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Flaxseed oil in the context of a weight loss programme ameliorates fatty liver grade in patients with non-alcoholic fatty liver disease: a randomised double-blind controlled trial

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Abstract

Long-chain *n*-3 fatty acids have been shown to regulate lipid metabolism and reduce fat accumulation in the liver. This trial investigated the effect of flaxseed oil, as a rich source of *α*-linolenic acid, on fatty liver and cardiometabolic risk factors in patients with non-alcoholic fatty liver disease (NAFLD). The randomised, double-blind, controlled trial was performed on sixty-eight NAFLD patients who were divided into flaxseed (*n* 34) and sunflower (*n* 34) oil groups. Patients were given a hypoenergetic diet (−2092 kJ/d) and 20 g/d of the corresponding oil for 12 weeks. Fatty liver grade, liver enzymes and cardiometabolic parameters were determined. The intention-to-treat approach was used for data analysis. Fatty liver grade significantly decreased in both groups (−0·68 in flaxseed *v.* −0·29 in sunflower, $P = 0{\cdot}002$). Alanine aminotransferase and aspartate aminotransferase decreased in both groups ($P < 0{\cdot}01$). Also, significant reduction was observed in blood glucose ($P = 0{\cdot}005$) and fat mass ($P = 0{\cdot}01$) in the flaxseed and muscle mass ($P = 0{\cdot}01$) in the sunflower group. However, none of these alterations was significantly different between the groups. Weight, waist circumference and blood pressure were significantly decreased in both groups but only weight change was significantly different between the groups ($P = 0{\cdot}01$). IL-6 did not significantly change in either group but showed a significant between-group difference ($P = 0{\cdot}03$). Overall, the results showed that in the context of a low-energy diet and moderate physical activity, flaxseed oil may benefit NAFLD patients to improve fatty liver grade, weight and IL-6 compared with sunflower oil.

Key words: **Non-alcoholic fatty liver disease: Flaxseed oil: Fatty liver grade: Weight: IL-6**

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease worldwide[1]. It is a mild form of liver disease with accumulation of fat in hepatocytes. But if left untreated, NAFLD can progress to non-alcoholic steatohepatitis and more severe diseases such as cirrhosis, hepatic failure and liver cancer[2]. NAFLD is strongly associated with obesity, insulin resistance, type 2 diabetes, dyslipidaemia and hypertension, co-morbidities which collectively termed as the metabolic syndrome[3–5]. Hence, NAFLD is a multisystem disease, affecting many extra-hepatic organs, including heart, systemic vessels and kidney, leading to considerable morbidity and mortality[1].

To date, there is no pharmacological treatment to control or improve NAFLD, but having a healthy diet and physical activity can reduce the risk of occurrence or progression of the disease[6]. In the diet, modification of the type or quantity of dietary fats can affect metabolic pathways involved in lipid metabolism and hepatic fat deposition[7]. Consumption of a high-fat diet, particularly if rich in saturated fats, increases the risk of obesity, insulin resistance and abnormalities in lipid metabolism and can therefore lead to NAFLD[8,9]. In contrast, substituting MUFA and PUFA for saturated fats may have beneficial consequences for the liver[9].

Among unsaturated fatty acids, *n*-3 fatty acids are advantageous for the liver due to the regulation of hepatic lipid metabolism and prevention of inflammation, as reviewed by Scorletti & Byrne[10]. According to animal and human studies, long-chain *n*-3 fatty acids present in fish reduce hepatic fat accumulation and liver enzyme levels, improve insulin sensitivity and decrease markers of inflammation, ballooning and fibrosis[11,12]. In addition, these fatty acids compete with *n*-6 fatty acids in the pathways that lead to the production of relevant eicosanoids[13]. Thus, consumption of *n*-3 fatty acids may improve NAFLD by

Abbreviations: ALT, alanine aminotransferase; NAFLD, non-alcoholic fatty liver disease.

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increasing the *n*-3:*n*-6 ratio in cells, resulting in decreased production of arachidonic acid-derived inflammatory eicosanoids and inhibiting hepatic fat accumulation[14].

Flaxseed oil is the richest dietary source of *n*-3 fatty acids after fish oil, with α-linolenic acid content of 40–60 %[15]. α-Linolenic acid is the precursor of long-chain *n*-3 fatty acids, EPA, DPA, and DHA. Nevertheless, contrary to long-chain *n*-3 fatty acids, health benefits of α-linolenic acid in NAFLD has been less recognised[13]. Limited number of animal studies investigated the effect of flaxseed oil on NAFLD and showed promising results[16,17]. In humans, there is only one pilot trial that examined the effect of milled flaxseed on NAFLD patients[18]. In that study, 12 weeks consumption of flaxseed powder decreased liver enzymes, fibrosis and steatosis to a greater extent than that observed in a control group. Since flaxseed oil is a better source of *n*-3 fatty acids than flaxseed (i.e. flax seeds), in the present study, we investigated the effect of flaxseed oil on fatty liver and liver enzymes along with cardiometabolic risk factors in NAFLD patients. As the control, we chose sunflower oil which has previously been used as a placebo in similar investigations on NAFLD patients[19]. In contrast to *n*-3 fatty acids, studies examining the effect of *n*-6 fatty acids on NAFLD are rather scarce but the available evidence has not yet shown either beneficial or detrimental effect of *n*-6 fatty acids on NAFLD[20].

Methods

Study design and participants

This was a randomised, double-blind, placebo-controlled clinical trial conducted in spring of 2016 in Shiraz, Iran. A sample size of thirty-four for each group was calculated based on a previous study[18] to detect a between-group difference of 0·45(SD 0·73) for fatty liver grade with assumption of a two-sided α level of 0·05, power of 80 %, and 20 % attrition rate. Participants were patients with NAFLD as diagnosed by a physician via abdominal ultrasound. The following inclusion criteria were used: age ≥ 18 years, BMI ≥ 25 kg/m^2 and consent to participate. Patients with type 2 diabetes, other metabolic diseases, cancer, organ failure, and malnutrition, those who were on special diets (e.g. vegetarian diet) or medications that affect blood pressure, glucose, lipid profile and fatty liver (e.g. methotrexate and valproic acid), or those who consumed alcohol were not included. Exclusion criteria were the loss of inclusion criteria during the intervention, for instance, taking medications that affect the measured biomarkers, consumption of fish oil or *n*-3 fatty acid supplements, development of infectious or inflammatory disease, low adherence to the trial protocol and retraction of consent.

Patients were recruited via posters and local newspaper advertisements. Upon recruitment, written informed consent was obtained from all participants following clarification of the study goal and procedure. The study was performed according to the guidelines of 1964 Declaration of Helsinki and its later amendments. The trial was approved by the ethics committee of the Shiraz University of Medical Sciences (Approval no. IR.SUMS.REC.1394.150) and registered in the Iranian Registry of Clinical Trials (IRCT 2016011125957 N1).

Intervention

Following selection of the participants and before randomisation, a 2-week run-in period was used, in which weight maintenance diets were given to patients. Afterwards, they were randomised through block randomisation with a block size of 4 into either flaxseed or sunflower oil groups. Patients and investigators were not aware of the treatment allocation. To keep blindness of the treatments, oils were packed in identical bottles. The intervention lasted for 12 weeks, during which participants were on a low-energy diet (−2092 kJ/d) in addition to consumption of 20 ml/d of either flaxseed or sunflower oil. Both oils were purchased from Verjen manufacturer. Measuring cups were provided for the participants, and they were asked to divide the 20 ml oil into two portions and eat them unheated, for instance with salad or cooked rice.

All participants received a 2092 kJ/d energy-deficit diet, composed of 50–55 % energy from carbohydrates, 10–15 % from protein and 30–35 % from fat. Registered dietitians calculated energy requirements of the participants individually using the Mifflin-St. Jeor equations and gave necessary recommendations for following the diets[21]. The American Dietetic Association food exchange list was given to the participants to ensure flexibility of the diet during the intervention[22]. Patients were also recommended to perform 30 to 40 min moderate physical activity per d. To minimise confounding effect of dietary *n*-3 fatty acids, participants were asked to abstain from fish, nuts, soya products and soya oil during the intervention. Also, they were recommended to consume low-fat meat and dairy products and use baking or grilling instead of frying as the method of cooking. Continual reminding for oil consumption and diet recommendations was performed, and possible adverse effects were explored by phone. Diary sheets were provided for the patients to mark them after each time of oil consumption in order to evaluate the compliance of the participants.

Fatty liver assessment

Fatty liver grade was assessed by liver ultrasound (Medison-Accuvix-V10) after 12-h fasting. The ultrasound was performed by a radiologist who was blinded to the group allocation. The severity of fatty liver (grades 1–3) was evaluated by the degree of echogenicity, visualisation of the diaphragm, borders of the liver vasculature and visualisation of the posterior portion of the right hepatic lobe.

Biochemical measures

Blood samples were obtained in fasting state before and end of the trial. Glucose, TAG, LDL-cholesterol, HDL-cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase and γ-glutamyl transferase were determined by using commercially available kits (Pars-Azmun) and an autoanalyser (BT 1500, Biotecnica Instruments). Insulin (Monobind Inc.), IL-6 (Diaclone) and total antioxidant capacity (ZellBio GmbH) were quantified with the ELISA method. Malondialdehyde was quantified with the thiobarbituric acid reactive substance method as described before[23].

British Journal of Nutrition

Anthropometric measures and blood pressure

Weight, waist circumference and blood pressure (Beurer BM 44, Beurer GmbH) were measured four times: At the beginning of the trial and the end of each month of the intervention. Weight was measured with minimal clothing to the nearest 0·1 kg using a digital scale (Glamor BS − 801, Hitachi)[24]. Height was estimated with the precision of 0·5 cm by using a non-stretchable tape fixed on a wall. Waist circumference was measured without pressure to the abdomen at the middle of the distance between the lowest rib and the iliac crest by using a non-stretchable tape. For measuring blood pressure, patients were seated quietly for at least 5 min and then blood pressure was measured twice with 5 min interval in between. The average of the two measurements was considered as the participant's blood pressure. Body composition including fat and skeletal muscle mass was estimated by a tetra-polar bioelectric impedance analyser with four electrodes attaching wrists and ankles (InBody, Biospace Co.) with CV ranging from 1·5 to 2·8 %.

Dietary intakes and physical activity

Dietary intakes were assessed by 3-d diet record. In the first week of the intervention and the end of each month, patients were asked to randomly select 2 weekdays and 1 weekend day to record all foods and drinks consumed. Food records were checked with the participants, and incomplete records were completed through interview (in person or by phone). Nutrient composition was determined with Nutritionist IV version 3.5.2 (Hearst Corp.). Physical activity was assessed by a validated international physical activity questionnaire and expressed as metabolic equivalent task in min/week (MET-min/week)[25].

Chemical analysis of oils

The fatty acid composition of flaxseed and sunflower oils was determined by HPLC[26]. The proportion of SFA, MUFA and PUFA was 10·6, 20·8 and 67·9 % in flaxseed and 11·2, 25·2 and 63·6 % in sunflower oil, respectively. Concentration of *n*-6 and *n*-3 fatty acids was 18·1 and 49·8 % in flaxseed oil and 63·4 and 0·22 % in sunflower oil, respectively.

Statistical analysis

Data were analysed with SPSS software version 19 (SPSS Inc.). Analysis was performed with the intention-to-treat approach, for which data of withdrawn participants were imputed using their baseline values. Normality of data was checked with the Shapiro–Wilk test, and abnormally distributed data were log-transformed before analysis. Baseline values between flaxseed and sunflower groups were compared with the independent *t* test. For comparison of baseline and post-intervention values in each group, the paired sample *t* test was used. For fatty liver grade, body composition and biochemical biomarkers (variables of Table 2), between-group comparisons were performed with repeated-measures ANOVA with age, sex and corresponding baseline values as confounders. *P* values of between-group differences were additionally calculated when the alteration in fat mass (i.e. fat loss) was added to the aforementioned confounders. Anthropometric and blood pressure variables were measured four times, for which repeated-measures ANOVA was used. Within-group comparison for anthropometric and blood pressure data was done with the Huynh–Feldt test because in all cases sphericity assumption was violated. For between-group comparisons for the same data, age, sex and the corresponding baseline values were entered in the test as the covariates. Statistical analysis was set at $P < 0·05$.

Results

Of a total of 235 patients who were assessed for eligibility, 127 met the inclusion criteria and sixty-eight patients agreed to participate in the trial. The patients were allocated to flaxseed (*n* 34) and sunflower (*n* 34) (control) oil groups. Eleven participants were excluded during the intervention because of irregular oil consumption, unwillingness and infection, and ultimately fifty-seven patients completed the study. However, all sixty-eight starting individuals were entered in final analysis. No harm or adverse effect of oils was reported. The compliance of the participants for consumption of oils was at least 80 %, with only four individuals consuming the oils between 80 and 90 % of the recommendation. The flow chart of the trial is shown in Fig. 1.

Baseline clinical and laboratory parameters of the participants in each group are given in Table 1. Except for age, flaxseed and sunflower groups did not differ in demographic, anthropometric, medical or biochemical parameters.

Fatty liver grade showed significant decrease in both groups, with flaxseed group being twice of that in sunflower (−0·68 in flaxseed *v.* −0·29 in sunflower) (Table 2). Similarly, serum concentrations of ALT (−4·6 and −5·6 U/l for flaxseed and sunflower groups, respectively) and aspartate aminotransferase (−2·2 and −3·0 U/l for flaxseed and sunflower groups, respectively) significantly decreased in both groups ($P < 0·01$). Significant reduction was also observed in fasting blood glucose (−0·43 mmol/l, $P = 0·005$) and fat mass (−2·8 kg, $P = 0·01$) of flaxseed and muscle mass (−0·7 kg, $P = 0·01$) of sunflower group. IL-6 did not significantly change in either group (−0·28 ng/l in flaxseed *v.* −0·24 ng/l in sunflower group, $P > 0·05$ for both). With age, sex, baseline values and fat loss as the covariates, only alteration in fatty liver grade was significantly different between the groups ($P = 0·002$). However, the between-group alteration was also significant for IL-6 ($P = 0·03$) when fat loss was removed from the covariates (Table 2).

The number of patients with each grade of steatosis before and after treatment is illustrated in Fig. 2. In both groups, mild (grade 1) cases of NAFLD increased, while moderate (grade 2) and severe (grade 3) cases decreased during the intervention but the alleviation of fatty liver severity was more pronounced in subjects who consumed flaxseed oil. Total cases of NAFLD decreased from thirty-four to twenty-eight in flaxseed oil and from thirty-four to thirty-three in sunflower oil group.

During 12-week intervention, weight, BMI, and waist circumference significantly decreased in both groups (−5·2 and −2·3 kg in weight, −1·9 and 0·86 kg/m^2 in BMI, and −8·9 and −7·4 cm in

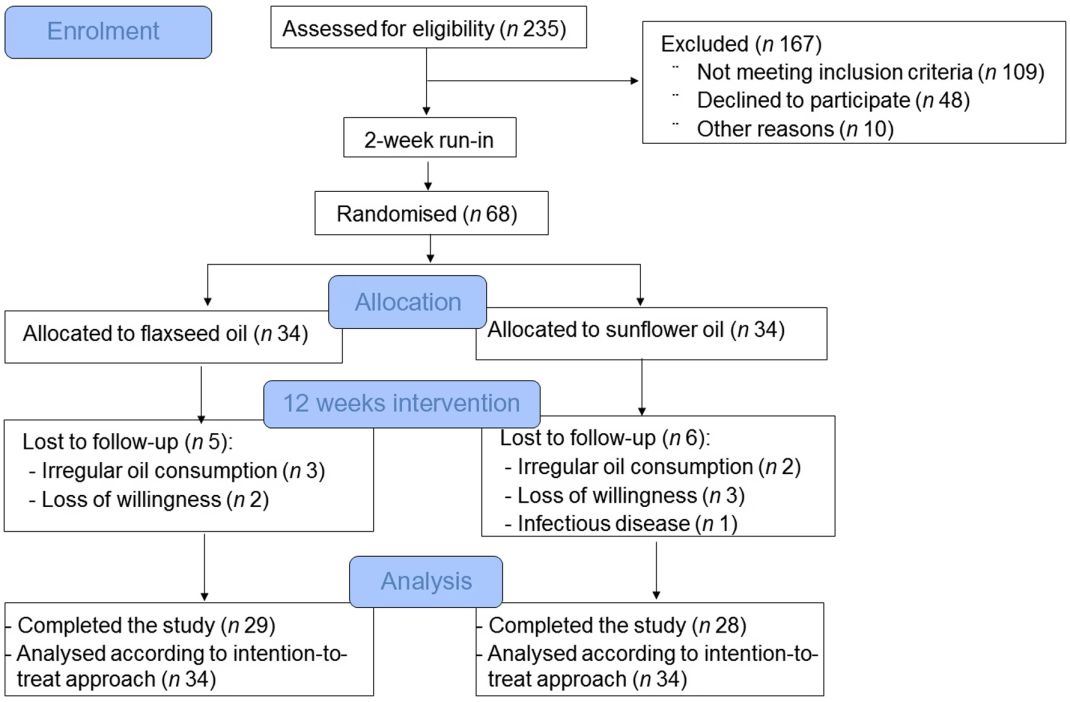

Fig. 1. CONSORT flow chart of the trial.

Table 1. Baseline characteristics of the participants
(Mean values and standard deviations; numbers and percentages)

	Flaxseed oil (*n* 34)		Sunflower oil (*n* 34)		
	Mean	SD	Mean	SD	*P**
Age (years)	45·5	8·7	40·8	8·7	0·03
Male sex					0·81
n	16		17		
%	47·1		50		
Smokers					1
n	4		4		
%	11·8		11·8		
Disease duration (years)	3·3	3·2	2·9	2·0	0·56
Fatty liver grade	1·65	0·54	1·71	0·58	0·67
AST (U/l)	16·6	9·2	18·5	8·1	0·38
ALT (U/l)	28·6	20·8	28·9	16·4	0·77
AST:ALT ratio	0·70	0·27	0·74	0·28	0·51
GGT (U/l)	28·9	16·4	30·1	14·2	0·76
Weight (kg)	83·2	13·1	80·7	11·8	0·41
BMI (kg/m^2)	30·1	4·1	29·6	3·9	0·61
Waist circumference (cm)	102·4	11·1	99·7	9·2	0·27
Systolic blood pressure (mmHg)	131·5	15·9	129·9	13·6	0·66
Diastolic blood pressure (mmHg)	88·6	8·4	88·8	10·5	0·92
Fasting blood glucose (mmol/l)	5·9	2·4	5·3	0·6	0·16
TAG (mmol/l)	1·90	1·60	1·71	0·99	0·56
Total cholesterol (mmol/l)	4·82	1·13	4·78	0·83	0·86
LDL-cholesterol (mmol/l)	2·88	0·93	2·83	0·78	0·83
HDL-cholesterol (mmol/l)	1·03	0·17	1·08	0·19	0·19

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl transferase.
* *P* was calculated with χ^2 for qualitative variables (sex and smoking) and independent *t* test for quantitative parameters (others).

waist circumference of flaxseed and sunflower groups, respectively) ($P < 0·001$ for all reductions) (Table 3). Likewise, systolic and diastolic blood pressure dropped in both groups (−14·3 and −9·5 mmHg in systolic and −8·4 and −6·4 mmHg in diastolic blood pressure of flaxseed and sunflower groups, respectively) ($P < 0·001$). Although decreases were greater in flaxseed group, only the difference in weight ($P = 0·01$) and BMI ($P = 0·009$) was statistically significant between the groups. Dietary intakes (excluding the prescribed oils) and physical activity were not different between the groups during the study (Table 4).

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Table 2. Fatty liver grade, biochemical parameters and body composition in the participants pre- and post-intervention based on the intention-to-treat analysis
(Mean values and standard deviations)

	Flaxseed oil (*n* 34)					Sunflower oil (*n* 34)						
	Baseline		After intervention			Baseline		After intervention				
	Mean	SD	Mean	SD	*P**	Mean	SD	Mean	SD	*P**	*P*†	*P*‡
Fatty liver grading	1·65	0·54	0·97	0·58	<0·001	1·71	0·58	1·41	0·56	0·001	0·001	0·002
ALT (U/l)	27·6	20·8	23·0	15·8	0·009	28·9	16·4	23·3	11·3	0·003	0·74	0·89
AST (U/l)	16·6	9·2	14·4	7·9	0·001	18·5	8·1	15·4	4·0	0·007	0·72	0·63
AST:ALT ratio	0·70	0·27	0·77	0·60	0·40	0·74	0·28	0·78	0·35	0·45	0·59	0·78
GGT (U/l)	28·9	16·5	26·3	18·3	0·22	30·1	14·2	27·2	12·7	0·07	0·81	0·95
FBG (mmol/l)	5·9	2·4	5·4	1·7	0·005	5·3	0·6	5·2	0·8	0·45	0·30	0·75
Insulin (μU/ml)	3·18	1·87	2·96	1·82	0·46	2·60	1·83	2·46	1·61	0·63	0·57	0·75
HOMA-IR	0·92	1·00	0·76	0·55	0·21	0·68	0·77	0·59	0·43	0·38	0·34	0·74
TAG (mmol/l)	1·90	1·60	1·66	1·24	0·10	1·71	0·99	1·61	1·00	0·40	0·54	0·81
Total cholesterol (mmol/l)	4·82	1·13	4·63	1·18	0·15	4·78	0·83	4·67	0·74	0·52	0·57	0·62
LDL-cholesterol (mmol/l)	2·88	0·93	2·69	0·93	0·10	2·83	0·78	2·72	0·88	0·42	0·69	0·48
HDL-cholesterol (mmol/l)	1·03	0·17	1·05	0·19	0·32	1·08	0·19	1·09	0·21	0·84	0·74	0·33
Muscle mass (kg)	31·6	8·0	31·1	6·9	0·60	29·1	6·1	28·4	5·9	0·01	0·08	0·26
Fat mass (kg)	27·3	9·1	24·6	8·4	0·01	26·5	11·9	25·6	11·1	0·22	0·30	–
Body fat (%)	33·0	10·1	30·5	8·8	0·09	33·1	11·6	33·5	10·7	0·71	0·14	–
MDA (μmol/l)	3·22	0·68	3·30	0·62	0·49	3·30	0·64	3·39	0·54	0·40	0·86	0·29
TAC (mmol/l)	0·28	0·07	0·27	0·05	0·64	0·26	0·05	0·27	0·04	0·69	0·74	0·77
IL-6 (ng/l)	7·63	1·29	7·35	0·89	0·10	8·54	4·55	8·29	3·27	0·43	0·03	0·17

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; FBG, fasting blood glucose; HOMA-IR, homeostatic model assessment for insulin resistance; MDA, malondialdehyde; TAC, total antioxidant capacity.
* Comparison of the variables between baseline and post-intervention was performed with the paired *t* test.
† Comparison of changes between the two groups was carried out by repeated-measures ANOVA with age, sex and corresponding baseline values as confounders.
‡ The same as the previous column but fat mass loss was added to the covariates.

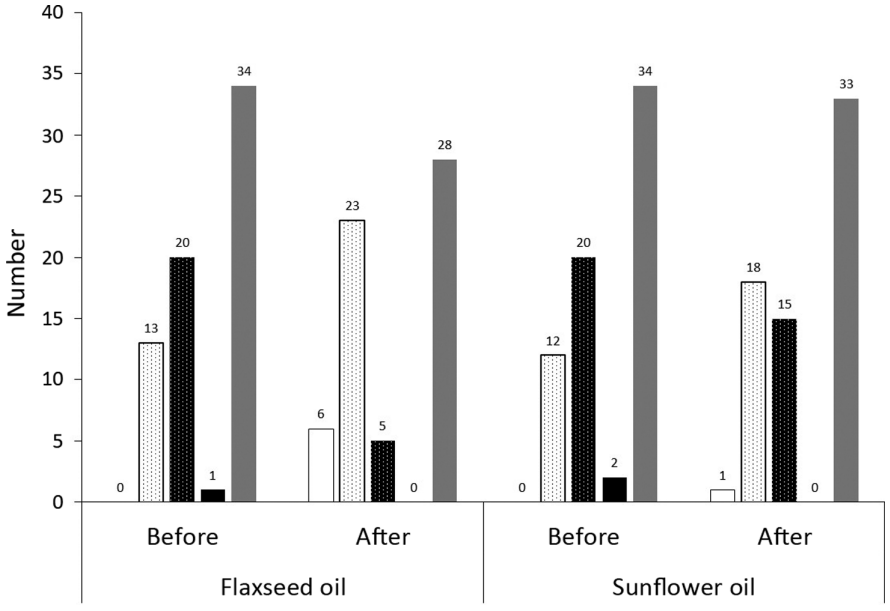

Fig. 2. Fatty liver grades pre- and post-intervention in the study groups. □, Normal; ⊡, grade 1; ▩, grade 2; ■, grade 3; ■, total non-alcoholic fatty liver disease.

Discussion

Our findings indicated that in the context of a weight loss programme (hypoenergetic diet plus physical activity), 12-week consumption of flaxseed oil decreased fatty liver grade compared with sunflower oil without changing liver enzymes and cardiometabolic risk factors. Both groups indicated significant reduction in weight, waist circumference and blood pressure, indicating that a part of these improvements has

Table 3. Anthropometric indices and blood pressure at baseline and after each month of the intervention according to the intention-to-treat analysis (Mean values and standard deviations)

	Flaxseed oil (*n* 34)									Sunflower oil (*n* 34)									
	Baseline		Month 1		Month 2		Month 3			Baseline		Month 1		Month 2		Month 3			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P**	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P**	*P*†
Weight (kg)	83·2	13·1	80·6	12·6	78·9	12·4	78·0	12·2	<0·001	80·7	110·8	79·4	12·1	78·5	12·2	78·4	12·2	<0·001	0·01
BMI (kg/m^2)	30·1	4·1	29·1	3·8	28·5	3·6	28·2	3·8	<0·001	29·6	3·9	29·1	4·0	28·8	3·9	28·7	3·9	<0·001	0·009
WC (cm)	102·4	11·1	98·6	10·5	96·0	10·8	93·6	10·1	<0·001	99·7	9·2	96·7	9·6	93·9	9·3	92·3	9·7	<0·001	0·44
SBP (mmHg)	131·5	15·9	124·3	13·1	123·5	10·9	117·2	9·1	<0·001	129·9	13·6	122·7	14·2	122·1	8·6	120·4	8·9	<0·001	0·84
DBP (mmHg)	88·6	8·4	84·1	8·6	85·4	8·0	80·1	6·8	<0·001	88·8	10·5	83·0	10·5	82·8	8·0	82·4	6·5	<0·001	0·45

WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure.
* Within-group comparisons were performed with repeated-measures ANOVA using the Huynh–Feldt test.
† For between-group comparisons, age, sex and the corresponding baseline values were used as the covariates in the repeated-measures test.

probably occurred due to the weight loss programme. In addition to fatty liver grade, flaxseed oil caused significant decrease in weight and IL-6 compared with sunflower oil.

Fatty liver

NAFLD is regarded as the hepatic manifestation of the metabolic syndrome. There is a close relationship between cardiometabolic risk factors, such as central obesity, insulin resistance, hyperglycaemia, hypertension, dyslipidaemia and NAFLD[27]. Thus, it is reasonable to expect that the attenuation of fatty liver is associated with improvement in these cardiometabolic risk factors. However, none of the cardiometabolic risk factors in this study was improved by flaxseed oil compared with sunflower oil. This suggests that the effect of flaxseed oil on liver steatosis was independent of correction of cardiometabolic risk factors and this may imply that flaxseed has a direct preventive effect on accumulation of fat in the liver. *n*-3 Fatty acids suppress hepatic *de novo* lipogenesis by inhibiting the transcription factor sterol regulatory element-binding protein-1, thus reducing activities of the lipogenic enzymes, acetyl-CoA carboxylase and fatty acid synthase[28]. *n*-3 Fatty acids also stimulate fatty acid oxidation in the liver and prevent fatty acid release from adipose tissue[28]. However, most of these benefits and mechanisms have been found in long-chain *n*-3 fatty acids such as EPA and DHA but literature on the effect of shorter chain *n*-3 fatty acids like *α*-linolenic acid is lacking. *α*-Linolenic acid is the most abundant *n*-3 fatty acid present in vegetable oils. In the flaxseed oil used in this study, we detected 49·8 % *α*-linolenic acid. By this way, patients in flaxseed oil group should have consumed about 10 g *α*-linolenic acid each day. Our results suggest that at this dose, *α*-linolenic acid was effective in improving liver steatosis.

Besides, the plausible direct effect of flaxseed oil on liver steatosis, the improvement in fatty liver grade may have resulted at least partly from weight reduction because both groups demonstrated significant decrease in weight and waist circumference. Weight loss has been recognised as the most effective strategy for treatment of NAFLD[29] and alleviation of more severe stages of the disease[30]. Even 5 % decrease in BMI has led to 25 % reduction in liver fat[31]. In addition, waist circumference and visceral adiposity can also determine NAFLD risk even in normal-weight individuals[32,33]. Thus, it is reasonable to presume that a part of improvement in fatty liver grade of either group has occurred consequent to reduction in weight and waist circumference. The decrease in ALT and aspartate aminotransferase levels in both groups may also have occurred as a result of weight and waist circumference reduction although such association has not been observed in all of previous studies[33].

Table 4. Dietary intakes excluding the prescribed oils and physical activity during the study period based on the intention-to-treat analysis (Mean values and standard deviations of 3-d food records obtained at start and the end of each month of the intervention)

	Flaxseed oil (*n* 34)		Sunflower oil (*n* 34)		
	Mean	SD	Mean	SD	*P**
Energy (kcal/d)†	1807	560·1	1781	596·8	0·97
Carbohydrate (g/d)	299·5	111·0	284·2	91·3	0·51
Protein (g/d)	59·5	18·1	59·3	20·6	0·99
Fat (g/d)	41·7	16·8	45·5	22·7	0·13
SFA (g/d)	12·4	4·3	12·9	5·3	0·68
PUFA (g/d)	17·7	6·9	19·3	9·7	0·36
MUFA (g/d)	11·2	4·6	13·4	6·6	0·11
Cholesterol (g/d)	220·3	127·2	254·6	142·2	0·34
Fibre (g/d)	19·9	9·0	19·9	10·6	0·98
Physical activity (MET-min/week)	563·0	567·7	744·5	806·9	0·29

MET, metabolic equivalent task.
* Comparisons were performed using the independent *t* test.
† To convert kcal to kJ, multiply by 4·184.

Liver enzymes and lipid profile

Flaxseed oil did not cause significant improvement in liver enzymes which could be due to the normal levels of these enzymes in our patients. Despite the common use of aminotransferases especially ALT as a non-invasive indicator of hepatosteatosis[34], these enzymes have low sensitivity for NAFLD diagnosis and thus a large number of NAFLD cases come with normal levels of liver enzymes[35,36]. Hence, aminotransferases are not appropriate indicators of fatty liver incidence and severity.

Our findings are in line with findings reported by Yari *et al.*[18] for liver steatosis but not for liver enzymes. In fact, Yari *et al.* reported that consumption of 30 g/d flaxseed powder for 12 weeks led to reduction of hepatic steatosis and fibrosis as well as serum concentrations of ALT, aspartate aminotransferase and γ-glutamyl transferase in NAFLD patients[18]. Although the concentration of *n*-3 fatty acids in flaxseed oil is much higher than

that in flax seeds (about 53 % *v.* about 22 %)[36], the difference in the results may have come from compounds that are removed during flaxseed oil extraction. These include fibre, antioxidants, phyto-oestrogens and lignans such as secoisolariciresinol diglucoside, which are suggested to have anti-inflammatory, antioxidative and lipid-modulating properties[37–39]. In agreement with this point, we did not observe beneficial effect of flaxseed oil on serum lipids but interventions with flaxseed have shown its benefits on TAG[18] and LDL-cholesterol[40]. A meta-analysis showed that flaxseed interventions reduced total and LDL-cholesterol when whole flaxseed or lignan was used but not when flaxseed oil was administered[41]. A systematic review revealed that α-linolenic acid alone has no effect on total cholesterol, TAG, LDL-cholesterol and VLDL[42].

Adiposity

The 2092 kJ energy deficit caused significant reduction in weight and waist circumference in both groups. However, flaxseed oil consumers showed more substantial weight reduction which was statistically different from sunflower group, indicating that flaxseed oil exerted an additional effect on weight and BMI. Accordingly, a meta-analysis suggested that consumption of flax seeds, flaxseed oil or flaxseed lignan results in significant decrease of weight, BMI and waist circumference[43]. Clinical trials have shown that flaxseed fibre may induce higher postprandial satiety and fullness without affecting ghrelin, cholecystokinin, glucagon like peptide-1 and energy intake[44]. Dietary assessment of this study also showed no difference in energy intake between groups. Hence, we presume that mechanisms like what was mentioned for hepatic fat accumulation may be involved. *n*-3 Fatty acids are known to decrease adipocyte lipogenesis and stimulate fatty acid mobilisation from adipose tissue[45]. Moreover, *n*-3 fatty acids decrease adipocyte lipoprotein lipase and increase muscle lipoprotein lipase, resulting in prevention of fat deposition in adipose tissue and augmentation of fatty acid oxidation in muscle and fat depots, the latter through increasing mitochondrial biogenesis in white adipose tissue[45]. Our results on fat mass confirm these justifications; although not significant between groups, fat mass of flaxseed oil consumers showed significant reduction, while in sunflower oil consumers, significant reduction was observed in muscle mass.

Blood pressure

During the intervention, significant decrease in blood pressure occurred in both groups, suggesting that an identical factor has caused such decrease. Since weight reduction has been introduced as an effective strategy for improving high blood pressure in overweight and obese individuals[46], we presume that the decrease in blood pressure has occurred due to weight reduction following taking the weight loss programme. Previous studies on the effect of flaxseed oil on blood pressure are quite conflicting. Even meta-analyses have indicated inconsistent results. In this regard, Urosoniu *et al.* reported that diastolic blood pressure decreased following consumption of both powder and oil preparations of flaxseed but flaxseed oil was not effective on systolic blood pressure[47]. However, Khalesi *et al.* reported beneficial effect of whole flaxseed on blood pressure but no effect from flaxseed oil was observed[48]. For α-linolenic acid, also, the results of meta-analyses are contradictory: while Takeuchi *et al.* suggested an antihypertensive effect[49], Wendland *et al.* did not find such a result[42].

Inflammation

Flaxseed oil caused significant reduction in IL-6 compared with sunflower oil. Circulating IL-6 is an important pro-inflammatory cytokine associated with non-alcoholic steatohepatitis development, systemic insulin resistance and diabetes[50]. *n*-3 Fatty acids present in flaxseed oil are precursors of anti-inflammatory eicosanoids and involved in the suppression of many aspects of inflammation[51]. However, a previous meta-analysis did not show effectiveness of flaxseed on IL-6[52]. In that meta-analysis, flaxseed significantly reduced circulating concentrations of high-sensitivity C-reactive protein and TNF-α but did not affect IL-6 and C-reactive protein[52]. Nonetheless, subgroup analysis of another meta-analysis revealed that flaxseed reduces C-reactive protein in subjects with BMI $\geq 30\ kg/m^2$ but not in normal-weight individuals. Since all of our patients had overweight or obesity (BMI $\geq 25\ kg/m^2$) and half of them had BMI $\geq 30\ kg/m^2$, the significant effect of flaxseed on IL-6 may be justified[53]. The results of repeated-measures ANOVA confirm this justification because after addition of fat loss to the covariates, the difference between two groups no longer existed, suggesting that fat mass loss is a crucial determinant of the anti-inflammatory effect of flaxseed. In other words, the plausible anti-inflammatory effect of flaxseed may be rendered through reduction in fat mass.

Strengths and limitations

One strength of this trial was allocation concealment for the specialist who performed the ultrasound assessment. Furthermore, we evaluated body composition which allowed us to assess the effect of flaxseed oil on fat and muscle mass. However, a bioelectric impedance analyser is sensitive to changes in body hydration, and since we did not prohibit water consumption before the test, the device may have overestimated body fat by 1·1 %[54]. Another limitation was the lack of information on circulating fatty acids; due to limited budget, we could not measure serum fatty acids. Relatively low sample size and short intervention duration were also drawbacks of this trial.

Conclusion

Overall, results of this trial suggest that in the context of a low-energy diet plus moderate exercise, 12-week consumption of 20 g/d flaxseed oil may mitigate fatty liver grade and decrease weight and IL-6 of NAFLD patients to a greater extent than sunflower oil. Decreasing aminotransferases, waist circumference, and systolic and diastolic blood pressure were benefits that came along with the weight loss programme as they changed similarly in both treatments. Since IL-6 is an important inflammatory cytokine in NAFLD, future studies with flaxseed oil are suggested to be conducted on patients with more severe cases of NAFLD and non-alcoholic steatohepatitis. Benefits of flaxseed oil may be

more substantial in patients with more severe cases of NAFLD and with higher levels of aminotransferases.

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