 Under what circumstances have you used your humidifier?		
a) Only when someone is ill – in their room	☐	☐
b) To humidify the whole house	☐	☐
c) To humidify one room or part of the house	☐	☐
d) Other _____	☐	☐

	No	Yes
43. Do you **usually** sleep with the windows open?	☐	☐

Air Pollution

44. During the working day is the traffic noise at home so intense that you have to close the windows in order not to be disturbed? *Tick one*

- a) Constantly ☐
- b) Frequently (4 or more days per week) ☐
- c) Seldom ☐
- d) Never ☐

45. During working days, how often do heavy vehicles (trucks, buses) pass your house? *Tick one*

- a) Constantly ☐
- b) Frequently (4 or more days per week) ☐
- c) Seldom ☐
- d) Never ☐

46. How much are you annoyed by outdoor air pollution (from traffic, industry etc) at home, if you keep the windows open? *(Indicate degree of annoyance on scale: 0 =no disturbance, 10 = intolerable annoyance).* *Number* ☐☐

Smoking

47. Do you currently smoke cigarettes, cigars, pipes or any other tobacco products? *Tick one*

- a) Daily ☐
- b) At least weekly (not daily) ☐
- c) Less often than weekly ☐
- d) Not at all ☐

If the answer is 'not at all' go to Q51 … … … … … .

48. I am now going to read out the names of some tobacco products, I want you to tell me whether you now smoke each; daily (1), at least weekly(2), less than weekly(3) or not at all(4). *Circle answer*

48.1 Manufactured cigarettes?	1	2	3	4
48.2 Roll your own cigarettes	1	2	3	4
48.3 Cigars	1	2	3	4
48.4 Pipes	1	2	3	4
48.5 Any other kind?	1	2	3	4

If person smokes manufactured cigarettes "at least weekly" or "daily", ask:

49. On average how many manufactured cigarettes do you smoke? 49.1 cigarettes per day *Number* ☐☐

or 49.2 cigarettes per week ☐☐

If person smokes roll-your-own cigarettes "at least weekly" or "daily", ask:

50. On average how many roll-your-own cigarettes do you smoke? 50.1 cigarettes per day *Number* ☐☐

or 50.2 cigarettes per week ☐☐

If person smokes any cigarettes or tobacco "at least weekly" or "daily",

go to Q52, else go to Q51

51. In your lifetime, have you ever smoked at least 100 cigarettes or similar amount of tobacco? *No Yes* ☐☐

If No; go to Q53

If yes: 51.1 In the past have you ever been a daily smoker? *No Yes* ☐☐

51.2 When did you finally stop smoking daily? *Day Month Year*

51.2.1 (Refers to most recent smoking date) ☐☐ ☐☐ ☐☐☐☐

or 51.2.2 weeks ago ☐ **or** months ago ☐ **or** years ago ☐

or 51.2.3 years old ☐☐

52. At what age did you first start smoking daily? *Years* ☐☐

53. Have you been regularly exposed to any tobacco smoke in the last 12 months? (Regularly means on most days or nights) *No Yes* ☐☐

If yes: 53.1 Not counting yourself how many people in your household smoke regularly? *Number* ☐☐

53.2. Do people smoke regularly in the room where you work? *No Yes* ☐☐

53.3 How many hours per day are you exposed to other people's tobacco smoke? *Hours* ☐☐

Medicines and Inhalers

	No	Yes
54. Have you used any inhaled medicines to help your breathing in the last 12 months?	☐	☐
If yes, which of the following have been used in the last 12 months?	No	Yes
54.1 **Short Acting beta-2-agonist inhalers**	☐	☐

If yes: a) Which one? _____

b) Type of inhaler _____

c) Dose per puff/vial? _____

		Number
54.1.1 In the last 12 months, how have you used them?		
a) Regularly, on a daily basis	Average no. puffs/day	☐☐
OR		
b) Only during exacerbations	No. exacerbations in last 12 mths	☐☐
or "attacks"	No. puffs/day	☐☐
	Average no. days	☐☐
54.1.2 In the last 3 months, how have you used them?		
a) Regularly, on a daily basis	Average no. puffs/day	☐☐
OR		
b) Only during exacerbations	No. exacerbations in last 3 mths	☐☐
or "attacks"	No. puffs/day	☐☐
	Average no. days	☐☐

	No	Yes
54.2 **Long Acting beta-2-agonists**	☐	☐

If yes: a) Which one? _____

b) Type of inhaler _____

c) Dose/puffs? _____

		Number
54.2.1 In the last 12 months, how have you used them?		
a) Regularly, on a daily basis	Average no. puffs/day	☐☐
OR		
b) Only during exacerbations	No. exacerbations in last 12 mths	☐☐
or "attacks"	No. puffs/day	☐☐
	Average no. days	☐☐
54.2.2 In the last 3 months, how have you used them?		
a) Regularly, on a daily basis	Average no. puffs/day	☐☐

OR

		Number
b) Only during exacerbations	No. exacerbations in last 3 mths	
or "attacks"	No. puffs/day	
	Average no. days	

	No	Yes
54.3 **Anticholinergic Inhalers**		

a) If used which one? _____

b) Type of inhaler? _____

c) Dose/puffs? _____

54.3.1 In the last 12 months, how have you used them?

		Number
a) Regularly, on a daily basis	Average no. puffs/day	
OR		
b) Only during exacerbations	No. exacerbations in last 12 mths	
or "attacks"	No. puffs/day	
	Average no. days	

54.3.2 In the last 3 months, how have you used them?

		Number
a) Regularly, on a daily basis	Average no. puffs/day	
OR		
b) Only during exacerbations	No. exacerbations in last 3 mths	
or "attacks"	No. puffs/day	
	Average no. days	

	No	Yes
54.4 **Inhaled Steroids**		

a) If used which one? _____

b) Type of inhaler? _____

c) Dose/puffs? _____

54.4.1 In the last 12 months, how have you used them?

		Number
a) Regularly, on a daily basis	Average no. puffs/day	
OR		
b) Only during exacerbations	No. exacerbationss in last 12 mths	
or "attacks"	No. puffs/day	
	Average no. days	

54.4.2 In the last 3 months, how have you used them? *Number*

a) Regularly, on a daily basis — Average no. puffs/day

OR

b) Only during exacerbations — No. exacerbations in last 3 mths *Number*

or "attacks" — No. puffs/day

Average no. days

If used regularly for the last 12 mths:

54.4.3 For how many years have you been taking them this way? *Years*

54.4.4 For how many years have you been taking inhaled steroids on most days?

54.5 **Inhaled cromoglycate/nedocromil?** *No* *Yes*

a) If used which one? _____

b) Type of inhaler _____

c) Dose/puffs? _____

54.5.1 In the last 12 months, how have you used them? *Number*

a) Regularly, on a daily basis — Average no. puffs/day

OR

b) Only during exacerbations — No. exacerbations in last 12 mths *Number*

or "attacks" — No. puffs/day

Average no. days

54.5.2 In the last 3 months, how have you used them? *Number*

a) Regularly, on a daily basis — Average no. puffs/day

OR

b) Only during exacerbations — No. exacerbations in last 3 mths *Number*

or "attacks" — No. puffs/day

Average no. days

54.6 **Inhaled Compound Bronchodilators?** *No* *Yes*

a) If used which one? _____

b) Type of inhaler _____

c) Dose/puffs? _____

54.6.1 In the last 12 months, how have you used them? *Number*

a) Regularly, on a daily basis — Average no. puffs/day

OR

Number

b) Only during exacerbations — No. exacerbations in last 12 mths
or "attacks" — No. puffs/day
Average no. days

54.6.2 In the last 3 months, how have you used them?

Number

a) Regularly, on a daily basis — Average no. puffs/day

OR

Number

b) Only during exacerbations — No. exacerbations in last 3 mths
or "attacks" — No. puffs/day
Average no. days

No Yes

54.7 **Inhaled combination of steroids and long acting beta 2 agonist?**

a) If used which one? _____

b) Type of inhaler _____

c) Dose/puffs? _____

54.7.1 In the last 12 months, how have you used them?

Number

a) Regularly, on a daily basis — Average no. puffs/day

OR

Number

b) Only during exacerbations — No. exacerbations in last 12 mths
or "attacks" — No. puffs/day
Average no. days

54.7.2 In the last 3 months, how have you used them?

Number

a) Regularly, on a daily basis — Average no. puffs/day

OR

Number

b) Only during exacerbations — No. exacerbations in last 3 mths
or "attacks" — No. puffs/day
Average no. days

No Yes

54.8 **Other inhaled medication?**

a) If used which one? _____

b) Type of inhaler _____

c) Dose/puffs? _____

54.8.1 In the last 12 months, how have you used them?

Number

a) Regularly, on a daily basis — Average no. puffs/day

OR

		Number
b) Only during exacerbations	No. exacerbations in last 12 mths	
or "attacks"	No. puffs/day	
	Average no. days	

54.8.2 In the last 3 months, how have you used them?

		Number
a) Regularly, on a daily basis	Average no. puffs/day	

OR

		Number
b) Only during exacerbations	No. exacerbations in last 3 mths	
or "attacks"	No. puffs/day	
	Average no. days	

55. Have you ever used inhaled steroids to help your breathing? *No* *Yes*

If yes 55.1 At what age did you start using inhaled steroids? *Years*

55.2 Are you currently taking inhaled steroids? *No* *Yes*

55.3 For how many months have you used inhaled steroids on most days in the past 5 years? *Months*

56. Have you used any pills, capsules, tablets or medicines, other than inhaled medicines, to help your breathing at any time in the last 12 months? *No* *Yes*

If yes, which of the following have been used? *No* *Yes*

56.1 **Oral theophyllines?**

a) If taken, which one? _____

56.1.1 In the last 12 months, how have you taken them? *No* *Yes*

a) Regularly, on a daily basis

Average daily dose in mgms *Mgms*

OR *No* *Yes*

b) In short courses

	Number
No. courses in last 12mths	
Average no. days per course	
Average daily dose in mgms	

No Yes

56.1.2 Have you taken oral theophylline in the last 3 months? ☐☐

56.1.3 Are you currently taking oral theophylline? ☐☐

No Yes

56.2 **Oral Steroids?** ☐☐

a) If taken, which one? _____

56.2.1 In the last 12 months, how have you taken them? *No Yes*

a) Regularly, on a daily basis ☐☐

Average daily dose in mgms ☐☐

OR

No Yes

b) In short courses ☐☐

Number

No. courses in last 12mths ☐☐

Average no. days per course ☐☐

Average daily dose in mgms ☐☐

No Yes

56.2.2 Have you taken oral steroids in the last 3 months? ☐☐

56.2.3 Are you currently taking oral steroids? ☐☐

No Yes

56.3 **Oral Anti-leukotrienes?** ☐☐

a) If taken, which one? _____

56.3.1. In the last 12 months, how have you taken them? *No Yes*

a) Regularly, on a daily basis ☐☐

Mgms

Average daily dose in mgms ☐☐

OR

b) In short courses ☐☐

Number

No. courses in last 12mths ☐☐

Average no. days per course ☐☐

Average daily dose in mgms ☐☐

No Yes

56.3.2. Have you taken oral anti-leukotrienes in the last 3 months? ☐☐

56.3.3 Are you currently taking oral anti-leukotrienes? ☐☐

No Yes

56.4 **Other Oral Medications?** ☐ ☐

56.4.1 List _____

_____

_____

No Yes

57. Have you ever been prescribed home oxygen therapy? ☐ ☐

If yes: 57.1 Are you currently using oxygen therapy at home? ☐ ☐

Years

57.2 For how many years have you been using oxygen therapy at home? ☐☐

No Yes

58. Have you ever had an influenza vaccination? ☐ ☐

If yes: 58.1 Have you been vaccinated for influenza in the last 12mths? ☐ ☐

59. Have you ever had a pneumonia vaccination? ☐ ☐

If yes: 59.1 Have you been vaccinated for pneumonia in the last 5 years? ☐ ☐

60. Have you ever been vaccinated or desensitised for allergy? ☐ ☐

If yes: 60.1 Have you been vaccinated for allergy in the last 12 months? ☐ ☐

61. Have you had any other injections to help your breathing at any time in the last 12 months? ☐ ☐

If yes: 61.1 What injections? _____

No Yes

62. Have you used any other remedies to help your breathing at any time in the last 12 months? ☐ ☐

If yes: 62.1 What remedies? _____

No Yes

63. Has your doctor ever prescribed medicines, including inhalers, for your breathing? ☐ ☐

If yes: 63.1 If you are prescribed medicines for your breathing, do you normally take: *Tick one*

a) All of the medicine? ☐

b) Most of the medicine? ☐

c) Some of the medicine? ☐

d) None of the medicine? ☐

63.2 When your breathing gets worse and you are prescribed medicine for your breathing, do you normally take: *Tick one*

a) All of the medicine? ☐

b) Most of the medicine? ☐

c) Some of the medicine? ☐

d) None of the medicine? ☐

63.3 Do you think it is bad for you to take medicine all the time for your breathing? *No Yes N/A* ☐☐☐

63.4 Do you think you should take as much medicine as you need to get rid of all your breathing problems? ☐☐☐

64. Have you ever visited a hospital emergency department because of breathing problems? *No Yes* ☐☐

If yes: 64.1 Was this due to asthma, chronic bronchitis or emphysema? ☐☐

64.2 Have you visited a hospital casualty department or emergency room because of asthma, chronic bronchitis or emphysema in the last 12 months? *No Yes* ☐☐

If yes: 64.2.1 If yes, how many times? *Number* ☐☐

65. Have you ever spent a night in hospital because of breathing problems? *No Yes* ☐☐

If yes: 65.1 Was this due to asthma, chronic bronchitis or emphysema? ☐☐

65.2 Have you spent a night in hospital because of asthma, chronic bronchitis or emphysema in the last 12 months? ☐☐

If yes: 65.3 How many nights and in what sort of ward? *Number*

a) General (no. days/nights) ☐☐
b) Chest/Respiratory ☐☐
c) Rehabilitation ☐☐
d) Intensive care unit ☐☐
e) Other _____ ☐☐

66. Have you ever been seen by a doctor because of breathing problems or because of shortness of breath? *No Yes* ☐☐

If yes: 66.1 Was this due to asthma, chronic bronchitis or emphysema? ☐☐

If yes: 66.1.1 Have you been seen by a GP for asthma, chronic bronchitis or emphysema in the last 12 months? *No Yes* ☐☐

If yes: 66.1.1.1 Where and how often? *Number*

a) At home (no. of times) ☐☐
b) In the GP's office or surgery ☐☐

66.1.2 Have you been seen by specialist (respiratory physician, allergy specialist, specialist physician) for asthma, chronic bronchitis or emphysema in the past 12 months? *No Yes* ☐☐

If yes: 66.1.2.1 How many times? *Number* ☐☐

66.1.3. Are you given regular appointments to be seen by a doctor for your asthma, chronic bronchitis or emphysema? (Regular = a further appointment is made at each visit) — No ☐ Yes ☐

If yes: 66.1.3.1 Are you given regular appts with a hospital doctor? ☐☐

66.1.3.2 Are you given regular appointments with your GP? ☐☐

67. Have you visited any of the following because of your breathing problems in the past 12 months? — No Yes

a) Nurse ☐☐

b) Physiotherapist ☐☐

c) Practitioner of alternate medicine? ☐☐

68. Have you had any clinical or laboratory tests because of your breathing problems in the last 12 months? — No ☐ Yes ☐

Number

If yes: 68.1 Which and how often?

a) Respiratory function test ☐☐

b) Skin test for allergy ☐☐

c) Blood test for allergy ☐☐

d) X-rays ☐☐

e) ECG or other heart tests ☐☐

69. Do you work? — No ☐ Yes ☐

If yes: 69.1 Have you lost any days of work because of your asthma, chronic bronchitis, emphysema in the last 12 months? ☐☐

Number

If yes: 69.1.1 How many days? ☐☐☐

If no: 69.2 Were you forced to give up working because of your asthma, chronic bronchitis, emphysema? — No ☐ Yes ☐

Year

If yes: 69.2.1 When? ☐☐☐☐

70. Whatever your working situation, have there been any days when you have had to give up activities (eg: leisure activities, household activities) because of your asthma, chronic bronchitis or emphysema in the last 12 months? — No ☐ Yes ☐

Number

If yes: 70.1 How many days? ☐☐☐

	No	Yes
71. Is blood being taken for fatty acid analysis	☐	☐
If yes: Please ask:		
a) Are you currently taking any fish oil/fatty acid dietary supplements?	☐	☐
b) Have you taken any fish oil/fatty acid supplements in the past 6 months?	☐	☐

If yes: c) Specify the brand, dose (in mg if possible) and when these supplements were commenced and/or ceased _____

_____

72. **Current Medications**:

Please detail routine medications volunteer is currently taking:

Medication	*Dose*	*No. Puffs/Inhalations/Nebulisers*	*No. Times per Day*

COPD STUDY – STAGE II **LUNG FUNCTION QUESTIONNAIRE** **PAGE 1**

Reference Number: ☐☐☐☐

Sample(*2001=201*): ☐☐☐

Date: ☐☐.☐☐.☐☐

1. How many times have you been woken at night with shortness of breath in the last 2 weeks? ☐☐

2. During the last 2 weeks, has your breathing been *TICK BOX*

a) worse than usual *1* ☐

b) the same as usual *2* ☐

c) better than usual *3* ☐

Have you had a cigarette in the last hour? ☐ *NO* ☐ *YES*

Have you used a puffer or inhaler in the last hour? ☐ ☐

IF YES: DELAY LUNG FUNCTION TESTS UNTIL ONE HOUR AFTER THE LAST CIGARETTE OR INHALER USE

NO *YES*

3. Have you had a respiratory infection in the last 3 weeks? *1* ☐ *2* ☐

DAYS

If yes: **3.1** How many days ago did it end? ☐☐

NO *YES*

4. Have you used a puffer or inhaler in the last 24 hours? *1* ☐ *2* ☐

If yes: **4.1** What inhaler(s) did you use and how many hours ago? *DRUG* *HOURS*

_____ ☐ ☐☐

_____ ☐ ☐☐

NO *YES*

5. Have you taken any other medication for breathing(other than inhalers) in the last 24 hours? *1* ☐ *2* ☐

COPD STUDY – STAGE II **LUNG FUNCTION QUESTIONNAIRE** **PAGE 2**

If yes: **5.1** Which medications did you take and how many hours ago? *DRUG* *HOURS*

_____ ☐ ☐☐

_____ ☐ ☐☐

NO *YES*

6. Have you taken any antihistamines or cough medicines in the last month? *1*☐ *2*☐

If yes: **6.1** Which medicines did you take and how many days ago? *DRUG* *DAYS*

_____ ☐ ☐☐

_____ ☐ ☐☐

NO *YES*

7. Have you taken any preparations of phenothiazine or imipramine in the last month? *1*☐ *2*☐

If yes: **7.1** Which medications did you take and how many days ago? *DRUG* *DAYS*

_____ ☐ ☐☐

_____ ☐ ☐☐

NO *YES*

8. Have you had a heart attack in the last 3 months? *1*☐ *2*☐

9. Are you currently taking any medication for your heart? *1*☐ *2*☐

10. Are you currently taking any medication for epilepsy? *1*☐ *2*☐

11. Are you currently taking any β-blocker medication? *1*☐ *2*☐

MALE *FEMALE*

12. Are you male or female *1*☐ *2*☐

NO *YES*

If female: **12.1** Are you pregnant? *1*☐ *2*☐

12.2 Are you breast feeding? *1*☐ *2*☐

IF YES to any questions 8-12 DO NOT CHALLENGE

FIELDWORKER NUMBER ☐

COPD STUDY – STAGE II LUNG FUNCTION DATA PAGE 1

Reference Number: ☐☐☐☐

Sample(*2001=201*): ☐☐☐

Date: ☐☐.☐☐.☐☐

CMS

1. Subject's height ☐☐☐

KGS

2. Subject's weight ☐☐☐

YEARS

3. Subject's age ☐☐

MALE *FEMALE*

4. Subject's sex ☐ ☐

5. Predicted FEV_1 ☐.☐☐

6. Initial FEV_1 and FVC

FEV_1	FVC	PEFR (L/s)
☐.☐☐	☐.☐☐	☐☐.☐
☐.☐☐	☐.☐☐	☐☐.☐
☐.☐☐	☐.☐☐	☐☐.☐
☐.☐☐	☐.☐☐	☐☐.☐
☐.☐☐	☐.☐☐	☐☐.☐

6.1 Number of rejected attempts ☐☐

7. Best Initial FEV_1 as % of predicted FEV_1 ☐☐☐

7a. Best FEV1/FVC ☐☐

7b. Best FVC/Predicted FVC ☐☐

COMPLETE TLCO MEASUREMENTS BEFORE PROCEEDING WITH METHACHOLINE CHALLENGE

COPD STUDY – STAGE II LUNG FUNCTION DATA PAGE 2

GAS TRANFER DATA – TLCO and HAEMOGLOBIN

YES NO

8. Does the subject smoke? ☐ ☐

HOURS

8.1 If yes: How many hours ago was the last cigarette/other tobacco smoked? ☐☐

YES NO

9. Has blood been collected and tested for haemoglobin? ☐ ☐

Test 1 gms/dL Test 2 gms/dL Mean gms/dL

9.1 If yes: Haemoglobin results: ☐☐.☐ ☐☐.☐ ☐☐.☐

10. Record best FVC from initial spirometry: ☐.☐☐ **11.1** Calculate 90% of best FVC: ☐.☐☐

11. Equipment used for TLCO measurement: Elite 6 ☐ Other ☐

	Test 1	Test 2	Test 3
12. BHT (secs)	☐☐.☐☐	☐☐.☐☐	☐☐.☐☐
13. VC_{insp}(L)	☐☐.☐☐	☐☐.☐☐	☐☐.☐☐
14. TLCO (ml/min/mmHg)	☐☐.☐☐	☐☐.☐☐	☐☐.☐☐
15. VA (L)	☐.☐☐	☐.☐☐	☐.☐☐

16. Average measurements:

$TLCO_{unc}$ (ml/min/mmHg) ☐☐.☐☐ %pred ☐☐☐

$TLCO_{Hb\ corr}$ (ml/min/mmHg) ☐☐.☐☐ %pred ☐☐☐

KCO or TLCO/VA (ml/min/mmHg/L) ☐.☐☐ %pred ☐☐☐

VA (L) ☐.☐☐ %pred ☐☐☐

COPD STUDY – STAGE II LUNG FUNCTION DATA PAGE 3

IF BEST INITIAL FEV_1 IS a) less than 70% predicted
OR b) less than 1.5 litres
DO NOT DO METHACHOLINE CHALLENGE .....GO TO BRONCHODILATOR CHALLENGE

Is the subject continuing with methacholine challenge ? ☐ NO ☐ YES

If NO, why ? _____

METHACHOLINE CHALLENGE

17. CONTROL FEV_1 following inhalation of diluent:

17.1 Record 2 technically satisfactory manoeuvres which measure within 100mls of each other ☐.☐☐

☐.☐☐

17.2 Number of rejected attempts ☐

18. Best Control FEV_1 (post-diluent) as % of Initial FEV_1 ☐☐☐

IF BEST CONTROL FEV_1 IS <89% OF BEST INITIAL FEV_1 STOP CHALLENGE
AND GO TO REVERSAL OF BRONCHOCONSTRICTION

19. Did the subject answer YES to questions 1 – 2 – 3 – 5 – 13 – 16 of the main questionnaire? ☐ NO ☐ YES

IF NO: FOLLOW SHORT PROTOCOL IF YES: FOLLOW LONG PROTOCOL

20. Will the long or short protocol be followed? ☐ LONG ☐ SHORT

Short Protocol: change to long protocol if FEV_1 falls to 90% of control FEV_1
stop methacholine challenge if FEV_1 falls to 80% of control FEV_1

90% of control FEV_1 ☐.☐☐

Long Protocol: stop methacholine challenge if FEV_1 falls to 80% of control FEV_1

80% of control FEV_1 ☐.☐☐

COPD STUDY – STAGE II	LUNG FUNCTION DATA	PAGE 4

METHACHOLINE BATCH NUMBER ☐☐☐

SESSION NUMBER ☐☐

ORDER IN SESSION ☐

21.

Dose Level	Cumulative Dose (mg)	FEV_1	FEV_1	No. rejected attempts
1	0.0078	☐.☐☐	☐.☐☐	☐
2	0.0156	☐.☐☐	☐.☐☐	☐
3	0.0312	☐.☐☐	☐.☐☐	☐
4	0.0625	☐.☐☐	☐.☐☐	☐
5	0.125	☐.☐☐	☐.☐☐	☐
6	0.25	☐.☐☐	☐.☐☐	☐
7	0.5	☐.☐☐	☐.☐☐	☐
8	1.0	☐.☐☐	☐.☐☐	☐
9	2.0	☐.☐☐	☐.☐☐	☐

FIELDWORKER NUMBER ☐

COPD STUDY – STAGE II	LUNG FUNCTION DATA	PAGE 5

22. Why was methacholine challenge stopped? *TICK BOX*

a) end of test reached (2mgs inhaled) 1 ☐

b) 20% fall in FEV_1 achieved 2 ☐

c) subject's technique not satisfactory 3 ☐

d) subject asked to stop 4 ☐

e) other (specify) _____ 5 ☐

REVERSAL OF BRONCHOCONSTRICTION

23. Record the first 2 technically satisfactory manoeuvres

FEV_1	FVC	PEFR(L/s)
☐.☐☐	☐.☐☐	☐☐.☐
☐.☐☐	☐.☐☐	☐☐.☐

23.1 Number of rejected attempts ☐

24. Best POST-BRONCHODILATOR FEV_1 as % of INITIAL FEV_1 ☐☐☐

25. Has the subject's FEV_1 returned to within 10% of initial measurement? ☐☐

NO YES

Ensure subject's lung function is within 10% of initial measurement before he/she leaves the testing centre

BRONCHODILATOR CHALLENGE ONLY

26. Record first 2 technically satisfactory manoeuvres

FEV_1	FVC	PEFR(L/s)
☐.☐☐	☐.☐☐	☐☐.☐
☐.☐☐	☐.☐☐	☐☐.☐

26.1 Number of rejected attempts ☐

FIELDWORKER NUMBER ☐

Appendix 5: LMM paper

Measuring bronchial responsiveness using the linear mixed effects model

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and accuracy of the analysis. The funding bodies had no direct role in the study design, data analysis, or manuscript preparation or approval.

Sources of funding: This study was supported by the National Health and Medical Research Council of Australia; Clifford Craig Foundation, Tasmania; Royal Hobart Hospital Research Foundation; and Asthma Australia.

Word count: 2692

Summary: We propose the use of the linear mixed effects model to examine predictors of bronchial responsiveness. It has greater power to detect associations than currently used methods and produces more accurate, reliable results that are easily interpreted.

Online Supplement: This article has an online supplement

Abstract

Rationale: Current methods used to assess risk factors for increased bronchial responsiveness (BR) have significant limitations.

Objectives: To demonstrate the use of a linear mixed model (LMM) to analyse factors associated with change in FEV_1 during a nonspecific bronchial provocation test and compare findings to those from regression of the log dose-response slope (logDRS).

Methods: We used cross-sectional data from the Tasmanian Longitudinal Health Study 2010 follow-up, in which an asthma and bronchitis enriched subsample (n=694) completed a questionnaire and methacholine challenge. Repeated FEV_1 measurements were modelled in an LMM with random intercepts and slopes, and interactions between dose and the predictors sex, age, smoking and asthma status. Linear regression was used to model the logDRS using the same predictors.

Results: Sex was not associated with change in FEV_1 in the LMM; however, it was a predictor of the logDRS, with FEV_1 falling faster in females compared to males. When height was added as a predictor, sex was not associated with BR using either statistical method. Asthma and smoking were consistently detected as risk factors for increased BR. In the LMM, mean fall in FEV_1 was 14ml, 37ml and 13ml higher per µmol methacholine for current smokers, and those with current and remitted asthma, respectively.

Conclusions: An LMM accurately detected no association between sex and BR in this sample by accounting for variation in initial FEV_1. This method has greater power, accuracy and fewer limitations than methods currently used to assess predictors of BR and should be used more widely.

Abstract word count: 250

MeSH terms: Bronchial Provocation Tests; Airway Hyper-responsiveness; Statistical Model.

Introduction

Bronchial hyperresponsiveness (BHR) is the "twitchy" irritable state of the airways associated with asthma and chronic obstructive pulmonary disease (COPD) (1-3). It is measured by a bronchial provocation challenge, which has been used clinically in the diagnosis of asthma (4).

Researchers investigating risk factors for increased bronchial responsiveness (BR) typically use linear or logistic regression (5-10), both of which require a single BR measurement per individual which summarises multiple FEV_1 measurements obtained in the test. Two commonly used summary methods are (1) defining BHR as positive if a pre-selected (e.g. 20%) fall in FEV_1 is achieved, and negative otherwise; or (2) estimating a dose-response "slope" using the initial and final FEV_1 measurements (11).

There are several limitations in using these summary measurements, the obvious being loss of information. Both methods use two FEV_1 measurement points to describe the fall in FEV_1, discarding most of the data collected during a challenge. A dichotomous outcome also ignores the variability in the response to methacholine between individuals and uses an arbitrary cut-off point which has not specifically been linked to disease risk, severity or mortality.

Estimation of the dose-response slope (DRS) ignores differences in baseline lung function between participants which are associated with BHR (12-19). To achieve a normally-distributed outcome and satisfy assumptions underlying regression, the DRS is often log transformed. Typically, the normality of the logDRS is not tested, potentially threatening the validity of the analyses. Finally, as zero gradient or even negative DRSs are possible and

cannot be log transformed, a small constant is added to the slope before transformation, rendering interpretation more difficult.

While acknowledging that slope is an appropriate way to characterize the dose-response curve from a provocation test (11), its determination poses some limitations. The use of %FEV_1 is problematic as those with a smaller baseline FEV_1 require less absolute change to achieve the same proportional change as those with higher baseline FEV_1. Ignoring the variation around the estimated slope may lead to inappropriately small standard errors of the effect estimates, artificially increasing power and identifying associations that may not exist.

Linear mixed modelling (LMM) (20) is a standard biostatistical tool that overcomes the limitations of a dichotomised BHR variable and the logDRS. LMM uses all the data obtained during the challenge in a multilevel analysis that estimates the individual change in FEV_1 with increasing cumulative dose. This well-established statistical method analyses change in a continuous outcome, and the results obtained are easy to interpret, yet has not been published for BR.

Here, we demonstrate the use of an LMM to identify factors associated with the change in FEV_1 during a bronchial provocation test and compare the results to those from analysis of the same data with logDRS as the outcome (11). Some of the results of this study have been reported previously in the form of an abstract (21).

Methods

Study sample

The Tasmanian Longitudinal Health Study (TAHS) began in 1968 with the enrolment of 8,583 Tasmanian school children born in 1961. The methods and previous results of TAHS are detailed elsewhere (22-24). In 2004-2006, as part of the 5th decade follow-up, 7,562 participants (88.1%) were traced and 5,729 (78.4%) responded to a postal survey (25). An asthma and bronchitis enriched subsample of these respondents was invited to a laboratory. Of those invited, 1,405 (58.9%) completed the full study, 346 (14.5%) partially completed, and 636 (26.6%) declined to take part or were uncontactable (25).

A small portion of participants who completed the full laboratory study later died (n=12) or withdrew from TAHS (n=18). The remaining 1,375 participants were invited to take part in this BHR study. Of those invited, 840 (61.1%) took part in the study, 696 of whom completed the full study including a questionnaire and methacholine bronchial provocation test, 286 (20.8%) declined participation and 249 (18.1%) were uncontactable. After exclusions due to missing asthma data (n=2), the final sample size was 694.

The BHR study was approved by the University of Melbourne Human Research Ethics Committee (HREC Ref. no. 040375.1). Written informed consent was obtained from all participants.

Lung function

Forced Expiratory Volume in one second (FEV_1) was measured using the EasyOne Pro® ultrasonic spirometer (ndd Medizintechnik, AG, Zürich, Switzerland). Acceptability and repeatability of spirometry testing was assessed according to American Thoracic Society and European Respiratory Society guidelines (26). If the best baseline FEV_1 was less than 1.5 litres or less than 70% of the predicted value (27), testing was discontinued.

The methacholine challenge was conducted as per American Thoracic Society guidelines (28). See Methods in online supplement for further details.

Definitions

LogDRS – Calculated as ln(DRS + 1.1). The DRS is calculated as FEV_1 decline (%) divided by the maximum dose of methacholine administered (μmol) (11).

Definitions of other variables are provided in the online supplement.

Statistical analysis

All FEV_1 measurements collected for each participant during the bronchial provocation test were modelled using an LMM. Predictors of FEV_1 were sex, methacholine dose, age, height, smoking and asthma status. Sex, age, smoking and asthma status were examined as predictors of the change in FEV_1 with increasing dose using interaction terms between each predictor and dose. Both random intercepts and slopes were considered to account for individual differences in baseline FEV_1 values and differing individual slopes.

A standard multivariable linear regression was used to model logDRS against the same predictors of change in FEV_1 used in the LMM.

Height is a strong predictor of baseline FEV_1. To explore the impact of assuming all subjects started at the same baseline FEV_1 when this was not the case, both analyses were repeated with height included as a predictor of slope.

The estimated effects from the two approaches were not directly comparable due to the transformations used in obtaining the logDRS. However, the direction and strength of the evidence of associations observed logDRS were compared. All statistical analyses were performed using Stata version 13.1 (StataCorp, College Station, TX, USA).

Results

The characteristics of the study sample are summarised in Table 1. There was a similar proportion of women (50.4%) and men. As expected, men, on average, were taller, had a higher baseline FEV_1, and were slightly more likely to be current smokers, whilst women were more likely to have current asthma.

Table 1 – Study characteristics of participants included in the analyses (N = 694)

Characteristics	**Males (N=344), n(%)**	**Females (N=350), n(%)**	**Total (N=694), n(%)**
Age (years)*	49.6 [0.60]	49.6 [0.60]	49.6 [0.60]
Height (cm)*	177.0 [5.99]	164.0 [5.62]	170.4 [8.70]
Asthma status			
Never asthma	197 (57.3)	174 (49.7)	371 (53.5)
Remitted asthma	81 (23.6)	87 (24.9)	168 (24.2)
Current asthma	66 (19.2)	89 (25.4)	155 (22.3)
Smoking			
Never smoker	166 (48.3)	163 (46.6)	329 (47.4)
Former smoker	119 (34.6)	134 (38.3)	253 (36.5)
Current smoker	59 (17.2)	53 (15.1)	112 (16.1)
Baseline FEV_1(L)	3.76 [0.56]	2.78 [0.40]	3.27 [0.69]

* mean [standard deviation] shown

Visual inspection of a random sample of dose-response curves confirmed that a linear relationship was a reasonable summary of the data for most participants (Figure 1). Figure 1 also illustrates the individual differences in initial FEV_1 and the change in FEV_1 during the methacholine challenge, demonstrating the need for random intercepts and slopes.

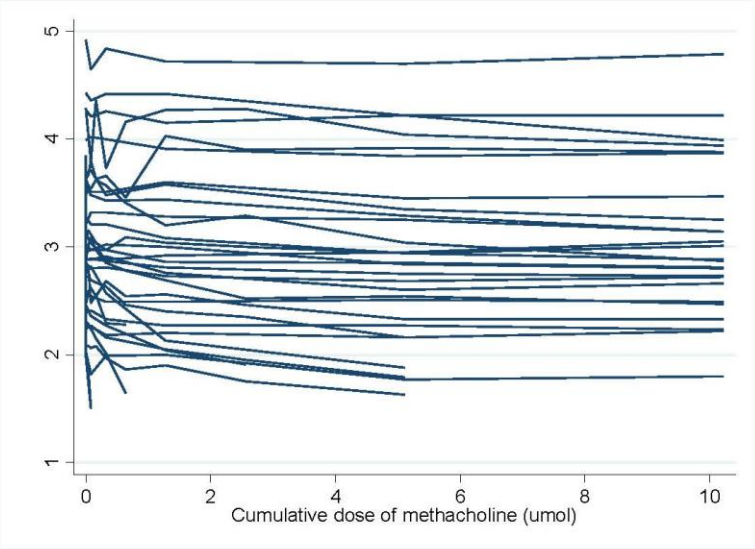

Figure 1: Dose-response curves from a random sample of study participants (n=37)

Associations between predictors and rate of change in FEV_1 per µmol methacholine, as determined using the LMM, and log %change in FEV_1 per µmol methacholine, as determined by regression of the logDRS, are displayed in Table 2. The LMM estimates correspond to the interaction terms between methacholine dose and each of the predictors, interpreted as the mean change in FEV_1 per µmol of methacholine with a one unit increase in the predictor variable. For a categorical variable, it is the difference in the mean change in FEV_1 per µmol of methacholine between the selected category and the reference category. Therefore, in the LMM omitting height (Model 1), the estimate corresponding to "Current Asthma" implies that the fall in FEV_1 for people with current asthma will be, on average, 37ml greater per µmol methacholine than that experienced by people who have never suffered from asthma, for individuals of the same age, sex and smoking status. For those with

remitted asthma, the estimated mean fall in FEV_1 will be 13ml greater per µmol methacholine compared to those who have never had asthma. Smoking was also identified as a risk factor of increased BR with current smokers having an estimated mean fall in FEV_1 14ml greater per µmol methacholine than never smokers, for individuals of the same age, sex and asthma status.

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Table 2: Associations between predictor variables and bronchial responsiveness to methacholine as determined by regression of the logDRS and change in FEV_1 modelled in an LMM

	Model 1				Model 2			
	LogDRS regression		LMM		LogDRS regression		LMM	
Predictor variable	**β-coefficient (SE)**	**p-value**	**β-coefficient (SE)**	**p-value**	**β-coefficient (SE)**	**p-value**	**β-coefficient (SE)**	**p-value**
Sex (female)	0.26 (0.09)	0.003	-0.0004 (0.003)	0.88	0.14 (0.13)	0.28	-0.003 (0.004)	0.51
Age (years)	0.26 (0.07)	<0.001	-0.010 (0.002)	<0.001	0.26 (0.07)	<0.001	-0.01 (0.002)	<0.001
Height (cm)	-	-	-	-	-0.009 (0.007)	0.21	-0.002 (0.0002)	0.45
Asthma status								
Never asthma	ref	-	ref	-	ref	-	Ref	-
Remitted asthma	0.44 (0.10)	<0.001	-0.013 (0.003)	<0.001	0.43 (0.10)	<0.001	-0.013 (0.003)	<0.001
Current asthma	1.16 (0.11)	<0.001	-0.037 (0.004)	<0.001	1.16 (0.11)	<0.001	-0.037 (0.004)	<0.001
Smoking								
Never smoker	ref	-	ref	-	ref	-	ref	-
Former smoker	0.013 (0.09)	0.89	0.0010 (0.003)	0.75	0.009 (0.09)	0.92	0.0009 (0.003)	0.76
Current smoker	0.26 (0.12)	0.036	-0.014 (0.004)	0.001	0.26 (0.12)	0.037	-0.014 (0.004)	0.001

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LogDRS - logarithm of the dose-response slope; LMM - linear mixed model; SE – standard error; M - male; F - female; ref - reference category

Units for LMMs – change in FEV_1(L)/µmol Methacholine

10

Models without height (Model 1 for each method) showed that sex was not associated with the change in FEV_1 in the LMM (β=-0.0004; p=0.88). However, it was a predictor of logDRS which was higher in females (β=0.26; p=0.003), indicating a faster drop in FEV_1 with increasing dose of methacholine.

Measures of association for all other variables were comparable in the two models - former smoking was not associated with logDRS in the regression or change in FEV_1 in the LMM. Increasing age, current smoking, remitted asthma and current asthma were associated with a decrease in FEV_1 in the LMM and a corresponding increase in logDRS with increasing methacholine. The strength of the evidence for an association for each predictor was similar in both models, except for current smoking where the strength of the evidence for an association was moderate in the logDRS regression (p=0.036) and strong in the LMM (p=0.001).

When height was added as a predictor of log %change in FEV_1 in the regression and change in FEV_1 in the LMM (Model 2 for each method), sex was no longer a predictor of logDRS (β=0.14; p=0.28), bringing the results in line with those seen in the LMM (β=-0.003; p=0.51). The direction and strength of all other associations remained unchanged and the difference in the strength of the evidence for an association with current smoking was still present.

We further checked whether using the log transformed DRS met the normality assumptions underlying linear regression. Figure 2 shows the plots of residuals versus fitted values using the multivariate linear regression model (Model 1). Under normality, the scatterplot of residuals versus fitted values is expected to show a random scatter of points above and below zero with the residuals falling relatively close to zero. The residuals deviate from zero with a decreasing trend. This implies that both the normality and independence of errors

assumptions for the linear regression have been violated, thus the results from the regression model may not be valid.

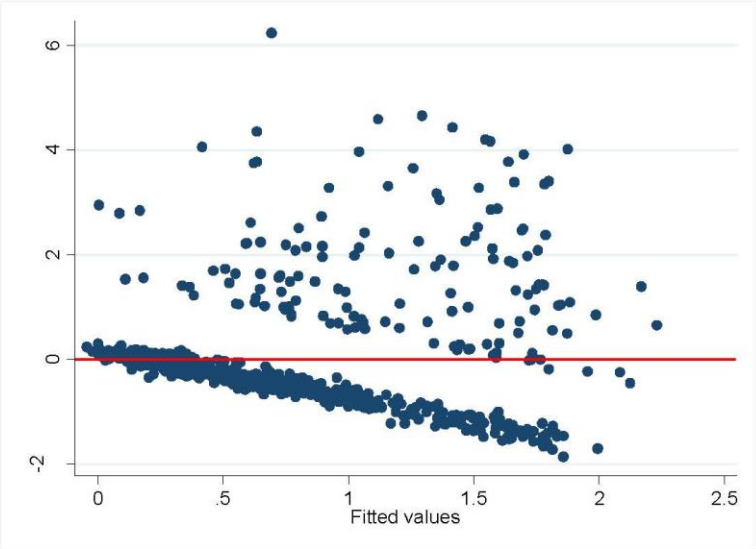

Figure 2: Plot of residuals vs fitted values for regression of the logDRS (Model 1)

Discussion

Our study showed that associations between relevant risk factors and BR, measured by a provocation challenge, can differ between analyses using the logDRS and those using the more reliable and better powered LMM. We used well-established risk factors for BHR (i.e. sex, age, smoking and asthma) to demonstrate this (5, 7, 14-19, 29). Our LMM analysis found that sex was not a predictor of BR. However, using logDRS, an association between sex and BR was noted in the model without height. The difference observed here may be attributed to the fact that the LMM accounted for the different baseline FEV_1 measurements, which were on average lower in women compared to men. This was due to

their lower height on average and consequently smaller lungs. By not accounting for different baseline FEV_1 values, the linear regression model captured a difference in slope between the two sexes which was merely due to females starting at a lower FEV_1 and thus recording a bigger relative fall in FEV_1 than males. Adding height to the model removed the association between sex and logDRS, further supporting that the difference in slope observed between sexes was due to differences in baseline FEV_1, which was partly corrected by adjusting for height. On the other hand, the LMM assumed random baseline FEV_1, thus concluding no difference in slope between the two sexes.

Other studies have investigated the relationship between gender and BR or BHR and observed associations that disappeared when baseline lung function was accounted for (30, 31). Wassmer and colleagues used various definitions of BHR, including a transformed DRS, and recommend using the DRS as the outcome measure in epidemiologic studies, transformed if necessary. They further advocated for including baseline FEV1 in the regression model (30). However, DRS is a mathematical function of baseline FEV1 and therefore strongly correlated. This may result in concluding no association for exposure variables that would otherwise be associated with the outcome.

Differences observed in the strength of the evidence for an association between current smoking and BR further demonstrates that the two models can produce different outcomes, even among the most strongly established risk factors. The LMM is likely to have greater power, as it uses all FEV_1 measurements for each individual, and greater accuracy as it accounts for differences in baseline FEV_1 and considers the error around the slope for each individual. Hence the results are more reliable. The smaller p-value for current smoking in the LMM is, therefore, likely to be the more reliable result and is consistent with smoking being a strong risk factor for BHR. For the first time, we also have estimates of the effects of

predictors on the change in FEV_1 during a provocation challenge which are easy to understand. The mean fall in FEV_1 in those with current asthma was 37ml greater per µmol of methacholine than those who have never suffered from asthma. The mean fall in FEV_1 in those with remitted asthma and current smokers was also greater by 13ml and 14ml, respectively, per µmol of methacholine compared to their respective reference categories. These differences are considerable given 10.2 µmol of methacholine is administered to those who reach the end of test. The interpretability of the estimates is a significant advantage of the LMM.

We have demonstrated that the log-transformed DRS in the linear regression did not achieve normality and violated the independence of errors assumption. The logDRS had a strongly positively skewed distribution in this asthma and bronchitis enriched sample. When the distribution of an outcome variable is skewed and assumptions of the linear regression method are violated, the results obtained are likely to be unreliable. Hence the importance of assessing the data from a provocation challenge using an appropriate statistical method such as the LMM we have used here.

We assumed a linear relationship between FEV_1 and methacholine dose based on a visual examination of plots from a random sample of participants. Other studies that examined this relationship in more detail some years ago also concluded that linearity is a reasonable summary of the individual plots. Cockcroft and Berschied found that a linear model summarised histamine dose-response curves better than a logarithmic model (32). Bellia and colleagues also found that either linear or exponential models were preferable to polynomial or logarithmic models when modelling the response to carbachol (a cholinergic agonist) assessed by specific airway conductance (33). Similarly, Orehek and colleagues also observed linear dose-response relationships to carbachol (34).

By comparing the findings of an LMM with those from regression of the logDRS using the same data, we demonstrated that factors identified as being associated with BR can depend on the statistical method used. Although the LMM is likely to have greater power and accuracy, this method has some limitations. We assumed linearity based on a visual inspection of a random sample of the data. This assumption may not apply to other datasets and we recommend checking the shape of the dose-response curves prior to using an LMM. The LMM is also a more complex statistical method and assistance from a statistician may be required to interpret the results. The generalisability of the findings from the LMM we have presented here are limited due to the narrow age range of the study sample, and enrichment with asthma and cough. However, the finding that asthma and smoking are risk factors for increased BR are consistent with other research (5, 7, 14-19, 29). This work suggests that sex is not associated with BR and previous research indicating it was may be due to an inherent limitation in the calculation of the slope. Further research is needed to confirm this.

In conclusion, we propose using an LMM to better assess risk factors of increased BR, measured with a bronchial provocation challenge. This well-established technique uses all the data collected, allows direct and meaningful interpretation, and guards against making invalid conclusions.

Acknowledgements

We acknowledge the TAHS study participants and previous investigators, Drs Heather Gibson, Bryan Gandevia, Harold Silverstone and Norelle Lickiss. We thank Professor John Hopper (University of Melbourne, VIC), Dr Iain Feather (Gold Coast Hospital, QLD) and the late Dr Stephen Morrison for their assistance with obtaining funds and data collection. We

also acknowledge the study site coordinators and respiratory scientists who collected data in the lung function laboratories; the research assistants who booked appointments, carried out interviews and entered data; and the organisational roles of Cathryn Wharton and Dr Desiree Mészáros.

References

1. Global Initiative for Chronic Obstructive Lung Disease. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease; 2018.
2. Postma DS, Rabe KF. The Asthma-COPD Overlap Syndrome. *The New England journal of medicine* 2015; 373: 1241-1249.
3. Sterk PJ, Bel EH. Bronchial hyperresponsiveness: the need for a distinction between hypersensitivity and excessive airway narrowing. *The European respiratory journal* 1989; 2: 267-274.
4. Global Initiative for Asthma. Global strategy for asthma management and prevention, 2018. Available from: www.ginasthma.org.
5. Peat JK, Salome CM, Woolcock AJ. Factors associated with bronchial hyperresponsiveness in Australian adults and children. *The European respiratory journal* 1992; 5: 921-929.
6. Arshad SH, Kurukulaaratchy RJ, Fenn M, Matthews S. Early life risk factors for current wheeze, asthma, and bronchial hyperresponsiveness at 10 years of age. *Chest* 2005; 127: 502-508.
7. Leynaert B, Bousquet J, Henry C, Liard R, Neukirch F. Is bronchial hyperresponsiveness more frequent in women than in men? A population-based study. *American journal of respiratory and critical care medicine* 1997; 156: 1413-1420.
8. Litonjua AA, Sparrow D, Weiss ST. The FEF25-75/FVC ratio is associated with methacholine airway responsiveness. The normative aging study. *American journal of respiratory and critical care medicine* 1999; 159: 1574-1579.
9. Kompauer I, Demmelmair H, Koletzko B, Bolte G, Linseisen J, Heinrich J. Association of fatty acids in serum phospholipids with lung function and bronchial hyperresponsiveness in adults. *European journal of epidemiology* 2008; 23: 175-190.

10. Lau S, Illi S, Sommerfeld C, Niggemann B, Bergmann R, von Mutius E, Wahn U. Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. *Lancet* 2000; 356: 1392.

11. O'Connor G, Sparrow D, Taylor D, Segal M, Weiss S. Analysis of dose-response curves to methacholine. An approach suitable for population studies. *The American review of respiratory disease* 1987; 136: 1412-1417.

12. Britton J, Pavord I, Richards K, Knox A, Wisniewski A, Wahedna I, Kinnear W, Tattersfield A, Weiss S. Factors influencing the occurrence of airway hyperreactivity in the general population: the importance of atopy and airway calibre. *The European respiratory journal* 1994; 7: 881-887.

13. Rijcken B, Schouten JP, Weiss ST, Speizer FE, van der Lende R. The relationship between airway responsiveness to histamine and pulmonary function level in a random population sample. *The American review of respiratory disease* 1988; 137: 826-832.

14. Trigg CJ, Bennett JB, Tooley M, Sibbald B, D'Souza MF, Davies RJ. A general practice based survey of bronchial hyperresponsiveness and its relation to symptoms, sex, age, atopy, and smoking. *Thorax* 1990; 45: 866-872.

15. Bakke PS, Baste V, Gulsvik A. Bronchial responsiveness in a Norwegian community. *The American review of respiratory disease* 1991; 143: 317-322.

16. Paoletti P, Carrozzi L, Viegi G, Modena P, Ballerin L, Di Pede F, Grado L, Baldacci S, Pedreschi M, Vellutini M, et al. Distribution of bronchial responsiveness in a general population: effect of sex, age, smoking, and level of pulmonary function. *American journal of respiratory and critical care medicine* 1995; 151: 1770-1777.

17. Sparrow D, O'Connor G, Colton T, Barry CL, Weiss ST. The relationship of nonspecific bronchial responsiveness to the occurrence of respiratory symptoms and decreased

levels of pulmonary function. The Normative Aging Study. *The American review of respiratory disease* 1987; 135: 1255-1260.

18. Schwartz J, Schindler C, Zemp E, Perruchoud AP, Zellweger JP, Wuthrich B, Leuenberger P, Ackermann-Liebrich U. Predictors of methacholine responsiveness in a general population. *Chest* 2002; 122: 812-820.
19. Cockcroft DW, Killian DN, Mellon JJ, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clinical allergy* 1977; 7: 235-243.
20. Verbeke G, Molenberghs G. Linear Mixed Models for Longitudinal Data. Springer; 2000.
21. Byrne S, Milanzi E, Burgess J, Hodge A, Matheson MC, Erbas B, Perret J, Thompson BR, Abramson MJ, Walters EH, Dharmage S, Jenkins M. Measuring bronchial hyperresponsiveness using a more sophisticated biostatistical method: the linear mixed effects model. *Respirology* 2017; 22: 37-37.
22. Gibson HB, Silverstone H, Gandevia B, Hall GJ. Respiratory disorders in seven-year-old children in Tasmania. Aims, methods and administration of the survey. *The Medical journal of Australia* 1969; 2: 201-205.
23. Nakajima K, Dharmage SC, Carlin JB, Wharton CL, Jenkins MA, Giles GG, Abramson MJ, Haydn Walters E, Hopper JL. Is childhood immunisation associated with atopic disease from age 7 to 32 years? *Thorax* 2007; 62: 270-275.
24. Perret JL, Dharmage SC, Matheson MC, Johns DP, Gurrin LC, Burgess JA, Marrone J, Markos J, Morrison S, Feather I, Thomas PS, McDonald CF, Giles GG, Hopper JL, Wood-Baker R, Abramson MJ, Walters EH. The interplay between the effects of lifetime asthma, smoking, and atopy on fixed airflow obstruction in middle age. *American journal of respiratory and critical care medicine* 2013; 187: 42-48.

25. Matheson MC, Abramson MJ, Allen K, Benke G, Burgess JA, Dowty JG, Erbas B, Feather IH, Frith PA, Giles GG, Gurrin LC, Hamilton GS, Hopper JL, James AL, Jenkins MA, Johns DP, Lodge CJ, Lowe AJ, Markos J, Morrison SC, Perret JL, Southey MC, Thomas PS, Thompson BR, Wood-Baker R, Haydn Walters E, Dharmage SC, TAHS investigator group. Cohort Profile: The Tasmanian Longitudinal Health STUDY (TAHS). *International journal of epidemiology* 2017; 46: 407-408i.

26. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J. Standardisation of spirometry. *The European respiratory journal* 2005; 26: 319-338.

27. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *American journal of respiratory and critical care medicine* 1999; 159: 179-187.

28. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, MacIntyre NR, McKay RT, Wanger JS, Anderson SD, Cockcroft DW, Fish JE, Sterk PJ. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *American journal of respiratory and critical care medicine* 2000; 161: 309-329.

29. Burney PG, Britton JR, Chinn S, Tattersfield AE, Papacosta AO, Kelson MC, Anderson F, Corfield DR. Descriptive epidemiology of bronchial reactivity in an adult population: results from a community study. *Thorax* 1987; 42: 38-44.

30. Wassmer G, Jörres RA, Heinrich J, Wjst M, Reitmeir P, Wichmann HE. The association between baseline lung function and bronchial responsiveness to methacholine. *European journal of medical research* 1997; 2: 47-54.31. Kanner RE, Connett JE,

Altose MD, Buist AS, Lee WW, Tashkin DP, Wise RA. Gender difference in airway hyperresponsiveness in smokers with mild COPD. The Lung Health Study. *American journal of respiratory & critical care medicine* 1994; 150: 956-961.

32. Cockcroft DW, Berscheid BA. Slope of the dose-response curve: usefulness in assessing bronchial responses to inhaled histamine. *Thorax* 1983; 38: 55-61.

33. Bellia V, Rizzo A, Amoroso S, Mirabella A, Bonsignore G. Analysis of dose-response curves in the detection of bronchial hyperreactivity. *Respiration; international review of thoracic diseases* 1983; 44: 10-18.

34. Orehek J, Gayrard P, Smith AP, Grimaud C, Charpin J. Airway response to carbachol in normal and asthmatic subjects: distinction between bronchial sensitivity and reactivity. *The American review of respiratory disease* 1977; 115: 937-943.

Online Data Supplement

Measuring bronchial hyperresponsiveness using the linear mixed effects model

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Overview

Dose-response to a provocative agent such as methacholine is commonly used as an outcome to assess airway sensitivity for different groups of interest in a population, e.g., asthmatic versus non-asthmatic. Individuals are given prescribed increment doses of the provocative agent until either the maximum dose or when FEV_1 drops by a pre-defined amount from the baseline FEV_1 (usually 20%). This implies that individuals have more than one measured value of FEV_1 which, when plotted against the cumulative dose, produces a dose response curve. In population studies, interest is in exploring factors that may be associated with the shape of the dose-response curve. Several methods have been suggested for how to summarise the curve such that it can be easily used as an outcome in statistical methods like linear or logistic regression which require a single outcome measure per individual. The next section gives an overview of such methods.

Logistic Regression using a Dichotomous Variable

To determine the value of the dichotomised variable, the percent change in FEV_1 relative to the initial FEV_1 is first obtained as

$$\%\ change\ in\ FEV_1 = \frac{FEV_{1i} - FEV_{1f}}{FEV_{1i}}\ x\ 100$$

where FEV_{1i} and FEV_{1f} are the initial and final FEV_1 measurements. An arbitrary cut-off point (usually a 20% drop in FEV_1) is then used to define BHR (yes/no). Due to the loss of information involved in this method, individuals with very different curves can be grouped together. This is demonstrated in Figure E1, developed using real data from four study participants. Using this method, persons 1 and 2 would be classified as not having BHR, and persons 3 and 4 as having BHR. The graph also illustrates the assumption that all persons start at the same point (an FEV_1 of 100%) and differences in baseline FEV_1 are not considered. This is a popular method; however, it involves vast information loss. Therefore, it will not be used in the comparison with the LMM.

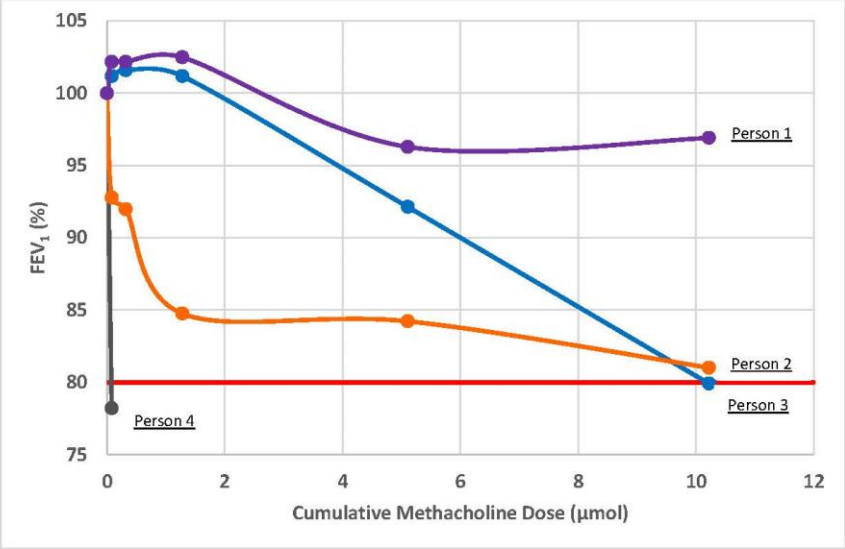

Figure E1: Methacholine dose-response curves of four participants in the TAHS BHR study

Two-stage Analysis Methods

Two-point Method

O'Connor (1) suggested that the slope adequately summarises the dose-response curve in population studies. He proposed to obtain the slope by dividing the percent change in FEV_1 (determined as above) by the cumulative dose i.e.,

$$\lambda_i = \frac{\%\ change\ in\ FEV_1}{Dose_f}$$

where λ_i is the dose-response slope and $Dose_f$ is the maximum dose. The estimated slope is then used as an outcome in standard statistical methods like linear regression. In most cases this is transformed to log scale. We will call this a two-point method and it is a popular approach. Again, using this method, persons with very different curves can have similar dose-response slopes (for example, persons 2 and 3 in figure 1) and differing baseline FEV_1 values are not considered.

Regression-slope Method

Abramson et al (2) noted that the two-point method underutilises information as it only uses two of the FEV_1 measurements per individual. He suggested to estimate the dose-response slope for individuals using linear regression. Specifically, the model

$$FEV_{1ij} = \alpha_{0i} + \alpha_{1i} d_{ij} + \epsilon_{ij}, \qquad \epsilon_{ij} \sim N(0, \sigma_i^2)$$

is fitted for each individual. FEV_{1ij} is the FEV_1 measurement for the i^{th} individual after the j^{th} dose, similarly d_{ij} is the cumulative dose. The parameter α_{1i} is interpreted as the change in FEV_1 when the dose changes by one unit for individual i. Note that this is a definition of slope. Hence, investigation of associations proceeds by using α_{1i} as an outcome in standard statistical methods. We will call this a regression-slope method. The

obvious advantage of this approach over the two-point methods is its ability to use all FEV_1 measurements from an individual. These two methods however share a drawback in that the analysis needs to be done in two stages, i.e., first estimate the slope and then perform the association analysis (regression against factors of interest). In general, two-stage analysis methods are associated with loss of information (3) as the summary statistic may not adequately describe the outcome.

Further, in the case of the regression-slope method, the slope for each individual is estimated with different precision, i.e., slopes for individuals with many outcome measurements are estimated with higher precision than those with fewer outcome measurements. In addition, the two stage analysis methods treat the slopes as measured values which ignores the fact that they have been estimated from the data and thus have variability around them. Ignoring these sources of variation can result in smaller standard errors for the measures of association, in which case we inflate the type I error and can conclude that there is an effect when none exists. Importantly, using little information can produce biased individual slope estimates which can lead to a biased average slope estimate. Note that there is no way to tell which issue will affect the results or whether the results have been affected at all.

The Linear Mixed Model

Linear mixed models (LMMs) have been used for decades in various fields of research, notably medical and agriculture. They are a direct extension of the regression-slope method. LMMs combine the two stages of analysis into one, that is, obtaining individual slopes and investigating their association with potential risk factors are done in one step. LMMs eliminate the need to first summarise the dose-response slope for each individual and thus avoids possible efficiency loss, inflating type I error and bias. Even in situations

where the regression-slope and LMM methods give similar results, the latter has other advantages, like, being used for prediction of individual dose response curves (which may be relevant for clinicians). Importantly, LMMs are valid under the missing at random mechanism (4) for missing data which is less strict compared to the missing completely at random (MCAR) mechanism assumed by the two-stage analysis methods.

Figure E2 illustrates some of the advantages of the LMM using the data from persons 2 and 3 from Figure E1. Firstly, the LMM allows for differing baseline FEV_1 values by including random intercepts. These values are notably different for persons 2 and 3. Secondly, the LMM considers the individual variation of the data around the trendline which can vary considerably. For person 3 the variation is small but for person 2 the variation is relatively large.

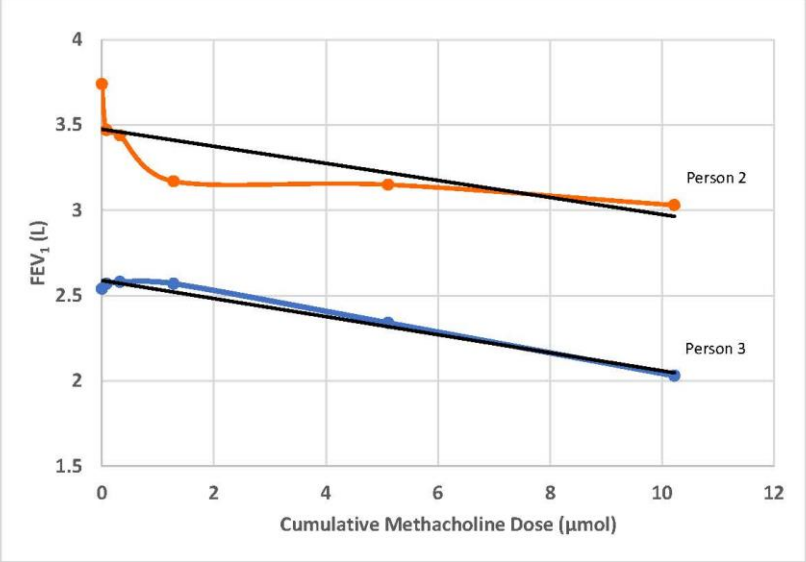

Figure E2: Methacholine dose-response curves and trend lines for two participants in the TAHS BHR study

Consider a simple example where the objective is to investigate whether the change in FEV_1 due to a unit change in methacholine dose is different for males and females, i.e., whether the average slopes of the dose response curves for males and females are different. The LMM can be specified as

$$FEV_{1ij} = \beta_0 + b_{0i} + (b_{1i} + \beta_1)d_{ij} + \beta_2 S_i + \beta_3 S_i \times d_{ij} + \epsilon_{ij}, \qquad (1)$$

FEV_{1ij} and d_{ij} are as defined in the regression-slope model, S_i is the sex for i^{th} individual, defined as 1 if female and 0 if male. Parameters b_{0i} and b_{1i} are known as random effects. They are added to acknowledge that FEV_1 measures within an individual are more similar (correlated) than measures between two different individuals, i.e., unlike in linear regression where we assume that any two FEV_1 measurements are not correlated. To see the interpretation of the parameters in LMM (1), let's define the model for each sex by substituting S_i with 1 for females and 0 for males. This results in the following models;

Female

$$FEV_{1ij} = \beta_0 + \beta_2 + b_{0i} + (b_{1i} + \beta_1 + \beta_3)d_{ij} + \epsilon_{ij}, \qquad (1a)$$

Male

$$FEV_{1ij} = \beta_0 + b_{0i} + (b_{1i} + \beta_1)\, d_{ij} + \epsilon_{ij}, \qquad (1b)$$

For females, $\beta_0 + \beta_2 + b_{0i}$ is the initial FEV_1 for individual i, $\beta_0 + \beta_2$ gives the average initial FEV_1 and b_{0i} indicates how individual i's initial FEV_1 deviates from the average initial FEV_1, it is referred to as the random intercept. This implies that if b_{0i} is positive the individual's

initial FEV_1 is above average otherwise it is below average. Similarly, for males β_0 is the average initial FEV_1 and b_{0i} indicates how individual i's initial FEV_1 deviates from the average. Notice that the difference between the average initial FEV_1 for males and females is β_2, thus a null hypothesis of $\beta_2 = 0$ tests whether average initial FEV_1 for males and females are equal.

In the same way, $b_{1i} + \beta_1 + \beta_3$ represents the change in FEV_1 for a unit change in the dose (slope) for the i^{th} female individual, $\beta_1 + \beta_3$ is the average slope for females, and b_{1i} shows how an individual's slope deviates from the average slope, referred to as the random slope. Positive values indicate that fall in FEV_1 for the individual will be less than average and negative values indicate it will be greater than average. For males, the average slope is given by β_1, and the difference between the average slopes for males and females is given by β_3. Thus, a null hypothesis of $\beta_3 = 0$ tests whether the average slopes for males and females are equal, recall that this was our objective. A positive difference indicates that the average fall in FEV_1 for females will be less than that of males. It's important to note that observations from all individuals are used in estimating the parameters.

It follows that when interest is in comparing slopes of the dose response curves between groups of interest, parameters corresponding to the interaction between dose and covariates in the LMM directly perform this comparison.

Comparison with Two-stage Methods

For the regression-slope method the comparison of slopes for males and females would be achieved by

$$\alpha_{1i} = \beta_1 + \beta_3 S_i + \epsilon_i, \qquad \epsilon_i \sim N(0, \sigma_\epsilon^2)$$

Recall that α_{1i} is the estimated slope for the dose-response curve for individual i. In this case β_1 is average slope for males and β_3 is the difference between the average slopes for males and females.

Similarly, for the two-point method we use the model

$$\lambda_i = \beta_1 + \beta_3 S_i + \epsilon_i, \qquad \epsilon_i \sim N(0, \sigma_\epsilon^2)$$

and the interpretation of the parameters is similar to the regression-slope method above. The two-stage analysis methods do not provide estimates for individual deviations from the average initial FEV_1. Therefore, these methods do not consider these differences in the determination of the slope.

Methods

Measurement and definition of variables

Anthropometry

Height (cm) was recorded using the Leicester Height Measure Mk II (Invicta Plastics Ltd, Oadby, Leicester, UK) with the participant shoeless.

Lung function

Forced Expiratory Volume in one second (FEV_1) was measured using the EasyOne Pro® ultrasonic spirometer (ndd Medizintechnik, AG, Zürich, Switzerland). Acceptability and repeatability of spirometry testing was assessed according to the 2005 ATS/ERS guidelines (5). If the best baseline FEV_1 was less than 1.5 litres or less than 70% of the predicted value(6), testing was discontinued.

For the methacholine challenge, methacholine chloride (Provocholine®, USP Methapharm Inc, Brantford, ON, Canada) was administered by a Mefar MB3 inhalation dosimeter (Mefar SRL, Bovezzi, Italy) in incremental doses until FEV_1 fell to less than 80% of the initial pre-

methacholine FEV_1 value or the maximum cumulative dose of 2mg (10.2 µmol) was administered (7). As per ATS guidelines, those with a history of asthma or recent respiratory symptoms were tested via a long protocol in which doses increased in doubling increments. A short protocol was utilised otherwise, in which doses increased in quadrupling increments. These subjects were changed to the long protocol if FEV_1 fell by 10% or more (7).

Definitions

LogDRS – Calculated as ln(DRS + 1.1). The DRS is calculated as FEV_1 decline (%) divided by the maximum dose of methacholine administered (µmol) (1).

Asthma status – categorised as never, remitted or current asthma, based on responses to the questions "Have you at any time in your life suffered from asthma or wheezy breathing?" and "Have you had an attack of asthma within the last 12 months?".

Smoking status was self-reported by participants and classified as never, former or current smoking.

References

1. O'Connor G, Sparrow D, Taylor D, Segal M, Weiss S. Analysis of dose-response curves to methacholine. An approach suitable for population studies. *The American review of respiratory disease* 1987; 136: 1412-1417.

2. Abramson MJ, Saunders NA, Hensley MJ. Analysis of bronchial reactivity in epidemiological studies. *Thorax* 1990; 45: 924-929.

3. Verbeke G, Molebergh G. Linear Mixed Models for Longitudinal Data. Springer; 2000.

4. Gilboy MB, Heinerichs S, Pazzaglia G. Enhancing Student Engagement Using the Flipped Classroom. *Journal of Nutrition Education and Behavior*; 47: 109-114.

5. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J. Standardisation of spirometry. *The European respiratory journal* 2005; 26: 319-338.

6. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *American journal of respiratory and critical care medicine* 1999; 159: 179-187.

7. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, MacIntyre NR, McKay RT, Wanger JS, Anderson SD, Cockcroft DW, Fish JE, Sterk PJ. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *American journal of respiratory and critical care medicine* 2000; 161: 309-329.

Appendix 6: Chapter 6 supplementary data

Table A1 – Unadjusted and adjusted associations of fruit intake with lung function outcomes

Lung function measure	Fruit intake (serves/day)					p_{trend}	$p_{linearity}$
	< 1	1	2	3	4 +		
FEV_1 (L)							
Model 1	Ref	0.09 (-0.04, 0.23)	-0.04 (-0.18, 0.10)	-0.06 (-0.25, 0.13)	-0.02 (-0.36, 0.32)	0.25	0.23
Model 2	Ref	0.01 (-0.08, 0.10)	0.05 (-0.04, 0.15)	0.04 (-0.08, 0.16)	0.05 (-0.18, 0.27)	0.29	0.92
Model 3	Ref	0.003 (-0.08, 0.09)	0.02 (-0.07, 0.12)	0.0003 (-0.13, 0.13)	-0.008 (-0.25, 0.23)	0.85	0.95
FVC (L)							
Model 1	Ref	0.12 (-0.05, 0.30)	-0.09 (-0.27, 0.10)	-0.11 (-0.36, 0.13)	0.03 (-0.41, 0.46)	0.16	0.12
Model 2	Ref	0.005 (-0.10, 0.11)	0.07 (-0.04, 0.18)	0.06 (-0.08, 0.21)	0.11 (-0.16, 0.37)	0.15	0.91
Model 3	Ref	0.003 (-0.10, 0.11)	0.04 (-0.07, 0.16)	0.03 (-0.12, 0.18)	0.04 (-0.24, 0.32)	0.49	0.94
FEV_1/FVC (%)							
Model 1	Ref	0.14 (-1.07, 1.36)	0.74 (-0.54, 2.03)	0.62 (-1.09, 2.32)	-0.83 (-3.88, 2.23)	0.47	0.62
Model 2	Ref	0.29 (-0.86, 1.43)	0.19 (-1.04, 1.41)	-0.09 (-1.70, 1.53)	-0.67 (-3.61, 2.27)	0.81	0.88
Model 3	Ref	0.15 (-1.00, 1.31)	-0.07 (-1.33, 1.19)	-0.35 (-2.03, 1.32)	-0.53 (-3.67, 2.61)	0.62	0.96

β-coefficient (95%CI) presented for each fruit category

Model 1: Unadjusted linear regression analysis

Model 2: Regression analysis adjusted for age, height, sex, smoking status, asthma status, BMI category, education, occupation

Model 3: Model 2 + fat score, vegetable intake

Table A2: Adjusted associations between fruit intake and lung function by sex

Fruit intake (serves/day)	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Male (n=394)	Female (n=400)	Male (n=394)	Female (n=400)	Male (n=394)	Female (n=400)
< 1	Ref	Ref	Ref	Ref	Ref	Ref
1	-0.004 (-0.12, 0.11)	0.03 (-0.11, 0.16)	-0.01 (-0.15, 0.12)	0.04 (-0.12, 0.20)	0.16 (-1.36, 1.68)	0.18 (-1.57, 1.94)
2	0.08 (-0.06, 0.22)	0.01 (-0.14, 0.12)	0.10 (-0.07, 0.26)	0.01 (-0.14, 0.16)	0.38 (-1.45, 2.21)	-0.37 (-2.08, 1.35)
3	0.13 (-0.06, 0.33)	-0.08 (-0.25, 0.09)	0.20 (-0.03, 0.43)	-0.07 (-0.27, 0.12)	-0.37 (-2.97, 2.23)	-0.38 (-2.56, 1.81)
4 +	-0.14 (-0.51, 0.23)	0.07 (-0.24, 0.37)	-0.06 (-0.49, 0.37)	0.09 (-0.27, 0.45)	-1.79 (-6.59, 3.02)	-0.23 (-3.79, 4.26)
p_{trend}	0.29	0.50	0.13	0.68	0.77	0.67
$p_{interaction}$	0.18		0.16		0.89	
$p_{linearity}$	0.55		0.57		0.96	

β-coefficient (95%CI) presented for each fruit category

Adjusted for age, height, sex, smoking status, asthma status, BMI category, education, occupation, vegetable intake, fat score

Table A3: Adjusted associations between fruit intake and lung function by smoking

Fruit intake (serves/day)	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never smoker (n=358)	Former smoker (n=294)	Current smoker (n=142)	Never smoker (n=358)	Former smoker (n=294)	Current smoker (n=142)	Never smoker (n=358)	Former smoker (n=294)	Current smoker (n=142)
< 1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
1	-0.06 (-0.20, 0.07)	-0.01 (-0.16, 0.14)	0.13 (-0.04, 0.31)	-0.09 (-0.25, 0.07)	-0.006 (-0.18, 0.17)	0.16 (-0.05, 0.37)	0.48 (-1.31, 2.26)	-0.43 (-2.42, 1.56)	0.45 (-1.88, 2.78)
2	-0.02 (-0.16, 0.13)	-0.004 (-0.16, 0.15)	0.13 (-0.09, 0.36)	-0.03 (-0.20, 0.14)	0.04 (-0.15, 0.22)	0.18 (-0.08, 0.45)	0.39 (-1.49, 2.27)	-0.77 (-2.81, 1.27)	0.26 (-2.70, 3.22)
3	-0.03 (-0.22, 0.15)	-0.03 (-0.23, 0.17)	0.07 (-0.28, 0.43)	-0.05 (-0.27, 0.17)	0.02 (-0.22, 0.25)	0.17 (-0.24, 0.59)	0.38 (-2.08, 2.84)	-0.93 (-3.53, 1.67)	-1.88 (-6.50, 2.75)
4 +	-0.01 (-0.30, 0.28)	-0.14 (-0.58, 0.30)	-0.01 (-0.30, 0.28)	0.05 (-0.29, 0.39)	-0.19 (-0.70, 0.33)	0.05 (-0.29, 0.39)	-0.74 (-4.49, 3.02)	0.38 (-5.38, 6.13)	-0.74 (-4.49, 3.02)
p_{trend}	1.00	0.70	0.26	0.91	0.92	0.13	1.00	0.52	0.74
$p_{interaction}$	0.80			0.67			0.96		
$p_{linearity}$	0.95			0.90			0.98		

β-coefficient (95%CI) presented for each fruit category

Adjusted for age, height, sex, smoking status, asthma status, BMI category, education, occupation, vegetable intake, fat score

Table A4. Adjusted associations between fruit intake and lung function by asthma status

Fruit intake (serves/day)	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never asthma (n=419)	Remitted asthma (n=200)	Current asthma (n=195)	Never asthma (n=419)	Remitted asthma (n=200)	Current asthma (n=195)	Never asthma (n=419)	Remitted asthma (n=200)	Current asthma (n=195)
< 1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
1	0.03 (-0.09, 0.15)	-0.04 (-0.21, 0.13)	0.01 (-0.18, 0.20)	-0.004 (-0.15, 0.14)	0.03 (-0.18, 0.23)	-0.009 (-0.23, 0.21)	0.64 (-0.93, 2.22)	-1.33 (-3.58, 0.91)	1.05 (-1.40, 3.49)
2	-0.02 (-0.15, 0.11)	0.02 (-0.17, 0.21)	0.13 (-0.07, 0.32)	0.05 (-0.11, 0.20)	-0.02 (-0.24, 0.20)	0.10 (-0.13, 0.33)	-1.30 (-3.00, 0.39)	0.62 (-1.83, 3.07)	2.09 (-0.46, 4.64)
3	0.03 (-0.14, 0.20)	-0.12 (-0.36, 0.13)	0.07 (-0.21, 0.34)	0.10 (-0.10, 0.29)	-0.12 (-0.41, 0.16)	0.03 (-0.29, 0.35)	-0.76 (-2.97, 1.45)	-1.19 (-4.37, 1.98)	1.84 (-1.72, 5.40)
4 +	-0.08 (-0.43, 0.27)	-0.02 (-0.58, 0.54)	0.11 (-0.28, 0.51)	-0.03 (-0.44, 0.38)	-0.11 (-0.76, 0.55)	0.20 (-0.26, 0.65)	-1.23 (-5.74, 3.28)	-1.54 (-5.70, 8.78)	0.25 (-4.84, 5.33)
p_{trend}	0.86	0.63	0.25	0.40	0.41	0.30	0.12	0.85	0.26
$p_{interaction}$	0.79			0.86			0.097		
$p_{linearity}$	0.95			0.99			0.36		

β-coefficient (95%CI) presented for each fruit category

Adjusted for age, height, sex, smoking status, asthma status, BMI category, education, occupation, vegetable intake, fat score

Table A5: Adjusted associations between fruit intake and lung function by BMI

Fruit intake (serves/day)	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	BMI <25 (n=221)	25≤BMI<30 (n=310)	BMI≥30 (n=263)	BMI <25 (n=221)	25≤BMI<30 (n=310)	BMI≥30 (n=263)	BMI <25 (n=221)	25≤BMI<30 (n=310)	BMI≥30 (n=263)
< 1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
1	0.15 (-0.02, 0.33)	-0.06 (-0.20, 0.08)	-0.02 (-0.17, 0.13)	0.12 (-0.09, 0.32)	-0.07 (-0.23, 0.09)	0.01 (-0.16, 0.19)	1.79 (-0.50, 4.08)	-0.20 (-2.00, 1.61)	-0.48 (-2.41, 1.45)
2	0.16 (-0.03, 0.35)	0.0008 (-0.15, 0.15)	-0.04 (-0.19, 0.12)	0.15 (-0.07, 0.37)	0.009 (-0.19, 0.17)	0.03 (-0.15, 0.21)	1.69 (-0.74, 4.12)	-0.10 (-2.07, 1.87)	-1.20 (-3.22, 0.82)
3	0.13 (-0.10, 0.35)	0.04 (-0.17, 0.25)	-0.14 (-0.36, 0.08)	0.05 (-0.21, 0.31)	0.07 (-0.17, 0.32)	-0.008 (-0.27, 0.25)	2.40 (-0.51, 5.31)	-0.33 (-3.05, 2.40)	-2.86 (-5.74, 0.02)
4 +	0.05 (-0.32, 0.41)	-0.02 (-0.39, 0.35)	0.08 (-0.47, 0.63)	0.02 (-0.41, 0.44)	0.09 (-0.35, 0.573)	0.07 (-0.57, 0.72)	1.65 (-3.11, 6.40)	-2.10 (-6.97, 2.78)	0.76 (-6.43, 7.94)
p_{trend}	0.38	0.67	0.35	0.70	0.49	0.87	0.17	0.63	0.082
$p_{interaction}$	0.51			0.85			0.43		
$p_{linearity}$	0.77			0.89			0.89		

β-coefficient (95%CI) presented for each fruit category

Adjusted for age, height, sex, smoking status, asthma status, BMI category, education, occupation, vegetable intake, fat score

Table A6: Adjusted associations between vegetable intake and lung function by sex

Vegetable intake (serves/day)	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Male (n=394)	Female (n=400)	Male (n=394)	Female (n=400)	Male (n=394)	Female (n=400)
< 1	Ref	Ref	Ref	Ref	Ref	Ref
1	0.02 (-0.16, 0.21)	-0.04 (-0.30, 0.22)	-0.04 (-0.26, 0.18)	-0.04 (-0.35, 0.26)	1.13 (-1.29, 3.54)	-0.16 (-3.54, 3.23)
2	0.16 (-0.04, 0.35)	-0.03 (-0.28, 0.22)	0.008 (-0.22, 0.24)	0.04 (-0.25, 0.33)	3.13 (0.58, 5.67)	-1.24 (-4.48, 1.99)
3	0.13 (-0.07, 0.34)	0.05 (-0.20, 0.29)	0.05 (-0.19, 0.29)	0.10 (-0.19, 0.38)	1.72 (-0.97, 4.41)	-0.34 (-3.48, 2.81)
4 +	0.18 (-0.13, 0.49)	0.02 (-0.23, 0.27)	0.10 (-0.26, 0.46)	0.09 (-0.21, 0.38)	2.70 (-1.30, 6.70)	-1.22 (-4.49, 2.05)
p_{trend}	0.018	0.30	0.22	0.12	0.045	0.52
$p_{interaction}$	0.63		0.99		0.060	
$p_{linearity}$	0.77		0.97		0.24	

β-coefficient (95%CI) presented for each vegetable category

Adjusted for age, height, sex, smoking status, asthma status, BMI category, education, occupation

Table A7: Adjusted associations between vegetable intake and lung function by BMI

Vegetable intake (serves/day)	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	BMI <25 (n=221)	25≤BMI<30 (n=310)	BMI≥30 (n=263)	BMI <25 (n=221)	25≤BMI<30 (n=310)	BMI≥30 (n=263)	BMI <25 (n=221)	25≤BMI<30 (n=310)	BMI≥30 (n=263)
< 1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
1	-0.17 (-0.47, 0.12)	0.003 (-0.29, 0.30)	0.05 (-0.17, 0.28)	-0.16 (-0.50, 0.19)	0.03 (-0.31, 0.37)	-0.06 (-0.32, 0.20)	-1.08 (-4.93, 2.78)	-0.33 (-4.17, 3.50)	1.91 (-1.01, 4.83)
2	-0.05 (-0.35, 0.25)	0.04 (-0.26, 0.34)	0.18 (-0.04, 0.41)	-0.05 (-0.40, 0.30)	-0.02 (-0.37, 0.33)	0.10 (-0.16, 0.36)	-0.08 (-4.02, 3.87)	0.93 (-3.00, 4.87)	2.16 (-0.76, 5.09)
3	-0.06 (-0.35, 0.23)	0.07 (-0.23, 0.37)	0.25 (0.02, 0.48)	-0.05 (-0.39, 0.29)	0.06 (-0.29, 0.41)	0.19 (-0.08, 0.46)	-0.51 (-4.31, 3.28)	0.41 (-3.51, 4.33)	2.54 (-0.46, 5.54)
4 +	0.009 (-0.29, 0.31)	0.04 (-0.30, 0.38)	0.15 (-0.12, 0.41)	0.01 (-0.34, 0.37)	0.08 (-0.31, 0.48)	0.10 (-0.21, 0.41)	0.07 (-3.87, 4.02)	-1.04 (-5.46, 3.37)	1.28 (-2.21, 4.76)
p_{trend}	0.20	0.42	0.029	0.29	0.61	0.042	0.56	0.87	0.43
$p_{interaction}$	0.74			0.70			0.86		
$p_{linearity}$	0.73			0.81			0.68		

β-coefficient (95%CI) presented for each vegetable category

Adjusted for age, height, sex, smoking status, asthma status, BMI category, education, occupation

Table A8 - Unadjusted and adjusted associations of vegetable intake with change in FEV1 during the methacholine challenge

Diet measure	Change in FEV1(L)/µmol methacholine		
Fruit intake (serves/day)	Model 1	Model 2	Model 3
<1	Ref	Ref	Ref
1	0.02 (0.008, 0.04)	0.01 (-0.003, 0.02)	0.01 (-0.002, 0.03)
2	0.02 (0.009, 0.04)	0.02 (0.003, 0.03)	0.02 (0.005, 0.03)
3	0.02 (0.005, 0.03)	0.01 (-0.001, 0.03)	0.02 (0.001, 0.03)
4 +	0.02 (-0.00009, 0.03)	0.009 (-0.006, 0.02)	0.01 (-0.003, 0.03)
p_{trend}	0.88	0.55	0.24
$p_{linearity}$	0.023	0.23	0.17

β-coefficient (95%CI) presented

Model 1: Unadjusted linear mixed model

Model 2: linear mixed model adjusted for age, sex, smoking status, asthma status, BMI category, education, occupation

Model 3: Model 2 + fat score, fruit intake

Table A9 - Adjusted associations of fruit intake with change in FEV1 during the methacholine challenge by sex, smoking status, asthma status and BMI

Fruit intake (serves/day)	Sex		Smoking			Asthma			BMI		
	Male (n=344)	Female (n=350)	Never smoker (n=329)	Former smoker (n=253)	Current smoker (n=112)	Never asthma (n=371)	Remitted asthma (n=168)	Current asthma (n=155)	Healthy weight (n=197)	Overweight (n=280)	Obese (n=217)
< 1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
1	-0.005 (-0.01, 0.004)	-0.002 (-0.01, 0.009)	0.002 (-0.008, 0.01)	-0.01 (-0.03, -0.002)	-0.0009 (-0.02, 0.01)	-0.001 (-0.01, 0.008)	-0.005 (-0.02, 0.009)	-0.01 (-0.03, 0.006)	-0.005 (-0.02, 0.009)	-0.0001 (-0.01, 0.01)	-0.009 (-0.02, 0.004)
2	-0.01 (-0.02, 0.0008)	-0.01 (-0.02, 0.0007)	-0.008 (-0.02, 0.003)	-0.01 (-0.02, 0.0004)	-0.02 (-0.04, -0.003)	-0.01 (-0.02, -0.002)	-0.004 (-0.02, 0.01)	-0.02 (-0.04, 0.00004)	-0.02 (-0.03, -0.001)	-0.008 (-0.02, 0.003)	-0.01 (-0.02, 0.003)
3	0.0008 (-0.01, 0.02)	-0.01 (-0.02, 0.003)	-0.0002 (-0.01, 0.01)	-0.01 (-0.03, 0.005)	-0.02 (-0.05, 0.01)	-0.01 (-0.02, 0.0009)	0.003 (-0.02, 0.02)	0.002 (-0.02, 0.02)	-0.008 (-0.03, 0.009)	0.0007 (-0.01, 0.02)	-0.01 (-0.03, 0.005)
4 +	-0.02 (-0.05, 0.01)	-0.02 (-0.05, 0.002)	-0.02 (-0.04, 0.005)	-0.03 (-0.07, 0.02)	-0.02 (-0.04, 0.005)	-0.02 (-0.05, 0.002)	-0.04 (-0.11, 0.02)	-0.01 (-0.05, 0.02)	-0.03 (-0.06, 0.002)	-0.02 (-0.05, 0.01)	-0.008 (-0.05, 0.04)
p_{trend}	0.28	0.011	0.13	0.15	0.049	0.003	0.80	0.62	0.045	0.27	0.11
$p_{interaction}$	0.63		0.31			0.33			0.88		
$p_{linearity}$	0.77		0.44			0.56			0.84		

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, education, occupation, vegetable intake, fat score

Table A10 - Adjusted associations of vegetable intake with change in FEV1 during the methacholine challenge by sex, smoking status and BMI

Vegetable intake (serves/day)	Sex		Smoking			BMI		
	Male (n=344)	Female (n=350)	Never smoker (n=329)	Former smoker (n=253)	Current smoker (n=112)	Healthy weight (n=197)	Overweight (n=280)	Obese (n=217)
< 1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
1	0.02 (0.002, 0.03)	0.0001 (-0.02, 0.02)	-0.004 (-0.01, 0.003)	0.02 (-0.004, 0.04)	0.03 (0.01, 0.06)	0.005 (-0.02, 0.03)	0.05 (0.02, 0.07)	0.002 (-0.02, 0.02)
2	0.03 (0.01, 0.04)	0.002 (-0.02, 0.02)	-0.01 (-0.02, -0.003)	0.02 (-0.0002, 0.04)	0.03 (0.009, 0.06)	0.004 (-0.02, 0.03)	0.06 (0.03, 0.08)	0.01 (-0.01, 0.03)
3	0.02 (0.003, 0.04)	0.003 (-0.02, 0.03)	-0.005 (-0.01, 0.005)	0.02 (0.0005, 0.04)	0.03 (0.004, 0.05)	0.01 (-0.01, 0.03)	0.05 (0.02, 0.08)	-0.0002 (-0.02, 0.02)
4 +	0.01 (-0.01, 0.04)	-0.0003 (-0.02, 0.02)	-0.02 (-0.04, 0.004)	0.02 (-0.006, 0.04)	0.04 (0.008, 0.07)	0.001 (-0.02, 0.03)	0.05 (0.02, 0.08)	-0.001 (-0.02, 0.02)
p_{trend}	0.091	0.92	0.97	0.28	0.13	0.83	0.023	0.76
$p_{interaction}$	0.30		0.13			0.067		
$p_{linearity}$	0.19		0.077			0.078		

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, education, occupation, fruit intake, fat score

Appendix 7: Chapter 7 supplementary data

Table A1: Demographic characteristics by quintile of pattern score for patterns 1 & 2

Characteristic	"High potassium & magnesium" pattern					"High protein & zinc" pattern				
	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
Participants (n)	239	237	237	236	238	239	235	238	237	238
Male, n (%)	162 (67.8)	115 (48.5)	124 (52.3)	106 (44.9)	105 (44.1)	157 (65.7)	127 (54.0)	103 (43.3)	109 (46.0)	116 (48.7)
Age (years)	57.0 [7.6]	57.8 [7.4]	58.1 [7.4]	58.8 [7.6]	59.1 [7.3]	58.2 [7.1]	58.6 [7.8]	58.0 [7.7]	58.0 [7.4]	58.1 [7.5]
Height (metres)*	1.71 [0.09]	1.68 [0.09]	1.68 [0.09]	1.68 [0.09]	1.67 [0.10]	1.71 [0.09]	1.69 [0.09]	1.67 [0.09]	1.68 [0.09]	1.68 [0.09]
Smoking, n (%)										
- Never	97 (40.6)	103 (43.5)	112 (47.3)	120 (50.9)	111 (46.6)	99 (41.4)	101 (43.0)	115 (48.3)	110 (46.4)	118 (49.6)
- Former	81 (33.9)	98 (41.4)	108 (45.6)	99 (42.0)	110 (46.2)	112 (46.9)	100 (42.6)	99 (41.6)	102 (43.0)	83 (34.9)
- Current	61 (25.5)	36 (15.2)	17 (7.2)	17 (7.2)	17 (7.1)	28 (11.7)	34 (14.5)	24 (10.1)	25 (10.6)	37 (15.6)
Energy intake (kj)	9082 [2821]	7777 [2606]	7830 [2591]	7797 [2462]	8765 [2711]	8905 [2823]	7923 [2422]	7815 [2608]	7818 [2524]	8793 [2868]
BMI category, n (%)*										
- Healthy	78 (32.6)	80 (33.9)	65 (27.4)	86 (36.4)	68 (28.6)	78 (32.6)	87 (37.0)	76 (31.9)	65 (27.5)	71 (29.8)
- Overweight	105 (43.9)	107 (45.3)	112 (47.3)	102 (43.2)	111 (46.6)	117 (49.0)	99 (42.1)	119 (50.0)	106 (44.9)	96 (40.3)
- Obese	56 (23.4)	49 (20.8)	60 (25.3)	48 (20.3)	59 (24.8)	44 (18.4)	49 (20.9)	43 (18.1)	65 (27.5)	71 (29.8)
Asthma status, n (%)										
- Never	200 (83.7)	180 (76.0)	202 (85.2)	187 (79.2)	185 (77.7)	206 (86.2)	190 (80.9)	181 (76.1)	193 (81.4)	184 (77.3)
- Remitted	17 (7.1)	35 (14.7)	16 (6.8)	19 (8.1)	22 (9.2)	16 (6.7)	23 (9.8)	23 (9.7)	21 (8.9)	26 (10.9)
- Current	22 (9.2)	22 (9.3)	19 (8.0)	30 (12.7)	31 (13.0)	17 (7.1)	22 (9.4)	34 (14.3)	23 (9.7)	28 (11.8)
COPD, n (%)	16 (6.7)	10 (4.2)	10 (4.2)	6 (2.5)	8 (3.4)	10 (4.2)	10 (4.3)	13 (5.5)	10 (4.2)	7 (2.9)
Atopy, n (%)^	122 (51.3)	143 (60.3)	119 (50.2)	123 (52.6)	115 (48.3)	120 (50.2)	118 (50.6)	139 (58.4)	120 (50.6)	125 (52.5)

All values presented as mean [standard deviation] unless otherwise stated; *n=1186, ^n=1184

Table A2 Demographic characteristics by quintile of pattern score for patterns 3 and 4

Characteristic	"High PUFAs and vitamin E" pattern					"High β-cryptoxanthin & vitamin C" pattern				
	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
Participants (n)	238	237	236	238	238	239	238	235	238	237
Male, n (%)	157 (66.0)	119 (50.2)	95 (40.3)	107 (45.0)	134 (56.3)	175 (73.2)	142 (59.7)	106 (45.1)	95 (39.9)	94 (39.7)
Age (years)	57.9 [7.1]	58.5 [7.3]	58.1 [7.6]	57.6 [7.7]	58.7 [7.7]	57.5 [7.5]	58.1 [7.6]	57.6 [7.5]	58.5 [7.4]	59.0 [7.4]
Height (metres)*	1.71 [0.09]	1.68 [0.09]	1.67 [0.10]	1.67 [0.09]	1.69 [0.09]	1.71 [0.09]	1.70 [0.10]	1.67 [0.09]	1.67 [0.08]	1.68 [0.09]
Smoking, n (%)										
- Never	74 (31.1)	100 (42.2)	114 (48.3)	124 (52.1)	131 (55.0)	84 (35.2)	116 (48.7)	110 (46.8)	114 (47.9)	119 (50.2)
- Former	119 (50.0)	101 (42.6)	100 (42.4)	86 (36.1)	90 (37.8)	127 (53.1)	89 (37.4)	93 (39.6)	93 (39.1)	94 (39.7)
- Current	45 (18.9)	36 (15.2)	22 (9.3)	28 (11.8)	17 (7.1)	28 (11.7)	33 (13.9)	32 (13.6)	31 (13.0)	24 (10.1)
Energy intake (kj)	9135 [2785]	7749 [2631]	7550 [2554]	7829 [2448]	8992 [2647]	9019 [2806]	7923 [2565]	7586 [2290]	7831 [2594]	8896 [2877]
BMI category, n (%)										
- Healthy	62 (26.1)	64 (27.0)	77 (32.8)	83 (34.9)	91 (38.2)	59 (24.7)	61 (25.7)	90 (38.3)	81 (34.0)	86 (36.3)
- Overweight	108 (45.4)	111 (46.8)	114 48.5)	101 (42.4)	103 (43.3)	109 (45.6)	124 (52.3)	94 (40.0)	108 (45.4)	102 (43.0)
- Obese	68 (28.6)	62 (26.2)	44 (18.7)	54 (22.7)	44 (18.5)	71 (29.7)	52 (21.9)	51 (21.7)	49 (20.6)	49 (20.7)
Asthma status, n (%)										
- Never	191 (80.3)	189 (79.8)	197 (83.5)	187 (78.6)	190 (79.8)	196 (82.0)	194 (81.5)	190 (80.9)	190 (79.8)	184 (77.6)
- Remitted	25 (10.5)	22 (9.3)	17 (7.2)	20 (8.4)	25 (10.5)	19 (8.0)	21 (8.8)	22 (9.4)	18 (7.6)	29 (12.2)
- Current	22 (9.2)	26 (11.0)	22 (9.3)	31 (13.0)	23 (9.7)	24 (10.0)	23 (9.7)	23 (9.8)	30 (12.6)	24 (10.1)
COPD, n (%)	11 (4.6)	10 (4.2)	8 (3.4)	12 (5.0)	9 (3.8)	9 (3.8)	7 (2.9)	9 (3.8)	14 (5.9)	11 (4.6)
Atopy, n (%)^	119 (50.0)	134 (56.5)	116 (49.6)	114 (48.1)	139 (58.4)	129 (54.2)	131 (55.3)	122 (52.1)	128 (53.8)	112 (47.3)

All values presented as mean [standard deviation] unless otherwise stated; *n=1186, ^n=1184

Table A3: Demographic characteristics by quintile of pattern score for patterns 5 and 6

Characteristic	"Low calcium and sugars" pattern					"High starch & lycopene" pattern				
	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
Participants (n)	238	238	236	238	237	238	238	237	236	238
Male, n (%)	130 (54.6)	109 (45.8)	112 (47.5)	125 (52.5)	136 (57.4)	164 (68.9)	126 (52.9)	90 (38.0)	97 (41.1)	135 (56.7)
Age (years)	58.8 [7.8]	59.4 [7.5]	57.2 [7.3]	58.4 [7.6]	57.0 [6.9]	60.3 [7.2]	58.7 [7.7]	58.1 [7.5]	57.1 [7.6]	56.7 [7.0]
Height (metres)*	1.69 [0.09]	1.67 [0.09]	1.68 [0.09]	1.69 [0.09]	1.69 [0.08]	1.72 [0.09]	1.69 [0.09]	1.67 [0.08]	1.67 [0.10]	1.68 [0.10]
Smoking, n (%) - Never - Former - Current	 107 (45.0) 94 (39.5) 37 (15.6)	 122 (51.3) 91 (38.2) 25 10.5)	 114 (48.3) 98 (41.5) 24 (10.2)	 98 (41.2) 111 (46.6) 29 (12.2)	 102 (43.0) 102 (43.0) 33 (13.9)	 85 (35.7) 125 (52.5) 28 (11.8)	 111 (46.6) 95 (39.9) 32 (13.5)	 125 (52.7) 83 (35.0) 29 (12.2)	 114 (48.3) 93 (39.4) 29 (12.3)	 108 (45.4) 100 (42.0) 30 (12.6)
Energy intake (kj)	8772 [2578]	8018 [2648]	7783 [2519]	8039 [2786]	8647 [2819]	8908 [2541]	7982 [2422]	7533 2550	7724 [2578]	9108 [2992]
BMI category, n (%)* - Healthy - Overweight - Obese	 81 (34.0) 109 (45.8) 48 (20.2)	 77 (32.5) 105 (44.3) 55 (23.2)	 77 (32.6) 105 (44.5) 54 (22.9)	 73 (30.7) 107 (45.0) 58 (24.4)	 69 (29.1) 111 (46.8) 57 (24.1)	 70 (29.4) 112 (47.1) 56 (23.5)	 94 (39.5) 107 (45.0) 37 (15.6)	 79 (33.3) 110 (46.4) 48 (20.3)	 81 (34.3) 97 (41.1) 58 (24.6)	 53 (22.4) 111 (46.8) 73 (30.8)
Asthma status, n (%) - Never - Remitted - Current	 195 (81.9) 21 (8.8) 22 (9.2)	 190 (79.8) 26 (10.9) 22 (9.2)	 200 (84.8) 19 (8.1) 17 (7.2)	 194 (81.5) 19 (8.0) 25 (10.5)	 175 (73.8) 24 (10.1) 38 (16.0)	 194 (81.5) 23 (9.7) 21 (8.8)	 183 (76.9) 25 (10.5) 30 (12.6)	 197 (83.1) 21 (8.9) 19 (8.0)	 186 (78.8) 20 (8.5) 30 (12.7)	 194 (81.5) 20 (8.4) 24 (10.1)
COPD, n (%)	13 (5.5)	15 (6.3)	8 (3.4)	6 (2.5)	8 (3.4)	15 (6.3)	11 (4.6)	9 (3.8)	8 (3.4)	7 (2.9)
Atopy, n (%)^	119 (50.2)	128 (54.0)	126 (53.6)	124 (52.1)	125 (52.7)	134 (56.5)	131 (55.0)	131 (55.5)	113 (47.9)	113 (47.7)

All values presented as mean [standard deviation] unless otherwise stated; *n=1186, ^n=1184

Table A4: Demographic characteristics by quintile of pattern score for patterns 7 & 8

Characteristic	"High vitamin C, low calcium" pattern					"High α-carotene, low lycopene" pattern				
	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
Participants (n)	239	237	238	237	236	239	238	237	235	238
Male, n (%)	113 (47.3)	116 (49.0)	112 (47.1)	124 (52.3)	147 (62.3)	145 (60.7)	112 (47.1)	111 46.8)	123 (52.3)	121 (50.8)
Age (years)	58.3 [7.3]	58.3 [7.3]	58.7 [7.7]	58.0 [7.6]	57.6 [7.4]	57.8 [7.2]	57.1 [7.2]	57.8 [7.6]	58.9 [7.7]	59.3 [7.6]
Height (metres)*	1.69 [0.09]	1.68 [0.09]	1.68 [0.09]	1.68 [0.09]	1.70 [0.09]	1.69 [0.09]	1.68 [0.09]	1.68 [0.09]	1.68 [0.09]	1.69 [0.09]
Smoking, n (%)										
- Never	113 (47.3)	104 (43.9)	110 (46.2)	109 (46.0)	107 (45.3)	106 (44.4)	122 (51.3)	106 (44.7)	98 (41.7)	111 (46.6)
- Former	99 (41.4)	100 (42.2)	106 (44.5)	93 (39.2)	98 (41.5)	106 (44.4)	92 (38.7)	106 (44.7)	108 (46.0)	84 (35.3)
- Current	27 (11.3)	33 (13.9)	22 (9.2)	35 (14.8)	31 (13.1)	27 (11.3)	24 (10.1)	25 (10.6)	29 (12.3)	43 (18.1)
Energy intake (kj)	9164 [2850]	7842 [2345]	7641 [2416]	7667 [2512]	8948 [2913]	9079 [2946]	7660 [2565]	7773 [2495]	7776 [2324]	8963 [2747]
BMI category, n (%)*										
- Healthy	74 (31.0)	86 (36.4)	75 (31.5)	69 (29.1)	73 (30.9)	72 (30.1)	70 (29.5)	70 (29.5)	76 (32.3)	89 (37.4)
- Overweight	115 (48.1)	105 (44.5)	107 (45.0)	105 (44.3)	105 (44.5)	108 (45.2)	113 (47.7)	114 (48.1)	112 (47.7)	90 (37.8)
- Obese	50 (20.9)	45 (19.1)	56 (23.5)	63 (26.6)	58 (24.6)	59 (24.7)	54 (22.8)	53 (22.4)	47 (20.0)	59 (24.8)
Asthma status, n (%)										
- Never	190 (79.5)	195 (82.3)	190 (79.8)	187 (78.9)	192 (81.4)	192 (80.3)	191 (80.3)	192 (81.0)	191 (81.3)	188 (79.0)
- Remitted	22 (9.2)	19 (8.0)	19 (8.0)	27 (11.4)	22 (9.3)	18 (7.5)	26 (10.9)	21 (8.9)	22 (9.4)	22 (9.2)
- Current	27 (11.3)	23 (9.7)	29 (12.2)	23 (9.7)	22 (9.3)	29 (12.1)	21 (8.8)	24 (10.1)	22 (9.4)	28 (11.8)
COPD, n (%)	17 (7.1)	9 (3.8)	11 (4.6)	6 (2.5)	7 (3.0)	10 (4.2)	6 (2.5)	5 (2.1)	11 (4.7)	18 (7.6)
Atopy, n (%)^	116 (48.7)	118 (49.8)	126 (52.9)	127 (54.0)	135 (57.2)	116 (48.7)	133 (55.9)	127 (54.0)	128 (54.5)	118 (49.6)

All values presented as mean [standard deviation] unless otherwise stated; *n=1186, ^n=1184

Table A5 – Unadjusted and adjusted associations between the "high protein & zinc" dietary pattern score and lung function

Lung function measure	"High protein & zinc" pattern quintiles					p_{trend}	$p_{linearity}$
	Q1	Q2	Q3	Q4	Q5		
FEV_1 (L)							
Model 1	Ref	-0.06 (-0.21, 0.08)	-0.21 (-0.36, -0.06)	-0.21 (-0.36, -0.07)	-0.21 (-0.35, -0.06)	0.001	0.28
Model 2	Ref	0.09 (-0.0003, 0.18)	0.06 (-0.03, 0.16)	0.02 (-0.07, 0.11)	0.03 (-0.06, 0.13)	0.87	0.18
FVC (L)							
Model 1	Ref	-0.06 (-0.24, 0.12)	-0.28 (-0.46, -0.10)	-0.27 (-0.45, -0.08)	-0.26 (-0.44, -0.08)	0.001	0.18
Model 2	Ref	0.13 (0.03, 0.23)	0.09 (-0.02, 0.19)	0.07 (-0.03, 0.17)	0.06 (-0.04, 0.16)	0.51	0.11
FEV_1/FVC (%)							
Model 1	Ref	-0.27 (-1.70, 1.15)	0.01 (-1.41, 1.43)	0.08 (-1.34, 1.50)	-0.09 (-1.51, 1.33)	0.97	0.96
Model 2	Ref	0.0008 (-1.28, 1.28)	0.04 (-1.24, 1.33)	-0.38 (-1.66, 0.90)	-0.11 (-1.39, 1.17)	0.72	0.93

β-coefficient (95%CI) presented for each quintile

Model 1: Unadjusted linear regression analysis

Model 2: Regression analysis adjusted for age, height, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A6 – Unadjusted and adjusted associations between the "high β-cryptoxanthin & vitamin C" dietary pattern score and lung function

Lung function measure	"high β-cryptoxanthin & vitamin C" quintiles					p_{trend}	$p_{linearity}$
	Q1	Q2	Q3	Q4	Q5		
FEV_1 (L)							
Model 1	Ref	-0.16 (-0.30, -0.01)	-0.27 (-0.42, -0.13)	-0.38 (-0.53, -0.24)	-0.36 (-0.50, -0.21)	<0.001	0.23
Model 2	Ref	-0.02 (-0.11, 0.08)	0.02 (-0.07, 0.12)	-0.02 (-0.11, 0.07)	-0.03 (-0.12, 0.07)	0.56	0.76
FVC (L)							
Model 1	Ref	-0.14 (-0.32, 0.04)	-0.40 (-0.58, -0.22)	-0.48 (-0.66, -0.30)	-0.46 (-0.64, -0.28)	<0.001	0.073
Model 2	Ref	0.06 (-0.04, 0.16)	0.01 (-0.09, 0.12)	0.007 (-0.10, 0.11)	-0.03 (-0.14, 0.07)	0.37	0.50
FEV_1/FVC (%)							
Model 1	Ref	-1.10 (-2.51, 0.32)	0.85 (-0.57, 2.27)	-0.67 (-2.09, 0.74)	-0.25 (-1.66, 1.17)	0.90	0.041
Model 2	Ref	-1.24 (-2.52, 0.04)	0.44 (-0.85, 1.74)	-0.74 (-2.04, 0.56)	-0.04 (-1.35, 1.26)	0.87	0.036*

β-coefficient (95%CI) presented for each quintile

Model 1: Unadjusted linear regression analysis

Model 2: Regression analysis adjusted for age, height, sex, smoking status, asthma status, BMI category, atopy, and energy intake

*p-value for likelihood ratio test comparing models with and without the dietary pattern = 0.073

Table A7 – Unadjusted and adjusted associations between the "low calcium and sugars" dietary pattern score and lung function

Lung function measure	"Low calcium and sugars" quintiles					p_{trend}	$p_{linearity}$
	Q1	Q2	Q3	Q4	Q5		
FEV_1 (L)							
Model 1	Ref	-0.09 (-0.24, 0.06)	0.08 (-0.07, 0.23)	-0.02 (-0.17, 0.13)	0.08 (-0.07, 0.23)	0.20	0.12
Model 2	Ref	0.04 (-0.05, 0.14)	0.08 (-0.01, 0.17)	0.007 (-0.08, 0.10)	0.03 (-0.06, 0.12)	0.65	0.29
FVC (L)							
Model 1	Ref	-0.17 (-0.35, 0.01)	-0.007 (-0.19, 0.18)	-0.07 (-0.26, 0.11)	0.06 (-0.13, 0.24)	0.37	0.11
Model 2	Ref	0.02 (-0.08, 0.13)	0.05 (-0.05, 0.15)	-0.02 (-0.12, 0.08)	-0.005 (-0.11, 0.10)	0.73	0.54
FEV_1/FVC (%)							
Model 1	Ref	1.23 (-0.19, 2.65)	2.02 (0.60, 3.44)	1.06 (-0.36, 2.47)	0.84 (-0.58, 2.26)	0.27	0.077
Model 2	Ref	0.99 (-0.28, 2.26)	0.97 (-0.32, 2.25)	0.79 (-0.48, 2.06)	0.96 (-0.31, 2.24)	0.19	0.65

β-coefficient (95%CI) presented for each quintile

Model 1: Unadjusted linear regression analysis

Model 2: Regression analysis adjusted for age, height, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A8 – Unadjusted and adjusted associations between the "high starch & lycopene" dietary pattern score and lung function

Lung function measure	"High starch & lycopene" pattern quintiles					p_{trend}	$p_{linearity}$
	Q1	Q2	Q3	Q4	Q5		
FEV_1 (L)							
Model 1	Ref	-0.05 (-0.20, 0.10)	-0.22 (-0.36, -0.07)	-0.18 (-0.32, -0.03)	-0.07 (-0.22, 0.08)	0.15	0.022
Model 2	Ref	0.10 (0.009, 0.19)	0.02 (-0.07, 0.12)	0.01 (-0.08, 0.10)	-0.01 (-0.11, 0.08)	0.36	0.087
FVC (L)							
Model 1	Ref	-0.13 (-0.32, 0.05)	-0.41 (-0.59, -0.23)	-0.36 (-0.54, -0.18)	-0.19 (-0.37, -0.009)	0.006	0.0004
Model 2	Ref	0.08 (-0.02, 0.18)	-0.01 (-0.12, 0.09)	-0.05 (-0.16, 0.05)	-0.04 (-0.14, 0.06)	0.12	0.14
FEV_1/FVC (%)							
Model 1	Ref	1.53 (0.11, 2.94)	2.20 (0.79, 3.62)	2.26 (0.84, 3.68)	1.89 (0.48, 3.31)	0.005	0.14
Model 2	Ref	1.26 (-0.02, 2.55)	0.87 (-0.44, 2.18)	1.18 (-0.13, 2.48)	0.50 (-0.80, 1.81)	0.52	0.21

β-coefficient (95%CI) presented for each quintile

Model 1: Unadjusted linear regression analysis

Model 2: Regression analysis adjusted for age, height, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A9: Adjusted associations between “high potassium & magnesium” dietary pattern quintiles and lung function by sex

Pattern quintiles	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Female (n=573)	Male (n=610)	Female (n=573)	Male (n=610)	Female (n=573)	Male (n=610)
Q1	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.03 (-0.18, 0.11)	-0.007 (-0.13, 0.11)	0.02 (-0.14, 0.18)	-0.08 (-0.21, 0.06)	-1.64 (-3.67, 0.38)	1.17 (-0.53, 2.87)
Q3	-0.02 (-0.17, 0.12)	-0.02 (-0.14, 0.10)	0.002 (-0.16, 0.16)	-0.11 (-0.25, 0.02)	-0.92 (-2.97, 1.13)	1.36 (-0.31, 3.03)
Q4	-0.02 (-0.17, 0.12)	0.13 (0.008, 0.26)	0.009 (-0.15, 0.17)	0.10 (-0.04, 0.24)	-1.00 (-3.01, 1.01)	1.58 (-0.16, 3.32)
Q5	0.05 (-0.09, 0.19)	0.17 (0.04, 0.29)	0.09 (-0.07, 0.24)	0.16 (0.03, 0.31)	-0.47 (-2.46, 1.52)	1.44 (-0.32, 3.20)
p_{trend}	0.32	0.002	0.27	0.005	0.83	0.078
$p_{interaction}$	0.31		0.13		0.24	
$p_{linearity}$	0.47		0.051		0.59	

β-coefficient (95% CI) presented for each quintile.

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A10: Adjusted associations between "high potassium & magnesium" dietary pattern quintiles and lung function by BMI category

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.14 (-0.02, 0.30)	-0.16 (-0.30, -0.03)	0.07 (-0.12, 0.27)	0.13 (-0.05, 0.30)	-0.15 (-0.30, 0.008)	-0.03 (-0.24, 0.18)	0.33 (-1.87, 2.53)	-1.31 (-3.25, 0.63)	2.47 (-0.23, 5.17)
Q3	0.13 (-0.03, 0.30)	-0.13 (-0.26, 0.007)	0.01 (-0.17, 0.20)	0.08 (-0.11, 0.26)	-0.15 (-0.30, -0.002)	-0.08 (-0.28, 0.13)	1.52 (-0.82, 3.85)	-0.63 (-2.53, 1.28)	1.57 (-1.01, 4.15)
Q4	0.15 (-0.004, 0.30)	-0.05 (-0.19, 0.09)	0.13 (-0.07, 0.32)	0.15 (-0.02, 0.32)	-0.05 (-0.20, 0.11)	0.07 (-0.14, 0.29)	0.94 (-1.24, 3.11)	-0.43 (-2.39, 1.54)	1.98 (-0.75, 4.71)
Q5	0.27 (0.10, 0.43)	0.004 (-0.13, 0.14)	0.12 (-0.07, 0.30)	0.28 (0.09, 0.46)	0.01 (-0.14, 0.17)	0.10 (-0.11, 0.30)	1.55 (-0.75, 3.84)	-0.08 (-2.00, 1.84)	1.48 (-1.12, 4.07)
p_{trend}	0.002	0.37	0.16	0.004	0.39	0.19	0.15	0.73	0.39
$p_{interaction}$	0.15			0.48			0.57		
$p_{linearity}$	0.24			0.25			0.73		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A11: Adjusted associations between "high potassium & magnesium" dietary pattern quintiles and lung function by smoking status

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.12 (-0.26, 0.02)	0.02 (-0.13, 0.17)	0.17 (-0.04, 0.38)	-0.12 (-0.28, 0.04)	0.02 (-0.15, 0.18)	0.10 (-0.13, 0.33)	-0.76 (-2.72, 1.20)	-0.05 (-2.14, 2.03)	1.94 (-0.97, 4.85)
Q3	-0.05 (-0.19, 0.08)	-0.02 (-0.17, 0.12)	0.10 (-0.17, 0.37)	-0.11 (-0.27, 0.04)	-0.02 (-0.18, 0.14)	-0.05 (-0.35, 0.25)	0.28 (-1.64, 2.19)	-0.08 (-2.12, 1.96)	2.58 (-1.22, 6.37)
Q4	0.01 (-0.12, 0.15)	0.04 (-0.11, 0.19)	0.24 (-0.04, 0.51)	-0.01 (-0.17, 0.14)	0.07 (-0.10, 0.23)	0.23 (-0.08, 0.53)	0.52 (-1.38, 2.42)	-0.33 (-2.40, 1.75)	2.47 (-1.41, 6.36)
Q5	-0.004 (-0.14, 0.13)	0.18 (0.04, 0.33)	0.23 (-0.04, 0.50)	0.05 (-0.10, 0.20)	0.19 (0.02, 0.35)	0.05 (-0.25, 0.36)	-0.86 (-2.79, 1.06)	1.38 (-0.65, 3.41)	4.40 (0.59, 8.21)
p_{trend}	0.48	0.006	0.046	0.20	0.010	0.46	0.68	0.18	0.014
$p_{interaction}$	0.27			0.74			0.16		
$p_{linearity}$	0.41			0.28			0.74		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A12: Adjusted associations between "high potassium & magnesium" dietary pattern quintiles and lung function by atopy

Pattern quintiles	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Non-atopic (n=562)	Atopic (n=621)	Non-atopic (n=562)	Atopic (n=621)	Non-atopic (n=562)	Atopic (n=621)
Q1	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.09 (-0.23, 0.04)	0.06 (-0.06, 0.18)	-0.08 (-0.23, 0.07)	0.02 (-0.12, 0.16)	-0.72 (-2.64, 1.20)	0.67 (-1.07, 2.40)
Q3	-0.03 (-0.16, 0.10)	0.008 (-0.12, 0.14)	-0.05 (-0.20, 0.09)	-0.07 (-0.21, 0.08)	0.34 (-1.49, 2.17)	0.82 (-0.98, 2.62)
Q4	0.08 (-0.05, 0.21)	0.05 (-0.08, 0.17)	0.05 (-0.10, 0.20)	0.05 (-0.10, 0.19)	1.26 (-0.61, 3.12)	0.03 (-1.76, 1.83)
Q5	0.05 (-0.08, 0.18)	0.19 (0.06, 0.32)	0.05 (-0.09, 0.20)	0.19 (0.04, 0.33)	0.72 (-1.09, 2.54)	0.94 (-0.88, 2.77)
p_{trend}	0.12	0.008	0.18	0.009	0.17	0.51
$p_{interaction}$	0.15		0.47		0.38	
$p_{linearity}$	0.18		0.15		0.66	

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A13: Adjusted associations between quintiles of the "high protein & zinc" dietary pattern and lung function by sex

Animal products pattern	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Female (n=573)	Male (n=610)	Female (n=573)	Male (n=610)	Female (n=573)	Male (n=610)
Q1	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.07 (-0.08, 0.21)	0.10 (-0.02, 0.22)	0.08 (-0.08, 0.24)	0.16 (0.03, 0.29)	0.08 (-1.95, 2.12)	-0.15 (-1.81, 1.51)
Q3	0.02 (-0.12, 0.16)	0.10 (-0.03, 0.22)	0.06 (-0.09, 0.22)	0.10 (-0.04, 0.24)	-0.48 (-2.42, 1.46)	0.55 (-1.21, 2.31)
Q4	-0.03 (-0.17, 0.11)	0.06 (-0.07, 0.18)	0.01 (-0.14, 0.17)	0.11 (-0.02, 0.25)	-0.46 (-2.43, 1.50)	-0.44 (-2.17, 1.29)
Q5	0.02 (-0.12, 0.16)	0.03 (-0.09, 0.16)	0.07 (-0.09, 0.22)	0.04 (-0.10, 0.17)	-0.59 (-2.58, 1.39)	0.27 (-1.43, 1.97)
p_{trend}	0.77	0.63	0.70	0.59	0.45	0.86
$p_{interaction}$	0.86		0.72		0.82	
$p_{linearity}$	0.43		0.23		0.96	

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A14: Adjusted associations between quintiles of the "high protein & zinc" dietary pattern and lung function by BMI category

Animal products pattern	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.18 (0.02, 0.33)	0.03 (-0.11, 0.16)	0.10 (-0.11, 0.30)	0.23 (0.06, 0.40)	0.07 (-0.09, 0.22)	0.11 (-0.12, 0.34)	0.22 (-1.94, 2.37)	-0.44 (-2.35, 1.46)	0.54 (-2.34, 3.42)
Q3	0.02 (-0.14, 0.18)	0.01 (-0.11, 0.14)	0.28 (0.07, 0.49)	0.11 (-0.06, 0.29)	0.003 (-0.14, 0.15)	0.27 (0.03, 0.51)	-1.50 (-3.74, 0.74)	0.31 (-1.50, 2.12)	2.04 (-0.94, 5.02)
Q4	0.10 (-0.06, 0.27)	-0.03 (-0.17, 0.10)	0.06 (-0.14, 0.25)	0.13 (-0.05, 0.32)	0.04 (-0.11, 0.18)	0.08 (-0.13, 0.30)	0.20 (-2.12, 2.53)	-0.95 (-2.81, 0.91)	0.31 (-2.40, 3.03)
Q5	-0.005 (-0.17, 0.16)	-0.03 (-0.17, 0.11)	0.21 (0.02, 0.40)	0.07 (-0.11, 0.25)	-0.05 (-0.20, 0.10)	0.24 (0.02, 0.45)	-1.20 (-3.48, 1.08)	0.30 (-1.60, 2.21)	0.79 (-1.88, 3.46)
p_{trend}	0.70	0.49	0.077	0.74	0.53	0.060	0.31	0.96	0.71
$p_{interaction}$	0.068			0.19			0.39		
$p_{linearity}$	0.074			0.13			0.55		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A15: Adjusted associations between quintiles of the "high protein & zinc" dietary pattern and lung function by asthma status

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.09 (-0.007, 0.19)	0.13 (-0.20, 0.45)	0.07 (-0.25, 0.39)	0.14 (0.03, 0.26)	0.18 (-0.18, 0.54)	-0.09 (-0.44, 0.27)	-0.42 (-1.82, 0.98)	1.36 (-3.14, 5.86)	3.34 (-1.12, 7.79)
Q3	0.07 (-0.03, 0.18)	0.24 (-0.08, 0.56)	-0.08 (-0.37, 0.21)	0.11 (-0.003, 0.22)	0.22 (-0.14, 0.58)	-0.20 (-0.52, 0.13)	-0.14 (-1.57, 1.28)	1.44 (-3.07, 5.94)	1.95 (-2.15, 6.05)
Q4	-0.001 (-0.10, 0.10)	0.02 (-0.31, 0.35)	0.21 (-0.11, 0.52)	0.06 (-0.05, 0.17)	0.04 (-0.33, 0.41)	0.15 (-0.20, 0.50)	-0.72 (-2.12, 0.68)	0.46 (-4.18, 5.10)	2.85 (-1.57, 7.26)
Q5	0.02 (-0.08, 0.12)	0.01 (-0.30, 0.32)	0.17 (-0.14, 0.47)	0.09 (-0.02, 0.20)	-0.04 (-0.39, 0.31)	-0.10 (-0.44, 0.24)	-0.82 (-2.23, 0.59)	1.06 (-3.34, 5.46)	5.36 (1.08, 9.64)
p_{trend}	0.87	0.69	0.15	0.33	0.45	1.00	0.22	0.82	0.022
$p_{interaction}$	0.23			0.23			0.34		
$p_{linearity}$	0.16			0.076			0.98		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A16: Adjusted associations between quintiles of the "high protein & zinc" dietary pattern and lung function by atopy

Pattern quintiles	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Non-atopic (n=562)	Atopic (n=621)	Non-atopic (n=562)	Atopic (n=621)	Non-atopic (n=562)	Atopic (n=621)
Q1	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.03 (-0.10, 0.16)	0.15 (0.02, 0.28)	0.09 (-0.05, 0.24)	0.16 (0.02, 0.31)	-1.10 (-2.91, 0.71)	1.09 (-0.71, 2.88)
Q3	0.11 (-0.02, 0.25)	0.03 (-0.09, 0.16)	0.14 (-0.01, 0.29)	0.05 (-0.09, 0.19)	-0.04 (-1.92, 1.85)	0.22 (-1.52, 1.95)
Q4	0.04 (-0.09, 0.17)	0.0008 (-0.13, 0.13)	0.10 (-0.05, 0.24)	0.04 (-0.10, 0.18)	-0.53 (-2.34, 1.28)	-0.22 (-2.02, 1.58)
Q5	0.004 (-0.13, 0.13)	0.06 (-0.07, 0.19)	0.05 (-0.09, 0.20)	0.06 (-0.08, 0.20)	-0.73 (-2.55, 1.09)	0.49 (-1.30, 2.29)
p_{trend}	0.92	0.90	0.47	0.83	0.58	0.97
$p_{interaction}$	0.20		0.57		0.41	
$p_{linearity}$	0.091		0.18		0.65	

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A17: Adjusted associations between quintiles of the "high PUFAs & vitamin E" dietary pattern and lung function by sex

Pattern quintiles	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Female (n=573)	Male (n=610)	Female (n=573)	Male (n=610)	Female (n=573)	Male (n=610)
Q1	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.02 (-0.13, 0.16)	0.06 (-0.06, 0.18)	0.03 (-0.13, 0.19)	0.04 (-0.09, 0.18)	-0.14 (-2.14, 1.86)	0.62 (-1.06, 2.31)
Q3	0.05 (-0.09, 0.18)	0.20 (0.07, 0.33)	0.07 (-0.09, 0.22)	0.12 (-0.03, 0.26)	0.007 (-1.94, 1.95)	2.33 (0.53, 4.14)
Q4	-0.02 (-0.16, 0.12)	0.05 (-0.08, 0.17)	-0.05 (-0.21, 0.11)	-0.06 (-0.20, 0.07)	0.71 (-1.25, 2.68)	1.99 (0.25, 3.73)
Q5	0.06 (-0.09, 0.21)	0.15 (0.03, 0.26)	0.11 (-0.06, 0.27)	0.12 (-0.01, 0.25)	-0.52 (-2.58, 1.54)	1.21 (-0.43, 2.84)
p_{trend}	0.58	0.021	0.42	0.21	0.87	0.067
$p_{interaction}$	0.55		0.98		0.46	
$p_{linearity}$	0.19		0.058		0.27	

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A18: Adjusted associations between quintiles of the "high PUFAs & vitamin E" dietary pattern and lung function by BMI category

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.01 (-0.17, 0.19)	-0.003 (-0.14, 0.13)	0.18 (0.005, 0.35)	-0.003 (-0.20, 0.19)	0.009 (-0.14, 0.16)	0.14 (-0.06, 0.33)	-0.25 (-2.70, 2.21)	0.09 (-1.79, 1.96)	1.75 (-0.68, 4.18)
Q3	0.12 (-0.05, 0.29)	0.08 (-0.05, 0.21)	0.23 (0.03, 0.42)	-0.02 (-0.21, 0.17)	0.08 (-0.07, 0.22)	0.28 (0.06, 0.49)	2.84 (0.47, 5.20)	0.53 (-1.34, 2.40)	0.18 (-2.50, 2.87)
Q4	0.002 (-0.16, 0.17)	-0.006 (-0.14, 0.13)	0.11 (-0.07, 0.29)	-0.07 (-0.25, 0.12)	-0.10 (-0.25, 0.06)	0.02 (-0.18, 0.22)	0.85 (-1.48, 3.17)	1.52 (-0.40, 3.45)	2.71 (0.19, 5.23)
Q5	0.10 (-0.07, 0.26)	0.13 (-0.007, 0.27)	0.09 (-0.11, 0.28)	0.07 (-0.11, 0.26)	0.16 (0.009, 0.31)	0.04 (-0.17, 0.25)	0.80 (-1.48, 3.07)	0.08 (-1.83, 1.98)	1.18 (-1.48, 3.85)
p_{trend}	0.30	0.081	0.43	0.48	0.13	0.89	0.49	0.60	0.24
$p_{interaction}$	0.67			0.33			0.25		
$p_{linearity}$	0.20			0.016			0.078		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A19: Adjusted associations between quintiles of the "high PUFAs & vitamin E" dietary pattern and lung function by atopy

Pattern quintiles	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Non-atopic (n=562)	Atopic (n=621)	Non-atopic (n=562)	Atopic (n=621)	Non-atopic (n=562)	Atopic (n=621)
Q1	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.05 (-0.08, 0.19)	0.05 (-0.07, 0.18)	0.03 (-0.12, 0.18)	0.05 (-0.09, 0.19)	0.83 (-1.05, 2.70)	0.17 (-1.57, 1.91)
Q3	0.11 (-0.02, 0.24)	0.14 (0.01, 0.27)	0.09 (-0.05, 0.24)	0.08 (-0.06, 0.23)	1.17 (-0.66, 2.99)	1.37 (-0.45, 3.18)
Q4	-0.06 (-0.19, 0.07)	0.12 (-0.009, 0.25)	-0.12 (-0.26, 0.02)	0.02 (-0.13, 0.16)	0.71 (-1.09, 2.51)	2.46 (0.64, 4.28)
Q5	0.10 (-0.04, 0.23)	0.13 (0.01, 0.26)	0.09 (-0.06, 0.24)	0.14 (-0.002, 0.27)	0.93 (-0.96, 2.82)	0.36 (-1.37, 2.09)
p_{trend}	0.49	0.024	0.74	0.089	0.39	0.32
$p_{interaction}$	0.24		0.63		0.34	
$p_{linearity}$	0.081		0.035		0.12	

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A20: Adjusted associations between quintiles of the "high β-cryptoxanthin & vitamin C" dietary pattern and lung function by sex

Pattern quintiles	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Female (n=573)	Male (n=610)	Female (n=573)	Male (n=610)	Female (n=573)	Male (n=610)
Q1	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.006 (-0.17, 0.15)	-0.03 (-0.14, 0.08)	0.009 (-0.17, 0.19)	0.07 (-0.05, 0.20)	-0.60 (-2.84, 1.63)	-1.64 (-3.22, -0.07)
Q3	-0.04 (-0.20, 0.11)	0.08 (-0.04, 0.21)	-0.09 (-0.25, 0.08)	0.09 (-0.05, 0.22)	0.40 (-1.71, 2.51)	0.58 (-1.13, 2.29)
Q4	-0.03 (-0.18, 0.12)	-0.03 (-0.16, 0.09)	-0.04 (-0.20, 0.13)	0.007 (-0.13, 0.15)	-0.70 (-2.78, 1.38)	-0.69 (-2.46, 1.07)
Q5	-0.06 (-0.21, 0.09)	-0.004 (-0.13, 0.12)	-0.11 (-0.27, 0.06)	0.009 (-0.13, 0.15)	-0.09 (-2.18, 2.00)	0.14 (-1.64, 1.91)
p_{trend}	0.36	0.98	0.15	0.95	0.92	0.76
$p_{interaction}$	0.46		0.55		0.87	
$p_{linearity}$	0.64		0.66		0.14	

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A21: Adjusted associations between quintiles of the "high β-cryptoxanthin & vitamin C" dietary pattern and lung function by BMI category

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.05 (-0.23, 0.14)	-0.02 (-0.16, 0.11)	0.03 (-0.15, 0.21)	-0.05 (-0.25, 0.15)	0.14 (-0.01, 0.28)	0.01 (-0.19, 0.21)	-0.009 (-2.53, 2.51)	-2.80 (-4.62, -0.98)	0.73 (-1.79, 3.24)
Q3	-0.05 (-0.22, 0.12)	0.04 (-0.10, 0.18)	0.09 (-0.10, 0.27)	-0.13 (-0.31, 0.06)	0.04 (-0.12, 0.20)	0.12 (-0.09, 0.32)	1.05 (-1.25, 3.36)	0.46 (-1.51, 2.42)	0.18 (-2.36, 2.71)
Q4	-0.04 (-0.21, 0.13)	0.005 (-0.13, 0.14)	-0.07 (-0.25, 0.12)	-0.13 (-0.32, 0.06)	0.09 (-0.06, 0.24)	-0.02 (-0.23, 0.18)	0.61 (-1.76, 2.98)	-1.42 (-3.31, 0.47)	-1.11 (-3.69, 1.46)
Q5	-0.09 (-0.26, 0.08)	-0.02 (-0.16, 0.12)	0.03 (-0.16, 0.22)	-0.25 (-0.43, -0.06)	0.05 (-0.10, 0.20)	0.08 (-0.12, 0.29)	2.21 (-0.12, 4.54)	-1.63 (-3.55, 0.29)	-0.17 (-2.75, 2.42)
p_{trend}	0.36	0.90	1.00	0.006	0.69	0.52	0.049	0.33	0.59
$p_{interaction}$	0.92			0.19			0.082		
$p_{linearity}$	0.92			0.72			0.045		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A22: Adjusted associations between quintiles of the "high β-cryptoxanthin & vitamin C" dietary pattern and lung function by smoking status

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.02 (-0.16, 0.12)	-0.03 (-0.17, 0.11)	0.02 (-0.24, 0.28)	0.09 (-0.07, 0.24)	0.03 (-0.12, 0.19)	0.006 (-0.28, 0.29)	-2.00 (-3.98, -0.03)	-1.12 (-3.03, 0.80)	0.98 (-2.62, 4.58)
Q3	0.02 (-0.13, 0.16)	-0.009 (-0.14, 0.13)	0.13 (-0.13, 0.39)	0.02 (-0.14, 0.18)	0.02 (-0.14, 0.17)	-0.01 (-0.30, 0.28)	-0.10 (-2.11, 1.91)	-0.08 (-1.98, 1.81)	3.73 (0.11, 7.36)
Q4	-0.05 (-0.20, 0.09)	-0.04 (-0.17, 0.10)	0.14 (-0.13, 0.40)	-0.03 (-0.19, 0.13)	-0.003 (-0.15, 0.15)	0.15 (-0.14, 0.44)	-0.92 (-2.92, 1.08)	-1.18 (-3.07, 0.70)	1.05 (-2.62, 4.71)
Q5	-0.12 (-0.26, 0.03)	0.06 (-0.07, 0.20)	-0.006 (-0.28, 0.27)	-0.11 (-0.27, 0.05)	0.03 (-0.12, 0.19)	0.05 (-0.26, 0.36)	-0.87 (-2.85, 1.11)	1.04 (-0.86, 2.93)	-0.84 (-4.71, 3.03)
p_{trend}	0.075	0.49	0.73	0.043	0.79	0.49	0.77	0.49	0.75
$p_{interaction}$	0.43			0.58			0.23		
$p_{linearity}$	0.78			0.84			0.030		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A23: Adjusted associations between quintiles of the "low calcium & sugars" dietary pattern and lung function by BMI category

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.03 (-0.13, 0.18)	0.11 (-0.02, 0.25)	-0.08 (-0.28, 0.11)	0.02 (-0.15, 0.20)	0.08 (-0.07, 0.23)	-0.09 (-0.31, 0.13)	1.04 (-1.15, 3.23)	1.42 (-0.48, 3.32)	-0.09 (-2.82, 2.64)
Q3	0.10 (-0.06, 0.26)	0.11 (-0.03, 0.24)	-0.01 (-0.21, 0.18)	0.07 (-0.10, 0.25)	0.05 (-0.10, 0.21)	0.003 (-0.22, 0.22)	1.25 (-0.95, 3.45)	1.34 (-0.57, 3.25)	-0.35 (-3.09, 2.40)
Q4	0.03 (-0.13, 0.19)	0.08 (-0.05, 0.22)	-0.18 (-0.38, 0.008)	0.07 (-0.11, 0.25)	-0.01 (-0.16, 0.14)	-0.16 (-0.37, 0.06)	-0.22 (-2.44, 2.01)	2.50 (0.61, 4.38)	-1.30 (-3.99, 1.39)
Q5	0.08 (-0.08, 0.24)	0.04 (-0.09, 0.17)	-0.05 (-0.24, 0.14)	0.02 (-0.16, 0.20)	-0.02 (-0.17, 0.13)	-0.03 (-0.25, 0.18)	1.31 (-0.95, 3.57)	1.34 (-0.53, 3.21)	-0.33 (-3.05, 2.38)
p_{trend}	0.36	0.64	0.41	0.62	0.55	0.64	0.48	0.081	0.59
$p_{interaction}$	0.55			0.79			0.43		
$p_{linearity}$	0.43			0.73			0.58		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A24: Adjusted associations between quintiles of the "low calcium & sugars" dietary pattern and lung function by smoking status

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.009 (-0.14, 0.12)	0.16 (0.02, 0.31)	-0.13 (-0.39, 0.13)	-0.02 (-0.16, 0.13)	0.09 (-0.08, 0.25)	0.005 (-0.28, 0.29)	0.48 (-1.35, 2.31)	2.94 (0.90, 4.98)	-3.39 (-6.97, 0.18)
Q3	0.04 (-0.09, 0.18)	0.12 (-0.02, 0.27)	0.10 (-0.16, 0.37)	0.03 (-0.12, 0.17)	0.07 (-0.09, 0.23)	0.12 (-0.17, 0.42)	0.25 (-1.62, 2.12)	2.15 (0.16, 4.14)	0.17 (-3.50, 3.83)
Q4	-0.02 (-0.16, 0.12)	0.06 (-0.08, 0.20)	-0.08 (-0.32, 0.17)	0.02 (-0.14, 0.17)	-0.04 (-0.20, 0.11)	-0.05 (-0.32, 0.23)	-0.23 (-2.16, 1.70)	2.59 (0.66, 4.52)	-1.41 (-4.85, 2.03)
Q5	0.02 (-0.12, 0.15)	0.07 (-0.07, 0.21)	-0.01 (-0.25, 0.22)	0.02 (-0.13, 0.17)	-0.009 (-0.17, 0.15)	-0.08 (-0.34, 0.19)	0.20 (-1.70, 2.11)	2.07 (0.09, 4.04)	0.36 (-2.96, 3.67)
p_{trend}	0.82	0.64	0.99	0.66	0.52	0.55	0.97	0.062	0.67
$p_{interaction}$	0.65			0.88			0.12		
$p_{linearity}$	0.38			0.83			0.18		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A25: Adjusted associations between quintiles of the "low calcium & sugars" dietary pattern and lung function by atopy

Pattern quintiles	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Non-atopic (n=562)	Atopic (n=621)	Non-atopic (n=562)	Atopic (n=621)	Non-atopic (n=562)	Atopic (n=621)
Q1	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.03 (-0.10, 0.16)	0.06 (-0.07, 0.19)	-0.05 (-0.19, 0.10)	0.10 (-0.04, 0.24)	1.48 (-0.35, 3.31)	0.55 (-1.22, 2.31)
Q3	-0.02 (-0.15, 0.11)	0.18 (0.05, 0.31)	-0.07 (-0.21, 0.08)	0.17 (0.03, 0.31)	0.63 (-1.21, 2.47)	1.24 (-0.55, 3.03)
Q4	-0.09 (-0.22, 0.04)	0.10 (-0.03, 0.23)	-0.15 (-0.29, -0.005)	0.11 (-0.03, 0.25)	0.64 (-1.17, 2.45)	0.92 (-0.86, 2.70)
Q5	0.002 (-0.13, 0.13)	0.07 (-0.06, 0.19)	-0.11 (-0.26, 0.03)	0.10 (-0.04, 0.24)	1.78 (-0.04, 3.61)	0.20 (-1.57, 1.98)
p_{trend}	0.53	0.22	0.056	0.17	0.13	0.70
$p_{interaction}$	0.092		0.082		0.41	
$p_{linearity}$	0.12		0.55		0.55	

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A26: Adjusted associations between quintiles of the "high starch & lycopene" dietary pattern and lung function by sex

Pattern quintiles	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Female (n=573)	Male (n=610)	Female (n=573)	Male (n=610)	Female (n=573)	Male (n=610)
Q1	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.02 (-0.12, 0.17)	0.16 (0.04, 0.28)	-0.005 (-0.17, 0.16)	0.15 (0.02, 0.28)	1.28 (-0.80, 3.37)	1.20 (-0.44, 2.85)
Q3	0.01 (-0.13, 0.15)	0.02 (-0.11, 0.15)	0.008 (-0.15, 0.17)	-0.06 (-0.20, 0.09)	0.63 (-1.36, 2.61)	1.15 (-0.68, 2.97)
Q4	-0.0008 (-0.14, 0.14)	0.006 (-0.12, 0.13)	-0.04 (-0.20, 0.12)	-0.07 (-0.21, 0.07)	1.11 (-0.90, 3.11)	1.18 (-0.61, 2.96)
Q5	-0.02 (-0.18, 0.13)	-0.01 (-0.13, 0.10)	-0.02 (-0.19, 0.15)	-0.05 (-0.18, 0.08)	0.36 (-1.78, 2.50)	0.58 (-1.05, 2.22)
p_{trend}	0.62	0.43	0.67	0.096	0.91	0.46
$p_{interaction}$	0.56		0.21		0.99	
$p_{linearity}$	0.15		0.092		0.59	

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A27: Adjusted associations between quintiles of the "high starch & lycopene" dietary pattern and lung function by BMI category

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.06 (-0.10, 0.21)	0.12 (-0.02, 0.25)	0.13 (-0.08, 0.34)	-0.02 (-0.19, 0.16)	0.10 (-0.05, 0.25)	0.19 (-0.04, 0.43)	1.70 (-0.49, 3.89)	1.49 (-0.38, 3.36)	-0.11 (-3.05, 2.82)
Q3	0.0006 (-0.16, 0.16)	0.02 (-0.12, 0.15)	0.07 (-0.13, 0.27)	-0.06 (-0.24, 0.12)	-0.03 (-0.18, 0.12)	0.07 (-0.15, 0.29)	0.75 (-1.53, 3.03)	1.21 (-0.68, 3.10)	0.42 (-2.32, 3.16)
Q4	0.01 (-0.15, 0.17)	0.01 (-0.13, 0.15)	0.007 (-0.18, 0.19)	-0.13 (-0.31, 0.05)	-0.03 (-0.18, 0.13)	0.02 (-0.19, 0.22)	2.47 (0.20, 4.74)	0.75 (-1.20, 2.69)	0.05 (-2.54, 2.64)
Q5	-0.11 (-0.29, 0.07)	-0.04 (-0.17, 0.10)	0.11 (-0.07, 0.29)	-0.11 (-0.31, 0.09)	-0.11 (-0.26, 0.04)	0.15 (-0.04, 0.35)	-1.23 (-3.75, 1.29)	1.46 (-0.42, 3.35)	0.14 (-2.32, 2.61)
p_{trend}	0.21	0.31	0.43	0.12	0.053	0.30	0.72	0.24	0.93
$p_{interaction}$	0.78			0.46			0.27		
$p_{linearity}$	0.44			0.49			0.15		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A28: Adjusted associations between quintiles of the "high starch & lycopene" dietary pattern and lung function by asthma status

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.11 (0.01, 0.21)	0.06 (-0.22, 0.35)	0.05 (-0.23, 0.34)	0.14 (0.02, 0.25)	-0.08 (-0.40, 0.23)	-0.23 (-0.54, 0.09)	0.57 (-0.86, 1.99)	2.76 (-1.24, 6.77)	5.78 (1.82, 9.75)
Q3	0.01 (-0.09, 0.11)	0.17 (-0.13, 0.47)	-0.02 (-0.34, 0.29)	0.02 (-0.09, 0.13)	-0.03 (-0.36, 0.31)	-0.37 (-0.72, -0.02)	0.05 (-1.38, 1.47)	3.60 (-0.57, 7.77)	5.88 (1.47, 10.29)
Q4	-0.03 (-0.13, 0.08)	0.42 (0.12, 0.73)	-0.05 (-0.34, 0.23)	-0.06 (-0.17, 0.06)	0.23 (-0.11, 0.57)	-0.31 (-0.63, 0.007)	0.45 (-1.00, 1.89)	5.27 (1.05, 9.49)	4.09 (0.12, 8.06)
Q5	-0.03 (-0.13, 0.08)	0.13 (-0.17, 0.44)	-0.06 (-0.36, 0.24)	-0.009 (-0.12, 0.10)	-0.08 (-0.42, 0.27)	-0.31 (-0.65, 0.02)	-0.37 (-1.80, 1.05)	4.64 (0.34, 8.94)	4.61 (0.44, 8.78)
p_{trend}	0.18	0.085	0.48	0.23	0.76	0.069	0.59	0.014	0.14
$p_{interaction}$	0.15			0.077			0.068		
$p_{linearity}$	0.12			0.045			0.28		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A29: Adjusted associations between quintiles of the "high starch & lycopene" dietary pattern and lung function by atopy

Pattern quintiles	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Non-atopic (n=562)	Atopic (n=621)	Non-atopic (n=562)	Atopic (n=621)	Non-atopic (n=562)	Atopic (n=621)
Q1	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.13 (-0.006, 0.27)	0.08 (-0.05, 0.20)	0.18 (0.03, 0.34)	-0.003 (-0.14, 0.13)	0.36 (-1.55, 2.27)	1.99 (0.28, 3.70)
Q3	0.06 (-0.08, 0.19)	-0.002 (-0.13, 0.12)	0.06 (-0.09, 0.22)	-0.08 (-0.22, 0.06)	0.37 (-1.56, 2.31)	1.27 (-0.46, 2.99)
Q4	0.01 (-0.12, 0.15)	0.01 (-0.12, 0.14)	-0.02 (-0.17, 0.13)	-0.07 (-0.21, 0.07)	0.66 (-1.21, 2.53)	1.60 (-0.19, 3.39)
Q5	0.002 (-0.13, 0.13)	-0.03 (-0.15, 0.10)	0.02 (-0.13, 0.17)	-0.09 (-0.23, 0.05)	-0.14 (-2.00, 1.71)	1.06 (-0.73, 2.86)
p_{trend}	0.52	0.50	0.45	0.14	0.93	0.32
$p_{interaction}$	0.95		0.39		0.79	
$p_{linearity}$	0.29		0.15		0.46	

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A30: Adjusted associations between quintiles of the "high vitamin C, low calcium" dietary pattern and lung function by BMI category

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)	BMI <25 (n=377)	25≤BMI<30 (n=534)	BMI≥30 (n=272)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.07 (-0.22, 0.09)	-0.02 (-0.16, 0.11)	0.10 (-0.10, 0.31)	-0.12 (-0.29, 0.06)	-0.006 (-0.16, 0.14)	0.06 (-0.17, 0.28)	0.68 (-1.51, 2.88)	-0.11 (-1.98, 1.77)	1.71 (-1.15, 4.57)
Q3	-0.03 (-0.19, 0.13)	-0.07 (-0.21, 0.06)	-0.08 (-0.27, 0.11)	-0.01 (-0.19, 0.17)	-0.03 (-0.17, 0.12)	-0.08 (-0.29, 0.14)	-0.55 (-2.81, 1.72)	-1.44 (-3.32, 0.44)	-0.21 (-2.92, 2.49)
Q4	-0.01 (-0.18, 0.16)	-0.17 (-0.31, -0.04)	0.03 (-0.16, 0.22)	-0.06 (-0.24, 0.13)	-0.14 (-0.29, 0.008)	0.05 (-0.16, 0.26)	0.63 (-1.69, 2.95)	-1.73 (-3.63, 0.16)	-0.02 (-2.66, 2.62)
Q5	-0.07 (-0.24, 0.09)	-0.13 (-0.26, 0.008)	-0.008 (-0.20, 0.18)	-0.04 (-0.22, 0.14)	-0.15 (-0.30, -0.001)	0.03 (-0.19, 0.24)	-0.42 (-2.70, 1.85)	-0.39 (-2.27, 1.48)	-0.41 (-3.08, 2.27)
p_{trend}	0.54	0.016	0.77	0.93	0.016	0.79	0.67	0.36	0.47
$p_{interaction}$	0.40			0.30			0.80		
$p_{linearity}$	0.61			0.72			0.44		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A31: Adjusted associations between quintiles of the "high vitamin C, low calcium" dietary pattern and lung function by smoking status

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.04 (-0.18, 0.09)	-0.01 (-0.15, 0.13)	0.08 (-0.17, 0.34)	-0.06 (-0.21, 0.09)	-0.06 (-0.22, 0.10)	0.12 (-0.16, 0.41)	0.38 (-1.51, 2.27)	0.96 (-1.01, 2.94)	-0.60 (-4.20, 2.99)
Q3	-0.07 (-0.21, 0.06)	-0.07 (-0.21, 0.06)	0.04 (-0.24, 0.32)	-0.04 (-0.19, 0.11)	-0.03 (-0.19, 0.12)	-0.0008 (-0.32, 0.31)	-1.05 (-2.92, 0.83)	-0.92 (-2.87, 1.03)	-0.17 (-4.14, 3.79)
Q4	-0.05 (-0.19, 0.08)	-0.11 (-0.25, 0.04)	-0.04 (-0.29, 0.22)	-0.07 (-0.21, 0.08)	-0.09 (-0.25, 0.07)	-0.03 (-0.31, 0.26)	-0.34 (-2.22, 1.55)	-1.17 (-3.18, 0.84)	-0.14 (-3.73, 3.45)
Q5	-0.13 (-0.26, 0.004)	0.02 (-0.12, 0.16)	-0.25 (-0.51, 0.007)	-0.11 (-0.26, 0.03)	0.05 (-0.10, 0.21)	-0.34 (-0.63, -0.05)	-0.53 (-2.40, 1.35)	-0.24 (-2.22, 1.73)	-0.80 (-4.44, 2.84)
p_{trend}	0.063	0.92	0.022	0.14	0.55	0.007	0.45	0.38	0.75
$p_{interaction}$	0.18			0.057			0.96		
$p_{linearity}$	0.54			0.54			0.63		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A32: Adjusted associations between quintiles of the "high alpha-carotene, low lycopene" dietary pattern and lung function by sex

Pattern quintiles	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Female (n=573)	Male (n=610)	Female (n=573)	Male (n=610)	Female (n=573)	Male (n=610)
Q1	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.07 (-0.20, 0.07)	0.08 (-0.04, 0.21)	-0.01 (-0.16, 0.14)	0.07 (-0.06, 0.21)	-1.36 (-3.24, 0.52)	0.71 (-1.04, 2.46)
Q3	0.08 (-0.06, 0.21)	0.10 (-0.03, 0.22)	0.14 (-0.008, 0.29)	0.15 (0.008, 0.29)	-1.28 (-3.16, 0.61)	-0.33 (-2.08, 1.41)
Q4	-0.01 (-0.15, 0.13)	-0.03 (-0.16, 0.09)	0.04 (-0.11, 0.19)	0.05 (-0.08, 0.19)	-1.37 (-3.30, 0.56)	-1.65 (-3.36, 0.06)
Q5	-0.05 (-0.19, 0.09)	-0.04 (-0.16, 0.08)	-0.02 (-0.17, 0.13)	0.02 (-0.11, 0.16)	-1.40 (-3.31, 0.51)	-1.48 (-3.19, 0.22)
p_{trend}	0.71	0.24	0.95	0.81	0.23	0.014
$p_{interaction}$	0.36		0.91		0.32	
$p_{linearity}$	0.059		0.069		0.52	

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A33: Adjusted associations between quintiles of the "high alpha-carotene, low lycopene" dietary pattern and lung function by asthma status

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)	Never asthma (n=952)	Remitted asthma (n=108)	Current asthma (n=123)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.006 (-0.11, 0.10)	-0.10 (-0.40, 0.21)	0.21 (-0.07, 0.50)	0.03 (-0.09, 0.14)	-0.14 (-0.48, 0.20)	0.23 (-0.08, 0.55)	-0.41 (-1.83, 1.02)	-0.84 (-5.12, 3.44)	1.01 (-2.95, 4.96)
Q3	0.06 (-0.04, 0.16)	0.05 (-0.27, 0.37)	0.34 (0.06, 0.61)	0.13 (0.01, 0.24)	0.06 (-0.29, 0.41)	0.39 (0.08, 0.69)	-0.91 (-2.33, 0.52)	-0.79 (-5.24, 3.65)	0.49 (-3.37, 4.34)
Q4	-0.003 (-0.10, 0.10)	-0.12 (-0.43, 0.19)	-0.10 (-0.38, 0.18)	0.06 (-0.05, 0.17)	-0.05 (-0.40, 0.30)	0.03 (-0.28, 0.34)	-1.13 (-2.56, 0.30)	-2.30 (-6.68, 2.07)	-3.62 (-7.53, 0.28)
Q5	-0.02 (-0.12, 0.08)	-0.08 (-0.40, 0.23)	-0.16 (-0.42, 0.10)	0.04 (-0.08, 0.15)	-0.10 (-0.45, 0.24)	-0.12 (-0.41, 0.17)	-1.10 (-2.53, 0.33)	-2.25 (-6.62, 2.13)	-2.71 (-6.37, 0.95)
p_{trend}	0.70	0.60	0.061	0.47	0.74	0.22	0.078	0.24	0.036
$p_{interaction}$	0.13			0.29			0.70		
$p_{linearity}$	0.035			0.033			0.86		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A34: Adjusted associations between quintiles of the "high alpha-carotene, low lycopene" dietary pattern and lung function by smoking status

Pattern quintiles	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.05 (-0.08, 0.18)	-0.003 (-0.14, 0.14)	-0.11 (-0.39, 0.17)	0.07 (-0.08, 0.21)	0.01 (-0.15, 0.17)	0.001 (-0.31, 0.31)	-0.17 (-2.02, 1.68)	0.02 (-1.96, 2.01)	-2.28 (-6.20, 1.64)
Q3	0.15 (0.01, 0.29)	0.08 (-0.06, 0.21)	-0.09 (-0.37, 0.19)	0.23 (0.07, 0.38)	0.10 (-0.05, 0.25)	0.02 (-0.29, 0.34)	-0.85 (-2.77, 1.06)	-0.25 (-2.15, 1.64)	-2.53 (-6.44, 1.39)
Q4	0.02 (-0.11, 0.16)	-0.04 (-0.17, 0.10)	-0.14 (-0.40, 0.13)	0.08 (-0.07, 0.24)	0.03 (-0.12, 0.18)	0.006 (-0.29, 0.30)	-1.20 (-3.14, 0.74)	-1.34 (-3.25, 0.57)	-3.15 (-6.89, 0.59)
Q5	0.02 (-0.12, 0.15)	-0.06 (-0.21, 0.08)	-0.19 (-0.44, 0.06)	0.07 (-0.08, 0.22)	-0.05 (-0.21, 0.11)	-0.05 (-0.33, 0.22)	-0.95 (-2.83, 0.92)	-1.49 (-3.51, 0.53)	-2.94 (-6.39, 0.52)
p_{trend}	0.97	0.33	0.16	0.40	0.66	0.67	0.19	0.065	0.12
$p_{interaction}$	0.92			0.96			0.97		
$p_{linearity}$	0.39			0.20			0.97		

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A35: Adjusted associations between quintiles of the "high alpha-carotene, low lycopene" dietary pattern and lung function by atopy

Pattern quintiles	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Non-atopic (n=562)	Atopic (n=621)	Non-atopic (n=562)	Atopic (n=621)	Non-atopic (n=562)	Atopic (n=621)
Q1	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.01 (-0.14, 0.12)	0.02 (-0.11, 0.14)	0.07 (-0.08, 0.22)	-0.002 (-0.14, 0.14)	-1.38 (-3.22, 0.47)	0.55 (-1.21, 2.31)
Q3	0.13 (-0.0008, 0.26)	0.05 (-0.07, 0.18)	0.18 (0.03, 0.33)	0.12 (-0.02, 0.26)	-0.15 (-1.98, 1.68)	-1.26 (-3.03, 0.51)
Q4	0.02 (-0.12, 0.15)	-0.05 (-0.18, 0.07)	0.07 (-0.08, 0.22)	0.03 (-0.12, 0.17)	-1.17 (-3.01, 0.67)	-1.71 (-3.47, 0.06)
Q5	-0.01 (-0.14, 0.11)	-0.07 (-0.20, 0.06)	0.04 (-0.10, 0.19)	-0.04 (-0.18, 0.11)	-1.46 (-3.24, 0.32)	-1.30 (-3.11, 0.50)
p_{trend}	0.92	0.14	0.60	0.74	0.15	0.023
$p_{interaction}$	0.71		0.94		0.18	
$p_{linearity}$	0.16		0.084		0.32	

β-coefficient (95% CI) presented for each quintile

Adjusted for age, sex, height, smoking status, energy intake, BMI category, asthma status and atopy

Table A36: Adjusted associations between quintiles for patterns 1-4 and change in FEV_1 during methacholine challenge

Pattern Quintiles	Change in FEV_1 (L) per mg Methacholine for each dietary pattern							
	High potassium & magnesium pattern		High protein & zinc pattern		High PUFAs and vitamin E pattern		High β-cryptoxanthin & vitamin C pattern	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.007 (-0.02, 0.03)	0.02 (-0.008, 0.04)	-0.02 (-0.04, 0.005)	-0.01 (-0.04, 0.01)	-0.001 (-0.02, 0.02)	0.0004 (-0.02, 0.02)	-0.005 (-0.03, 0.02)	-0.008 (-0.03, 0.01)
Q3	0.01 (-0.01, 0.03)	0.007 (-0.02, 0.03)	-0.004 (-0.03, 0.02)	0.004 (-0.02, 0.03)	0.005 (-0.02, 0.03)	0.006 (-0.02, 0.03)	-0.01 (-0.03, 0.01)	-0.01 (-0.04, 0.01)
Q4	0.01 (-0.009, 0.04)	0.01 (-0.01, 0.03)	-0.007 (-0.03, 0.02)	-0.004 (-0.03, 0.02)	0.006 (-0.02, 0.03)	0.01 (-0.01, 0.03)	-0.004 (-0.03, 0.02)	0.0009 (-0.02, 0.02)
Q5	0.007 (-0.02, 0.03)	0.01 (-0.01, 0.04)	-0.0005 (-0.02, 0.02)	0.01 (-0.01, 0.03)	0.008 (-0.01, 0.03)	0.008 (-0.01, 0.03)	0.005 (-0.02, 0.03)	0.006 (-0.02, 0.03)
p_{trend}	0.47	0.40	0.80	0.22	0.38	0.35	0.66	0.45
$p_{linearity}$	0.029*	0.54	0.25	0.022^	0.073	0.34	0.48	0.62

β-coefficient (95%CI) presented for each quintile

Model 1: Unadjusted analysis

Model 2: Adjusted for sex, age, BMI category, asthma status, smoking status, atopy and energy intake

*p-value for likelihood ratio test comparing models with and without the dietary pattern as a predictor of change in FEV_1= 0.79 (i.e. no relationship)

^p-value for likelihood ratio test comparing models with and without the dietary pattern as a predictor of change in FEV_1= 0.29 (i.e. no relationship)

Table A37 - Adjusted associations of quintiles of the "high potassium & magnesium" dietary pattern with change in FEV_1 during the methacholine challenge by sex, BMI category and asthma status

Pattern quintiles	sex		BMI category			Asthma status		
	Female (n=498)	Male (n=532)	BMI <25 (n=336)	25≤BMI<30 (n=473)	BMI≥30 (n=221)	Never asthma (n=846)	Remitted asthma (n=90)	Current asthma (n=94)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.008 (-0.03, 0.04)	0.02 (-0.01, 0.05)	-0.004 (-0.04, 0.03)	0.02 (-0.009, 0.06)	0.03 (-0.02, 0.07)	0.02 (-0.009, 0.04)	0.007 (-0.07, 0.08)	0.003 (-0.11, 0.11)
Q3	0.0008 (-0.04, 0.04)	0.01 (-0.02, 0.04)	-0.01 (-0.05, 0.03)	0.0006 (-0.03, 0.03)	0.04 (-0.002, 0.09)	0.007 (-0.02, 0.03)	0.008 (-0.08, 0.10)	0.001 (-0.12, 0.12)
Q4	0.007 (-0.03, 0.04)	0.01 (-0.02, 0.04)	0.01 (-0.03, 0.05)	0.01 (-0.02, 0.05)	0.009 (-0.04, 0.06)	0.02 (-0.008, 0.04)	-0.03 (-0.11, 0.05)	-0.03 (-0.14, 0.09)
Q5	0.009 (-0.03, 0.04)	0.01 (-0.02, 0.04)	0.01 (-0.03, 0.05)	0.02 (-0.009, 0.06)	-0.006 (-0.05, 0.04)	0.02 (-0.008, 0.04)	-0.02 (-0.10, 0.07)	-0.02 (-0.12, 0.08)
p_{trend}	0.72	0.41	0.47	0.29	0.58	0.20	0.48	0.54
$p_{interaction}$	0.99		0.28			0.96		
$p_{linearity}$	0.81		0.33			0.93		

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A38 - Adjusted associations of quintiles of the "high potassium & magnesium" dietary pattern with change in FEV_1 during the methacholine challenge by smoking status and atopy

Pattern quintiles	Smoking			Atopy	
	Never smoker (n=478)	Former smoker (n=427)	Current smoker (n=125)	Non-atopic (n=477)	Atopic (n=553)
Q1	Ref	Ref	Ref	Ref	Ref
Q2	0.02 (-0.01, 0.06)	0.02 (-0.02, 0.05)	-0.008 (-0.06, 0.04)	0.01 (-0.02, 0.05)	0.01 (-0.02, 0.04)
Q3	0.03 (-0.0003, 0.07)	-0.02 (-0.05, 0.02)	0.03 (-0.04, 0.10)	0.02 (-0.01, 0.05)	-0.004 (-0.04, 0.03)
Q4	0.02 (-0.01, 0.06)	0.006 (-0.03, 0.04)	0.0006 (-0.07, 0.07)	0.03 (-0.0005, 0.06)	-0.01 (-0.04, 0.02)
Q5	0.02 (-0.01, 0.06)	0.01 (-0.02, 0.05)	-0.03 (-0.10, 0.05)	0.02 (-0.01, 0.05)	0.009 (-0.02, 0.04)
p_{trend}	0.36	0.61	0.75	0.21	0.94
$p_{interaction}$	0.33			0.34	
$p_{linearity}$	0.32			0.47	

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A39 - Adjusted associations of quintiles of the "high protein & zinc" dietary pattern with change in FEV_1 during the methacholine challenge by sex, BMI category and asthma status

Pattern quintiles	Sex		BMI category		
	Female (n=498)	Male (n=532)	BMI <25 (n=336)	25≤BMI<30 (n=473)	BMI≥30 (n=221)
Q1	Ref	Ref	Ref	Ref	Ref
Q2	0.02 (-0.02, 0.06)	-0.04 (-0.07, -0.01)	0.01 (-0.02, 0.05)	-0.03 (-0.07, -0.002)	-0.01 (-0.06, 0.04)
Q3	0.002 (-0.03, 0.04)	0.01 (-0.02, 0.04)	-0.007 (-0.05, 0.03)	0.01 (-0.02, 0.04)	0.004 (-0.05, 0.06)
Q4	0.001 (-0.03, 0.04)	-0.005 (-0.03, 0.02)	-0.01 (-0.05, 0.03)	-0.01 (-0.04, 0.02)	0.02 (-0.03, 0.07)
Q5	0.005 (-0.03, 0.04)	0.02 (-0.008, 0.05)	-0.004 (-0.04, 0.04)	0.01 (-0.02, 0.05)	0.03 (-0.02, 0.08)
p_{trend}	0.87	0.083	0.58	0.27	0.085
$p_{interaction}$	0.008		0.20		
$p_{linearity}$	0.002		0.030		

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A40 - Adjusted associations of quintiles of the "high protein & zinc" dietary pattern with change in FEV_1 during the methacholine challenge by smoking status and atopy

Pattern quintiles	Smoking			Atopy	
	Never smoker (n=478)	Former smoker (n=427)	Current smoker (n=125)	Non-atopic (n=477)	Atopic (n=553)
Q1	Ref	Ref	Ref	Ref	Ref
Q2	-0.003 (-0.04, 0.03)	-0.03 (-0.07, 0.00)	0.02 (-0.05, 0.08)	0.005 (-0.03, 0.04)	-0.03 (-0.07, -0.0005)
Q3	0.01 (-0.02, 0.05)	0.007 (-0.03, 0.04)	-0.05 (-0.12, 0.02)	0.004 (-0.03, 0.04)	0.002 (-0.03, 0.03)
Q4	0.009 (-0.02, 0.04)	-0.007 (-0.04, 0.03)	-0.05 (-0.12, 0.03)	0.007 (-0.02, 0.04)	-0.02 (-0.05, 0.02)
Q5	0.02 (-0.02, 0.05)	0.003 (-0.03, 0.04)	0.03 (-0.04, 0.09)	0.01 (-0.02, 0.04)	0.01 (-0.02, 0.04)
p_{trend}	0.25	0.64	0.64	0.42	0.34
$p_{interaction}$	0.16			0.36	
$p_{linearity}$	0.010			0.024	

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A41 - Adjusted associations of quintiles of the "high PUFAs & vitamin E" dietary pattern with change in FEV_1 during the methacholine challenge by sex, BMI category and asthma status

Pattern quintiles	Sex		BMI category			Asthma status		
	Female (n=498)	Male (n=532)	BMI <25 (n=336)	25≤BMI<30 (n=473)	BMI≥30 (n=221)	Never asthma (n=846)	Remitted asthma (n=90)	Current asthma (n=94)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.01 (-0.05, 0.02)	0.008 (-0.02, 0.04)	-0.02 (-0.07, 0.02)	0.02 (-0.02, 0.05)	-0.005 (-0.05, 0.04)	-0.004 (-0.03, 0.02)	0.01 (-0.06, 0.09)	0.09 (-0.02, 0.20)
Q3	-0.001 (-0.04, 0.03)	0.006 (-0.02, 0.04)	-0.02 (-0.06, 0.03)	0.01 (-0.02, 0.05)	0.02 (-0.03, 0.07)	0.005 (-0.02, 0.03)	-0.005 (-0.10, 0.09)	0.05 (-0.05, 0.16)
Q4	0.005 (-0.03, 0.04)	0.01 (-0.02, 0.04)	-0.01 (-0.05, 0.03)	0.03 (-0.002, 0.06)	-0.005 (-0.05, 0.04)	-0.002 (-0.03, 0.02)	0.03 (-0.04, 0.11)	0.18 (0.08, 0.28)
Q5	-0.02 (-0.05, 0.02)	0.02 (-0.00, 0.05)	-0.003 (-0.04, 0.04)	0.02 (-0.01, 0.05)	-0.01 (-0.06, 0.04)	0.001 (-0.02, 0.03)	0.02 (-0.06, 0.09)	0.16 (0.04, 0.27)
p_{trend}	0.60	0.10	0.81	0.16	0.76	0.86	0.55	0.001
$p_{interaction}$	0.37		0.75			0.032		
$p_{linearity}$	0.43		0.56			0.31		

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A42 - Adjusted associations of quintiles of the "high PUFAs & vitamin E" dietary pattern with change in FEV_1 during the methacholine challenge by smoking status and atopy

Pattern quintiles	Smoking			Atopy	
	Never smoker (n=478)	Former smoker (n=427)	Current smoker (n=125)	Non-atopic (n=477)	Atopic (n=553)
Q1	Ref	Ref	Ref	Ref	Ref
Q2	-0.005 (-0.04, 0.03)	0.007 (-0.03, 0.04)	-0.02 (-0.08, 0.04)	0.02 (-0.009, 0.05)	-0.02 (-0.06, 0.01)
Q3	-0.01 (-0.05, 0.02)	0.02 (-0.02, 0.05)	0.03 (-0.03, 0.10)	0.02 (-0.01, 0.05)	-0.01 (-0.04, 0.02)
Q4	-0.007 (-0.04, 0.03)	0.03 (-0.002, 0.06)	-0.008 (-0.07, 0.05)	0.02 (-0.01, 0.05)	0.004 (-0.03, 0.04)
Q5	0.0002 (-0.03, 0.04)	0.03 (-0.008, 0.06)	-0.07 (-0.15, 0.003)	0.02 (-0.02, 0.05)	-0.001 (-0.03, 0.03)
p_{trend}	0.88	0.062	0.21	0.42	0.58
$p_{interaction}$	0.22			0.33	
$p_{linearity}$	0.35			0.25	

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A43 - Adjusted associations of quintiles of the "high β-cryptoxanthin & vitamin C" dietary pattern with change in FEV_1 during the methacholine challenge by sex, BMI category and asthma status

Pattern quintiles	Sex		BMI category			Asthma status		
	Female (n=498)	Male (n=532)	BMI <25 (n=336)	25≤BMI<30 (n=473)	BMI≥30 (n=221)	Never asthma (n=846)	Remitted asthma (n=90)	Current asthma (n=94)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.002 (-0.04, 0.04)	-0.009 (-0.04, 0.02)	0.004 (-0.04, 0.05)	-0.02 (-0.05, 0.01)	0.01 (-0.03, 0.05)	-0.009 (-0.03, 0.02)	0.001 (-0.09, 0.09)	0.002 (-0.10, 0.10)
Q3	0.005 (-0.03, 0.04)	-0.02 (-0.05, 0.005)	0.01 (-0.03, 0.05)	-0.02 (-0.05, 0.01)	-0.02 (-0.07, 0.02)	-0.008 (-0.03, 0.02)	-0.04 (-0.12, 0.04)	-0.07 (-0.17, 0.04)
Q4	0.01 (-0.03, 0.05)	-0.006 (-0.04, 0.02)	0.01 (-0.03, 0.05)	-0.02 (-0.05, 0.02)	0.04 (-0.01, 0.08)	0.002 (-0.02, 0.03)	-0.02 (-0.10, 0.06)	0.01 (-0.07, 0.10)
Q5	0.01 (-0.03, 0.05)	0.01 (-0.02, 0.04)	0.04 (-0.0009, 0.08)	-0.005 (-0.04, 0.03)	-0.02 (-0.07, 0.03)	0.005 (-0.02, 0.03)	0.005 (-0.07, 0.08)	0.02 (-0.06, 0.11)
p_{trend}	0.39	0.78	0.037	0.80	0.70	0.50	0.95	0.59
$p_{interaction}$	0.62		0.15			0.93		
$p_{linearity}$	0.67		0.40			0.84		

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A44 - Adjusted associations of quintiles of the "high β-cryptoxanthin & vitamin C" dietary pattern with change in FEV_1 during the methacholine challenge by smoking status and atopy

Pattern quintiles	Smoking			Atopy	
	Never smoker (n=478)	Former smoker (n=427)	Current smoker (n=125)	Non-atopic (n=477)	Atopic (n=553)
Q1 Q2 Q3 Q4 Q5	Ref -0.007 (-0.04, 0.03) -0.006 (-0.04, 0.03) 0.01 (-0.02, 0.05) 0.005 (-0.03, 0.04)	Ref -0.02 (-0.05, 0.01) -0.03 (-0.06, 0.008) -0.0002 (-0.03, 0.03) 0.005 (-0.03, 0.04)	Ref 0.04 (-0.03, 0.10) 0.02 (-0.05, 0.09) -0.02 (-0.09, 0.05) 0.03 (-0.05, 0.10)	Ref -0.008 (-0.04, 0.02) 0.002 (-0.03, 0.03) -0.0007 (-0.03, 0.03) 0.001 (-0.03, 0.03)	Ref -0.007 (-0.04, 0.02) -0.03 (-0.06, 0.005) 0.002 (-0.03, 0.03) 0.01 (-0.02, 0.05)
p_{trend}	0.46	0.64	0.91	0.80	0.39
$p_{interaction}$	0.51			0.45	
$p_{linearity}$	0.48			0.54	

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A45 - Adjusted associations of quintiles of the "low calcium & sugars" dietary pattern with change in FEV_1 during the methacholine challenge by sex, BMI category and asthma status

Pattern quintiles	Sex		BMI category		
	Female (n=498)	Male (n=532)	BMI <25 (n=336)	25≤BMI<30 (n=473)	BMI≥30 (n=221)
Q1	Ref	Ref	Ref	Ref	Ref
Q2	0.002 (-0.03, 0.03)	-0.01 (-0.04, 0.02)	0.02 (-0.02, 0.06)	-0.02 (-0.05, 0.01)	-0.01 (-0.06, 0.04)
Q3	-0.02 (-0.05, 0.01)	-0.04 (-0.07, -0.01)	-0.02 (-0.06, 0.01)	-0.02 (-0.06, 0.008)	-0.05 (-0.10, -0.006)
Q4	-0.02 (-0.06, 0.01)	-0.0001 (-0.03, 0.03)	-0.004 (-0.04, 0.04)	-0.004 (-0.04, 0.03)	0.03 (-0.08, 0.02)
Q5	-0.001 (-0.04, 0.03)	0.006 (-0.02, 0.04)	0.01 (-0.03, 0.05)	-0.005 (-0.04, 0.03)	-0.004 (-0.04, 0.05)
p_{trend}	0.53	0.65	0.95	0.98	0.85
$p_{interaction}$	0.37		0.63		
$p_{linearity}$	0.006		0.012		

β-coefficient (95%CI) presented for each quintile

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A46 - Adjusted associations of quintiles of the "low calcium & sugars" dietary pattern with change in FEV_1 during the methacholine challenge by smoking status and atopy

Pattern quintiles	Smoking			Atopy	
	Never smoker (n=478)	Former smoker (n=427)	Current smoker (n=125)	Non-atopic (n=477)	Atopic (n=553)
Q1	Ref	Ref	Ref	Ref	Ref
Q2	0.01 (-0.02, 0.04)	-0.03 (-0.06, 0.01)	0.02 (-0.04, 0.08)	0.005 (-0.03, 0.04)	-0.01 (-0.05, 0.02)
Q3	-0.02 (-0.05, 0.02)	-0.05 (-0.08, -0.01)	-0.03 (-0.09, 0.04)	-0.02 (-0.06, 0.07)	-0.04 (-0.07, -0.005)
Q4	-0.009 (-0.04, 0.02)	-0.01 (-0.05, 0.02)	0.001 (-0.06, 0.06)	-0.008 (-0.04, 0.02)	-0.01 (-0.04, 0.02)
Q5	0.01 (-0.02, 0.05)	-0.009 (-0.04, 0.03)	0.001 (-0.06, 0.07)	0.005 (-0.03, 0.04)	0.0004 (-0.03, 0.03)
p_{trend}	0.80	0.80	0.82	0.92	0.99
$p_{interaction}$	0.84			0.93	
$p_{linearity}$	0.024			0.016	

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A47 - Adjusted associations of quintiles of the "high starch & lycopene" dietary pattern with change in FEV_1 during the methacholine challenge by sex, BMI category and asthma status

Pattern quintiles	Sex		BMI category			Asthma status		
	Female (n=498)	Male (n=532)	BMI <25 (n=336)	25≤BMI<30 (n=473)	BMI≥30 (n=221)	Never asthma (n=846)	Remitted asthma (n=90)	Current asthma (n=94)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.02 (-0.02, 0.06)	0.01 (-0.02, 0.04)	0.03 (-0.008, 0.07)	0.008 (-0.02, 0.04)	0.02 (-0.03, 0.07)	0.01 (-0.01, 0.04)	0.01 (-0.07, 0.09)	0.07 (-0.04, 0.18)
Q3	0.004 (-0.03, 0.04)	-0.01 (-0.04, 0.02)	0.01 (-0.03, 0.05)	0.002 (-0.03, 0.03)	-0.04 (-0.09, 0.01)	-0.005 (-0.03, 0.02)	-0.01 (-0.10, 0.07)	0.06 (-0.07, 0.19)
Q4	-0.002 (-0.04, 0.03)	-0.009 (-0.04, 0.02)	0.007 (-0.03, 0.05)	-0.001 (-0.04, 0.03)	-0.03 (-0.07, 0.02)	-0.007 (-0.03, 0.02)	-0.03 (-0.11, 0.06)	0.06 (-0.05, 0.18)
Q5	-0.009 (-0.05, 0.03)	0.007 (-0.02, 0.04)	0.009 (-0.04, 0.05)	-0.00003 (-0.03, 0.03)	-0.009 (-0.05, 0.04)	0.001 (-0.02, 0.03)	-0.01 (-0.10, 0.08)	0.01 (-0.11, 0.14)
p_{trend}	0.29	0.98	0.88	0.84	0.38	0.64	0.51	0.81
$p_{interaction}$	0.74		0.80			0.95		
$p_{linearity}$	0.43		0.45			0.59		

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A48 - Adjusted associations of quintiles of the "high starch & lycopene" dietary pattern with change in FEV_1 during the methacholine challenge by smoking status and atopy

Pattern quintiles	Smoking			Atopy	
	Never smoker (n=478)	Former smoker (n=427)	Current smoker (n=125)	Non-atopic (n=477)	Atopic (n=553)
Q1	Ref	Ref	Ref	Ref	Ref
Q2	0.02 (-0.01, 0.05)	0.01 (-0.02, 0.04)	0.02 (-0.04, 0.09)	0.01 (-0.02, 0.04)	0.02 (-0.01, 0.05)
Q3	-0.004 (-0.04, 0.03)	-0.02 (-0.05, 0.02)	0.03 (-0.03, 0.10)	-0.0003 (-0.03, 0.03)	-0.006 (-0.04, 0.03)
Q4	-0.01 (-0.05, 0.02)	0.005 (-0.03, 0.04)	-0.01 (-0.08, 0.06)	-0.003 (-0.04, 0.03)	-0.007 (-0.04, 0.03)
Q5	-0.01 (-0.05, 0.03)	0.01 (-0.02, 0.05)	-0.03 (-0.10, 0.05)	0.003 (-0.03, 0.03)	-0.003 (-0.04, 0.03)
p_{trend}	0.21	0.48	0.29	0.87	0.44
$p_{interaction}$	0.45			0.95	
$p_{linearity}$	0.38			0.50	

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A49 - Adjusted associations of quintiles of the "high vitamin C, low calcium" dietary pattern with change in FEV_1 during the methacholine challenge by sex, BMI category and asthma status

Pattern quintiles	Sex		BMI category			Asthma status		
	Female (n=498)	Male (n=532)	BMI <25 (n=336)	25≤BMI<30 (n=473)	BMI≥30 (n=221)	Never asthma (n=846)	Remitted asthma (n=90)	Current asthma (n=94)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.007 (-0.02, 0.04)	-0.03 (-0.07, -0.002)	-0.006 (-0.04, 0.03)	-0.03 (-0.06, 0.006)	0.006 (-0.05, 0.06)	-0.01 (-0.04, 0.01)	0.006 (-0.08, 0.09)	-0.04 (-0.13, 0.05)
Q3	-0.01 (-0.04, 0.02)	-0.02 (-0.05, 0.01)	-0.02 (-0.06, 0.02)	-0.01 (-0.05, 0.02)	-0.006 (-0.06, 0.05)	-0.01 (-0.03, 0.01)	-0.04 (-0.12, 0.04)	-0.07 (-0.16, 0.02)
Q4	-0.02 (-0.05, 0.01)	-0.006 (-0.04, 0.03)	0.006 (-0.03, 0.05)	-0.02 (-0.06, 0.01)	-0.006 (-0.05, 0.04)	-0.005 (-0.03, 0.02)	-0.008 (-0.09, 0.07)	-0.18 (-0.31, -0.06)
Q5	-0.002 (-0.04, 0.03)	-0.02 (-0.05, 0.008)	-0.02 (-0.06, 0.02)	-0.02 (-0.06, 0.007)	0.02 (-0.03, 0.06)	-0.008 (-0.03, 0.02)	-0.03 (-0.11, 0.05)	-0.05 (-0.14, 0.04)
p_{trend}	0.50	0.50	0.57	0.19	0.58	0.70	0.47	0.10
$p_{interaction}$	0.15		0.76			0.26		
$p_{linearity}$	0.48		0.95			0.66		

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A50 - Adjusted associations of quintiles of the "high vitamin C, low calcium" dietary pattern with change in FEV_1 during the methacholine challenge by smoking status and atopy

Pattern quintiles	Smoking			Atopy	
	Never smoker (n=478)	Former smoker (n=427)	Current smoker (n=125)	Non-atopic (n=477)	Atopic (n=553)
Q1	Ref	Ref	Ref	Ref	Ref
Q2	-0.02 (-0.05, 0.01)	-0.01 (-0.05, 0.02)	0.01 (-0.05, 0.08)	-0.004 (-0.03, 0.03)	-0.02 (-0.06, 0.009)
Q3	-0.03 (-0.06, 0.006)	-0.02 (-0.05, 0.02)	0.05 (-0.02, 0.12)	-0.009 (-0.04, 0.02)	-0.02 (-0.06, 0.009)
Q4	-0.02 (-0.05, 0.01)	-0.009 (-0.04, 0.03)	0.02 (-0.04, 0.08)	-0.02 (-0.06, 0.007)	0.003 (-0.03, 0.04)
Q5	-0.04 (-0.07, -0.006)	0.004 (-0.03, 0.04)	0.03 (-0.04, 0.10)	0.01 (-0.02, 0.04)	-0.03 (-0.07, -0.004)
p_{trend}	0.029	0.71	0.36	0.77	0.10
$p_{interaction}$	0.46			0.021	
$p_{linearity}$	0.97			0.24	

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A51 - Adjusted associations of quintiles of the "high α-carotene, low lycopene" dietary pattern with change in FEV_1 during the methacholine challenge by sex, BMI category and asthma status

Pattern quintiles	Sex		BMI category			Asthma status		
	Female (n=498)	Male (n=532)	BMI <25 (n=336)	25≤BMI<30 (n=473)	BMI≥30 (n=221)	Never asthma (n=846)	Remitted asthma (n=90)	Current asthma (n=94)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.004 (-0.04, 0.03)	-0.03 (-0.05, 0.004)	-0.02 (-0.06, 0.02)	-0.03 (-0.07, -0.001)	0.03 (-0.02, 0.07)	-0.02 (-0.04, 0.005)	0.008 (-0.07, 0.09)	0.0002 (-0.10, 0.10)
Q3	-0.008 (-0.04, 0.03)	-0.01 (-0.04, 0.02)	-0.02 (-0.06, 0.02)	-0.02 (-0.05, 0.01)	0.03 (-0.01, 0.08)	-0.02 (-0.04, 0.007)	0.03 (-0.05, 0.11)	0.05 (-0.04, 0.14)
Q4	0.005 (-0.03, 0.04)	0.006 (-0.02, 0.04)	-0.002 (-0.04, 0.04)	0.001 (-0.03, 0.03)	0.02 (-0.03, 0.07)	0.002 (-0.02, 0.03)	-0.06 (-0.15, 0.03)	0.13 (0.03, 0.22)
Q5	0.001 (-0.03, 0.04)	0.01 (-0.02, 0.04)	-0.03 (-0.06, 0.01)	0.0005 (-0.03, 0.03)	0.06 (0.02, 0.11)	-0.004 (-0.03, 0.02)	0.04 (-0.04, 0.12)	0.13 (0.03, 0.22)
p_{trend}	0.78	0.21	0.37	0.44	0.017	0.79	0.59	0.001
$p_{interaction}$	0.73		0.13			0.015		
$p_{linearity}$	0.34		0.24			0.14		

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A52 - Adjusted associations of quintiles of the "high α-carotene, low lycopene" dietary pattern with change in FEV_1 during the methacholine challenge by smoking status and atopy

Pattern quintiles	Smoking			Atopy	
	Never smoker (n=478)	Former smoker (n=427)	Current smoker (n=125)	Non-atopic (n=477)	Atopic (n=553)
Q1	Ref	Ref	Ref	Ref	Ref
Q2	-0.02 (-0.05, 0.009)	-0.01 (-0.04, 0.02)	-0.005 (-0.07, 0.06)	0.001 (-0.03, 0.03)	-0.03 (-0.06, -0.0006)
Q3	-0.04 (-0.07, -0.003)	0.009 (-0.02, 0.04)	0.03 (-0.04, 0.10)	0.007 (-0.02, 0.04)	-0.03 (-0.06, 0.004)
Q4	-0.008 (-0.04, 0.02)	0.01 (-0.02, 0.05)	0.03 (-0.03, 0.10)	0.02 (-0.01, 0.05)	-0.008 (-0.04, 0.02)
Q5	-0.01 (-0.05, 0.02)	0.03 (-0.0005, 0.07)	-0.01 (-0.07, 0.05)	0.01 (-0.02, 0.04)	-0.003 (-0.04, 0.03)
p_{trend}	0.72	0.025	0.84	0.24	0.68
$p_{interaction}$	0.28			0.52	
$p_{linearity}$	0.28			0.25	

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Appendix 8: Chapter 8 supplementary data

Table A1 - Adjusted associations between E-DII quintiles and lung function by sex

E-DII quintile	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Female (n=573)	Male (n=610)	Female (n=573)	Male (n=610)	Female (n=573)	Male (n=610)
Q1	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.05 (-0.16, 0.07)	0.02 (-0.12, 0.16)	-0.007 (-0.14, 0.12)	-0.04 (-0.20, 0.12)	-0.99 (-2.60, 0.63)	0.46 (-1.51, 2.43)
Q3	-0.02 (-0.14, 0.10)	-0.05 (-0.19, 0.09)	-0.02 (-0.16, 0.11)	-0.15 (-0.30, 0.003)	0.09 (-1.57, 1.74)	0.92 (-1.01, 2.85)
Q4	-0.15 (-0.27, -0.02)	-0.23 (-0.36, -0.09)	-0.14 (-0.28, -0.007)	-0.23 (-0.38, -0.07)	-1.31 (-3.02, 0.41)	-1.65 (-3.57, 0.27)
Q5	-0.11 (-0.26, 0.03)	-0.16 (-0.29, -0.02)	-0.17 (-0.33, -0.01)	-0.15 (-0.30, -0.002)	0.38 (-1.60, 2.37)	-1.58 (-3.45, 0.29)
p_{trend}	0.035	0.001	0.008	0.015	0.97	0.005
$P_{interaction}$	0.61		0.61		0.11	
$p_{linearity}$	0.092		0.29		0.065	

β-coefficient (95%CI) presented for each E-DII quintile

Adjusted for age, height, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A2 - Adjusted associations between E-DII quintiles and lung function by BMI category

E-DII quintile	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Healthy weight (n=377)	Overweight (n=534)	Obese (n=272)	Healthy weight (n=377)	Overweight (n=534)	Obese (n=272)	Healthy weight (n=377)	Overweight (n=534)	Obese (n=272)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	-0.08 (-0.24, 0.07)	0.02 (-0.11, 0.15)	0.03 (-0.17, 0.23)	-0.11 (-0.28, 0.06)	0.02 (-0.12, 0.17)	0.05 (-0.17, 0.26)	-0.26 (-2.42, 1.89)	-0.21 (-2.05, 1.64)	-0.55 (-3.26, 2.17)
Q3	-0.004 (-0.16, 0.15)	-0.02 (-0.15, 0.12)	-0.10 (-0.29, 0.09)	-0.03 (-0.21, 0.14)	-0.06 (-0.21, 0.08)	-0.17 (-0.38, 0.04)	0.32 (-1.85, 2.49)	0.91 (-0.93, 2.75)	0.06 (-2.62, 2.75)
Q4	-0.19 (-0.35, -0.03)	-0.18 (-0.31, -0.05)	-0.19 (-0.39, 0.003)	-0.13 (-0.31, 0.04)	-0.19 (-0.33, -0.04)	-0.21 (-0.42, 0.007)	-2.31 (-4.52, -0.09)	-1.01 (-2.85, 0.84)	-1.32 (-4.03, 1.40)
Q5	-0.28 (-0.44, -0.12)	-0.05 (-0.19, 0.09)	-0.10 (-0.29, 0.09)	-0.26 (-0.43, -0.08)	-0.06 (-0.22, 0.09)	-0.13 (-0.34, 0.08)	-2.29 (-4.52, -0.05)	-0.29 (-2.24, 1.66)	-0.60 (-3.26, 2.05)
p_{trend}	<0.000	0.079	0.094	0.006	0.070	0.057	0.012	0.55	0.57
$P_{interaction}$	0.30			0.26			0.91		
$p_{linearity}$	0.076			0.12			0.470		

β-coefficient (95%CI) presented for each E-DII quintile

Adjusted for age, height, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A3 - Adjusted associations between E-DII quintiles and lung function by smoking

E-DII quintile	FEV_1 (L)			FVC (L)			FEV_1/FVC (%)		
	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)	Never smoker (n=542)	Former smoker (n=495)	Current smoker (n=146)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.03 (-0.09, 0.16)	-0.04 (-0.18, 0.09)	-0.28 (-0.65, 0.08)	0.04 (-0.10, 0.19)	-0.03 (-0.18, 0.11)	-0.39 (-0.79, 0.01)	-0.30 (-2.07, 1.47)	-0.63 (-2.50, 1.24)	0.33 (-4.69, 5.35)
Q3	-0.02 (-0.15, 0.11)	-0.009 (-0.14, 0.12)	-0.39 (-0.74, -0.03)	-0.05 (-0.19, 0.09)	-0.06 (-0.21, 0.09)	-0.48 (-0.87, -0.08)	0.47 (-1.33, 2.28)	0.55 (-1.29, 2.39)	-0.59 (-5.54, 4.35)
Q4	-0.10 (-0.23, 0.03)	-0.28 (-0.42, -0.14)	-0.45 (-0.79, -0.11)	-0.11 (-0.25, 0.04)	-0.27 (-0.42, -0.11)	-0.38 (-0.75, -0.005)	-0.52 (-2.33, 1.28)	-2.45 (-4.37, -0.52)	-3.42 (-8.12, 1.28)
Q5	-0.02 (-0.16, 0.12)	-0.16 (-0.30, -0.02)	-0.55 (-0.88, -0.23)	-0.07 (-0.22, 0.09)	-0.16 (-0.32, -0.01)	-0.47 (-0.83, -0.11)	0.47 (-1.49, 2.44)	-1.10 (-3.06, 0.87)	-4.75 (-9.22, -0.29)
p_{trend}	0.30	0.001	0.001	0.114	0.003	0.072	0.75	0.095	0.001
$P_{interaction}$	0.067			0.31			0.090		
$p_{linearity}$	0.067			0.17			0.28		

β-coefficient (95%CI) presented for each E-DII quintile

Adjusted for age, height, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A4 - Adjusted associations between E-DII quintiles and lung function by atopy

E-DII quintile	FEV_1 (L)		FVC (L)		FEV_1/FVC (%)	
	Non-atopic (n=562)	Atopic (n=621)	Non-atopic (n=562)	Atopic (n=621)	Non-atopic (n=562)	Atopic (n=621)
Q1	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.04 (-0.09, 0.17)	-0.06 (-0.19, 0.06)	0.03 (-0.12, 0.17)	-0.06 (-0.19, 0.08)	0.04 (-1.78, 1.85)	-0.59 (-2.31, 1.12)
Q3	-0.06 (-0.19, 0.07)	-0.01 (-0.14, 0.11)	-0.08 (-0.23, 0.06)	-0.08 (-0.22, 0.06)	-0.40 (-2.21, 1.41)	1.30 (-0.42, 3.01)
Q4	-0.18 (-0.31, -0.06)	-0.19 (-0.32, -0.06)	-0.17 (-0.31, -0.03)	-0.18 (-0.33, -0.04)	-1.81 (-3.57, -0.05)	-1.21 (-3.01, 0.59)
Q5	-0.08 (-0.22, 0.05)	-0.18 (-0.30, -0.05)	-0.07 (-0.22, 0.08)	-0.20 (-0.34, -0.06)	-1.37 (-3.28, 0.54)	-0.69 (-2.45, 1.07)
p_{trend}	0.016	0.001	0.059	0.001	0.040	0.34
$P_{interaction}$	0.38		0.60		0.44	
$p_{linearity}$	0.046		0.33		0.090	

β-coefficient (95%CI) presented for each E-DII quintile

Adjusted for age, height, sex, smoking status, asthma status, BMI category, atopy, and energy intake

Table A5 - Unadjusted and adjusted associations of E-DII with change in FEV_1 during the methacholine challenge

E-DII quintiles	Change in FEV_1(L)/mg methacholine	
	Model 1	Model 2
Q1	Ref	Ref
Q2	0.03 (0.002, 0.05)	0.02 (-0.002, 0.05)
Q3	0.02 (-0.005, 0.04)	0.02 (-0.006, 0.04)
Q4	0.01 (-0.01, 0.03)	0.008 (-0.02, 0.03)
Q5	0.004 (-0.02, 0.03)	0.003 (-0.02, 0.03)
p_{trend}	0.73	0.69
$p_{linearity}$	0.053	0.022*

β-coefficient (95%CI) presented

Model 1: Unadjusted linear mixed model

Model 2: Linear mixed model adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake

* p-value for likelihood ratio test comparing models with and without E-DII as a predictor of BR = 0.30

Table A6 - Adjusted associations of E-DII with change in FEV_1 during the methacholine challenge by smoking status, asthma status, BMI category, and atopy

E-DII quintiles	Smoking			Asthma			BMI			Atopy	
	Never smoker (n=478)	Former smoker (n=427)	Current smoker (n=125)	Never asthma (n=846)	Remitted asthma (n=90)	Current asthma (n=94)	Healthy weight (n=336)	Overweight (n=473)	Obese (n=221)	Non-atopic (n=477)	Atopic (n=553)
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	0.01 (-0.02, 0.04)	0.03 (-0.007, 0.06)	0.14 (0.03, 0.24)	0.02 (-0.005, 0.04)	-0.01 (-0.10, 0.07)	0.06 (-0.03, 0.15)	0.009 (-0.03, 0.05)	0.03 (-0.001, 0.06)	0.02 (-0.03, 0.07)	0.02 (-0.02, 0.05)	0.03 (-0.005, 0.06)
Q3	0.02 (-0.01, 0.05)	0.004 (-0.03, 0.04)	0.13 (0.03, 0.24)	0.01 (-0.01, 0.04)	0.07 (-0.01, 0.14)	0.03 (-0.05, 0.11)	0.007 (-0.03, 0.05)	0.01 (-0.02, 0.05)	0.04 (-0.008, 0.09)	0.02 (-0.01, 0.05)	0.02 (-0.02, 0.05)
Q4	0.003 (-0.03, 0.04)	0.009 (-0.03, 0.04)	0.10 (-0.0001, 0.20)	-0.001 (-0.03, 0.02)	0.08 (-0.003, 0.16)	0.05 (-0.04, 0.15)	-0.03 (-0.07, 0.01)	0.02 (-0.02, 0.05)	0.05 (-0.005, 0.10)	-0.0009 (-0.03, 0.03)	0.02 (-0.02, 0.05)
Q5	-0.02 (-0.05, 0.02)	0.009 (-0.03, 0.05)	0.12 (0.02, 0.21)	0.0008 (-0.03, 0.03)	0.02 (-0.06, 0.09)	-0.03 (-0.14, 0.09)	0.01 (-0.03, 0.05)	-0.01 (-0.05, 0.02)	0.02 (-0.03, 0.07)	-0.008 (-0.04, 0.03)	0.01 (-0.02, 0.05)
p_{trend}	0.32	0.95	0.52	0.49	0.38	0.99	0.93	0.29	0.45	0.38	0.77
$p_{interaction}$	0.25			0.27			0.16			0.78	
$p_{linearity}$	0.022			0.023			0.016			0.072	

β-coefficient (95%CI) presented

Adjusted for age, sex, smoking status, asthma status, BMI category, atopy, and energy intake