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Olive oil lessened fatty liver severity independent of cardiometabolic correction in patients with non-alcoholic fatty liver disease: A randomized clinical trial

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ABSTRACT

Objectives: Olive oil has health benefits for the correction of metabolic diseases. We aimed to evaluate the effect of olive oil consumption on the severity of fatty liver and cardiometabolic markers in patients with non-alcoholic fatty liver disease.
Methods: This randomized, double-blind, clinical trial was conducted on 66 patients with non-alcoholic fatty liver disease. Patients were divided to receive either olive or sunflower oil, each 20 g/d for 12 wk. A hypocaloric diet (−500 kcal/d) was recommended to all participants. Fatty liver grade, liver enzymes, anthropometric parameters, blood pressure, serum lipid profile, glucose, insulin, malondialdehyde, total antioxidant capacity, and interleukin-6 were assessed pre- and postintervention.
Results: Fatty liver grade, weight, waist circumference, and blood pressure significantly decreased in both groups. Sunflower oil significantly reduced serum aspartate and alanine aminotransferases and olive oil only decreased serum aspartate aminotransferase. Fat-free mass and skeletal muscle mass significantly reduced after the consumption of sunflower oil and serum triacylglycerols and fat mass significantly declined after the ingestion of olive oil. Among these variables, only changes in fatty liver grade (-0.29 ± 0.46 in sunflower oil versus -0.75 ± 0.45 in olive oil; $P < 0.001$), skeletal muscle mass (-0.71 ± 1.36 in sunflower oil versus $+0.45 \pm 2.8$ in olive oil; $P = 0.04$), and body fat percentage ($+0.38 \pm 5.2\%$ in sunflower oil versus $-3.4 \pm 5.5\%$ in olive oil; $P = 0.04$) were significantly different between the groups.
Conclusions: Olive oil may alleviate the severity of fatty liver independent of correcting cardiometabolic risk factors. Low-calorie diets may benefit patients with non-alcoholic fatty liver disease additionally through mitigation of obesity, blood pressure, and liver enzymes.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the rapidly growing pathologic conditions of the liver whereby >5% of liver weight is from fat [1]. Mild cases of NAFLD are not life-threatening but if left untreated, the disease could progress to more severe cases that are associated with inflammation (i.e., non-alcoholic steatohepatitis) and subsequently liver injury, cirrhosis, and in some cases hepatocellular carcinoma [2].

Much like type 2 diabetes and metabolic syndrome, the major characteristics of NAFLD are obesity, hyperglycemia, insulin resistance, dyslipidemia, and hypertension [3]. The increase in prevalence of NAFLD concomitant with the soaring prevalence of obesity and other aforementioned metabolic disorders further demonstrates the tight link between these pathologic states [4]. Because of the multifactorial essence of the disease, various pharmacologic and nutritional agents have been suggested for the treatment of NAFLD but no successful consensus has been reached [5]. To date, the core strategy for the correction of NAFLD is lifestyle modification, the most important of which is through the correction of diet and physical activity [4,6].

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A large body of evidence that is mainly from epidemiologic investigations emphasizes the health benefits of the Mediterranean diet in the cardiovascular system and metabolism [7,8]. Many of these health effects are attributed to olive oil, which is one of the essential components of the Mediterranean diet. To act as a functional food, olive oil is indebted to its high monounsaturated fatty acids (MUFAs) and small amounts of highly bioactive compounds. The most important compounds are polyphenols, which are antioxidant chemicals with abundant biological effects [8,9].

Similar to the beneficial effects of the Mediterranean diet, epidemiologic and experimental studies have demonstrated an association between olive oil consumption and reduced morbidity and mortality from cardiometabolic diseases and some types of cancer [9]. In particular, the protective role of olive oil against obesity, diabetes, cardiovascular diseases, and other similar metabolic diseases has been numerously reported. However, the number of human clinical trials on the issue is scarce and particularly with regard to fatty liver. Only one group of investigators has examined the effect of olive oil, canola oil, or soybean/safflower oil (as a control) on male patients with NAFLD and found improvements in severity of fatty liver, insulin resistance, and lipid profile [10].

Our study was the second of its kind to examine the effect of olive oil on NAFLD. However, what distinguishes this study from the one by Nigam et al. [10] is that we did not limit our participants to one sex. Moreover, we used sunflower oil as the control because this oil seems to be a better control for olive oil than soybean/safflower oils as used by Nigam et al. Soybean oil contains considerable amounts of omega-3 fatty acids (6.8%), which by themselves can appear to have a protective effect for the liver in NAFLD as reported in previous studies [11].

Methods

Design

This study was a randomized, double-blind, parallel-group, controlled, clinical trial that was conducted in the spring of 2016 in Shiraz, Iran. The sample size was estimated at 33 on the basis of the change in mean of weight (5.4 kg) and a standard deviation of 7.9 as reported in a previous investigation [10]. The assumption of power was 80% with an error of 5% and an attrition rate of 20%. Although our primary outcome was fatty liver grade, we could not calculate the sample size on the basis of fatty liver grade because Nigam et al. [10] reported the fatty liver grade numerically.

The trial was approved by the ethics committee of the Shiraz University of Medical Sciences (Approval no. IR.SUMS.REC.1394.103) and registered in the Iranian Registry of Clinical Trials (IRCT2016011825957 N2; February 9, 2016). All participants gave their written informed consent upon recruitment.

Patients

Patients were recruited via posters and local newspaper advertisements. Patients were included in the study upon diagnosis of NAFLD by a physician on the basis of an ultrasound examination, body mass index (BMI) $\geq$ 25 kg/m^2, age $\geq$18 y, and willingness to participate. Patients were excluded in cases of liver disease other than NAFLD (e.g., alcoholic fatty liver, viral hepatitis, cirrhosis, and bile duct obstruction), severe illnesses (e.g., cancer and organ failure), pregnancy and lactation, diabetes, malnutrition, special diets or dietary limitations, any type of alcohol consumption, medications to correct hypertension or dyslipidemia, and medications that induce fatty liver (e.g., methotrexate, tamoxifen, and valproic acid). In addition, participants who had a low adherence to diet and physical activity recommendations for >15 d during the 12-wk intervention period, incidence of acute diseases or infections, hospitalization, and no longer met the inclusion criteria were excluded from the study.

Intervention

After a 2-wk run-in period, participants were assigned by random block procedure to consume either olive oil (Verjen, Gorgan, Iran) or sunflower oil (control group; Oila, Tehran, Iran). The random allocation was performed by an investigator who was not directly involved in the investigation. By means of computer-generated random numbers and a block size of four, six possible sequences of arms (A or B) were ordered and recorded. The allocation was performed according to this order and continued until all participants were specified to an arm.

The oils were provided to the participants in identical bottles. Neither participants nor the investigators who carried out the experiments nor the physician who performed the liver ultrasound were aware of the type of oils consumed until the study was terminated. Patients were instructed to consume 20 g of the provided oil each day for a course of 12 wk. Ten-mL measuring cups were provided for the participants and they were asked to add two cups of oil per day to salads or rice (at the time of cooking or consumption). This amount of oil made up almost one third of the daily oil consumption. The rest of the regimen oil was obtained from low-fat dairy and meats as well as oil used for cooking purposes.

A 500-kcal deficit diet (10–15% of energy from protein, 30–35% from fat, and 50–55% from carbohydrates) was designed for patients in both groups as well as advice to have 30 to 40 min of moderate physical activity per day. The diet was explained and an exchange list was given to each patient to enable flexibility of the diet during the 12-wk intervention. Because omega-3 fatty acids have shown beneficial effects on fatty liver, participants were asked to minimize the consumption of fish and nuts and avoid taking omega-3 supplements. Also, patients in both groups were asked to use sunflower oil for cooking purposes, use the least amount of oil during cooking, avoid the consumption of other sources of oil and especially canola and soy oil, avoid fried foods, and consume low-fat meat and dairy. Patients were contacted biweekly to emphasize recommendations on diet and physical activity, receive consultation on possible difficulties in dieting and oil consumption, and be evaluated for cases of noncompliance. Also, diary sheets were provided for patients to mark after each consumption of the prescribed oil.

Anthropometric measures and blood pressure

All measurements were performed by trained dietitians. Weight (Glamor BS-801, Hitachi, China), waist circumference, and blood pressure (Beurer BM 44, Beurer GmbH, Germany) were measured four times: At the beginning and end of each month of the intervention. To measure blood pressure, patients were seated and blood pressure was measured twice with at least 1 min interval. The mean of two measurements was considered the patient's blood pressure. Body composition including fat mass, fat-free mass, and skeletal muscle mass was estimated by bioelectric impedance analyzer (InBody, Biospace Co., South Korea).

Biochemical parameters

Blood samples were collected in the morning after a 12-h overnight fast at baseline and the end of the study. Glucose, triacylglycerols, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), alanine transaminase (ALT), aspartate transaminase, and gamma-glutamyl transferase were determined by using commercially available kits (Pars-Azmun, Iran) and an auto-analyzer (BT 1500, Biotecnica Instruments, Italy). Insulin (Monobind Inc, Lake Forest, CA), interleukin-6 (Diaclone, France), and total antioxidant capacity (ZellBio GmbH, Germany) were quantified with the enzyme-linked immunosorbent assay method. Malondialdehyde was quantified with the thiobarbituric acid reactive substance method as described before [12].

Liver ultrasound

Fat infiltration was evaluated by liver ultrasound (Medison-Accuvix-V10) in 12-h fasting state before and after the intervention. The ultrasound was performed by a single expert radiologist. The severity of fatty liver (grades 1–3) was evaluated by the degree of echogenicity, visualization of the diaphragm, borders of the liver vasculature, and visualization of the posterior portion of the right hepatic lobe. The radiologist was blinded to the participants' group allocation.

Dietary intakes and physical activity

Diet was evaluated with a 3-d diet record at the beginning and end of each month of the intervention. Nutrient composition was determined with Nutritionist IV version 3.5.2 (Hearst Corp., San Bruno, CA). Physical activity was assessed by a validated international physical activity questionnaire and expressed as metabolic equivalent task in minutes per week (Met-min/wk) [13].

Chemical analysis of oils

The fatty acid compositions of olive and sunflower oils were determined using high-performance liquid chromatography [14]. The main difference between the

fatty acid composition of olive and sunflower oil as used in this study was the percentage of oleic acid (64.3% in olive oil versus 25.0% in sunflower oil), linoleic acid (15.4% in olive oil versus 63.2% in sunflower oil), and palmitic acid (15.2% in olive oil versus 7.4% in sunflower oil). Omega-3 fatty acids constituted 1% and 0.22% of olive and sunflower oil, respectively.

Statistical analysis

The statistical analyses were performed with SPSS version 19 (SPSS Inc., Chicago, IL). The normality of the data distribution was examined by visual examination of the histogram curve of data frequencies and in case of abnormality, the data were log transformed. The baseline characteristics of the participants were compared between groups with independent samples *t* test (or χ^2 for sex and smoking status). Pairwise data were tested by paired sample *t* test. An analysis of covariance was used to examine the significance of the difference in changes of variables between the two groups using age, sex, BMI, waist circumference, and baseline values of the corresponding variables as covariates. For the variables that were measured four times (i.e., baseline and weeks 4, 8, and 12), changes of values in each group were evaluated by repeated measures and between-group differences were assessed with repeated measures using age, sex, BMI, waist circumference, and baseline values of the corresponding variables as covariates. A P value < 0.05 was considered statistically significant.

Results

Of the 192 patients who were assessed for inclusion criteria, 87 patients passed the first eligibility step and 66 patients gave informed written consent and participated in the study. Twelve patients were excluded during the intervention due to reluctance to continue, illness, travel, and low adherence to the recommendations (Fig. 1). Therefore, 66 and 54 patients were entered in the intent-to-treat and per-protocol analyses, respectively (supplementary materials).

The baseline characteristics of the participants are presented in Table 1. Except for waist circumference and total antioxidant capacity, there was no significant difference between the groups at baseline. Fatty liver grade significantly decreased in both groups (Table 2). The reduction in the olive oil group was more than twice as that in the sunflower group (-0.75 ± 0.45 versus -0.29 ± 0.46), which rendered a significant difference between the groups ($P < 0.001$). The serum concentrations of aspartate transaminase were significantly reduced in both groups and ALT only reduced in the sunflower group but none of these liver enzyme changes differed significantly between the two groups. Similarly, changes in serum lipids (i.e., triacylglycerols, and total, LDL-, and HDL-cholesterol), fasting glucose, insulin, homeostasis model assessment of insulin resistance (marker of insulin resistance), malondialdehyde (oxidative stress indicator), interleukin-6, and total antioxidant capacity did not differ between the two groups.

Fat-free mass and skeletal muscle mass decreased significantly in the sunflower group and body-fat mass and percentage significantly decreased in the olive group, which resulted in a between-group difference for skeletal muscle mass ($P = 0.04$) and body-fat percentage ($P = 0.05$).

The frequency of fatty liver grades in the sunflower and olive oil groups is depicted in Figure 2. In both groups, mild (grade 1) cases of fatty liver increased but moderate (grade 2) and severe (grade 3) cases decreased during the 12-wk intervention. In the olive oil group, normal and grades 1, 2, and 3 cases of fatty liver were 0%, 28.1%, 62.5%, and 9.4% at baseline and 12.5%, 68.8%, 18.8%, and 0% at the end of the intervention, respectively. In the sunflower oil

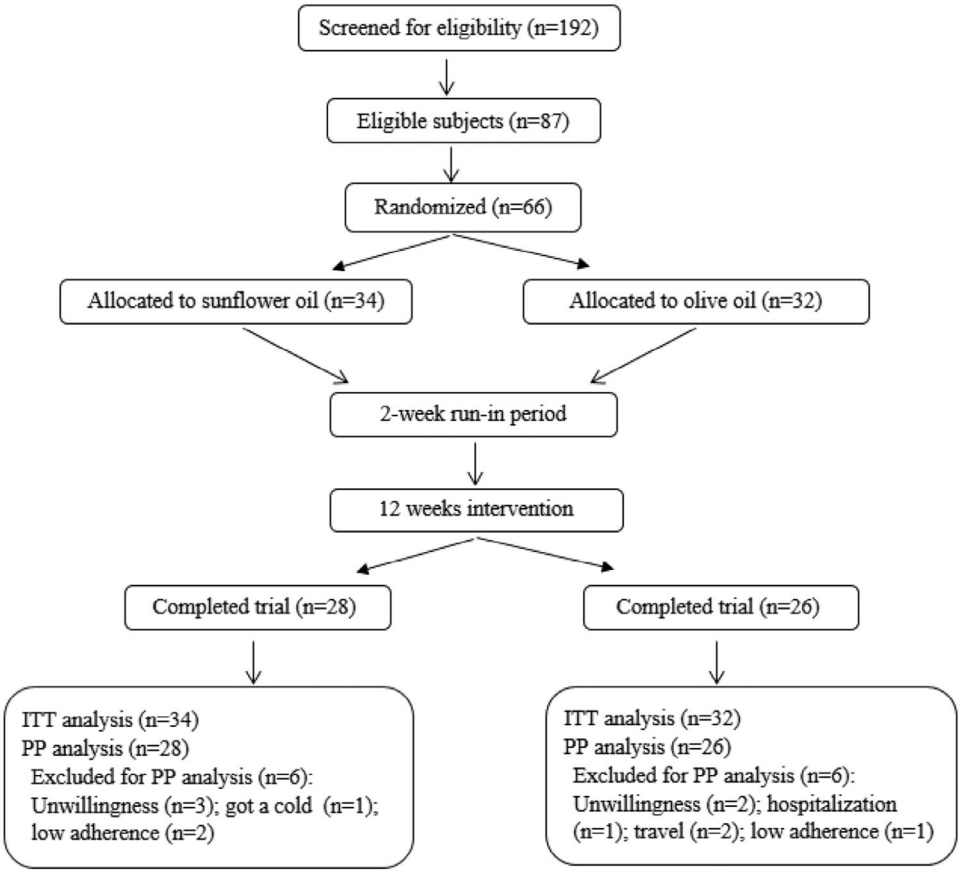

Fig. 1. Study flowchart. ITT, intent-to-treat; PP, per-protocol.

Table 1
Baseline characteristics of participants by intent-to-treat analysis*

	Sunflower oil (n = 34)	Olive oil (n = 32)	*P* value†
Age, y	40.8 ± 8.7	46.3 ± 13.6	0.06
Male, n (%)	17 (50.0)	12 (37.5)	0.3
Smoker, n (%)	4 (11.8)	2 (7.7)	0.4
NAFLD duration, y	2.8 ± 2.0	2.8 ± 2.2	0.9
Alanine aminotransferase, U/L	28.9 ± 16.4	27.2 ± 18.6	0.7
Aspartate aminotransferase, U/L	18.5 ± 8.1	18.7 ± 10.5	0.9
Gamma-glutamyl transferase, U/L	30.1 ± 14.2	31.3 ± 17.0	0.8
Fatty liver grade	1.7 ± 0.58	1.8 ± 0.59	0.5
Weight, kg	80.7 ± 11.8	82.4 ± 13.3	0.6
Body mass index, kg/m^2	29.6 ± 3.9	30.6 ± 3.6	0.3
Waist circumference, cm	99.7 ± 9.2	104.8 ± 9.4	0.03
Glucose, mg/dL	94.8 ± 11.2	96.2 ± 10.9	0.5
Fat-free mass, kg	51.5 ± 8.9	53.4 ± 11.1	0.5
Skeletal muscle mass, kg	29.1 ± 6.1	29.6 ± 6.8	0.8
Fat mass, kg	26.5 ± 11.9	30.7 ± 8.5	0.2
Body fat, %	33.1 ± 11.6	37.2 ± 6.0	0.1
Insulin, μU/mL	2.6 ± 1.8	2.7 ± 1.9	0.8
Triacylglycerols, mg/dL	151.3 ± 87.5	158.0 ± 111.7	0.8
Total cholesterol, mg/dL	184.8 ± 31.9	196.4 ± 54.9	0.3
LDL-cholesterol, mg/dL	109.5 ± 30.3	113.0 ± 28.9	0.6
HDL-cholesterol, mg/dL	41.9 ± 7.3	42.7 ± 9.8	0.7
Malondialdehyde, μmol/L	3.3 ± 0.64	3.2 ± 0.94	0.8
TAC, mmol/L	0.26 ± 0.05	0.30 ± 0.07	0.03
Interleukin-6, ng/L	8.5 ± 4.5	7.5 ± 1.6	0.2
Systolic BP, mmHg	129.9 ± 13.6	129.9 ± 11.3	1
Diastolic BP, mmHg	88.8 ± 10.5	88.9 ± 8.5	1

BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAFLD, non-alcoholic fatty liver disease; TAC, total antioxidant capacity.

* Data are either means ± standard deviation or n (%).

† Qualitative variables were examined with χ^2 test and tested with independent *t* test.

group, these cases were 0%, 35.3%, 58.8%, and 5.9% at baseline and 2.9%, 52.9%, 44.1%, and 0% at the end, respectively. The alleviation of fatty liver severity was more pronounced in patients who consumed olive oil.

Anthropometric characteristics and blood pressure were measured at baseline and the end of weeks 4, 8, and 12. Weight, BMI, waist circumference, and systolic and diastolic blood pressure decreased significantly in both groups without a significant difference in between (Table 3). The energy and dietary intakes of macronutrients did not differ between the groups during the intervention except for MUFAs, polyunsaturated fatty acids (PUFAs), and omega-3 fatty acids (Table 4). Compared with sunflower oil, olive oil consumers showed a significantly higher intake of MUFAs and omega-3 fatty acids and a lower intake of PUFAs. Physical activity also did not differ between the groups.

Discussion

Our results showed that the 12-wk consumption of olive oil alleviated the severity of fatty liver but this alleviation was not associated with significant improvements in liver enzymes, cardiometabolic risk factors, and inflammatory and oxidative stress markers although a significant reduction in body fat percentage and an improvement in skeletal muscle mass was observed in the olive oil group compared with the sunflower oil group.

Liver

We observed a significant improvement in fatty liver grade in both groups with a more remarkable betterment in the olive oil group ($P < 0.001$). Our findings with regard to the grade of fatty liver is in line with those by Nigam et al. [10] who reported that the consumption of 20 g olive oil or canola oil for 6 mo improved the fatty liver grade compared with soybean/safflower oil. However, the difference between the two studies is the extent of beneficial effects of olive oil. Nigam et al. [10] reported improved fatty liver that was associated with reduced weight and insulin resistance but we did not observe such an association. The discrepancy in the results of the two studies could be because of the difference in the length of the intervention (3 mo versus 6 mo), recommendations for energy intake (−500 kcal/d versus daily energy requirements), and baseline levels of the biochemical measures, which were higher in study by Nigam et al. [10].

NAFLD is considered the hepatic form of metabolic syndrome in which the disease is associated with a wide spectrum of inflammation, oxidative stress, and metabolic disturbances, with the latter ranging from insulin resistance and hyperglycemia to dyslipidemia and hypertension [15]. Since there are interrelationships between these disorders and NAFLD, a correction of these disarrangements may be assumed to come along with the alleviation of hepatic steatosis. However, the results of this study were not in accordance with this hypothesis because the consumption of olive oil reduced the extent of fat deposition in the liver but was unable to correct metabolic disturbances that are associated with NAFLD.

Olive oil is likely able to reduce hepatocyte fat deposition independently from metabolic routs, possibly through increased fatty acid oxidation. There are reports from other investigators that support this justification. For instance, the consumption of diets that are rich in MUFAs (28% to total calories) for 8 wk by patients with type 2 diabetes reduced liver fat by 30% and this decline was associated with increased postprandial β-oxidation of fatty acids [16]. Similarly, in prediabetic patients, high MUFA intake (28%, half as olive oil) for 12 wk decreased hepatic fat and improved hepatic and total insulin sensitivity [17]. Such an effect has also been observed in animal studies where genes that are involved in fat lipolysis and oxidation were induced after the consumption of MUFAs. For instance, the elevation of fatty acid oxidation by MUFAs [18] or olive oil [19] has been reported in non-hepatic tissues such as adipose tissue [18] and the heart [19] of animal species.

According to proteomic investigations, this hepatic protection may be exerted through antioxidant effects and the regulation of hepatocytes' activity and metabolism [20]. Decreased body-fat percentage after the consumption of olive oil in this study supports this justification that olive oil may have the potential to improve fatty liver grade directly through enhanced fatty acid oxidation and reduced fat deposition in various tissues including adipocytes and hepatocytes. A part of the metabolic and cytoprotective effects of olive oil is suggested to be exerted by hydroxytyrosol, which is the most abundant phenolic compound in olive oil [21]. Of note, the amount of olive oil that is consumed needs to be calculated along with other fat sources in the diet and should be inside the recommended dietary fat range because, like any other source of dietary fat, high doses of olive oil can result in adverse effects including hepatic steatosis [22].

The alleviated fatty liver grade in the olive oil group was not associated with a greater reduction in liver enzymes compared with the sunflower oil group although the levels of aminotransferases decreased in both groups. However, the average levels of aminotransferases were not higher than the normal ranges and the lack of more effectiveness in the olive oil group may be the result of such normality. In fact, although the elevation of aminotransferases and particularly ALT is usually used as a common non-invasive detection of hepatosteatosis [23], these tests have a low sensitivity and as a result, many NAFLD cases with normal levels of liver enzymes generally exist [23–26]. Therefore, the levels of aminotransferases cannot precisely indicate the extent of fatty liver.

Table 2
Fatty liver grade, liver enzymes, cardiometabolic and oxidative stress parameters, and body composition measures pre- and postintervention periods by intent-to-treat analysis*

	Wk 0	Wk 12	Change	*P* value†	*P* value‡
Fatty liver grade§					
Sunflower	1.7 ± 0.58	1.4 ± 0.56	−0.29 ± 0.46	0.001	<0.001
Olive	1.8 ± 0.59	1.1 ± 0.56	−0.75 ± 0.45	<.001	
Alanine aminotransferase, U/L					
Sunflower	28.9 ± 16.4	23.3 ± 11.3	−5.6 ± 10.3	0.003	0.2
Olive	27.2 ± 18.6	24.3 ± 14.1	−2.8 ± 8.9	0.08	
Aspartate aminotransferase, U/L					
Sunflower	18.5 ± 8.1	15.4 ± 4.0	−3.0 ± 6.1	0.007	0.2
Olive	18.7 ± 10.5	16.9 ± 9.4	−1.8 ± 4.1	0.02	
Gamma-glutamyl transferase, U/L					
Sunflower	30.1 ± 14.2	27.2 ± 12.7	−2.9 ± 9.1	0.07	0.8
Olive	31.3 ± 17.0	29.1 ± 18.4	−2.2 ± 11.8	0.3	
Triacylglycerols, mg/dL					
Sunflower	151.3 ± 87.5	142.2 ± 88.6	−9.1 ± 62.9	0.4	0.5
Olive	158.0 ± 111.7	141.0 ± 112.0	−17.0 ± 34.9	0.01	
Total cholesterol, mg/dL					
Sunflower	184.4 ± 31.9	180.5 ± 28.6	−4.3 ± 38.6	0.5	0.8
Olive	196.4 ± 54.9	186.3 ± 48.4	−10.1 ± 31.3	0.08	
LDL-cholesterol, mg/dL					
Sunflower	109.5 ± 30.3	105.0 ± 34.0	−4.4 ± 31.9	0.4	0.9
Olive	113.0 ± 28.9	106.7 ± 32.5	−6.2 ± 27.8	0.2	
HDL-cholesterol, mg/dL					
Sunflower	41.9 ± 7.3	42.1 ± 8.1	0.16 ± 4.4	0.8	0.9
Olive	42.7 ± 9.8	42.2 ± 10.1	0.51 ± 8.2	0.7	
Glucose, mg/dL					
Sunflower	94.8 ± 11.2	93.0 ± 14.9	−1.7 ± 13.2	0.5	0.3
Olive	96.2 ± 10.9	93.5 ± 12.6	−2.7 ± 11.5	0.2	
Insulin, μU/mL					
Sunflower	2.6 ± 1.8	2.5 ± 1.6	−0.14 ± 1.7	0.6	0.8
Olive	2.7 ± 1.9	2.7 ± 2.1	−0.06 ± 2.2	0.9	
HOMA-IR					
Sunflower	0.68 ± 0.77	0.59 ± 0.43	−0.09 ± 0.63	0.4	0.9
Olive	0.63 ± 0.44	0.58 ± 0.42	−0.05 ± 0.51	0.6	
Malondialdehyde, μmol/L					
Sunflower	3.3 ± 0.64	3.4 ± 0.54	0.09 ± 0.63	0.4	0.3
Olive	3.2 ± 0.94	3.2 ± 1.0	−0.05 ± 0.66	0.7	
TAC, mmol/L					
Sunflower	0.26 ± 0.05	0.27 ± 0.04	0.01 ± 0.04	0.7	0.8
Olive	0.30 ± 0.07	0.29 ± 0.08	0.01 ± 0.08	0.5	
Interleukin-6, ng/L					
Sunflower	8.5 ± 4.5	8.3 ± 3.3	−0.24 ± 1.7	0.4	0.3
Olive	7.5 ± 1.6	7.7 ± 1.8	0.17 ± 1.2	0.4	
Fat-free mass, kg					
Sunflower	51.5 ± 8.9	50.5 ± 9.2	−1.0 ± 2.2	0.02	0.7
Olive	53.4 ± 11.1	52.5 ± 11.5	−0.86 ± 3.6	0.2	
Skeletal muscle mass, kg					
Sunflower	29.1 ± 6.1	28.4 ± 5.9	−0.71 ± 1.36	0.01	0.04
Olive	29.6 ± 6.8	30.0 ± 6.5	0.45 ± 2.8	0.4	
Fat mass, kg					
Sunflower	26.5 ± 11.9	25.6 ± 11.1	−0.83 ± 3.4	0.2	0.3
Olive	30.7 ± 8.5	27.5 ± 7.9	−3.1 ± 6.3	0.02	
Body fat, %					
Sunflower	33.1 ± 11.6	33.5 ± 10.7	0.38 ± 5.2	0.7	0.05
Olive	37.2 ± 6.0	33.8 ± 7.3	−3.4 ± 5.5	0.004	

HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; TAC, total antioxidant capacity.
* Data are means ± standard deviation (n = 34 for sunflower group; n = 32 for olive oil group).
† Paired *t* test used for determination of the difference between baseline and endpoint values in each group.
‡ Analysis of covariance used to examine the significance of the difference in changes of variables between the two groups with age, sex, body mass index, waist circumference, and baseline values as covariates.
§ Values are mean and standard deviation of fatty liver grade when coded as 0 (no fatty liver), 1 (mild fatty liver), 2 (moderate fatty liver), and 3 (severe fatty liver).

Obesity and weight

Weight, BMI, and waist circumference significantly decreased in both groups, probably as a result of consuming a hypocaloric diet. Although the difference between the two groups was not statistically significant, the larger decrease in the obesity-related anthropometric measures in the olive oil group may have contributed to the observed improvement in fatty liver intensity in this group. This suggests that, despite the possible mechanisms that were described for the direct effect of olive oil on liver steatosis, the indirect beneficial effect of this oil on fatty liver is also plausible through its impact on weight and waist circumference.

Besides the decreases in body weight and waist circumference, olive oil caused significant reductions in fat mass and body fat

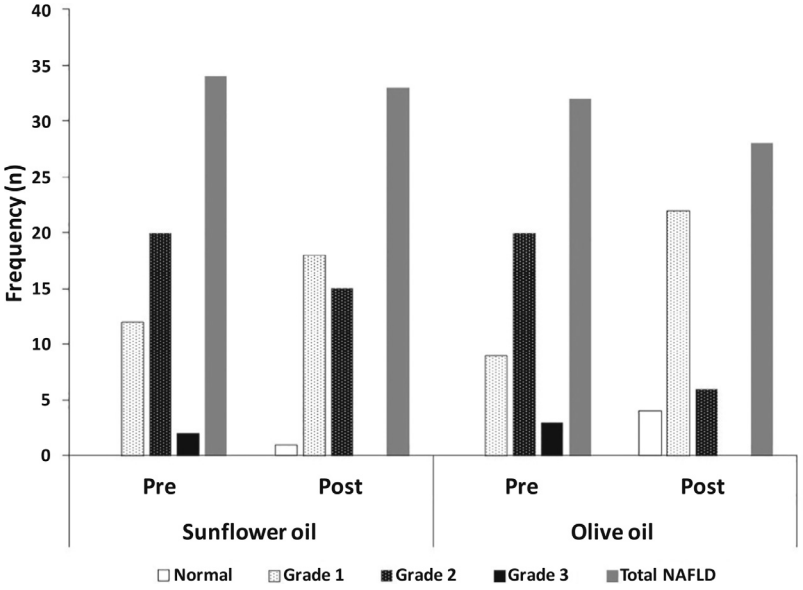

Fig. 2. Fatty liver grade as assessed by ultrasound in the pre- and postintervention periods by study group. NAFLD, non-alcoholic fatty liver disease.

percentage and sunflower oil resulted in significant declines in fat-free mass and skeletal muscle mass, which caused significant between-group differences in skeletal muscle mass ($P = 0.04$) and body fat percentage ($P = 0.05$). Since the maintenance of muscle mass is one of the drawbacks of weight loss diets, the preservation of muscles and enhancement of fat-tissue loss after the consumption of olive oil is valuable.

Little information is available about the effect of olive oil on obesity-related anthropometric measures and the present scant data are very inconsistent. Data from a cross-sectional study showed a positive relationship between olive oil consumption and BMI, which was associated with increased energy intake that was likely the result of a lack of compensation upon the consumption of olive oil [27]. However, in a prospective cohort, the high consumption of olive oil was not associated with a higher weight gain and risk of becoming overweight and obese [28]. Similarly, the incidence of obesity was higher in people who consumed sunflower oil compared with those who consumed olive oil over a 6-y period in a prospective cohort [29].

In clinical trials, in the context of hypocaloric diets, olive oil induced more weight loss than canola oil in an 8-wk interventional study [30]. Likewise, diets that are high in MUFAs reduced central obesity and improved metabolic syndrome risk factors compared with PUFAs [31]. A part of the discrepancies may be explained by differences in the dose of MUFAs and genetic issues. In this regard, Warodomwichit et al. [32] reported that the effect of MUFA intake on weight may depend on the amount of MUFAs that are consumed and the presence of adiponectin gene polymorphism. For instance, the ingestion of ≥13% of daily energy as MUFAs and being the carrier of −11391A allele was required to see a remarkable antiobesity effect of MUFAs in white Americans [32].

Blood lipid profile

In this study, no significant difference in blood lipid fractions was observed between the groups. Although serum triacylglycerols decreased in the olive oil group, no significant difference was

Table 3
Anthropometric measures and blood pressure values during the study period by intent-to-treat analysis*

	Wk 0	Wk 4	Wk 8	Wk 12	Change	*P* value†	*P* value (treatment × time)‡
Weight, kg							
Sunflower	80.7 ± 11.8	79.4 ± 12.1	78.5 ± 12.2	78.4 ± 12.2	−2.3 ± 2.3	<0.001	0.2
Olive	82.4 ± 13.3	80.1 ± 13.3	79.4 ± 13.4	79.1 ± 13.3	−3.4 ± 2.5	<0.001	
BMI, kg/m^2							
Sunflower	29.6 ± 3.9	29.1 ± 4.0	28.8 ± 3.9	28.7 ± 3.9	−0.86 ± 0.85	<0.001	0.2
Olive	30.6 ± 3.6	29.7 ± 3.7	29.5 ± 3.6	29.3 ± 3.7	−1.3 ± 0.88	<0.001	
Waist circumference, cm							
Sunflower	99.7 ± 9.2	96.7 ± 9.6	93.9 ± 9.3	92.3 ± 9.7	−7.4 ± 3.8	<0.001	0.8
Olive	104.8 ± 9.4	101.5 ± 9.7	98.0 ± 9.5	96.2 ± 10.2	−8.6 ± 5.3	<0.001	
Systolic BP, mmHg							
Sunflower	129.9 ± 13.6	122.7 ± 14.2	122.1 ± 8.6	120.4 ± 8.9	−9.5 ± 14.2	<0.001	0.8
Olive	129.9 ± 11.3	124.7 ± 10.5	123.6 ± 8.1	120.7 ± 8.0	−9.2 ± 11.1	<0.001	
Diastolic BP, mmHg							
Sunflower	88.8 ± 10.5	83.0 ± 10.5	82.8 ± 8.0	82.4 ± 6.5	−6.4 ± 11.3	<0.001	0.9
Olive	88.9 ± 8.5	84.6 ± 9.3	84.5 ± 10.0	83.7 ± 9.0	−5.1 ± 11.3	<0.001	

BMI, body mass index; BP, blood pressure.
* Data are means ± standard deviation (n = 34 for sunflower group; n = 32 for olive oil group).
† Within-group changes evaluated by repeated measures.
‡ Between-group differences assessed by repeated measures with age, sex, BMI, waist circumference, and baseline values of the corresponding variables as covariates.

Table 4
Dietary intakes and physical activity of participants during the intervention by intent-to-treat analysis*

	Sunflower oil	Olive oil	*P* value†
Energy, kcal/d	1587 ± 499	1545 ± 399	0.7
Carbohydrate, g/d	255 ± 78	255 ± 76	1
Protein, g/d	53.5 ± 18.0	51.2 ± 16.5	0.6
Fat, g/d	57.8 ± 4.6	56.2 ± 4.5	0.2
SFA, g/d	13.7 ± 4.7	16.9 ± 2.8	0.3
MUFA, g/d	16.6 ± 5.1	22.9 ± 2.9	<0.001
PUFA, g/d	27.3 ± 5.9	16.4 ± 4.7	<0.001
Omega-3 fatty acid, g/d	0.27 ± 0.21	0.36 ± 0.14	0.05
Fiber, g/d	16.5 ± 5.5	19.1 ± 6.3	0.1
Physical activity, Met-min/wk	744 ± 807	640 ± 794	0.6

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.
* Data are means of all dietary records during the intervention ± standard deviation.
† Determined by independent *t* test.

observed between the two groups. The lack of beneficial effect of olive oil on blood lipids may may be due to the relatively optimal levels of lipids in our participants. Similar to the aforementioned issues, previous investigations on the effect of olive oil or MUFAs have produced conflicting results. For instance, PUFAs showed a more beneficial effect than MUFAs on total cholesterol [33] and triacylglycerols [33,34] in patients with hypercholesterolemia [33] or metabolic syndrome [34]. Accordingly, Maki et al. [35] reported on the benefits of corn oil over extra virgin olive oil due to its ability to reduce total, LDL, very LDL, and non-HDL cholesterol. In contrast to these reports, Liu et al. [31] found that decreased triacylglycerols were associated with decreased abdominal fat mass after the consumption of high oleic-acid canola oil but no such effect was observed after the consumption of corn/safflower oil or flax/safflower oil.

Glucose

Both groups showed non-significant reductions in fasting blood glucose. A meta-analysis of clinical trials showed that in patients with type 2 diabetes, high-MUFA diets cause significant reductions in fasting plasma glucose when compared with high-PUFA diets [36]. Since we excluded patients with type 2 diabetes, the reason for the discrepancy in the results of this study and those of the meta-analysis may be the levels of fasting glucose, which might not have been high enough to allow for the beneficial effect of MUFAs in olive oil to appear. In agreement with this justification, Brunerova et al. [37] reported reductions in blood glucose and glycated hemoglobin of patients with type 2 diabetes who consumed a high-fat diet that was enriched with MUFAs for 3 mo; however, the same effect was not observed in obese, non-diabetic individuals [37].

The lack of effectiveness of olive oil on metabolic parameters despite of its benefit on fatty liver and discrepancies that are observed in the results of different studies may be partly due to the amount of polyphenols in olive oil. Polyphenols are neutraceuticals with the potential to prevent NAFLD and other related metabolic disorders [15]. In the context of NAFLD, this protection is exerted against oxidation, inflammation, insulin resistance, dyslipidemia, and blood pressure [15]. In high-fat diet-fed rats, polyphenol-rich virgin olive oil prevented high-fat diet-induced insulin resistance, inflammation, and hepatic oxidative stress more than polyphenol-poor olive oil [38]. However, for the study to remain blinded, we could not use virgin olive oil because the special smell and pungent taste of virgin olive oil [39] informed participants of the type of the oil.

Oxidative stress and inflammation

Phenolic compounds in olive oil not only help in the stability of olive oil against oxidative products but are also supposed to be responsible for the antioxidant and antiinflammatory potential of olive oil [35,36]. However, olive oil did not significantly change serum malondialdehyde, IL-6, and total antioxidant capacity in this study, probably because we did not use polyphenol-containing virgin olive oil to keep the intervention blinded. Nonetheless, a meta-analysis of clinical trials did not show a benefit from high-phenolic olive oil on serum malondialdehyde although in the same study, a beneficial effect was observed on oxidized LDL [40].

Strengths and limitations

The strength of this study is its assessment of body composition in addition to other measurements of obesity, which helped with the interpretation of the results. Also, the radiologist was blinded to the group allocation of the patients, which reduced unwanted errors in the grading of fatty liver. Nonetheless, ultrasounds are not very accurate to detect mild cases of fatty liver but reasonably accurate to identify moderate-to-severe cases. Bioelectric impedance analysis is also subject to error by factors such as exercise, ambient and skin temperature, hydration status, and recent ingestion of food or beverages. Other limitations include the relatively short duration of the intervention period and normal levels of aminotransferases that may have prevented the observation of the best benefit from the administered oils. Also, the low sample size likely hindered the detection of beneficial effects from either oil in some parameters such as weight and serum aminotransferases.

Conclusions

The 12-wk intake of 20 g/d olive oil attenuated fatty liver grade and reduced body-fat percentage in patients with NAFLD but did not affect liver enzymes and cardiometabolic risk factors. The reductions in weight, waist circumference, blood pressure, and serum aminotransferases occurred in both groups and was likely the result of the hypocaloric diet because no between-group differences were observed in any of these measurements. Future studies are needed to examine the effect of olive oil on fatty liver in context of an isocaloric diet and compare high- and low-phenolic olive oils.

Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.nut.2018.02.021.

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