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Metabolic dysfunction-associated liver disease in Australia: epidemiology and therapeutic applications of medical weight management

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Abstract

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common cause of liver disease worldwide, with its rise mirroring the obesity epidemic. It represents a growing cause of morbidity and mortality, yet estimates of disease prevalence within Australia, and identification of those at risk of developing complications, remain lacking. Furthermore, therapeutic options are limited, with weight loss fundamental to management but difficult to achieve and maintain. This thesis explores the epidemiology of MASLD in Australia and investigates whether medical weight management approaches developed for obesity are effective, and whether they can be safely and effectively adapted for the treatment of MASLD.

To provide estimates of MASLD in Australia, a cross-sectional analysis of a large nationwide cohort (n=4,747) study was performed, and found elevated ALT in 13%, and MASLD in 37%. 61% of those with MASLD also had an elevated BARD score, suggesting that a large proportion of Australian adults with MASLD may warrant further investigation for risk of advanced fibrosis.

Real-world weight loss outcomes were examined in a retrospective cohort study (n=411) from a publicly-funded specialist weight management service. Using a very low energy diet (VLED) based approach, 50% achieved clinically significant weight loss (≥ 10% total body weight loss (TBWL)) at 12 months, with a mean 10.5% TBWL. However, clinic retention was challenging, with 29% patient retention at 12 months. Metabolic phenotype (specifically presence of MASLD and insulin resistance) and early treatment response were found to be predictors of weight loss outcomes at 12 months.

The DARWIN randomised pilot study compared a 12-week ketogenic VLED to a Mediterranean diet (MD). The VLED achieved significantly greater hepatic steatosis reduction than a Mediterranean diet (77% vs 14% relative reduction), and resulted in greater weight loss (13% TBWL vs 4%). Low-dose semaglutide (0.5mg weekly) maintained these benefits to week 24 at which point superior histological improvement was also demonstrated in the VLED group. Extension follow-up to week 48 (24 weeks after cessation of all active intervention) found that the VLED group maintained clinically significant improvements in weight (10% TBWL) and hepatic steatosis (55% relative reduction), despite some weight regain and modest liver fat increase.

Collectively, these findings establish the substantial burden of MASLD in Australia, and demonstrated that the VLED-based medical weight management approach achieves clinically meaningful hepatic and metabolic benefits in MASLD. These results support the broader use of VLED-based programs as effective, accessible and a pragmatic first-line treatment for MASLD, but also highlight the need for improved engagement strategies in publicly-funded weight management services.

Declaration

This is to certify that this thesis:

I. Contains no material that has been accepted for the award of any other degree or diploma at any university or institution.

II. Comprises only my original work except where indicated in the preface.

III. Includes due acknowledgement in the text to all other materials used.

IV. Is fewer than 100,000 words in length, exclusive of tables, figures, bibliographies, and appendices.

Ann Farrell, February 2026

Preface

The work presented in this thesis was carried out under the supervision of Associate Professor Marno Ryan, alongside Professor Alexander Thompson, Professor Jessica Howell and Dr Catherine Croagh at St Vincent's Hospital Melbourne and the University of Melbourne, with Professor Paul Desmond acting as advisory chair. Ethical approvals for the original research conducted was obtained from St Vincent's Hospital Melbourne Human Research Ethics Committee (HREC Ref: 277/18; 071/19; 225/20).

This is a thesis with publication. A narrative review included in Chapter 1 and two of the four main results chapters have been published or accepted for publication by peer-reviewed journals. The two remaining results chapters have been prepared for publication and are in the process of submission to peer-reviewed publications. For the narrative review, which is included in the literature review as the final published version, I was the lead researcher and principal author and undertook the literature review, data analysis and primary draft of the paper. Marno Ryan and Jessica Howell assisted in the visualisation of the paper and in reviewing and editing the drafts. For all results chapters I was the lead researcher and principal author, and was responsible for all of the data analyses, project administration and the original and final drafts of the papers. The co-authors on each paper contributed to study design, study execution, data collection and review and editing of manuscripts.

Chapter three is a cross-sectional analysis of the AusDiab study to estimate the frequency of ALT elevation and MAFLD in a large nationwide prospective cohort study. Marno Ryan, Alexander Thompson and Catherine Croagh assisted with conceptualisation of the study, contributed to study design and assisted with writing (review & editing). Dianna Magliano and Jonathan Shaw are lead investigators of the AusDiab study and were responsible for design, recruitment, data collection, project administration, and assisted with writing (review & editing) of this paper. Jessica Howell helped to conceptualise the study, contributed to study design, helped to develop the statistical analysis plan and was the primary supervising author of this project.

Chapter four is a retrospective cohort analysis to determine real world-outcomes of medical weight management. Hayden Green, James Williams and Han Goh assisted with data collection. Catherine Croagh, Jessica Howell and Alexander Thompson assisted in

conceptualising the study design and contributed to writing (review & editing). Marno Ryan was involved in conceptualisation of the study, developing the study design, assisted with writing (review & editing), and was the overall primary supervising author of the project.

Chapters five and six present the outcomes from a pilot randomised trial comparing the use of a ketogenic very low energy diet to a Mediterranean diet for the treatment of MASLD. Tonya Paris contributed to recruitment, data collection, analysis of food-diaries, writing (review & editing) and project administration. Evelyn Parr provided access to and help with analysis of DEXA for body composition and physical activity monitors, as well as writing (review & editing). Elena George developed the Mediterranean diet meal program and assisted in writing (review & editing). Jessica Howell was involved in developing the study design and helped to develop the statistical analysis plan, contributed to recruitment and writing (review & editing). Catherine Croagh was involved in developing the study design, contributed to recruitment and writing (review & editing). Tom Sutherland and Mark Page contributed to data collection through reporting of all the study MRIs, and assisted with interpretation of results. Penny McKelvie contributed to data collection through analysis of all of the liver histology. Alexander Thompson was involved in conceptualisation of the study, developing the study design, and contributed to writing (review & editing). Marno Ryan was involved in conceptualisation of the study, developing the study design, assisted with writing (review & editing), helped with funding acquisition, project administration, and was the overall primary supervising author of the project.

Publications and presentations arising from this thesis

Journal publications

- **Farrell A**, Paris T, Parr E, George E, Howell J, Croagh C, Sutherland T, Page M, McKelvie P, Thompson A, Ryan M. Very Low Energy Ketogenic Diet vs Mediterranean Diet for MASLD: Superior Steatosis Reduction in a Randomised Pilot Study. (2026) JHep Reports. In Press. DOI: 10.1016/j.jhepr.2026.101787

- **Farrell A**, Magliano D, Shaw J, Thompson A, Croagh C, Ryan M and Howell J. A problem of proportions: estimates of metabolic associated fatty liver disease and liver fibrosis in Australian adults in the nationwide 2012 AusDiab Study. (2022) Scientific Reports, DOI: 10.1038/s41598-022-05168-0

- **Farrell A**, Ryan M, Howell J. Epidemiology of non-alcoholic fatty liver disease-related hepatocellular carcinoma: a western perspective. (2020) Hepatoma Res 2020;6:18. DOI: 10.20517/2394-5079.2019.019

Abstract oral presentations

- Very low energy diet (VLED) shows sustained benefits in weight and hepatic steatosis: A 48 week analysis of the DARWIN study comparing the VLED vs Mediterranean Diet (MD) in MASLD
  Ann Farrell, Tonya Paris, Evelyn Parr, Elena George, Jessica Howell, Catherine Croagh, Tom Sutherland, Mark Page, Penny McKelvie, Alexander Thompson, Marno Ryan
  Australian Gastroenterology Week 2025
  St Vincent's Hospital Melbourne Research Week 2025: Junior Investigator Oral Presentation
- Effects of a very low energy ketogenic diet versus a Mediterranean diet on hepatic and metabolic outcomes in metabolic dysfunction associated fatty liver disease: A randomised controlled trial
  Ann Farrell, Tonya Paris, Evelyn Parr, Elena George, Jessica Howell, Catherine Croagh, Tom Sutherland, Mark Page, Leah Brennan, Penny McKelvie, Alexander Thompson, Marno Ryan
  Australian Gastroenterology Week 2024
- NAFLD is a big problem in Australia: Analysis from the AusDiab cohort
  Ann Farrell, Dianna Magliano, Jonathan Shaw, Alexander Thompson, Catherine Croagh, Marno Ryan & Jessica Howell

Australian Gastroenterology Week 2020

- Adapting medical weight loss strategies to NAFLD – is it effective?
  Ann Farrell, Hayden Green, James Williams, Jessica Howell, Catherine Croagh, Alexander Thompson, Marno Ryan
  American Association for the Study of Liver Diseases – The Liver Meeting 2019

- Medical weight management using the very low energy diet is very effective but compliance remains challenging.
  Ann Farrell, Hayden Green, James Williams, Jessica Howell, Catherine Croagh, Alexander Thompson, Marno Ryan
  Australian Gastroenterology Week 2019

Abstract publications

- Effects of the very low energy diet compared to a mediterranean diet on hepatic and metabolic outcomes in metabolic associated steatotic liver disease: A randomised control trial
  Ann Farrell, Tonya Paris, Evelyn Parr, Elena George, Jessica Howell, Catherine Croagh, Tom Sutherland, Mark Page, Penny McKelvie, Alexander Thompson, Marno Ryan
  AASLD The Liver Meeting 2024. Poster presentation.
- Obesity management during the rise of NAFLD: real world outcomes of the medical weight loss approach using a very low energy diet
  Ann Farrell, Hayden Green, James Williams, Jessica Howell, Catherine Croagh, Alexander Thompson, Marno Ryan
  EASL International Liver Conference 2020. Poster presentation.

Prizes

- Effects of the very low energy diet compared to a Mediterranean Diet on hepatic and metabolic outcomes in metabolic associated steatotic liver disease: A randomised control trial
  Ann Farrell, Tonya Paris, Evelyn Parr, Elena George, Jessica Howell, Catherine Croagh, Tom Sutherland, Mark Page, Penny McKelvie, Alexander Thompson, Marno Ryan
  St Vincent's Research Week 2025: Best clinical poster

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I would like to acknowledge the financial support received from the St Vincent's Hospital Melbourne Research Endowment Fund (Grant #91940), and in-kind contributions of food products that were accepted from Cobram estates, Australian Almond Board, Safcol and Heinz for the DARWIN study. I would also like to acknowledge the support I received from an Australian Government Research Training Program (RTP) Scholarship in order to complete this thesis.

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List of abbreviations

AASLD	American association for the study of liver diseases
ABS	Australian Bunhanesreau of Statistics
AHS	Australian Health Survey
AIHW	Australian Institute of Health and Welfare
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
APASL	Asian Pacific Association for the study of the liver
APD	Accredited practising dietitian
AST	Aspartate aminotransferase
AUC	Area under the curve
AUROC	Area under the receiver operating characteristic curve
BMI	Body mass index
CAP	Controlled attenuation parameter
CCK	Cholecystokinin
CI	Confidence interval
CRN	Clinical Research Network
CRP	C-reactive protein
CVD	Cardiovascular disease
DEXA	Dual-energy X-ray absorptiometry
EASD	European association for the study of diabetes
EASL	European association for the study of the liver
EASO	European association for the study of obesity
ELF	Enhanced Liver Fibrosis score
FFA	Free fatty acid
FFQ	Food frequency questionnaire
FLI	Fatty liver index
FXR	Farnesoid X receptor
GCKR	Glucokinase regulator
GESA	Gastroenterological society of Australia
GGT	Gamma-glutamyl transferase
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
GLP-1 RA	Glucagon-like peptide-1 receptor agonist

GORD	Gastroesophageal reflux disease
HbA1c	Glycated haemoglobin
HCV	Hepatitis C virus
HDL-C	High-density lipoprotein
HOMA-IR	Homeostatic model assessment of insulin resistance
HIS	Hepatic steatosis index
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IL-6	Interleukin-6
IQR	Interquartile range
LDL-C	Low-density lipoprotein cholesterol
LFD	Low-fat diet
LFHC	Low-fat/High-carbohydrate diet
LPS	Lipopolysaccharide
LSM	Liver stiffness measurement
MAFLD	Metabolic dysfunction-associated fatty liver disease
MASH	Metabolic dysfunction-associated steatohepatitis
MASLD	Metabolic dysfunction-associated steatotic liver disease
MD	Mediterranean diet
MEDAS	Mediterranean diet adherence screener
MEN2	Multiple endocrine neoplasia type 2
MRI	Magnetic resonance imaging
MRI-LFF	Magnetic resonance imaging – liver fat fraction
MRI-PDFF	Magnetic resonance imaging – proton density fat fraction
MRS	Magnetic resonance spectroscopy
NAFL	Nonalcoholic fatty liver
NAFLD	Nonalcoholic fatty liver disease
NAION	Non-arteritic anterior ischaemic optic neuropathy
NAS	NAFLD activity score
NASH	Nonalcoholic steatohepatitis
NCD	Non-communicable diseases
NFS	NAFLD fibrosis score
NHMRC	National Health and Medical Research Council
NHANES	National Health and Nutrition Examination Survey
OLT	Orthotopic liver transplantation

OR	Odds ratio
PBS	Pharmaceutical Benefits Scheme
PCOS	Polycystic ovarian syndrome
PPAR	Peroxisome proliferator-activated receptor
PYY	Peptide YY
RCT	Randomised controlled trial
ROS	Reactive oxygen species
SAT	Subcutaneous adipose tissue
SCFA	Short-chain fatty acid
SD	Standard deviation
SHR	Sub-hazard ratio
SLD	Steatotic liver disease
SNP	Single-nucleotide polymorphism
SRR	Summary relative risk
T2DM	Type 2 diabetes mellitus
TBWL	Total body weight loss
TGA	Therapeutic Goods Administration
TGR5	Takeda G protein-coupled receptor 5
THR-β	Thyroid hormone receptor-beta
TLR4	Toll-like receptor 4
TNF-α	Tumour necrosis factor-alpha
UNGA	United Nations General Assembly
VAT	Visceral adipose tissue
VCTE	Vibration controlled transient elastography
VLCD	Very low calorie diet
VLCKD	Very low calorie ketogenic diet
VLDL	Very low density lipoprotein
VLED	Very low energy diet
WC	Waist circumference
WHO	World Health Organisation

Chapter 1. Introduction and Literature Review

1.0 Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common cause of liver disease worldwide, affecting approximately a third of the global population (1). MASLD represents the hepatic manifestation of the obesity and type 2 diabetes (T2DM) epidemics and has increasingly become a major public health concern (2). In Australia, where 32% of adults are obese, and metabolic syndrome is present in approximately one-third, MASLD prevalence is growing and is an increasing cause of morbidity and mortality (3, 4).

MASLD comprises a spectrum of disease states encompassing simple hepatic steatosis, steatohepatitis, progressive fibrosis, cirrhosis and hepatocellular carcinoma (5). While many individuals with simple steatosis experience stable disease and a more indolent disease course, a proportion will progress to advanced fibrosis, which is the primary determinant of clinical outcomes (6). Advanced fibrosis substantially increases the risk of cirrhosis and associated complications such as hepatocellular carcinoma and liver-related mortality (6). Beyond the liver, MASLD is associated with the metabolic syndrome and metabolic comorbidities such as T2DM and cardiovascular disease (7). MASLD is independently associated with increased risk of cardiovascular disease, which represents the leading cause of death in individuals with MASLD (8).

There is currently no approved pharmacotherapy for the treatment of MASLD in Australia and lifestyle modification through diet and exercise remains the cornerstone of treatment (9). Weight loss has been demonstrated to have a clear dose-response relationship to histological improvement in MASLD with loss of 5% shown to reduce steatosis and 10% to be associated with fibrosis regression (10). International clinical practice guidelines recommend dietary energy restriction as first-line therapy with a Mediterranean dietary pattern favoured for its hepatic and metabolic benefits (9, 11). However, achieving and maintaining clinically significant weight loss (≥10% total body weight) through conventional dietary counselling alone is difficult (12). Physiological adaptations to weight loss including changes in appetite-regulating hormones, reduced basal metabolic rate, and increased hunger, push the body towards weight regain, making sustained weight loss difficult (13).

Medical weight management strategies developed for the treatment of obesity offer potential solutions for the treatment of MASLD, however have not been systematically adapted or rigorously evaluated in these cohorts. Very low energy diets (VLEDs), which provide approximately 450-800

calories per day induce rapid and substantial weight loss and have demonstrated efficacy in achieving T2DM remission (14). The development of long-acting Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) which have been shown to induce significant loss of weight, have also been shown to have hepatic benefits in MASLD in clinical trials (15, 16). Despite these advances, obesity and MASLD are mostly treated as separate entities, by different disciplines and knowledge gaps remain.

The VLED approach as used for obesity has not been widely used for the treatment of MASLD, or directly compared the Mediterranean diet (MD), which is more commonly favoured in this setting. Furthermore, optimal strategies for maintaining VLED-induced weight loss are undefined, and real-world implementation challenges are poorly characterised. Additionally, within the Australian context, there is limited data regarding MASLD prevalence. No population-based studies have applied contemporary diagnostic criteria with systematic fibrosis risk stratification, and so disease prevalence and identification of high-risk populations remain uncertain (17, 18). This thesis addresses these gaps through epidemiological, observational, and interventional research. The following literature review provides the basis for these works by examining MASLD pathophysiology, epidemiology, and clinical outcomes, including current treatment strategies. It also explores obesity physiology and management, as a basis for potential shared treatment strategies with MASLD, and establishes the rationale for the original research presented in Chapters 3-6.

1.0.1 MASLD nomenclature within this thesis

The terminology for steatotic liver disease has evolved considerably during the period in which this thesis was undertaken. In 2020, an international consensus proposed the term metabolic dysfunction-associated fatty liver disease (MAFLD) to replace the traditionally used non-alcoholic fatty liver disease (NAFLD), emphasising the metabolic basis of the condition, and this term is endorsed locally by the Gastroenterological Society of Australia (GESA) (19). Subsequently, a 2023 multi-society Delphi consensus introduced metabolic dysfunction-associated steatotic liver disease (MASLD) as the preferred terminology, which has since been adopted by major European and North American hepatology societies, and is the terminology required for publication into their associated journals. This thesis uses the updated MASLD nomenclature in the literature review and general discussion, acknowledging that historical studies published prior to these consensus statements use NAFLD and are cited using their original terminology where appropriate.

Individual chapters within this thesis use the nomenclature that was current at the time of their publication, or appropriate for regional differences in preferred terminology. Within this literature review (section 1.5.2), a narrative review published in 2019 uses NAFLD terminology. Chapter 2, an

epidemiological analysis of the AusDiab cohort published in 2022 uses MAFLD, and the remaining chapters use MASLD in accordance with current standards. Despite these terminology differences, the patient populations described across these chapters are largely overlapping and the findings should be interpreted as contributing to a singular body of evidence on steatotic liver disease.

1.1 Nomenclature and Diagnostic Criteria

As previously outlined, the nomenclature of steatotic liver disease has evolved significantly over the past five years, reflecting improved understanding of disease pathophysiology and concerns about patient understanding and stigmatisation. Historically known as non-alcoholic fatty liver disease (NAFLD), this was a diagnosis of exclusion, requiring the presence of hepatic steatosis (≥ 5% of hepatocytes) in the absence of significant alcohol consumption, viral hepatitis, medications or other secondary causes of liver disease (20). This negative framing, defining the disease by what it was not, rather than what it is, was confusing and was thought to hinder patient acceptance and awareness of a rapidly growing disease.

In 2020, an international expert panel proposed renaming NAFLD to metabolic dysfunction-associated fatty liver disease (MAFLD), which used positive diagnostic criteria (19). Under MAFLD criteria, the diagnosis could be made if there was hepatic steatosis plus at least one of three metabolic conditions: overweight/obesity (as per ethnicity-specific BMI cut-offs), T2DM, or evidence of metabolic dysregulation (defined by two or more metabolic risk factors) (19). A key difference in the new criteria was that it did not require the exclusion of other liver diseases, acknowledging that metabolic dysfunction can coexist with other liver diseases including alcohol-related liver disease (19). Traditional NAFLD criteria required alcohol intake to be below defined thresholds (<20g/day for women, <30g/day for men). The newer approach better reflects clinical practice where patients may have both metabolic risk factors and moderate alcohol intake and dual diagnoses enables each to be addressed individually with less stigma.

However, in 2023 following extensive multi-stakeholder consultation, a Delphi consensus process involving multiple international liver societies recommended a further nomenclature change to metabolic dysfunction-associated steatotic liver disease (MASLD) (5). This change addressed concerns that the term ‘fatty’ carried stigma, potentially discouraging patients from seeking care and perpetuating weight bias (21, 22). The new nomenclature introduced an overarching category of ‘steatotic liver disease’ (SLD), which encompasses MASLD, MASLD and increased alcohol intake (MetALD), alcohol-related liver disease, and specific steatotic liver disease aetiologies (5).

MASLD diagnostic criteria closely mirror those of MAFLD, requiring hepatic steatosis alongside cardiometabolic risk factors (5). These are outlined in Table 1.1. The evolution from NAFLD to MAFLD and MASLD reflects growing recognition of metabolic dysfunction being the core pathophysiological driver of this disease, while also addressing concerns about stigmatisation. As previously noted, MASLD has been adopted by the North American and European liver and gastroenterology societies and the use of this nomenclature is required for publication into their associated journals (23). Despite this MAFLD is still the preferred term endorsed by the Gastroenterological Society of Australia (GESA) and the Asian Pacific Association for the Study of the Liver (APASL) (17).

1. Table 1.1. Comparison of NAFLD, MAFLD and MASLD Diagnostic criteria

	NAFLD	MAFLD	MASLD
Hepatic steatosis	≥5% hepatocytes	≥5% hepatocytes	≥5% hepatocytes
Diagnostic approach	Diagnosis of exclusion	Positive diagnostic criteria	Positive diagnostic criteria with exclusions
Metabolic criteria	None	Either T2DM OR overweight/obesity OR 2 of the following: • ↑ waist circumference • Hypertension or antihypertensive treatment • ↑ triglyceride levels or lipid-lowering treatment • ↓ HDL-cholesterol levels or lipid-lowering treatment • Prediabetes • HOMA-IR score ≥2.5 • High sensitivity C-reactive protein >2mg/l	At least 1 of the following: • Overweight/obesity or ↑ waist circumference • Prediabetes or diabetes • Hypertension or antihypertensive treatment • ↑ triglyceride levels or lipid-lowering treatment • ↓ HDL-cholesterol levels or lipid-lowering treatment
Alcohol threshold	<20g/day women <30g/day men	No threshold	<20g/day women, <30g/day men for MASLD Higher allowed for MetALD
Concurrent liver disease	Must be excluded	Can coexist	Must be excluded (separate category for overlap)
Terminology rationale	Defined by absence of alcohol	Emphasizes metabolic basis	Non-stigmatising metabolic focus

Abbreviations: HOMA-IR: Homeostasis model of assessment of insulin resistance score, HDL: high-density lipoprotein

1.2 Pathophysiology of MASLD

The pathogenesis of MASLD represents a complex interplay of metabolic, inflammatory, genetic and environmental factors, which are incompletely understood.

1.2.1 Insulin resistance

Insulin resistance, which is the central process underlying the metabolic syndrome, plays a central role in MASLD pathophysiology and is the main driver of its metabolic complications (24). When insulin levels are low during fasting, skeletal muscle depends on free fatty acids (FFAs) from adipose tissue as the primary energy source. Low insulin promotes triglyceride lipolysis in adipose tissue, and the release of FFAs into the circulation. Low insulin, combined with increased glucagon secretion, encourages liver glycogen breakdown and gluconeogenesis to maintain hepatic glucose production and normal fasting plasma glucose levels. When insulin levels rise after eating, adipose tissue lipolysis is suppressed which reduces circulating plasma FFA levels. This promotes glucose uptake and utilisation by muscle and liver, resulting in glucose becoming the primary energy source (24).

Insulin resistance, which is characterised by persistently high insulin levels, causes a disruption to this cycle. Insulin-resistant adipose tissue promotes a chronic excess of FFAs, which can lead to lipotoxicity (25). The liver disposes of FFAs through mitochondrial β-oxidation, re-esterification into triglycerides, and storage in lipid droplets or release as very low density lipoprotein (VLDL) cholesterol into the systemic circulation (24). When these mechanisms are overwhelmed through impaired cellular glucose uptake in response to insulin, excess influx of energy as FFAs from increased lipolysis, and increased hepatic de novo lipogenesis, this leads to hepatic steatosis (26). Oversecretion of VLDL cholesterol in insulin-resistant individuals causes atherogenic dyslipidaemia and is often proportional to hepatic triglyceride excess (24). Conversely, even small increases in liver triglycerides are associated with the development of insulin resistance, atherogenic dyslipidaemia, lower plasma adiponectin, and an unfavourable metabolic profile (26, 27). Intrahepatic triglyceride accumulation may represent a protective response, limiting the formation of more toxic lipid intermediates, endoplasmic reticulum stress, and the formation of reactive oxygen species which are processes that intensify in the progression from simple steatosis to steatohepatitis (24, 28).

The relationship between insulin resistance and hepatic steatosis is bidirectional. MASLD, by definition, requires the presence of at least one metabolic risk factor, but the presence of multiple metabolic syndrome features correlates with liver disease severity and is associated with risk of

increased mortality (29, 30). Conversely, MASLD itself contributes to systemic insulin resistance through the release of hepatokines such as fetuin-A, which inhibits insulin receptor signalling and selenoprotein P, which impairs insulin-mediated glucose uptake in muscle (31, 32). This hepatocyte-driven worsening of peripheral insulin resistance further promotes adipose tissue lipolysis and FFA flux to the liver, further contributing to metabolic dysfunction.

1.2.2 Adipose tissue dysfunction

Adipose tissue plays an important role, as its capacity to safely store excess energy determines whether lipid overflows to ectopic sites such as the liver. Adipose tissue is the main lipid storage location in the body and has a key role in buffering the daily influx of dietary fat entering the circulation. In obesity, subcutaneous adipose tissue is unable to expand to store the excess incoming energy, which can lead to ectopic fat deposition in other organs such as the liver, but also into skeletal muscle and in visceral adipose tissues (33). Prolonged excess energy intake can also lead to adipocyte hypertrophy, which impairs lipid storage capacity and can lead to tissue hypoperfusion, resulting in hypoxia, dysregulated adipokine secretion and increased macrophage infiltration (34). This results in a decreased production of insulin-sensitising adipokines (e.g. adiponectin and omentin) and an increase in pro-inflammatory mediators including tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and leptin (35).

It is hypothesised that the body's capacity to store excess energy in subcutaneous adipose tissue varies between individuals and that once this limit is exceeded, lipids are directed to ectopic sites such as the liver (36). This individual variability may explain the occurrence of 'lean MASLD', in which individuals with MASLD have a normal BMI using ethnicity-specific thresholds (37). Lipolysis from insulin-resistant adipose tissue releases FFAs into the circulation, with 59% of hepatic triglycerides coming from the portal circulation, 15% from dietary fats, and the remainder from de novo lipogenesis (38).

1.2.3 Hepatic lipotoxicity

The simple accumulation of triglycerides in the liver is relatively benign and triglycerides themselves serve a protective function by sequestering potentially harmful FFAs (39). It is the development of toxic lipid intermediates that causes hepatocellular injury and inflammation (40). Lipid species such as saturated FFAs (e.g. palmitate), trigger mitochondrial dysfunction, endoplasmic reticulum stress, and activation of inflammatory signalling cascades (41, 42). This mitochondrial dysfunction also contributes to lipotoxicity. In MASLD, mitochondria undergo adaptive responses including increased β-oxidation and proliferation to accommodate the elevated influx of FFAs (43). However, sustained

lipid overload eventually overwhelms mitochondrial capacity, leading to incomplete fatty acid oxidation and excessive reactive oxygen species (ROS) generation (25). Mitochondrial ROS also further cause dysfunction by causing damage to mitochondrial DNA, proteins and membranes (25). Oxidative stress from excess ROS production plays a key role in the inflammation associated with progressive phenotypes of MASLD. ROS mediate lipid peroxidation which triggers activation of stellate cells and promotes fibrogenesis (44).

1.2.4 The Gut-Liver Axis

There is increasing evidence that the gut-liver axis plays an important role in MASLD pathogenesis. Increased intestinal permeability, often described as 'leaky gut', allows translocation of bacterial products including lipopolysaccharides (LPS) via the portal circulation (45). Circulating LPS levels are elevated in MASLD and correlate with disease severity (46). In the liver LPS activates toll-like receptor-4 (TLR4) on hepatocytes and Kupffer cells, triggering inflammatory pathways that contribute to steatohepatitis (46).

There are several factors that contribute to impaired intestinal barrier function in MASLD. Dysbiosis reduces short-chain fatty acid (SCFA) production, which serves as the primary energy source for colonocytes and maintains tight junction integrity (47). The gut microbiome composition in MASLD is altered and characterised by reduced bacterial diversity and increased Proteobacteria and Enterobacteriaceae (48). Additionally, some bacterial taxa produce ethanol and acetaldehyde which may directly contribute to hepatic injury (49).

Bile acids within the gut help to regulate lipid and glucose metabolism and their regulatory activities are mediated by farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5) (50). In MASLD bile acid production is elevated and altered gut microbiota causes a shift in the bile acid pool composition with increased FXR-antagonistic species, suppressing FXR mediated signalling and potentially contributing to metabolic dysfunction (51).

1.2.5 Genetic Factors

Whilst environmental and lifestyle factors influence MASLD development, genetic variation contributes to disease susceptibility and progression. The strongest genetic association involves PNPLA3 (patatin-like phospholipase domain-containing 3), with the I148M variant (rs738409 C>G) being significantly associated with increased risk for hepatic steatosis, MASH, fibrosis, cirrhosis and hepatocellular carcinoma (52, 53). The I148M variant impairs triglyceride hydrolase activity and evades ubiquitin-mediated degradation which leads to hepatic fat retention (54). The highest

prevalence of the PNPLA3 I148M variant has been noted in individuals of Hispanic ancestry (approx. 49%), and is also seen in approximately 23% of those with European ancestry, but is lowest in those with African ancestry (approx. 17%) (52, 55).

Other genetic variants associated with MASLD include TM6SF2 (transmembrane 6 superfamily member 2) E167K which causes accelerated protein degradation and impaired VLDL secretion, and GCKR (glucokinase regulator) P446L which enhances hepatic glucose uptake and de novo lipogenesis (56, 57). Genome-wide association studies continue to identify novel susceptibility loci, but these have not been associated with as significant an effect as the PNPLA3 I148M variant (58).

1.3 Natural History of MASLD

1.3.1 Simple steatosis to Steatohepatitis

Simple steatosis is hepatic fat accumulation (≥5% hepatocytes) without significant associated hepatocyte injury or inflammation (20). Longitudinal studies suggest that the majority of patients with simple steatosis will follow a relatively benign course (59). Despite its relatively indolent nature, simple steatosis is not completely benign, with simple steatosis being associated with increased all-cause mortality, with cardiovascular disease being the leading cause of death in MASLD (60, 61). A large Swedish population-based study demonstrated that patients with simple steatosis had a 71% higher mortality risk compared to matched controls, highlighting that extrahepatic complications can arise even in those with early-stage disease (62).

Metabolic dysfunction-associated steatohepatitis (MASH, formerly NASH) represents a more aggressive phenotype and histologically is characterised by hepatocyte ballooning, lobular inflammation and variable degrees of fibrosis in addition to steatosis (63). MASH is progressive and is associated with fibrosis development and worse liver-associated outcomes (59). Factors associated with progression from simple steatosis to MASH include T2DM (which approximately doubles risk), metabolic syndrome severity, genetic variants, and sustained obesity (24, 26, 64). Each additional metabolic syndrome component approximately doubles the odds of at-risk MASH (MASLD activity score ≥4 and fibrosis stage ≥2 on liver biopsy), with T2DM, central adiposity and low HDL identified as the most significant independent predictors (65). The PNPLA3 I148M is the strongest genetic determinant with homozygous carriers having approximately 3.5-fold increased risk of advanced fibrosis and of note, obesity has been found to amplify this genetic predisposition (52, 66).

Not all patients with simple steatosis will develop MASH, and paired biopsy studies show the dynamic nature of MASLD histology. A meta-analysis of paired biopsy studies found that approximately 31% of patients with simple steatosis progressed to MASH after a median of 4.7 years (incidence rate 7.4/100 person-years), whilst 29% of MASH patients demonstrated spontaneous resolution after a median of 3.5 years, demonstrating that the disease trajectory remains modifiable (67).

1.3.2 Fibrosis development

Hepatic fibrosis is characterised by excessive deposition of extracellular matrix proteins, predominantly collagens, and occurs when production in response to chronic liver injury exceeds degradation (68). In MASLD, fibrosis develops through activation of hepatic stellate cells (HSCs), which transform from quiescent, vitamin A storing cells to proliferative, collagen-producing myofibroblasts, in response to inflammatory and lipotoxic signals (68). This response is initially a wound-healing function, but when it becomes chronic, leads to progressive architectural distortion and then to cirrhosis (68). In MASLD, fibrosis staging is performed histologically using the NASH Clinical Research Network (CRN) system which classifies fibrosis from F0 (no fibrosis) through to F4 (cirrhosis) (63). Clinically, stages F3 and F4 are often grouped as ‘advanced fibrosis’ and carry substantially worse prognosis than earlier stages (69).

The rate of fibrosis progression in MASLD is variable. While simple steatosis progresses slowly, MASH progresses at rates comparable to hepatitis C (59). Pooled data from paired biopsy studies estimate that patients with simple steatosis progress approximately one fibrosis stage per 14.3 years, and those with MASH progress one stage per 7.1 years (59). Similar to MASH, factors associated with fibrosis progression include T2DM, hypertension and PNPLA3 I148M polymorphism (59). Higher stages of fibrosis also progress more rapidly (59). Fibrosis staging is important as it is the strongest histological predictor of liver-related mortality and overall survival in MASLD (70, 71).

1.3.3 Progression to cirrhosis

Cirrhosis represents the end-stage of fibrosis and is clinically significant as once it develops, prognosis significantly worsens (72). Population-based studies suggest that approximately 3-5% of individuals with MASLD will develop cirrhosis over 15-20 years, and this proportion is higher among those with baseline MASH or advanced fibrosis (73). The annual incidence of cirrhosis development in patients with MASH is estimated to be 1.4% (74). Modelling studies project that MASLD-related cirrhosis prevalence will increase substantially in the near future, reflecting both the continuing obesity epidemic, but also the aging population in whom metabolic comorbidities is high (73).

Cirrhosis can remain compensated with relatively stable liver function, or progress to decompensation which can manifest as ascites, variceal haemorrhage, encephalopathy or jaundice (72). Compensated MASLD cirrhosis has an annual decompensation rate of approximately 8%, and each decompensation event significantly worsens survival (6, 75). In a Swedish nationwide cohort, five-year survival in compensated cirrhosis was approximately 44%, falling to 25% after first decompensation (76). Similarly US Medicare data demonstrated a cumulative mortality of 31% in compensated MASLD cirrhosis, and 51% in decompensated disease over 8 years of follow-up (77). Pooled meta-analysis data estimated all-cause mortality in MASLD cirrhosis to be 7.9 per 100 person-years (78). Interestingly, the pattern of mortality in MASLD cirrhosis differs to other aetiologies, with patients more commonly dying from cardiovascular disease and extrahepatic malignancies than liver-related causes, in contrast to alcohol-related cirrhosis which carries the highest liver-related mortality (79, 80). Following decompensation however, liver-related mortality becomes the leading cause of death (80).

1.3.4 Fibrosis regression

An important aspect of the natural history of MASLD in the potential for disease regression. This reversibility provides the basis for current treatment guidelines recommending weight loss and lifestyle intervention (9). Histologic resolution of steatohepatitis and regression of fibrosis has been demonstrated post weight loss in lifestyle intervention and bariatric surgery studies. Bariatric surgery achieves MASH resolution in approximately 83% of patients at 1-year follow up, with sustained benefit and progressive fibrosis improvement through 5 years post surgery (81). This study found that 70% demonstrated fibrosis regression and almost half of those with F3 stage fibrosis regressed to lower stages (81). Intensive lifestyle interventions have demonstrated similar results with achieving ≥10% total body weight loss (TBWL) associated with MASH resolution in approximately 90% of patients, and 45% demonstrating fibrosis regression at 1-year (10). Based on these findings, the threshold for fibrosis regression appears to be ≥10% TBWL, with greater weight loss associated with higher probability of fibrosis stage reduction (10). Even cirrhosis may regress to non-cirrhotic fibrosis in some patients following bariatric surgery, though complete normalisation of liver architecture at this stage is uncommon (81).

These observations reinforce our understanding that MASLD is not an inevitably progressive disease, and that intervention at any stage can alter the natural history, with weight loss a disease-modifying therapy capable of reversing established disease. Despite this, the durability of the hepatic benefits associated with weight loss are contingent on the maintenance of these changes. Pooled data from the

PIVENS and FLINT trials demonstrated that weight change was independently associated with histological outcomes, with each kilogram of weight loss increasing the odds of MASH resolution by 7% and fibrosis improvement by 5% (82). Conversely, weight gain was associated with worsening of both biochemical and histological disease activity (82). Longitudinal data further confirms that even modest weight regain (≥1.5kg) following MASLD remission significantly increases the risk of recurrence (83).

1.3.5 Hepatocellular carcinoma

MASLD is an increasingly important cause of hepatocellular carcinoma (HCC). The epidemiology of MASLD-related HCC in western populations has been reviewed in detail elsewhere in this chapter (Section 1.5.2)(84). In brief, while the overall annual incidence of HCC in MASLD (approximately 1-2%) is lower than other liver diseases such as viral hepatitis, MASLD now represents the fastest-growing indication for HCC-related liver transplantation and accounts for 10-35% of HCC cases in western cohorts due to the significant burden of disease (85-87). Notably MASLD-related HCC can occur even in the absence of cirrhosis (20-50% of cases), however there is currently no evidence to support HCC surveillance outside of those with cirrhosis (9).

1.4 Epidemiology of MASLD

1.4.1 Global MASLD prevalence

MASLD has emerged as the most prevalent chronic liver disease globally, affecting an estimated 38% of the worlds adult population (2). This represents a substantial increase from earlier estimates of approximately 25% prevalence in 2016, reflecting both a genuine increase in disease burden driven by the obesity epidemic, but also improved diagnostic methods and patient identification (80). The global prevalence of MASLD has been continuing on a clear upward trajectory with prevalence increasing from around 25% in analyses conducted before 2005, to 38% in studies from 2016 onwards (2, 88). This acceleration in prevalence parallels the global increases in obesity, which has nearly tripled since 1980, and T2DM, which has quadrupled over the same period (89).

MASH affects approximately 5-7% of the global population, representing roughly 15-20% of all individuals with MASLD (1). The proportion of MASLD patients with advanced fibrosis (F3-F4) is estimated to be 5-7%, while cirrhosis is present in around 2-3% (73, 78). Due to the significant burden of disease, these figures translate into substantial absolute numbers, with current estimates suggesting that over 2 billion people have MASLD worldwide (1).

There is definite geographic variation in MASLD prevalence, largely driven by differences in obesity and metabolic syndrome rates, dietary patterns, and genetic background. Latin America has the highest prevalence at 44%, followed by the Middle East and North Africa at 37%, south Asia 34%, South-East Asia 33%, North America 31%, East Asia 30%, Asia-Pacific 28% and Western Europe 25% (2). However there is also variation between countries within each region. In Asia, Japan has a low prevalence of 18% compared to 40% in urban Southeast Asian populations (90). Similar variation is also seen within Europe, with some meta-analyses suggesting Mediterranean countries have lower rates compared to non-Mediterranean Europe (24% vs 29%), possibly reflecting dietary influences, although this difference did not reach statistical significance (91).

Modelling studies predict that prevalence will continue to rise. In the US, MASLD cases are expected to increase from 83 million in 2015 to 101 million by 2030, with MASH cases projected to increase from 17 million to 27 million (73). Without the introduction of effective interventions, the hepatic disease burden attributable to MASLD is projected to more than double over the coming decades (21, 73).

1.4.2 MASLD prevalence in Australia

Australia has a high prevalence of obesity and metabolic risk factors, however epidemiological data on MASLD remains limited. Furthermore, no Australian population-based studies have applied contemporary MAFLD or MASLD diagnostic criteria, alongside a risk assessment for advanced fibrosis. Previous estimates of MASLD have relied primarily on surrogate markers particularly elevated alanine aminotransferase (ALT), with Australian population studies finding ALT elevation in 9-11% of adults (92, 93). This likely underestimates true disease prevalence as the majority of individuals with MASLD have normal ALT levels (94, 95).

The Crossroads study from regional Victoria demonstrated high fatty liver disease prevalence, reporting age and sex standardised NAFLD prevalence of 36% using the fatty liver index diagnostic criteria (96). Application of MAFLD criteria to this cohort increased prevalence to 47%, as a result of capturing those with dual liver pathologies (97). While informative, these data are derived from rural and regional communities in the Goulburn Valley and may not be generalisable to the broader Australian population. The Raine study, a Western Australian birth cohort, identified ultrasound-defined MASLD in 15% of adolescents at age 17, suggesting significant disease burden even in younger age groups (98).

Modelling studies using Australian obesity prevalence data have estimated MASLD affects approximately 5.5 million Australian adults (22-25% prevalence), consistent with global estimates for similarly developed nations (4). However these estimates remain imprecise given the limitations of indirect assessment methods and the absence of population-based studies directly measuring hepatic steatosis or applying contemporary diagnostic criteria.

1.4.3 Obesity and Diabetes Prevalence in Australia

Given the established links between MASLD and obesity, the prevalence of MASLD in Australia is likely to mirror that of obesity, T2DM and the metabolic syndrome.

The Australian Diabetes, Obesity and Lifestyle (AusDiab) study was a nationally representative population-based cohort study initiated in 1999-2000 and designed to provide comprehensive assessment of the epidemiology of metabolic disease in Australian (99). This study provides the basis for the epidemiological analysis presented in this thesis, and as such Australian metabolic disease prevalence at the time of the 2012 AusDiab survey, when the hepatic assessment that is presented in Chapter 3 was undertaken is outlined below.

At the time of the 2012 AusDiab survey T2DM prevalence in Australia was 4-5%, with 16% classified as having prediabetes (100, 101). MASLD is thought to affect up to 70% of those with T2DM and current estimates indicate that T2DM prevalence has risen to 5.3%, suggesting MASLD burden has also risen proportionally (102). The metabolic syndrome affected 23-30% of Australian adults, exceeding 50% in those over 65 years of age (101, 103). Given that insulin resistance drives both the metabolic syndrome and hepatic steatosis, these data indicate a likely similar MASLD burden in one-quarter to one-third of Australian adults, consistent with global estimates (2).

1.4.4 Social determinants, inequity and public policy

Similar to obesity and diabetes, MASLD prevalence is impacted by social determinants of health, with socioeconomic disadvantage, lower educational attainment, ethnicity and geographical location influencing disease prevalence, progression and access to care (2, 104). Many of these biopsychosocial factors influence dietary choices, which are an important risk factor for MASLD. Food insecurity is defined as the lack of consistent access to enough safe and nutritious food for an active and healthy lifestyle, and has been identified as an important upstream determinate of health and an emerging risk factors for MASLD. A large analysis of low-income adults in the US National Health and Nutrition Examination Survey (NHANES) cohort, food insecurity was associated with MASLD (adjusted OR 1.38) and advanced fibrosis (adjusted OR 2.20), adjusted for demographic,

socioeconomic and behavioural health characteristics, and among those with MASLD and advanced fibrosis, food insecurity was also associated with higher all-cause mortality (105, 106).

Food insecurity is also a growing problem in Australia, with data from the Australia bureau of statistics (ABS) estimating that 13.2% of Australian households were food insecure in 2023 (107). The rates were highest in single-parent households with dependent children and Aboriginal and Torres Strait islander communities, and affected a greater proportion of those in the lowest tax bracket compared to the highest tax bracket (107). Given the links between food insecurity and MASLD, the future burden of MASLD is likely to continue to grow in these particularly disadvantaged communities.

Inequality also extends to the diagnosis and treatment of MASLD; in particular, access to non-invasive fibrosis assessment, specialist hepatology services and emerging pharmacotherapies is uneven, with those in regional and rural areas, socioeconomically disadvantaged groups and First Nations communities particularly affected (104). As the body of evidence grows regarding the use of incretin-based therapies in MASH, the high cost and absence of public subsidy risks widening these disparities as outlined in the World Health Organisation (WHO) global guideline on the use of GLP-1 medicines in the treatment of obesity and obesity-related conditions, with benefits impacting more advantaged patients first (108).

Despite its prevalence and shared risk factors with other non-communicable diseases (NCD), MASLD has largely been absent from global NCD health policy strategies (109). In the face of the considerable global burden of disease and subsequent increase in associated complications such as cirrhosis and MASLD-related HCC, this has begun to change. For the first time, the United Nations General Assembly (UNGA) meeting in December 2025 formally recognised liver disease and MASLD within the global NCD agenda (110). Additionally, the 2026 World Health Assembly (WHA) adopted the first global resolution dedicated to MASLD, ‘Steatotic liver disease: a missing piece in the global noncommunicable disease response’ (111). The resolution calls on member nations to integrate steatotic liver disease into national NCD strategies, strengthen primary care approaches, improve surveillance and awareness, and pursue action on risk factors such as unhealthy diets, physical inactivity and harmful alcohol use (111). These recommendations provide a mandate for Australia, which currently lacks a dedicated national MASLD strategy.

1.5 Clinical outcomes and disease burden

1.5.1 Liver-related outcomes

Fibrosis stage as predictor of outcomes

Fibrosis stage is the most important predictor of liver-related and overall mortality (70, 71). A meta-analysis incorporating 17,301 patients from 14 studies found that hazard ratios for all-cause mortality were 1.46 for F2, 1.96 for F3 and 3.66 for F4 when compared to F0 (no fibrosis) (112). Liver-related mortality was even higher with hazard ratios of 4.07 for F2, 7.59 for F3, and 15.1 for F4. Ten year all-cause mortality rose from 7.7% in patients with F0-F2 fibrosis to 41.5% in those with cirrhosis, highlighting the importance of identifying patients with advanced disease. These findings have also been prospectively evaluated in a large longitudinal study from the NASH Clinical Research Network (CRN), which followed 1,773 participants with biopsy-proven MASLD for a median of 4 years and found that all-cause mortality increased from 0.32 deaths per 100-person years in F0-F2 to 0.89 in F3 and 1.76 in F4 (6). They also found that any hepatic decompensation event was independently associated with subsequent mortality (adjusted hazard ratio 6.8), identifying decompensation as a critical event in disease trajectory (6). As a result of these findings, fibrosis assessment is clinically important in the diagnostic work up for MASLD in order to prognosticate risk for further health-related complications (9).

Complications of Cirrhosis

As previously mentioned, MASLD-related cirrhosis carries substantial morbidity and mortality, particularly after the development of liver decompensation through complications such as ascites, variceal haemorrhage and hepatic encephalopathy (6). Ascites is the most common complication from cirrhosis and develops in 5-10% of patients with compensated cirrhosis per year, with up to 60% developing ascites within 10 years of diagnosis (72). Variceal haemorrhage occurs at a rate of 10-15% per year in patients with portal hypertension and varices, and carries a six-week mortality of 15-20% per bleeding episode (113). Hepatic encephalopathy affects 30-45% of patients with cirrhosis and independently predicts mortality (114, 115). These complications are associated with significant morbidity and mortality, highlighting the importance of risk stratification in MASLD patients with the aim to prevent the development of cirrhosis and further complications, in MASLD considering its potentially reversible disease course.

There are some factors which differentiate MASLD-related cirrhosis from other aetiologies for cirrhosis. Portal hypertension-related decompensation may occur at lower hepatic venous pressure gradient levels than in other aetiologies (116). This may relate to differences in hepatic architecture, such as the heterogenous distribution of steatosis and fibrosis through the liver. While the risk of hepatic decompensation appears comparable between MASLD and alcohol-related cirrhosis, mortality following decompensation is higher in MASLD, possibly due to older age and increased burden of additional metabolic comorbidities in the MASLD cohort (117). Sarcopaenia and frailty

are particularly prevalent in MASLD cirrhosis, affecting 40-70% of patients with end-stage liver disease (118). The coexistence of obesity and muscle wasting, termed sarcopaenic obesity is 6-times more common in MASLD-related cirrhosis than other aetiologies and is associated with worse transplant outcomes and complicates nutritional management (119). The management of MASLD-related cirrhosis requires ongoing management of metabolic comorbidities alongside standard cirrhosis care. Current guidelines recommend sarcopenia prevention, surveillance for portal hypertension and hepatocellular carcinoma (9).

Liver transplantation

MASLD is an increasingly important indication for liver transplantation in Australia, with transplant registry data showing that the proportion of patients with MASH activated for transplantation increased from 2% in 2003 to 11% in 2017, making MASH the third leading indication for liver transplantation (120). Modelling studies estimate that approximately 15% of liver transplants in Australia are currently attributable to MASLD, with this projected to increase over the coming decades (4). Post-transplant outcomes in Australia and New Zealand are excellent with 5-year survival approximately 84% (121). However recurrent MASLD following transplant is common, with meta-analysis data indicating incidence rates of approximately 60% at 1-year and over 80% by 5-years (122). MASH was also found to recur in 40-57% of recipients, however progression to graft cirrhosis is uncommon (122).

1.5.2 Hepatocellular carcinoma

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Review

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Epidemiology of non-alcoholic fatty liver disease related hepatocellular carcinoma: a western perspective

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is now the most common cause of liver disease worldwide, and represents an increasingly important cause of hepatocellular carcinoma (HCC). As the prevalence of NAFLD has increased, the burden of NAFLD-related HCC has been rising in parallel. This is particularly evident in Western countries, where NAFLD is estimated to account for 10%-59% of all HCC. NAFLD-related HCC can occur in the presence or absence of cirrhosis, and, while those with cirrhosis remain at the greatest risk, factors such as steatohepatitis, age, genetic polymorphisms, type 2 diabetes mellitus and obesity also appear have an impact on the risk of developing HCC in NAFLD. In this review, we present the epidemiology of NAFLD-related HCC from a Western perspective, highlighting gaps in current knowledge and future directions for research in this field.

Keywords: Non-alcoholic fatty liver disease, steatohepatitis, cirrhosis, hepatocellular carcinoma, epidemiology

Introduction

Over the past two decades, non-alcoholic fatty liver disease (NAFLD) has rapidly become the most common cause of liver disease worldwide, affecting approximately a quarter of the global population[1]. It is considered to be a hepatic end organ effect of the metabolic syndrome and its rise to prominence has

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coincided with the onset of the obesity and type 2 diabetes mellitus (T2DM) epidemics[2]. Previously, due to the strong association between NAFLD and the metabolic syndrome, NAFLD was considered a disease of affluent nations; however, with the epidemiological transition and increasing urbanisation in low and middle income countries, prevalence of the metabolic syndrome and NAFLD is now increasing rapidly across the globe[3-5]. This high prevalence and subsequent burden of progressive liver disease has resulted in NAFLD becoming an increasingly important cause of hepatocellular carcinoma (HCC)[6-8]. This review outlines the epidemiology of NAFLD-related HCC, with a focus on Western countries.

Methods

We conducted a search of the Medline, PubMed and Cochrane databases to identify English language, original research articles conducted in adults over the age of 18, and published between 1 January 1990 and 1 September 2019 using the Medical Subject Headings "Nonalcoholic Fatty Liver Disease" and "Hepatocellular carcinoma". Duplicate results were removed using Endnote X9, before screening by title and abstract was performed to find research relating to HCC in patients with NAFLD (single reviewer - AF). Original research articles were eligible for selection, including meta-analyses, controlled trials, cohort studies and case-series, which clearly described their study population including their diagnostic criteria for NAFLD, and described the incidence or prevalence of NAFLD-related HCC.

NAFLD, nonalcoholic steatohepatitis and the natural history of disease

NAFLD is a broad term used to describe a spectrum of liver diseases characterised by excessive hepatic fat accumulation with associated insulin resistance, in the absence of a secondary cause or significant alcohol consumption[9,10]. More precisely, NAFLD is defined as the presence of steatosis in > 5% of hepatocytes histologically or by proton density fat fraction on magnetic resonance imaging (MRI-PDFF), and it can be subclassified into nonalcoholic fatty liver (NAFL) or nonalcoholic steatohepatitis (NASH)[9,10]. While NAFL refers to simple steatosis, NASH is characterised by hepatic lobular inflammation and is associated with an increased risk of progression to fibrosis, cirrhosis and hepatic decompensation[9]. NASH can only reliably be differentiated from NAFL histologically, and the prevalence of NASH in liver biopsies from NAFLD patients is estimated to be 59.10% (95%CI: 47.55%-69.73%) from pooled NASH prevalence data[1]. This estimate is higher than previous estimates, suggesting NASH occurred in approximately one third of NAFLD patients; however, retrospective biopsy studies are inherently affected by selection bias of patients in whom there is high clinical suspicion for steatohepatitis[11,12].

Fibrosis is an important prognostic factor in NAFLD. Fibrosis stage is independently associated with mortality, liver transplantation and liver-related events, with fibrosis occurring at twice the rate in patients with NASH compared with NAFL[13,14]. Furthermore, liver-related mortality increases significantly with increasing fibrosis stage[15]. In a meta-analysis of 411 patients from 11 cohort studies with biopsy proven NAFLD (2145.5 person-years of follow-up), the annual fibrosis progression rate in patients with NAFL and stage 0 fibrosis at baseline was 0.07 stages per year (95%CI: 0.02-0.11 stages/year), and 0.14 stages per year in NASH (95%CI: 0.07-0.21 stages per year)[16]. This is the equivalent of one stage of progression over 14.3 years (95%CI: 9.1-50 years) in NAFL *vs.* 7.1 years (95%CI: 4.8-14.3 years) for NASH. In patients with NASH, approximately 20% of those with advanced

(F3) fibrosis will progress to cirrhosis, and 20% of compensated NASH cirrhotics will develop hepatic decompensation over a two-year period[17,18].

Current global trends in HCC

Liver cancer, of which HCC is the main subtype, has the sixth highest cancer incidence, and is the fourth leading cause of cancer-related death worldwide[19]. There are significant geographic variations in the incidence of HCC, reflecting the differences in aetiology of liver diseases in each region[20]. In Asian countries where the highest incidence rates of HCC are reported (20 per 100,000 in men and 6.9 in women), viral hepatitis, particularly hepatitis B, accounts for the majority of cases with the population attributable fraction due to hepatitis B approximately 51%-57%[21,22]. Comparatively, in high income regions of the world such as Western Europe, North America and Australasia, where the predominant aetiologies are hepatitis C and alcohol, HCC incidence rates are much lower[8]. In Europe, the age-standardised incidence rate is estimated at 6.8 per 100,000 population in men and 2.2 in women, while, in the US, the overall incidence is 11.6 per 100,000 population in men and 4.3 per 100,000 in women[21,23]. This is on the background of an almost three-fold increase in HCC cases in the United States over the past two decades[24,25]. Australia and New Zealand have also demonstrated a significant increase in HCC with a 7.5-fold increase between 1982 and 2014[26]. In 2015, the Australian incidence of HCC was 7.6 per 100,000 persons, again with higher rates in men (12 per 100,000) than women (3.9 per 100,000), which is expected to have increased to 8.6 cases per 100,000 persons in 2019[27]. This increase is likely due in part to an aging population, with a high prevalence of hepatitis C; however, the rise of the obesity epidemic, and an associated increase in the prevalence of NAFLD-related HCC, may also be contributing[28,29]. Furthermore, within these Western countries, particularly in the US, NAFLD, fibrosis and HCC are more prevalent in some ethnic sub-populations. This has been seen in Hispanic, Pacific Islander and subcontinental Indian groups, whereas other sub-populations, such as African Americans, appear to have lower rates despite similar risk factors[30-32]. Whether this difference is due to disparities in socioeconomic status or diet remains to be seen; however, there may also be inherent biological differences with a higher prevalence of the PNPLA3 genetic polymorphism noted in the Hispanic population[33].

Prevalence of NAFLD-related HCC

NAFLD has become the most rapidly increasing cause of HCC in Western countries, with the proportion of HCC attributable to NAFLD increasing significantly over the past two decades[34,35]. This shift has mirrored the rise of obesity and the metabolic syndrome, while also coinciding with the development of effective treatments for viral hepatitis and greater coverage of hepatitis B vaccination programmes[6]. This increase in NAFLD-related HCC is most pronounced in Western nations: in the BRIDGE study, a multiregional, large-scale longitudinal cohort study of consecutive newly diagnosed HCC cases, NAFLD accounted for 10%-12% of HCC cases in North America and Europe, but only 1%-6% in Asian countries[7]. In North America, NAFLD is a common cause of HCC. In a large early cohort study of HCC patients identified from a healthcare claims database in the USA, NAFLD was found to be the most common aetiology, accounting for 59% of their 4406 cases[36]. However, subsequent similar US-based longitudinal cohort studies have reported a much lower proportion of NAFLD-related HCC. The Surveillance, Epidemiology, and End Results (SEER) cancer registry database found that NAFLD was the predominant aetiology in 14.1% of their 4929 HCC cases; this was even lower in the Veteran Affairs (VA) Hospitals cohort, where 8% of 1500 HCCs were attributable to NAFLD[8,37]. This may reflect differences in the diversity of the groups studied here, with the VA group enriched with older males with hepatitis C. NAFLD is now the second leading cause of liver transplantation for HCC in the United States after Hepatitis C virus (HCV), and is the most rapidly growing indication for HCC-related liver transplantation, having increased from 8.3% in 2002 to 13.5% in 2012[38]. Moreover, the number of people with NAFLD-related HCC on liver transplant waiting lists in the United States has also risen from 2.1% in 2002 to 17.9% in 2017[38,39].

European and Australasian studies have also described an increase in the prevalence of NAFLD-related HCC. In the UK, between 2000 and 2010 there was a 10-fold increase in NAFLD-related HCC, with histologically or radiologically proven NAFLD accounting for 34.8% of all HCC cases in 2010 in one cohort of 633 patients[34]. Data from the European Transplant Registry also show an increase in transplantation for NAFLD-related HCC from 0.2% in 2007 to 1.2% in 2017[40]. In Australia and New Zealand, NAFLD is the third leading cause of HCC in those who underwent transplantation, and an Australian cohort of 272 HCC patients found NAFLD to be the underlying aetiology with 14% of cases[41,42]. A summary of these cohorts is outlined in Table 1.

Table 1. Overview of trials

Author	Country	Design/Method	Number of participants	Definition of NAFLD	Results	Proportion of NAFLD with cirrhosis (%)	Limitations/Bias
Marrero *et al*.[69]	USA	Single centre retrospective cohort of consecutive HCC cases	105	Histology	Cryptogenic cirrhosis 2nd leading cause of HCC (*n* = 30, 29%) and 6 of those cases had histological features of NASH, while 8 had clinical features of NAFLD	N/A	Retrospective study design
Sanyal *et al*.[47]	USA	Single centre prospective cohort of patients with NASH cirrhosis	152	Histologic features of steatohepatitis and cirrhosis	NASH had a lower risk of developing hepatocellular carcinoma compared to those with HCV cirrhosis (10/149 patients *vs*. 25/147 patients at risk; *P* < 0.01)	100%	Small cohort, with low numbers of HCC and comparator group was those with treatment naïve HCV or treatment non-responders in pre DAA therapy era
Paradis *et al*.[70]	France	Retrospective review of HCC liver resection specimens between 1995 and 2007	128	N/A	65.5% of cases with Met Sy associated HCC had F0-2 fibrosis	N/A	Surgical resection bias could account for high proportion of patients without significant fibrosis
Yatsuji *et al*.[46]	Japan	Prospective cohort study of patients with NASH and HCV cirrhosis	68	Histologic features of steatohepatitis and cirrhosis	5-year HCC rate was 11.3% for NASH and 30.5% for HCV	100%	Small cohort, with low numbers of HCC and comparator group was those with treatment naïve HCV or treatment non-responders in pre DAA therapy era
Ascha *et al*.[44]	USA	Prospective analysis of patients with NASH and HCV cirrhosis between 2003 and 2007	195	Histologic features of NASH or cryptogenic cirrhosis in the presence of metabolic syndrome and without a history of significant alcohol intake	NASH Cirrhotics had a yearly cumulative incidence of HCC 2.6% c/w 4% in HCV cirrhosis NASH patients were older at diagnosis of cirrhosis (56.6 years *vs*. 48.2 years)	100%	Small cohort, with low numbers of HCC and comparator group was those with treatment naïve HCV or treatment non-responders in pre DAA therapy era
Sanyal *et al*.[36]	USA	Retrospective review of HCC cases identified from a large healthcare claims database between 2002 and 2008	4406	Documentation of diagnosis based on ICD-9 coding	NAFLD (59%) most common aetiology of HCC followed by diabetes (36%) Of 729,018 patients with NAFLD/NASH codes, 19,217 (2.7%) had HCC at one time (incident or prevalent case)	46%	Use of coding criteria likely underestimates NAFLD cases when estimating incidence or prevalence
Bhala *et al*.[45]	Australia, USA, UK, Italy	Prospective multicentre natural history study of NAFLD patients with advanced fibrosis or Child's Pugh A cirrhosis	247	Histologic features of advanced fibrotic NAFLD as well as elevated aminotransferases for at least 6 months	HCC occurred more commonly in patients with HCV cirrhosis than in NAFLD [*n* = 18 (6.8%) *vs.* 6 (2.4%); P = 0.03]	52.2%	Small cohort, with low numbers of HCC and comparator group was those with treatment naïve HCV or treatment non-responders in pre DAA therapy era
Ertle *et al*.[50]	Germany	Single centre review of new HCC cases within a 12-month period between 2007 and 2008	162	NAFLD/NASH was defined according to the histological features of NASH, when available or cryptogenic cirrhosis in the presence of MetSy and without a history of significant alcohol intake	NAFLD and NASH most common aetiology 24% followed by HCV 23.3% but cryptogenic made up 15.3%	58.3%	Small cohort. All NASH patients were cirrhotic with no non-cirrhotic NASH controls

Table

Wong *et al*.[38]	USA	Retrospective cohort study of OLT recipients on organ registry for HCC in US from 2002 to 2012.	10,061	Documented diagnosis of NASH or obese patients with cryptogenic/ unknown aetiology of liver disease	NASH was 2nd leading cause of OLT for HCC (8.3% in 2002 *vs*. 10.3% in 2007 *vs*. 13.5% in 2012) behind HCV but the most rapidly growing indication (4× increase in NASH, 2× increase in HCV) Non-NASH-related HCC patients were more likely to be men (79.2% *vs*. 70.6%) and were younger at time of OLT (57.2 ± 7.6 *vs*. 59.3 ± 7.3) NASH-related patients had higher BMI (33.6 ± 4.3 *vs*. 27.3 ± 4.9) and higher rates of diabetes (42.8% *vs*. 20.8%)	N/A	Used a modified diagnostic criterion for NASH. Did not present proportion of cirrhotic patients. Transplant patients tend to have high adherence and engagement with medical care, which may account for the older and heavier patients undergoing transplant with NASH
Dyson *et al*.[34]	UK	Retrospective longitudinal cohort of consecutive HCC cases between 2000 and 2010	632	Evidence of a fatty liver on biopsy or imaging, with an otherwise negative liver screen, drinking < 21 or < 14 units of alcohol per week respectively for at least 5 years prior to their first presentation with liver disease	NAFLD accounted for 34.8% of HCC cases NAFLD patients were older (71.3 *vs*. 67) Survival similar to other aetiologies but higher incidental presentation (38.2%) and lower prevalence of cirrhosis (77.2%)	77.2%	Retrospective single centre study in a region which high rates of obesity and diabetes
Park *et al*.[7]	Multi-regional	The BRIDGE study. Observational longitudinal cohort in 42 countries between 2005 and 2012	18,301 (Asia 67% of patients, Europe 20% and North America 13%)	Medical chart review	Proportion of HCC attributable to NAFLD: North America 12%, Europe 10%, China 1%, Taiwan 5%, South Korea 6%	N/A	Multiple centres in different regions without a clear definition of NAFLD used
Younossi *et al*.[8]	USA	Longitudinal cohort study using SEER registries to identify patients with HCC between 2004 and 2009	4929	ICD codes NAFLD also defined by clinical diagnosis (cryptogenic cirrhosis, obese-diabetics with cryptogenic liver disease)	14.1% of HCC linked to NAFLD and a 9% annual increase in NAFLD-related HCC NAFLD HCC were older, had shorter survival time, more heart disease and more likely to die from primary liver cancer Only 5% who received transplant post HCC were for NAFLD	N/A	Coding information can underestimate NAFLD and obesity
Mittal *et al*.[37]	USA	Retrospective cohort analysis of patients with HCC in US Veterans Affairs hospitals between 2005 and 2010	1500	Histology or presence of the metabolic syndrome in the absence of other causes of liver disease	NAFLD HCC accounted for 8% of HCC 1-year survival comparable between aetiologies	58.3%	The vast majority of the cohort were male, which does not represent the general population
Perumpail *et al*.[71]	USA	Case series of HCC patients	44	Radiological or histological evidence of hepatic steatosis, with no causes for secondary hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication or hereditary disorders	9 cases of HCC in the absence of cirrhosis, and 6 were attributable to NAFLD/NASH	N/A	Very small case series

Piscaglia *et al.*[49]	Italy	Multicentre observational prospective cohort of patients with NAFLD-related HCC	145	NAFLD if all other known aetiologies of liver disease could be ruled out and if consistent present or past histological or ultra sonographic features of fatty liver and alcohol intake < 30 g/day	46.2% of NAFLD associated HCC were non-cirrhotic compared to 2.8% in those with HCV 73.1% of NAFLD-related HCC patients have diabetes mellitus compared with only 24.9% of HCV-related HCC patients NAFLD HCC detected at later stage, had larger volume and more infiltrative pattern compared to HCV-related HCC Non-significant survival difference NAFLD 28.5 months *vs*. 35 months in HCV	53.8%	Median follow up of 13 months so longer term survival results may not be truly reflective of the general population
Mohamad *et al.*[48]	USA	Retrospective analysis of patients diagnosed with HCC and NAFLD at the Cleveland Clinic between 2003 and 2012	83	Histologically	43.4% of NAFLD-related HCC occurred with a non-cirrhotic liver NAFLD HCC were older and more likely to present with a single nodule, larger tumour and more likely to undergo resection, but did have higher rates of recurrence	56.6%	Retrospective case series with selection bias for patients who underwent liver biopsy
Kanwal *et al.*[32]	USA	Prospective longitudinal cohort of NAFLD patients with matched controls	296,707	2 or more elevated ALT values (> 40 IU/mL for men and > 31 IU/mL for women) in the outpatient setting in the absence of other causes of liver disease or any ICD codes for alcohol use	Risk of HCC 0.21/1000 PYs in those with NAFLD HR: 7.62; 95%CI: 5.76-10.09 NAFLD with cirrhosis a/w higher risk 10.6/1000 PYs 20% of NAFLD associated HCC were not cirrhotic	80%	ALT is not always an accurate diagnostic marker for NAFLD and this method may be underestimate prevalence in both NAFLD and control group
Hong *et al.*[42]	Australia	Multicentre prospective cohort of newly diagnosed HCC between 2012 and 2013	272	Medical chart review	NAFLD associated HCC accounted for 14% of cases	N/A	No clear definition of NAFLD used
Bengtsson *et al.*[52]	Sweden	Single centre retrospective cohort study of all HCC patients at Karolinska University Hospital Stockholm from 2004 to 2017	225	NAFLD defined by no other cause of liver disease and one of: (1) Liver biopsy supporting NAFLD; (2) Radiological features supporting steatosis; (3) BMI ≥ 25 and T2DM or BMI ≥ 30	Non-cirrhotic NAFLD-HCC were older (74 years *vs*. 70 years), had a lower prevalence of T2DM (66% *vs*. 80%), larger tumours, less frequently underwent liver transplantation (0% *vs*. 11%) and more frequently underwent resection (35% *vs*. 8%) compared to those with cirrhosis Mortality was similar (aHR for non-cirrhotic NAFLD-HCC *vs*. cirrhotic NAFLD-HCC 0.93, 95%CI: 0.58-1.51)	63%	Retrospective design and cohort derived from a tertiary referral centre which may be affected by referral bias, with those not suitable for curative intervention not referred for review

NASH: nonalcoholic steatohepatitis; HCC: hepatocellular carcinoma; NAFLD: non-alcoholic fatty liver disease; BMI: body mass index; HCV: hepatitis C virus; SEER: surveillance, epidemiology, and end results; HR: hazard ratio; aHR: adjusted hazard ratio; OLT: orthotopic liver transplantation; T2DM: type 2 diabetes mellitus; ALT: alanine aminotransferase; LT: in Wong USA 2012 please change the 'LT' in the methods column to OLT; DAA: direct-acting antiviral agents; ICD: international classification of diseases; N/A: not applicable; PYs: person years

Incidence of HCC in NAFLD

Although NAFLD represents an increasing proportion of HCC cases, the true risk of HCC in NAFLD patients with and without cirrhosis is not clear. As HCC is an infrequent outcome of chronic liver disease, there are limited studies with the adequate cohort size and duration of follow up required to accurately assess incidence rates. As a result, several meta-analyses have been performed to pool data and increase the sample size. A systematic review by White *et al*.[43] found the risk of HCC in NAFLD patients to be in the range of 0%-38% over a median of 5-10 years of follow up. More recently, Younossi *et al*.[1] performed a meta-analysis to evaluate the global prevalence and outcomes of NAFLD, and calculated an annual HCC incidence of 0.44 per 1000 person years (95%CI: 0.29-0.66) in patients with NAFLD, with much higher rates in those with NASH, 5.29 per 1000 person years (95%CI: 0.75-37.56). These meta-analyses are somewhat limited by the heterogeneity of the included studies, with small retrospective cohorts and inconsistent methods of defining NAFLD. As a result, a large United States longitudinal study led by Kanwal *et al*.[32] compared 296,707 patients with NAFLD to 296,707 matched healthy controls to determine the incidence of HCC in these cohorts. NAFLD was defined by persistently elevated transaminases (> 40 IU/mL for men and > 31 IU/mL for women, with a minimum of two results at least six months apart), in the absence of any significant alcohol intake or another cause of liver disease. The healthy controls had normal liver function tests, no other history of liver disease and minimal alcohol intake. The overall incidence of HCC in the NAFLD patients was low, 0.21 per 1000 patient years; however, HCC incidence was much higher in patients with NAFLD cirrhosis, 10.6 per 1000 patient years. Overall, cirrhosis in NAFLD appears to be the most significant risk factor for HCC [Figure 1], with approximately 6%-13% of patients with NAFLD cirrhosis developing a HCC during 3-10 years of follow up in a number of prospective observational cohorts[44-47].

NAFLD-related HCC in the absence of cirrhosis

NAFLD patients without liver cirrhosis are also at increased risk of HCC compared to those without liver disease[32,43]. This is particularly concerning given the high population prevalence of NAFLD, and the implications for HCC screening and increasing disease burden[1]. In the US, Sanyal *et al*.[36] found that, among patients with NAFLD-related HCC, as many as 54% were not known to be cirrhotic based on health care coding information. The proportion of non-cirrhotic patients was similarly high in the smaller Cleveland clinic cohort, which found that 43.4% of cases occurred in the absence of cirrhosis[48]. Those patients tended to be older (67.5 ± 12.3 years *vs*. 62.7 ± 8.1 years), and less likely to be obese (52% *vs*. 83%) or have type 2 diabetes (38% *vs*. 83%), than their cirrhotic counterparts. Similar findings have also been reported from European cohort studies, with a high proportion of NAFLD-related HCC occurring in noncirrhotic patients: 46.2% in a multicentre Italian study, 41.7% in a single centre German study and 22.7% in a UK-based cohort[34,49,50]. In the cohort study by Kanwal *et al*.[32], the risk of HCC in non-cirrhotic NAFLD was higher than in the healthy controls, but the overall incidence rates were low (0.08 *vs*. 0.02 per 1000 person years). Despite this, due to the high global prevalence of NAFLD, the absolute burden of NAFLD related HCC, including those with and without cirrhosis, remains significant.

Clinical outcomes in NAFLD-related HCC

Patients with NAFLD-related HCC tend to present at an older age compared to patients with HCC due to other aetiologies[8,28,31]. In the SEER cohort, NAFLD-related HCC patients were older (73 ± 8 years *vs*. 66 ± 11 years), had more heart disease (35.1% *vs*. 7%-27%) and had a shorter mean survival time (14.17 ± 17.14 months *vs*. 17.85 ± 21.47 months), compared to other aetiologies; and 84.3% died of their primary liver cancer[8]. This group found higher odds of one-year mortality (OR: 1.21, 95%CI: 1.01-1.45) in those with NAFLD-related HCC, although other large HCC cohorts have found no difference in overall survival[34,37,49]. Furthermore, a Canadian study of 929 patients who underwent a transplant for HCC, including 60 with NAFLD-related HCC, also found that these patients had similar one-, three- and five-year survival outcomes (98%, 96% and 80%, respectively) compared to non-NAFLD HCC

(95%, 84% and 78%, respectively), with no difference in tumour recurrence (13.3% *vs.* 14%)[51]. As patients with NAFLD-related

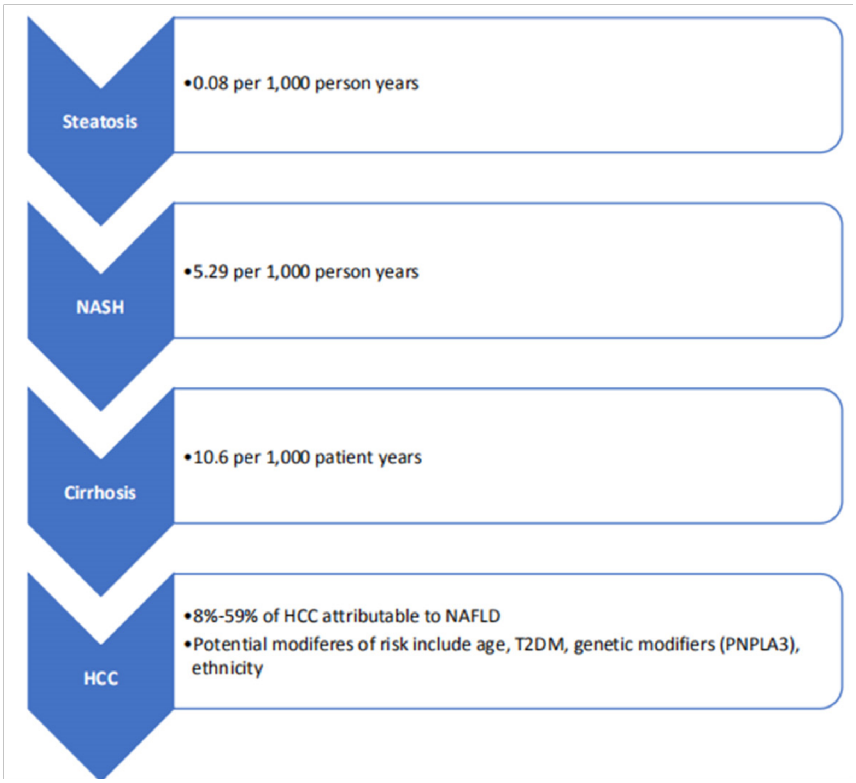

Figure 1. Increasing risk of HCC with stage of disease[1,7,32,36-38]. NASH: nonalcoholic steatohepatitis; HCC: hepatocellular carcinoma; NAFLD: non-alcoholic fatty liver disease; T2DM: type 2 diabetes mellitus

HCC tend to be older, with greater metabolic and vascular comorbidities than those presenting with HCC from other causes, the relative equivalence in survival may be due to the proportion of patients without cirrhosis in some of these cohorts (22.8% and 41.7% in the studies by Dyson and Mittal, respectively)[8,34,37].

Although non-cirrhotic and cirrhotic NAFLD-related HCC may present differently, overall survival rates between these two groups appear to be the same. A review of NAFLD-related HCC cases at the Cleveland clinic found that HCC in non-cirrhotic patients was more likely to present as a single nodule (80.6% *vs.* 52.2%) and with a larger nodule size (> 5 cm) (77.8% *vs.* 10.6%)[48]. From a treatment perspective, they were more likely to receive hepatic resection (66.7% *vs.* 17%) but less likely to receive loco-regional therapy (22.3% *vs.* 61.7%) or orthotopic liver transplantation (OLT) (0% *vs.* 72.3%). They also found that HCC in non-cirrhotic NAFLD had a higher rate of recurrence than in those with cirrhosis (86% *vs.* 14%); however, after adjusting for age or OLT, there was no difference in overall survival between the two groups. This is consistent with data from a large European cohort of NAFLD-related HCC patients in Sweden, which found non-cirrhotic patients to be older (median 74 years *vs.* 70 years), with larger tumours[52]. Similarly, in this group, those without cirrhosis were more likely to have a resection (35% *vs.* 8%), and less frequently underwent OLT (0% *vs.* 11%), but no significant difference was found in the overall mortality rates [adjusted hazard ratio (aHR): 0.93, 95%CI: 0.58-1.51, $P = 0.78$]. On further analysis, parameters independently associated with a higher risk for overall mortality in non-cirrhotic patients included; Barcelona Clinic Liver Cancer class C or D (aHR compared with class 0:7.03, 95%CI: 2.89-17.11, $P < 0.001$), the presence of four or more tumours (aHR compared with one tumour: 9.26, 95%CI: 3.20-26.81, $P < 0.001$), higher serum albumin (aHR

0.29 per increased g/dL, 95%CI: 0.15-0.56, $P < 0.001$) and the presence of T2DM (aHR: 3.65, 95%CI: 1.69-7.89, $P = 0.001$).

Obesity and type 2 diabetes and HCC risk

Obesity and T2DM are metabolic comorbidities closely associated with NAFLD, which have also been independently linked to the development of HCC[53,54]. T2DM has been found to be associated with increased HCC risk, particularly in patients with non-viral liver disease. A 2012 meta-analysis including 17 case-control studies and 32 cohort studies showed a statistically significant increased risk of HCC prevalence among diabetic individuals (RR: 2.31, 95%CI: 1.87-2.84)[53]. The pooled risk estimate from 17 case-control studies (OR: 2.40, 95%CI: 1.85-3.11) was slightly higher than that from the 25 cohort studies included (RR: 2.23, 95%CI: 1.68-2.96). This analysis was limited by lower patient numbers, which prevented adequate subgroup analysis, and, while there was some adjustment for the presence of viral hepatitis and cirrhosis, the possible presence of underlying NAFLD was not accounted for. In a study from the Mayo Clinic, the hazard ratio of HCC in cirrhotic patients without HCV infection was found to be two times higher in patients with T2DM compared to those without [Hazard Ratio (HR): 2.1, 95%CI: 1.1-4.1][55]. Additionally, T2DM has also been found to be an independent risk factor for HCC in patients with cryptogenic cirrhosis; however, these patients also demonstrated features of NAFLD based on markers of insulin resistance and lipid profiles[56]. Overall, while T2DM does appear to be associated with an increased risk of HCC in those with or without known liver disease, current studies are limited by confounding and the difficulty of excluding underlying NAFLD as a contributing factor.

Obesity is associated with an increased risk of many types of malignancy, and several epidemiological studies suggest a modest increase in the relative risk of HCC in obese populations[57]. A 2012 meta-analysis of 26 prospective cohort studies, including 25,337 individuals, found excess body weight [body mass index (BMI) ≥ 25 kg/m^2] and obesity (BMI ≥ 30 kg/m^2) to be associated with an increased risk of primary liver cancer [Summary Relative Risks (SRR) 1.48, 95%CI: 1.31-1.67; and SRR 1.83, 95%CI: 1.59-2.11, respectively][54]. While this evidence is somewhat limited by heterogeneity, on subgroup analysis, there was an increased risk in males, those with HCV and cirrhosis from any aetiology compared with the general population, suggesting that obesity may be an important cofactor in the development of HCC.

Genetic markers in NAFLD-related HCC

Several host genetic polymorphisms have been linked to the presence of NAFLD, risk of fibrosis progression and the development of NAFLD-related HCC[58]. These are particularly relevant as they may present novel biomarkers to help triage those at most risk of HCC and therefore in need of screening, particularly among those with NAFLD and no cirrhosis. The most studied of these is a single-nucleotide polymorphism (SNP) in PNPLA3. The association between PNPLA3 (rs738409) and hepatic steatosis was first identified in the genome-wide survey performed on the > 2000 participants of the Dallas Heart Study who underwent Magnetic Resonance Spectroscopy of the liver for quantification of liver fat[59]. The presence of this SNP has since been found to be associated with an increased risk of advanced fibrosis in all patients with liver disease, and a 2014 meta-analysis confirmed that it was also associated with an increased risk of HCC in patients with NASH and alcohol-related cirrhosis (OR: 1.67, 95%CI: 1.27-2.21), but not in other aetiologies (OR: 1.33, 95%CI: 0.96-1.82)[60]. Moreover, in a large well-characterised Northern European cohort of patients with histologically proven NAFLD patients, 100 of whom had NAFLD-related HCC, it was shown that PNPLA3 rs738409, especially the C > G polymorphism, was associated with an increased risk of HCC

independent of other traditional risk factors including age, gender, BMI, presence of T2DM or the presence of advanced fibrosis or cirrhosis[61]. Despite this evidence, the potential use of this SNP as a predictive biomarker to identify high-risk individuals for HCC screening has not yet been validated in larger cohorts, and it is not widely used.

Other potential genetic risk modifiers of interest include a variant in the *MBOAT7* gene, which was associated with an increased risk of NAFLD-related HCC in an Italian cohort of NAFLD patients (OR: 1.65, 95%CI: 1.08-2.55; n = 765), particularly in those without advanced fibrosis (P < 0.001), and TERT promoter mutations that can be found in low and high grade dysplastic lesions, and in increased frequency in early HCC[62-64]. The *TM6SF2* genetic variant on chromosome 19 has also been shown to be associated with an increased risk of NAFLD and NAFLD-related hepatic fibrosis and cirrhosis, independent of other known traditional risk factors including PNPLA3 status[65]. In one cohort of 99 biopsy-confirmed HCC patients, homozygous carriage of the *TM6SF2* rs58542926 minor allele was associated with an increased risk of NAFLD-related HCC. However, this was not shown to be significant on multivariate analysis when adjusting for traditional risk factors such as age, gender or cirrhosis[66]. Further investigation is required to determine the clinical significance of these associations and establish whether they may be of prognostic or diagnostic significance in the future. Consideration of cost, access to and acceptability of genetic sequencing as a screening or prognostic tool for patients with NAFLD must also be explored.

Should patients with NAFLD undergo HCC surveillance?

Routine HCC surveillance is recommended for all patients with cirrhosis regardless of the aetiology of their liver disease[9,10], and as such all patients with NAFLD cirrhosis should be enrolled in a screening program. While HCCs that are detected by surveillance programs are found at an earlier stage than those found incidentally, patients with NAFLD-related HCC are less likely to have recognised liver disease, and as such have a lower proportion of HCCs detected through screening compared with other causes of liver disease[67]. In the UK Newcastle cohort and the HCC NAFLD Italian groups, only 22.8%-47.6% of NAFLD-related HCC cases were detected during surveillance compared with 46.2%-63.3% in HCV[34,49]. Additionally, even in high risk patients who have NAFLD cirrhosis, screening is less likely to detect early stage HCCs compared with other aetiologies[34,37,49]. This is likely attributable to the current recommended screening modality of ultrasound, which can be technically challenging in obese patients with excess visceral adiposity, and is highly operator dependent[68]. Cross-sectional imaging such as MRI may be used as an alternative in patients in whom adequate views of the liver are unable to be obtained via ultrasound, however the suitability and cost-effectiveness of this approach has yet to be determined. Concern remains regarding whether NAFLD patients without cirrhosis should also be screened, considering that 23%-54% of NAFLD-related HCC occurs in non-cirrhotic patients. However, due to the large volume of NAFLD patients and low annual HCC incidence of less than 2% in this cohort, routine screening is not currently recommended[34,36,37,49]. The development of risk stratification tools may be of use in identifying high-risk patients who would benefit from screening, and may include the use of genetic markers.

Limitations in current estimates and Future directions

While these observational studies are informative in reviewing trends, there are several limitations to this method of determining the true burden of disease. The largest cohorts have typically identified NAFLD using coded registry data, or blood tests, which can be inconsistent and will not always reflect whether there is underlying fibrosis or steatohepatitis. Additionally, HCC registry information tends to be more representative of tertiary care and patients who have been referred for active management. As NAFLD related HCC patients tend to be older and more comorbid, it is possible that not all cases are referred to these specialist centres, and they may have remained in community care settings.

Similarly, as the SEER cohort demonstrated, patients with NAFLD-related HCC are less likely to be transplanted compared to HCC due to other causes, thus transplant registry information is also likely to underestimate the true burden of disease. Another limitation of cross-sectional retrospective studies is the evolving nature of NAFLD and NASH. These are not static disease states, but can progress or regress over time with changes in weight, which is often difficult to assess. While these studies have examined the risk associated with NAFLD alone, the increasing prevalence of obesity and diabetes in HCC of all aetiologies suggests that these metabolic factors including NAFLD could be an important co-factor in hepatic carcinogenesis[34]. In the future, the development of a simple, cost-effective population-based diagnostic test would be of benefit to improve the detection rate of NAFLD and trigger screening for underlying fibrosis and cirrhosis. Additionally, the development of prognostic markers or algorithms to identify high-risk patients for HCC surveillance is also an area of ongoing research. Further, as treatment for NAFLD progresses, and weight management becomes a greater focus of therapy, the ongoing risk of HCC in individuals with NAFLD who have subsequently lost weight or undergone therapy will need to be evaluated.

Conclusion

NAFLD is an important cause of liver disease worldwide and is associated with an increased risk of developing HCC, particularly in the presence of liver cirrhosis. While the absolute risk appears to be low, prevalence of NAFLD-related HCC is increasing in parallel with the prevalence of NAFLD worldwide, particularly in the West. The future global burden of NAFLD-related HCC represents a major public health threat and further research to identify cost-effective prevention and treatment strategies are urgently required.

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Authors' contributions

Performed the literature review and generated the manuscript: Farrell A

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1.5.3 Cardiovascular disease

Cardiovascular disease (CVD) is the leading cause of death in patients with MASLD, reflecting both the high baseline prevalence of established CVD and cardiovascular risk factors in this cohort, as well as direct contributions to atherogenesis (61). A meta-analysis of 36 longitudinal studies including over 5.8 million individuals, demonstrated that MASLD was associated with a 45% increased risk of fatal or non-fatal CVD events (pooled hazard ratio 1.45, 95% CI 1.31-1.61), independent of age, sex, adiposity measures, diabetes, and other cardiovascular risk factors (123). These findings were consistent across studies using imaging, ICD codes or liver biopsy for MASLD diagnosis. Due to this strong association, the American Heart Association has recognised MASLD as a risk factor for atherosclerotic cardiovascular disease and recommended that clinicians consider MASLD when assessing CVD risk (8).

Advanced fibrosis in MASLD is associated with a higher risk of cardiovascular disease compared to simple steatosis, with a prospective study of 285 patients with biopsy-proven MASLD showing that advanced fibrosis (F3-F4) was an independent predictor of incident cardiovascular disease after adjustment for traditional CVD risk factors (SHR 2.86, 95%CI 1.36 - 6.04) (124). Advanced fibrosis has also been shown to independently predict the development of arterial hypertension and T2DM, suggesting that progressive hepatic fibrogenesis is associated with metabolic dysfunction (125).

While CVD is the leading absolute cause of death in MASLD due to its high prevalence, a Swedish national registry study following 10.568 patients with biopsy-proven MASLD found that the excess mortality seen in MASLD compared with matched controls was driven primarily by extrahepatic cancer (aHR 2.16, 95%CI 2.03-2.30), followed by cirrhosis (aHR=18.15, 95% CI=14.78 to 22.30), then CVD (aHR=1.35, 95% CI=1.26 to 1.44) (62). Regardless, cardiovascular risk assessment and management in all patients with MASLD, regardless of the stage of their liver disease remains important.

MASLD is thought to contribute to CVD risk via mechanisms beyond their shared metabolic risk factors, with the liver having direct effects in systemic metabolism and inflammation (61). The steatotic liver has altered lipoprotein metabolism, producing an atherogenic lipid profile which is characterised by increased smaller dense low-density lipoprotein (LDL) particles, elevated triglyceride-rich lipoproteins, and reduced high-density lipoprotein (HDL) cholesterol (126). Additionally, hepatic production of pro-inflammatory and prothrombotic mediators contributes to systemic inflammation and endothelial dysfunction, with C-reactive protein, fibrinogen and plasminogen activator inhibitor-1 elevated in MASLD and correlating with both liver disease severity

and CVR risk markers (61). Altered hepatokine secretion, including fetuin-A and fibroblast growth factor 21, may also influence insulin sensitivity and vascular function contributing to CVD risk (127).

1.5.4 Extrahepatic manifestations

Type 2 Diabetes Mellitus

The relationship between MASLD and T2DM is bidirectional, with MASLD both predisposing to and being exacerbated by T2DM. A meta-analysis of 19 observational studies involving 296, 439 individuals demonstrated that MASLD is associated with a 2.2-fold increased risk of incident T2DM (HR 2.22, 95%CI 1.84-2.60), independent of obesity and other metabolic parameters (128). This risk increases further with disease severity, with one study reporting a nearly 5-fold increased risk among those with high MASLD fibrosis scores (HR 4.74, 95%CI 3.54-5.94). Conversely, T2DM accelerates MASLD progression, with diabetic patients demonstrating higher rates of MASH and advanced fibrosis (26). This close relationship highlights the role of the liver in metabolic dysfunction and supports therapeutic approaches that target shared pathways.

Other metabolic conditions

Other metabolic conditions are similarly overrepresented in MASLD patients. Chronic kidney disease is strongly associated with MASLD, with pooled data from a meta-analysis including over 1 million individuals found a 1.43-fold increased risk of incident CKD stage ≥3 (HR 1.43, 95% CI 1.33-1.54), independent of traditional renal risk factors (129). Women with polycystic ovarian syndrome, which is also associated with insulin resistance, have a 2.5-fold increased risk of MASLD (OR 2.54, 95% CI 2.19-2.95) (130). Obstructive sleep apnoea is roughly twice as prevalent in MASLD patients, and may also contribute to liver disease progression through chronic intermittent hypoxia (131). Extrahepatic cancers, particularly gastrointestinal malignancies, also demonstrate increased incidence in MASLD, potentially mediated by systemic inflammation, hyperinsulinaemia and the association with obesity (132). Recognition of these extrahepatic manifestations highlights that MASLD management requires a holistic, multisystem approach, rather than a solely liver-centric focus to ensure good patient outcomes.

1.6 Diagnosis and assessment of MASLD

1.6.1 Detection of MASLD

MASLD, particularly in the early stages is a relatively asymptomatic disease and as such, the diagnosis requires an index of suspicion regarding underlying risk. As previously outlined, MASLD

is closely associated with other metabolic conditions, affecting 70-90% of those with obesity, and up to 70% of those with T2DM (133-135). Current guidelines recommend screening for MASLD in individuals with obesity, T2DM, the metabolic syndrome or persistently elevated liver enzymes (9, 11).

The key component of diagnosing MASLD is detecting hepatic steatosis. This can be done a number of different ways with varying degrees of accuracy. A common clinical method used to identify likely underlying steatosis is elevated liver transaminases in the presence of metabolic risk factors such as T2DM (136). While pragmatic, the main limitation of this method is that ALT is normal in the majority of individuals with MASLD, even with MASH or advanced disease (94, 95).

Ultrasound is the most commonly used diagnostic tool to detect steatosis, and offers high specificity (95%), but lower sensitivity (85%) and can be limited by the operator dependent nature of the scan and is less accurate if steatosis is <33% (136). Controlled Attenuation Parameter (CAP), measured via transient elastography (Fibroscan, Echosens), quantifies hepatic fat content in decibels per metre (dB/m), and CAP values correlate with histological steatosis grade, with the threshold of approximately 248 dB/m correlating with steatosis grade 1 or greater(137). CAP values plateau at higher steatosis grades, and as such, its utility in monitoring treatment response is unclear. Magnetic resonance imaging (MRI) can provide a quantification of hepatic fat through a proton density fat fraction (PDFF) and is highly accurate compared to liver histology, however MRI is expensive and PDFF is not widely available (138, 139). Liver biopsy remains the gold standard in the diagnosis and grading of steatosis, however it is invasive, expensive and can be affected by sampling error (140).

Several clinical prediction scores have been developed to estimate hepatic steatosis using more commonly available parameters. The most extensively validated and widely used, is the Fatty Liver Index (FLI), a diagnostic algorithm that incorporates BMI, GGT, WC and triglycerides(141). A score of <30 is used to rule out hepatic steatosis and ≥60 to rule in steatosis, and validation studies have confirmed its performance in European and Asian populations (141-143). Pooled meta-analysis data from several validation studies found that at the lower threshold of FLI 30, FLI demonstrated a sensitivity of 81% and specificity of 65% for detecting hepatic steatosis, and at the higher cut off of FLI 60, the sensitivity was 44% with specificity of 90% with a positive predictive value of 67% (144). Despite some limitations in its accuracy, FLI still remains useful as a screening tool in primary care and particularly in epidemiological research, as it uses readily available parameters that are often collected in population health surveys, and is endorsed by both European and Asia-Pacific clinical practice guidelines in this setting (9, 145).

FLI may provide prognostic value beyond hepatic steatosis detection, with UK biobank analyses demonstrating that high FLI (≥60) was associated with increased risk of ischaemic heart disease (adjusted HR 2.14, 95%CI 1.94-2.36) and T2DM (adjusted HR 4.55, 95%CI 4.04-5.12) (146). Additionally, longitudinal studies using serial FLI measurements have demonstrated that persistent elevation, as well as incident elevation, is associated with increased all-cause mortality (adjusted HR, 1.86; 95%CI 1.75-1.98) and incidence of myocardial infarctions (adjusted HR, 1.3; 95%CI 1.21-1.40), while FLI regression was associated with improved outcomes (147).

The Hepatic Steatosis Index (HSI) and NAFLD Liver Fat Score are similar diagnostic stratification algorithms with comparable accuracy to the FLI but are less commonly used (148, 149).

4. Table 1.6.1 Diagnostic methods for MASLD

Method	**Criteria**	**Accuracy**	**Limitations**
Clinical	T2DM or 2 metabolic risk factors AND Elevated transaminases	N/A	Abnormal LFTs do not always reflect underlying steatosis/fibrosis
Ultrasound	Radiologically evident hepatic steatosis	Sensitivity 85%	Operator dependent More accurate if >33% steatosis
Fatty Liver Index (FLI)	Score > 60 (BMI, GGT, WC, Triglycerides)	AUROC 0.83	Epidemiological research tool, may have different cut offs in different ethnicities
Hepatic Steatosis Index (HIS)	ALT:AST ratio, BMI, T2DM, sex	AUROC 0.81	Less well validated in different populations than FLI
NAFLD Liver Fat score	Metabolic syndrome, T2DM, fasting insulin, AST, AST:ALT ratio	AUROC 0.86-0.87	Less well validated in different populations than FLI, fasting insulin not routinely performed
Controlled Attenuation Parameter (CAP)	>250dB/m	AUROC 0.80 – 0.88	Not widely available, doesn't correlate well with degree of steatosis

MRI Proton Density Fat Fraction (PDFF)	>5% steatosis	AUROC 0.9 – 0.98	Expensive, not widely available
Liver biopsy	>5% steatosis	Considered Gold standard	Invasive, expensive, sampling errors

1.6.2 Fibrosis Risk Stratification

As outlined in section 1.6.1, fibrosis stage is the most important predictor of outcomes in MASLD, and after making the diagnosis, fibrosis assessment is the next step in clinical workup.

Liver biopsy remains the gold standard for MASLD diagnosis, distinguishing MASH from simple steatosis and, most importantly, for fibrosis staging. Histologically, fibrosis is staged using the NASH CRN system, which grades fibrosis from F0 (normal) to F4 (cirrhosis) (63). This is the scoring system that has been used for natural history studies and clinical trials, and formed much of our understanding for the basis of this disease. However, there are a number of limitations which restrict its use as a diagnostic tool. Most importantly, it is an invasive procedure with risk of complications such as pain or significant bleeding. The incidence of serious complications is approximately 0.3-0.57% and incidence of mortality 0.01% (150). Sampling variability is also a potential drawback, with only 1/50,000 of the whole liver tissue sampled within a typical 15-20mm sample from a biopsy, and studies have demonstrated that up to 25-30% of paired liver biopsies from different lobes show discordance in fibrosis stage (150, 151). Furthermore, it is resource-intensive, costly, and inter- and intra-observer variability in histological scoring is well documented, even among expert pathologists (152). As a result, liver biopsy is restricted to patients in whom there is diagnostic uncertainty, where alternative liver diseases cannot be excluded non-invasively, in suspected advanced fibrosis/cirrhosis where histological staging would change management, and in clinical trials requiring histological endpoints (9).

Subsequently, current guidelines advocate a non-invasive approach to fibrosis risk-stratification with a sequential two-tier approach with initial screening using non-invasive fibrosis scores, simple serum-based diagnostic algorithms to stratify risk, followed by confirmatory testing with imaging-based techniques in intermediate/indeterminate and high-risk patients (9, 17).

Serum-marker scores use readily available information and have been used extensively in viral hepatitis, and more recently MASLD specific scores have been developed. The FIB-4 is the most widely used and validated of these screening tools and is the score recommended by the Australian GESA guidelines (17). It requires a few readily available clinical parameters (Age, AST, ALT and

platelets) and can be calculated using a number of easily accessible, free online calculators and has an excellent negative predictive value (>90%) at lower cut off values and pooled meta-analysis data has determined an AUC statistic of 0.76 (153, 154). A low FIB-4 score of <1.3 classifies patients as low risk of advanced fibrosis, >2.7 as high risk of advanced fibrosis and between these values patients are considered of indeterminate risk (17). Local Australian data has also shown an association with longer term liver-related outcomes, with low FIB-4 score (<1.3) demonstrating a low likelihood of developing HCC or hepatic decompensation (≤1% over a median follow up of 5 years) (155). In MASLD patients with FIB-4 scores >2.67, 21% developed liver decompensation or HCC over a median of 57 months in a meta analysis of 2518 patients, supporting the use of this threshold as identifying those who should be referred for specialist management (17, 156).

An alternative to FIB-4 is the disease specific NAFLD Fibrosis Score (NFS), which requires more variables for calculation (Age, BMI, diabetes, AST:ALT, platelets and albumin) than FIB-4, but offers fairly comparable performance (157, 158). Although FIB-4 and NFS have similar overall accuracy, the FIB-4 performs slightly better in MASLD subgroups such as those with T2DM for fibrosis stratification (158). Both FIB-4 and NFS score perform poorly in those < 35 years old, and different cut offs have been proposed for FIB-4 when used in those >65 years old to improve accuracy in that cohort (9). The BARD score is very simple risk score combining BMI, AST:ALT ratio and diabetes status which is easy to calculate but demonstrates slightly lower accuracy than FIB-4 or NFS, with an area under the receiver curve (AUROC) of 0.67-0.77, however a low score is particularly useful for ruling out advanced fibrosis (159). The BARD score also classifies patients more simply as low or high risk, whereas both FIB-4 and NFS stratify patients as low, high or indeterminate risk which adds a degree of diagnostic uncertainty, necessitating second-line tests such as imaging-based modalities.

5. Table 1.6.2 Tests for fibrosis assessment

Modality	Thresholds	Accuracy	Notes
First-line tests for liver fibrosis assessment			
FIB-4	Low risk <1.3 High risk >2.7	AUROC 0.77-0.80	• Age, AST, ALT, platelets • First line recommended by guidelines • Performs less well in <35y.o and >65y.o.
NAFLD Fibrosis score	Low risk < -1.455 High risk >0.676	AUROC 0.75-0.78	• Age, BMI, T2DM, AST, ALT, platelets, albumin • Performs slightly less well than FIB-4 in some MASLD subgroups

			• Performs less well in <35y.o.
BARD	Low risk 0-1 High risk 2-4	AUROC 0.67-0.77	• BMI ≥28, AST:ALT ratio ≥0.8, T2DM • Simple to calculate with binary outcome
Second-line tests for liver fibrosis assessment			
Vibration-controlled transient elastography (Fibroscan, Echosens)	Low risk <8kPa ≥ 8.2kPa risk of significant fibrosis ≥ 12kPa risk of advanced fibrosis	AUC 0.85	• Imaging based test • Not widely available outside metropolitan areas • Less accurate with increasing BMI
Shear-wave elastography	High risk >8kPa	AUC 0.84-0.86	• Imaging based test • Not widely available and high rate of unreliable readings
MRI elastography	High risk >5kPa	AUROC 0.92-0.95	• Imaging based test • Very expensive, not widely available
ELF score	High risk >9.8	AUROC 0.80-0.83	• Serum based test • hyaluronic acid, procollagen III, amino-terminal peptide, tissue inhibitor of matrix metalloproteinase-1 • Some cost to patient, not widely available
Hepascore	Low risk <0.6 High risk ≥0.6	AUROC 0.88	• Serum based score • Age, sex, GGT, bilirubin, α-2-macroglobulin, hyaluronic acid • Some cost to patient, not widely available
Definitive testing for liver fibrosis			
Liver biopsy	Fibrosis score F0 – no fibrosis F1 – mild fibrosis F2 – perisinusoidal and periportal fibrosis F3 – bridging fibrosis F4 – cirrhosis	Gold standard	• Risk of sampling error and interobserver variability • Resource intensive and risk of serious complications

Sources: (9, 11, 17, 153, 158, 159)

Vibration-controlled transient elastography (VCTE) performed via Fibroscan (Echosens), measures liver stiffness measurement (LSM) as a surrogate marker for underlying fibrosis. It is measured using a sonographic probe held onto the skin over the liver and is more accurate than a serum based test such as FIB-4 for predicting advanced fibrosis in MASLD (17). Factors which may falsely elevate LSM include recent food intake, hepatic inflammation or congestion, cholestasis and elevated BMI.

VCTE measurements can also be highly variable, and high measurements >7.9kPa may not be reproducible in about one-third of patients (160). It is the most widely used second-line fibrosis assessment tool used in clinical practice however there is no Medicare rebate associated with the scan and machines are expensive, limiting its availability to predominantly specialist centres, typically in metropolitan areas (17). As outlined in Table 1.6.2, other second-line fibrosis assessment tools are available, including other imaging modalities such as shear-wave or MRI elastography, however these are less consistently available, but can be used interchangeably with Fibroscan. Direct serum fibrosis tests which incorporate serum markers of fibrogenesis or fibrinolysis into their prognostic algorithm can also be used as second-line tests however are not covered by Medicare in Australia and so are associated with some cost to the patient, and are not widely available (Table 1.6.2) (17).

Following this sequential approach, the majority of patients at risk of advanced fibrosis and worse long-term outcomes will be identified, with liver biopsy reserved for those in whom the non-invasive approach is inconclusive or discordant with clinical assessment, and in whom accurate fibrosis staging would change ongoing management.

1.7 Current Treatment Approaches for MASLD

1.7.1 Lifestyle Modification

Evidence for weight loss targets

A strong base of evidence demonstrates the disease modifying impact of weight loss in MASLD. The relationship between weight loss and histological improvement follows a clear dose-response relationship, with even 3-5% TBWL shown to improve steatosis, 5-7% TBWL improving lobular inflammation and more substantial weight loss of ≥ 10% associated with improved hepatocyte ballooning and fibrosis (10). The seminal study establishing these thresholds was a paired-biopsy, longitudinal study of 293 patients with biopsy-proven MASH, who underwent a 52-week lifestyle intervention in order to lose weight(10). 58% of patients who achieved ≥ 5% TBWL had MASH resolution, but in patients who achieved ≥ 10% TBWL, there was a much more significant improvement with 90% having MASH resolution and 45% achieving fibrosis regression of at least one stage. These findings have been confirmed in subsequent studies and form the basis for current treatment guidelines, with weight loss recommended as first line therapy for MASLD, with a target of 7-10% TBWL for MASH and fibrosis reduction in those with overweight and/or obesity(9, 11). Dietary energy restriction to induce weight loss forms the basis of first line treatment in MASLD and is discussed further in Section 1.8.2.

Physical activity

While energy restriction plays a larger role in weight loss than exercise, physical activity is still important in the management of MASLD, with meta-analysis data demonstrating that exercise without dietary modification can improve hepatic steatosis by approximately 2-4% absolute reduction even in the absence of weight loss (161). The mechanisms by which exercise improves MASLD include improved skeletal muscle insulin sensitivity (reducing hepatic lipid influx), enhanced hepatic fatty acid oxidation, reduced inflammation, and improved mitochondrial function (162). MASLD guidelines recommend minimising sedentary time, with recommendations tailored to the individuals preferences and ability but overall recommending at least 150 minutes per week of moderate-intensity (e.g. brisk walking), or 75 minutes per week of vigorous-intensity exercise (9). Resistance training at least twice weekly is also recommended to help preserve lean mass during weight loss.

Challenges in implementing lifestyle changes

Despite strong evidence supporting lifestyle modification for MASLD, achieving and maintaining clinically significant weight loss remains extremely challenging for most patients. Meta-analyses of behavioural weight loss interventions demonstrate average weight loss of 5-8.5kg (5-9%) at 6 months, with weight plateaus and subsequent regain such that 3-6kg (3-6%) is maintained at 24-48 months (12). In the previously mentioned Vilar-Gomez lifestyle intervention paired biopsy study, only 30% of the 293 participants achieved ≥ 5% TBWL at 52 weeks, despite intensive dietary counselling and monitoring, highlighting the difficulties of actually achieving the weight loss targets recommended by guidelines (10).

Barriers to lifestyle modification include physiological adaptations to weight loss which favour weight gain (outlined further in section 1.8.1), psychosocial factors (food environment, emotional and behavioural eating triggers, cultural expectations), socioeconomic constraints (cost of healthy foods, time for exercise), and comorbidities limiting physical activity (musculoskeletal disease, cardiovascular disease) (13). These challenges have increased interest in more intensive medical weight management approaches, including very low energy diets and pharmacotherapy, to augment lifestyle modification in patients with MASLD.

1.7.2 Dietary interventions

Energy restriction through dietary modification is the primary basis of weight loss and a fundamental aspect of MASLD management, with current guidelines recommending a 500-1000 kcal/day deficit to achieve 7-10% weight loss, with some evidence suggesting that the pattern of dietary composition may provide additional hepatic benefits independent of energy balance (9).

Mediterranean Diet

The MD has emerged as the dietary pattern with the strongest evidence base for MASLD management and is recommended in the European EASL-EASD-EASO guidelines (9). A landmark randomised crossover trial in 12 patients with biopsy-proven MASLD demonstrated a 39% relative reduction in hepatic steatosis measured by magnetic resonance spectroscopy (MRS) following 6-weeks of MD, compared with only 7% reduction with a low-fat/high-carbohydrate diet, despite equivalent weight loss between the interventions (163). Insulin sensitivity, assessed by hyperinsulinaemic-euglycaemic clamp also improved significantly with MD but not with the control low fat diet.

Population-level observational data reinforces these findings. In an Italian cross-sectional analysis of 584 consecutive outpatients with cardiovascular risk factors who were screening using ultrasound and asked to complete a dietary survey, MASLD prevalence showed an inverse dose-response relationship with MD adherence scores, falling from 97% in those with low MD scores to 71% in high adherers (164). Prospective data from the Framingham Heart Study also confirmed these associations with longitudinal data from 1,521 participants followed up for a median of 6-years, each one standard deviation improvement in MD score was associated with a 26% reduction (95% CI 10-39%) in incident fatty liver (165). The ATTICA cohort provided similar observations with follow-up to 10-years, demonstrating inverse associations between MD and markers of both steatosis and fibrosis, with MD additionally protecting against incident diabetes and cardiovascular disease among participants with existing MASLD (166).

The cardiovascular benefits of the MD were established by the PREDIMED trial, which demonstrated an approximately 30% reduction in incidence of cardiovascular disease in high-risk individuals randomised to MD supplemented with extra virgin olive oil or nuts, compared to a control diet (167). This cardiovascular benefit is particularly relevant for MASLD patients, given the high risk of cardiovascular morbidity and mortality.

For the treatment of MASLD however, recent trials have provided more nuanced findings. The Australian MEDINA study comparing an ad libitum MD to ad libitum low-fat diet, found that both diets reduced intrahepatic lipid but there was no statistical difference between groups (168). The DIRECT-PLUS trial demonstrated that a polyphenol-enriched ‘green MD’ achieved superior hepatic fat reduction (38.9%) compared with standard MD (19.6%) and healthy dietary guidelines (12.2%), suggesting that specific components may enhance benefit more than others (169). Meta-analysis data supports MD efficacy in MASLD with pooled data demonstrating significant reductions in FLI,

insulin resistance, hepatic steatosis and liver stiffness compared to control diets (170, 171). However when compared directly, MD and low-fat diets with matched energy intake have shown equivalent efficacy for hepatic endpoints in pooled data, potentially suggesting that energy restriction is the main driver of benefit, however studies to date have had relatively short follow-up and the relative contributions of energy restriction versus dietary composition remain incompletely resolved (172).

Any hepatoprotective effects of the MD are likely mediated by multiple mechanistic pathways. The high monounsaturated fatty acid content, primarily from olive oil, reduces hepatic lipogenesis and improves insulin sensitivity (173). Polyphenols from olive oil, wine and plant foods have anti-inflammatory and antioxidant effects, and omega-3 polyunsaturated fatty acids from fish reduce hepatic triglyceride synthesis and inflammatory cytokine production (173). Additionally, the high fibre content helps to modulate the gut microbiome, reducing bacterial translocation and hepatic exposure to endotoxins (173).

6. Table 1.7.2.1. Summary of key trials for Mediterranean diet in MASLD

Study	**Design**	**Population**	**Intervention**	**Comparison**	**Hepatic outcomes**	**Metabolic outcomes**
Ryan et al JHep 2013 (163)	Randomised crossover 6 weeks each diet N=12	MASLD • Biopsy proven, non-cirrhotic	Mediterranean diet (MD)	Low fat/High carbohydrate diet (LFHC)	• MRS liver fat ↓ 39% vs ↓ 7% in comparison	• No difference in weight change b/w groups • Insulin sensitivity improved with MD not LFHC
Properzi et al Hepatology 2018 (174)	Parallel RCT 12 weeks N=48	MRS confirmed MASLD + BMI 25 or greater	Ad libitum MD	Ad libitum Low fat diet (LFD)	• Both MD and LFD reduced liver fat (↓ 32% vs 25%)with no difference between groups	• Similar weight loss (-2.1kg vs -1.6kg) b/w groups • Similar improvements in metabolic profile
George et al Liver Int 2022 (MEDINA) (168)	Parallel RCT 12 weeks N=42	MASLD	Ad libitum MD	LFD	• No b/w group difference in MRS liver fat	• HOMA-IR improved in LFD but not MD
Katsagoni et al Br J Nutr 2018 (175)	3-arm parallel RCT 6 months N=63	MASLD	MD only Vs MD + exercise + sleep counselling	Control (written guidance only)	• US steatosis grade and shear-wave elastography reduced in both MD groups vs control	• Greater weight loss in both MD groups vs control • HOMA-IR and lipids improved in MD groups

Yaskolka Meir et al Gut 2021 (DiRECT PLUS) (169)	3-arm parallel RCT 18 months N=294	Abdominal obesity and dyslipidaemia	Green MD (high polyphenols, mankai, green tea) or standard MD	Healthy dietary guidelines	• Greater liver fat loss greatest in green MD (-39%) > MD (-20%) > control (-12%)	• -3.7kg weight loss green MD, -2.7kg MD, -0.4kg control • Improved metabolic profile in MD groups
Abenavoli et al Nutrients 2017 (176)	3-arm RCT 6 months N=50	MASLD	MD vs MD + antioxidant supplement (silybin-vit E-phospholipid complex)	Standard care	• Improved US steatosis grade, ALT, AST and GGT in MD groups	• Improved BMI, lipid profile and glucose metabolism in MD groups

Low-carbohydrate and Ketogenic diets

Low-carbohydrate diets which restrict carbohydrate intake to <130g/day or <26% of total energy have been shown to reduce hepatic steatosis. A randomised trial comparing 2 weeks of carbohydrate restriction (<20g/day) to calorie restriction (1200-1500 kcal/day) in 18 patients with MASLD found significantly greater reductions in hepatic triglycerides in the low carbohydrate group (55% vs 28% relative reduction) despite similar weight loss (~4.3% TBWL), suggesting a metabolic benefit independent of energy restriction (177). A multi-omics study using a 14-day isocaloric low-carbohydrate diet with increased protein content in MASLD patients with obesity found that despite no energy restriction, liver fat decreased from 16 to 3.2% accompanied by marked decreases in hepatic de novo lipogenesis, enhanced mitochondrial β-oxidation and down regulation of fatty acid synthesis pathways (178).

These findings have not been consistently demonstrated across clinical trials. A 3-arm parallel randomised trial comparing low-carbohydrate high-fat diet, intermittent fasting (5:2 diet) and standard care in 74 patients with MASLD found both intervention diets were superior for reducing hepatic steatosis and body weight over 12 weeks, with no significant difference between the two active interventions (179). Despite promising results in some trials and mechanistic plausibility, weight loss rather than dietary composition has been consistently shown to be the strongest factor in hepatic improvements in MASLD.

Very low energy diets

Very low energy diets (VLEDs) provide 2500-3350 kJ/day (450-800kcal/day), typically using meal replacement products, to achieve rapid and substantial weight loss with corresponding improvements in hepatic steatosis. The counterpoint study demonstrated that an 8-week 600kcal/day diet reduced liver fat by 30% at week 1 and 70% at week 8, with normalisation of hepatic insulin sensitivity within

7 days preceding improvement in pancreatic β-cell function (180). The DiRECT trial demonstrated that primary-care delivered VLED programmes could achieve T2DM remission in 46% of participants at 12 months, with benefits maintained in 36% at 24 months (14, 181).

Pre-bariatric surgery studies have demonstrated that 2-6 weeks of VLED reduces liver volume by 14-16% and hepatic fat content by 43-51%, facilitating safer surgical access to improve peri-operative outcomes (182, 183). A review of 19 studies including 968 patients with MASLD including with decompensated cirrhosis (on transplant list) found consistent improvements in liver function tests, hepatic steatosis indices, and liver size with adherence rates of 69-93% and only minor adverse events (184).

7. Table 1.7.2.2. Summary of key trials for very low energy diets in MASLD

Study	**Design**	**Population**	**Intervention**	**Duration**	**Hepatic outcomes**	**Metabolic outcomes**
Lim et al Diabetologia 2011 (180)	Prospective cohort	T2DM with obesity N=11	600kcal/day diet	8 weeks	• MRI Liver fat ↓ 70%	• 15% TBWL • HbA1c ↓ 7.4% to 6%
Scragg et al Clin Transl Gastro 2020 (185)	Prospective cohort	MASLD and BMI >27 N=30	~800kcal/day	8-12 weeks VLED +6 months follow up	• Improved ALT, AST, GGT • Liver stiffness ↓ 1.3kPa • Changes maintained at follow-up	• 13.4% TBWL • 67% retained weight loss at 9 months
Zhyzhneuskaya et al Diabet Med 2025 (186)	Substudy of DiRECT RCT	T2DM and obesity N=42	~850kcal/day	12-20 weeks VLED then structure reintroduction and maintenance	• MRI liver fat ↓ 86% • MASLD remission 74% • Improved ALT and GGT	• 11.9kg weight loss • Improved fasting glucose
Rayner et al Int J Obesity 2019 (187)	Prospective cohort RCT	Obesity N=25 vs 15 controls	800 kcal/day VLED	8 weeks	• MRI liver fat ↓ 55%	• 12kg weight loss • HOMA-IR improved42% at week 1
Schwenger et al Obes Surg 2018 (188)	Prospective cohort	Morbid obesity pre-bariatric surgery N=21 with pre and post VLED assessment	900kcal/day	2-3 weeks pre surgery	• Decreased ALP but increased AST and ALT	• Improved BMI, fasting glucose and insulin, HbA1c, HDL and LDL cholesterol

Lewis et al Obes Surg 2006 (182)	Prospective cohort	Morbid obesity pre-bariatric surgery N=18	450-800 kcal/day	6 weeks	• MRI liver fat ↓ 43%	• 9.2kg weight loss

Intermittent fasting

Intermittent fasting diets, including the 5:2 diet (significant energy restriction on 2 non-consecutive days per week) and time restricted eating (confining eating to 4-10 hr wonder per day) have gained popularity as alternatives to continuous energy restriction. A meta-analysis of 6 studies found intermittent fasting significantly improved body weight, BMI, ALT and AST compared to control groups, though effects on lipid profile was inconsistent (189). A subsequent larger meta-analysis of 20 RCTs comparing isocaloric intermittent fasting to caloric restriction found no difference in metabolic outcomes, suggesting that previously demonstrated benefits may be related to energy restriction more so than specific to the fasting pattern itself (190).

1.7.3 Pharmacotherapy for MASLD

Emerging MASLD specific therapies

In Australia, no pharmacological therapies have received TGA approval specifically for the treatment of MASLD or MASH. Internationally, however the therapeutic landscape has evolved significantly in recent years. Resmetirom, a thyroid hormone receptor-β (THR-β) agonist, became the first FDA-approved therapy for MASH in March 2024, receiving approval for non-cirrhotic MASH with moderate to advanced fibrosis (F2-F3) based on the MAESTRO-NASH phase 3 trial demonstrating MASH resolution without fibrosis worsening in 26-30% (80mg and 100mg dose respectively) vs 10% with placebo , and fibrosis improvement without MASH worsening in 24-26% vs 14% with placebo at 12 months (191). THR-β activation enhances hepatic lipid oxidation and reduces hepatic fat content without the cardiovascular effects associated with non-selective thyroid hormone activation (192). Semaglutide (Wegovy 2.4mg weekly) subsequently received FDA approval for MASH in August 2025, becoming the first GLP-1 RA approved for this indication, based on the phase-3 ESSENCE trial which demonstrated MASH resolution in 62.9% vs 34.3% with placebo, and fibrosis improvement in 36.8% vs 22.4% with placebo at 72 weeks (193). Despite favourable but modest fibrosis improvement in fibrosis in clinical trials, FXR agonist obeticholic acid was rejected by the FDA due to concerns about drug-induced liver injury and unfavourable risk-benefit profile due to pruritis and dyslipidaemia (194, 195).

New MASH-specific drugs continue to be developed, with promising phase 2b trial results for pan-PPAR agonists including lanifibranor and FGF21 analogues such as pegozafermin and efruxifermin, with phase 3 studies underway (196). Lanifibranor resulted in MASH resolution in 49% vs 22% for placebo and improvement in fibrosis in 48% vs 29% at the dose of 1200mg (197). In the ENLIVEN trial Pegozafermin achieved MASH resolution in 37% and fibrosis improvement in 27% at its highest dose vs 2% and 7% with placebo respectively (198). Efruxifermin (HARMONY trial) also outperformed placebo with fibrosis improvement in 41% vs 20% with placebo, and resolution of MASH in 76% vs 15% in placebo (199). These positive results are promising and directly acting MASLD drugs have the potential to change the therapeutic landscape for the treatment of MASLD, which is projected to continue to be the most common cause of liver disease worldwide (196).

Diabetes and obesity medications with hepatic benefit

Several medications commonly used for T2DM have demonstrated hepatic benefits, relevant to the treatment of MASLD. Pioglitazone, a thiazolidinedione PPAR-γ agonist, improves MASH histology in both diabetic and non-diabetic patients, with pooled meta-analysis data demonstrating steatohepatitis resolution and fibrosis improvement (200). However, recommendations regarding its use in MASLD remain mixed due to associated weight gain (2-4kg), fluid retention, cardiovascular risks and bone density reduction, with the American AASLD guidelines recommending consideration in MASH patients with T2DM, but other guidelines remaining more cautious (9, 11, 196). Sodium-glucose cotransporter-2 (SGLT2) inhibitors may also be beneficial in MASLD, although the level of evidence is insufficient to recommend its use specifically for MASLD (9).

As previously discussed, GLP-1 RAs have demonstrated significant hepatic benefits in MASLD. Mechanistic studies suggest there may be direct effects on hepatic lipogenesis and inflammatory pathways, although the extent to which these contribute independently of weight loss remains unclear (201, 202). Dual incretin agonists primarily developed for the treatment of obesity and T2DM, have shown promise in phase 2 studies for the treatment of MASH. In the SYNERGY-NASH trial, tirzepatide (GIP/GLP-1 dual receptor agonist) achieved MASH resolution in 44-62% vs 10% with placebo, and over half have fibrosis improvement (16). Survodutide, a glucagon/GLP-1 dual receptor agonist, showed similar efficacy for MASH resolution, 47-62% vs 14% placebo, and phase 3 trials for both agents are underway (203). In patients with compensated MASH cirrhosis, semaglutide has demonstrated an acceptable safety profile with no hepatic decompensation events, supporting its use for weight and metabolic management in this cohort (204).

Other pharmacotherapy

Statins are recommended in patients with MASLD and dyslipidaemia given the associated elevated cardiovascular risk, with evidence supporting the safety of their use even in the presence of elevated liver enzymes (11). Statins do not substantially reduce hepatic steatosis but may reduce cardiovascular events and possibly liver-related outcomes through anti-inflammatory and antifibrotic mechanisms (205). Other lipid-lowering agents such as ezetimibe and omega-3 fatty acid supplements have shown inconsistent effects on markers of liver disease and are not recommended as primary MASLD therapy (9).

Vitamin E (α-tocopherol) 800IU daily demonstrated improvement in steatohepatitis in the PIVENS trial, with MASH resolution in 43% vs 19% with placebo in patients without T2DM (206). Guidelines suggest vitamin E may be considered in non-diabetic patients with biopsy-proven MASH, although guidelines recommend caution given concerns about long-term safety, including possible increased all-cause mortality and prostate cancer risk at high doses, and vitamin E is not recommended in patients with diabetes or cirrhosis (9, 11).

1.7.4 Bariatric Surgery

Bariatric surgery produces the greatest and most durable weight loss of any weight loss intervention, with proportional results seen in MASLD. Roux-en-Y gastric bypass and sleeve gastrectomy can achieve 50-70% excess weight loss at 12 months, with trials reporting similar outcomes between the two surgeries (207, 208). A meta-analysis of 32 cohort studies demonstrated that bariatric surgery resolved steatosis in 66% and improved or resolved fibrosis in 40% of patients (209). The BARICAN study was a French prospective cohort which demonstrated MASH resolution in 84% and progressive fibrosis improvement, with 70% demonstrating improvement at 5 years post bariatric surgery (81). The BRAVES randomised trial confirmed superiority of bariatric surgery over lifestyle intervention plus best medical care, with MASH resolution of 56-57% vs 16% at 12 months (210).

Current guidelines recommend consideration of bariatric surgery in MASLD patients with BMI $\geq$ 35 kg/m^2 (9). Compensated cirrhosis is not an absolute contraindication when surgery is performed at an experienced tertiary centre, with multidisciplinary support but careful patient evaluation with regards to type of surgery and presence of portal hypertension is recommended (9, 211). Decompensated cirrhosis, would generally preclude a patient from bariatric surgery due to the high risk of morbidity and mortality, although sleeve gastrectomy at the same time or after liver transplant can be considered, with the goal of improved long term post-transplant outcomes (212). Weight regain post bariatric surgery affects approximately 20-25% of patients to varying degrees and is associated with

histological worsening of MASLD, highlighting the importance of post-surgical lifestyle maintenance (213).

1.8 Medical Weight Management

Obesity and MASLD are overlapping disease processes and weight loss is the mainstay of treatment for MASLD, so understanding the biological mechanisms of weight regulation is essential for developing effective treatment strategies for MASLD. Obesity is a progressive, chronic, relapsing and remitting condition that is most commonly defined by body mass index (BMI) ≥30 kg/m^2 (214). Obesity develops slowly over time, with long-term positive energy balance and is the result of a complex mix of genetics and environmental factors. The rise in obesity is largely attributed to the ready availability of high-energy foods alongside our increasingly sedentary lifestyles, however genetics is thought to play the key role in determining who becomes overweight (BMI 25-30) in a high-energy environment versus who becomes obese. As excess fat accumulates in the body, leptin (the 'satiety hormone') also increases to prevent further accumulation, but genetic susceptibility is thought to impair this negative feedback loop, explaining why some people struggle with weight while others do not; with twin and adoption studies demonstrating this genetic predisposition to weight gain being more important than environment (215). Despite this, the rapid increase in obesity over recent decades cannot be explained by genetic changes alone, which would take much longer to accrue. There is increasing evidence regarding epigenetic modification in response to early-life, particularly in utero, nutrition potentially playing a role in the increasing predisposition to obesity (216).

1.8.1 Physiology of weight regulation

Set point theory

Weight loss is difficult to achieve and maintain due to homeostatic mechanisms that defend a biologically determined body weight. This regulatory system is thought to be a result of evolutionary adaptation to environments with unpredictable food supply, and is a complex system of hormone signals from the gastrointestinal tract, adipose tissue, and other sites to coordinate appetite and energy expenditure (13). This "defence of body weight" presents a significant clinical challenge, making both weight loss and maintenance of weight loss difficult to achieve.

Classical set point theory proposes that body weight is regulated around a biologically determined level, similar to a thermostat (217). When body weight decreases below the 'set point', compensatory

responses increase hunger, reduce satiety, and decrease energy expenditure to promote weight regain. This theory has evolved more recently to the concept of "settling point", acknowledging that the defended weight can vary in response to sustained environmental changes (e.g. food availability, activity patterns), but returns towards the previous established point when that environmental pressure is removed, suggesting that weight varies within upper and lower boundaries influenced by environmental factors, but that physiological processes counteract changes beyond these limits (218).

Hormonal control of appetite

Multiple hormonal systems, coordinated primarily through the hypothalamus, regulate energy intake and expenditure. Leptin, which is secreted by adipose tissue in proportion to fat mass, provides a signal of energy stores to the brain, providing a negative loop with elevated levels suppressing appetite and increasing energy expenditure (219). Ghrelin, the only known appetite stimulating hormone, is secreted by gastric cells and rises before meals, triggering hunger sensations (220). Peptide YY (PYY) and cholecystokinin (CCK), and GLP-1 are released from intestinal cells post prandially, and suppress appetite and promote meal cessation through central effects on the hypothalamus and through peripheral mechanisms, with GLP-1 also slowing down gastric emptying (221, 222). Together these hormones work to maintain energy balance under normal physiological conditions.

Hormonal adaptation to weight loss

A landmark Australian study by Sumithran, Proietto and colleagues, demonstrated that weight loss induces changes to appetite regulating hormones that persist long after the acute weight loss phase, explaining the high rates of weight regain observed clinically (223). In this study, 50 overweight or obese participants undertook a 10-week VLED and achieved a mean weight loss of 13.5kg. Circulating levels of appetite-regulating hormones were measured at baseline, at the end of the diet/weight loss phase (week 10), and at 1-year after completing diet (week 62). At 1-year, despite partial weight regain, multiple hormonal changes persisted, with ghrelin remaining elevated and leptin, PYY, CCK and amylin remaining suppressed compared to baseline. These findings were important in demonstrating that hormonal changes post weight loss that favour weight regain, are not transient responses to acute energy deficit, but persist for prolonged periods after weight loss. Furthermore, the Biggest Loser study, which followed contestants who achieved substantial weight loss (mean 58kg) through a televised intensive lifestyle intervention, demonstrated that suppressed leptin levels persisted at 6-years post weight loss (224). This persistent hormonal pressure towards positive energy balance explains why weight loss is so hard to maintain and that ongoing support is required beyond just the initial weight loss stage.

Metabolic adaptations to weight loss

Beyond hormonal changes, weight loss triggers adaptive reductions in energy expenditure that exceed what would be predicted from reduced body mass alone, a phenomenon termed "metabolic adaptation" or "adaptive thermogenesis" (225, 226). Following 10% TBWL, total daily energy expenditure decreases by approximately 10-15% below predicted based on body composition, which is thought to be secondary to reduced resting metabolic rate, reduced thermic effect of food, and improved skeletal muscle efficiency (226). This effect has been demonstrated, in individuals who have lost weight requiring approximately 300-400 fewer calories per day than never-obese individuals of similar body composition (227). This was also seen in the Biggest Loser cohort, with resting metabolic rate suppressed by approximately 500 calories below predicted values per day at 6 years of follow-up, and most participants had regained substantial weight despite ongoing efforts (224). Interestingly, those who successfully maintained weight loss at 6 years showed the greatest persistent metabolic adaptation, potentially suggesting that there is ongoing biological resistance to the reduced weight state that persists long term. This cohort were compared to a matched cohort of patients who underwent Roux-en-Y bariatric surgery, with both groups found to have metabolic adaptation proportional to weight loss at 6 months but by 1 year the surgical group had no detectable metabolic adaptation despite continued weight loss and maintenance (228). However the mechanisms behind this are unclear.

Other compensatory responses to weight loss

The compensatory responses to weight loss extend beyond hormones and metabolism, and include alterations in neural reward processing and eating behaviour. Functional neuroimaging studies demonstrate that following weight loss, brain regions involved in the emotional, cognitive and sensory control of food intake show enhanced responses to visual food cues, making foods more enticing and harder to resist (229). At a behavioural level, weight-reduced individuals take longer to reach satiation, report greater hunger and disinhibition, and perceive food as more rewarding than at their pre-weight loss baseline (230). Weight-reduced individuals also demonstrate decreased non-resting energy expenditure, including reduced spontaneous physical activity, that persists beyond the active weight loss period (231).

All of these findings highlight that effective long-term obesity management must address the biological drive towards weight regain after initial weight loss, and strategies that may help attenuate or counteract these compensatory mechanisms such as pharmacotherapy, structured maintenance

programs or potentially bariatric surgery, should be considered as part of a comprehensive obesity management plan.

1.8.2 Very Low Energy Diets

Definition and Composition

VLEDs, also termed very low calorie diets (VLCDs), are nutritionally complete meal replacement programs that typically use commercial products (shakes, bars, soups etc) that replace all/most conventional meals and provide approximately 450-800 kcal/day (232). The macronutrient composition of VLEDs (50-70g protein (0.8-1.0 g/kg ideal body weight), <50g carbohydrate, and 7-15g fat per day) ensure nutritional adequacy despite severe energy restriction, with adequate protein intake to help attenuate the loss of lean mass (233). The low carbohydrate content induces nutritional ketosis, which suppresses appetite and facilitates adherence to an otherwise challenging energy deficit (234).

Role of ketosis

Carbohydrate restriction to <50-80 g/day depletes hepatic glycogen stores within 24-48 hours, which triggers ketogenesis as the liver converts fatty acids to ketone bodies (β-hydroxybutyrate, acetoacetate, acetone) to use as an energy supply (235). Contrary to popular belief, this does not result in targeted fat loss, nor does it result in acceleration of the weight loss achieved. Ketosis is used to aid tolerability of energy-restricted diets though effects on appetite regulating hormones and centrally in the hypothalamus. Sumithran and colleagues demonstrated that when individuals are in ketosis, the weight loss induced increase in ghrelin was suppressed, and subjective appetite ratings were lower compared with after reintroduction of carbohydrates (234). These effects appear to be dose-dependent, with higher β-hydroxybutyrate concentrations associated with greater suppression of ghrelin and hunger (236). A meta-analysis confirmed that individuals adhering to ketogenic VLEDs report less hunger and greater satiety than those on equivalent non-ketogenic energy restriction (237). This appetite suppression is important while following an intensive energy restricted diet such as a VLED, but the effects cease once no longer following a very low carbohydrate diet and as such structured transition and weight maintenance strategies to help with the increase in hunger are required.

VLEDs in the management of obesity

VLEDs produce greater initial weight loss than conventional dietary restriction, typically achieving weight loss of 10-15 kg over 8-16 weeks (238). A meta-analysis of randomised trials demonstrated

short-term weight loss of 16.1% with VLED compared to 9.7% with conventional low-calorie diets, although long-term weight loss was similar between the two approaches (239).

The DiRECT trial demonstrated that a VLED-based intervention in patients with T2DM achieved 10 kg mean weight loss at 12 months in a primary care setting, with T2DM remission in 46% of participants, which far exceeds standard diabetes care (14). Two-year follow-up showed maintenance of 7.6kg in weight loss and 36% sustained diabetes remission, and further extension study data found that 26% of those in remission at year 2 remained so at 5 years (181, 240).

A common misconception is that rapid weight loss results in more rapid or greater weight regain in the longer term. This has been disproven, with a randomised trial comparing rapid weight loss via a VLED (12 weeks) to gradual weight loss via conventional diet (36 weeks); with both the rate and proportion of weight regain at 144 weeks (approx. 3 years) similar between the two groups (241). Rapid weight loss, however, is associated with a higher likelihood of early success, which is the strongest predictor of long-term treatment outcome (242, 243). With the VLED, significantly more participants achieved the target weight loss, and achieved this 'early success' compared to the gradual approach (81% vs 50%) (241). This was also seen in other studies in which those with fast initial weight loss, were 5.1 times more likely to achieve clinically significant weight loss at 18 months than those who lost weight slowly (244). Maintenance strategies further improve outcomes with a systematic review finding that anti-obesity drugs, exercise, and high-protein diets each improved weight maintenance after VLED compared to control (245).

Clinical practice guidelines recommend VLEDs for the treatment of obesity as part of a structured program comprising an intensive weight loss period (typically 8-16 weeks of VLED), followed by transition off the VLED with gradual reintroduction of portion-controlled food alongside long-term maintenance strategies including behavioural support and where appropriate, pharmacotherapy (214, 246). The Australian obesity management algorithm recommends VLED as an initial treatment option for individuals with BMI 30-40 kg/m^2 with obesity-related complications, or BMI >40 kg/m2, with under medical supervision required (214).

Safety considerations

Commercially available VLEDs are nutritionally complete and have demonstrated acceptable safety when used under medical supervision (239). Common side effects, particularly during the initial 1-2 weeks include fatigue, headache, dizziness and constipation; however these are typically minor and resolve once ketogenic adaptation occurs (239). More significant adverse effects are uncommon but

include gallstone formation, hair thinning and very rarely, cardiac arrhythmias in predisposed individuals (239). The risk of gallstone formation with rapid weight loss is well documented but can be attenuated by ensuring adequate fat intake and by gradually transitioning off the diet when stopping the VLED (247). Contraindications to the VLED include pregnancy and breastfeeding, eating disorders, type 1 diabetes, unstable cardiac disease, porphyria, and significant hepatic or renal impairment (214). Close monitoring of patients with T2DM is essential, with pre-emptive reduction of insulin and sulfonylurea doses to prevent hypoglycaemia (214).

Cost and access considerations

Many brands of VLED products are readily available from pharmacies, however costs range from AUD $6.50 to $8.50 per day (3 products per day) for meal replacement shakes, and more for other formulations such as bars, which may be cost-prohibitive for some patients. A modified version of a VLED can be undertaken without using commercial meal replacement products, although very close monitoring and education is required to avoid nutritional deficiencies while also maintaining energy restriction. Other potential barriers to VLED use include the need for medical supervision from a medical practitioner or dietitian, which may be difficult to access in non-metropolitan areas, and there are limited Medicare rebates for allied health appointments, adding to the patient associated costs.

1.8.3 Pharmacotherapy for Weight Management

1.8.3.1 Incretin-Based therapies – GLP-1 receptor agonists

GLP-1 RAs have transformed the obesity pharmacotherapy landscape, with new long acting agents inducing weight loss approaching levels achieved by bariatric surgery. These drugs reduce body weight through multiple mechanisms, with peripheral GLP-1 effects including delayed gastric emptying and more centrally, through hypothalamic appetite circuits to reduce hunger and increase satiety(248). Two GLP-1 agents are currently approved for weight management and available in Australia; semaglutide (Wegovy, NovoNordisk) a long-acting GLP-1RA and tirzepatide (Mounjaro, Eli Lilly) a dual glucose-dependent insulinotropic polypeptide (GIP)/GLP-1 RA.

Evidence in obesity

The STEP studies established the efficacy of semaglutide 2.4mg weekly as a highly effective anti-obesity medication (Table 1.8.3.1.1). In STEP 1, participants with obesity (BMI ≥27 kg/m^2 with one weight related condition or BMI >30 kg/m^2) without T2DM, achieved 14.9% TBWL at 68 weeks, with 69.1% achieving ≥10% TBWL and 50.1% ≥15% TBWL (249). The SURMOUNT studies (Table 1.8.3.1.2) established tirzepatide as even more effective than semaglutide, with 15mg weekly

achieving 20.9% TBWL at 72 weeks in SURMOUNT 1, a cohort similar to STEP 1 (obesity without T2DM), with 57% achieving ≥20% TBWL, a threshold similar to that achieved through bariatric surgery (250). Weight loss is attenuated in patients with T2DM but significant weight loss can still be achieved with GLP-1s. STEP 2 and SURMOUNT 2 evaluated the efficacy of semaglutide and tirzepatide respectively, in participants with obesity and T2DM. STEP 2 demonstrated 9.6% TBWL at 68 weeks with semaglutide 2.4mg weekly, while SURMOUNT 2 found 14.7% TBWL with tirzepatide 15mg weekly (251, 252). SURMOUNT 5 was a head to head study comparing semaglutide to tirzepatide for the treatment of obesity in those without T2DM, using the highest tolerated doses of each (1.7-2.4mg/week semaglutide vs 10-15mg/week tirzepatide), and tirzepatide was found to have significantly greater weight loss of 20.2% vs 13.7% with semaglutide, without any significant difference in side effect profile (253).

While these medications are extremely effective, ongoing use is required to maintain their beneficial effects. STEP 4 followed patients who had been on semaglutide (2.4mg/week) for 20 weeks with participants then randomised to either continued semaglutide or switched to placebo with the placebo group experiencing a 6.9% weight gain after 48 weeks, compared to those who continued semaglutide who had a further weight loss of 7.9% (254). SURMOUNT 4 also demonstrated this loss of effect post cessation vs continuation, with those switched to placebo after a 36 week run in with highest tolerated dose tirzepatide, experiencing 14% weight regain, while those who continued tirzepatide had an additional 5.5% weight reduction after 52 weeks(255). These findings confirm that ongoing treatment is required to maintain weight loss in the majority of cases.

8. Table 1.8.3.1.1 Summary of STEP trials – Semaglutide for treatment of Obesity

Trial	**Population**	**Design**	**Duration**	**Weight change (Drug)**	**Weight change (Comparator)**	**Reference**
STEP 1	Obesity without T2DM (n=1961)	Semaglutide 2.4mg weekly vs placebo	68 weeks	-14.9%	-2.4% (placebo)	Wilding et al NEJM 2021 (249)
STEP 2	Obesity with T2DM (n=1210)	Semaglutide 2.4mg weekly vs placebo	68 weeks	-9.6%	-3.4% (placebo)	Davies et al Lancet 2021 (251)
STEP 3	Obesity without T2DM (n=611)	Semaglutide 2.4mg weekly + intensive behavioural therapy vs placebo + intensive	68 weeks	-16%	-5.7% (placebo +intensive behavioural therapy))	Wadden et al JAMA 2021 (256)

		behavioural therapy				
STEP 4	Obesity without T2DM (n=803)	Semaglutide 2.4mg 20-week run-in, then randomised to semaglutide continuation or switch to placebo for 48 weeks	68 weeks (including run-in period)	-7.9% (from point of randomisation)	+6.9% (from point of randomisation)	Rubino et al JAMA 2021 (254)
STEP 5	Obesity without T2DM (n=304)	Semaglutide 2.4mg vs placebo	104 weeks	-15.2%	-2.6% (placebo)	Garvey et al Nat Med 2022 (257)

9. Table 1.8.3.1.2 Summary of SURMOUNT trials – Tirzepatide for the treatment of Obesity

Trial	**Population**	**Design**	**Duration**	**Weight change (Drug)**	**Weight change (Comparator)**	**Reference**
SURMOUNT 1	Obesity without T2DM (n=2539)	Tirzepatide 15mg vs placebo	72 weeks	-20.9%	-3.1% (placebo)	Jastreboff et al NEJM 2022 (250)
SURMOUNT 2	Obesity with T2DM (n=938)	Tirzepatide 15mg vs placebo	72 weeks	-14.7%	-3.2% (placebo)	Garvey et al Lancet 2023 (252)
SURMOUNT 3	Obesity without T2DM (579)	12-week intensive lifestyle run-in then tirzepatide 15mg vs placebo	84 weeks (including run-in period)	-18.4% (from point of randomisation)	+2.5% (from point of randomisation)	Wadden et al Nat Med 2023 (258)
SURMOUNT 4	Obesity without T2DM (n=670)	Tirzepatide 10-15mg 36-week run-in, then randomised to tirzepatide continuation or switch to placebo for 48 weeks	88 weeks (including run-in period)	-5.5% (from point of randomisation)	14%% (from point of randomisation)	Aronne et al JAMA 2024 (255)
SURMOUNT 5	Obesity without T2DM (n=751)	Tirzepatide 10-15mg vs Semaglutide 1.7-2.4mg	72 weeks	20.2%	-13.7% (semaglutide)	Aronne et al NEJM 2025 (253)

Metabolic and cardiovascular benefits

Beyond weight loss, GLP-1s produce clinically significant improvements in cardiometabolic parameters, with the STEP and SURMOUNT trials demonstrating improvements in waist circumference, blood pressure, triglycerides, HbA1c and improvements in markers of inflammation. The SELECT trial demonstrated significant benefit with semaglutide in secondary prevention of cardiovascular events in patients with obesity and pre-existing cardiovascular disease (but without T2DM) (259). Semaglutide 2.4mg weekly reduced major adverse cardiovascular events by 20% compared with placebo over a mean follow-up of 39.8 months (hazard ratio 0.80, 95% CI 0.72 to 0.90) (259). This demonstrates that treating obesity pharmacologically not only reduces weight, but also improves cardiovascular morbidity and mortality. Evidence is still emerging as to whether this is a class effect of all GLP-1 obesity medications, or if semaglutide is more effective than tirzepatide for this specific endpoint.

The effects of incretin-based therapies on the liver in MASLD have previously been outlined in section 1.7.3.

Safety, tolerability and contraindications

The most common adverse effects of GLP-1 RAs are gastrointestinal, and include nausea (40-50%, vomiting (20-30%), diarrhoea (30%) and constipation (20-25%). These are typically mild to moderate in severity and occur predominantly during the initiation and dose-escalation stage of treatment, with the majority of side effects noted to resolve with continued treatment(248). In STEP 1, 4.5% of semaglutide patients discontinued due to gastrointestinal side effects vs 0.8% in placebo, and in SURMOUNT 1, 6.2% discontinued tirzepatide due to adverse effects vs 2.6% in placebo (249, 250). Less common but clinically important adverse effects include cholelithiasis and cholecystitis which occur at around twice the rate of placebo and acute pancreatitis which is rare and occurs in approximately 0.2-0.3% (260). There is also a theoretical concern regarding thyroid C-cell tumours based on animal models, however human relevance is uncertain but GLP-1 medications are generally avoided in patients with multiple endocrine neoplasia type 2 (MEN2) due to possible risk of medullary thyroid carcinoma (260).

Worsening of diabetic retinopathy has been reported inconsistently with GLP-1 use, and no harmful retinal effects have been found in those without T2DM (261). An association between nonarteritic anterior ischaemic optic neuropathy (NAION) and GLP-1s has been observed, but further evidence is required to determine a clear link to GLP-1 use (262).

Access and cost in Australia

No anti-obesity medication is currently listed on the Pharmaceutical Benefits Scheme (PBS) for weight management, meaning patients must pay the full private prescription cost, which remains the largest barrier to access of these medications in Australia. Semaglutide (Wegovy) costs up to $400 per month, and tirzepatide (Mounjaro) $700 per month for the highest doses. This substantially limits equitable access to effective treatment, particularly for patients from lower socioeconomic backgrounds who bear the greatest burden of obesity-related complications (108).

1.8.3.2 Other anti-obesity medications

Several other medications are TGA-approved or used off-label for weight management in Australia, and like Wegovy and Mounjaro are available via a non-subsidised private prescription. These agents produce more modest weight loss than GLP-1 RAs but may still be appropriate when cost, access or contraindications preclude GLP-1 use. These are outlined in Table 1.8.3.2.

10. Table 1.8.3.2. Non GLP-1 obesity medications available in Australia

Medication	Mechanism	Mean weight loss in clinical trials	Approx. monthly cost	Key considerations
Orlistat (Xenical)	Gastrointestinal lipase inhibitor (reduces fat absorption)	3-5%	$100	• Needs to be taken with every main meal • Available without prescription • SE: gastrointestinal side effects (steatorrhoea, faecal urgency), can cause fat soluble vitamin malabsorption
Phentermine (Duromine)	Sympathomimetic appetite suppressant	5-10%	$90-150	• Potential for dependence • Contraindicated in uncontrolled hypertension, CVD, arrhythmias • SE: insomnia, tachycardia, dry mouth
Naltrexone/bupropion (Contrave)	Opioid antagonist + dopamine/noradrenaline reuptake inhibitor	5-8%	$250	• Contraindicated with opioid use, uncontrolled hypertension, seizure disorders, eating disorder • May increase blood pressure, concerns about potential worsening of mood
Topiramate	Anticonvulsant, thought to have appetite suppressing effect, causes altered taste	5-9%	$10-25	• SE: cognitive impairment 'brain fog', paraesthesia, nephrolithiasis, metabolic acidosis • Teratogenic – contraception required • Some evidence for helping binge eating disorder

Source: Markovic 2022 (214)

Emerging therapies

The therapeutic landscape for obesity continue to evolve rapidly. Oral GLP-1 formulations are in development for obesity, potentially improving convenience and patient uptake compared to the current subcutaneously administered formulations. Next-generation agents combining GLP-1 with additional receptor targets, including amylin analogues (e.g. cagrilintide/semaglutide), glucagon co-agonists (e.g. survodutide), and triple agonists (e.g. GLP-1/GIP/glucagon agents like retatrutide), are progressing through phase 3 studies, with phase 2 data suggesting even greater weight loss potential than with currently available agents. With the continued development of multiple effective agents, it is hoped that a more competitive market may make these medications more affordable and accessible for those who would benefit most.

Chapter 2. Aims and Hypothesis

2.0 Knowledge gaps identified

The evidence reviewed in Chapter 1 highlights several gaps in current knowledge regarding MASLD diagnosis and treatment. Australian epidemiological data on MASLD prevalence and fibrosis risk are limited, with no nationwide estimates using contemporary diagnostic criteria. Furthermore, current treatment guidelines emphasise lifestyle modification without adequate acknowledgement of the physiology of obesity, or incorporation of evidence-based weight management approaches that have demonstrated efficacy in metabolic disease. Addressing these gaps formed the basis of the research presented in Chapters 3-6.

2.1 Aims and Hypothesis

2.1.1 Epidemiological analysis of MASLD

As previously outlined in Section 1.4.3, the AusDiab (Australian Diabetes, Obesity and Lifestyle) study is a nationwide population-based cohort study designed to investigate the prevalence of diabetes, obesity, hypertension and kidney disease in Australian adults. In the third follow-up survey of the cohort, which was performed in 2012, liver enzymes were assessed for the first time, representing an opportunity to describe MASLD in a large and well-characterised cohort.

The aim of this study (Chapter 3) was to describe the frequency of ALT elevation and MASLD in the AusDiab study, a nationwide Australian cohort, using validated non-invasive diagnostic markers, and identify factors independently associated with both. A secondary aim was to describe the proportion of those with MASLD at risk of advanced fibrosis using the BARD score. It was hypothesised that MASLD would be highly prevalent in this cohort and that ALT would be associated with T2DM and features of the metabolic syndrome.

2.1.2 Real world medical weight management

St Vincent's Hospital Melbourne weight management clinic is one of a limited number of publicly-funded specialist obesity services offering medical weight management and staffed by physicians from gastroenterology, endocrinology and general medicine, alongside support from a dietitian. The clinic follows the medical obesity management approach recommended by Australian clinical practice guidelines. A retrospective audit was performed to determine the effectiveness of this approach in a real-world setting (Chapter 4).

This study aimed to describe the cohort seeking medical weight management through a publicly-funded specialist weight management service, and determine weight loss outcomes achieved. Secondary outcomes included examining predictors of weight loss outcomes and loss to follow-up. It was hypothesised that the medical weight loss methods were effective, and that clinically significant weight loss would be achievable in this real-world setting, but that patient engagement and clinic retention were challenging.

2.1.3 Adapting medical weight management strategies to MASLD

The DARWIN (The Effect of Diet on Appetite Regulation, Weight and Steatosis in Individuals with Nonalcoholic Fatty Liver Disease) trial was a randomised pilot study conducted at St Vincent's Hospital Melbourne, designed to compare a ketogenic VLED to a MD in adults with biopsy-proven MASLD and overweight or obesity (BMI 27-35kg/m^2). The trial comprised a 12-week dietary intervention weight loss phase, followed by a 12-week maintenance phase in which the VLED group transitioned to a portion-controlled diet alongside low-dose semaglutide (0.5mg weekly). Outcomes were assessed again at week 48, 24 weeks after cessation of all active intervention to assess for durability of treatment response.

The primary aim of the DARWIN study was to determine the efficacy of a 12-week ketogenic VLED compared to a 12-week MD in reducing hepatic steatosis defined by MRI-liver fat fraction (MRI-LFF) in adults with MASLD and overweight or obesity (Chapter 5). Secondary aims included the evaluation of weight loss, body composition and metabolic profile including liver enzymes. Effect of the semaglutide maintenance strategy and change in liver histology was also assessed as a secondary aim at week 24. The subsequent extension study (Chapter 6) aimed to evaluate the durability of these benefits to week 48.

It was hypothesised that the VLED group would achieve greater hepatic steatosis and weight reduction than the MD and that low-dose semaglutide would assist in maintaining these benefits during the transition off the diet. It was also hypothesised that while more modest, the MD would also result in improvements to hepatic steatosis and metabolic profile with the MD considered to be a more enduring dietary pattern with benefits persisting in the longer term extension study.

Chapter 3. A problem of proportions: estimates of metabolic associated fatty liver disease and liver fibrosis in Australian adults in the nationwide 2012 AusDiab Study

This chapter is presented as the final published version of a manuscript published in the peer reviewed journal Scientific Reports.

This paper estimates the frequency of elevated ALT and MAFLD in Australia by examining a large Australian cohort. It uses MAFLD terminology throughout, as appropriate for the time of publication and regionally preferred nomenclature.

Reference:

Farrell A, Magliano D, Shaw J, Thompson A, Croagh C, Ryan M and Howell J.
A problem of proportions: estimates of metabolic associated fatty liver disease and liver fibrosis in Australian adults in the nationwide 2012 AusDiab Study. (2022) Scientific Reports, DOI: 10.1038/s41598-022-05168-0

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A problem of proportions: estimates of metabolic associated fatty liver disease and liver fibrosis in Australian adults in the nationwide 2012 AusDiab Study

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3.0 Abstract

Metabolic Associated Fatty Liver Disease (MAFLD) is the most common cause of liver disease in Australia, but prevalence data are limited. We aimed to describe the frequency of alanine aminotransferase (ALT) elevation, and MAFLD within a large prospective Australian cohort. Crosssectional analysis of the 2012 survey of the Australian Diabetes, Obesity and Lifestyle (AusDiab) study which included 4747 Australian adults (aged 34–97 yrs) was performed. Frequency of ALT elevation (men ≥ 40 IU/L, women ≥ 30 IU/L) and MAFLD (Fatty Liver Index (FLI) > 60 alongside metabolic risk factors) was determined and risk of advanced fibrosis stratified using the BARD score. Elevated ALT was found in 13% of the cohort, including 22% of people with diabetes, 18% with obesity, and 17% with the metabolic syndrome. 37% of the cohort had MAFLD, and those with MAFLD were more likely to be older (OR 1.01 per 1 year (95% CI 1.00–1.02)), male (OR 1.37 (95% CI 1.17–1.59)), have ALT elevation (OR 3.21 (95% CI 2.59–3.99)), diabetes (OR 3.39 (95% CI 2.61–4.39)), lower HDL-C (OR 0.15 per 1 mmol/L (95% CI 0.12–0.19)), higher diastolic blood pressure (OR 1.05 per 10 mmHg (95% CI 1.05– 1.06)), a sedentary lifestyle (OR 1.99 (95% CI 1.59–2.50)) and less likely to have tertiary education (OR 0.81 (95% CI 0.7–0.94) compared to those without MAFLD. Of those with MAFLD, 61% had a BARD score suggesting risk of advanced fibrosis and 22% had an elevated ALT. Over 10% of this Australian cohort had elevated ALT, and 37% had MAFLD, with many at risk for advanced fibrosis.

Metabolic associated fatty liver disease (MAFLD) is the most common cause of liver disease, with a quarter of the global population estimated to be affected [1]. Due to the high global prevalence and rapid rise in the incidence of MAFLD in low-middle and high income countries, MAFLD is emerging as a major threat to national and global health [1,2]. The term MAFLD encompasses a spectrum of liver diseases characterised by excessive hepatic fat

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accumulation in the absence of significant alcohol use or other secondary causes of steatosis[3]. Hepatic steatosis can lead to steatohepatitis (MASH), and progress to hepatic fibrosis and cirrhosis [1,2]. MAFLD has also become the most rapidly increasing cause of hepatocellular carcinoma (HCC)[4–6]. Despite this, to date there are no approved medical treatments for MAFLD, with weight loss the only intervention that has been proven to be of benefit[3,7].

3.1 Introduction

MAFLD is the most common cause of elevated aminotransferase levels in developed and developing countries, especially in primary care[7–10]. In particular, elevated serum alanine aminotransferase (ALT) levels account for a significant proportion of the indication for referrals to specialist liver services[11]. While elevated ALT indicates liver damage, it has also been shown in cross-sectional studies to correlate with obesity and features of the metabolic syndrome[12,13]. Additionally, prospective cohort studies have consistently demonstrated that elevated ALT levels are associated with increased risk of developing the metabolic syndrome and type 2 diabetes (T2DM)[14–17].

To date, there are limited data regarding the prevalence of elevated ALT and MAFLD within the Australian population. Previous Australian cohort studies have estimated the burden of MAFLD based on the frequency of ALT elevation, which occurred in 9–11% of adults and was associated with obesity, younger age, and features of higher cardiometabolic risk [12,13]. The recent Crossroads study performed in a single community in regional Victoria found MAFLD prevalence to be 36% using the fatty liver index, affecting a higher proportion of men and those with metabolic risk factors [18].

In this cross sectional analysis, we describe the frequency of ALT elevation and MAFLD, using non-invasive diagnostic markers of hepatic steatosis in a large population-based Australian cohort. Additionally, we aimed to identify factors independently associated with an elevated ALT and to describe the proportion of MAFLD patients at risk of having advanced fibrosis.

3.2 Methodology

Study design. We undertook a cross-sectional analysis of individuals who had liver function tests (LFTs) performed in the 2012 survey (3rd follow up) of the Australian Diabetes, Obesity and Lifestyle (AusDiab) study. AusDiab is a nationwide population-based study, designed to investigate the prevalence of diabetes, obesity, hypertension and kidney disease in Australian adults(age ≥ 25 years). The original cohort were selected to be representative of the general Australian population at the time. The AusDiab study design, and methodology has been detailed elsewhere, with the baseline data collection performed in 2000, and follow up surveys in 2005 and 2012[19,20]. The sampling selection of the 2012 cohort is outlined in Supplementary Figure 1. This research was approved by the Human Research Ethics Committee of St Vincent's Hospital Melbourne and all participants of the AusDiab study provided informed consent. All methods were conducted in accordance with the relevant guidelines and regulations.

LFTs were performed for the first time in this cohort in 2012, and other variables obtained in the 2012 survey included measures of obesity (body mass index (BMI) and waist circumference (WC)), and markers of metabolic health including blood pressure, lipid profiles, oral glucose tolerance tests and haemoglobin A1c (HbA1c).

Data collection. Blood sampling was performed following an overnight fast (≥ 8 h) and blood specimens were collected in fluoride/oxalate tubes for glucose, and serum separator tubes for LFTs and lipids, which were centrifuged with serum/plasma separated on-site. The samples were transported to the central laboratory that day, however when not possible, were immediately frozen for transport. All analyses were conducted at a central laboratory (Healthscope Pathology, Victoria).

Diabetes was classified as a fasting plasma glucose of ≥ 7.0 mmol/l or two-hour post-load plasma glucose level of ≥ 11.1 mmol/l or receiving treatment with an antidiabetic agent as per the World Health Organization (WHO) guidelines [21]. Metabolic syndrome was defined using the International Diabetes Federation (IDF) guidelines[22]. Overweight and obesity were defined using the WHO classification for BMI (overweight 25–29 kg/ m^2, obese > 30 kg/m^2) and WC (overweight F > 80 cm/M > 94 men, obese F > 88/M > 102 cm), and physical activity and sedentary behaviour were assessed by questionnaire [23]. Alcohol intake was obtained from self-administered Food Frequency Questionairres (FFQ) which was developed and validated by the cancer council of Victoria [24]. Excess alcohol intake was defined as ≥ 30 g per day for men and ≥ 20 g per day for women as per the EASL guidelines[3].The definition of elevated ALT is not consistently defined in the literature, and can vary significantly between studies. In the largest Australian cohort to date (the Australian Health Survey (AHS) study), ALT elevation was defined as ≥ 40 IU/L in men and ≥ 30 IU/L in women[13]. We defined ALT elevation by this same threshold in order to present comparable local data.

Definition of MAFLD and risk of advanced fibrosis. MAFLD was defined using the diagnostic criteria proposed by Eslam et al., with hepatic steatosis determined using the Fatty Liver Index (FLI) and MAFLD diagnosed in those with steatosis alongside at least one of the following: BMI ≥ 25, presence of T2DM or BMI < 25 with at least two metabolic risk factors[25]. Using the Fatty Liver Index (FLI), a diagnostic algorithm incorporating BMI, WC, serum triglyceride and gamma-glutamyl transferase (GGT), score of > 60 had been determined as the threshold for detecting hepatic steatosis in Caucasian cohorts (AUROC 0.84)[26–29].Within the criteria for metabolic risk factors, data were not available for two of the seven criteria: high-sensitivity C-reactive protein (hs-CRP) and the homeostasis model assessment of insulin resistance score (HOMA-IR). The BARD score (BMI ≥ 28 = 1 point, AST/ALT ratio (AAR) of ≥ 0.8 = 2 points, type 2 diabetes mellitus (T2DM) = 1 point) was used to identify those with MAFLD at low risk of advanced fibrosis. A score of ≥ 2 is associated with advanced fibrosis, while a score of < 2 has a high negative predictive value for ruling out significant fibrosis of 96%[30]. **Statistical analysis.** Baseline (2012) characteristics were compared between those with and without an elevated ALT. The distribution of data was determined using the Shapiro-Francia test, with non-parametric data presented as median with interquartile range, proportions compared using the chi-squared test, and comparison of continuous variables performed using Wilcoxon rank sum test for those with a non-parametric distribution. For variables with highly skewed distribution (serum triglycerides), log transformation was performed. Odds ratios were calculated for the association between elevated ALT levels and age, gender, smoking status, BMI, WC, diabetes, systolic and diastolic blood pressure, triglycerides, low-density lipoprotein cholesterol (LDL-C), highdensity lipoprotein cholesterol (HDL-C), metabolic syndrome and exercise levels. An explanatory multivariable logistic regression model was developed using backward elimination and likelihood ratio testing, including

	Normal ALT	Elevated ALT †	Crude OR (95% CI)	Adj OR (95% CI) ‡

Age (years/OR per 1 year)	61 (53–69)	57 (51–63)	0.97 (0.96–0.97)	0.96 (0.95–0.96)
Gender				
Female	55% (n = 2290)	57% (n = 338)	1 (reference)	
Male	45% (n = 1860)	43% (n = 259)	0.94 (0.79–1.12)	0.64 (0.53–0.78)
Smoker (vs non-smoker)	6% (n = 230)	5% (n = 26)	0.77 (0.51–1.17)	
BMI (kg/m^2/OR per 1 kg/m^2)	27 (24–30)	29 (26–33)	1.08 (1.06–1.09)	§
Waist circ (cm/OR per 1 cm)	93.5 (84–103)	99.3 (90–111)	1.03 (1.02–1.04)	1.02 (1.02–1.03)
T2 Diabetes (vs no T2DM)	9% (n = 360)	17% (n = 104)	2.22 (1.75–2.82)	1.89 (1.43–2.49)
Systolic BP (mmHg/OR per 10 mmHg)	128 (116–142)	130 (118–142)	1.00 (1.00–1.01)	
Diastolic BP (mmHg/OR per 10 mmHg)	72 (65–79)	76 (69–83)	1.03 (1.02–1.04)	1.01 (1.01–1.02)
Triglycerides¶ (mmol/L/OR per 1 mmol/L)	1.1 (0.8–1.6)	1.4 (1–1.9)	2.23 (1.89–2.64)	1.69 (1.39–2.06)
LDL (mmol/L/OR per 1 mmol/L)	3 (2.4–3.6)	3 (2.4–3.7)	1.05 (0.95–1.16)	
HDL (mmol/L/OR per 1 mmol/L)	1.5 (1.2–1.8)	1.4 (1.1–1.7)	0.51 (0.41–0.63)	
Metabolic syndrome (vs no metabolic syndrome)	38% (n = 1436)	53% (n = 297)	2.03 (1.7–2.45)	§
Exercise				
Sufficient	64% (n = 2585)	58% (n = 336)	1 (reference)	1 (reference)
Insufficient	25% (n = 1019)	26% (n = 152)	1.15 (0.94–1.41)	0.97 (0.78–1.21)
Sedentary	11% (n = 440)	16% (n = 92)	1.61 (1.07–1.25)	1.32 (1.01–1.73)

Table 3.1. Metabolic and biochemical profiles of participants according to ALT level: the AusDiab study. † Elevated ALT defined at > 40 IU/L in men and > 30 IU/L in women. ‡ Multivariable analysis performed by logistic regression examining ALT elevation adjusted for age, gender, WC, T2DM,diastolic blood pressure, triglycerides and exercise. § Not included in multivariable model due to collinearity: BMI collinear with WC, metabolic syndrome collinear with multiple included variables (WC, lipids, blood pressure). ¶ Log transformed variable used for crude and adjusted OR.

variables that were associated with elevated ALT levels (p value < 0.1) on univariable analysis. Gender was not included in the multivariable ALT models due to implied bias of the gender specific ALT thresholds used. BMI and diagnosis of the metabolic syndrome were not included due to collinearity with other included variables (BMI collinear with WC and metabolic syndrome with the individual components of this diagnosis e.g. blood pressure). Where a linear test for trend was confirmed, ordinal variables were included as continuous variables in the model. The same modelling steps were performed for the association between MAFLD and age, gender, smoking, diabetes, systolic and diastolic blood pressure, LDL-C, HDL-C, ALT elevation at the higher threshold and exercise levels. Analysis was performed using Stata 15 software (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC).

Ethics. This study has ethical approval through St Vincent's Hospital Melbourne (LRR 225/20, project ID 69047).

3.3 Results

Baseline cohort and characteristics. The AusDiab study recruited 11,247 adults aged 25 and older for the baseline survey performed in 1999/2000, with 4747 returning for the 3rd follow up in 2012. Among these 4747 participants, the median age was 60 years (IQR 53–68), 55% were female, 10% had T2DM, and two-thirds were overweight (41%) or obese (27%) according to BMI. The majority of participants identified as Caucasian ethnicity (93%), were born in Australasia or the United Kingdom including Northern Ireland (90%), and 66% lived in metropolitan capital cities.

Elevated ALT is associated with many features of the metabolic syndrome.

Elevated ALT (≥ 40 IU/L in men and ≥ 30 IU/L in women) was found in 13% of the cohort (n = 597), including 22% of people with diabetes, 20% of obese participants, and 17% of those with the metabolic syndrome. Distribution of clinical variables between those with and without elevated ALT levels is shown in Table 3.1. Those with elevated ALT were younger (57 years vs 61 years, $p \leq 0.001$), with a higher median BMI (29 vs 27, $p \leq 0.001$), more T2DM (17% vs 9%, $p \leq 0.001$), and a greater proportion met the criteria for the metabolic syndrome (53% vs 38%, $p \leq 0.001$) compared to those without ALT elevation.

On multivariable logistic regression, female gender (OR 1.56, 95% CI 1.27–1.9), higher diastolic blood pressure (OR 1.01 per 10 mmHg, 95% CI 1.01–1.02), elevated triglyceride levels (OR 1.69 per 1 mmol/L, 95% CI 1.39–2.06), T2DM (OR 1.89, 95% CI 1.43–2.49), sedentary lifestyle (OR 1.32, 95% CI 1.01–1.73) and greater WC (OR 1.02 per cm, 95% CI 1.02–1.03), were independently associated with an elevated ALT. The relationship between age and ALT was non-linear, with ALT elevation occurring most commonly in middle age (Fig. 1).

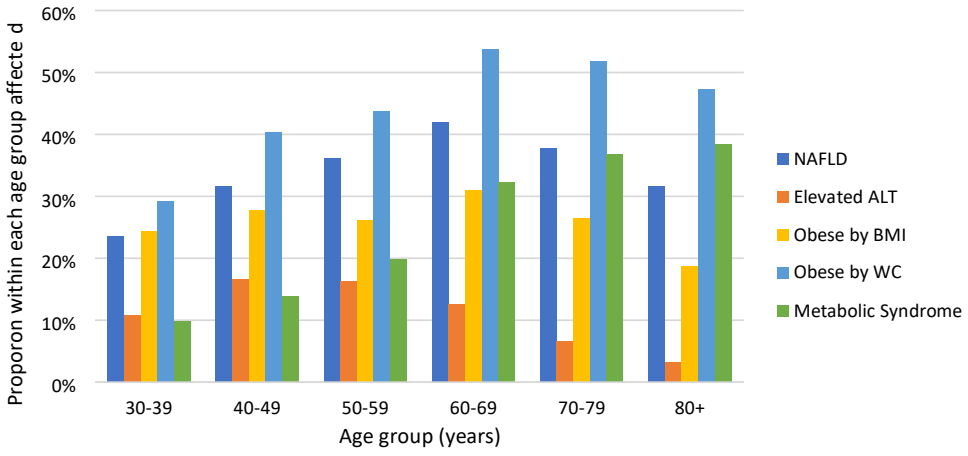

Figure 3.1. Frequency of mafld, alt elevation, obesity and the metabolic syndrome by age groups.

	MAFLD	No MAFLD	Crude OR (95% CI)	Adj OR (95% CI)†
Age (years/OR per 1 year)	61 (54–68)	60 (52–68)	1.01 (1.00–1.01)	1.01 (1.00–1.02)

Male gender (vs female)	58%	37%	2.32 (2.05–2.62)	1.37 (1.17–1.59)
Smoker (vs non-smoker)	6%	5%	1.20 (0.93–1.57)	
T2 Diabetes (vs no T2DM)	19%	4%	5.43 (4.37–6.76)	3.39 (2.61–4.39)
Systolic BP (mmHg/OR per 10 mmHg)	133 (122–146)	123 (113–137)	1.02 (1.02–1.03)	
Diastolic BP (mmHg/OR per 10 mmHg)	76 (70–83)	69 (63–76)	1.06 (1.06–1.07)	1.05 (1.05–1.06)
LDL (mmol/L/OR per 1 mmol/L)	3 (2.3–3.7)	3 (2.4–3.6)	1 (0.93–1.07)	
HDL (mmol/L/OR per 1 mmol/L)	1.3 (1.1–1.5)	1.6 (1.3–1.9)	0.10 (0.09–0.13)	0.15 (0.12–0.19)
ALT elevation	22%	7%	3.57 (2.98–4.28)	3.21 (2.59–3.99)
(≥ 40 IU/L and ≥ 30 IU/L vs no ALT elevation)				
Exercise				
Sufficient	55%	69%	1 (reference)	1 (reference)
Insufficient	29%	23%	1.57 (1.36–1.81)	1.43 (1.21–1.69)
Sedentary	16%	8%	2.34 (1.93–2.83)	1.99 (1.59–2.50)
Post-secondary education (vs primary/secondary education only)	32%	40%	0.72 (0.63–0.81)	0.81 (0.7–0.94)

Table 3.2. Crude and adjusted odds ratios of variables associated with mafld. † Multivariable analysis performed by logistic regression examining MAFLD by FLI adjusted for age, gender, diastolic BP, T2DM, HDL cholesterol, ALT elevation, exercise and level of education. NB: BMI, waist circumference, triglycerides and GGT not included in the model due to collinearity as these variables are included in the FLI diagnostic algorithm.

MAFLD and markers of advanced fibrosis. 37% of the cohort had MAFLD. Those with MAFLD were older (OR 1.01 per year, 95% CI 1.01–1.02), more likely to be male (OR 1.37, 95% CI 1.17–1.59), have T2DM (OR 3.39, 95% CI 2.61–4.39), lower HDL-C (OR 0.15 per 1 mmol/L, 95% CI 0.12–0.19) and a higher diastolic blood pressure (OR 1.06 per 10 mmHg, 95% CI 1.05–1.06) compared to those without MAFLD. A sedentary lifestyle (OR 1.99, 95% CI 1.59–2.50) and not completing any post-secondary education (OR 1.24, 95% CI 1.06– 1.44) were also independently associated with MAFLD on multivariable logistic regression analysis (Table 3.2). There was no difference in the frequency of MAFLD between those living in metropolitan and regional or rural areas (36% vs 38%, p = 0.171).

Participants with ALT elevation (≥ 40 IU/L in men and ≥ 30 IU/L in women), were over 3 times more likely to have MAFLD (OR 3.57, 95% CI 2.98–4.28) compared to those without ALT elevation. Elevated ALT was found in 22% of those with MAFLD. The diagnostic criteria for MAFLD correlated strongly with the presence of hepatic steatosis by FLI, with only two participants with hepatic steatosis not meeting criteria for MAFLD due to BMI < 25 and the presence of only one metabolic risk factor. Excess alcohol consumption (≥ 30 g/day men, ≥ 20 g/ day women) was found in 22% of those with MAFLD and 20% of the total cohort.

In those with MAFLD, the BARD score ruled out advanced fibrosis in 38%. 61% had a BARD score ≥ 2 identifying them as at possible risk of advanced fibrosis and a high BARD score was also found in 35% of those with MAFLD who also had an elevated ALT.

3.4 Discussion

In this large Australian sample we found a high frequency of ALT elevation (13%) and MAFLD (37%); with ALT elevation more common than has previously been described among Australian adults in the community. The Australian Health Survey (AHS), and Busselton cohorts found the frequency of ALT elevation to be lower at 11% and 9% respectively, with the AHS study noting the same independent relationship with younger age, diabetes, higher triglycerides and greater WC, consistent with the existing international literature[12,13]. All three cohorts examined ALT in a cross-sectional manner at a single timepoint, however these previous cohorts were younger (mean ages being; 43–46 years in AHS, 52yrs in Busselton, 60yrs in AusDiab), with fewer participants with diabetes (4.3–6.9% in AHS vs 10% in AusDiab), or obesity (17% in Busselton vs 27% in AusDiab), all factors likely to affect the frequency of ALT elevation in each cohort. Additionally, the Busselton study used a higher ALT threshold, > 40 IU/L for both men and women, which may also contribute to these differences.

The proportion of adults with elevated ALT was shown to decrease with advancing age independent of gender, alcohol use and the metabolic syndrome in a large longitudinal study, which was thought to be the result of diminishing liver and skeletal muscle mass associated with aging [31]. Similarly, in our sample the frequency of ALT elevation decreased in those over the age of 60, despite features of the metabolic syndrome increasing, and MAFLD remaining constant in this age stratum. This age disparity shows that while ALT elevation is associated with similar metabolic risk factors such as diabetes, hypertension and dyslipidaemia, it is an imperfect surrogate marker for MAFLD as it may under recognise MAFLD in older patients and additional diagnostic methods are often required[17,32,33].

Estimating the prevalence of MAFLD is difficult, partly due to the absence of a widely available, non-invasive and accurate diagnostic test. MAFLD has a reported global prevalence of 25%, with estimates based on composite data from a range of studies with heterogenous diagnostic criteria that include radiological methods such as ultrasound, ALT elevation or clinical scoring systems such as the FLI [1]. To date, there are limited data about the prevalence of MAFLD in Australia. We present the first MAFLD estimates using the criteria proposed by Eslam et al. alongside a validated diagnostic biomarker to detect hepatic steatosis in an Australia-wide cohort [28]. Our results are consistent with those of the Crossroads study, performed in a single area in regional Victoria, which found the age- and sex- standardized prevalence of MAFLD to be 36% using FLI[18]. Our cohort predominantly lived in metropolitan capital cities (66%), with no difference found in the rates of MAFLD compared to those living in regional and rural Australia, further informing our understanding of the frequency of MAFLD throughout the Australian population. Like the Crossroads study, our cohort was predominantly Caucasian, and the European threshold of FLI > 60 was used in both studies. FLI uses a combination of clinical and biochemical factors to develop a score with good accuracy for predicting the presence of radiologically-evident hepatic steatosis with an AUC-ROC of 0.85[26]. While FLI does not predict the degree of liver fat, and has predominantly been validated against ultrasound and MRI, it is a useful diagnostic tool in epidemiologic research, having been widely used in this setting. The MAFLD nomenclature has arisen due to the current variability in the diagnosis of fatty liver in clinical practice and the desire to recognise it as a spectrum of disease which can co-exist alongside other liver diseases such as alcohol. When applying the MAFLD diagnostic criteria to our cohort, data was not available for two of the seven criteria for metabolic risk in those with BMI < 25 (hs-CRP and HOMA-IR), however these are not widely used screening tests in clinical practice and are unlikely to alter our findings. These data offer useful clinical information about the degree of MAFLD in a large Australian

cohort, enriched with older participants, in the absence of radiological population-based screening studies in Australia [34].

The high proportion of MAFLD in our study, is likely to be reflective of the advanced age and high frequency of overweight and obesity (69%) and diabetes (10%) in our cohort. While this is higher than that found in the general Australian population aged 18 years and over (63% and 4% respectively), it was similar to those of similar age strata in the nationally representative Australian National Health Survey which was also performed in 2012[35]. The high frequency of ALT elevation and MAFLD in our cohort is concerning from a public health perspective. How we best identify individuals at greatest risk of advanced fibrosis, and therefore at the greatest risk of morbidity and mortality, is a priority. Non-invasive fibrosis estimation algorithms, are widely used to identify patients at risk of advanced fibrosis who should be referred for specialist hepatology review. We used the BARD score which was able to be performed with the information available, and has been validated in multiple cohorts to perform similarly to the more widely known MAFLD fibrosis score [36,37]. These non-invasive markers tend to be sensitive but less specific for advanced fibrosis, and MAFLD alongside elevation of ALT is a more commonly used clinical trigger for specialist referral, as ALT elevation in MAFLD is associated with a higher risk of developing cirrhosis[11,38]. A significant proportion of our cohort were found to have MAFLD with an elevated ALT (8%), although the BARD score ruled out advanced fibrosis in 38% of those with MAFLD. Those with an elevated BARD score would benefit from further investigation with another non-invasive marker of fibrosis or a second-line investigation such as elastography. Additionally, as the obesity epidemic continues to grow, with rates of overweight and obesity in Australia increasing by 4% between 2012 and the most recent national health survey data in 2018, the associated MAFLD burden is also likely to increase even further [35]. Future studies examining the true prevalence of MAFLD and advanced fibrosis in the Australian population incorporating multiple noninvasive markers of fibrosis, alongside radiological or histological criteria to validate these findings are warranted.

There are some limitations to this study. This is a cross-sectional analysis of a follow up survey conducted as part of the larger prospective AusDiab study, and a predominantly Caucasian cohort. As a result, there may be selection and survival bias inherent within the cohort, and our findings may not be generalisable to the wider Australian population or other more ethnically diverse groups. Additionally, information about other known liver disease was not collected in 2012. However this is less relevant when the definition of MAFLD is applied, which allows for co-existing liver diseases. Furthermore, the community prevalence of viral hepatitis in Australia is relatively low (1–2%), with high risk populations such as migrants from endemic regions and people who inject drugs likely under-represented in this cohort[39]. A strength of this study was the ability to incorporate diagnostic and prognostic MAFLD algorithms, and to our knowledge this is the first large Australia-wide study to estimate MAFLD and risk of advanced fibrosis using validated clinical algorithms. This cohort is older than previously reported population-based cohorts examining ALT elevation, contributing to our current understanding of the association between metabolic health and liver enzymes in the Australian population across the lifespan. This information is important in informing the public health response to the growing healthcare burden associated with MAFLD.

3.5 Conclusion

The frequency of MAFLD and elevated ALT was high in this large nationwide Australian cohort, with these figures expected to rise with the growing obesity epidemic. These numbers are important in healthcare resource planning, as the demand is likely to exceed the capacity of specialist liver services in the future.

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Author contributions

A.F., J.H., M.R., A.T. and C.C. conceived of the presented idea. D.M. and J.S. are lead investigators of the AusDiab study. A.F., D.M., J.S., A.T., C.C., M.R. and J.H. contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

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Chapter 4. Metabolic predictors of weight loss response in severe obesity: a retrospective cohort study from an Australian specialist weight management clinic

This research examines real-world outcomes of medical weight management techniques through a retrospective audit of a specialist weight management service. It also explores predictors of weight loss outcomes and loss to follow-up.

This chapter is presented as a manuscript which is currently in the process of submission to peer-reviewed publications.

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4.0 Abstract

Background and Aims: New-generation GLP-1 receptor agonists can achieve 15-22% weight loss in clinical trials for the treatment of obesity, but less than 10% of those who could benefit are projected to have access by 2030. Understanding patient and system factors influencing the treatment of severe obesity can help shape the public health response. We examined weight loss outcomes and predictors of response in a publicly-funded Australian specialist obesity clinic.

Methods: A retrospective cohort study of 411 patients attending St Vincent's Hospital Melbourne Weight Management Clinic (July 2015 - February 2019). Treatment centred around a very low-energy diet (VLED) with optional additional pharmacotherapy. The primary outcome was proportion achieving ≥10% total body weight loss (TBWL) at 3, 6 and 12 months. Exploratory analyses examined associations between metabolic phenotype (Fatty Liver Index (FLI), insulin resistance by HOMA-IR) and outcomes.

Results: This cohort had severe obesity (mean BMI 46.5 ± 8.4 kg/m²) and was predominantly female (67%). Retention was 58% at 3 months, 47% at 6 months, and 29% at 12 months. Mean TBWL was 6.7% (± 5.6) at 3 months (n=237), 9.1% (± 8.5) at 6 months (n=191), and 10.5% (± 10.3) at 12 months (n=120), with 50% achieving ≥10% weight loss at 12 months. Metabolic phenotype predicted response: At 12 months patients without hepatic steatosis (FLI <30) achieved ≥10% TBWL in 78% vs 44% with FLI ≥30 (p=0.01); those without insulin resistance (HOMA-IR <2.5) achieved ≥10% TBWL in 82% vs 39% with insulin resistance (p=0.02).

Conclusions: This public health service achieved clinically significant weight loss in patients with severe obesity, although clinic retention was challenging. Metabolic phenotype predicted response with the absence of hepatic steatosis and insulin resistance identifying patients more likely to achieve clinically meaningful weight loss. Patients with metabolic dysfunction may benefit from earlier treatment escalation.

4.1 Introduction

Effective obesity treatment is often limited by the gap between evidence-based interventions and their accessibility in clinical practice. New-generation GLP-1 receptor agonists represent a significant therapeutic advance, achieving 15-22% weight loss in clinical trials for the treatment of obesity, yet fewer than 10% of those who could benefit are projected to have access by 2030(1-3). This disparity recently prompted the World Health Organisation (WHO) to caution that, without policy intervention, these therapies could exacerbate rather than reduce health inequities(3). Understanding outcomes in publicly-funded weight management services, which serve populations facing greater barriers to care, is essential for shaping the public health response.

These access concerns are particularly relevant in Australia, where almost one-third of adults are now classified as having obesity, with 4.6% meeting criteria for class III obesity (BMI ≥40 kg/m²)(4). Severe obesity reduces life expectancy by 8-10 years and is associated with substantial cardiometabolic morbidity(5, 6). The treatment of severe obesity is challenging: weight loss achieved through lifestyle measures is difficult to achieve and maintain due to persistent maladaptive hormonal changes that favour weight regain(7).

Bariatric surgery, although effective, carries procedural risks and access through public healthcare is limited(8, 9). New-generation GLP-1 receptor agonists such as semaglutide and tirzepatide remain prohibitively expensive(3). In Australia, these medications are not listed on the Pharmaceutical Benefits Scheme (PBS) for obesity, resulting in them being inaccessible to most patients attending publicly-funded services(10). Appetite-suppressing pharmacotherapy has been available but not PBS-

subsidised for obesity, limiting use to those able to afford them(11). For many years, medical weight management has therefore centred on very low-energy diets (VLED), which achieve rapid weight loss through dietary energy restriction and ketosis-induced appetite suppression(12, 13). Although VLEDs are not without cost, particularly for meal-replacement products, they represent a substantially more affordable intervention than new-generation GLP-1 receptor agonists(14, 15).

The WHO guideline emphasises that pharmacotherapy should be delivered within comprehensive care models that incorporate structured behavioural interventions(3). Understanding treatment outcomes and barriers to care is essential for the development of equitable weight loss programs moving forward but data from publicly-funded weight management services remain limited, despite these settings serving populations at the greatest risk of exclusion from these therapeutic advances.

This study aimed to characterise weight loss outcomes and identify predictors of treatment outcomes, including markers of metabolic dysmetabolism such as hepatic steatosis and insulin resistance, in a publicly-funded specialist weight management clinic which serves a catchment with high socioeconomic disadvantage, during the period preceding the availability of new-generation GLP-1 medications.

4.2 Methods

This single-centre retrospective cohort study used prospectively collected clinical data from the St Vincent's Hospital Melbourne Weight Management Clinic, a publicly-funded specialist service within a tertiary referral centre in metropolitan Melbourne, Australia. The clinic is staffed by physicians from gastroenterology, endocrinology, and general medicine, with dietitian support and referral pathways to exercise physiology and psychology. There is no cost for patients to attend; however, meal replacements and pharmacotherapy are patient-funded.

The study was approved by the St Vincent's Hospital Melbourne Human Research Ethics Committee (HREC 071/19). Individual consent was waived due to the retrospective design and use of de-identified data.

Participants

All consecutive patients attending at least one clinic appointment between 23 July 2015 and 21 February 2019 were included. Referral criteria to the clinic were BMI ≥35 kg/m² with at least one obesity-related comorbidity. There was no exclusion criteria. Follow-up data were collected until March 2020, ensuring a minimum 12-month follow-up window. This study period was selected as it preceded the onset of COVID-19 pandemic-associated restrictions in Melbourne, which resulted in

all outpatient services being transitioned to telehealth unless medically necessary, which prevented standardised in-person weight measurement and potentially introduced confounders related to prolonged stay-at-home orders, including reduced mobility, altered dietary pattern and disrupted access to healthcare (16).

Intervention

Patients were prescribed a modified ketogenic VLED, which consisted of two meal replacement products (e.g. Optifast or Optislim) and one low-carbohydrate meal per day (approximately 3150 kJ/day). Appetite-suppressing pharmacotherapy (topiramate, phentermine, or GLP-1 receptor agonists) was prescribed at the discretion of the treating clinician to assist with weight loss or for maintenance of weight loss as required, with all costs funded by the patients. Suitable patients were referred for bariatric surgery based on patient preference or inadequate response to medical management. The primary treatment target of the clinic is ≥10% total body weight loss (TBWL), a threshold associated with clinically meaningful improvements in obesity-related comorbidities.

Outcomes

The primary outcome was to determine weight loss achieved at 3, 6, and 12 months, as well as the proportion achieving the ≥10% TBWL target. Weight was assessed at clinic visits within ± 4 weeks of target dates, and was measured using calibrated digital scales using a standardised clinic protocol. Secondary outcomes included characterisation of the cohort, review of weight loss methods utilised, and clinic retention rates at 3, 6 and 12 months. Clinic retention was calculated as the proportion of patients still attending the clinic (appointment ± 4 weeks from each timepoint), out of all patients who attended at least one clinic appointment. Loss to follow-up was defined as failure to attend 2 consecutive scheduled appointments despite standard clinic recall procedures. Exploratory analysis was performed to determine factors associated with weight loss outcomes and loss to follow up; including sex, baseline BMI, treatment type, early treatment response (≥5% TBWL at 3 months), and markers of metabolic dysfunction (Fatty Liver Index (FLI) and insulin resistance by HOMA-IR).

The Fatty Liver Index (FLI) was used as a biomarker for underlying hepatic steatosis, the defining feature of metabolic dysfunction-associated steatotic liver disease (MASLD), and was calculated in patients with available baseline biochemical data (triglycerides, BMI, gamma-glutamyl transferase, and waist circumference) (17). FLI was categorised as no steatosis (<30), indeterminate (30-59), or probable hepatic steatosis (≥60). MASLD was defined as FLI ≥60 plus at least one cardiometabolic criterion as per the 2023 consensus guidelines(18). Insulin resistance was calculated using HOMA-IR score (fasting glucose × fasting insulin / 22.5), with HOMA-IR ≥2.5 defining insulin resistance in those with available baseline data (19, 20).

Data collection

Baseline demographic and clinical variables including age, sex, weight, height, waist circumference, comorbidities, previous obesity treatments and family history were collected prospectively for all clinic patients as part of the standard clinic protocol. Data were extracted retrospectively from the electronic medical record. Body weight was recorded at each visit. Baseline laboratory data collected included liver function tests, fasting glucose and insulin and lipid profiles but these tests were ordered at the discretion of the treating clinician and were not available for all patients. Fasting insulin, required for HOMA-IR calculation, is not rebatable under the Medicare Benefits Schedule and was therefore not routinely ordered.

Statistical analysis

Descriptive data are presented as mean ± SD for parametric variables, median (IQR) for non-parametric variables, and % (n) for categorical variables. Continuous variables were compared using independent t-tests or one-way ANOVA; categorical variables using Pearson chi-square tests. Multivariable logistic regression was used to examine predictors of loss to follow-up, with covariates selected based on clinical relevance. Early treatment response as a predictor of 12-month weight loss outcomes was also examined using multivariable logistic regression, adjusting for age, sex and baseline BMI. Exploratory subgroup analysis examining metabolic predictors of weight loss outcomes (FLI and HOMA-IR) used unadjusted logistic regression due to small sample size. Analysis was per-protocol, including patients with data at each timepoint. All statistical analyses were performed using Stata version 18 (StataCorp. 2023; Texas, USA).

4.3 Results

Participant characteristics

411 patients attended at least one appointment between July 2015 and February 2019 (Figure 4.1). The cohort had mean age 54 ± 12 years, was predominantly female (67%, n=277) and had a mean BMI of 46.5 kg/m² (± 8.4), with 78% (n=320) having class III obesity (BMI ≥40) (Table 4.1). Female patients were younger (52 vs 57 years, $p<0.01$) with lower body weight (122.9 ± 25 vs 144.2 ± 30 kg, $p<0.01$), but BMI was comparable between sexes ($p=0.51$). 95% had at least one obesity-related comorbidity. Most patients (81%) had a family history of obesity and 42% reported childhood obesity (Table 4.1).

After initial assessment, 92% were commenced on the VLED. Pharmacotherapy was prescribed to 43% (n=175). Topiramate was the most commonly prescribed agent (75%), followed by phentermine

(48%) and GLP-1 agonists (44%), 54% being prescribed multiple agents. 30% (n=125) were referred for consideration of bariatric surgery.

13. Table 4.1. Baseline characteristics of study participants

Characteristic	Total (n=411)	Female (n=277)	Male (n=134)	P-value
Demographics				
Age, years, mean (SD)	53.5 (12.4)	52.0 (12.5)	56.6 (11.5)	**<0.01**
Age, years, median (IQR)	54 (44-63)	52 (42-62)	56 (49-65)	
Anthropometrics				
Weight, kg, mean (SD)	129.9 (28.7)	122.9 (25.2)	144.2 (30.2)	**<0.01**
BMI, kg/m², mean (SD)	46.5 (8.4)	46.8 (8.7)	46.1 (7.7)	0.42
BMI category, % (n)				0.50
Overweight (25-29.9 kg/m²)	0.2% (1)	0.4% (1)	0% (0)	
Class I (30-34.9 kg/m²)	5% (20)	6% (16)	3% (4)	
Class II (35-39.9 kg/m²)	17% (69)	16% (44)	19% (25)	
Class III (≥40 kg/m²)	78% (320)	78% (215)	78% (105)	
Metabolic parameters				
Fasting glucose, mmol/L, median (IQR)	5.8 (5.0-6.8)	5.7 (4.9-6.5)	6.2 (5.2-8.2)	**0.03**
Fasting insulin, mU/L, median (IQR)	18 (12-24)	18 (11-24)	19 (14-26)	0.69
HOMA-IR, median (IQR)	4.4 (2.8-6.6)	3.6 (2.6-6.0)	5.4 (4.0-8.3)	0.19
HbA1c, %, median (IQR)	5.8 (5.5-6.8)	5.7 (5.4-6.7)	6.1 (5.6-7.0)	0.67
Triglycerides, mmol/L, median (IQR)	1.5 (1.1-2.1)	1.4 (1.0-2.0)	1.6 (1.2-2.4)	0.15
LDL cholesterol, mmol/L, median (IQR)	2.9 (2.2-3.7)	2.9 (2.4-3.6)	2.7 (1.8-3.8)	0.15
HDL cholesterol, mmol/L, median (IQR)	1.0 (0.9-1.3)	1.1 (0.9-1.4)	1.0 (0.9-1.1)	**<0.01**
ALT, U/L, median (IQR)	26 (19-42)	24 (17-36)	35 (23-58)	**<0.01**
AST, U/L, median (IQR)	23 (19-31)	22 (18-30)	25 (19-36)	0.12
GGT, U/L, median (IQR)	34 (21-60)	30 (19-51)	44 (26-80)	**<0.01**
Ferritin, µg/L, median (IQR)	92 (47-169)	72 (38-128)	156 (80-286)	**<0.01**
CRP, mg/L, median (IQR)	8 (5-12)	8 (5-12)	8 (4-15)	0.81
Cardiometabolic comorbidities, % (n)				
Type 2 diabetes	28% (116)	21% (59)	43% (57)	**<0.01**
IGT/IFG	16% (64)	16% (45)	14% (19)	0.59
Dyslipidaemia	44% (181)	40% (111)	52% (70)	**0.02**
Hypertension	50% (207)	43% (120)	65% (87)	**<0.01**

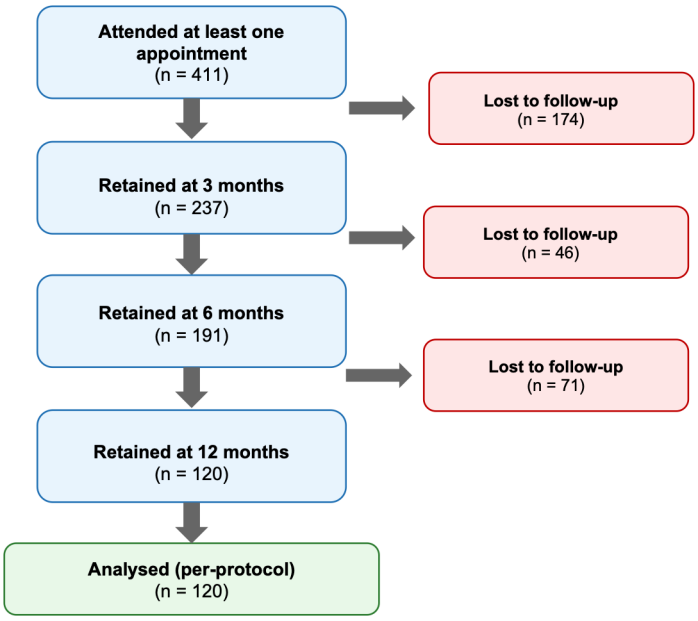
Ischaemic heart disease	6% (26)	3% (9)	13% (17)	**<0.01**
Cerebrovascular disease	1% (5)	0.4% (1)	3% (4)	**0.02**
MASLD	22% (90)	22% (60)	23% (30)	0.84
Chronic kidney disease	30% (125)	31% (85)	30% (40)	0.86
Gout	7% (28)	3% (8)	15% (20)	**<0.01**
PCOS	24% (56)	24% (56)		
Non-metabolic comorbidities, % (n)				
Obstructive sleep apnoea	49% (200)	47% (129)	53% (71)	0.22
Osteoarthritis	39% (160)	38% (104)	42% (56)	0.41
GORD	37% (152)	39% (108)	33% (44)	0.23
Depression	24% (98)	24% (67)	23% (31)	0.81
Comorbidity burden, % (n)				
Cardiometabolic comorbidities				**0.02**
None	15% (63)	18% (49)	10% (14)	
One	24% (99)	26% (73)	19% (26)	
Two	23% (95)	23% (64)	23% (31)	
Three or more	38% (154)	33% (91)	47% (63)	
Non-metabolic comorbidities				0.53
None	19% (77)	20% (56)	16% (21)	
One	34% (141)	34% (94)	35% (47)	
Two or more	47% (193)	46% (127)	49% (66)	
Other characteristics, % (n)				
Current or former smoker	22% (87)	22% (57)	23% (30)	0.78
Alcohol use	26% (102)	21% (56)	35% (46)	**0.01**
Childhood obesity	42% (173)	42% (115)	43% (58)	0.73
Family history of obesity	81% (317)	82% (218)	78% (99)	0.31

Baseline characteristics of included participants. P values from independent t-tests (continuous) or chi-square tests (categorical). BMI = body mass index; SD = standard deviation; IQR = interquartile range; HOMA-IR = homeostatic model assessment for insulin resistance; IGT/IFG = impaired glucose tolerance/impaired fasting glucose; MASLD = metabolic dysfunction-associated steatotic liver disease; PCOS = polycystic ovary syndrome; GORD = gastro-oesophageal reflux disease; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; CRP = C-reactive protein

Clinic Retention

Patient retention in the clinic was 58% (n=237) at 3 months, 47% (n=191) at 6 months, and 29% (n=120) at 12 months. Of the 411 patients, 136 (33%) attended only one appointment before being lost to follow-up. In multivariable logistic regression, type 2 diabetes was associated with loss to follow-up (OR 1.80, 95% CI 1.09-2.97, p=0.02). Previous weight loss medication use showed a trend toward better retention but did not reach statistical significance (OR 0.60, 95% CI 0.36-1.01, p=0.054). No other baseline characteristics, including referral for bariatric surgery, were significantly associated with loss to follow-up (supplementary material).

3. Figure 4.1 Patient flow diagram



Patients attending St Vincent's Hospital Melbourne Weight Management Clinic between July 2015 and February 2019. Retention at each timepoint defined as attendance within ± 4 weeks of each timepoint. Loss to follow-up defined as failure to attend two consecutive appointments despite standard recall procedures.

Weight loss outcomes

Progressive weight loss was observed across 3, 6 and 12 months (Table 4.2). Mean weight loss was 6.7% (± 5.6) at 3 months (n=237), 9.1% (± 8.5) at 6 months (n=191), and 10.5% (± 10.3) at 12 months (n=120), with 50% achieving ≥10% weight loss at 12 months.

Baseline BMI category was not associated with weight loss outcomes. Patients with class III obesity achieved similar weight loss to those with class II or I obesity (10.6% (±10.3) TBWL vs 10.1% (±10.6) vs 14.3% (±9.3), p=0.795). Weight loss did not differ by sex (females 10.7% (±10.6) vs males 10.2% (±9.9), p=0.80) or age category (p=0.51)(supplementary material).

Early weight loss response was associated longer term outcomes, with patients who achieved ≥5% TBWL at 3 months achieving significantly greater TBWL at 12 months (13.9% (±9.9) vs 2.4% (±7.2); $p < 0.01$). This group were also more likely to achieve ≥10% TBWL (63% vs 19%, adjusted OR 7.18, 95% CI 2.44-21.12, $p<0.01$) and had higher clinic retention (57% vs 28%, $p<0.01$) at 12 months.

Patients prescribed adjunctive pharmacotherapy achieved greater weight loss than those on VLED alone at all timepoints. At 3 months, mean TBWL was 7.6% (±5.7) in the pharmacotherapy group compared with 5.5% (±5.3) with VLED alone ($p<0.01$). This persisted at 6 months (10.4% (±8.8) vs 7.1% (±7.7), $p=0.01$) and 12 months (11.9% (±10.3) vs 7.2% (±9.9), $p=0.02$). Patients receiving pharmacotherapy were also more likely to achieve ≥10% TBWL at 12 months (57% (48/84) vs 34% (12/35), $p=0.02$).

14. Table 4.2. Weight loss outcomes across timepoints

Outcome	**3 months** (n=237)	**6 months** (n=191)	**12 months** (n=120)
Weight loss			
Absolute, kg, median (IQR)	7.9 (3.2-12.6)	11 (2.6-17.8)	12.6 (2-19.8)
Percentage, mean (SD)	6.7% (5.6)	9.1% (8.5)	10.5% (10.3)
Clinical thresholds achieved, n (%)			
Achieved ≥5% TBWL, % (n)	60% (141)	65% (124)	63% (76)
Achieved ≥10% TBWL, % (n)	29% (68)	45% (85)	50% (60)
Clinic retention			
Retention from baseline cohort, % (n)	58% (237/411)	47% (191/411)	29% (120/411)

Per-protocol analysis of outcomes at each timepoint across the whole cohort. P values from independent t-tests (continuous) or chi-square tests (categorical).mean (SD) or median (IQR). SD=standard deviation; IQR = interquartile range.

Metabolic dysfunction-associated steatotic liver disease

FLI was able to be calculated in 52% of the baseline cohort (n=214), with hepatic steatosis (FLI ≥60) present in 54% (n=116/214). All patients with FLI ≥60 met at least one cardiometabolic criteria for a diagnosis of MASLD, resulting in a substantially higher rate of MASLD than clinically documented diagnoses (22%, n=89). Compared to those with FLI <60, those with MASLD by FLI were more likely to be male (42% vs 24%, $p<0.01$), have a higher mean BMI (51.5 vs 40.3 kg/m², $p<0.01$), have type 2 diabetes (44% vs 26%, $p<0.01$), and hypertension (61% vs 39%, $p<0.01$).

Absence of hepatic steatosis (FLI <30) was associated with greater weight loss at each timepoint compared to those with possible and probable steatosis (FLI ≥30), with an effect that strengthened over time (Table 4.3). At 3 months, differences in reaching the target weight loss did not reach significance (p=0.20), however by 6 months, patients with FLI <30 were more likely to achieve ≥10% TBWL (63% vs 40%, p=0.04), with this difference even greater at 12 months (78% vs 44%, OR 4.41, 95% CI 1.28-15.23, p=0.01). Clinic retention did not differ by FLI status (35% vs 32%, p=0.74). A sensitivity analysis restricted to patients with definitive FLI classification (FLI <30 vs FLI ≥60) showed consistent results (supplementary material).

15. Table 4.3. Weight loss outcomes by Fatty Liver Index

Outcome	FLI <30	FLI ≥30	Unadjusted OR (95% CI)	P value
3 months (n=134)				
Weight loss, %, mean (SD)	-7.6 (6.5)	-6.2 (5.4)		0.21
Achieved ≥10% TBWL, % (n)	31% (10/32)	25% (25/102)	0.71 (0.30-1.71)	0.45
6 months (n=105)				
Weight loss, %, mean (SD)	-11.5 (8.9)	-8.9 (7.9)		0.16
Achieved ≥10% TBWL, % (n)	63% (17/27)	40% (31/78)	2.58 (1.04-6.36)	**0.04**
12 months (n=70)				
Weight loss, %, mean (SD)	-13.0 (10.1)	-10.0 (9.6)		0.27
Achieved ≥10% TBWL, % (n)	78% (14/18)	44% (23/52)	4.41 (1.28-15.23)	**0.01**
Clinic retention				
Retained at 12 months, % (n)	35% (18/52)	32% (52/162)		0.74

Weight loss outcomes by Fatty Liver Index (FLI) across timepoints. FLI <30 rules out hepatic steatosis; FLI ≥30 indicates possible or probable steatosis. Per-protocol analysis including patients with baseline FLI data retained at each timepoint. P values from independent t-tests (continuous) or unadjusted logistic regression. CI=confidence interval; SD=standard deviation; TBWL=total body weight loss.

Insulin resistance

HOMA-IR data were available for 133 patients (32%). Insulin resistance (HOMA-IR ≥2.5) was associated with reduced weight loss, with patients without insulin resistance achieving greater mean TBWL at 3, 6, and 12 months, and similar to FLI, this effect increased over time (Table 4.4). The proportion achieving ≥10% TBWL was consistently higher in those without insulin resistance at all timepoints, but was greatest at 12 months where 82% of those without insulin resistance achieved ≥10% TBWL, compared to 39% with insulin resistance (OR 0.14 (0.03-0.75), p=0.02). Clinic

retention did not differ by insulin resistance status (p=0.32). In contrast, type 2 diabetes status was not associated with weight loss outcomes at any timepoint (≥10% TBWL at 12 months 43% vs 54%, p=0.26).

16. Table 4.4. Weight loss outcomes by insulin resistance status across timepoints

Outcome	No Insulin Resistance	Insulin resistance	Unadjusted OR (95% CI)	P value
3 months (n=78)				
Weight loss, %, mean (SD)	-7.8 (6.6)	-5.3 (5.3)		0.11
Achieved ≥10% TBWL, % (n)	44% (7/16)	23% (14/62)	0.38 (0.12-1.19)	0.10
6 months (n=62)				
Weight loss, %, mean (SD)	-12.8 (9.3)	-7.5 (7.4)		**0.04**
Achieved ≥10% TBWL, % (n)	69% (9/13)	39% (19/49)	0.28 (0.08-1.04)	0.06
12 months (n=47)				
Weight loss, %, mean (SD)	-15.5 (11.2)	-8.1 (8.2)		**0.02**
Achieved ≥10% TBWL, % (n)	82% (9/11)	39% (14/36)	0.14 (0.03-0.75)	**0.02**
Clinic retention				
Retained at 12 months, % (n)	44% (11/25)	33% (36/108)		0.32

Weight loss outcomes by insulin resistance status across timepoints. Insulin resistance defined as HOMA-IR ≥ 2.5. Per-protocol analysis including patients with HOMA-IR data retained at each timepoint. P values from independent t-tests (continuous) or unadjusted logistic regression. Odds ratios reflect odds of achieving ≥10% TBWL in the insulin resistant group relative to the no insulin resistance group. CI=confidence interval; SD=standard deviation; TBWL=total body weight loss.

4.4 Discussion

This study from a publicly-funded Australian specialist weight management clinic shows that VLED-based medical weight management delivered in an outpatient setting to a cohort with severe obesity is effective, leading to clinically significant weight loss, which increases over time in the clinic. However, it also highlights that patient engagement with weight loss services is challenging, with 29% patient retention at 12 months.

Metabolic phenotype, specifically dysmetabolism defined by hepatic steatosis and insulin resistance, were identified as predictors of weight loss success in this cohort. These findings have implications for patient counselling and resource allocation as new-generation GLP-1 therapies become available. Absence of hepatic steatosis by FLI was associated with greater likelihood of achieving clinically meaningful weight loss (Table 4.3). This association was evident by 6 months and strengthened at 12

months. Similarly, the absence of insulin resistance by HOMA-IR predicted greater weight loss with a consistent trend across all timepoints (Table 4.4). Importantly, clinic retention rates did not differ by FLI (p=0.74) or insulin resistance status (p=0.32), indicating that these associations were not impacted by differential attrition across groups.

These findings suggest that underlying metabolic dysfunction may slow response to weight loss interventions. The compensatory hyperinsulinaemia of insulin-resistant states may impair weight loss through inhibition of lipolysis, and reduced mobilisation of adipose tissue, and impaired weight loss in type 2 diabetes is well documented (21-23). MASLD and insulin resistance are closely correlated, reflecting overlapping pathophysiology, and both may serve as markers for identifying patients who may struggle to achieve clinically significant weight loss with dietary intervention alone(24). The substantial disparity between MASLD by FLI (54% with FLI ≥60) and clinically documented diagnosis of MASLD (22%) highlights under-recognition of MASLD in obesity services and supports routine biochemical and/ or radiological screening(25). Early identification of MASLD in patients with obesity is important, with weight loss directly impacting disease progression, supporting earlier consideration of treatment escalation in this cohort.

BMI did not predict weight loss outcomes in our cohort, consistent with evidence that BMI is a poor predictor of percentage weight loss across lifestyle interventions (26). However, individuals with the same BMI can have markedly different metabolic profiles. In our cohort, hepatic steatosis and insulin resistance were associated with poorer outcomes, suggesting that metabolic phenotype may better inform treatment selection than obesity severity alone (27). These findings suggest that metabolic phenotype could help identify patients most likely to respond to intensive dietary intervention, and that those with metabolic dysfunction may benefit from earlier consideration of pharmacotherapy or bariatric surgery, although this requires validation in prospective studies.

Australian data from similar services provide additional context. The Darwin based 'Top End Weight Management Service' reported median 8.5% weight loss with 48% retention at 12 months, and 44% achieving ≥10% weight loss(28). Notably, that service subsidised pharmacotherapy, which may partly explain their higher retention. The Austin Health clinic (also in Melbourne), and the Canberra Obesity Management Service (COMS) are also both publicly-funded services using VLED-based treatment with adjunctive pharmacotherapy as appropriate. Austin Health reported mean 6.2% weight loss with 42% retention at 12 months, while COMS reported ≥10% weight loss in 27.9% of patients with any follow-up attendance(29, 30). Variation in reported metrics limited comparisons however our higher weight loss (mean 10.5%, compared to Darwin and Austin) but lower retention (29% at

12 months), may reflect retention bias favouring more engaged patients, or other differences in our clinic processes and cohorts. The populations served by these services differ considerably. Our clinic, based in inner Melbourne, serves a catchment characterised by socioeconomic disadvantage and high cultural and linguistic diversity, whereas the Darwin service is regional and 40% of patients identified as Aboriginal and/or Torres Strait Islander. The Austin clinic, while also Melbourne-based, is situated in a suburban catchment with a less disadvantaged and more culturally homogenous demographic profile. These differences in patient demographics and pharmacotherapy subsidisation limit direct comparison of outcomes across services; however, the consistently low retention rates of 29-48% across Australian publicly-funded services indicate that engagement is a systemic challenge, not unique to our setting.

Type 2 diabetes was the only baseline characteristic significantly associated with loss to follow-up (OR 1.80, p=0.02). This may reflect the additional burden of managing a complex chronic disease, competing medical appointments, or the psychological impact of multiple diagnoses (31). Previous weight loss medication use showed a trend toward better retention, potentially reflecting greater familiarity with or preference for medical weight management approaches.

Pharmacotherapy was prescribed to 43% of patients, despite none of the available agents; topiramate (~AU$6/month), phentermine (~AU$77/month), or first-generation GLP-1 agonists e.g. liraglutide (~AU$300/month); being PBS-subsidised for obesity(11). This suggests cost may have limited even broader uptake. The WHO guideline explicitly identifies cost as a barrier to access, with new-generation GLP-1 therapies costing ~AU$400-500/month without subsidy(3). As access was already limited at lower price points, utilisation of these medications will be further limited without policy intervention.

This study has several limitations. Due to the retrospective nature of the study, we were unable to capture the peak weight loss achieved during the treatment period if this did not coincide with a designated timepoint. Alcohol intake was recorded as part of clinical assessment but was not systematically quantified, precluding detailed analysis of its contribution to hepatic steatosis or weight loss outcomes. Additionally, it was difficult to adequately capture patient-related factors, such as financial burden, patient preferences and expectations that may have contributed to disengagement. The per-protocol analysis may overestimate treatment effects, as retained patients may represent a more engaged or responsive subgroup. Additionally, as a retrospective cohort study, the sample size was determined by clinic throughput rather than a priori power calculation. The full cohort provided adequate power for descriptive weight loss outcomes, however exploratory subgroup analyses were

limited by smaller sample sizes, particularly FLI and HOMA-IR data which were available for only 52% and 32% of patients respectively, which may have limited power to detect associations and the generalisability of these findings. The study period for enrolment (July 2015 to February 2019) was selected to ensure standardised in-clinic weight measurement of 12 month outcomes, as after March 2020, the COVID-19 pandemic necessitated transition to telehealth, and reliable weight measurements were no longer available. Furthermore, generalisability of this data may be limited by the nature of the single-centre design and specific clinic model integrating medical and surgical referral pathways.

This study has several strengths. It represents one of the larger published cohorts from an Australian weight management service and provides real-world outcome data from a population facing significant barriers to care. The exploration of metabolic phenotype as a predictor of treatment response is clinically relevant as new-generation pharmacotherapies become available and risk stratification is required for resource allocation.

4.5 Conclusion

This publicly-funded outpatient VLED-based medical weight loss service achieved clinically significant weight loss in a cohort with severe obesity. Metabolic phenotype predicted response, with patients without hepatic steatosis (FLI <30) or insulin resistance (HOMA-IR <2.5) more likely to achieve ≥10% weight loss. These findings from the pre- long-acting GLP-1 era suggest that patients with metabolic dysfunction may benefit more from pharmacotherapy or treatment intensification, and that engagement challenges for this group must be addressed or expanding GLP-1 access may exacerbate rather than reduce inequalities, leaving this cohort further behind.

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Chapter 5. Very Low Energy Ketogenic Diet vs Mediterranean Diet for MASLD: Superior Steatosis Reduction in a Randomised Pilot Study

This chapter presents the results of the DARWIN (The Effect of Diet on Appetite Regulation, Weight and Steatosis in Individuals with Nonalcoholic Fatty Liver Disease) study, a randomised pilot study comparing a ketogenic VLED to a MD in adults with biopsy-proven MASLD and overweight or obesity (BMI 27-35kg/m^2).

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Conflict of Interest statement

The authors declare the following financial and non-financial competing interests: AF, TP, EBP, EG, JH, CC, TS, MP, PM, AT: no competing interests. MR: has received speaking honorarium from Novo Nordisk

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5.0 Abstract

Background & Aims: Weight loss is the cornerstone of treatment for metabolic dysfunction associated steatotic liver disease (MASLD). This pilot study compared the efficacy and safety of a very low energy ketogenic diet (VLED) versus a Mediterranean diet (MD) in improving hepatic steatosis and liver histology in individuals with overweight or obesity and MASLD.

Methods: We conducted a pilot randomised controlled trial in adults with histologically confirmed MASLD and BMI 27-35 kg/m^2. Participants were assigned to either a 12-week VLED (3151 kJ/day) or MD program (8950 kJ/day) and monitored for 24 weeks. The VLED group received low-dose semaglutide (0.5 mg/weekly) from week 13 for weight maintenance. Primary outcome was change in hepatic steatosis by MRI liver-fat-fraction (MRI-LFF) at 12 weeks. Secondary outcomes included total body weight loss (TBWL) and change in liver histology over 24 weeks.

Results: The VLED group (n=14) achieved significantly greater reduction in MRI-LFF (-77% relative reduction (IQR 51, 88)) compared to the MD group (n=11, -14% (IQR 0, 30), p<0.01). The VLED also produced TBWL at week 12 (-13% (IQR -17, -9) vs -4% (-4.4, -0.2), p<0.01). At 24 weeks, the VLED/semaglutide group maintained a -14% TBWL (IQR -17,-10) from baseline vs -3% TBWL (IQR -4, 0) in the MD. Liver histology improved in both groups, with greater improvements in the VLED group (NAFLD activity score reduction VLED: -2 (IQR -3.5, -2) vs MD: -1 (IQR -1, -1), p<0.01).

Conclusions: The very low energy diet resulted in significantly greater reduction in hepatic steatosis and weight loss compared to Mediterranean diet. The very low energy diet is widely available, easily accessible and should be more commonly considered for MASLD patients with overweight/obesity.

5.1 Introduction

Metabolic dysfunction associated steatotic liver disease (MASLD) is the most common liver disease worldwide, characterised by excessive hepatic fat accumulation and strong associations with obesity and the metabolic syndrome(1). MASLD carries a significant disease burden, ranging from hepatic-related complications (liver cirrhosis, decompensated liver disease and hepatocellular carcinoma), to extra-hepatic events (including type 2 diabetes mellitus (T2DM) and major adverse cardiovascular events(2, 3). Weight loss remains the cornerstone of MASLD management. However, treatment practices are heterogeneous, and compensatory hormonal changes make diet-induced weight loss difficult to achieve and maintain(4, 5). Guidelines recommend at least 5% weight loss to improve hepatic steatosis, and 10% or greater for hepatic fibrosis regression(6). Weight loss is primarily achieved through an energy deficit facilitated by dietary energy restriction. The Mediterranean diet (MD) is often recommended for MASLD as it can improve liver fat content and cardiometabolic health, even without significant weight loss(7), with some arguing that the MD may be easier to maintain in the long term (6).

The medical management of obesity utilises a ketogenic Very Low Energy Diet (VLED), which induces rapid weight loss, is associated with remission of T2DM, and is often used short-term before bariatric surgery to reduce hepatic steatosis(8-10). The carbohydrate restriction on the VLED leads to fatty acid oxidation in the liver, resulting in the production of ketones which reduce appetite through the suppression of appetite-regulating hormones, thus helping patients to maintain the strict energy restriction(11). Expert obesity guidelines recommend the VLED to achieve target weight loss, before transitioning to a portion-controlled weight maintenance diet to help prevent weight regain(12). Due to hormonal and metabolic changes after weight loss which increase hunger, many obesity experts argue that pharmacotherapy is required to prevent weight regain during the maintenance phase(4, 13, 14). This approach has been shown to improve weight maintenance post-VLED versus control and is a practice followed within local weight management services(15-17). Semaglutide is a Glucagon-like peptide-1 receptor agonist (GLP-1RA) and has been shown to induce weight loss, improve markers of hepatic steatosis and inflammation, and, more recently, hepatic fibrosis(18-20). GLP-1RAs have recently become the preferred medication in weight management; however they are expensive at high doses, and use of low-dose semaglutide presents a pragmatic approach to weight maintenance in clinical practice.

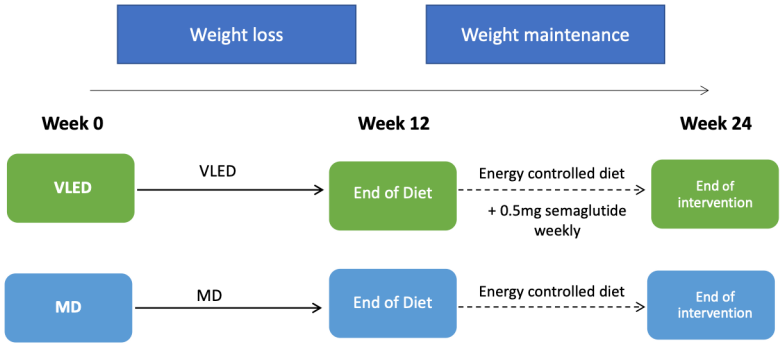
In this study, we aimed to determine the effectiveness of a 12-week ketogenic very low-energy diet (VLED) in reducing hepatic steatosis in overweight and obese individuals with MASLD, compared to the Mediterranean diet (MD). Secondary aims included evaluating the transition to a weight maintenance phase (with low-dose semaglutide commenced in the VLED group and persistence of

MD principles assessed in the MD group), and changes in body weight, body composition, cardiometabolic profile, and liver histology at 24 weeks.

5.2 Methods

Study design

We conducted a single-centre, unblinded, randomised controlled trial, comparing a 12-week ketogenic VLED versus prescribed MD in overweight and obese MASLD patients. Participants were randomised 1:1, with stratification for T2DM and sex, using computerised randomisation managed by REDCap electronic data capture tools(21, 22). The study design is outlined in Figure 5.1. This study was approved by the Human Research Ethics Committee of St Vincent's Hospital Melbourne (HREC 277/18, Project ID Number 50048), and registered in the Australian Registry of Clinical trials (www.anzctr.org.au ACTRN12623000756628).



4. Figure 5.1. Study design and intervention timeline

Participants were randomised to either very low energy diet (VLED) or Mediterranean diet (MD) for a 12-week weight loss phase. During the subsequent 12-week weight maintenance phase (weeks 12–24), the VLED group transitioned to an energy-controlled diet with weekly subcutaneous semaglutide 0.5mg, while the MD group continued with an energy-controlled diet alone. MD = Mediterranean diet, VLED = very low energy diet.

Primary outcome was change in hepatic steatosis on magnetic resonance imaging liver-fat-fraction (MRI-LFF) at the end of the 12-week diet. Secondary outcomes included change in body weight and metabolic profile as well as paired liver biopsies at baseline and week 24. Participants and participant-facing trial staff were not blinded, but intervention allocation was concealed from outcome assessors.

Participants

Patients were screened from liver clinics at St Vincent's Hospital Melbourne, between April 2021 - July 2023 (28 months), to recruit adults 18-70 years old with body mass index (BMI) 27-35 kg/m^2 and MASLD defined by elevated ALT and/or GGT, ≥10% hepatic steatosis on MRI and Metavir fibrosis score of ≥F1 on liver histology. Key exclusions: excessive alcohol (>20 g/day men, >10 g/day women), concomitant liver disease, current GLP-1RA or insulin use, recent weight loss (>5% total body weight (TBW)) or previous bariatric surgery. Participants continued their usual medications throughout the study period.

Intervention

All participants had dietitian consultations at baseline and week 6, with telephone support at weeks 2, 4 and 9. Both groups were advised to perform 150-300 minutes of weekly exercise per EASL guidelines(6).

Ketogenic VLED: Two meal replacement products (Optifast VLCD shake (Nestlé) provided) plus one very low-carbohydrate, low-fat meal daily (3151 kJ total, 40% protein, 27% fat, 31% carbohydrates) were advised for the VLED group. A booklet containing nine recipes and resource guides outlining suitable foods and beverages was provided.

***Prescribed MD*:** Modified MD providing up to 8950 kJ daily (17% protein, 44% fat (50% of total fat from monounsaturated fats), 30% carbohydrate) was advised, adjusted from a previously published ad-libitum MD to allow participants to remain eucaloric(23). A two-week rotating meal plan with recipes was provided alongside essential components of the MD, such as extra virgin olive oil, tinned fish, baked beans and almonds.

***Maintenance phase (Weeks 13-24)*:** The dietitian provided individualized energy requirement counselling week 13 onwards, but no meal plans were provided. The VLED group commenced semaglutide (Ozempic, Novonordisk) at week 13 (0.25 mg weekly via subcutaneous injection for 4 weeks, then 0.5 mg weekly for 8 weeks). The MD group were encouraged to maintain MD dietary habits.

Adherence

Dietary adherence was assessed via 3-day food diaries (patient record on paper) at baseline, week 12 and 24. Nutritional analysis was conducted using FoodWorks version 10 (Xyris, Brisbane, QLD). The VLED group were assessed for presence of ketosis (serum 3-hydroxybutyrate measured at week

2 and 6). Urinary ketone testing strips were provided to participants to self-monitor compliance. MD adherence was assessed using the PREDIMED MEDAS score, with >7 indicating adequate compliance(24). At week 24, PREDIMED MEDAS score was calculated from food diaries. Medication adherence was monitored through self-reporting, pharmacy records and returned medication devices.

Outcomes

Hepatic steatosis measured using magnetic resonance imaging liver-fat-fraction (MRI-LFF) on 3T magnet (Siemens Skyra and Vida) using Siemens Liver lab (software version XA50) protocol. Liver stiffness measurement (LSM) and controlled attenuation parameter (CAP) obtained using Fibroscan (Echosens, France). Liver biopsies obtained under ultrasound guidance by radiologists and liver histology was reviewed by a pathologist using the NASH Clinical Research Network (CRN) scoring(25). Body composition assessed via whole body dual-energy x-ray absorptiometry scan (GE Lunar iDXA Pro, enCORE software version 18) and physical activity was measured using an activPAL (PAL technologies, Scotland).

Sample size

Based on previous MD studies in MASLD patients, which found an MRI liver fat reduction of 12-25%, we anticipated a 15% reduction in liver fat on the MD with a standard deviation (SD) of 8%(7, 26). This was a pilot study and 20 participants, with 10 in each arm would provide an 80% power with 0.05 alpha to detect a difference of 20%. We aimed to recruit 24 participants to allow for 20% drop out.

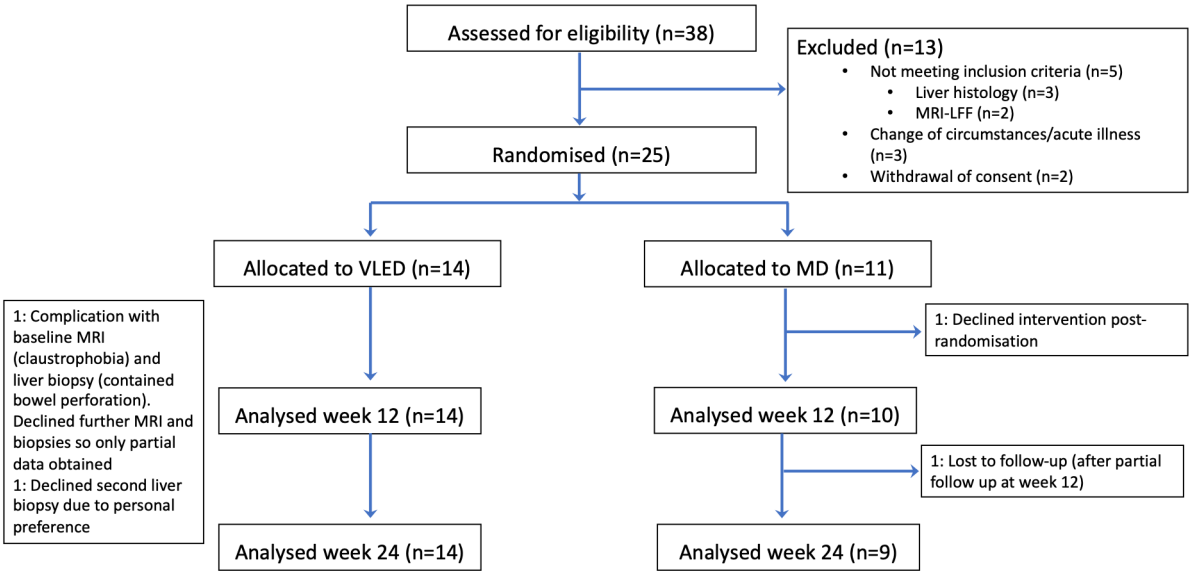
Statistical analysis

Data normality was assessed using the Shapiro-Wilk test. Normally distributed data are presented as mean±SD, non-parametric data as median(IQR). Between-group comparisons used Fisher's exact test or chi-squared test, t-test, or Wilcoxon rank sum as appropriate. Signed rank test was used for within-group differences, and Spearman correlation to assess the relationship between two continuous variables. Per-protocol analysis was performed, including participants with available data at each time point; no imputation was performed for missing data. Intention-to-treat analysis is provided in supplementary material (Appendix E). Subgroup and adjusted analyses were not performed due to the small sample size of this pilot study. All statistical analysis was performed using Stata version 18 (StataCorp. 2023; Texas USA).

5.3 Results

Study population

Twenty-five participants were randomised, with 24 completing the 12-week intervention and 23 completing the full 24-week study (Figure 5.2). The cohort was predominantly middle-aged Caucasian (76%, n=19), with a mean BMI of 31.7±3.1 kg/m². Baseline median MRI-LFF was 21% (IQR 14.95, 28.7), and only one participant had advanced liver fibrosis histologically (F3-4) (Table 5.1).



5. Figure 5.2 CONSORT flow diagram of participant enrolment, allocation and follow-up

Flow of participants through the trial from screening to final analysis.
MD = Mediterranean diet, VLED = very low energy diet, MRI-LFF = magnetic resonance imaging liver fat fraction.

17. Table 5.1. Baseline characteristics of study participants randomised to Very Low Energy Diet (VLED) or Mediterranean Diet (MD) interventions

Characteristics	Cohort n= 25	VLED (n = 14)	MD (n= 11)	P-value
Male sex, n (%)	14 (56%)	9 (64%)	5 (45%)	0.35
Age, y (IQR)	49 (42, 57)	45 (35, 59)	50 (44, 57)	0.53
Type 2 diabetes mellitus, n (%)	2 (8%)	1 (7%)	1 (7%)	0.86
Hypertension, n (%)	7 (28%)	4 (29%)	3 (27%)	0.94
Dyslipidaemia	6 (24%)	3 (21%)	3 (27%)	0.73

Ethnicity, n (%) - Oceanian Caucasian - South + Central Asian - South + East European - Pacific Islander	15 (60%) 5 (20%) 4 (16%) 1 (4%)	7 (50%) 5 (36%) 2 (14%) 0 (0%)	8 (73%) 0 (0%) 2 (18%) 1 (9%)	0.12
Smoking, n (%)	4 (16%)	4 (29%)	0 (0%)	0.05
Daily energy intake (kJ)	7454 (5960, 9653)	8944 (6326, 11607)	7308 (6116, 10534)	0.54
Daily step count (total steps)	6075 (4909, 7839)	5629 (4856, 7534)	6348 (5235, 8144)	0.62
Sedentary time (min/day)	537 (439, 641)	529 (489, 621)	546 (401, 716)	0.79
MRI Liver-fat-fraction, % (IQR)	21.2 (14.95, 28.7)	19.4 (16.1, 29.6)	21.4 (13.8, 28)	0.84
Liver NAS score (IQR)	4 (4, 5)	4 (4, 5)	4 (4, 5)	0.34
Liver fibrosis score, n (%) - grade 1-2 - grade 3-4	23 (96%) 1 (4%)	12 (92%) 1 (8%)	11 (100%) 0	0.53
Liver stiffness measurement, kPa (IQR)	5.6 (4.9, 6.4)	5.7 (4.6, 7.3)	5.6 (4.9, 6)	0.64
CAP, dB/m (IQR)	322 (295, 333)	327 (295, 341)	317 (292, 325)	0.32
ALT, IU/L (IQR)	51 (37, 67)	46.5 (37, 58)	52 (30, 99)	0.66
GGT, IU/L (IQR)	55 (37, 114)	49.5 (30, 86)	67 (40, 140)	0.11
Body weight, kg (SD)	91.9 (±13.6)	90.4 (±13.1)	93.9 (±14.5)	0.44
BMI, kg/m^2 (SD)	31.7 (±3.1)	31.2 (±3.2)	32.3 (±3.1)	0.34
Waist circumference, cm (SD)				
- Women	108.6 (±7.7)	107.7 (±10.0)	109.4 (±6.2)	0.86
- Men	108.8 (±8.9)	105.6 (±8.3)	114.6 (±7.3)	0.053
HOMA-IR score (IQR)	4.1 (2.8, 6.4)	5.1 (3.2, 6.6)	3.8 (2.3, 6.3)	0.34

Data presented as median (interquartile range) for non-parametric continuous variables, mean (±standard deviation) for parametric continuous variables, and number (percentage) for categorical variables. P-values were calculated using Fisher's exact test or chi-square test for categorical variables, Wilcoxon rank sum test for non-normally distributed continuous variables, and t-test for normally distributed continuous variables. P<0.05 considered statistically significant. VLED = Very Low Energy Diet; MD = Mediterranean Diet; MRI = magnetic resonance imaging; NAS = non-alcoholic fatty liver disease activity score; CAP = controlled attenuation parameter; ALT = alanine aminotransferase; GGT = gamma-glutamyl transferase; BMI = body mass index; HOMA-IR = homeostatic model assessment for insulin resistance.

Primary outcome: Liver fat fraction (0-12 weeks)

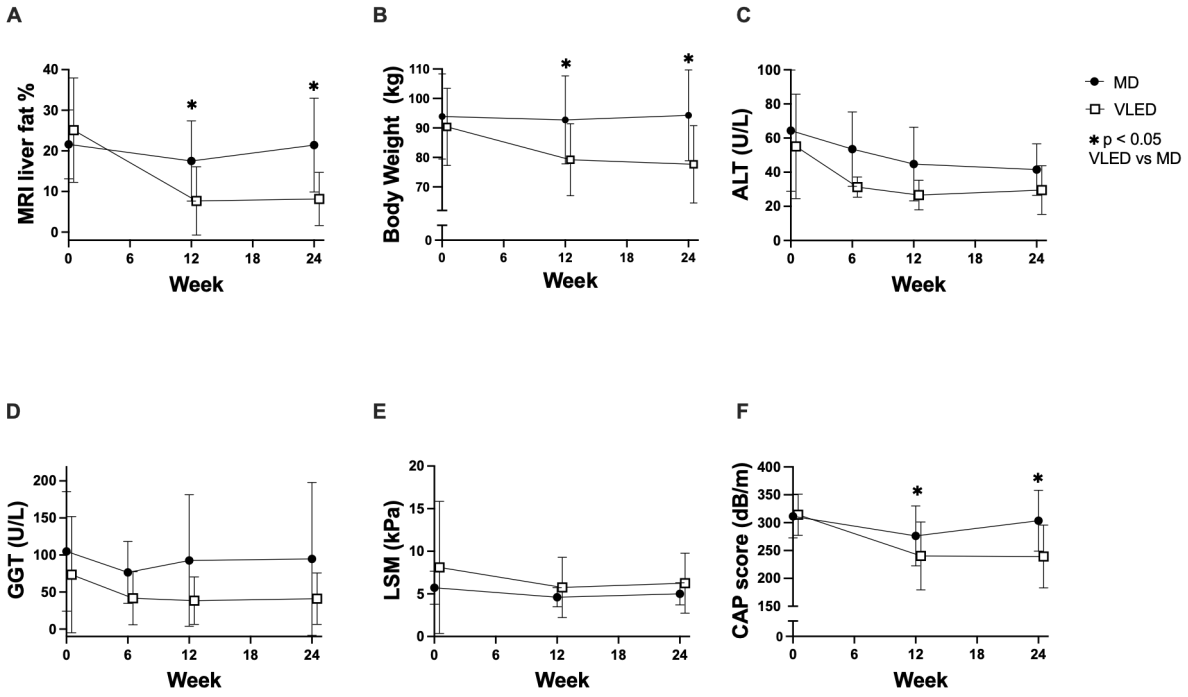
The VLED resulted in a significant improvement in MRI-LFF compared to the MD at 12 weeks (Table 5.2/Figure 5.3). The VLED achieved an absolute reduction in MRI-LFF of -18% (IQR -24, -10), representing a relative reduction of -77% (IQR -88, -55) from baseline ($p<0.01$), with 69% (n=9/13) achieving normalisation (≤5%) of hepatic steatosis (HS). Comparatively, the MD group had a non-significant absolute reduction of -5% (IQR -8, 0) from baseline ($p=0.15$) (Table 5.2). Similar results were observed using intention-to-treat analysis of the entire cohort (supplementary material).

18. Table 5.2. Comparison of clinical outcomes between Very Low Energy Diet (VLED) and Mediterranean Diet (MD) interventions at 12 and 24 weeks

	Week 12			**Week 24**		
	VLED	MD	P-value^	VLED	MD	P-value^
Relative reduction in MRI liver-fat-fraction (MRI-LFF)(%)	-77 (-88, -55)*	-14 (-30, 0)	< 0.01	-64 (-83, -57)*	-4 (-22, 2)	<0.01
Absolute reduction in LFF (% steatosis)	-18 (-24, -10)*	-5 (-8, 0)	< 0.01	-16 (-25, -9)*	-0.6 (-5, 0.6)	<0.01
Normalisation of hepatic steatosis (≤5%)	69% (n=9)	10% (n=1)	< 0.01	46% (n=6)	0% (n=0)	<0.01
Weight (kg)	-12 (± 5.8)*	-3.5 (± 3.9)*	< 0.01	-13 (± 4.4)*	-2 (± 5.4)	<0.01
Total body weight loss (%)	-13 (± 6)*	-4 (± 4)*	< 0.01	-15 (± 5)*	-2 (± 5)	<0.01
Clinically significant weight loss (>10%TBWL)	71% (n=10)	10% (n=1)	<0.01	79% (n=11)	10% (n=1)	<0.01
BMI (kg/m^2)	-4 (± 1.8)*	-1.2 (± 1.3)*	< 0.01	-4.5 (± 1.7)*	-0.6 (± 1.8)	<0.01
LSM (kPa)	-1.1 (-2, 0)*	-0.25 (-1.3, 0.25)	0.32	-0.9 (-1.6, 0.1)	-0.1 (-1.5, 0.5)	0.56
CAP (dB/m)	-82.5 (-123, -41.5)*	-34.5 (-48, 4)	0.049	-85 (-98, -40)*	-16 (-37, 11)	<0.01
ALT (IU/l)	-26 (-39, -3)*	-13.5 (-45, -5)*	0.88	-24 (-43, -6)*	-18 (-30, -5)*	0.61
GGT (IU/l)	-25 (-32, -14)*	-13 (-19, -1)	0.21	-23 (-31, -4)*	-13 (-18, -8)	0.38
Albumin (g/L)	1 (0, 1)*	1 (-1, 3)	0.59	0 (-3, 3)	-2 (-4, 1)	0.34
HOMA-IR score	-1.8 (-2.9, -1.3)*	-1.2 (-4.4, -0.6)*	0.74	-1.5 (-2.8, 0.1)*	-0.1 (-0.2, 1.2)	0.36
HbA1C (%)	-0.4 (-0.6, -0.1)*	-0.3 (-0.3, -0.1)*	0.35	-0.5 (-0.6, -0.3)*	-0.3 (-0.3, 0.1)	0.055

LDL-cholesterol (mmol/l)	0 (-0.2, 0.35)	0.3 (-0.05, 0.7)	0.35	0 (-0.3, 0.4)	0.1 (-0.4, 0.7)	0.74
HDL-cholesterol (mmol/l)	0.05 (-0.02, 0.2)	-0.01 (-0.05, 0.2)	0.86	0.19 (0.05, 0.3)*	0.03 (-0.01, 0.14)	0.44
Triglycerides (mmol/l)	-0.3 (-0.6, -0.1)*	-0.8 (-1.2, 0.1)	0.78	-0.4 (-0.6, 0.1)	-0.2 (-1.2, 0.1)	0.82
Diastolic BP (mmHg)	-9 (-11, -2)*	-1 (-9, 4)	0.14	-6.5 (-11, -5)*	-5 (-11, -1)	0.3
Systolic BP (mmHg)	-8 (-17, 6)	7 (-10, 19)	0.12	-14 (-18, -6)*	-3 (-11, 4)	0.09

*Data presented as median (interquartile range) for non-parametric variables, mean (standard deviation) for parametric variables, or %(n) for categorical variables and represents change from baseline at each time point. Per protocol analysis performed. P-values calculated for between-group comparisons were performed using Wilcoxon rank-sum test for continuous variables and chi-square test for categorical variables. Within-group changes from baseline were assessed using Wilcoxon signed-rank test. P<0.05 considered statistically significant. *P-value<0.05 in-group change from baseline for continuous variables. ^P-value VLED vs MD. VLED = Very Low Energy Diet; MD = Mediterranean Diet; MRI = magnetic resonance imaging; LFF = liver-fat-fraction; BMI = body mass index; LSM = liver stiffness measurement; CAP = controlled attenuation parameter; ALT = alanine aminotransferase; GGT = gamma-glutamyl transferase; HOMA-IR = homeostatic model assessment for insulin resistance; HbA1c = glycated haemoglobin; LDL = low-density lipoprotein; HDL = high-density lipoprotein; BP = blood pressure; TBWL = total body weight loss.*



6. Figure 5.3. Comparison of liver-related biomarkers and metabolic parameters between Very Low Energy Diet (VLED) and Mediterranean Diet (MD) over 24 weeks

Time course of (A) MRI liver-fat-fraction, (B) total body weight (C) alanine aminotransferase (ALT), (D) gamma-glutamyl transferase (GGT), (E) liver stiffness measurement (LSM), and (F) controlled attenuation parameter (CAP) score in participants randomised to VLED or Mediterranean Diet interventions. Data

*presented as median with interquartile range. P values were calculated using Wilcoxon rank sum test. P<0.05 considered statistically significant. *P <0.05 for between-group comparison (VLED vs MD) at that timepoint.*

Secondary outcomes (0 -12 weeks)

Weight loss

Greater weight loss was achieved by the VLED compared to MD at week 12 (-13% vs -4 % TBWL p<0.01), with both groups achieving statistically significant weight loss compared to baseline (p<0.01 VLED and p=0.01 MD) (Table 5.2/Figure 5.3). Clinically significant weight loss (>10% TBWL) was achieved in 71% (n=10/14) of VLED participants versus 10% (n=1/10) in the MD group (p<0.01). There was a strong positive correlation between TBWL and relative liver fat reduction (Spearman's rho=0.68, R^2=0.46, p<0.01).

Liver and metabolic parameters

The VLED was associated with significant improvement in GGT, LSM and CAP scores (GGT p<0.01, LSM p=0.03; CAP p<0.01 from baseline; Table 5.2/Figure 5.3). Both groups showed improvements in ALT (VLED p<0.01; MD p<0.01), with no between-group difference (p=0.88).

Both diets significantly improved markers of insulin sensitivity at week 12 (HOMA-IR: VLED p<0.01, MD p=0.04; HbA1c:VLED: p<0.01, MD p=0.02 from baseline; Table 5.2), with no significant between-group differences. The VLED also showed a significant reduction in triglycerides (p=0.03) and diastolic blood pressure (p=0.02) at week 12 vs baseline (Table 5.2).

Dietary adherence

The VLED group showed good adherence to the diet, with 13/14 participants able to demonstrate ketosis during the diet intervention period (12 via serum ketones, 1 via urinary ketones). All MD participants demonstrated good adherence to the prescribed diet (week 12 MD score >7). Analysis of participant food diaries confirmed VLED participants achieved significant energy restriction (4791 kJ/day (IQR 3745, 5377) at week 12 vs 8944 kJ/day (IQR 6326, 11607) at baseline, p<0.01), while MD participants remained eucaloric (7440 kJ/day (IQR 6038, 10788) at week 12 vs 7308 kJ/day (IQR 6116, 10534) at baseline, p=0.51), with macronutrient composition changes in line with dietary prescriptions (supplementary material).

Maintenance phase (12-24 weeks)

The VLED/semaglutide group maintained a stable MRI-LFF between week 12 and 24 (-0.1% (IQR -1.8, 3.7), p=0.75), while the MD group showed a small but significant increase in MRI-LFF (+3.3% (IQR 1.7, 6.5), p=0.03) (Figure 5.3). Both groups remained weight stable during this maintenance

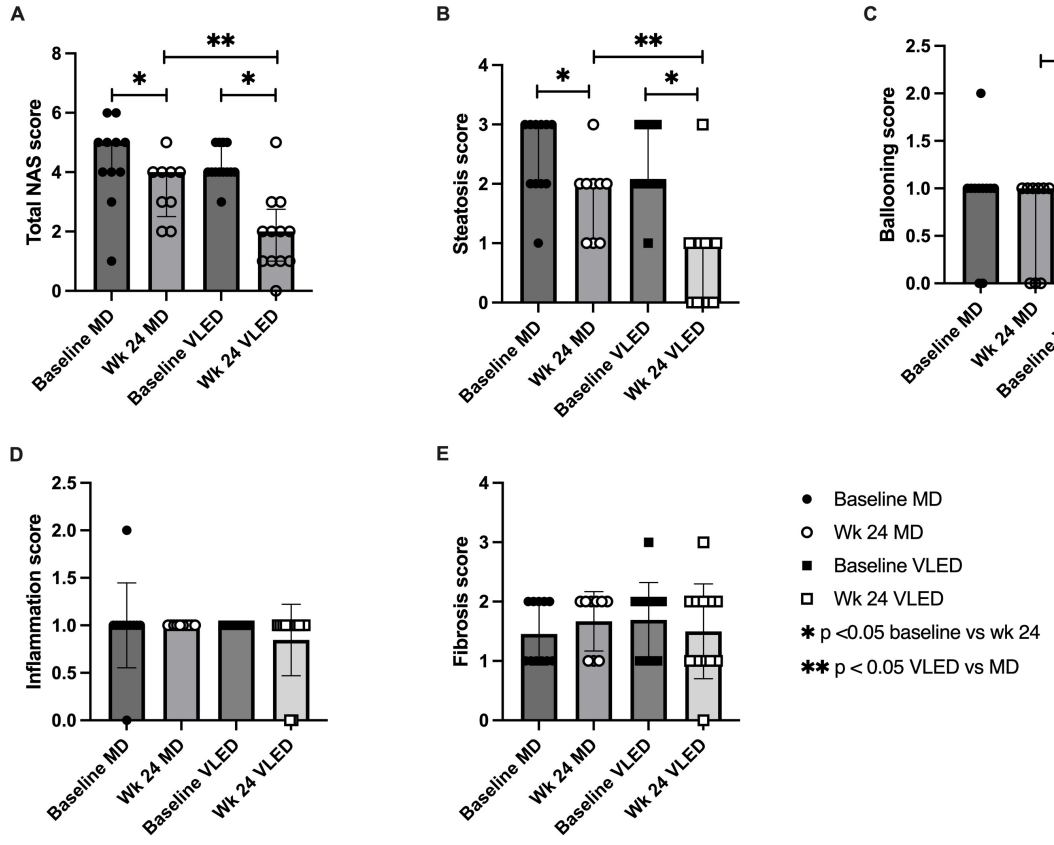
period (VLED/semaglutide: -0.8kg (SD 4.2), p=0.33; vs MD: +1.9kg (SD 2.9), p=0.09). No change was seen in Fibroscan LSM, CAP, or liver enzymes between week 12 and 24 in either group (Table 5.2).

When compared to baseline, at week 24 the VLED/semaglutide group maintained a significant -15% TBWL (SD 5.5, p<0.01), with 79% (n=11/14) achieving clinically significant (>10% TBWL) weight loss. They also maintained a significant relative reduction in LFF of -64% ((IQR -83, -57), p<0.01). The MD group showed no significant changes from baseline in weight (-2% TBWL (SD 5.3), p=0.41) or LFF (-4% (IQR -22, 2), p=0.41).

The VLED/semaglutide group increased their dietary energy intake by 1208 kJ/day (IQR -224, 3680, p=0.047) between week 12 and 24 during the maintenance phase, while the MD group maintained consistent intake (-256 kJ/day (IQR -1022, 505), p=0.68). There was a trend towards a lower energy intake compared to baseline in the VLED/semaglutide group (-517 kJ/day (IQR -3119, 710, p=0.13) at week 24, but this was not statistically significant, and no change was seen in the MD group (191 kJ/day (IQR 7, 1209), p=0.31). MD scores remained >7 at week 24 in n=7/8 MD participants. Both groups' macronutrient intake at week 24 returned towards their baseline levels (supplementary material).

Liver histology: improvement at week 24 vs baseline

Paired liver biopsies obtained at baseline and week 24 (n=12 VLED, n=9 MD) showed significant improvements in NAFLD activity score (NAS) in both groups, with greater benefits seen in the VLED/semaglutide arm (Figure 5.4). The VLED/semaglutide group demonstrated significant improvements in hepatic steatosis (-1.5 stage (IQR -2, -1), p<0.01) and hepatocellular ballooning (-1 stage (IQR -1, 0), p=0.02). The MD also resulted in an improvement in steatosis (-1 stage (IQR -1, 0), p=0.01), but to a lesser degree than the VLED (p=0.02) (Figure 5.4). No change in histological fibrosis stage was noted within either group (VLED: F score change 0 (IQR -1, 0), p=0.18; vs MD: 0 (IQR 0, 0), p=0.16), though participants achieving >10% TBWL regardless of intervention were more likely to show fibrosis improvement than those who did not (40% (n=4/10) vs 0% (n=0/11), p=0.02).



7. Figure 5.4. Histological assessment of liver biopsy samples showing changes in NAFLD Activity Score components and fibrosis between baseline and 24 weeks

*Comparison of (A) total NAFLD Activity Score (NAS), (B) steatosis score, (C) ballooning score, (D) inflammation score, and (E) fibrosis score in participants receiving Mediterranean Diet (MD) or Very Low Energy Diet (VLED) interventions. Data shown at baseline and week 24 for each treatment group. Individual data points are overlaid on bar graphs showing median and interquartile range. P-values were calculated using Wilcoxon rank-sum test for between group comparisons and Wilcoxon signed-rank test for within group changes. P<0.05 considered statistically significant.*P<0.05 for within-group change from baseline to week 24; **P<0.05 for between-group comparison (VLED vs MD) at week 24.*

Body composition

At week 12, the VLED resulted in reductions from baseline in fat mass (p<0.01), including both visceral (p<0.01) and subcutaneous adipose tissue (p<0.01), and a reduction in lean mass (p<0.01), which was noted in both the appendicular and trunk regions (Table 5.3). Lean mass loss accounted for 24% of the TBWL achieved at week 12. The MD group showed modest fat mass reduction from baseline (p=0.03), specifically visceral adipose tissue (p=0.01), with no change in lean mass (p=0.09). Across all participants weight loss was predominantly fat-driven, with TBWL strongly correlated with fat mass reduction (Spearman's rho=0.94, R^2=0.88, p<0.01) but also associated with lean mass loss (rho=0.66, R^2=0.44, p<0.01).

During the maintenance phase, lean mass remained unchanged in both groups from week 12. The MD group showed a trend toward fat mass gain (p=0.051), while the VLED/semaglutide group maintained stable fat mass (p=0.09). No changes were measured in physical activity in either group at any time point during the trial (supplementary material).

19. Table 5.3. Changes in body composition from baseline at 12 and 24 weeks in participants receiving Very Low Energy Diet (VLED) or Mediterranean Diet (MD) interventions

	Week 12			Week 24		
	VLED	MD	P-value^	VLED	MD	P-value^
Body fat (%)	-4.2 (-7.8, -3.1)*	-2.0 (-2.4, -0.3)*	0.01	-7.4 (-9.8, -3.2)*	-0.9 (-2.3, 0.0)	<0.01
Fat mass (kg)	-8.4 (-11.7, -5.8)*	-2.9 (-3.8, -1.7)*	0.01	-11.1 (-13.0, -5.7)*	-2.0 (-3.5, -0.1)*	<0.01
Lean mass (kg)	-2.7 (-3.7, -2.0)*	-0.2 (-1.5, -0.2)	<0.01	-2.6 (-3.6, -1.4)*	-0.4 (-0.7, 0.7)	0.01
-Appendicular	-1.7 (-2.4, -1.1)*	-0.1 (-0.6, 0.1)	<0.01	-1.8 (-2.3, -0.7)*	-0.1 (-0.4, 0.1)	<0.01
-Trunk	-0.8 (-1.5, -0.3)*	-0.2 (-0.4, 0.1)	0.15	-0.8 (-1.0, -0.5)	0.1 (-0.5, 0.4)	0.16
VAT mass (g)	-661.7 (-1066.0, -513.0)*	-393.8 (-626.1, -116.9)*	0.15	-691.5 (-873.6, -547.2)*	-325.5 (-430.6, -71.0)*	0.01
SAT mass (g)	-637.2 (-864.8, -491.3)*	-97.9 (-136.0, -28.9)	0.01	-809.6 (-967.2, -651.2)*	-175.9 (-344.1, 6.9)	<0.01

*Data presented as median (interquartile range) and represents change from baseline at each time point. Per protocol analysis performed. P-values were calculated using Wilcoxon rank-sum test for between group comparisons and Wilcoxon signed-rank test for within group changes. P<0.05 considered statistically significant. * P<0.05 for within-group change from baseline. ^P-values for between-group comparisons (VLED vs MD) at each time point. VLED = Very Low Energy Diet; MD = Mediterranean Diet; VAT = visceral adipose tissue; SAT = subcutaneous adipose tissue.*

Adverse events

Five adverse events (AE) were recorded during the study, including three liver-biopsy-related complications. The biopsy-related adverse events (3 of 25 participants) were predominantly minor (pain, minor bleeding) and resolved without sequelae. Mild gastrointestinal side effects were reported

in both diet groups, predominantly constipation in the VLED group and bloating in the MD (supplementary material). No additional side effects were reported with the use of semaglutide.

5.4 Discussion

This pilot randomised controlled trial demonstrates that a 12-week ketogenic VLED is significantly more effective than a prescribed MD in reducing hepatic steatosis and achieving clinically significant weight loss in overweight/obese individuals with MASLD. The VLED achieved a 77% relative reduction in MRI-LFF compared to 14% with the MD ($p<0.01$), with 69% of VLED participants normalising hepatic steatosis (<5% MRI-LFF) versus none in the MD group. Weight loss was also significantly greater with the VLED (13% vs 4% TBWL, $p<0.01$), with 71% achieving clinically significant weight loss (>10% TBWL) compared to only 10% of MD participants. These benefits were maintained through to week 24 with low-dose semaglutide, and were accompanied by greater histological improvements including reduced hepatocellular ballooning.

We compared the VLED to MD, which is recommended in international guidelines(6). While MD did not achieve statistically significant reductions in hepatic steatosis by MRI-LFF, it did demonstrate improvements in ALT, HOMA-IR, and HbA1c. Participants in the MD group remained largely eucaloric, which limited significant weight change. Histological improvement in steatosis in the MD despite modest weight loss was consistent with previous studies and suggests benefits beyond weight reduction, likely related to the anti-inflammatory effects of the MD(7).

The data highlights the importance of clinically significant weight loss in managing patients with overweight/obesity and MASLD, with the VLED an effective strategy to achieve this. The magnitude of weight loss and hepatic fat reduction after our 12-week VLED exceeded previously reported lifestyle interventions and was comparable to phase 2 high-dose GLP-1RA studies(27). In MASH with F2 or 3 fibrosis, 0.4 mg semaglutide daily achieved 13% weight loss at 72 weeks, while in MASH cirrhosis, 2.4 mg semaglutide weekly for 48 weeks resulted in a 31% relative hepatic steatosis reduction on MRI-PDFF with 8.8% weight loss(19, 28). Pemvidutide (GLP-1RA/glucagon dual receptor agonist) induced a 69% relative reduction in MRI liver fat after 12 weeks in MASLD and BMI ≥28 kg/m^{2}[29]. Comparatively, the VLED offers a viable non-pharmacological alternative as a safe, rapid, and effective initial weight loss strategy in MASLD.

The histological findings at 24 weeks are noteworthy and demonstrate benefits of the VLED-semaglutide approach extending beyond steatosis reduction to improvements in hepatocellular

ballooning, a marker of liver cell injury, likely reflective of the substantial weight loss achieved by the VLED. The maintenance phase with semaglutide may have consolidated these benefits by preventing weight regain, potentially complemented by indirect hepatic effects of GLP-1 receptor agonists, including reduced de novo lipogenesis and anti-inflammatory properties, that appear to be at least partially independent of weight loss(30, 31). Regardless, the net clinical effect of the combined intervention on histology represents a pragmatically relevant outcome. Participants achieving >10% TBWL were more likely to have fibrosis improvement, regardless of intervention, reiterating the importance of substantial weight loss in MASLD. This aligns with current guidelines recommending ≥10% TBWL and supports a dose-response relationship between weight loss and histological improvement. The importance of sustained weight loss is further demonstrated by the phase 3 ESSENCE study of high-dose (2.4 mg weekly) semaglutide resulting in 11% weight loss and 37% achieving fibrosis regression vs 22% on placebo after 72 weeks(20). Our study did not detect fibrosis change, but was not powered to do so and the follow-up interval was not optimised to capture this.

Lean mass loss occurred in the VLED, not the MD; however this occurred to an expected degree, with 25-30% loss of lean mass typical for dietary weight loss interventions(32, 33). This is thought to be at least partly reflective of the reduction in axial load required to be carried, necessitating less lean mass(32). While lean mass loss can be a concern in patients with chronic liver disease, this was a young cohort without cirrhosis, with no functional impact on physical activity(34).

Mediterranean-style diets are optimal for MASLD; however are difficult to translate into clinical practice as replication of the diets that have shown benefit in research studies require comprehensive education from a dietitian(7, 23, 35, 36). In contrast, the VLED can be utilised by any trained healthcare provider, including general practitioners, nurses and allied health practitioners(12, 37). VLED programs using meal replacement products are accessible and can be readily administered in an outpatient setting. The VLED guidelines are simple to follow and evidence-based, utilising ketosis for appetite suppression, assisting adherence(11). The greater metabolic benefits observed with the VLED vs MD may reflect the greater weight loss achieved, making it a better option in patients for whom >5% weight loss is desirable.

The maintenance of weight loss and hepatic steatosis reduction observed in the VLED/semaglutide group through week 24 addresses a critical issue in managing MASLD patients with overweight or obesity after weight loss – weight regain. While weight change during the maintenance phase did not differ significantly between groups, the VLED group had achieved substantially greater weight loss

(>10% TBWL), and therefore faced greater physiological drive towards weight regain due to metabolic adaptation(4, 13). Despite this, and despite increased energy intake and macronutrient changes observed in the VLED group during the maintenance phase, low-dose semaglutide effectively maintained the benefits achieved by the VLED, providing a practical weight maintenance strategy. While GLP-1RAs alone are effective for weight loss, side-effect tolerability and high cost can be prohibitive to more widespread use(38). Low-dose semaglutide has been utilised in the medical management of obesity when higher doses are not required to achieve weight loss goals, or not tolerated due to side effects or from a cost perspective(39, 40). This pragmatic use of the VLED to induce clinically significant weight loss followed by low-dose semaglutide to prevent weight regain presents a more affordable alternative to high-dose GLP-1RA monotherapy with lower medication costs and improved tolerability.

While there are initial costs associated with this approach (meal replacement products and semaglutide), they should be weighed against the significant long-term burden of progressive liver disease. If this strategy can prevent progression to advanced fibrosis, the long-term cost-effectiveness might be favourable despite higher upfront costs. A formal health economic evaluation, which is beyond this pilot study's scope, will be crucial to guide implementation and health policy.

This study has several important strengths that differentiate it from previous work. This is the first randomised trial to directly compare the ketogenic VLED to the MD for the management of MASLD, a clinically relevant comparison with both approaches recommended for metabolic health but without any direct head-to-head comparisons to guide treatment selection. Unlike previous small uncontrolled VLED studies in MASLD, which used heterogeneous diagnostic criteria, we employed paired liver biopsies with standardised histological scoring (NASH-CRN), demonstrating improvements not only in steatosis but also in hepatocellular ballooning – a key marker of liver injury(41-43). Another key strength is the comprehensive assessment of liver outcomes, including both non-invasive (MRI-LFF, Fibroscan) and invasive (histology) measures. Both groups received matched levels of dietitian support throughout, minimising contact bias that can confound dietary comparisons. Finally, the sequential strategy of VLED induction followed by low-dose GLP-1RA maintenance represents a novel, pragmatic approach to sustained weight management in MASLD that has not been previously evaluated.

Several limitations should be acknowledged. The relatively low prevalence of T2DM and advanced fibrosis in our cohort may limit generalizability to populations with more severe metabolic dysfunction or liver disease; however the significant improvements in early-stage MASLD seen may be beneficial in preventing disease progression. The 24 week duration, may not fully capture the long-

term sustainability of the observed benefits, and the study occurred during Covid outbreaks with periods of prolonged community lockdown in Melbourne, which may have impacted our results(44). This was a pilot study with a small sample size, so while it was designed with sufficient power to detect the primary outcome, study power is limited for some secondary analyses. The small sample size precluded subgroup analysis based on factors such as sex and smoking status, however the magnitude of the primary outcome difference (77% vs 14% relative reduction in MRI-LFF) substantially exceeds what could be plausibly attributed to baseline differences between groups.

Our findings have important clinical implications. The superior efficacy of the VLED approach, combined with its good tolerability, minimal adverse events, wide applicability and low cost, supports the consideration of VLED as a first-line intervention for patients with overweight/obesity and MASLD. The ability to achieve and maintain substantial weight loss with a dual diet and medication approach addresses a critical unmet need in MASLD management. Future studies with a larger cohort, including more advanced liver disease and a longer period of follow-up are warranted.

5.6 Conclusion

A ketogenic 12-week VLED is more effective than a prescribed MD in reducing hepatic steatosis and achieving clinically meaningful weight loss in patients with obesity and MASLD, with greater improvements in liver enzymes, liver histology and cardiometabolic parameters. The VLED followed by low-dose semaglutide offers an effective strategy to address both the initial weight loss and subsequent weight maintenance challenges in MASLD and demonstrates the benefits of adapting the medical weight loss approach to the management of overweight and obese patients with MASLD.

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Chapter 6. Long-term Durability of Ketogenic Very Low Energy Diet Benefits in MASLD: A 48-Week Extension Study

This chapter presents the final outcomes of the DARWIN study, exploring the trends in hepatic steatosis and body weight 24 weeks after the active intervention finished.

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6.0 Abstract

Background and Aim: Achieving and maintaining weight loss in metabolic dysfunction-associated steatotic liver disease (MASLD) remains challenging. Our previous study demonstrated that a 12-week very low energy diet (VLED) achieved superior hepatic steatosis reduction compared to a Mediterranean diet (MD), with low-dose semaglutide maintaining benefits to week 24. This extension study evaluates the durability of these benefits 24 weeks after cessation of active intervention.

Methods: Adults with biopsy-confirmed MASLD and BMI 27-35 kg/m2 were randomised to VLED (3151 kJ/day) or MD (8950 kJ/day) for 12 weeks, followed by a 12-week maintenance phase (weeks 13-24) with portion-controlled diet and low-dose semaglutide 0.5mg weekly in the VLED group. From week 25 onwards, no dietary counselling or pharmacotherapy was provided. At week 48,

participants underwent repeat assessment of hepatic steatosis by MRI-liver-fat-fraction (MRI-LFF), as well as body weight, liver enzymes, liver elastography and metabolic parameters.

Results: At week 48 the VLED group (n=14) maintained clinically significant improvements from baseline including -10.4% total body weight loss (TBWL, IQR -13, -4) and -55% relative reduction in MRI-LFF (IQR -75, -31) vs MD (n=8) -2.1% TBWL (IQR -3, 1, p<0.01) and +5% relative change in MRI-LFF (IQR -23, 40, p<0.01). 50% of VLED participants maintained ≥ 10% TBWL, and 31% maintained MRI-LFF ≤ 5%. During weeks 25-48, both groups showed upward trends in weight (VLED +5.3% (IQR 3, 8) vs MD +0.1% (IQR -0.6, 2.5), p=0.02) and MRI-LFF (VLED +4.2% (IQR 0, 10) vs MD +1.4% (IQR 0, 10), p=0.94). Despite this, the VLED group maintained improvements in ALT, GGT, HbA1c, liver stiffness measurement and HOMA-IR from baseline (all p<0.05).
Conclusion: The VLED group maintained clinically significant improvements in weight, hepatic steatosis, and metabolic parameters at week 48, despite some weight regain after cessation of the active intervention.

6.1 Introduction

Weight loss remains the cornerstone of treatment for Metabolic dysfunction-associated steatotic liver disease (MASLD), which is the most prevalent liver disease worldwide (1, 2). Guidelines recommend aiming for at least 5% total body weight loss (TBWL) in order to improve hepatic steatosis, and ≥10% TBWL for histological improvement, including fibrosis regression (2). However, achieving and maintaining clinically significant weight loss presents substantial challenges due to compensatory metabolic and hormonal adaptations that promote weight regain (3-5).

Long-term maintenance of weight loss and its associated metabolic benefits following dietary intervention remains a critical yet understudied aspect of MASLD management. Previous studies have demonstrated that weight regain is common after diet-based weight loss interventions, and the durability of hepatic and metabolic improvements beyond the active treatment period is uncertain (6-8). A 5-year follow-up of a large post-bariatric surgery cohort demonstrated that sustained weight loss produces progressive, time-dependent improvements in liver histology, with fibrosis regression continuing over time in those maintaining significant weight loss (9). This highlights that duration of weight loss maintenance, not just weight loss itself, is critical in long-term management of MASLD, however the durability of weight loss and hepatic benefits after intensive dietary interventions is less well documented.

In our previously published 24-week study, we demonstrated that a 12-week ketogenic very low energy diet (VLED) achieved superior reduction in hepatic steatosis (77% relative reduction) and greater weight loss (13% TBWL) compared to a prescribed Mediterranean diet (MD) (14% relative reduction in steatosis, 4% TBWL)(10). Following the intensive dietary phase, participants in the VLED group received low-dose semaglutide (0.5 mg weekly) for weight maintenance from weeks 13-24, which successfully maintained the achieved benefits through to week 24.

The key clinical question is whether these benefits persist after cessation of the intervention. If durable, time-limited intensive interventions represent a practical, resource-efficient approach to managing MASLD. If not, ongoing maintenance strategies are required. This extension study evaluated outcomes 24 weeks after cessation of intervention (week 48 total), comparing the durability of VLED-induced benefits compared to MD in MASLD.

6.2 Methods

Study Design and Participants

This study represents an extension of the DARWIN (The effect of Diet on Appetite Regulation, Weight and steatosis in Individuals with Nonalcoholic fatty liver disease) study, a single-centre, unblinded randomised pilot study conducted at St Vincent's Hospital Melbourne. The study methods, baseline characteristics, and 24-week outcomes are outlined in chapter 5. Briefly, adults aged 18-70 years with BMI 27-35 kg/m² and histologically confirmed MASLD (Metavir fibrosis score ≥F1) were randomised 1:1 with stratification for sex and type 2 diabetes mellitus (T2DM), to receive either a 12-week ketogenic VLED or prescribed MD intervention. The study was approved by the Human Research Ethics Committee of St Vincent's Hospital Melbourne (HREC 277/18, Project ID Number 50048) and registered in the Australian Registry of Clinical Trials (www.anzctr.org.au ACTRN12623000756628).

Original Intervention (Weeks 0-24)

Full details of the dietary interventions have been previously outlined (Chapter 5). The VLED group consumed two meal replacement products (Optifast VLCD shake, Nestlé) plus one very low carbohydrate, low-fat meal daily (3151 kJ/day; 40% protein, 27% fat, 31% carbohydrates). The MD group followed a prescribed Mediterranean diet (8950 kJ/day; 17% protein, 44% fat with 50% from monounsaturated fats, 30% carbohydrate). From week 13 onwards during the maintenance phase (weeks 13-24), individualised energy requirement counselling was provided without meal plans, and

the VLED group commenced semaglutide 0.25 mg weekly for 4 weeks, then 0.5 mg weekly for 8 weeks.

Extension Phase (Weeks 25-48)

Following completion of the 24-week active intervention, participants entered an observational extension phase with no further dietary counselling, meal plans, or pharmacotherapy provided. All participants were reviewed at week 36 for monitoring but no dietary advice was provided to either group during the extension phase (weeks 25-48). At week 48, participants underwent comprehensive liver and metabolic assessment.

Outcomes

Hepatic steatosis was assessed by magnetic resonance imaging liver-fat-fraction (MRI-LFF) and measured on a 3T magnet (Siemens Skyra and Vida) using the Siemens Liver Lab protocol (software version XA50). Liver stiffness measurement (LSM) and controlled attenuation parameter (CAP) were obtained using Fibroscan (Echosens, France). Body weight was measured using calibrated electronic scales, and body composition was assessed via whole-body dual-energy x-ray absorptiometry scan (GE Lunar iDXA Pro, enCORE software version 18). Total body weight loss (TBWL) was calculated as percentage change from baseline. Clinically significant weight loss was defined as ≥10% TBWL. Fasting blood samples were collected for measurement of liver enzymes (ALT, GGT), lipid profile, HbA1c, and calculation of insulin resistance (HOMA-IR). Blood pressure was measured using a calibrated electronic sphygmomanometer.

Three-day food diaries were collected at baseline, week 24 and week 48 and analysed using FoodWorks version 10 (Xyris, Brisbane, QLD). Mediterranean diet adherence was assessed using the PREDIMED MEDAS score, with a score ≥7 indicating adequate compliance. Physical activity was measured using an activPAL device worn continuously for 7 consecutive days (PAL Technologies, Scotland).

Statistical Analysis

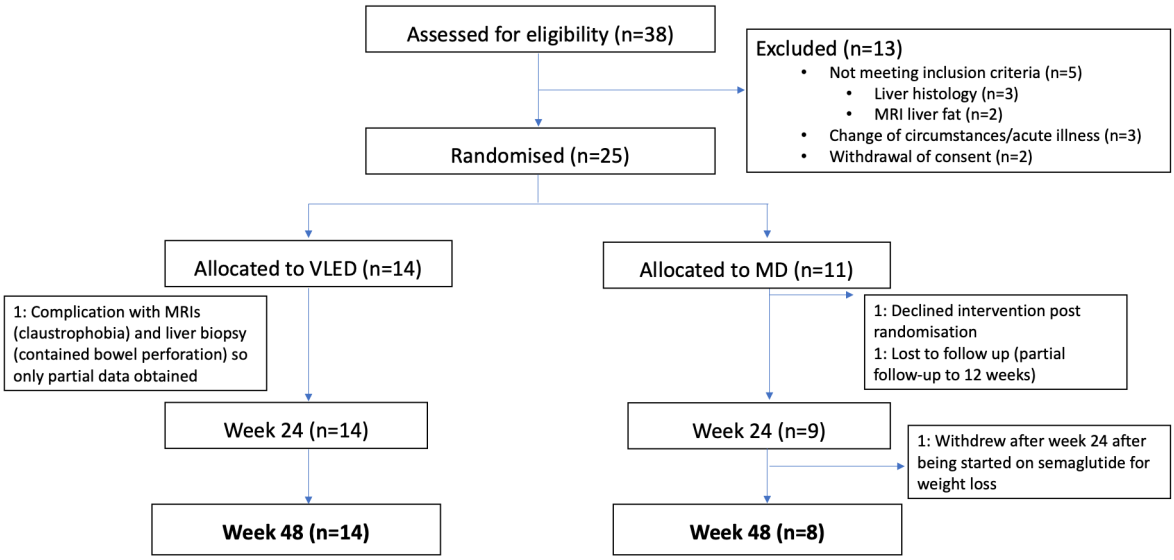
Distribution of data was assessed using the Shapiro-Wilk test. Normally distributed data are presented as mean ± standard deviation (SD), and non-parametric data as median (interquartile range, IQR). Between-group comparisons used Fisher's exact test, t-test, or Wilcoxon rank-sum test as appropriate. Within-group changes were assessed using paired t-test or signed rank test as appropriate. Spearman correlation was used to assess relationships between continuous variables. Per-protocol analysis was performed including all participants who provided data at week 48. All statistical analyses were

performed using Stata version 18 (StataCorp, Texas, USA). A p-value <0.05 was considered statistically significant.

6.3 Results

Study Population and Follow-up

25 participants were randomised, and 23 completed the 24-week intervention phase (VLED n=14, MD n=9). At week 48, 22 participants completed follow-up assessments (VLED n=14, MD n=8), representing 88% retention from the original cohort (Figure 6.1). 1 participant from the MD group was lost to follow-up between weeks 24 and 48 as they withdrew to start semaglutide for weight loss. Baseline characteristics of participants completing week 48 assessments are presented in Table 6.1. The cohort was predominantly middle-aged (median age 47 years (IQR 37, 59)), Caucasian (73%), with mean BMI 31.7 ± 3.1 kg/m². There were no significant baseline differences between groups.



8. Figure 6.1. Participant flow from screening through 48-week follow-up.

38 individuals were assessed, 25 were randomised to VLED (n=14) or MD (n=11). Retention at week 48: 100% (14/14) VLED and 73% (8/11) MD. Reasons for exclusion and withdrawal outlined in figure. VLED = very low energy diet; MD = Mediterranean diet; MRI = magnetic resonance imaging.

20. Table 6.1. Baseline characteristics of study participants completing 48-week follow-up

Characteristics	VLED (n=14)	MD (n=8)	P-value
Age (years)	45 (35, 59)	49 (43, 57)	0.67
Male sex, % (n)	64% (9)	50% (4)	0.51
Caucasian ethnicity, % (n)	64% (9)	88% (7)	0.24
BMI (kg/m²)	31.5 (± 3.2)	32.0 (± 3.0)	0.37
Weight (Kg)	90 (± 13)	94.3 (± 14)	0.41
Waist circumference (cm) - Women - Men	 107.7 (±10.0) 105.6 (±8.3)	 109.5 (±7.9) 114.6 (±8.5)	 0.62 0.06
Type 2 diabetes, % (n)	7% (1)	13% (1)	0.67
MRI-LFF (%)	19.4 (16, 30)	21.5 (17, 27)	0.94
Histological NAS score	4 (4, 5)	5 (4, 5)	0.22
Liver fibrosis score	2 (1, 2)	1.5 (1, 2)	0.51
Liver Stiffness Measurement (kPa)	5.7 (4.6, 7.3)	5.4 (5.0, 5.8)	0.61
CAP (dB/m)	327 (295, 341)	315 (294, 327)	0.47
ALT (IU/L)	46.5 (37, 58)	54.5 (36, 97)	0.54
GGT (IU/L)	49.5 (30, 86)	58 (40, 140)	0.22
HOMA-IR	5.1 (3.2, 6.6)	3.4 (1.9, 5.5)	0.25

Baseline characteristics of participants who completed week 48 follow-up, stratified by intervention group. Continuous variables presented as median (interquartile range) for non-parametric data or mean (± standard deviation) for parametric data. Categorical variables are presented as percentage % (number). Between-group comparisons were performed using Wilcoxon rank-sum test for non-parametric continuous variables, independent t-test for parametric continuous variables, and Fisher's exact test for categorical variables. ALT = alanine aminotransferase; BMI = body mass index; CAP = controlled attenuation parameter; GGT = gamma-glutamyl transferase; HOMA-IR = homeostatic model assessment for insulin resistance; MD = Mediterranean diet; MRI-LFF = magnetic resonance imaging- liver fat fraction; NAS = NAFLD activity score; VLED = very low energy diet.

Primary Outcome: Hepatic Steatosis at Week 48

At week 48, the VLED group demonstrated a sustained and significant improvement in hepatic steatosis compared to baseline, with a median -55% relative reduction in MRI-LFF (IQR -75, -31; p<0.01), which was an absolute reduction of -13.9% (IQR -21, -3) from baseline. 31% (n=4) achieved sustained normalisation (≤5% MRI-LFF) of hepatic steatosis. In contrast, the MD group showed minimal change from baseline, with a median +5% relative change (IQR -23, 40; p=0.67) in MRI-LFF (absolute change +1.3% (IQR -5, 9) from baseline and 0% of participants achieved normalisation of hepatic steatosis (Table 6.2).

Between weeks 24 and 48, both groups showed upward trends in MRI-LFF during the monitoring extension phase. The VLED group had a median absolute increase of +4.2% MRI-LFF (IQR 0, 10; p=0.01), vs +1.4% (IQR 0, 10; p=0.09) in the MD group (p=0.94 between groups). The change in MRI-LFF from baseline to week 48 was strongly correlated to change in body weight during the same period (Spearman's rho = 0.73, p<0.01) (Figure 6.2).

21. Table 6.2. Changes from baseline at week 48 in hepatic, anthropometric, and metabolic outcomes

	VLED (n=14)				**MD (n=8)**				**Between-group**
VARIABLE	**Baseline**	**Week 48**	**Δ BL→48**	**Δ wk24→48**	**Baseline**	**Week 48**	**Δ BL→48**	**Δ wk24→48**	**p-value^**
MRI-LFF (%)	19.4 (16, 30)	13.1 (4, 19)	-13.9 (-21, -3)*	4.2 (0, 10)	21.5 (17, 27)	28.3 (15, 34)	1.3 (-5, 9)	1.4 (0, 10)	<0.01
MRI-LFF ≤5% (n)	-	31% (4)	-	-	-	0% (0)	-	-	0.08
Weight (kg)	90.0 (84, 99)	81.8 (72, 93)	-10.6 (-11.5, -2.9)	+3.8 (2,6.5)	94.3 (87,104)	95.0 (85,107)	-2.2 (-3.2, 1.2)	-0.1 (-0.6,2.1)	0.01
TBWL (%)	-	-	-10.4 (-13, -4)*	+5.3 (3, 8)*	-	-	-2.1 (-3, 1)	+0.1 (-0.6, 2.5)	<0.01
≥10% TBWL (n)	-	50% (7)	-	-	-	0% (0)	-	-	0.02
≥5% TBWL (n)	-	71% (10)	-	-	-	0% (0)	-	-	<0.01
ALT (IU/L)	46.5 (37, 58)	34.5 (23, 40)	-18 (-28, -6)*	+1 (-5, 12)	54.5 (36, 97)	59 (34,75)	-7 (-21, 6)	+10 (0, 26)	0.29
GGT (IU/L)	49.5 (30, 86)	26 (20, 36)	-21 (-29, -7)*	+2 (-3, 4)	58 (40, 140)	68 (40, 136)	-4 (-12, 28)	+20 (2.5, 35)	0.01
LSM (kPa)	5.7 (4.6, 7.3)	4.4 (4.0, 5.8)	-1.5 (-2, -0.1)*	-0.45 (-2, 0)*	5.4 (5.0, 5.8)	6.8 (6.2, 7.8)	+0.9 (0.7, 2.9)*	+2.0 (1.2, 2.8)*	<0.01

CAP (dB/m)	327 (295, 341)	279 (234, 320)	-36 (-44, -12)*	+30 (5, 59)	315 (294, 327)	316 (276, 343)	+14 (-66, 46)	-7 (-38, 23)	0.19
HOMA-IR	5.1 (3.2, 6.6)	3.1 (1.6, 3.7)	-1.6 (-2.9, -0.7)*	0 (-0.8, 0.4)	3.4 (1.9, 5.5)	3.3 (2.0, 4.5)	-0.4 (-2.9, 0.7)	0 (-1.9, 1)	0.28
HbA1c (%)	5.6 (5.6, 6.1)	5.6 (5.3, 5.9)	-0.3 (-0.5, 0)*	+0.2 (0.1, 0.4)*	5.7 (5.4, 6.1)	5.7 (5.4, 5.8)	-0.15 (-0.4, 0)	+0.1 (-0.1, 0.5)	0.49
Lean mass (kg)	52.3 (46.1, 59.5)	52.2 (44.4, 57.7)	-1.9 (-2.3, -1.3)*	+1.1 (-0.7, 1.3)	55.2 (45.8, 59.1)	55.9 (45.0, 59.2)	-0.2 (-0.9, 0.5)	-0.3 (-1.1, 0.1)	<0.01
Fat mass (kg)	33.7 (28.1, 39.2)	27.2 (20.3, 36.7)	-8.1 (-9.4, -4.6)*	+3.8 (2.5, 5.1)*	38.4 (34.6, 43.6)	38.5 (33.5, 41.1)	-2.1 (-2.3, 1)	+1.2 (0.2, 2.7)	<0.01
Body fat (%)	39 (32, 45)	32 (27, 38)	-4 (-7, -2)*	+3 (3, 4)*	41 (37, 47)	40 (38, 48)	-0.8 (-1.3, 0.6)	+1 (0.4, 2)	<0.01

*Outcomes at baseline and week 48 in participants completing follow-up, by intervention group. Data are median (IQR). Changes shown as absolute Δ BL→48 (baseline to week 48) and absolute Δ wk24→48 (week 24 to week 48) except where % noted. Between-group comparisons performed using Wilcoxon rank-sum test (continuous) or Fisher's exact test (categorical). Note 1 VLED participant did not have MRI-LFF performed due to claustrophobia but completed all other outcome measures. Per-protocol analysis of those with available data for each outcome presented. *Within-group change P < 0.05 (Wilcoxon signed-rank test). ^Between-group P-value for Δ BL→48. ALT = alanine aminotransferase; CAP = controlled attenuation parameter; GGT = gamma-glutamyl transferase; HbA1c = glycated haemoglobin; HOMA-IR = homeostatic model assessment for insulin resistance; LSM = liver stiffness measurement; MD = Mediterranean diet; MRI-LFF = magnetic resonance imaging- liver fat fraction; TBWL = total body weight loss; VLED = very low energy diet.*

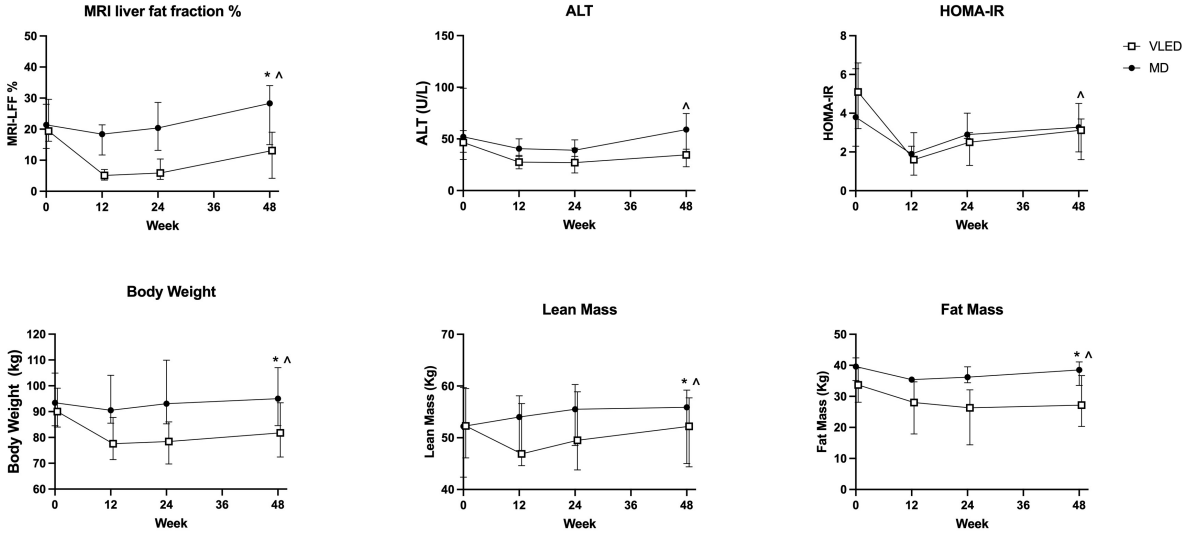


Figure 1. Hepatic, anthropometric and metabolic outcomes over 48 weeks of monitoring.
* p <0.05 VLED vs MD from baseline ^ p <0.05 VLED in-group difference from baseline + p <0.05 MD in-group difference from baseline

9. Figure 6.2. Trajectory of outcomes over 48 weeks.

*Changes in MRI liver fat fraction, alanine aminotransferase (ALT), homeostatic model assessment for insulin resistance (HOMA-IR), body weight, lean mass, and fat mass from baseline through 48 weeks. Data are median with interquartile range. * P < 0.05 VLED vs MD at that timepoint (Wilcoxon rank-sum test). ^ P < 0.05 within-group change from baseline in VLED group (Wilcoxon signed-rank test). + P < 0.05 within-group change from baseline in MD group. VLED = very low energy diet; MD = Mediterranean diet.*

Body Weight Outcomes

The VLED group maintained significant weight loss from baseline with a median -10.4% TBWL (IQR -13, -4; p<0.01), compared to -2.1% TBWL (IQR -3, 1; p=0.34) in the MD group at week 48 (p<0.01 between groups). Clinically significant weight loss (≥10% TBWL) was maintained by 50% (n=7) of VLED participants compared to 0% of the MD group (p=0.02). During the monitoring extension phase (weeks 24-48), the VLED group experienced some weight regain. The VLED group gained a median +5.3% (IQR 3, 8; p<0.01) from week 24 to 48, while the MD group showed minimal change +0.1% (IQR -0.6, 2.5; p=0.78) (p=0.02 between groups).

Liver parameters

Significant improvements in liver enzymes were maintained in the VLED group at week 48, with ALT reduced by -18 IU/L (IQR -28, -6; p<0.01), and GGT -21 IU/L (IQR -29, -7; p<0.01) compared to baseline. In comparison, the MD group showed a non-significant median change in ALT of -7 IU/L (IQR -21, 6, p=0.21) and GGT -4 IU/L (IQR -12, 28, p=0.67), with the between-group difference significant for GGT (p=0.01), but not ALT (p=0.29, Table 6.2).

Fibroscan measurements at week 48 also showed continued improvement in the VLED group, with LSM change of -1.5 kPa (IQR -2, -0.1; p<0.01) from baseline, vs +0.9 kPa (IQR 0.7, 2.9; p=0.02) in the MD group (between-group difference p<0.01). Additionally, the VLED group had a -36 dB/m (IQR -44, -12; p=0.01) reduction in CAP score from baseline, compared to +14 dB/m (IQR -66, 46; p=0.58) in the MD group, though the between-group difference was not significant (p=0.19).

Metabolic Parameters

In addition to improvements in weight and liver parameters, the VLED group maintained improvements in cardiometabolic profile with significant improvements in markers of insulin resistance and glycaemic control. HOMA-IR improved by -1.6 (IQR -2.9, -0.7; p<0.01) versus -0.4 (IQR -2.9, 0.7; p=0.48) in the MD group, and HbA1c decreased by -0.3% (IQR -0.5, 0; p=0.03),

versus in the MD group (-0.15% (IQR -0.4, 0.05) p=0.21). Despite this, no significant difference was noted between groups for these outcomes at week 48 (Table 6.2).

Body Composition

Body composition at week 48 reflected the sustained weight loss in the VLED group and revealed sustained fat mass reduction from baseline. The VLED group showed a median reduction in fat mass (-8.1 kg (IQR -9.4, -4.6) p<0.01), and lean mass (-1.9 kg (IQR -2.3, -1.3) p<0.01) from baseline, while the MD group showed non-significant changes in both (fat mass -2.1 kg (IQR -2.3, 1.0; p=0.58), lean mass (-0.2 kg (IQR -0.9, 0.5; p=0.40)). These fat mass and lean mass changes at week 48 were significantly different between groups (p<0.01 for both) (Table 6.2).

During the monitoring extension phase, the VLED group showed an increase in both fat mass (+3.8 kg (IQR 2.5, 5; p<0.01)) and lean mass (+1.1 kg (IQR -0.7, 1.3; p=0.31)) from week 24. This represents a fat:lean regain ratio of 3.5:1, indicating that 78% of weight regained was fat mass. The MD group had non-significant changes in body composition, but there was a trend towards increase in fat mass (+1.2 kg (IQR 0.2, 2.7; p=0.07)) but no significant change in lean mass (-0.3 kg (IQR -1.1, 0.1; p=0.33)) from week 24. These between-group changes were significant for fat mass (p=0.02) but not lean mass (p=0.25).

Dietary Intake and Adherence

Analysis of three-day food diaries at week 48 revealed that neither group showed significant changes in total energy intake compared to baseline. The VLED group's median energy intake at week 48 was 7655 kJ/day (IQR 5292, 8631), which was not significantly different from baseline (8944 kJ/day (IQR 6326, 11607); p=0.09). Similarly, macronutrient intake at week 48 largely returned towards baseline in the VLED group with 21% of daily energy intake from protein (IQR 16, 26; p=0.09), 35% from fat (IQR 30, 38; p=0.86), and 41% carbohydrate (IQR 33, 49; p=0.48).

The MD group maintained good adherence to Mediterranean diet principles at week 48, with 83% (n=5/6) of participants achieving PREDIMED MEDAS scores ≥7, similar to adherence rates at weeks 12 and 24. There was no change in median daily energy intake at week 48 compared with baseline, but a lower proportion of energy intake came from protein and carbohydrates, with a trend towards higher fat intake, consistent with MD principles (Table 6.3).

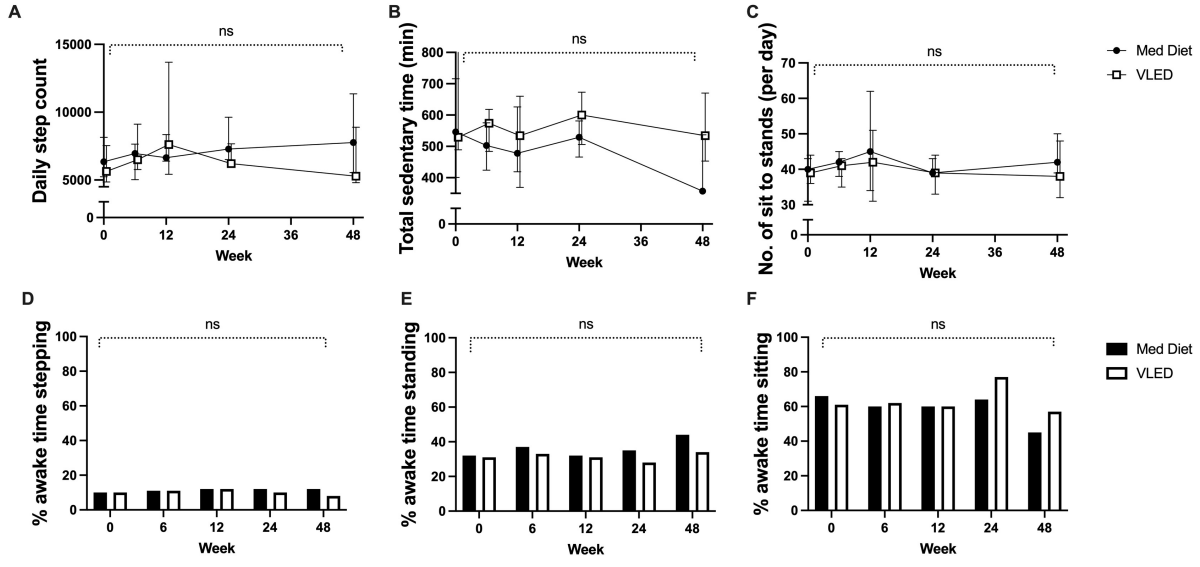
22. Table 6.3. Dietary composition at baseline and week 48 by treatment group

	VLED			MD		
Variable	**Baseline**	**Week 48**	**p-value**	**Baseline**	**Week 48**	**p-value**
n	13	11		11	6	
Energy (kJ/day)	8944 (IQR 6326, 11607)	7655 (IQR 5292, 8631)	0.09	7308 (IQR 6116, 10534)	7727 (IQR 5853, 7950)	0.75
Protein (% energy)	18 (IQR 16, 19)	21 (IQR 16, 26)	0.09	21 (IQR 18, 23)	17 (IQR 16, 21)	0.046
Fat (% energy)	34 (IQR 31, 40)	35 (IQR 30, 38)	0.86	34 (IQR 30, 40)	41 (IQR 37, 46)	0.07
Carbohydrate (% energy)	45 (IQR 39, 48)	41 (IQR 33, 49)	0.48	38 (IQR 32, 46)	30 (IQR 25, 34)	0.046

Data presented as median (IQR). Per protocol analysis, including participants who completed the diet diary at each timepoint. Within-group p-values calculated using Wilcoxon signed-rank test comparing baseline to week 48. VLED = very low energy diet; MD = Mediterranean diet.

Physical Activity

There was no significant change in measures of physical activity at week 48 compared to baseline or week 24 (Figure 6.3), suggesting that physical activity did not contribute to the observed changes in weight and hepatic steatosis.



10. Figure 6.3. Physical activity and sedentary behaviour outcomes over 48 weeks by intervention group.

Daily step count (A), total sedentary time (B), number of sit-to-stand transitions per day (C), and percentage of awake time spent stepping (D), standing (E), and sitting (F) in participants randomised to Mediterranean diet (Med Diet) or very low energy diet (VLED). Data are presented as median with interquartile range (A–C) or percentage (D–F). Between-group comparisons were performed using Wilcoxon rank sum test; within-

group comparisons were performed using Wilcoxon signed rank test. Statistical significance was set at P < 0.05. ns = not significant.

6.4 Discussion

This extension study demonstrates that intensive dietary intervention with a VLED can achieve durable metabolic benefits in MASLD that persist beyond the active treatment period. At week 48, 24 weeks after the cessation of dietary support and pharmacotherapy, the VLED group maintained clinically significant improvements with -10.4% TBWL and a -55% relative reduction in hepatic steatosis from baseline. 31% of the VLED group achieved sustained hepatic steatosis normalisation (MRI-LFF ≤5%) and 50% maintained ≥ 10% TBWL. While weight and hepatic steatosis began to increase after semaglutide cessation at week 24, the benefits that persisted at week 48 still represent clinically meaningful changes.

Intensive dietary intervention with the VLED has been established as effective for metabolic disease, providing the basis for its application to the treatment of MASLD. The DiRECT trial demonstrated that a comparable 3-month VLED delivered in primary care (306 participants with T2DM across 49 primary care practices in the UK), achieved a mean 10kg weight loss at 12 months with 46% T2DM remission(11). The 2-year outcomes confirmed durability of these changes, with the VLED group maintaining a mean 7.6 kg weight loss from baseline and 36% maintaining T2DM remission at 24 months (12). Additionally, 64% of those maintaining ≥ 10% TBWL achieved T2DM remission (12). These findings demonstrate that intensive dietary intervention with the VLED is feasible in routine care, and capable of producing sustained metabolic benefits. Our study applies this approach to MASLD, with the VLED-based treatment resulting in sustained hepatic benefits.

Despite this, weight regain following VLED-induced weight loss is a well-characterised challenge in obesity management, as the significant weight loss achieved triggers physiological weight defence mechanisms such as reduced energy expenditure and sustained elevation of appetite-regulating hormones, which predisposes towards weight regain(4, 5, 13). With weight regain occurring in our study after the cessation of semaglutide at the end of week 24, this suggests that low-dose GLP-1 receptor agonist therapy was helpful in counteracting these compensatory mechanisms during the maintenance phase. This is consistent with meta-analysis data from Johansson et al of randomised controlled trials evaluating post-VLED maintenance strategies that show that anti-obesity drugs improved weight maintenance by 3.5 kg over a median of 18 months(14).

The optimal duration and intensity of post-VLED maintenance interventions remain unclear. Our 12-week course of semaglutide 0.5 mg weekly was considerably shorter than the pharmacotherapy duration in previous studies that demonstrated sustained benefit for weight maintenance, raising the question of whether extended pharmacotherapy treatment, potentially at higher doses, might improve durability(14). This contrasts with the DiRECT trial's approach of structured monthly support for 24 months without pharmacotherapy (12). Our findings highlight the contrasting concepts of weight management interventions in MASLD pursuing effective short-term treatment with the goal of durable remission, against the need for ongoing support which may be more resource-intensive. Our data support both possibilities, with substantial benefits persisting 24 weeks after intervention, however the increasing weight trajectory suggests additional longer-term follow-up data is required to determine optimal maintenance strategies in this cohort.

Our findings support the principle that the degree of weight loss, rather than specific dietary composition, is the main driver of hepatic steatosis reduction in MASLD. We demonstrated a strong correlation between weight change and hepatic steatosis reduction ($r=0.73$) from baseline to week 48, followed by a proportional regain during the extension phase (+5.3% weight change, +4.2% change in MRI-LFF in the VLED group). In contrast, despite adherence to Mediterranean diet principles (83% PREDIMED ≥7 at week 48), the MD group showed no hepatic improvement (MRI-LFF +5% from baseline) with minimal weight loss (−2.1% TBWL). This does not diminish the Mediterranean diet's benefits for cardiovascular health or diabetes prevention, but emphasises that caloric restriction and substantial weight loss are required to reverse hepatic steatosis in MASLD. We demonstrated that the Mediterranean diet can be successfully implemented long-term with minimal monitoring. This raises the potential for a combined strategy using a VLED for rapid initial weight loss and hepatic steatosis reduction, followed by the Mediterranean diet for long-term weight maintenance and cardiovascular disease prevention, although this requires formal prospective evaluation.

Body composition analysis revealed important differences between the weight loss and regain phases. During the intervention (baseline to week 24), weight loss comprised 72% fat and 28% lean mass (fat:lean ratio 2.6:1), indicating preferential fat loss and preserving metabolically active lean mass. However, weight regain during the extension phase was accompanied by a compositional shift to 78% fat mass and 22% lean mass (fat:lean ratio 3.5:1), indicating that regained weight was predominantly fat rather than lean mass restoration. The VLED group retained 88% of fat mass reduction at week 48 despite 34% weight regain, although there was significant individual heterogeneity (range 7.5-106%). Lean mass, particularly skeletal muscle, contributes to resting energy expenditure and insulin sensitivity(15, 16). The less favourable weight regain composition

suggests that interventions specifically targeting lean mass preservation during the maintenance and extension phases, i.e. resistance training or higher protein intake, may improve long-term metabolic outcomes.

Durable disease modification was seen in 31% of VLED participants, who maintained hepatic steatosis normalisation (≤5% MRI-LFF) at week 48, 24-weeks after cessation of all active intervention (2). Additionally, liver enzyme changes suggested sustained metabolic improvements, with GGT remaining significantly improved at week 48 despite weight regain and partial increase in steatosis. GGT has been shown to be a marker of oxidative stress and metabolic dysfunction, and is independently associated with insulin resistance, metabolic syndrome and cardiovascular disease mortality(17-20). Comparatively, ALT, which is more closely linked to hepatocyte injury, remained improved but showed an uptrend in parallel with increasing steatosis, suggesting ALT may be more sensitive to fluctuations in steatosis than GGT(21).

This study has several strengths. The 48-week follow-up provides important insight into long-term weight and hepatic outcomes post weight loss intervention in MASLD, which addresses a critical gap in the literature. This is a well-characterised cohort with comprehensive assessment, including MRI-LFF, Fibroscan, body composition, and metabolic parameters at all timepoints. Additionally, the high retention rate (88% from original cohort, 96% from those who completed to week 24) reduces the risk of retention bias, which can be a limitation of extension studies. Limitations of this study include the pilot design, and small sample size which limits statistical power for sub-group analysis. Additionally, this was a predominantly Caucasian cohort with minimal patients with advanced fibrosis included, which limits generalisability to the wider MASLD population.

Regardless, these findings have demonstrated that a 12-week VLED followed by short-term low-dose semaglutide during the transition off the diet, can produce durable MASLD benefits that persist beyond treatment cessation. This approach could serve as a first-line intervention in patients with MASLD and overweight or obesity, without decompensated disease. This approach of using a time-limited intervention offers practical advantages over indefinite lifestyle modification or prolonged pharmacotherapy; however the trend towards weight regain suggests that further investigation is required to determine optimal long-term maintenance strategies. Larger studies with longer follow-up are required, which could include prolonged GLP-1 therapy, intermittent VLED cycles, or sequential VLED followed by a maintenance MD. Cost-effectiveness analyses and pragmatic real-world implementation studies would also support the translation of this approach into clinical practice.

6.5 Conclusion

A 12-week VLED followed by 12 weeks of low-dose semaglutide for weight loss and maintenance in the treatment of MASLD, resulted in sustained improvements in hepatic steatosis and weight at week 48. Despite some increases after semaglutide cessation, lasting improvements in weight, hepatic steatosis and metabolic parameters persisted 24 weeks after the intervention. These findings also confirm the role of low-dose semaglutide in weight maintenance, counteracting the expected biological pressures towards weight regain. Larger, more diverse cohorts with longer follow-up are required to optimise maintenance approaches and establish long-term efficacy.

6.6 References

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Chapter 7. Summary and Future Directions

7.0 Thesis Overview

Metabolic dysfunction-associated steatotic liver disease (MASLD) represents a significant global health challenge, being both highly prevalent and difficult to treat despite good evidence for the disease-modifying effect of weight loss. Closely linked with obesity and insulin resistance, it is the most common cause of liver disease worldwide, and a growing cause of both liver-related morbidity and mortality and a contributing factor to cardiometabolic disease.

Despite its prevalence and clinical impact, MASLD remains underappreciated compared to other obesity-associated conditions such as T2DM, with public awareness and treatment programs lacking in both funding and support prioritisation. This thesis sought to address this gap by defining the scope of the disease in Australia through its epidemiology and exploring pragmatic treatment approaches through the application of medical weight management to MASLD.

The epidemiology of MASLD, and in particular MASLD-associated hepatocellular carcinoma within Western populations was reviewed (Chapter 1, Section 1.5.2), leading into the original epidemiological analysis establishing the substantial burden of MASLD in Australia, providing estimates of prevalence and fibrosis risk using validated non-invasive markers (Chapter 3). The retrospective cohort study provided real-world data that medical weight management utilising VLEDs can achieve clinically significant weight loss in Australian practice (Chapter 4). These observational findings provided the foundation for the DARWIN randomised controlled trial which adapted this medical weight management approach to the treatment of MASLD and compared it to the commonly used Mediterranean diet. The primary analysis of the DARWIN trial (Chapter 5), demonstrated that a ketogenic VLED achieves substantially greater reductions in hepatic steatosis and total body weight than the MD, with low-dose semaglutide post VLED effective for maintaining these benefits. The extension data from the DARWIN study (Chapter 6) revealed the persistence of clinically meaningful improvements to weight and hepatic steatosis despite partial weight regain, 24-weeks after the cessation of semaglutide in the VLED group. As a whole, these findings established both the scale of MASLD as a public health challenge in Australia, and the potential of a VLED-based weight management approach as an effective and accessible treatment strategy; from real-world clinical practice to randomised trial evidence in MASLD.

The following sections discuss the key findings and clinical implications of each study, before outlining priorities for future research.

7.1 Summary and clinical implications

7.1.1 Epidemiology of MASLD

Key findings

In the cross-sectional analysis of 4,747 Australian adults from the nationwide AusDiab study, 13% had elevated ALT and 37% had MAFLD. Only 22% of those with MAFLD also had an elevated ALT, and 61% had an elevated BARD score, suggesting a significant proportion of Australians with MAFLD may be at risk of advanced fibrosis. T2DM was the strongest predictor of MAFLD (OR 3.39), and while elevated ALT was associated with features of the metabolic syndrome, it was an unreliable surrogate marker for MAFLD, particularly in older adults.

Clinical implications

This was the first Australia-wide study to provide MAFLD estimates using the updated diagnostic criteria, and the high prevalence of MAFLD demonstrated the significant burden of disease in Australia and the need for appropriate identification and diagnosis of those with MAFLD in the community. The high proportion of those with MAFLD at risk of advanced fibrosis (BARD ≥2) also has important implications. While the BARD score used in this study is more sensitive than specific, performing slightly less accurately that the more commonly used FIB-4, it still has a high negative predictive value for underlying advanced fibrosis and has been associated with all-cause mortality and cardiovascular outcomes in those with T2DM in large population-based studies (1, 2). This signals that a large proportion of those with MAFLD in Australia require additional second-line fibrosis assessment and may benefit from specialist hepatology services. This highlights the need for clear referral pathways and has direct implications on resource planning, with service capacity likely insufficient to meet the projected demand as obesity rates continue to rise.

Although pragmatic, the poor sensitivity of ALT as a surrogate marker for MAFLD or risk of advanced fibrosis, further supports the use of composite diagnostic algorithms such as FLI or radiological techniques if there is an index of suspicion, over ALT alone in primary care screening for MAFLD. The strong independent association between T2DM and MAFLD (OR 3.39) supports routine screening in all patients with T2DM, consistent with current guidelines (3). Additionally, the association between sedentary lifestyle and lower educational attainment in those with MAFLD also raises equity concerns, suggesting that MAFLD may disproportionately affect socioeconomically disadvantaged populations who may face greater barriers to care.

7.1.2 Real world outcomes of medical weight management

Key findings

A retrospective cohort analysis of 411 patients attending a publicly funded specialist weight management clinic found that 50% achieved clinically significant weight loss (≥ 10% TBWL) at 12 months, with a mean 10.5% TBWL, although retention was challenging (29% at 12 months). Metabolic phenotype was a significant predictor of weight loss response, with patients without hepatic steatosis (FLI <30) or insulin resistance (HOMA-IR <2.5), more likely to achieve greater weight loss. Early treatment response was also a strong predictor of longer-term outcomes, with patients who achieved at least 5% TBWL at 3 months more likely to achieve ≥ 10% TBWL at 12 months and had higher clinic retention. T2DM was the only predictor of loss to follow-up.

Clinical implications

This real-world outcomes data demonstrated clinically significant weight loss using a VLED-based approach, supporting the use of this model of care as recommended by obesity management guidelines (4). Its effectiveness was the basis for its application to the treatment of MASLD as prospectively analysed in the DARWIN study. Despite this being a high-risk obese cohort, MASLD was clinically documented in only 22%, compared to 54% identified by FLI, illustrating that MASLD is an under-recognised obesity-related complication and greater liver-directed screening in obesity care is required. The identification of hepatic steatosis and insulin resistance as predictors of poorer weight loss response and T2DM as a predictor of loss to follow-up, supports metabolic phenotyping to identify those who may need additional support (e.g. pharmacotherapy, intensified follow-up) in order to achieve weight loss targets. The finding that early treatment response is a strong predictor of 12-month weight loss outcomes, supports the shift away from the conventional step-up care approach, in which intensive interventions are reserved for those who fail less structured approaches, towards the earlier commencement of structured programs such as VLED or pharmacotherapy.

Despite the benefits demonstrated, the poor clinic retention in this service highlights that structural and patient-level barriers to care in obesity remain substantial in the public health system, and likely include cost of meal-replacement products and pharmacotherapy, cultural and health literacy issues, mental health comorbidities and differing patient expectations around weight loss.

7.1.3 Adapting medical weight management to MASLD.

Key findings

In the DARWIN randomised pilot study, a 12-week ketogenic VLED achieved significantly greater hepatic steatosis reduction than a Mediterranean diet (77% vs 14% relative reduction in MRI-LFF),

with 69% of VLED participants normalising liver fat. The VLED also resulted in greater weight loss (13% vs 4% TBWL). Low-dose semaglutide (0.5mg weekly) maintained these benefits to week 24 when superior histological improvement was also seen in the VLED group.

The extension study (week 48 follow-up) found that the VLED group maintained clinically significant improvements in weight (10% TBWL) and hepatic steatosis (55% relative reduction in MRI-LFF) as well as in metabolic parameters such as ALT, GGT, HbA1c and HOMA-IR, despite some weight regain and modest liver fat increase after cessation of the active intervention.

Clinical implications

The DARWIN trial is the first randomised trial to directly compare a ketogenic VLED to MD in patients with biopsy-proven MASLD using gold-standard hepatic outcome measures including MRI-LFF and histological outcomes. This direct comparison can be used to guide treatment recommendations and inform clinical guidelines. The results support the use of the VLED medical weight management approach in MASLD, with the VLED achieving superior hepatic steatosis reduction compared to the MD, and the magnitude of benefit seen was comparable to phase 2 GLP-1 receptor agonist trials, supporting its consideration as a first-line weight loss intervention and viable non-pharmacological alternative.

The VLED is accessible and can be administered or supervised by any trained healthcare provider. While there is some cost to the patient for meal replacement products, they represent a more affordable option for most patients compared to unsubsidised pharmacotherapy. The benefits of VLED-based programs are also largely reproducible in real-world settings, as demonstrated in the retrospective audit study in Chapter 4, and can be delivered in both specialist and primary care settings. While the MD is appropriate for general cardiovascular health and sustainable as an eating pattern, as seen in our week 48 data, it may be insufficient as a first line MASLD treatment when substantial weight loss is required, with MD without significant energy restriction, producing modest hepatic improvement. The dose-response relationship between weight loss and liver improvement support weight loss, rather than dietary composition alone, as being the key driver of liver and metabolic improvements and should be the basis of patient recommendations for treatment.

After significant weight loss was achieved with the VLED, low-dose semaglutide was shown to assist with the transition off the diet, maintaining the effects of the VLED a further 12 weeks despite expected biological pressures towards weight regain. This offers a pragmatic and effective approach to weight maintenance post VLED, with low-dose GLP-1 more affordable and better tolerated than

higher doses. Notably, no participants in the VLED group withdrew due to adverse effects or intolerance to the diet or semaglutide, suggesting that it is well tolerated in this population.

The efficacy of this approach was reinforced by the observation that weight and hepatic steatosis started increasing after semaglutide cessation. Although durable benefits were seen at week 48 (50% maintaining ≥ 10% TBWL, 31% sustaining hepatic steatosis normalisation), supporting the VLED as capable of producing clinically meaningful longer-term benefits, this is likely to be insufficient long term, and raises the possibility of weight maintenance pharmacotherapy being required indefinitely. The weight regain seen disproportionately composed of fat mass, compared to the proportion lost during the weight loss phase, highlighting the clinical importance of both preventing weight regain but also implementing measures to assist with lean mass preservation and maintenance.

Overall, the medical weight management approach of VLED for weight loss, followed by low-dose pharmacotherapy for weight maintenance is effective, accessible, moderately priced and should be more widely considered as a first-line treatment for patients with MASLD who are overweight or obese.

7.2 Future directions

This thesis identifies several areas for future research.

7.2.1 Epidemiological analysis of MASLD

The AusDiab analysis provided nationwide estimates of MASLD prevalence and fibrosis risk, but several important gaps remain. While use of the FLI and BARD scores is pragmatic in an epidemiological setting, prevalence estimates require validation against radiological and clinical gold standards in an Australian population-based cohort. Future studies incorporating second-line non-invasive markers of fibrosis risk such as FIB-4 or VCTE would provide more precise estimates of the proportion of Australians with significant fibrosis requiring specialist review.

At present, while multiple markers of metabolic risk are incorporated into routine national health survey data collection, such as through the Australian Health Survey, this has traditionally not incorporated hepatic steatosis or fibrosis biomarkers. Implementation of routine collection of platelet count, alongside liver enzymes would enable calculation of markers such as FIB-4. This would enable more accurate and timely depiction of prevalence and incidence trends and facilitate more analysis of

high-risk subgroups including those of lower socioeconomic status and more diverse populations who were underrepresented in the predominantly Caucasian AusDiab cohort.

Alongside better epidemiological characterisation, there is a need for targeted awareness campaigns and implementation of more systematic screening in high-risk groups (particularly in T2DM and obesity), which would represent a practical step towards addressing the under-recognition of MASLD demonstrated across studies in this thesis. Future work should also examine how social determinants shape MASLD in Australia, including the role of food insecurity, and further explore the disease burden within socioeconomically disadvantaged, rural and Aboriginal and Torres Strait Islander communities. Existing screening and care pathways also need evaluation to ensure they reach the groups at greatest risk, rather than widening existing inequities. This information would help to inform Australia's response to the recent WHA resolution on steatotic liver disease, and to guide health policy and equitable resource allocation.

7.2.2 Real world medical weight management

Medical weight management is rapidly being transformed by the emergence of new-generation incretin therapies for obesity. As these therapies become more widely available, the focus of future research in real-world settings should shift towards understanding how to best integrate pharmacotherapy into existing models of care. The VLED remains a viable non-pharmacological treatment, but future research could determine the cost-effectiveness and relative efficacy of differing treatment approaches, including either VLED or pharmacotherapy alone; sequentially as used in the DARWIN study, or in combination with low-dose pharmacotherapy, in a 'medication-sparing' approach, to inform evidence-based treatment selection in clinical practice.

Particularly within publicly funded services, cost remains a significant barrier to access of effective obesity treatments. Urgent health economic analyses are required to evaluate the cost-effectiveness of PBS-listing of anti-obesity medications, considering the growing healthcare expense related to obesity-associated health complications. Beyond cost, the poor retention observed highlights the need for better understanding of barriers to sustained engagement with the role of mental health comorbidities, weight stigma, and health literacy worth exploring. Prospective qualitative studies would help to inform more of the patient related factors associated with this, and ideally would include baseline interviews exploring patients' weight journey to date, perceived barriers to care, attitudes towards different weight loss methods and expectations of medical care. Follow-up interviews should be performed by an investigator independent to the treating team to assess patients understanding of proposed treatment, satisfaction with therapeutic relationship, assess ongoing

barriers to care and evolving expectations and goals of care. This would ideally include interviews with patients who have decided to no longer attend the clinic in order to avoid bias. This level of information would help inform clinical practice and best use of the limited resources available to engage these difficult to engage patients.

7.2.3 Adapting medical weight management strategies to MASLD

The application of medical weight management to MASLD is an area that will also evolve rapidly with the progress made in obesity pharmacotherapy. The DARWIN pilot study demonstrated the efficacy of a VLED followed by low-dose semaglutide in a cohort with biopsy confirmed MASLD and obesity, however larger studies, with more diverse populations, including those with compensated cirrhosis are required to confirm the efficacy and safety of this approach. Furthermore, studies evaluating whether this approach is feasible when delivered with minimal monitoring in Australian primary care settings would also broaden the reach and inform implementation of this treatment strategy.

The DARWIN study also confirmed the benefits of the MD although these were more modest compared to the changes associated with significant weight loss with the VLED. As such the role of the MD warrants further investigation, including the feasibility of using energy restricted MDs, or potential use as a weight maintenance diet following VLED-induced weight loss, or as a primary intervention in lean MASLD where aggressive energy restriction is not appropriate.

Long-term weight management remains a significant unresolved challenge in weight and MASLD management and future research should evaluate optimal weight maintenance strategies; including extended high versus low-dose GLP-1 therapy, intermittent VLED cycles to address weight regain, or as previously mentioned sequential or concurrent VLED and low-dose pharmacotherapy.

7.3 Conclusion

MASLD represents a growing public health challenge in Australia, with this thesis demonstrating that its burden is both substantial and likely underestimated. This thesis provides evidence that medical weight management, in particular a ketogenic VLED based approach, is an effective, accessible and pragmatic treatment strategy for MASLD. Real-world outcome data demonstrates that significant weight loss is able to be achieved with this approach, in an Australian publicly funded outpatient-based service, while the DARWIN study provided the first direct evidence that a VLED achieves superior hepatic and metabolic benefits compared to a MD. These findings support the broader

adoption of VLED-based medical weight management as an effective and accessible first-line treatment for MASLD, and also highlight the need for systematic MASLD screening, improved access to care, and continued research into long-term weight management strategies as the therapeutic landscape evolves.

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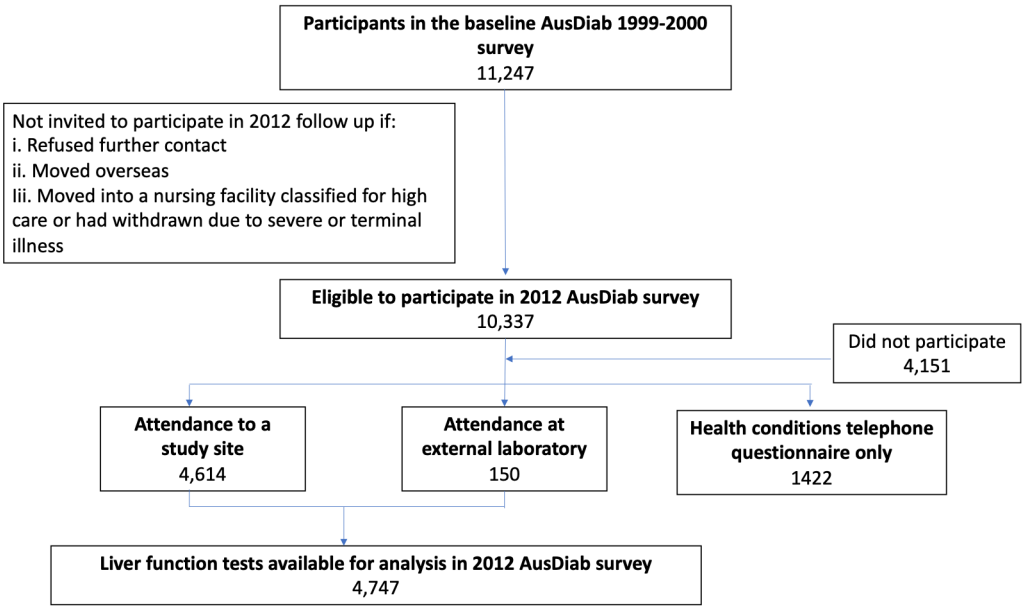
Appendices

Appendix I. Chapter 3 Supplementary material

Supplementary figure 1: Sampling frame for AusDiab follow-up in 2012



Appendix II. Chapter 4 Supplementary Material

Variable	Univariate P value	Adjusted Odds Ratio	95% CI	Multivariable P value
Age, years	0.14	1.00	0.98–1.02	0.87
Male sex	0.17	0.96	0.59–1.56	0.87
Baseline BMI, kg/m²	0.14	1.02	0.99–1.05	0.12
Current smoker	0.15	0.74	0.41–1.31	0.29
Type 2 diabetes	**0.02**	**1.80**	**1.09–2.97**	**0.02**
Referred for bariatric surgery	0.72	0.89	0.54–1.49	0.67
Previous weight loss medication	0.02	0.60	0.36–1.01	0.054

Table S1. Baseline factors associated with loss to follow-up at 12 months. *Multivariable logistic regression model. Loss to follow-up defined as absence of weight data at 12 months. Univariate P values from simple logistic regression; adjusted odds ratios and multivariable P values from model including all listed variables. BMI=body mass index; CI=confidence interval.*

Outcome	Female	Male	P value
Baseline (n=411)			
Sample, % (n)	67% (277)	33% (134)	
3 months (n=237)			
Sample with data, % (n)	67% (159)	33% (78)	
Weight loss, %, mean (SD)	-6.3 (5.4)	-7.4 (5.9)	0.14
Achieved ≥5% TBWL, % (n)	57% (91)	64% (50)	0.31
Achieved ≥10% TBWL, % (n)	28% (44)	31% (24)	0.62
6 months (n=191)			
Sample with data, % (n)	65% (124)	35% (67)	
Weight loss, %, mean (SD)	-8.7 (8.7)	-9.8 (8.2)	0.38
Achieved ≥5% TBWL, % (n)	62% (77)	70% (47)	0.27
Achieved ≥10% TBWL, % (n)	44% (54)	46% (31)	0.72
12 months (120)			
Sample retained, % (n)	27% (75)	34% (45)	
Weight loss, %, mean (SD)	-10.7 (10.6)	-10.2 (9.9)	0.80
Achieved ≥5% TBWL, % (n)	63% (47)	64% (29)	0.85
Achieved ≥10% TBWL, % (n)	53% (40)	44% (20)	0.35
Clinic retention			
Retained at 12 months, % (n)	62.5% (75/120)	38% (45/120)	0.17

Table S2. Weight loss outcomes by sex across timepoints. *Per-protocol analysis including patients with weight data at each timepoint. P values from independent t-tests (continuous) or chi-square tests (categorical). SD=standard deviation; TBWL=total body weight loss.*

Outcome	<40 years	40–54 years	55–64 years	≥65 years	P value
3 months (n=237)					
Weight loss, %, mean (SD)	-5.5 (5.4)	-6.8 (6.0)	-7.2 (5.9)	-6.9 (4.6)	0.53
Achieved ≥5% TBWL, % (n)	59% (23/39)	57% (51/89)	58% (30/52)	65% (37/57)	0.81
Achieved ≥10% TBWL (n)	21% (8/39)	36% (32/89)	25% (13/52)	26% (15/57)	0.25
6 months (n=191)					
Weight loss, %, mean (SD)	-7.2 (9.6)	-10.3 (9.7)	-8.7 (7.8)	-8.8 (6.9)	0.44
Achieved ≥5% TBWL, % (n)	61% (14/23)	66% (45/68)	64% (32/50)	66% (33/50)	0.97
Achieved ≥10% TBWL, % (n)	44% (10/23)	47% (32/68)	40% (20/50)	46% (23/50)	0.89
12 months (n=120)					
Weight loss, %, mean (SD)	-9.4 (12.1)	-10.9 (11.3)	-8.2 (8.7)	-12.1 (9.4)	0.51
Achieved ≥5% TBWL, % (n)	50% (8/16)	67% (28/42)	54% (14/26)	72% (26/36)	0.30
Achieved ≥10% TBWL, % (n)	44% (7/16)	48% (20/42)	39% (10/26)	64% (23/36)	0.21
Clinic retention					
Retained at 12 months, % (n)	25% (16/63)	27% (42/156)	26% (26/99)	39% (36/93)	0.15

Table S3. Weight loss outcomes by age category across timepoints. *Per-protocol analysis including patients with weight data at each timepoint. P values from one-way ANOVA (continuous), or Pearson chi-squared test (categorical). SD=standard deviation; TBWL=total body weight loss.*

Outcome	No steatosis (FLI <60)	Probable steatosis (FLI ≥60)	P value
3 months (n=134)			
Weight loss, %, mean (SD)	-7.0 (6.1)	-6.1 (5.3)	0.35
Achieved ≥10% TBWL, % (n)	27% (17/63)	25% (18/71)	0.83
6 months (n=105)			
Weight loss, %, mean (SD)	-10.3 (8.2)	-8.9 (8.2)	0.35
Achieved ≥10% TBWL, % (n)	54% (28/52)	38% (20/53)	0.10
12 months (n=70)			
Weight loss, %, mean (SD)	-11.4 (10.1)	-10.2 (9.6)	0.61

Achieved ≥10% TBWL, % (n)	63% (20/32)	45% (17/38)	0.12
Clinic retention			
Retained at 12 months, % (n)	33% (32/98)	33% (38/116)	0.99

Table S4. Weight loss outcomes by MASLD (FLI ≥60) vs FLI <60. *Per-protocol analysis including patients with baseline FLI data retained at each timepoint. P values from independent t-tests (continuous) or chi-square tests (categorical). FLI=Fatty Liver Index; SD=standard deviation; TBWL=total body weight loss*

Outcome	Low FLI (<30)	Intermediate FLI (30–59)	High FLI (≥60)	P value
3-month outcomes (n=134)				
Weight loss, %, mean (SD)	-7.6 (6.5)	-6.4 (5.6)	-6.1 (5.3)	0.45
Achieved ≥10% TBWL, % (n)	31% (10/32)	23% (7/31)	25% (18/71)	0.72
6-month outcomes (n=105)				
Weight loss, %, mean (SD)	-11.5 (8.9)	-9.1 (7.3)	-8.9 (8.2)	0.36
Achieved ≥10% TBWL, % (n)	63% (17/27)	44% (11/25)	38% (20/53)	0.10
12-month outcomes (n=70)				
Weight loss, %, mean (SD)	-13.0 (10.1)	-9.4 (10.1)	-10.2 (9.6)	0.53
Achieved ≥10% TBWL, % (n)	78% (14/18)	43% (6/14)	45% (17/38)	**0.048**
Clinic retention				
Retained at 12 months, % (n)	35% (18/52)	30% (14/46)	33% (38/116)	0.91

Table S5. Weight loss outcomes by Fatty Liver Index categories across timepoints. *Per-protocol analysis including patients with baseline FLI data retained at each timepoint. P-values from one-way ANOVA (continuous) and Pearson chi-square (categorical). FLI=Fatty Liver Index; SD=standard deviation; TBWL=total body weight loss.*

Outcome	FLI <30	FLI ≥60	P value
3-month outcomes (n=103)			
Weight loss, %, mean (SD)	-7.6 (6.5)	-6.1 (5.3)	0.21
Achieved ≥5% TBWL, % (n)	69% (22/32)	51% (36/71)	0.09
Achieved ≥10% TBWL, % (n)	31% (10/32)	25% (18/71)	0.53
6-month outcomes (n=80)			
Weight loss, %, mean (SD)	-11.5 (8.9)	-8.9 (8.2)	0.18
Achieved ≥5% TBWL, % (n)	74% (20/27)	59% (31/53)	0.17
Achieved ≥10% TBWL, % (n)	63% (17/27)	38% (20/53)	**0.03**
12-month outcomes (n=56)			

Weight loss, %, mean (SD)	-13.0 (10.1)	-10.2 (9.6)	0.33
Achieved ≥5% TBWL, % (n)	78% (14/18)	68% (26/38)	0.47
Achieved ≥10% TBWL, % (n)	78% (14/18)	45% (17/38)	**0.02**
Program retention			
Retained at 12 months, % (n)	35% (18/52)	33% (38/116)	0.81

Table S6. Weight loss outcomes by Fatty Liver Index: ruled-out vs MASLD by FLI. *Sensitivity analysis excluding patients with intermediate FLI (30–59). Per-protocol analysis including patients with baseline FLI data retained at each timepoint. P-values from independent t-tests (continuous) and Pearson chi-square (categorical). FLI=Fatty Liver Index; SD=standard deviation; TBWL=weight loss*

Characteristic	FLI <60 (n=98)	MASLD (FLI ≥60) (n=116)	P value
Demographics			
Age, years, mean (SD)	54.3 (12.9)	55.0 (11.4)	0.68
Male sex, % (n)	23% (23)	42% (49)	**<0.01**
Anthropometrics			
Weight, kg, mean (SD)	110.2 (17.0)	144.6 (23.6)	**<0.01**
BMI, kg/m², mean (SD)	40.3 (5.0)	51.5 (7.4)	**<0.01**
Waist circumference, cm, mean (SD)	117.5 (11.6)	139.9 (12.9)	**<0.01**
Metabolic parameters			
ALT, U/L, median (IQR)	22.5 (18-40)	34 (22-45)	**<0.01**
GGT, U/L, median (IQR)	26 (18-42)	52 (27-86)	**<0.01**
HbA1c*, %, median (IQR)	5.7 (5.4-6.3)	6.2 (5.7-8.1)	**<0.01**
Comorbidities, % (n)			
Type 2 diabetes	26% (25)	44% (51)	**<0.01**
Hypertension	39% (38)	61% (71)	**<0.01**
Dyslipidaemia	46% (45)	52% (60)	0.40
Clinical MASLD documented	12% (12)	30% (35)	**<0.01**
FIB-4 category**, % (n)			
- Low risk (<1.3)	74% (48)	73% (45)	
- Indeterminate (1.30-2.67)	22% (14)	19% (12)	
- High risk (>2.67)	5% (3)	8% (5)	

Table S7. Baseline characteristics by MASLD (FLI ≥60) vs FLI <60. *The Fatty Liver index is calculated using BMI, GGT, waist circumference and triglycerides so their distributions differ between groups by definition. P values from independent t-tests (continuous parametric variables), Mann-Whitney test (continuous non-parametric variables), or Pearson chi-square test (categorical variables). Note HbA1c available for 150/214 patients with FLI data. FLI=Fatty Liver Index; MASLD=metabolic-dysfunction associated steatotic liver disease; SD=standard deviation; IQR=interquartile range; BMI=body mass index; ALT=alanine aminotransferase; GGT=gamma-glutamyl transferase. *HbA1c available for 150/214 patients with FLI data, and **FIB-4 calculable in 127/214 patients with FLI data; findings should be interpreted with caution given incomplete data.*

Appendix III: Chapter 5 Supplementary Material

Supplementary Text

Inclusion and Exclusion criteria

Inclusion criteria

1. Individuals aged 18 – 70 years, who are able to provide consent
2. BMI ≥27kg/m^2 and ≤35kg/m^2
3. Elevated ALT and/or GGT (above laboratory reference range)
4. ≥ F1 fibrosis on liver biopsy with ≥ Grade 2 steatosis
5. Liver fat on MRS ≥10%

Exclusion criteria

1. Alcohol >2 standard drink/day for men and >1 standard drink/day for women (>20g/day men, >10g/day women)
2. Other liver disease, i.e. viral hepatitis, autoimmune liver disease, haemochromatosis
3. Decompensated cirrhosis or cirrhosis with Fibroscan >25kPa
4. Other significant medical or psychological issue that would preclude a diet; such as cardiac disease, lung disease or malignancy.
5. Antibiotics, probiotics, corticosteroids, hormonal therapies or methotrexate within three months of baseline data collection, or current use of a GLP-1 agonist
6. Already lost >5% body weight or previous bariatric surgery
7. Participation in another interventional trial, or participation in another interventional trial within the last 6 months

Dietary Intervention

All participants underwent a 30 minute face-to-face dietary education session at baseline, and 6-week timepoints with study Accredited Practising Dietitian (APD). Consultations included provision of meal plans and printed educational material which was explained by the study APD (T.P). Phone support was provided during weeks 2, 4 and 9 by the study APD. A face-to-face review at week 6 was conducted for 30 minutes with 20-minute follow-up phone calls at weeks 2, 4, and 9 scheduled to address any questions or concerns. Participants were also able to contact the study team at any time outside of the above scheduled sessions if required. Both groups were given general advice recommending 30-60minutes of moderate physical activity 3-5 times per week (ref).

After completing 12 weeks of their assigned diet, all participants underwent a further face to face 30 minute education session with the study APD for the maintenance phase.

Ketogenic VLED group

Participants followed a diet which consisted of two meal replacement products and one very low carbohydrate, low fat meal per day. The VLED meal plan provided a daily energy intake of 3150.6kJ, and comprised 74g protein (40% total energy), 23g fat (27% total energy), and 56g carbohydrates (31% total energy). Participants were also given written resources to assist with adherence to the VLED that contained:

1. a lists of suggested suitable foods (e.g., non-starchy vegetables) and beverages (e.g., sugar free-soft drinks, herbal tea) that could be consumed on the diet
2. nine recipes ideas for the low-carbohydrate, low-fat meal developed by an APD

Participants were provided with the required meal replacement products for the diet (Optifast VLCD shake (Nestlé)), which were provided at baseline and week 6.

During the maintenance phase, participants in the VLED group had their individual energy requirements calculated using the Schofield equation by the study APD using height, weight, age, sex and activity factor. Participants were encouraged to meet their personal energy requirements, and taught how to track their dietary intake using mobile apps. No specific meal plans were provided, but participants received a handout on the Australian Guide to Healthy Eating to explain and encourage consuming the recommended serving sizes (e.g., 1 cup of leafy greens) and food groups (e.g., fruits, vegetables, grains and cereals, meat and meat alternatives, dairy and discretionary foods). Potential meal ideas and food group combinations were discussed for guidance, with the option for participants to decide how much to follow these recommendations.

Isoenergetic MD group

The isoenergetic Mediterranean diet was based on a previously published ad libitum Mediterranean diet (George nutrients 2018) and modified to be isoenergetic. The diet provided a daily energy intake of 8950kJ, comprising 90.4g protein (17% total energy), 105.9g total fat (44% total energy), 162.1g of carbohydrates (30% total energy), and 50% of total fat from monounsaturated fats. MD participants were provided with a written handout to assist with adherence to the diet that contained:

1. A two-week rotating meal plan with set meals and recipes for breakfast, lunch, and dinner, and snacks
2. MedDiet food pyramid diagram outlining food groups and recommended servings for each group

3. 'No Cooking' and quick meal ideas; pairing vegetables (e.g., green salad mix), carbohydrate (e.g., wholegrain crackers), protein (e.g., canned lentils) and condiments (e.g., extra virgin olive oil)

Essential components of the MD such as extra virgin olive oil, tinned salmon and tuna, baked beans and almonds were provided at baseline and week 6 to participants.

During the maintenance phase, participants in the MD group were encouraged to maintain improved dietary intake and habits based on the MD but were given flexibility to adapt these habits to their personal preferences.

Outcome measures

Outcome	**Measurement**
Primary Outcome	
Change in liver fat fraction from baseline MRI	Magnetic resonance imaging to obtain a liver fat fraction (MRI-LFF) on 3T magnet (Siemens Skyra and Vida) using Siemens Liver lab (software version XA50) protocol
Secondary Outcomes	
Change in liver histology from baseline liver biopsy	Histological NAFLD activity score and Fibrosis score on liver biopsy samples as assessed by a single pathologist
Body weight (kg)	Measured on same digital scales without shoes or excess clothing
Waist circumference (cm)	Measured twice at the midpoint between the bottom of ribs and top of hip bone using non-elastic tape measure without excess clothing underneath. The average measure of the two was used. Assessed change from baseline.
Blood Pressure (mmHg)	Measured twice with participant in seated position using an electronic sphygmomanometer with the lowest reading used. Assessed change from baseline.
Liver enzymes (ALT, AST, GGT, ALP, Bilirubin, Albumin)	On blood samples obtained via venesection and processed by St Vincent's Hospital Melbourne Pathology. Analysed change from baseline.
Lipid profile (LDL-cholesterol, HDL-cholesterol, Triglycerides)	On fasting (>8hrs) blood samples obtained via venesection and processed by St Vincent's Hospital Melbourne Pathology. Analysed change from baseline.

Blood ketones (3-hydroxybutyrate)	On fasting (>8hrs) blood samples collected via venesection and immediately placed on ice and then processed by St Vincent's Hospital Melbourne Pathology. Presence of blood ketones at any level used as measure of ketosis in VLED group.
Insulin resistance assessed via HOMA-IR score	Calculated from fasting glucose and insulin levels on fasting blood samples processed by St Vincent's Hospital Melbourne Pathology. Analysed change from baseline.
HbA1c	On blood samples processed by St Vincent's Hospital Melbourne Pathology. Analysed change from baseline.
Liver stiffness measurement (kPa)	Vibration-controlled transient elastography via a Fibroscan (Echosens, France) performed on fasted patient by a trained technician. Assessed change from baseline.
Controlled attenuation parameter (dB)	Obtained on Vibration-controlled transient elastography via a Fibroscan (Echosens, France) performed in fasted patient by a trained technician. Assessed change from baseline.
Body composition	Performed on a fasted patient with excess clothing and shoes removed by a trained technician. Whole body dual-energy x-ray absorptiometry scan (GE Lunar iDXA Pro, enCORE software version 18). Assessed change from baseline.
Physical activity	Measured using an activPAL (PAL technologies, Scotland) thigh-worn monitor which was worn continuously by participants for 7 consecutive days. Assessed change from baseline.

Diet side effects

	VLED	**MD**	**P value**
Constipation	93% (n=13)	0%	<0.01
Nausea	7% (n=1)	10% (n=1)	0.80
Bloating	29% (n=4)	70% (n=7)	0.05
Abdo pain/discomfort	14% (n=2)	30% (n=3)	0.35

16 participants reported side effects while undergoing the dietary intervention, 10 on the VLED and 6 on the MD. 64% of participants on the VLED reported constipation (n=9/14) with a small number complaining of

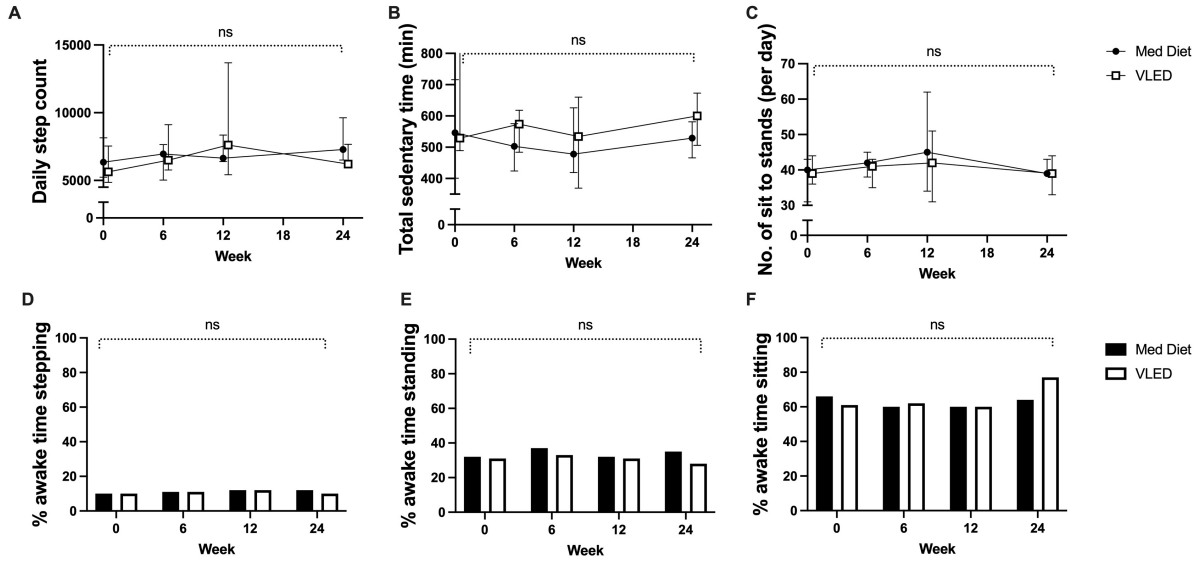
bloating (n=3), abdominal pain or discomfort (n=2) and nausea (n=1). In the MD, bloating was the main reported side effect (60% n=6) and small proportion experiencing abdominal pain or discomfort (n=3) and nausea (n=1). All side effects were reported as mild, and the majority resolved without intervention. One participant on the VLED required regular aperients for constipation.

Adverse Events

Throughout the study five adverse events (AE) reported. There were three liver biopsy related AEs. One participant had a contained bowel perforation that was managed conservatively but required an overnight admission, one participant had a febrile inflammatory reaction post biopsy thought to be associated with the gelfoam used, and another participant had a vasovagal response peri-procedure and required a short period of monitoring. One participant on the VLED arm was referred to psychiatry for worsening of mood and body weight related issues. At the end of the study, one participant on the VLED arm was incidentally found to have a gastric mucosa-associated lymphoid tissue lymphoma, which was not thought to be related to the intervention.

Supplementary Figures

Figure S1: Physical activity across 24 weeks



Physical activity and sedentary behaviour in participants receiving Very Low Energy Diet (VLED) or Mediterranean Diet (MD) interventions over 24 weeks

Comparison of (A) daily step count, (B) total sedentary time, (C) number of sit-to-stand transitions per day, (D) percentage of awake time stepping, (E) percentage of awake time standing, and (F) percentage of awake time sitting at baseline and weeks 6, 12, and 24. Data presented as median with interquartile range. Open bars/squares represent VLED group; filled bars/circles represent MD group. No significant between-group differences were observed for any physical activity parameter (ns = not significant).

Supplementary Tables

Table S1: Schedule of study assessments

Week Number	0	2	4	6	9	12	18	24
Clinic review	x			x		x	x	x
Phone call		x	x		x			
Dietitian review	x					x		x
3-day diet diary	x					x		x
Demographic data	x							
Anthropometry	x			x		x	x	x
MRI	x					x		x
Venesection for pathology	x	x		x		x	x	x
Fibroscan + CAP	x					x		x
Liver Biopsy (US guided)	x							x
DEXA (body composition)	x					x		x
Activity monitor	x			x		x		x

Schedule of study assessments
Overview of clinical assessments and data collection time points throughout the 24-week study period. MRI = magnetic resonance imaging; CAP = controlled attenuation parameter; US = ultrasound; DEXA = dual-energy X-ray absorptiometry.

Table S2: Intention to treat analysis of MRI-LFF change at week 12

	VLED	MD	p-value
Per protocol Relative reduction in MRI-LFF (%)	-77 (IQR -88, -55)	-14 (IQR -30, 0)	< 0.01
Per protocol Absolute reduction in MRI-LFF (% steatosis)	-18 (IQR -24, -10)	5 (IQR -8, 0)	< 0.01
Intention to treat Relative reduction in MRI-LFF (%)	-73 (IQR -89, -49)	-9 (IQR -30, 0)	< 0.01
Intention to treat Absolute reduction in MRI-LFF (% steatosis)	-17 (IQR -24, -8)	-2 (IQR -8, 0)	<0.01

Comparison of per-protocol and intention-to-treat analyses for change in hepatic steatosis at week 12

Data presented as median (interquartile range). Intention-to-treat analysis was performed assuming no change from baseline for participants with missing MRI-LFF data (n=1 VLED, n=1 MD). P-values calculated using Wilcoxon rank-sum test, p<0.05 considered significant. MRI-LFF = magnetic resonance imaging liver fat fraction; VLED = very low energy diet; MD = Mediterranean diet; IQR = interquartile range.

Table S3: Liver histology at baseline and end of intervention

	VLED (n=12)		MD (n=9)		
	Baseline	Week 24	Baseline	Week 24	P-value
NAFLD activity score (NAS)	4 (IQR 4, 5)	2 (IQR 1, 2.5)	5 (IQR 4, 5)	4 (IQR 3, 4)	<0.01
Steatosis score	2 (IQR 2, 3)	1 (IQR 0, 1)	3 (IQR 2, 3)	2 (IQR 1, 2)	0.02
Ballooning score	1 (IQR 1, 1)	0 (IQR 0, 1)	1 (IQR 1, 1)	1 (IQR 0, 1)	0.04
Lobular Inflammation score	1 (IQR 1, 1)	1 (IQR 1, 1)	1 (IQR 1, 1)	1 (IQR 1, 1)	0.41
Fibrosis score	2 (IQR 1, 2)	1.5 (IQR 1, 2)	1 (IQR 1, 2)	2 (IQR 1, 2)	0.14

Liver histology at baseline and week 24 in participants with paired liver biopsies
Data presented as median (interquartile range). P-values represent between-group comparison of change from baseline, calculated using Wilcoxon rank-sum test, $p<0.05$ considered significant. Histological scoring performed using the NASH Clinical Research Network (CRN) criteria. VLED = very low energy diet; MD = Mediterranean diet; NAS = NAFLD activity score; IQR = interquartile range.

Table S4: Dietary macronutrient composition across 24 weeks

	VLED			MD		
	Baseline	Week 12	Week 24	Baseline	Week 12	Week 24
Daily energy (kJ)	8944 (IQR 6326, 11607)	4791 (IQR 3745, 5377)	6036 (IQR 5072, 8264)	7308 (IQR 6116, 10534)	7440 (IQR 6038, 10788)	7410 (IQR 6791, 11352)
Daily carbohydrate (g)	237 (IQR 143,274)	62 (IQR 52, 74)	152 (IQR 110, 223)	176 (IQR 135, 224)	128 (IQR 109, 193)	127 (IQR 110, 181)
Protein (% total daily energy intake)	18 (IQR 16, 19)	34 (IQR 27, 39)	20 (IQR 18, 21)	21 (IQR 18, 23)	19 (IQR 16, 20)	19 (IQR 18, 20)
Fat (% of total daily energy intake)	34 (IQR 31, 40)	41 (IQR 35, 43)	35 (IQR 32, 38)	34 (IQR 30, 40)	47 (IQR 45, 51)	41 (IQR 37, 49)
Carbohydrate (% of total daily energy intake)	45 (IQR 39, 48)	25 (IQR 17, 27)	44 (IQR 37, 44)	38 (IQR 32, 46)	28 (IQR 26, 33)	34 (IQR 28, 37)

Dietary macronutrient composition at baseline, week 12 and week 24
Data presented as median (interquartile range), derived from 3-day diet diaries. VLED = very low energy diet; MD = Mediterranean diet; IQR = interquartile range; kJ = kilojoules.

Table S5: Micronutrient changes pre and post dietary intervention

	VLED			MD		
	Baseline	Week 12	P-value	Baseline	Week 12	P-value
Mg (mmol/L)	0.8 (IQR 0.75, 0.82	0.82 (IQR 0.78, 0.85)	0.07	0.8 (IQR 0.8, 0.83)	0.83 (IQR 0.8, 0.91)	0.8
Alb (g/L)	41 (IQR 39, 42)	41.5 (IQR 40, 43)	0.046	41 (IQR 40, 44)	42.5 (IQR 40, 44)	0.38
Vit D (mmol/L)	61 (IQR 30, 84)	92 (IQR 57, 102)	<0.01	48 (IQR 35, 60)	58 (IQR 31, 80)	0.14
Ferritin (µg/L)	285 (IQR 149, 376)	166 (IQR 128, 304)	0.2	211 (IQR 125, 382)	164 (IQR 134, 319)	0.01
Transferrin Sat (%)	24 (IQR 18, 31)	23 (IQR 18, 25)	0.26	24 (IQR 18, 40)	27 (IQR 24, 28)	0.76
B12 (pmol/L)	356 (IQR 284, 379)	454 (IQR 398, 567)	<0.01	333 (IQR 174, 409)	269 (IQR 177, 358)	0.19
Folate (nmol/L)	25 (IQR 19, 33)	27 (IQR 21, 33)	0.71	30 (IQR 22, 36)	29 (IQR 21, 35)	0.31

Micronutrient profile at baseline and week 12
Data presented as median (interquartile range). P-values represent within-group change from baseline, calculated using Wilcoxon signed-rank test, $p<0.05$ considered significant. VLED = very low energy diet; MD = Mediterranean diet; Mg = magnesium; Alb = albumin; Vit D = vitamin D; Transferrin Sat = transferrin saturation; IQR = interquartile range

Appendix IV. DARWIN study protocol

Full Project Title	The Effect of Diet on Appetite Regulation, Weight and Steatosis in Individuals with Nonalcoholic Fatty Liver Disease
Short Title	The DARWIN studies
Primary Investigator	Assoc. Professor Marno Ryan
Coordinating Site	Department of Gastroenterology St Vincent's Hospital Fitzroy VIC 3065
Supporting Site	Centre for Exercise and Nutrition Australian Catholic University Fitzroy VIC 3065

Introduction

Obesity and NAFLD

The Australian Bureau of Statistics National Health Survey (2014-2015) estimates that over one quarter of Australians are obese. Obesity is associated with multiple metabolic comorbidities including Nonalcoholic Fatty Liver Disease (NAFLD), which is the most prevalent liver disease in Australia. NAFLD occurs in up to 80% of individuals with morbid obesity (BMI >35kg/m2), and a proportion of individuals can progress to fibrosis and cirrhosis. At present the only approved therapy for NAFLD is loss of weight.

Weight homeostasis and the GIT

Diet induced weight loss is difficult to maintain due to compensatory changes that encourage energy balance and weight gain. These mechanisms include alterations in Appetite Regulating hormones (AR hormones) i.e. reduction in hormones that regulate satiety (CCK, GLP-1), and an increase in hormones that promote hunger (ghrelin).

The gastrointestinal tract (GIT) is important in weight homeostasis. The AR hormones from the GIT (CCK, GLP-1, and peptide YY) are released from the duodenum and jejunum in response to fatty acids with chain length >12 carbon atoms. These play a role in the suppression of appetite, and in obesity these responses are attenuated. Small intestinal Free Fatty Acid (FFA) sensors located in the duodenum and jejunum mediate the fat-induced release of GI hormones, and obesity has been shown to be associated with altered duodenal expression of a number of these (FFAR1, FFAR4 and CD36)[1]. It is unclear however what the effect weight loss has on the expression of these receptors, and whether changes to this expression could play a role in weight regain following weight loss.

The gut microbiome is also thought to play a role in the pathogenesis of obesity and evidence suggests that dysbiosis is closely related to the onset and progression of NAFLD[2,3]. Individuals with a low gut bacterial diversity have been noted to have increased overall adiposity, insulin resistance and dyslipidaemia as well as a more inflammatory phenotype (elevated C-Reactive Protein and higher White Cell Count)[4]. A dietary intervention study in 49 obese and overweight individuals, found that a low energy, high protein diet for 6 weeks could improve this lack of bacterial diversity but found

no improvement in low grade inflammation during this short intervention[5]. It is not yet evident how this dysbiosis responds to sustained weight loss or weight loss via different mechanisms, and the resulting impact on inflammation.

The role of diet

Diet is a key factor in loss of weight and at present the only dietary intervention that has been shown to improve hepatic steatosis without weight loss is the Mediterranean diet (MD)[6]. Recently there has been increasing interest in the Very Low Energy ketogenic Diet (VLED) in the management of obesity however the impact on hepatic steatosis is not well defined. The VLED is a rapid weight loss diet, often leading to greater weight loss than low fat diets[7]. During the VLED, the carbohydrate restriction leads to fatty acid oxidation in the liver, resulting in the production of ketones. These have been associated with a reduction in appetite during ketosis, thus potentially helping participants to maintain their diet. A study by Proietto and colleagues confirmed this; 39 non-diabetic overweight/obese individuals completed an eight-week VLED followed by a 2 week re-feeding phase. They demonstrated that during the eight-week VLED, ghrelin levels were kept suppressed, CCK levels did not fall, and participants' hunger ratings were lower than in the re-feeding phase, post diet[8].

The ketogenic VLED offers rapid weight loss and thus health benefits for individuals with NAFLD and other metabolic co-morbidities. A recent study examined the effect of a short term (2 week) modified ketogenic diet on liver histology in individuals prior to bariatric surgery and found a reduction in liver fat without any negative or unintended outcomes[9].

Use of appetite suppressing medications

To counteract the body's hormonal adaptations to weight loss and prevent weight regain, pharmacotherapy can be used to help with appetite suppression. Semaglutide is a Glucagon-like peptide-1 analogue and has been shown to induce a modest amount of short to medium term loss of weight, and improvement in markers of hepatic steatosis and hepatic inflammation[10,11]. The evidence to date suggests that Semaglutide can be used as an adjunct in the treatment of NAFLD, however lifestyle induced weight loss, rather than pharmacotherapy is still the recommended treatment approach. We propose that the role for this pharmacotherapy in NAFLD is not to induce weight loss,

but as an aid for weight maintenance, an approach commonly used in the medical management of obesity.

Bariatric surgery

The prevalence of NAFLD in individuals undergoing the laparoscopic sleeve gastrectomy (LSG) or laparoscopic Roux-en-Y gastric bypass (LRYGB) has been reported to be as high as 90%[12]. Many studies have demonstrated that bariatric surgery in these individuals is associated with improvement in liver histology, resolution of NASH and reduction in hepatic fibrosis[13; 14; 15].

Furthermore, the sustained weight reduction that can be achieved with bariatric surgery is significant. In a recent meta-analysis, the percentage excess weight loss was more than 50% at 10 years post-surgery for both the LSG and the LRYGB [16]. The mechanisms for this resistance to weight regain are still being elucidated.

During the LSG, the fundus is removed, which is the primary site of ghrelin production, and the sustained weight loss is partially attributed to a loss of hunger, but the mechanisms are not clear. During the LRYGB, the duodenum is isolated, and theoretically GLP-1 secretion is boosted by an increase in bile salt delivery to the distal ileum.

Study Rationale

We plan to examine the effects of two different dietary approaches on liver steatosis and histology, as well as on AR hormones, duodenal GI receptor expression and the gut microbiome. This is an attempt to examine the mechanisms of dietary mediated changes to the liver and weight regulation.

In addition, we plan to examine the changes in microbiome and duodenal AR hormone receptor expression in individuals undergoing the LSG, to examine the mechanisms behind the sustained weight loss seen in these patients.

Hypothesis

1. The VLED will be safe and well tolerated in individuals with NAFLD.
2. More participants on the VLED will achieve a significant weight loss, due to the ketosis

3. At the end of the intervention period the VLED group will have a greater loss of hepatic steatosis than the MD
4. Improvement in insulin sensitivity will be greater per unit body weight loss for those on the MD than those on the VLED.
5. Participants on the VLED will have a loss of diversity in their gut microbiome whilst on the VLED, which will normalise by 48weeks. In contrast, those on the MD will have an improvement in gut microbiome diversity.
6. The MD will be better tolerated with a higher degree of adherence than the VLED, and will be associated with an improvement in mood and QoL.
7. The use of appetite suppressing medications will improve weight maintenance post VLED
8. The abnormal expression of duodenal FFA sensors in obesity will be modified following loss of weight, particularly after Bariatric Surgery

Aims

1. In individuals with NAFLD compare the effects of two different dietary approaches on:

i. Weight loss
    a. Change in weight, BMI, waist circumference, body composition
ii. Liver outcomes:
    a. Change in liver fat as measured by MRI
    b. Change in liver stiffness as measured by elastography
    c. Change in histological inflammation/steatosis/fibrosis in a subset who will undergo a liver biopsy
iii. Insulin sensitivity
iv. Changes in AR hormones – ghrelin, peptide YY, GLP-1, CCK
v. Diversity of the gut microbiome
vi. Adherence to the diet and Quality of life
vii. Effect on daily physical activity

2. To examine changes seen in the gut microbiome and AR hormone expression and AR hormone receptor expression in individuals undergoing Bariatric Surgery.

Methods

Overview

This research will comprise two different trials which will run in parallel.

Study 1: Dietary intervention randomised control trial

This single centre randomised dietary intervention study will assess the effects of two different dietary management approaches in overweight and obese individuals with NAFLD. The two interventions are:

1. The Very Low Energy Diet (VLED) at 800 kCal followed by Semaglutide
2. The Mediterranean diet (MD)

Study 2: Prospective cohort bariatric surgery study

A prospective cohort study will follow obese patients with NAFLD undergoing bariatric surgery, assessing for change in liver fat and loss of body weight, comparing baseline parameters to post surgical changes at 1 year. Expression of AR hormones and AR hormone receptors at baseline and 12 months will be examined.

Study 1: Dietary Intervention

Inclusion criteria

6. Individuals aged 18 – 75 who are able to provide consent
7. BMI ≥ 27kg/m^2 and ≤ 35kg/m^2
8. Elevated ALT and/or GGT
9. ≥ F1 fibrosis on liver biopsy (with ≥ Grade 2 steatosis)
10. Liver fat on MRS ≥ 10%

Exclusion criteria

8. Alcohol >2 SD/day for men and >1 SD/day for women
9. Other liver disease, i.e. viral hepatitis, autoimmune liver disease, haemochromatosis
10. Decompensated cirrhosis or cirrhosis with Fibroscan > 25kPa

11. Other significant medical or psychological issue that would preclude a diet; such as cardiac disease, lung disease or malignancy
12. Antibiotics, probiotics, corticosteroids, hormonal therapies or methotrexate within three months of baseline data collection, or current use of a GLP-1 agonist or insulin
13. Already lost >5% body weight or previous bariatric surgery
14. Participation in another interventional trial, or participation in another interventional trial within the last 6 months

Recruitment

Participants will be recruited from the Liver, Diabetes and Obesity Clinics at St Vincent's Hospital Melbourne. The individual would be randomised on the day of recruitment to either:

1. VLED
2. MD

Randomisation

Once enrolled, participants will be allocated a trial number which will serve as their identifier throughout the course of the study, and will be used to generate a computerised randomisation.

Participants will undergo 1:1 randomisation to the two groups with stratification for diabetes mellitus and gender.

Baseline assessment

The baseline assessment will be as follows:

Demographic information: Age, sex, ethnicity

Anthropometry: Weight, height, waist and hip circumference

Assessment of nutritional and metabolic state:

i. Blood tests: FBE, UEC, LFT inc AST, fasting glucose, HbA1c, lipid profile (LDL-cholesterol, HDL-cholesterol, Triglycerides, total cholesterol), hs-CRP, Iron studies, vitamin D, B12, folate

Appetite regulating hormone assessment (ghrelin, peptide YY, GLP-1, CCK, leptin)

i. Fasting
ii. Post prandial: 30min, 60min, 120min
iii. Visual analogue scale (VAS) of appetite

NAFLD assessment

i. MR Liver
ii. Ultrasound guided Liver biopsy
iii. Hepatic non-invasive transient elastography (Fibroscan)

Diet and activity assessment

ii. Diet diary for 3 days
iii. Activity monitor for 1 week

Stool collection for microbiota assessment

Quality of life and mood questionnaires

i. Primary goals for weight loss questionnaire (PGWLQ)
ii. Nutrition quality of life (NQoL)
iii. Eating disorder examination questionnaire (EDEQ)
iv. Diet related quality of life (DR-QoL)
v. Depression anxiety stress scale (DASS 21)
vi. Clinical impairment assessment (CIA)
vii. Assessment of quality of life (AQoL)

Dietary intervention

Following the data collection, the participants will be commenced on the diet they have been assigned:

a. **VLED:** Consists of two meal replacement products (Optifast) and one very low carbohydrate meal per day, to a total of 800kCal The meal replacement products will be provided and a meal plan provided for the daily meal.

b. **MD:** This is an ad libitum, predominantly plant based diet supplemented with monounsaturated fatty acids, and will consist of a two-weekly rotating meal plan.. Olive oil, almonds, canned legumes and canned fish will be supplied during the 12 week intervention period.

The dietary interventions will go for 12 weeks and at this point they will undergo end of diet assessments. A clinical assessment will be performed halfway through the diet at 6 weeks and phone contact will be made at 2, 4 and 9 weeks to monitor progress.

Monitoring Phase

After the 12 week dietary intervention those in the VLED group will be advised to follow an ad libitum but calorie controlled diet and will be commenced on Semaglutide to assist in weight maintenance. The Semaglutide will be commenced at 0.25mg weekly and uptitrated to a dose of 0.5mg weekly after four weeks. Participants will continue on 0.5mg weekly until week 24. The Semaglutide as well as consumables such as needles and sharps disposal bins will be supplied to the participants at no personal cost.

In this phase of the trial the MD group will no longer receive supplemented food products but will be advised to continue to follow the principles of the MD without restriction.

At the end of the monitoring phase at week 24, participants will undergo a repeat MR liver as well as a liver biopsy to assess for change in steatosis and/or inflammation. In participants who elected to have a baseline gastroscopy with duodenal biopsy, these will be repeated at this point in time.

Weight Maintenance Phase

Participants will continue to be monitored until week 48 to assess for weight maintenance and durability of the hepatic response to the diets.

Final data collection

At the end of the trial at 48 weeks, there will be a final data collection, with the baseline investigations repeated, including body anthropometry, MR liver, as well as diet and activity assessments.

Compliance

This will be assessed using diet diaries and by assessing ketone levels in the VLED group. In those receiving Semaglutide, medication compliance will be monitored by counting the medication supply.

Participant Interviews

The study dietitian will conduct one-on-one online recorded interviews using Zoom, with each individual, consenting participant who has taken part in this study. Participants will download a free version of Zoom on their smartphone or laptop/desktop computer.

These participant interviews recorded through Zoom and deidentified once transcribed using Otter AI will be saved in an encrypted password protected secure server database in a St Vincents Hospital drive.

The one-to-one interview questions were developed using Proctors framework [17], a implementation science framework strategy. This framework will obtain information to support the implementation of outcomes. Questions will be related to acceptability, adoption, appropriateness, costs, feasibility, fidelity and sustainability in this RCT.

In implementation science, implementation strategies such as Proctors, are of importance as they establish the 'how to' component of changing healthcare practice [17]. They are also required to understand the drivers of differences and how to bring effective interventions to those most in need [17]. To eliminate bias, one question (question 4) will ask participants to rate how helpful (from 0-100) staff members and the study dietitian was during the 12-week intervention. This question will be completed by each study participant anonymously using an online survey tool (Qualitrics). A study link will be sent to each participant to answer this question. During the online one-on-one recorded interview through Zoom, Otter AI will be installed which uses artificial intelligence in real-time to transcribe each recorded interview. The interviews will take approximately 20-30 minutes to complete.

The purpose of conducting participant interviews is to apply the Proctors implementation framework to;

1. determine study participants' opinions and factors associated with reinventing improved methodologies for future dietary research trials;
2. and whether participants can sustain positive dietary changes in the long term after following a 12-week MD or VLCD intervention

Figure 1. Timeline of investigations in Diet Cohort

Week Number	0	2	4	6	9	12	18	24	36	48
All participants										
Clinic review	x			x		x	x	x	x	x
Phone call		x	x		x					
Dietician review	x					x		x		x
3-day diet diary	x					x		x		x
Demographic data	x									
Anthropometry	x			x		x	x	x	x	x
Blood tests	x	x		x		x	x	x	x	x
Stool collection	x			x		x		x		x
AR hormones	x					x				x
Fibroscan + CAP	x					x		x		x
Liver ultrasound	x									
Liver Biopsy (US guided)	x							x		
MRI	x					x		x		x
DEXA (body composition)	x					x		x		x
Quality of life and mood questionnaires	x					x		x		x
Activity monitor	x			x		x		x		x

Study 2: Bariatric Surgery

Inclusion criteria

Individuals undergoing bariatric surgery who have at least 2 metabolic risk factors and an elevated ALT/GGT.

Exclusion criteria

1. Alcohol >2 SD/day for men and >1 SD/day for women
2. Other liver disease, i.e. viral hepatitis, autoimmune liver disease, haemochromatosis
3. Antibiotics, probiotics, corticosteroids, hormonal therapies or methotrexate within three months of baseline data collection.
4. Participation in another interventional trial

Recruitment

Participants will be recruited through the Bariatric Surgery clinics at St Vincent's Hospital Melbourne.

Baseline assessment

The baseline assessment will be as follows:

Demographic information: Age, sex, ethnicity

Anthropometry: Weight, height, waist and hip circumference

Assessment of metabolic state:

i. Blood tests: FBE, UEC, LFT inc AST, fasting glucose, HbA1c, lipid profile (LDL-cholesterol, HDL-cholesterol, Triglycerides, total cholesterol), hs-CRP, Iron studies
ii. Nutritional status: B12, folate, Iron studies, vitamin D, selenium, zinc
iii. Dietary diary for 1 week
iv. Activity monitor for 1 week
v. Hepatic non-invasive transient elastography (Fibroscan)
vi. Appetite regulating hormones (ghrelin, peptide YY, GLP-1, CCK)
vii. MRI liver

Diet and activity assessment

Stool collection for microbiota assessment

Quality of life questionnaires

Gastroscopy and duodenal biopsy

Intraoperative liver biopsy (Optional)

End of trial assessment (52 weeks)

As for baseline data collection, including a repeat Gastroscopy and duodenal biopsy. Participants who had an intra-operative liver biopsy at baseline will be offered the option of having a repeat liver biopsy under ultrasound guidance.

Week Number	0	12	26	52
Clinic review	x	x	x	x
Demographic data	x			
Anthropometry	x	x	x	x
Routine bloods	x	x	x	x
Stool collection	x			x
AR hormones	x	x		x
Fibroscan + CAP	x	x	x	x
MRI	x			x
Liver ultrasound	x			
DEXA (body composition)	x	x	x	x
Activity monitor	x	x	x	x
Quality of life and mood questionnaires	x	x	x	x
Gastroscopy + duodenal Bx	x			x
Optional				
Liver biopsy	x (intra-operative)			x (US guided)

Figure 3: Bariatric surgery prospective cohort study

Patient information and Informed consent

The patient information sheet will explain the nature of the study, its objectives and the potential risks and benefits. Written consent will be obtained in writing prior to the start of the study and those unable to consent will not be included in the trial.

Laboratory techniques

- Standard of care pathology tests will be processed via St Vincent's Pathology.
- Hepatic elastography will be performed using Fibroscan ®, with 10 readings taken from the right side of the abdomen, using either the M or the XL probe.
- The MRI and ultrasound scans will be performed at St Vincent's Radiology
- DEXA scans will be performed at the Australian Catholic University, Fitzroy campus
- Activity monitors will be supplied and analysed by the Australian Catholic University Centre for Exercise and Nutrition
- Expression of lipid and glucose receptors will be assessed using immunohistochemistry and RT PCR techniques.
- Liver biopsies will be performed by the St Vincent's radiology department and the histology will be assessed by a single specialist pathologist in the St Vincent's pathology department who will be blinded to the intervention.
- Endoscopy will be performed by Gastroenterologists and Upper Gastrointestinal Surgeons at St Vincent's Hospital and St Vincent's Private Hospital Fitzroy.

Results

Primary end-point

- Hepatic steatosis as measured by MR at 12 and 24 weeks
- Weight loss at 12 and 24 weeks

Secondary end-points

- Change in liver histology; steatosis grade, fibrosis stage and NAFLD Activity Score (NAS)
- Weight loss (as % of baseline body weight) at 48 weeks
- Hepatic steatosis as measured by MRI at 48 weeks
- Liver Function Tests

- Liver stiffness – measured by Fibroscan
- Insulin resistance
- Changes in anthropometric measures, fat and lean muscle mass
- Changes in AR hormones and AR hormone receptors in the duodenum
- Diversity of gut microbiome
- Tolerance of the diets including mood and quality of life

Sample size

Study 1

Based on previous MD studies in NAFLD patients, which found an MRI liver fat reduction of 12-25%, we anticipated a 15% reduction in liver fat on the MD with a standard deviation (SD) of 8%. As a pilot study, 20 participants with 10 in each arm would provide an 80% power with 0.05 alpha to detect a difference of 20%. We aim to recruit 24 participants to allow for 20% drop out.

Study 2

This cohort will be used to compare the metabolic changes in the dietary group to those undergoing bariatric surgery, and is also a pilot study to assess the impact of surgery on AR hormones and AR hormone receptors. For this group we aim to recruit 20 participants.

Statistical methods / Analysis

Intention-to-treat (ITT) will be used to analyse the primary and secondary end-points and all statistical comparisons will be made with a 5% level of significance and a 95% confidence interval.

Baseline demographic information such as age, ethnicity, gender, and co-morbidities will be reported as descriptive statistics (mean, percentages, standard deviation and ranges). Similarly liver stiffness measurements, change in weight, hormone profile and expression of duodenal AR hormone receptors will also be expressed as descriptive statistics.

For quantitative end-points t-test and linear regression will be used to compare the groups.

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