Archives of Physiology and Biochemistry
The Journal of Metabolic Diseases

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iarp20

Effects of lacto-ovo-vegetarian diet *vs.* standard-weight-loss diet on obese and overweight adults with non-alcoholic fatty liver disease: a randomised clinical trial

Nazila Garousi, Babak Tamizifar, Makan Pourmasoumi, Awat Feizi, Gholamreza Askari, Cain C. T. Clark & Mohammad Hasan Entezari

To cite this article: Nazila Garousi, Babak Tamizifar, Makan Pourmasoumi, Awat Feizi, Gholamreza Askari, Cain C. T. Clark & Mohammad Hasan Entezari (2021): Effects of lacto-ovo-vegetarian diet *vs.* standard-weight-loss diet on obese and overweight adults with non-alcoholic fatty liver disease: a randomised clinical trial, Archives of Physiology and Biochemistry, DOI: 10.1080/13813455.2021.1890128

To link to this article: https://doi.org/10.1080/13813455.2021.1890128

Published online: 09 Mar 2021.

ARCHIVES OF PHYSIOLOGY AND BIOCHEMISTRY
https://doi.org/10.1080/13813455.2021.1890128

Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

Effects of lacto-ovo-vegetarian diet *vs.* standard-weight-loss diet on obese and overweight adults with non-alcoholic fatty liver disease: a randomised clinical trial

Nazila Garousi[a], Babak Tamizifar[b], Makan Pourmasoumi[c], Awat Feizi[d], Gholamreza Askari[e], Cain C. T. Clark[f] and Mohammad Hasan Entezari[a]

[a]Department of Clinical Nutrition, Food Security and Nutrition Research Center, School of Nutrition and Food Sciences, Isfahan University of Medical Sciences, Isfahan, Iran; [b]Gastroenterology and Hepatology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran; [c]Gastrointestinal and Liver Diseases Research Center, Guilan University of Medical Sciences, Rasht, Iran; [d]Department of Epidemiology and Biostatistics, School of Public Health, Cardiac Rehabilitation Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran; [e]Department of Community Nutrition, Food Security and Nutrition Research Center, School of Nutrition and Food Sciences, Isfahan University of Medical Sciences, Isfahan, Iran; [f]Centre for Intelligent Healthcare, Coventry University, Coventry, UK

ABSTRACT

The aim of the study was to compare the effects of a lacto-ovo-vegetarian diet (LOV-D) *vs.* a standard weight-loss diet (SWL-D) on obese/overweight adults with NAFLD. Present randomised clinical trial recruited 75 overweight/obese adults with NAFLD, who were randomly assigned into LOV-D and SWL-D groups for 3 months. The LOV-D was designed based on eliminating meat, poultry, and fish; while including dairy products and eggs. The SWL-D was planned according to the standard food pyramid, which was free in all sources of food. Adherence to LOV-D significantly outperformed SWL-D in reducing levels of alanine aminotransferase (ALT), body weight, waist circumference, BMI, fasting blood sugar, insulin, homeostasis model assessment of insulin resistance (HOMA-IR), triacylglycerol (TG), cholesterol, low-density lipoprotein cholesterol (LDL-C), and systolic blood pressure (SBP). Furthermore, ultrasonography revealed a higher alleviation in NAFLD grade among LOV-D, compared with SWL-D. This study suggests that adherence to LOV-D for 3 months has beneficial effects on NAFLD improvement, anthropometric measures, glycaemic-related markers, and lipid profiles.

ARTICLE HISTORY
Received 5 October 2020
Revised 13 January 2021
Accepted 7 February 2021
Published online 8 March 2021

KEYWORDS
Lacto-ovo-vegetarian diet; standard weight-loss diet; non-alcoholic fatty liver; randomised clinical trial

Background

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of liver disease worldwide, with NAFLD being defined as evidence of excessive hepatic fat accumulation in the absence of remarkable alcohol consumption and secondary causes of hepatic steatosis (Chalasani *et al*. 2012, Mansour-Ghanaei *et al*. 2019). NAFLD affects nearly 14–30% of the general population in different parts of the world, and it appears that it will dramatically increase over the next two decades (Bellentani 2017, Mansour-Ghanaei *et al*. 2018). The exact aetiology of NAFLD is not clearly understood. However, several non-modifiable factors, such as age, race, genetic background, and baseline histology, as well as a number of modifiable factors including inappropriate diet, physical inactivity, weight gain, insulin resistance, and drug abuse have been suggested as the most common contributors in the onset and progression of NAFLD (Abenavoli *et al*. 2016, Hsu *et al*. 2017). The NAFLD spectrum ranges from simple hepatic steatosis without evidence of hepatocyte injury to non-alcoholic steatohepatitis (NASH) with an associated hepatocellular injury. In this regard, if NAFLD is not managed appropriately, it may progress to chronic liver damage, including cirrhosis and hepatocellular carcinoma (Angulo 2002, Loomba and Sanyal 2013). Thus, determining the proper treatment is an important issue faced by healthcare systems globally. Currently, lifestyle modifications (in the form of a healthy diet, physical exercise, and weight loss) remain the cornerstone of NAFLD management (Liver and Diabetes 2016). Implementing a weight-loss diet in the form of caloric restriction, which is standard for omnivorous people, is the most important intervention for NAFLD treatment, and can reduce visceral, subcutaneous, and hepatic fat, as well as promoting weight loss (Clark and Diehl 2003, Fontana *et al*. 2004). Researches have shown that NAFLD patients who lose 5–7% of their total body weight show improvements in liver enzymes, hepatic fat, insulin sensitivity, and histological changes (Huang *et al*. 2005, Clark 2006, Ratziu 2017).

A lacto-ovo-vegetarian diet (LOV-D), which characterised by variable servings of dairy and eggs as well as vegetables and grains, has long been posited to be beneficial for general health and, in particular, cardiovascular health (Rajaram 2003, Rosell 2006). Adherence to this diet is associated with

CONTACT Mohammad Hasan Entezari entezari@hlth.mui.ac.ir Department of Clinical Nutrition, School of Nutrition and Food Sciences, Isfahan University of Medical Sciences, Isfahan, Iran; Makan Pourmasoumi makan.pourmasoumi@gmail.com Gastrointestinal and Liver Diseases Research Center, Guilan University of Medical Sciences, Rasht, Iran

improved lipid profiles (Yang *et al*. 2012) and lower weight gain (Burke *et al*. 2008) in comparison with the western diet. Additionally, studies have suggested that a vegetarian diet is more acceptable among many people, who are more satisfied to follow it for a longer period in compare with other weight-loss diets (Smith *et al*. 2000, Burke *et al*. 2007). As a vegetarian diet is usually high in calcium, magnesium, antioxidant constituents, and fibre, it can improve human insulin sensitivity, regardless of weight loss or physical activity (Ard *et al*. 2004). Although a standard weight-loss diet (SWL-D) has long been suggested as a front-line treatment for NAFLD, there is a lack of evidence regarding the effects of LOV-D on NAFLD in comparison with SWL-D. Given all the effectiveness of LOV-D on metabolic markers that involve in NAFLD development/progression, a hypothesis regarding the beneficial clinical application of LOV-D can be laid down. Therefore, this study was conducted to compare and determine the effects of LOV-D and SWL-D on obese and overweight adults with NAFLD.

Methods

Study participants

Patients were identified and recruited from February to April 2018. Inclusion criteria required participants to have been diagnosed with NAFLD, to be motivated to participate in the study, aged between 20 and 55 years, obese or overweight (BMI $>$ 25 kg/m^2), and non-consumers of alcohol ($<$40 g/week). NAFLD was diagnosed by an ultrasonography scan and associated with a persistently elevated alanine aminotransferase (ALT) concentration higher than 19 U/L for women and 30 U/L for men (Prati *et al*. 2002). Participants were excluded from the study if they were following a special diet, pregnant, breastfeeding, or suffering from viral hepatitis, diabetes mellitus, untreated hypothyroidism or other causes of chronic liver disease. The sample size was calculated based on the type one error (α) =0.05 and power = 0.80%. Considering 20% potential dropout, 80 patients were estimated as the final sample size. This study was a single-centre, randomised clinical trial. The study protocol was approved by the Ethics Committee of Isfahan University of Medical Sciences (IR.MUI.REC.1396.3.249) and retrospectively registered at the Iranian Registry clinical trial (IRCT20140208016529N2). This study was also conducted in accordance with the CONSORT guidelines.

Study design

All participants who were eligible to study were entered into a two-week run-in period. They were requested to provide their demographic information and a signed written informed consent. After the two-week run-in period, 80 NAFLD patients were randomly assigned to the LOV-D or SWL-D groups for three months. Randomisation was performed using SPSS software version 21 (SPSS Inc, Chicago, IL) and was stratified according to age, sex, and BMI. Patients were instructed to follow their routine physical activity regime, and not to consume any supplements and/or medication which might affect the outcomes of interest. All subjects received their dietary plan at the beginning of each month for 3 months. The participants' adherence to the administration of diets was monitored weekly by phone interview. In addition, subjects were asked to complete a 3-d dietary record in order to determine compliance with the study's dietary goals.

Diet intervention

Both diets were caloric-restricted and involved 500 kcal less than the energy requirements computed individually for each subject. The total energy requirements of each patient were calculated by using the Mifflin-St. Jeor equation, according to resting energy expenditure and the individual's physical activity levels (Mifflin *et al*. 1990). Both diets were planned to consist of approximately 50–55% energy from carbohydrates, 15–20% from protein, and 25–30% from total fat. The LOV-D was designed based on restraining in the consumption of meat and meat products, poultry, fish and seafood, and flesh of any other animal; but, consisted of dietary products and eggs as well as all the other food groups. In this regard, LOV-D nearly included protein sources from egg (24%), dairy (19%), gluten (26%), soy (16%), nuts (8%), vegetables, and fruits (7%). The SWL-D was planned according to the food pyramid, in which all sources of food were freely available. Approximately 18% of protein sources in SWL-D provided from meat and meat products, poultry, fish and seafood, and flesh of any other animal.

Blood sampling and biochemical analyses

A 10 mL blood sample was drawn from participants after a 12-h overnight fast to determine the concentration of liver enzymes, lipid profiles, and glycaemic status markers. All samples were well-stored in the laboratory at −80 °C before analysis. Fasting plasma glucose (FBS), fasting insulin, lipid profile including triacylglycerol (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C), and liver enzymes, such as ALT and aspartate aminotransferase (AST) were measured by an enzymatic method (Pars Azmoon Co. kit, Tehran, Iran); serum insulin concentration was measured by an ELISA kit (Lise-Meitner-Straße, Kiel, Germany). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by a previously described formula (Pisprasert *et al*. 2013).

Anthropometric measurements and ultrasonographic assessment

Anthropometric indices, including body weight, height, and waist circumference were measured by the expert staff at the beginning of the study and after the 12-week intervention. The weight and height of participants were measured while wearing light clothing and no shoes to a precision of 0.1 cm (Seca, Hamburg, Germany). Waist circumference was quantified midway between the last rib and the iliac crest.

BMI was calculated as weight in kg divided by height in metres squared. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured 3 times after 5 min of rest using a mercury sphygmomanometer (ALPK2, Zhejiang, China; Datis Co, Tehran, Iran). The average of the three measurements was then recorded and used in the analyses.

An ultrasonography blinded assessment was made to determine the extent of hepatic steatosis at the beginning and end of the study. Fatty infiltration of the liver was graded from 0 to III, which indicated normal liver, mild, moderate, and severe steatosis, respectively, based on several parameters, including the attenuation of echogenicity, blurred vessels, the liver-to-kidney contrast ratio, and the brightness of the liver. As this approach is observational, each assessment was repeated by another radiologist to confirm the result.

Dietary and physical activity assessments

In order to evaluate dietary intakes, participants received a 3-d food record at the beginning, middle, and end of the trial. The subjects were instructed to record their daily dietary intake for three non-consecutive days (two weekdays and one weekend day). The data of the food record then was analysed using Nutritionist version 4 software (First Databank, San Bruno, CA), modified for Iranian foods. In order to enhance the accuracy of the portion size assessment, food scales, and models, as well as national food composition tables, were used as a reference guide for participants (Ghaffarpour *et al*. 1999). Physical activity was also examined by using the metabolic equivalent of task (MET) questionnaire (Ainsworth *et al*. 2000) at the beginning and end of the trial.

Primary and secondary outcomes

The primary outcome was a significant decrease in ALT and AST blood levels. The secondary outcome measurements included changes in anthropometric values, insulin metabolic markers, lipid profiles, blood pressure, and NAFLD grade among participants.

Statistical analysis

Statistical analyses were performed by using SPSS software version 21 (SPSS Inc, Chicago, IL). The Kolmogorov–Smirnov test was applied to assess the normality of variables distribution. Based on the normality of data distribution, a paired-sample t-test was used to evaluate the change of each variable from baseline values. Due to the comparison of changes between the LOV-D and SWL-D groups, group comparison procedures (including an analysis of variance (ANOVA) test, repeated measures ANOVA, independent t-test, or the Chi-square test) were performed, with respect to the type of variables. An analysis of covariance (ANCOVA) test was applied to compare the effects of LOV-D and SWL-D on the main outcomes while controlling for identified covariates. All results are expressed as means and standard deviation. $p < .05$ is considered as statistically significant.

Results

Participant characteristics

A total of 80 NAFLD patients who met the eligibility criteria were randomly assigned into the LOV-D or SWL-D groups. During the 3 months follow-up, three participants were excluded due to having over 10% discontinued intervention ($n = 2$) and emigration ($n = 1$) from the LOV-D group. Also, two patients dropped out of the SWL-D group because of over 10% discontinued intervention ($n = 1$) and travel ($n = 1$). The attrition rate was not significantly different between the two groups. Finally, 75 subjects were included in the final analysis (Figure 1).

Table 1 displays the demographic characteristics of the two groups at the beginning of the study. Except grade of NAFLD, there were no significant differences between individuals following the LOV-D and SWL-D in terms of anthropometric measurements and demographic information.

Based on the 3-d dietary records, no significant difference was found between the two groups at the trial baseline. In addition, physical activity was not significantly different between the two groups pre- and post-intervention (Table 2). No adverse effect was reported among participants throughout the study.

Primary outcomes

The liver enzyme concentrations, including ALT and AST, were significantly reduced after the 3-month intervention in comparison with the baseline values in both groups. The ALT/AST was significantly decreased in the LOV-D group ($p < .001$), whereas the reduction of ALT/AST did not reach significance in the SWL-D group ($p = .078$). Adherence to LOV-D resulted in a more significant reduction in ALT (-21.32 ± 19.77 *vs.* -10.15 ± 20.30; $p = .04$), but not in and AST (-8.51 ± 11.25 *vs.* -4.15 ± 9.82; $p = .08$) and ALT/AST ($p = .47$), in comparison with SWL-D (Table 3).

Secondary outcomes

Bodyweight, BMI, and waist circumference significantly decreased in both groups. In comparison with SWL-D, a more significant reduction in body weight (-5.85 ± 3.09 *vs.* -2.20 ± 2.14; $p < .001$), BMI (-2.13 ± 1.04 *vs.* -0.73 ± 0.75; $p < .001$) and waist circumference (-7.54 ± 3.64 *vs.* -2.38 ± 1.72; $p < .001$) were observed in the LOV-D group. Within-group differences demonstrated significant reductions in TG, TC, LDL-C, FBS, insulin, and HOMA-IR in the LOV-D group. Adherence to the LOV-D diet resulted in significant decreases in TG (-48.00 ± 78.20 *vs.* 10.07 ± 78.54; $p = .006$), TC (-23.29 ± 29.21 *vs.* 2.18 ± 44.67; $p < .001$), LDL-C (-21.24 ± 24.30 *vs.* 3.36 ± 40.81; $p < .001$), FBS (-8.40 ± 6.20 *vs.* -1.86 ± 8.50; $p = .001$), insulin (-4.94 ± 5.40 *vs.* 0.81 ± 8.35;

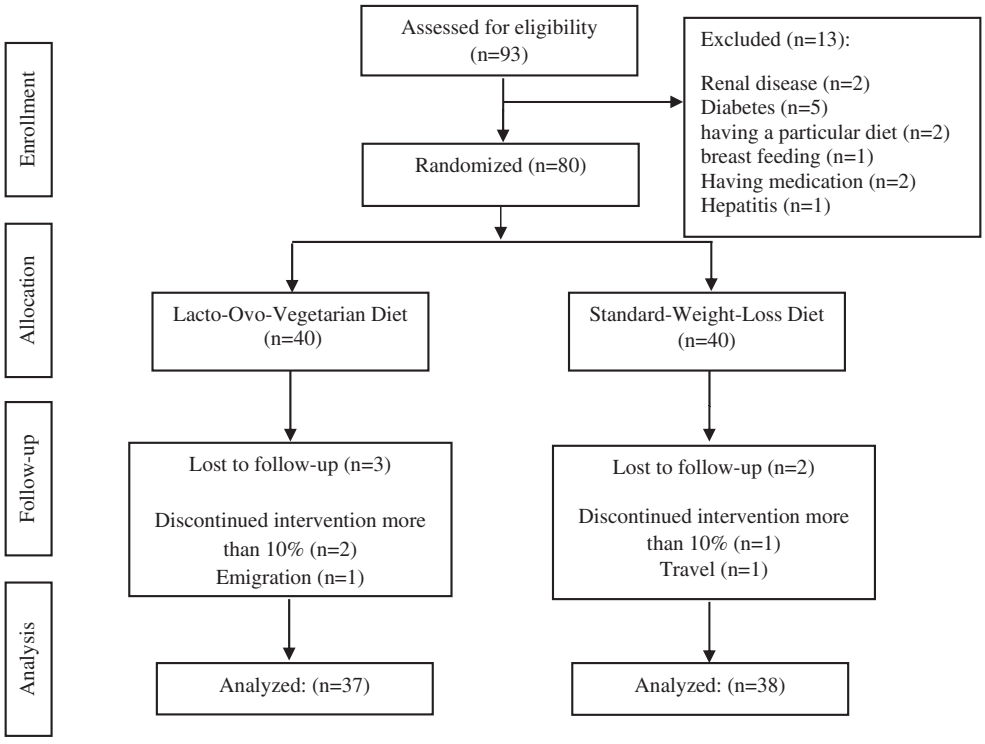

Figure 1. Participant flow diagram.

Table 1. Baseline characteristics of study participants.

Variables	LOV-D number (37)	SWL-D number (38)	*p* Value[a]
Grade of fatty liver (%) based on US			
Grade I	8	18	.02[b]
Grade II	29	20	
Grade III	–	–	
Age (year)	43.51 ± 9.85	42.84 ± 9.85	.769
Sex			
Woman (%)	22 (59.5%)	17 (44.7%)	.202[b]
Man (%)	15 (40.5%)	21 (55.3%)	
Smoking	5 (13.5%)	6 (15.8%)	.781
Physical activity (met)	37.14 ± 3.28	37.19 ± 4.10	.680
Weight (kg)	87.16 ± 18.15	85.97 ± 15.46	.234
BMI (kg/m^2)	32.02 ± 4.57	30.06 ± 3.81	.105
Waist (cm)	109.97 ± 12.29	106.01 ± 10.81	.240

Data are means ± SDs.
[a]Obtained from independent *t*-test.
[b]Obtained from Chi-square test.
LOV-D: lacto-ovo-vegetarian diet; SWL-D: standard-weight-loss diet; BMI: body mass index; US: ultrasonography

$p = .006$), and HOMA-IR (-1.62 ± 1.48 *vs.* 0.02 ± 2.14; $p < .001$), compared with the SWL-D group. Significant decreases in SBP (-0.66 ± 1.06 *vs.* -0.31 ± 0.99; $p = .023$) were also found in the LOV-D group, compared with the control group. However, there was no difference in HDL (1.56 ± 10.15 *vs.* -1.23 ± 15.89; $p = .77$), DBP (-0.29 ± 1.03 *vs.* -0.30 ± 0.89; $p = .31$), and MAP (-0.41 ± 0.88 *vs.* -0.30 ± 0.76; $p = .13$) between the two groups (Table 3).

Liver Doppler sonography

The findings from the liver ultrasonography revealed a more significant improvement in the LOV-D group, compared to the SWL-D group ($p = .01$). The rate of improvement in LOV-D subjects was 67%, while the rate of alleviation in the SWL-D group was 21%. The frequencies of increased NAFLD grade were 0.02 and 0% in the SWL-D and LOV-D groups, respectively (Figure 2).

Discussion

This study was a randomised clinical trial to determine the effect of LOV-D on obese/overweight adults with NAFLD independently and in comparison with SWL-D. The results revealed that adherence to LOV-D for 3 months had greater beneficial effects on anthropometrics measures, ALT, TG, TC, LDL-C, FBS, insulin metabolic markers, and SBP in comparison with SWL-D. However, no significant difference was observed between LOV-D and SWL-D in terms of AST, ALT/AST, HDL, MAP, and DBP.

Diet modification has been widely known as a first-line management strategy for NAFLD (Liver and Diabetes 2016). However, there is a lack of knowledge about the possible beneficial effects of LOV-D on this disease. In accordance with this study's results, the favourable impact of other diets with high plant-based food content has been previously shown. Mediterranean diet, which is rich in vegetables and fruits, has been suggested as a way that can improve hepatic insulin sensitivity (Abenavoli *et al.* 2014), liver enzymes (Kontogianni *et al.* 2014, Razavi Zade *et al.* 2016), and hepatic steatosis (Ryan *et al.* 2013). The Dietary Approach to Stop Hypertension (DASH – which is characterised by a higher content of vegetables, fruits, whole grains, low-fat dairy products, fish, poultry, nuts and legumes, while being limited in sodium, total fat, saturated fat, cholesterol, and added sugars intake (Sacks *et al.* 2001)) is a different vegetable- and fruit-rich diet which has been the subject of investigations into NAFLD. In this regard, Razavi Zade *et al.* (2016) showed

Table 2. Energy, macronutrient intakes, and physical activity at all-time points.

Variables	Group	Baseline	Middle of study	End of study	*p* Value[a]
Energy (kcal)	LOV-D	2332.07 ± 366.50	2055.50 ± 312.94	1911.02 ± 290.04	.061
	SWL-D	2429.17 ± 323.87	2255.27 ± 338.05	2075.54 ± 314.68	
	p Value[b]	.228	.010	.001	
Protein (g)	LOV-D	80.40 ± 12.01	79.31 ± 10.57	81.28 ± 10.161	.540
	SWL-D	80.83 ± 10.50	78.79 ± 12.17	77.09 ± 11.33	
	p Value[b]	.868	.845	.96	
Carbohydrate (g)	LOV-D	354.75 ± 69.88	299.68 ± 63.24	278.44 ± 49.11	.009
	SWL-D	376.23 ± 57.36	335.39 ± 55.38	326.54 ± 58.72	
	p Value[b]	.242	.003	<.001	
Fat (g)	LOV-D	70.34 ± 10.27	66.31 ± 8.71	60.36 ± 8.62	.408
	SWL-D	69.27 ± 10.69	68.11 ± 10.89	64.81 ± 10.67	
	p Value[b]	.659	.432	.051	
Cholesterol (mg)	LOV-D	332 ± 119	280 ± 124	298 ± 133	.397
	SWL-D	287 ± 136	303 ± 140	264 ± 122	
	p Value[b]	.131	.459	.252	
SFA (g)	LOV-D	22.56 ± 3.52	19.32 ± 3.08	18.38 ± 3.11	.004
	SWL-D	23.14 ± 4.54	22.49 ± 4.36	21.69 ± 4.46	
	p Value[b]	.544	.001	<.001	
MUFA (g)	LOV-D	23.32 ± 3.61	23.09 ± 3.38	20.65 ± 3.33	.991
	SWL-D	22.90 ± 4.09	22.57 ± 3.92	21.61 ± 3.90	
	p Value[b]	.642	.543	.256	
PUFA (g)	LOV-D	17.70 ± 4.34	17.67 ± 3.04	15.26 ± 2.39	.131
	SWL-D	16.33 ± 3.28	16.30 ± 3.37	15.16 ± 3.11	
	p Value[b]	.128	.069	.877	
Vitamin C (mg)	LOV-D	3466.51 ± 4254	7533.25 ± 9050	9430.13 ± 10548	.004
	SWL-D	2630.75 ± 2205	3840.82 ± 5744	3035.47 ± 3453	
	p Value[b]	.287	.038	.001	
Calcium (mg)	LOV-D	770.58 ± 190.48	1075.84 ± 155.31	1081.95 ± 170.88	<.001
	SWL-D	847.53 ± 177.96	828.32 ± 195.24	792.90 ± 171.34	
	p Value[b]	.075	<.001	<.001	
Magnesium (mg)	LOV-D	365.84 ± 73.33	470.61 ± 76.43	482.88 ± 78.67	<.001
	SWL-D	370.24 ± 62.16	382.32 ± 58.69	397.40 ± 53.41	
	p Value[b]	.352	<.001	<.001	
Fibre total (g)	LOV-D	27.18 ± 6.05	40.72 ± 7.95	42.32 ± 7.02	<.001
	SWL-D	28.21 ± 4.96	29.24 ± 4.99	27.56 ± 6.31	
	p Value[b]	.421	<.001	<.001	
Physical activity (met)	LOV-D	37.14 ± 3.28	–	38.53 ± 3.95	.721[b]
	SWL-D	37.19 ± 4.10	–	37.14 ± 3.27	
	p Value[b]	.219	–	.101	

[a]Obtained from repeated measured ANOVA test.
[b]Obtained from ANOVA test.

that a DASH diet can reduce liver enzyme concentrations, body weight, inflammatory factors, some lipid profiles, and improve insulin sensitivity, in comparison with a control group.

This study revealed that LOV-D has a greater weight reduction effect compared with SWL-D. Although the energy intake in LOV-D was less than SWL-D, and it seems that this difference may be the cause of the greater weight reduction in LOV-D, the trend of change in energy intake during 3 months was not significant between them. In addition, after controlling for energy intake and other potential confounders, the weight loss effect of diet was more pronounced in LOV-D groups. Only sparse data has investigated whether a vegetarian diet differs in terms of weight-loss efficacy. In line with the current results, a study (Valachovičová *et al*. 2006) which matched vegetarians and non-vegetarians revealed that non-vegetarians had higher insulin, glucose, and homeostasis model assessment measurements than vegetarians. Also, this study showed that vegetarianism has a protective effect on BMI. In contrast with the current results, Burke *et al*. (2007) indicated that adherence to LOV-D has no prior effect on anthropometry measurements, compared with SWL-D in overweight and obese adults. The cause of this inconsistency might be due to declining adherence in abstaining from meat and meat-related products over time, as implicated in the study (Burke *et al*. 2007).

The main difference between LOV-D and SWL-D is in terms of its prohibition of meat. Previous studies have shown that high meat intake is associated with a higher risk of diabetes type 2, through increases in glucose intolerance and impaired insulin sensitivity. Although diabetes and insulin resistance are well-known as NAFLD risk factors, the direct effect of meat on NAFLD has not been investigated. A cross-sectional study suggested that meat intake, such as lamb, beef, liver, sausage, chicken, and turkey was higher in NAFLD patients, in comparison with healthy adults (Zelber-Sagi *et al*. 2007). However, more studies are needed to investigate the direct effects of meat and related products on NAFLD and its metabolic parameters, especially liver enzymes.

The role of higher energy intake on NAFLD risks has been well-documented (Wehmeyer *et al*. 2016). However, the relationship between NAFLD and diet is complicated and is not solely limited to energy intake. Several studies have shown that NAFLD patients have higher carbohydrate intake, in comparison to healthy controls (Cortez-Pinto *et al*. 2006, Volynets *et al*. 2012). In addition, results have indicated that protein intake in NAFLD patients is higher than in healthy participants (Cortez-Pinto *et al*. 2006, Volynets *et al*. 2012,

Table 3. Metabolic profiles, anthropometrics measures, and blood pressure at baseline and after 12-week intervention in patients with NAFLD.

Variables	Before	After	MD	*p* Value[a]	*p* Value[b]
Weight (kg)					
LOV-D	87.16 ± 18.15	81.30 ± 17.20	−5.85 ± 3.09	<.001	<.001[c]
SWL-D	85.97 ± 15.46	83.77 ± 15.40	−2.20 ± 2.14	<.001	
BMI (kg/m^2)					
LOV-D	32.02 ± 4.57	29.88 ± 4.54	−2.13 ± 1.04	<.001	<.001[c]
SWL-D	30.06 ± 3.8	29.33 ± 3.98	−0.73 ± 0.75	<.001	
Waist circumference (cm)					
LOV-D	109.97 ± 12.29	102.43 ± 11.68	−7.54 ± 3.64	<.001	<.001[c]
SWL-D	108.39 ± 10.91	106.01 ± 10.81	−2.38 ± 1.72	<.001	
ALT (IU/L)					
LOV-D	46.02 ± 28.58	24.70 ± 15.82	−21.32 ± 19.77	<.001	0.04
SWL-D	42.26 ± 23.37	32.10 ± 18.55	−10.15 ± 20.30	.004	
AST (IU/L)					
LOV-D	30.94 ± 13.20	22.43 ± 6.93	−8.51 ± 11.25	<.001	.08
SWL-D	29.94 ± 11.14	25.78 ± 9.36	−4.15 ± 9.82	.013	
ALT/AST					
LOV-D	1.470 ± 0.51	1.08 ± 0.46	−0.38 ± 0.51	<.001	.47
SWL-D	1.39 ± 0.52	1.21 ± 0.48	−0.17 ± 0.60	.078	
Triacylglycerol (mg/dL)					
LOV-D	169.43 ± 82.95	121.43 ± 49.42	−48.00 ± 78.20	.001	.006
SWL-D	171.84 ± 74.45	181.92 ± 81.82	10.07 ± 78.54	.434	
Cholesterol (mg/dL)					
LOV-D	189.24 ± 26.93	165.94 ± 26.81	−23.29 ± 29.21	<.001	<.001
SWL-D	205.97 ± 44.76	208.15 ± 39.38	2.18 ± 44.67	.765	
LDL-C (mg/dL)					
LOV-D	114.16 ± 25.75	92.91 ± 24.33	−21.24 ± 24.30	<.001	<.001
SWL-D	123.42 ± 38.48	126.78 ± 30.96	3.36 ± 40.81	.614	
HDL-C (mg/dL)					
LOV-D	44.75 ± 8.61	46.32 ± 10.37	1.56 ± 10.15	.354	.77
SWL-D	47.91 ± 12.17	46.68 ± 11.96	−1.23 ± 15.89	.635	
FBS (mg/dL)					
LOV-D	99.05 ± 8.43	90.62 ± 8.77	−8.40 ± 6.20	<.001	.001
SWL-D	97.00 ± 10.16	95.13 ± 8.01	−1.86 ± 8.50	.184	
Insulin (μU/mL)					
LOV-D	22.75 ± 17.70	17.80 ± 17.47	−4.94 ± 5.40	<0.001	.006
SWL-D	26.77 ± 24.63	27.59 ± 22.59	0.81 ± 8.35	.550	
HOMA-IR					
LOV-D	5.59 ± 4.42	3.97 ± 3.75	−1.62 ± 1.48	<.001	<.001
SWL-D	6.53 ± 6.46	6.56 ± 5.56	0.02 ± 2.14	.932	
SBP (mmHg)					
LOV-D	12.52 ± 1.34	11.86 ± 1.14	−0.66 ± 1.06	.001	.023
SWL-D	13.03 ± 1.37	12.72 ± 1.28	−0.31 ± 0.99	.058	
DBP (mmHg)					
LOV-D	9.82 ± 0.71	9.52 ± 0.75	−.29 ± 1.03	.090	.31
SWL-D	10.03 ± 0.97	9.73 ± 0.87	−.30 ± 0.89	.045	
MAP (mmHg)					
LOV-D	10.72 ± 0.84	10.30 ± 0.78	−0.41 ± 0.88	.007	.13
SWL-D	11.03 ± 1.00	10.73 ± 0.93	−0.30 ± 0.76	.018	

[a]Obtained from paired t-test.
[b]Obtained from ANCOVA. Adjusted for baseline measures, BMI, physical activity, carbohydrate, and difference in before/after energy intake.
[c]Adjusted for baseline measures, carbohydrate, and energy.
LOV-D: lacto-ovo-vegetarian diet; SWL-D: standard-weight-loss diet; BMI: body mass index; TG: triacylglycerol; TC: total-cholesterol; LDL-C: low-density lipoprotein; HDL-C: high-density lipoprotein; FPG: fasting plasma glucose; HOMA-IR: homeostasis model assessment of insulin resistance; SBP: systolic blood pressure; DBP: diastolic blood pressure; ALT: alanine aminotransferase; AST: aspartate aminotransferase

Wehmeyer *et al*. 2016). Diet with higher vegetables and fruits content provides lower energy density and can act as an essential factor in weight management. However, the role of vegetables and fruits is not limited to their associations with lower energy density. They also contain polyphenols and carotenoids, which can improve metabolic homeostasis, act as anti-inflammatory factors, and suppress hepatic stellate cell activation (Salomone *et al*. 2016).

A possible mechanism underlying the efficacy of LOV-D on NAFLD might be its high vegetable and fruit content, along with the elimination of meat consumption. Obesity and insulin resistance are the important risk factors for the onset of NAFLD, which can increase excess fat deposition in hepatocytes and elevate hepatocyte lipotoxicity and oxidative stress (Yang *et al*. 2010). Impaired insulin signalling, due to compensatory hyperinsulinemia, also provides more

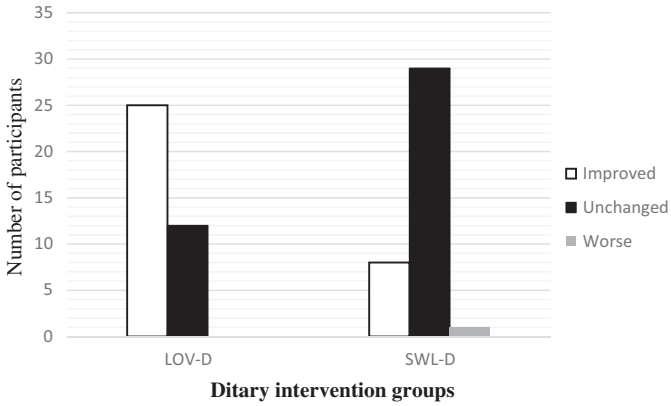

Figure 2. Comparison of the changes in liver improvement after a 3-month intervention with LOV-D and SWL-D. Between-group *p* values were obtained from the Chi-square test. LOV-D: lacto-ovo-vegetarian diet; SWL-D: standard-weight-loss diet.

substrate for lipogenesis, elevates hepatic FFAs uptake, decreases β-oxidation, and inhibits glycogen synthesis (Martín-Domínguez *et al*. 2013, Hardy *et al*. 2016). Furthermore, higher calcium and magnesium intake have favourable effects on improving insulin sensitivity, stimulating anti-inflammatory response (Almoznino-Sarafian *et al*. 2007, Pikilidou *et al*. 2009), suppressing the peroxidation of lipids cells, and enhancing microsomal triglyceride transfer protein hepatic cells (Cho *et al*. 2005, King *et al*. 2009).

Recently, the role of the gut microbiome in many diseases has been investigated (Saad *et al*. 2013). It has been suggested that the gut microbiome population is fundamentally different in individuals with obesity, diabetes, metabolic syndrome, and NAFLD (Parnell *et al*. 2012). The pivotal role of the gut-liver axis in the onset and progress of NAFLD is well-documented (Eslamparast *et al*. 2013). Gut-derived lipopolysaccharides, which are produced by gram-negative bacteria, can induce liver injury. It can result in metabolic deregulation, gut permeability, elevated hepatic oxidative stress, and fibrosis (Jirillo *et al*. 2002, Abu-Shanab and Quigley 2010). The effect of LOV-D on NAFLD might therefore also be related to its fibre content, and how it acts as a prebiotic. Prebiotics can modify gut microbiota, inhibit pathogen growth, stimulate the bacterial products of short-chain fatty acids, and improve lipid and glucose metabolism (Parnell *et al*. 2012). Also, the prebiotics can improve liver function and alleviate levels of ALT and AST (Daubioul *et al*. 2005).

With regard to the expected difficulties in encouraging subjects to adhere to specific diets and the high projected rate of attrition during the study, there was excellent retention after 3 months in the trial. In addition, adjustments were made in the results to account for major potential confounders. However, there are several limitations that should be considered when interpreting the data. First, due to the nature of dietary interventions, blinding was not applicable, which might have resulted in reporting bias. Second, the study duration was relatively short. However, even over this time period, it was possible to show the prominent beneficial effects of LOV-D on NAFLD treatment. Third, the patients' adherence to the diet relied on self-reporting, which might have resulted in misstatements. To manage this bias, the patients' diet was assessed by 3-d food record and checked against the participant's compliance with the advised diet. Furthermore, the subjects in control group consumed more kcal than those in intervention group which may impact on the results. Due to this reason, the results were adjusted energy intake as well as other confounders to minimise such unfavourable effect and enhance accuracy of conclusion. Lastly, due to the limitations of the study's resources, ultrasonography was used to diagnose and detect NAFLD improvement instead of a fibroscan test. Although ultrasonography is a validated method for NAFLD detection (Hernaez *et al*. 2011), there are more precise tests in terms of the quantification of hepatic fat content (Barchetta *et al*. 2016). In this regard, liver enzyme levels, which have been used clinically as surrogate diagnostic and monitoring markers of NAFLD (Eliades *et al*. 2013), were measured to enhance the overall accuracy of the results. Also, due to the small number of participants, the results cannot be generalised to large-scale population groups.

Conclusion

This study suggested that LOV-D is more effective in treating NAFLD, compared with SWL-D. Although SWL-D has been known as the first-line treatment for NAFLD, the results revealed LOV-D can be a promising therapy, having a greater impact in comparison with SWL-D. In addition, following the LOV-D resulted in several beneficial impacts on general health, including promoting human health and warding off chronic disease risk factors. Future studies of longer duration are warranted to confirm these results.

Acknowledgements

The authors thank all the study participants. They are also grateful to the staff of Khorshid Hospital for their support and cooperation.

Author contributions

Nazila Garousi, Makan Pourmasoumi, Gholamreza Askari, and Mohammad Hasan Entezari contributed to the conception and design of the research; Nazila Garousi, Babak Tamizifar, and Awat Fezi contributed to the acquisition and analysis of the data; Nazila Garousi and Makan Pourmasoumi drafted the initial manuscript. All authors critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

Disclosure statement

The authors declare no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

ORCID

Makan Pourmasoumi http://orcid.org/0000-0001-9335-7276

Data availability statement

The data that support the findings of this study are available on request from the corresponding author, M-H.E.

References

Abenavoli, L., *et al.*, 2016. Metabolic aspects of adult patients with nonalcoholic fatty liver disease. *World journal of gastroenterology*, 22 (31), 7006–7016.

Abenavoli, L., *et al.*, 2014. Alimentary regimen in non-alcoholic fatty liver disease: Mediterranean diet. *World journal of gastroenterology*, 20 (45), 16831–16840.

Abu-Shanab, A. and Quigley, E.M., 2010. The role of the gut microbiota in nonalcoholic fatty liver disease. *Nature reviews gastroenterology and hepatology*, 7 (12), 691–701.

Ainsworth, B.E., *et al.*, 2000. Compendium of physical activities: an update of activity codes and MET intensities. *Medicine and science in sports and exercise*, 32 (9), S498–S504.

Almoznino-Sarafian, D., *et al.*, 2007. Magnesium and C-reactive protein in heart failure: an anti-inflammatory effect of magnesium administration? *European journal of nutrition*, 46 (4), 230–237.

Angulo, P., 2002. Nonalcoholic fatty liver disease. *The new England journal of medicine*, 346 (16), 1221–1231.

Ard, J.D., *et al.*, 2004. The effect of the PREMIER interventions on insulin sensitivity. *Diabetes care*, 27 (2), 340–347.

Barchetta, I., *et al.*, 2016. No effects of oral vitamin D supplementation on non-alcoholic fatty liver disease in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. *BMC medicine*, 14 (1), 92.

Bellentani, S., 2017. The epidemiology of non-alcoholic fatty liver disease. *Liver international*, 37, 81–84.

Burke, L.E., *et al.*, 2007. Effects of a vegetarian diet and treatment preference on biochemical and dietary variables in overweight and obese adults: a randomized clinical trial. *The American journal of clinical nutrition*, 86 (3), 588–596.

Burke, L., *et al.*, 2008. A randomized clinical trial of a standard *versus* vegetarian diet for weight loss: the impact of treatment preference. *International journal of obesity*, 32 (1), 166–176.

Chalasani, N., *et al.*, 2012. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology*, 142 (7), 1592–1609.

Cho, H. J., *et al.*, 2005. The possible role of Ca2+ on the activation of microsomal triglyceride transfer protein in rat hepatocytes. *Biological and pharmaceutical bulletin*, 28 (8), 1418–1423.

Clark, J.M., 2006. Weight loss as a treatment for nonalcoholic fatty liver disease. *Journal of clinical gastroenterology*, 40, S39–S43.

Clark, J.M. and Diehl, A.M., 2003. Nonalcoholic fatty liver disease: an underrecognized cause of cryptogenic cirrhosis. *JAMA*, 289 (22), 3000–3004.

Cortez-Pinto, H., *et al.*, 2006. How different is the dietary pattern in non-alcoholic steatohepatitis patients? *Clinical nutrition*, 25 (5), 816–823.

Daubioul, C., *et al.*, 2005. Effects of oligofructose on glucose and lipid metabolism in patients with nonalcoholic steatohepatitis: results of a pilot study. *European journal of clinical nutrition*, 59 (5), 723–726.

Eliades, M., *et al.*, 2013. Meta-analysis: vitamin D and non-alcoholic fatty liver disease. *Alimentary pharmacology and therapeutics*, 38 (3), 246–254.

Eslamparast, T., *et al.*, 2013. Probiotics and nonalcoholic fatty liver disease. *Middle east journal of digestive diseases*, 5 (3), 129–136.

Fontana, L., *et al.*, 2004. Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. *Proceedings of the national academy of sciences*, 101 (17), 6659–6663.

Ghaffarpour, M., Houshiar-Rad, A., and Kianfar, H., 1999. *The manual for household measures, cooking yields factors and edible portion of foods*. Tehran: Nashre Olume Keshavarzy.

Hardy, T., *et al.*, 2016. Nonalcoholic fatty liver disease: pathogenesis and disease spectrum. *Annual review of pathology*, 11, 451–496.

Hernaez, R., *et al.*, 2011. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. *Hepatology*, 54 (3), 1082–1090.

Hsu, C.C., Ness, E., and Kowdley, K.V., 2017. Nutritional approaches to achieve weight loss in nonalcoholic fatty liver disease. *Advances in nutrition*, 8 (2), 253–265.

Huang, M.A., *et al.*, 2005. One-year intense nutritional counseling results in histological improvement in patients with non-alcoholic steatohepatitis: a pilot study. *The American journal of gastroenterology*, 100 (5), 1072–1081.

Jirillo, E., *et al.*, 2002. The role of the liver in the response to LPS: experimental and clinical findings. *Journal of endotoxin research*, 8 (5), 319–327.

King, J.L., *et al.*, 2009. Inadequate dietary magnesium intake increases atherosclerotic plaque development in rabbits. *Nutrition research*, 29 (5), 343–349.

Kontogianni, M.D., *et al.*, 2014. Adherence to the Mediterranean diet is associated with the severity of non-alcoholic fatty liver disease. *Clinical nutrition*, 33 (4), 678–683.

Liver, E.A.F.T.S.O.T. and Diabetes, E.A.F.T.S.O., 2016. EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. *Obesity facts*, 9 (2), 65–90.

Loomba, R. and Sanyal, A.J., 2013. The global NAFLD epidemic. *Nature reviews gastroenterology and hepatology*, 10 (11), 686–690.

Mansour-Ghanaei, F., *et al.*, 2018. Green tea as a safe alternative approach for nonalcoholic fatty liver treatment: a systematic review and meta-analysis of clinical trials. *Phytotherapy research*, 32 (10), 1876–1884.

Mansour-Ghanaei, F., *et al.*, 2019. Efficacy of curcumin/turmeric on liver enzymes in patients with non-alcoholic fatty liver disease: a systematic review of randomized controlled trials. *Integrative medicine research*, 8 (1), 57–61. doi:

Martín-Domínguez, V., *et al.*, 2013. Pathogenesis, diagnosis and treatment of non-alcoholic fatty liver disease. *Revista espanola de enfermedades digestivas*, 105 (7), 409–420.

Mifflin, M.D., *et al.*, 1990. A new predictive equation for resting energy expenditure in healthy individuals. *The American journal of clinical nutrition*, 51 (2), 241–247.

Parnell, J.A., *et al.*, 2012. The potential role of prebiotic fibre for treatment and management of non-alcoholic fatty liver disease and associated obesity and insulin resistance. *Liver international*, 32 (5), 701–711.

Pikilidou, M., *et al.*, 2009. Insulin sensitivity increase after calcium supplementation and change in intraplatelet calcium and sodium–hydrogen exchange in hypertensive patients with Type 2 diabetes 1. *Diabetic medicine*, 26 (3), 211–219.

Pisprasert, V., *et al.*, 2013. Limitations in the use of indices using glucose and insulin levels to predict insulin sensitivity: impact of race and gender and superiority of the indices derived from oral glucose tolerance test in African Americans. *Diabetes care*, 36 (4), 845–853.

Prati, D., *et al.*, 2002. Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Annals of internal medicine*, 137 (1), 1–10.

Rajaram, S., 2003. The effect of vegetarian diet, plant foods, and phytochemicals on hemostasis and thrombosis. *The American journal of clinical nutrition*, 78 (3), 552S–558S.

Ratziu, V., 2017. Non-pharmacological interventions in non-alcoholic fatty liver disease patients. *Liver international*, 37, 90–96.

Razavi Zade, M., *et al.*, 2016. The effects of DASH diet on weight loss and metabolic status in adults with non-alcoholic fatty liver disease: a randomized clinical trial. *Liver international*, 36 (4), 563–571.

Rosell, M., *et al.*, 2006. Weight gain over 5 years in 21 966 meat-eating, fish-eating, vegetarian, and vegan men and women in EPIC-Oxford. *International journal of obesity*, 30 (9), 1389–1396.

Ryan, M.C., *et al.*, 2013. The Mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease. *Journal of hepatology*, 59 (1), 138–143.

Saad, N., *et al.*, 2013. An overview of the last advances in probiotic and prebiotic field. *LWT – food science and technology*, 50 (1), 1–16.

Sacks, F.M., *et al.*, 2001. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. *New England journal of medicine*, 344 (1), 3–10.

Salomone, F., Godos, J., and Zelber-Sagi, S., 2016. Natural antioxidants for non-alcoholic fatty liver disease: molecular targets and clinical perspectives . *Liver international*, 36 (1), 5–20.

Smith, C.F., Burke, L.E., and Wing, R.R., 2000. Vegetarian and weight-loss diets among young adults. *Obesity research*, 8 (2), 123–129.

Valachovičová, M., *et al.*, 2006. No evidence of insulin resistance in normal weight vegetarians. *European journal of nutrition*, 45 (1), 52–54.

Volynets, V., *et al.*, 2012. Nutrition, intestinal permeability, and blood ethanol levels are altered in patients with nonalcoholic fatty liver disease (NAFLD). *Digestive diseases and sciences*, 57 (7), 1932–1941.

Wehmeyer, M.H., *et al.*, 2016. Nonalcoholic fatty liver disease is associated with excessive calorie intake rather than a distinctive dietary pattern. *Medicine*, 95 (23), e3887.

Yang, L., *et al.*, 2010. Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. *Cell metabolism*, 11 (6), 467–478.

Yang, S. Y., *et al.*, 2012. Chinese lacto-vegetarian diet exerts favorable effects on metabolic parameters, intima-media thickness, and cardiovascular risks in healthy men. *Nutrition in clinical practice*, 27 (3), 392–398.

Zelber-Sagi, S., *et al.*, 2007. Long term nutritional intake and the risk for non-alcoholic fatty liver disease (NAFLD): a population based study. *Journal of hepatology*, 47 (5), 711–717.