Title: *Ad libitum* Mediterranean and Low Fat Diets both Significantly Reduce Hepatic Steatosis: a Randomized Controlled Trial

Authors:

Catherine Properzi¹, Therese A. O'Sullivan¹, Jill L. Sherriff², Helena L. Ching^{3, 4}, Garry P. Jeffrey^{3, 4}, Rachel F. Buckley^{6, 7, 8}, Jonathan Tibballs⁵, Gerry C. MacQuillan^{3, 4}, George Garas^{3, 4}, Leon A. Adams^{3, 4}.

Affiliations:

1. School of Medical and Health Science, Edith Cowan University, Joondalup, Australia;
2. School of Public Health, Curtin Health Innovation Research Institute, Curtin University, Bentley, Australia;
3. Medical School, The University of Western Australia, Nedlands, Australia;
4. Department of Hepatology, Sir Charles Gairdner Hospital, Nedlands, Australia;
5. Department of Radiology, Sir Charles Gairdner Hospital, Nedlands, Australia;
6. Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, United States;
7. Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, Australia;
8. Melbourne School of Psychological Sciences, University of Melbourne, Parkville, Australia.

Keywords: NAFLD, Mediterranean, low fat, diet, randomized controlled trial

Contact:

Assoc. Professor Adams, M503, Medical School, University of Western Australia
QEII Medical Centre, Nedlands WA 6009, Australia

Ph: (+619) 6151 0835 Fax: (+618) 6151 1028 Email: leon.adams@uwa.edu.au

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1002/hep.30076](https://doi.org/10.1002/hep.30076)

This article is protected by copyright. All rights reserved

Abbreviations:

AQoL / QoL Assessment of Quality of Life/ Quality of Life

AIx Aortic augmentation index

ANCOVA Analysis of covariance

AST Aspartate aminotransferase

BMI Body mass index

CVD / CV Cardiovascular disease/ cardiovascular

GGT Gamma-glutamyl transferase

FRS Framingham Risk Score

HbA1c Glycated haemoglobin

HOMA_IR Homeostatic model assessment of insulin resistance

HTGC Hepatic triglyceride content

INR International normalised ratio

IPAQ International Physical Activity Questionnaire

LF Low Fat diet

LSM Liver stiffness measurement

MD Mediterranean diet

MET Metabolic equivalent

MRS Magnetic resonance spectroscopy

NAFLD Non-alcoholic fatty liver disease

NASH Non-alcoholic steatohepatitis

PREDIMED *Prevención con Dieta Mediterránea*

PWV Pulse wave velocity

PRESS Point-resolved spectroscopy sequence

Financial Support: Catherine Properzi received an Australian Government Research Training Program Scholarship.

Cobram Estate Olive Oil donated a portion of the olive oil supplied on the Mediterranean intervention.

Abstract

Although diet induced weight loss is first-line treatment for patients with non-alcoholic fatty liver disease (NAFLD), long-term maintenance is difficult. The optimal diet for either improvement in NAFLD or associated cardio-metabolic risk factors regardless of weight loss, is unknown. We examined the effect of two *ad libitum* isocaloric diets [Mediterranean (MD) or Low Fat (LF)] on hepatic steatosis and cardio-metabolic risk factors. Subjects with NAFLD were randomized to a 12-week blinded dietary intervention (MD vs LF). Hepatic steatosis was determined via magnetic resonance spectroscopy (MRS). From a total of 56 subjects enrolled, 49 subjects completed the intervention and 48 were included for analysis. During the intervention, subjects on the MD had significantly higher total and monounsaturated fat but lower carbohydrate and sodium intakes compared to LF subjects ($p < 0.01$). At week 12, hepatic steatosis had reduced significantly in both groups ($p < 0.01$) and there was no difference in liver fat reduction between the groups ($p = 0.32$), with mean (SD) relative reductions of 25.0% ($\pm 25.3\%$) in LF and 32.4% ($\pm 25.5\%$) in MD. Liver enzymes also improved significantly in both groups. Weight loss was minimal and not different between groups [$-1.6 (\pm 2.1)$ kg in LF vs $-2.1 (\pm 2.5)$ kg in MD, ($p = 0.52$)]. Within-group improvements in the Framingham risk score, total cholesterol, serum triglyceride, and HbA1c were observed in the MD (all $p < 0.05$) but not with the LF diet. Adherence was higher for the MD compared to LF (88% vs. 64%, $p = 0.048$). **Conclusions:** *Ad libitum* low fat and Mediterranean diets both improve hepatic steatosis to a similar degree.

Non-alcoholic fatty liver disease (NAFLD) represents abnormal hepatic lipid deposition, related to increasing peripheral adipose accumulation and insulin

resistance. With the world-wide epidemics of obesity and diabetes, NAFLD is now the most prevalent liver disease worldwide, affecting up to 25% of the adult population.(1) Subjects with NAFLD may develop liver injury, termed non-alcoholic steatohepatitis (NASH), with a subset developing progressive fibrosis, cirrhosis and complications including end-stage liver failure and hepatocellular carcinoma.(2) Patients with NAFLD also have an increased risk of cardiovascular disease (CVD), which is the leading cause of mortality in this population.(3) Thus the morbidity and mortality associated with this condition represents an increasing health burden in an aging population.

Treatment for NAFLD is through lifestyle modification consisting of caloric restriction and exercise, with an emphasis on weight loss.(4, 5) The applicability, tolerability, cost and benefits of lifestyle modification mean it is a powerful intervention that could greatly reduce the societal morbidity and mortality related to NAFLD. Unfortunately, the success and longevity of lifestyle changes that focus on weight loss, are poor.(6, 7) In the PREDIMED study (median follow-up duration of 4.8 years), an *ad libitum* Mediterranean dietary approach emphasizing change in nutrient composition rather than weight loss, was used successfully to reduce cardiovascular events in older adults with type 2 diabetes or major cardiovascular risk factors.(8) This approach therefore seems suitably efficacious and may provide appropriate levels of long-term adherence to intervention diets.

Within the setting of dietary intervention for NAFLD, the role of diet type and nutrient profile has received limited attention. While the evidence around weight loss in NAFLD shows a strong correlation between reduction in body weight and improvement in NAFLD(9), the confounding influence of weight loss means that effects or benefits of nutrient composition remains relatively unexplored. Practice guidelines now reflect a small body of evidence highlighting the advantages of a Mediterranean style of eating(5, 10) and while this has been suggested as the optimal therapeutic approach in situations where weight loss is not achieved,(11) this requires confirmation.

Dietary patterns, such as Mediterranean and low-fat diets are associated with low rates of CVD and a reduction in general cardiovascular risk.(8) Consequently, a Mediterranean dietary pattern with predominantly unsaturated fat has been suggested by the American Heart Association (12-14) and the National Heart Foundation of Australia (15) to reduce cardiovascular events. As CVD is the leading cause of death among patients with NAFLD, these diets are therefore logical recommendations for this population. Nevertheless, evidence from high-quality trials relating to Mediterranean diets and NAFLD is limited. One pilot study of 12 subjects has provided evidence of reductions in hepatic steatosis with a Mediterranean diet, (16) however the small cohort makes it difficult to generalize these findings. Clearly, more evidence is required to strengthen recommendations about optimal evidence-based care.

In order to guide optimal nutritional treatment for NAFLD and to examine the efficacy of focusing upon altering nutrient profiles without body weight loss, we performed a randomized controlled trial comparing Mediterranean and low fat diets using an *ad libitum* approach to energy intake. Our primary outcome was hepatic steatosis, with secondary outcomes of CV risk, arterial stiffness, HbA1c and insulin resistance measures, liver enzymes, compliance and health-related quality of life (QoL).

Methods

Subjects

From April 2013 to June 2016, adult patients were recruited from NAFLD clinics at a Perth tertiary hospital and from private clinics, by participating gastroenterologists. Subjects provided written informed consent for the study prior to completing any assessments. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, was approved the Sir Charles Gairdner and Osborne Park Hospital Group Human Research Ethics Committee (No. 2012-113) and registered on Australia New Zealand Clinical Trials Registry (ACTRN12612000841875).

Inclusion and exclusion criteria

Subjects required a diagnosis of NAFLD, with hepatic steatosis quantified as >5.5%

as determined by magnetic resonance spectroscopy (MRS) and an average alcohol consumption of <20 g/day or 140 g/week for females or <30 g/day or 210 g/week for males.

Exclusion criteria were: secondary causes of NAFLD (eg. medication induced), unstable body weight (variation >5% within the preceding 3-month period), current use of weight loss medications (eg. Orlistat), current use of pioglitazone, other liver disease (viral hepatitis, auto-immune or cholestatic liver disease, Wilson's disease, hemochromatosis, alpha-1 anti-trypsin deficiency), unstable diabetes (HbA1c >8.5%), decompensated cirrhosis (INR >1.3, platelets <100x10⁹/mm, bilirubin >20 mmol/l, albumin <35 g/l, ascites or hepatic encephalopathy), renal failure, malignancy (aside from skin cancer), inability to provide informed consent, claustrophobia preventing MRS examination, current smoking, atrial fibrillation preventing SphygmoCor® assessment and pregnancy or lactation.

Study Design and Outcomes

A 12-week, prospective, parallel group, single-blinded randomized controlled trial of subjects with NAFLD was conducted. The primary outcome was percentage of hepatic steatosis at the end of week 12, determined by MRS. Secondary outcomes were CV risk measures consisting of: 1) Framingham Risk Score (FRS), 2) arterial stiffness assessed by pulse wave velocity (PWV) and aortic augmentation index (AI_x) using Sphygmocor®, 3) HbA1c and insulin resistance measures (HOMA-IR) 4) quality of life and 5) liver function tests, and 6) compliance.

Randomization

At baseline, subjects were randomized in a single-blinded fashion into one of two dietary intervention groups; Low Fat (LF) or Mediterranean (MD) diet. Subjects were randomized in a 1:1 fashion using randomly selected envelope-concealed allocations in blocks of four, which were prepared prior to trial commencement. Stratification by diabetes status was used, due to the prognostic effect on NAFLD severity and vascular risk. Following individual subject enrollment and consent by the trial Nurse, the next sequential envelope was drawn.

Dietary Intervention

The LF diet was based on National Health and Medical Research Council (17) and American Heart Association Dietary recommendations.(13) Target macronutrient energy contributions for the LF diet were 50% from carbohydrate; 30% from fat (with <10% of energy as saturated fat); 20% from protein. The MD was based on analysis of actual foods consumed in traditional Cretan diets (18) with alterations to allow for standardization of protein intake with the LF diet. Target macronutrient energy contributions were 40% from carbohydrate; 35-40% from fat (with <10% of energy as saturated fat); 20% of energy as protein.

Dietary interventions were standardized in terms of education, counseling and dietary care. Education materials included diet-specific summaries of the patterns of food intake, and a food-group list specifying preferred choices and approximate numbers and size of serves to consume per day based on dietary modeling and individual requirements. Recipe books, designed specifically for each diet in this study, were provided.

To minimize financial disadvantage to subjects consuming core foods in the MD, all subjects were provided with two food supplements appropriate to their diet. At each 4-weekly visit, the foods provided were 750g of nuts (almonds or walnuts) and 750ml of olive oil for the MD and 1kg of natural muesli and 200g of low fat snack bars for the LF diet.

Education and dietary prescription was individualized by the study dietitian within the diet-specific recommendations, to allow for personal food preferences. All subjects received equivalent intensity of care in terms of opportunities for contact, availability of individual dietary counseling, type and amount of written resources, and the number of food items provided. Subjects were aware of the number (1 or 2) of their individual dietary allocation, however, the diet types were not disclosed at any point during the screening, informed consent or during the trial.

Assessment and Monitoring

Subjects underwent assessment at baseline and end of study for dietary intake and

composition, NAFLD, cardiovascular risk, anthropometry, fasting biochemistry, physical activity and quality of life. The study dietitian contacted participants by phone on a weekly basis for the first four weeks, to assess compliance and provide support in making dietary change. Contact was then every four weeks at scheduled review visits, or through additional patient-initiated contact. At each scheduled visit, subjects completed a standardized compliance questionnaire (19) and underwent fasting blood draw and anthropometric measures. Subjects received further food supplies, support and could ask questions. Non-compliance was defined as less than 70% compliance with scored items. Subjects also completed daily self-assessed checklists of compliance with food group targets.

Dietary Assessment

Dietary intake and composition data were collected using a modified-Burke diet history interview carried out by a single Accredited Practising Dietitian (CP) experienced in dietary intervention trials and analyzed using nutritional analysis software (Foodworks Professional version 8, Xyris Software, Australia).

Clinical Assessment

Subjects completed a standardized interviewer-administered questionnaire which recorded smoking, hypertension, diabetes, family history, dyslipidemia, alcohol intake, medications and history of vascular disease (cerebral, coronary, peripheral).

Anthropometry and Body Composition

Height and body mass (nearest 0.05 kg in light clothing, no shoes) were measured using a single set of calibrated electronic medical scales with stadiometer. Waist circumference was measured using flexible steel girth tape (W606PM, Lufkin, USA) at the narrowest point between the 10th rib border and iliac crest. If narrowing was not obvious then the midpoint was used.(20) Resting blood pressure was assessed after a five-minute, seated rest. The mean of three valid measurements was used for each assessment.

Physical Activity Assessment

Physical activity was assessed at both baseline and end of study using the International Physical Activity Questionnaire (IPAQ) long form.(21) Subjects were

advised to maintain their usual level of activity for the duration of the intervention.

NAFLD Assessment

Hepatic triglyceride content (HTGC) was quantified by MRS-Proton Density Fat Fraction (MRS-PDFF) using a two-voxel point-resolved spectroscopy sequence (PRESS) performed on a Philips 3T Ingenia scanner (Philips Medical Systems, the Netherlands). PDFF methods are recommended to assess treatment efficacy for clinical trials in NAFLD.(22) For each patient an e-THRIVE sequence was run before the MRS sequence to obtain sagittal, coronal and axial slices through the entire liver in order to position the volume of interest away from tissue boundaries, major blood vessels or intrahepatic bile ducts. In order to minimize the influence of abdominal motion on field homogeneity, iterative shimming around the volume of interest was used. The HTGC was measured in two different locations across the liver to account for the heterogeneity of fat deposition within the liver using voxels of size 20 mm x 20 mm x 20 mm. Co-localization of voxels was performed using screen captures of voxel placement at baseline so as to replicate the positioning at study end. The acquisition of spectra was initiated on expiration (where chest movement is minimized) using the following parameters: echo time (TE) = 50 ms, repetition time (TR) = 1800 ms, flip angle = 90°, 4 averages, 8 individual dynamic scans. Quantitative analysis of HTGC was performed using the AMARES non-linear least square quantitation algorithm in version 4.0 of the jMRUI software package (available at: <http://www.jmrui.eu/>). HTGC was calculated as described in Longo *et al.*(23)

Standard liver function tests (ALP, AST, ALT bilirubin and GGT) were assessed at baseline and week 12 by the state reference laboratory (Pathwest, Nedlands WA, Australia). Normal ALT levels were defined according to this laboratory (<40 IU/l for males, <35 IU/l for females). Non-invasive assessment of hepatic fibrosis was undertaken using Fibroscan® and Hepascore. Hepascore is a serum-based model consisting of age, sex, bilirubin, GGT, alpha-2 macroglobulin and hyaluronic acid, which has been validated as a predictive model for fibrosis in NAFLD.(24) Fibroscan was performed following an overnight fast by an experienced assessor with >200 acquisitions.

Metabolic Assessment

Twelve-hour fasting blood samples were collected for analysis of: plasma glucose, insulin (with calculated HOMA1-IR and HOMA2-IR), HbA1c, sodium, potassium, urea, creatinine, liver function tests, cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol levels.

Vascular Assessment

Arterial stiffness using applanation tonometry (SphygmoCor®) was used to measure the aortic augmentation index [AIx] and pulse wave velocity [PWV]. Carotid-femoral PWV is considered to be the “gold standard” non-invasive assessment of arterial stiffness which predicts future cardiovascular events and mortality.(25) Patients had carotid-femoral PWV and AIx assessed by a single operator under standardized conditions as per consensus recommendations.(26)

Quality of Life Assessment

Health-related quality of life (HRQL) was assessed using The Assessment of Quality Of Life (AQoL-8D) tool.(27) The AQoL-8D was developed in an Australian population, is valid and reliable and has a higher correlation than other instruments with subjective well-being.(28)

Power Calculation and Statistical Analysis

We aimed to recruit 55 subjects. Allowing for 10% drop-out, this would provide 25 subjects per treatment group to detect diet-induced differences in hepatic triglyceride content (HTC) of $\geq 13\%$ (absolute value 2.5%), based on a significance level of 5%, power of at least 80% and mean (SD) for IHTC in NAFLD patients of 19.5% (3.1%).(29)

Endpoints were analyzed on an intention-to-treat basis at study completion (week 12). Repeated measures analysis of covariance (ANCOVA) was used to examine differences in outcomes between diet groups at week 12 after adjusting for baseline values. Statistical differences within groups were analyzed by paired t-tests or non-parametric Wilcoxon signed rank tests. ANCOVA was also used to investigate the effect of measured adherence on hepatic steatosis. Significance was determined

using a value of 0.05. Statistical analyses were carried out with SPSS statistical analysis software for Mac version 24.0.

Results

A total of 56 subjects were recruited with 5 subjects subsequently deemed ineligible after MRS examination showed insufficient steatosis (<5.5%). Of the 51 randomized subjects, one was excluded at week two for failing to implement any recommended dietary changes, and another subject left the study at week eight for personal reasons. Forty-nine subjects completed the full 12 weeks of intervention. One subject was excluded from end-point analysis due to excess alcohol consumption that was not declared at baseline, but found on analysis of the dietary interview data. A flow diagram of study participation is shown in Figure 1 and the baseline characteristics of the subjects can be found in Table 1. The cohort was middle-aged with an even sex distribution. Approximately one third had diabetes or hypertension. No subjects were cirrhotic. Subjects randomized to the MD group had a higher mean hepatic steatosis percentage and lower HDL-cholesterol. Groups did not differ on body mass index (BMI), blood pressure, total cholesterol, triglycerides or HbA1c.

Dietary Intervention

Total daily energy and macronutrient intake was not different between groups at baseline (Table 2), however fibre intake was higher ($p=0.015$) and energy (%) from saturated fat was lower ($p=0.012$) in the MD group. A significant alteration in diet was achieved between groups at week 12, and from baseline within each group (Table 2). Measured macronutrient intakes of both groups were comparable with predicted intakes for the diets. At completion, energy, fibre, saturated fat and alcohol intakes across the two groups were not significantly different. Carbohydrate and sugar intakes were both significantly higher in the LF diet. Intakes of total fat and monounsaturated fat were significantly higher and sodium was significantly lower in the Mediterranean diet. Saturated fat intake was not different between diets [9.3 (2.9)% vs. 9.5 (1.9)% of energy for LF and MD, respectively, $p=0.54$].

Hepatic Steatosis and Liver Measures

Hepatic triglyceride content reduced significantly from baseline in both groups ($p<0.001$) with relative change of -25.0% (25.3%) in the LF group and -32.4 (25.5)%

in the MD group (Table 3 and Figure 2). After adjustment for baseline, hepatic triglyceride was not significantly different between the groups at completion ($p=0.28$) (Table 4). The proportion of subjects with NAFLD resolution was higher in the LF group (37.5% vs 12.5%, $p=0.046$). This was likely to be related to a lower baseline hepatic fat within LF group, as there was no significant difference in NAFLD resolution following adjustment for baseline hepatic fat content (odds ratio 0.2, 95% confidence interval 0.05 - 1.3, $p=0.11$). At end of treatment, there were no significant differences in liver function tests, Hepascore or LSM between the two groups. LSM and Hepascore did not change significantly during the period of the intervention. Within groups however, ALT reduced significantly in both groups with GGT falling significantly in the MD group ($p<0.001$) and trended towards improvement in the LF group ($p=0.055$). Five subjects with elevated ALT at baseline normalized their ALT, with no difference between LF and MD (8% vs 21%, $p=0.6$).

Cardiovascular Risk

The FRS did not differ between diet groups at week 12, however there was a significant improvement in FRS within the MD group, which was not observed in the LF group (Tables 3 and 4). Total cholesterol, plasma triglycerides, and HbA1c all improved significantly from baseline in the MD group but not the LF group (all $p<0.05$). End of treatment values of fasting lipids and measures of insulin resistance were not significantly different between the two groups. No differences were seen between or within groups in arterial stiffness measures of PWV or AI_x .

Anthropometry and Activity

Despite the *ad libitum* nature of the diet and instruction to subjects that weight maintenance was preferred, both groups lost small amounts of body weight with resultant reductions in BMI and waist measurements. Weight loss was similar between groups [1.6 (2.1) kg vs. 2.1 (2.5) kg, $p=0.52$, in LF and MD, respectively] and represents a relative reduction of 2.1% and 2.3% from baseline, respectively. Physical activity remained equivalent between the two groups and did not change significantly over the course of the study.

Quality of Life and Compliance

Both the LF and the MD subjects experienced significant improvements in overall QoL scores (Table 3). The LF group experienced significant improvements in five of the eight domains (independent living, mental health, self-worth, coping and relationships) and the MD reported significantly improved scores in three (mental health, coping and relationships).

Nine subjects (36%) were categorized as non-compliant with the intervention diet in the LF group, compared with three (16%) from the MD group ($p=0.048$).

Improvement in hepatic steatosis was equivalent among subjects who adhered to either diet (Figure 3).

Discussion

Our study addresses a gap in evidence about the effect of diets on hepatic steatosis and cardiovascular risk when weight loss is not achieved or appropriate, by demonstrating that both Mediterranean and low fat diets lead to a significant improvement in hepatic steatosis and measures of liver function. This finding complements the clear evidence of the effectiveness of diet-induced weight loss as the primary treatment for NAFLD.(5, 9, 10) The disease-related burden of NAFLD advanced-fibrosis and NASH-related cirrhosis has been increasing at rates beyond that of diabetes and obesity in the last decade(30) and diet represents a low-cost, low-risk strategy to reduce this.

Cardiovascular risk measures were not different between diet groups at the end of the study however, within group improvements in cardiovascular risk measures were only seen within the MD group. Within this group, a mean 0.5% reduction in the FRS from baseline was observed, representing a 0.5% reduction in the estimated 10-year risk of a major cardiovascular event or death.(31) This is consistent with previous findings that the Mediterranean diet lowers the risk of future cardiovascular events and death in patients with established cardiovascular risk factors.(32) As cardiovascular disease is the leading cause of death in patients with NAFLD, this study supports practice guidelines which recommend the adjustment of medical nutrition therapy in line with the Mediterranean diet.(5, 10)

We demonstrated in a relatively short timeframe, that both a Mediterranean diet rich in olive oil, and a low fat diet could induce significant changes in hepatic fat with only a 2% body weight loss. This is less than the 3-5% body weight loss required to improve hepatic fat.(9, 33) In addition, the slight increase in energy intake we observed over the intervention, with no change to physical activity, suggests that weight loss depends on factors other than just caloric deficit.(34) Both groups had significant reductions in saturated fat intake over the duration of the trial; saturated fat ingestion has been demonstrated to increase hepatic triglyceride content and hepatic insulin resistance.(35, 36) Both groups also significantly increased dietary fibre consumption, which has been associated with reduced hepatic fat (37). The increase in total fibre and the concomitant increase in prebiotic factors within the diet may also influence the microbiota and therefore gut-liver axis, which is implicated in NAFLD development and progression.(37, 38) In addition, the Mediterranean diet has high levels of polyphenols found in fruit and vegetables, and high levels of monounsaturated fats found in olive oil. These compounds have been implicated in having wide ranging benefits including inhibiting *de novo* hepatic lipogenesis, improving peripheral insulin sensitivity and reducing cardiovascular risk.(37, 39) Thus, there is potential for significant improvement in hepatic steatosis in the absence of significant weight loss in patients with NAFLD, and for preferential improvements in cardiometabolic risk with the Mediterranean diet.

Our findings of the preferential impact of the Mediterranean diet on cardiometabolic risk are supported by two smaller studies,(16, 40) where an isocaloric Mediterranean diet led to improved hepatic insulin sensitivity in 12 patients with NAFLD over six weeks(16), and reduced HbA1c levels in 36 patients with type 2 diabetes.(40) A greater reduction in hepatic steatosis levels in subjects consuming Mediterranean diets in comparison to low-fat, high-carbohydrate control diets, was observed in these studies but not in our study. This may be due to the shorter duration of these interventions (6 and 8 weeks) which was perhaps insufficient time to see a reduction in hepatic steatosis with the low fat arm.(41) The greater proportion of energy from carbohydrates in the low fat diet administered to subjects with type 2 diabetes(40) [$53\% \pm 2.1$ compared to the $48.0\% \pm 5.4\%$ in our trial] may be implicated in increasing hepatic *de novo* lipogenesis.(42) Another longer trial found subjects following a low carbohydrate Mediterranean diet had a greater

reduction in hepatic steatosis than those following a low fat diet. However, the absolute magnitude of this difference was small (1.5%).⁽⁴³⁾ The significant caloric restriction, very low carbohydrate intake in the Mediterranean arm, greater weight loss and inclusion criteria (only half of subjects had NAFLD), are all meaningful differences from our trial.

Importantly, our trial established the ability of subjects to achieve nutrient intakes comparable to documented traditional Mediterranean diets. The *ad libitum* approach and the achievement of the Mediterranean nutrient profile without the limitations of consuming a local-style cuisine, means that the results of this randomized controlled trial are readily translatable to a clinical setting and prescription within medical nutrition therapy. Moreover, the high level of compliance and improved quality of life suggest that it may be associated with greater long-term efficacy.

The strengths of our study lie in the randomized design and the ability to achieve nutrient targets within personal food preferences. Dietary change has been noted as having poor long-term adherence,⁽⁴⁴⁾ an essential factor in achieving meaningful outcomes and reducing health-care costs.⁽⁴⁵⁾ Our examination of adherence, especially in the context of usual food preferences and availability, provides real insight into the relevance of these diets as clinical treatment tools.

The comprehensive assessment of hepatic steatosis and cardiovascular risk and the monitoring of potential confounders such as activity, further strengthen our results. Although this study is similar in duration to previous dietary studies, (36, 40, 46) more long-term trials are required to determine whether these diets are efficacious treatments for NAFLD. The short duration of the intervention may also explain why there were no changes to Hepascore, liver stiffness and markers of vascular disease.

The use of multiple methods for assessment of dietary compliance (self and investigator administered), the *ad-libitum* approach and the use of a single study

dietitian for collection and analysis of all data, are all means by which bias was addressed. We expect that bias would be concentrated in reporting of discretionary foods. As these were limited to an equal extent in both diets, we assume the potential bias would be similar across groups. The small and non-significant increases in both energy intakes and activity levels within our populations are within expected variability considering the small cohort.

In summary, we have demonstrated that there is no difference in the reduction in hepatic steatosis produced by a Mediterranean or a low fat diet over 12 weeks, when energy intake is not significantly altered from baseline. Both diets led to a similar degree of reduction (25-32%) in hepatic steatosis and resolution of NAFLD. The dietary patterns are easily transferrable to practice and treatment is relatively inexpensive. Our results show that a Mediterranean diet can be adhered to by subjects consuming from a local Western food supply. We suggest that from the evidence presented, Mediterranean and low fat diets prescribed within the framework of individualized dietary care are viable and efficacious even without clinically significant body weight loss. The improvement in cardiovascular risk seen in the Mediterranean diet builds on evidence suggesting it may be the preferred dietary pattern for people with NAFLD.

Acknowledgements:

Dr Briohny Smith and Dr Wendy Cheng for assistance with recruitment. Jeanette Atkinson, Thomas Greig, Department of Medical Technology & Physics, Stephanie Hopa and the MRI Department, Sir Charles Gairdner Hospital, all for assistance with MRS.

References

1. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016;64:73-84.
2. Adams LA, Lymp JF, Sauver JS, Sanderson SO, Lindor KD, Feldstein A, Angulo P. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 2005;129:113-121.

3. Adams LA, Anstee QM, Tilg H, Targher G. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. *Gut* 2017;66:1138-1153.
4. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, et al. The Diagnosis and Management of Nonalcoholic Fatty Liver Disease: Practice Guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2017.
5. European Association for the Study of the L, European Association for the Study of D, European Association for the Study of O. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016;64:1388-1402.
6. Pownall HJ, Bray GA, Wagenknecht LE, Walkup MP, Heshka S, Hubbard VS, Hill J, et al. Changes in body composition over 8 years in a randomized trial of a lifestyle intervention: the look AHEAD study. *Obesity (Silver Spring)* 2015;23:565-572.
7. Dudekula A, Rachakonda V, Shaik B, Behari J. Weight loss in nonalcoholic Fatty liver disease patients in an ambulatory care setting is largely unsuccessful but correlates with frequency of clinic visits. *PLoS One* 2014;9:e111808.
8. Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Aros F, Gomez-Gracia E, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med* 2013;368:1279-1290.
9. Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, Friedman SL, et al. Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis. *Gastroenterology* 2015;149:367-378 e365; quiz e314-365.
10. Italian Association for the Study of the L. AISF position paper on nonalcoholic fatty liver disease (NAFLD): Updates and future directions. *Dig Liver Dis* 2017;49:471-483.
11. Eslamparast T, Tandon P, Raman M. Dietary Composition Independent of Weight Loss in the Management of Non-Alcoholic Fatty Liver Disease. *Nutrients* 2017;9.
12. Sacks FM, Lichtenstein AH, Wu JHY, Appel LJ, Creager MA, Kris-Etherton PM, Miller M, et al. Dietary Fats and Cardiovascular Disease: A Presidential Advisory From the American Heart Association. *Circulation* 2017;136:e1-e23.

13. Eckel RH, Jakicic JM, Ard JD, de Jesus JM, Miller NH, Hubbard VS, Lee IM, et al. 2013 AHA/ACC Guideline on Lifestyle Management to Reduce Cardiovascular Risk. *Circulation* 2014;129:S76-S99.
14. Van Horn L, Carson JA, Appel LJ, Burke LE, Economos C, Karmally W, Lancaster K, et al. Recommended Dietary Pattern to Achieve Adherence to the American Heart Association/American College of Cardiology (AHA/ACC) Guidelines: A Scientific Statement From the American Heart Association. *Circulation* 2016;134:e505-e529.
15. Collins C BT, Rollo M. . Dietary Patterns and Cardiovascular Disease Outcomes: an Evidence Check rapid review brokered by the Sax Institute (www.saxinstitute.org.au) for the National Heart Foundation of Australia. 2017.
16. Ryan MC, Itsiopoulos C, Thodis T, Ward G, Trost N, Hofferberth S, O'Dea K, et al. The Mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease. *J Hepatol* 2013;59:138-143.
17. Eat for Health: Educators Guide. In. Canberra: National Health and Medical Research Council; 2013.
18. Kafatos A, Verhagen H, Moschandreas J, Apostolaki I, Van Westerop J. Mediterranean diet of Crete: Foods and nutrient content. *Journal of the American Dietetic Association* 2000;100:1487-1493.
19. Estruch R, Martinez-Gonzalez MA, Corella D, Salas-Salvado J, Ruiz-Gutierrez V, Covas MI, Fiol M, et al. Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. *Ann Intern Med* 2006;145:1-11.
20. Marfell-Jones M, Olds T, Stewart A, Carter L, editors. ISAK manual, International standards for Anthropometric Assessment: International Society for the Advancement of Kinanthropometry; 2012.
21. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 2003;35:1381-1395.
22. Caussy C, Reeder SB, Sirlin CB, Loomba R. Non-invasive, quantitative assessment of liver fat by MRI-PDFF as an endpoint in NASH trials. *Hepatology* 2018.
23. Longo R, Pollesello P, Ricci C, Masutti F, Kvam BJ, Bercich L, Croce LS, et al. Proton MR spectroscopy in quantitative in vivo determination of fat content in human liver steatosis. *J Magn Reson Imaging* 1995;5:281-285.

24. Adams LA, George J, Bugianesi E, Rossi E, De Boer WB, van der Poorten D, Ching HLI, et al. Complex non-invasive fibrosis models are more accurate than simple models in non-alcoholic fatty liver disease. *Journal of Gastroenterology and Hepatology* 2011;26:1536-1543.
25. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol* 2010;55:1318-1327.
26. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* 2006;27:2588-2605.
27. Hawthorne G, Richardson J, Osborne R. The Assessment of Quality of Life (AQoL) instrument: a psychometric measure of health-related quality of life. *Qual Life Res* 1999;8:209-224.
28. Richardson J, Iezzi A, Khan MA, Maxwell A. Validity and reliability of the Assessment of Quality of Life (AQoL)-8D multi-attribute utility instrument. *Patient* 2014;7:85-96.
29. Belfort R, Harrison SA, Brown K, Darland C, Finch J, Hardies J, Balas B, et al. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med* 2006;355:2297-2307.
30. Kabbany MN, Conjeevaram Selvakumar PK, Watt K, Lopez R, Akkas Z, Zein N, Carey W, et al. Prevalence of Nonalcoholic Steatohepatitis-Associated Cirrhosis in the United States: An Analysis of National Health and Nutrition Examination Survey Data. *Am J Gastroenterol* 2017;112:581-587.
31. MDCalc.com. In: MD Aware; 2005-2017.
32. Estruch R, Ros E, Martinez-Gonzalez MA. Mediterranean diet for primary prevention of cardiovascular disease. *N Engl J Med* 2013;369:676-677.
33. Patel NS, Doycheva, I., Peterson, M. R., Hooker, J., Kisselva, T., Schnabl, B., . . . Loomba, R. Effect of weight loss on magnetic resonance imaging estimation of liver fat and volume in patients with nonalcoholic steatohepatitis. *Clinical Gastroenterology and Hepatology : The Official Clinical Practice Journal of the American Gastroenterological Association* 2015;13:561-568.
34. Hafeekost K, Lawrence D, Mitrou F, O'Sullivan TA, Zubrick SR. Tackling overweight and obesity: does the public health message match the science? *BMC Med* 2013;11:41.

35. Hernandez EA, Kahl S, Seelig A, Begovatz P, Irmeler M, Kupriyanova Y, Nowotny B, et al. Acute dietary fat intake initiates alterations in energy metabolism and insulin resistance. *J Clin Invest* 2017;127:695-708.
36. Bjermo H, Iggman D, Kullberg J, Dahlman I, Johansson L, Persson L, Berglund J, et al. Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial. *Am J Clin Nutr* 2012;95:1003-1012.
37. Zelber-Sagi S, Salomone F, Mlynarsky L. The Mediterranean dietary pattern as the diet of choice for non-alcoholic fatty liver disease: Evidence and plausible mechanisms. *Liver Int* 2017;37:936-949.
38. Federico A, Dallio M, Godos J, Loguercio C, Salomone F. Targeting gut-liver axis for the treatment of nonalcoholic steatohepatitis: translational and clinical evidence. *Transl Res* 2016;167:116-124.
39. Rienks J, Barbaresco J, Nothlings U. Association of Polyphenol Biomarkers with Cardiovascular Disease and Mortality Risk: A Systematic Review and Meta-Analysis of Observational Studies. *Nutrients* 2017;9.
40. Bozzetto L, Prinster A, Annuzzi G, Costagliola L, Mangione A, Vitelli A, Mazzarella R, et al. Liver fat is reduced by an isoenergetic MUFA diet in a controlled randomized study in type 2 diabetic patients. *Diabetes Care* 2012;35:1429-1435.
41. Kirk E, Reeds DN, Finck BN, Mayurranjan MS, Patterson BW, Klein S. Dietary Fat and Carbohydrates Differentially Alter Insulin Sensitivity During Caloric Restriction. *Gastroenterology* 2009;136:1552-1560.
42. Chong MF, Hodson L, Bickerton AS, Roberts R, Neville M, Karpe F, Frayn KN, et al. Parallel activation of de novo lipogenesis and stearoyl-CoA desaturase activity after 3 d of high-carbohydrate feeding. *Am J Clin Nutr* 2008;87:817-823.
43. Gepner Y, Shelef I, Schwarzfuchs D, Zelicha H, Tene L, Yaskolka Meir A, Tsaban G, et al. Effect of Distinct Lifestyle Interventions on Mobilization of Fat Storage Pools: The CENTRAL MRI Randomized Controlled Trial. *Circulation* 2017:CIRCULATIONAHA.117.030501.
44. Brownell KD CL. Adherence to dietary regimens 1: An overview of research. *Behavioral Medicine* 1995;Vol. 20
45. Adherence to long-term therapies: evidence for action. In. Geneva World Health Organization; 2003.

46. Promrat K, Kleiner DE, Niemeier HM, Jackvony E, Kearns M, Wands JR, Fava JL, et al. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology* 2010;51:121-129.

Author Manuscript

hep_30076_f1.tif

hep_30076_f2.tif

hep_30076_f3.tif

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Properzi, C;O'Sullivan, TA;Sherriff, JL;Ching, HL;Jeffrey, GP;Buckley, RF;Tibballs, J;MacQuillan, GC;Garas, G;Adams, LA

Title:

Ad Libitum Mediterranean and Low-Fat Diets Both Significantly Reduce Hepatic Steatosis: A Randomized Controlled Trial

Date:

2018-11-01

Citation:

Properzi, C., O'Sullivan, T. A., Sherriff, J. L., Ching, H. L., Jeffrey, G. P., Buckley, R. F., Tibballs, J., MacQuillan, G. C., Garas, G. & Adams, L. A. (2018). Ad Libitum Mediterranean and Low-Fat Diets Both Significantly Reduce Hepatic Steatosis: A Randomized Controlled Trial. *HEPATOLOGY*, 68 (5), pp.1741-1754. <https://doi.org/10.1002/hep.30076>.

Persistent Link:

<http://hdl.handle.net/11343/283957>