

Replacing ghee with rapeseed oil on NAFLD outcomes

The effects of replacing ghee with rapeseed oil on liver steatosis and enzymes, lipid profile, insulin resistance, and anthropometric measurements in patients with non-alcoholic fatty liver disease: A randomized controlled clinical trial

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Abstract

Non-alcoholic fatty liver disease, which is a prevalent hepatic condition worldwide, is expected to develop into the leading reason for end-stage fatty liver in the forthcoming decades. Incorporating Rapeseed oil into a balanced diet may be beneficial in improving NAFLD. The goal of this trial was to evaluate the impact of substituting ghee with rapeseed oil on primary outcomes such as fatty liver and liver enzymes, as well as on secondary outcomes including glycemic variables, lipid profile, and anthropometric measurements in individuals with NAFLD. Over 12 weeks, 110 patients [70 men and 40 women; BMI (mean \pm SD): 28.2 ± 1.6 kg/m²; mean age: 42 ± 9.6 y], who daily consumed ghee, were assigned to the intervention or control group through random allocation. The intervention group, was advised to substitute ghee with rapeseed oil in the same amount. The control group continued consumption of ghee and was instructed to adhere a healthy diet. Results showed a significant reduction in the steatosis in the intervention group in comparison to the control group ($P < 0.001$). However, a significant change in the levels of ALT (-14.4 IU/l), GGT (-1.8 IU/l), TG (-39.7 mg/dl), TC (-17.2 mg/dl), LDL (-7.5 mg/dl), FBS (-7.5 mg/dl), Insulin (-3.05 mU/l), HOMA-IR (-0.9), QUICKI (+0.01), weight (-4.3 kg), BMI (-0.04 kg/m²), waist (-5.6 cm) and waist to height ratio (-0.04) was seen in the intervention group. The consumption of rapeseed oil instead of ghee caused improvements in liver steatosis and enzymes, glycemic variables, and anthropometric measurements among individuals with NAFLD.

Keywords: Canola oil, Clarified butter, Ghee, Rapeseed Oil, Non-alcoholic fatty liver disease, Insulin resistance

Introduction

In the global population, Non-alcoholic fatty liver disease (NAFLD) prevalence is estimated to be around 25%, with the lowest rates in Africa (13%) and the highest rates in Southeast Asia (42%) ⁽¹⁾. According to a study in 2016, the proportion of NAFLD in Iranian population was reported to be 33.9% ⁽²⁾. NAFLD is a clinical diagnosis, in which at least 5% liver steatosis exists as determined by liver imaging or biopsy in the absence of any other known causes of liver dysfunction, and probably presents with elevated liver enzymes ^(3, 4). It is noteworthy that NAFLD has been linked to a lot of metabolic diseases like insulin resistance (IR), and obesity which are the key properties of the metabolic syndrome (MS) ⁽⁵⁾. Therefore NAFLD is often thought to be its hepatic manifestation ⁽⁶⁾. Additionally, according to recent studies, there exists a correlation between smoking and the risk of developing non-alcoholic fatty liver disease ⁽⁷⁾. Treating of individuals who are suffering from NAFLD usually includes multiple modes of treatment that targets various facets, such as weight reduction, lifestyle adjustments, and optimization of drug therapy ⁽⁸⁾. There are many drugs on the pipeline that are reckoned as good candidates to cure NAFLD/NASH, as evident in various recent papers, for example pioglitazone, vitamin E, and semaglutide ⁽⁹⁾. NAFLD pathogenesis is defined by the triglyceride accumulation in the liver ⁽¹⁰⁾. The role of fatty acid obtained through dietary intake as a key contributor to liver fat accumulation, is widely recognized ⁽¹⁰⁾. Indeed the various dietary lipids have some unique characteristics, namely different degrees of saturation, which are divided into saturated, mono- and poly-unsaturated fatty acids ⁽¹¹⁾. However, some dietary habits, like ‘western dietary pattern’ with low fiber and high saturated fat, are considered crucial in the commencement and development of NAFLD ⁽¹²⁻¹⁴⁾. Ghee contains 60.4% SFAs, 31.4% MUFAs, 4% PUFA, and 1.5% trans fatty acids and that is clear with utilizing a diet abundant in the SFA; liver fat increases ⁽¹⁵⁻¹⁸⁾. On the other hand, based on researches, the butyrate of butter could induce insulin sensitivity, and also the Conjugated linoleic acid (CLA) of that has beneficial impacts on metabolic illnesses ^(19, 20). Additionally that is evident excessive consumption of Trans fatty acids (TFAs) leads to notable hepatic steatosis, characterized by an increase in hepatic lipogenic gene expressions, heightened influx of free fatty acids into the liver, and the accumulation of lipid peroxide ⁽²¹⁾. The lipidomic properties involve the hepatic accumulation of TFAs and a reduction in arachidonic acid content. These lipid species, including TFAs, alongside their potential to induce

local cytokines by Kupffer cells, may play a crucial role in the commencement and development of non-alcoholic fatty liver disease ⁽²¹⁾.

Rapeseed oil, also known as Canola oil, has the lowest amount of SFA (7.1 percents of fatty acids content) and the highest concentration of n-3 fatty acid and MUFA (61% Oleic acid, 21% Linoleic acid, and 11% Linolenic acid) of all the oils that are most popular in the USA ⁽²²⁾. According to the myriad recommendations by public health organizations like the American Heart Association (AHA) and National Cholesterol Education Program (NCEP), which advised limiting the consumption of saturated and trans fatty acids (TFAs), one way to meet this recommendation is to lowering consumption of oils that are rich in SFA ⁽²³⁻²⁵⁾. Given the mentioned details, canola has the most tremendous potential to reduce SFA usage by substituting oils in the diet ⁽²⁵⁾. Considering the crucial influence of the gut microbiota in the development of fatty liver disease, altering the gut microbiota through nutritional supplements like probiotics or prebiotics, omega-3 polyunsaturated fatty acids (PUFA), or other functional foods as complementary therapies can potentially reverse metabolic disorders associated with NAFLD ^(26, 27). Consequently, reports indicate simultaneous positive effects of a plant oil rich in omega-3 and a prebiotic (like rapeseed oil that is rich in sinapine and omega-3) in individuals with NAFLD ⁽²⁸⁾. Furthermore, certain studies have indicated that the consumption of oils abundant in omega-3, such as *Camelia sativa* oil or rapeseed oil, may enhance glycemic control, alleviate inflammation, and reduce oxidative stress in individuals with NAFLD ⁽²⁹⁾. Numerous curative agents have been attempted for the management of NAFLD, in any case compelling treatment is still unavailable. Also, many physicians are attracted by using natural products to alleviate this very common liver disease, due to their safety, large availability and low-cost, as evident in a lot of literature data ⁽³⁰⁾. Although researchers recognize the significance of oils in the diet, there is inadequate testimony regarding the relationship between substituting ghee with rapeseed oil in the management of NAFLD. Starting from this background, the aim of this trial is to assess the impacts of substituting ghee with rapeseed oil on liver steatosis and enzymes, lipid profile, glycemic variables, and anthropometric measurements in patients with non-alcoholic fatty liver disease.

Material and Methods:

Recruitment and eligibility screening

This parallel randomized controlled trial was conducted with the objective of studying the impact of substituting ghee with rapeseed oil for a period of three months on the outcomes of NAFLD. The primary objectives of this study involved assessing liver function including both fatty liver and liver enzyme levels. Additionally, secondary objectives encompassed the evaluating of glycemic variables, lipid profile, and anthropometric measurements. The procedure of the survey was approved by the Ethics Committee at Urmia University of Medical Sciences and was registered at the Iranian Registry of Clinical Trials website (www.irct.ir) (IRCT20170206032417N5). The sample size was established based on the trial of Nigam et al. and the mean change of HOMA-IR (effect size= 1.3), and the $1-\alpha/2$, and $1-\beta$ were considered equal to 1.96 and 0.84, respectively (31). The equation used to estimate the sample size was as follows; $n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 \times (S_1^2 + S_2^2)}{(u_1 - u_2)^2}$. The timeframe was chosen based on earlier research that demonstrated the favorable impacts of the Dash diet on patients with NAFLD ⁽³²⁾. The study included 125 adult patients detected with non-alcoholic fatty liver disease referred to the gastrointestinal and liver clinic in Imam Khomeini University Hospital in Urmia, Iran, during the months of February and August 2022, in order of diagnosis were recruited in the trial. NAFLD was diagnosed by gastroenterology and liver specialist via ultrasonography showing fatty liver, without another type of liver disease. All NAFLD patients were invited to contribute in the study and were enrolled if they satisfied the eligibility criteria and consented to take part. Before entry into the study, each patient was requested to provide written informed consent. The usual treatment in these patients was using pioglitazone and vitamin E medications, which was kept constant throughout the study. At the beginning of the study, people were selected who were consuming 3 to 8 servings of ghee daily, and 5gr ghee considered as a serving. As well as the usual treatment, the control group's patients were instructed to keep up their ghee intake in the same amount. Also, in the intervention group, in parallel with the usual treatment, participants were recommended to change their consumed ghee to rapeseed oil in the same amount. To be included, participants had to be older than 18 years from both sexes, have first the visit to the hospital for NAFLD, consume 3 to 8 servings of ghee daily, and have a body mass index (BMI) under 30 kg/m² with steatosis grade of 2 or 3. Patients with being on a particular diet, viral

hepatitis, diabetes mellitus, psychiatric conditions, untreated hypothyroidism, kidney disease, heart disorders, bone disease, gastrointestinal illnesses (like celiac), alpha-1 antitrypsin deficiency, using alcoholic beverages, failure to adhere to our recommendations, taking herbal medicines, nonsteroidal anti-inflammatory drugs, cholesterol-lowering drugs, barbiturates, antiepileptic drug, pregnant, breastfeeding, and menopause women, and smokers were not included in the research. The research was conducted with a group of 110 participants, consisting of 70 men (35 participants were allocated to each group) and 40 women (20 participants were allocated to each group). The stratified block randomization was designed by an independent statistics specialist based on the steatosis grade, gender, and age. The intervention and control groups were formed through a random allocation process of the patients by a blinded person. The block randomization method was employed to randomize the participants, ensuring homogenized individuals allocate to each group. The laboratory personnel, radiologists, and statisticians were blinded to the group allocation until the completion of the research. For controlling the intake of other foods, participants of the pair of groups were asked to pursue the guidelines provided by the Food and Agriculture Organization (FAO) for Iranian⁽³³⁾. Initially, data related to gender, age, level of education, physical activity, calorie intake, drug and supplements type and dosage, plant-based medicine, income, past chronic medical condition, and familial history of NAFLD was obtained through a general questionnaire. In addition, anthropometric measurements and ultrasound imaging were conducted at the beginning and conclusion of the research. Participants were followed up by telephone weekly, and essential suggestions were made.

Biochemical measurements

At the start and end of the survey, after an overnight fast, patients got 5 mm of venous blood specimen drawn to execute biochemical assessments. Blood samples were subjected to centrifugation at a rate of 4000 revolutions per minute for a duration of 10 minutes. The resulting serum samples were then stored at a temperature of -80° C until biochemical examination. Enzyme-linked immunosorbent assay (ELISA) kits (Pars Azmoon Co, Tehran, Iran) were used to estimate serum fasting insulin levels. Analysis of the liver enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT)), lipid profile (total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL-c), and high-density lipoprotein (HDL-c)), and fasting blood glucose levels were conducted using BT1500 autoanalyzer (Biotechnica Instrument SpA, Rome, Italy). The

suggested formulas were utilized to compute Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and also to calculate Quantitative Insulin-Sensitivity Check Index (QUICKI) ^(34, 35). As mentioned earlier, we conducted measurements for liver enzymes, lipid profile, fasting blood glucose, and insulin levels. Additionally, HOMA-IR and QUICKI were calculated using the respective formulas.

Liver ultrasonography

At the beginning and end of the study, the assessment of fatty liver grade was performed by abdominal ultrasonography by a single operator and one expert radiologist (Siemens ACUSON S2000 Siemens Healthcare, Erlangen, Germany) with patients in a fasted state, before and end of the research project. NAFLD was identified based on the existence of a sonographic pattern in accordance with the subsequent criteria: including liver-kidney echo discrepancy, attenuated echo penetration, visibility of the diaphragm, and narrowing of the lumen of the hepatic veins, as observed on ultrasonography. Fatty liver was further classified into normal, grades 1, 2, and 3, following the modified criteria outlined by Kurtz et al ⁽³⁶⁾. Although this imaging technique is low-cost and well-accepted, nevertheless the case of diagnosing mild steatosis and steatosis in obese patients has low performance and sensitivity. As a result, patients with grade 1 NAFLD and those with a BMI higher than 30 were not included in this study.

Anthropometric measurements

The measurements of height and weight were taken through the utilizing of digital scale and stadiometer with a precision of 0.1 cm and 100 gr, respectively, at baseline and week 12 ⁽³⁷⁾. While measuring the participants were with negligible clothes and no footwear. The following formula was used to calculate BMI: kg/m^2 , in this equation, kg represents the individual's weight in kilograms and m^2 is the square of their height in meters. The measurement of waist circumference (WC) was made just after the patient breathed out, by placing a flexible tape between the hip bones and the lowest rib. It was ensured that the tape was horizontal around the waist and did not compress the skin. The waist-height ratio is determined by dividing the waist measurement by the height measurement, both expressed in centimeters. For reliability, the measurements were conducted triplicate, and the mean of the three readings was employed. As outlined previously, we obtained measurements for waist circumference (WC) and height. Additionally, we calculated the Body Mass Index (BMI) and waist-height ratio using the appropriate formula.

Dietary intake and physical activity assessment

To evaluating the consumption of rapeseed oil and ghee and the usage of other food groups, including cereal, dairy, vegetables, fruit, grains, meat, and sugar, before intervention and each month, four 3-day 24-hour recalls (one weekend day and two non-consecutive week day) totally in 12 days were performed. The metabolic equivalent of task (MET) questionnaire was used to evaluate the physical activities before intervention and each month ⁽³⁸⁾.

Primary and secondary outcomes

The principal purposes of our investigation were to evaluate the liver function including the levels of liver enzymes in the blood and the degree of liver steatosis as primary outcomes. Additionally, we examined the serum concentration of lipid profile, glycemic variables, and anthropometric measurements as secondary outcomes.

Statistical analysis

The statistical analysis was conducted using SPSS software version 26 (IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY), and the threshold for accepting the statistical significance of the results was established as $p\text{-value} < 0.05$. The homogeneity of individuals before and after the study remained relatively unchanged, and as a result, we conducted the analysis based on the by protocol analysis. General characteristics between control and intervention groups before intervention were compared by independent sample T-test for quantitative and chi-square for qualitative variables and were reported as mean \pm SD and frequency (%), respectively. To compare the differences within groups, the paired samples T-tests were applied. Using the Kolmogorov–Smirnov test, we assessed the normality of the continuous values. To analyze the changes in dietary intakes and MET at baseline, 1st, 2nd, and 3rd months, repeated measures of ANOVA was used. To evaluating the effects of replacing ghee with rapeseed oil on serum levels of lipid profile, liver enzymes, glycemic index, and anthropometric measurements, we applied ANCOVA test by adjusting weight changes and baseline value of the outcome. Ordinal Generalized Linear Models were employed to evaluate changes in severity of fatty liver (without change, aggravation, or improvement) within the study population over the course of study.

Results

A random assignment process was used to divide 125 individuals into 2 groups. Following randomization, 15 participants were removed from the study because of not meeting inclusion criteria ($n=5$) and lost to follow-up ($n=10$). In total, the study was completed with 110 patients: the intervention group ($n=55$) and the control group ($n=55$) (Figure 1). The age range of the patients was between 18 and 67 years. Regarding basic characteristics, no statistically significant variations were noted between the groups (Table 1). According to the 3-d 24-h dietary recalls obtained during the intervention, no significant differences were seen between the groups in terms of energy intake and food groups (Figure 2). Using the MET questionnaire, the results did not indicate any significant differences between control and intervention groups (Figure 2). Furthermore, (Figure 3) illustrates the composition of oils consumed by patients both before and during the study. Regarding the figure 3, Ghee and Rapeseed consumption between the control and intervention group was significant ($P_{\text{group}} = <0.001$, $P_{\text{time}} = <0.001$, $P_{\text{group.time}} = <0.001$). About the other oils consumption there was no significant difference between groups, before and during the study ($P_{\text{group}} = 0.075$, $P_{\text{time}} = 0.231$, $P_{\text{group.time}} = 0.652$).

Primary outcomes

The study concluded with a significant reduction in the serum levels of ALT ($P=0.014$) and GGT ($P=0.024$) in contrast the control group. Adjustments for the impacts of the baseline value of the outcome and mean weight change did not affect the results. In regard to AST, there was a significant reduction in the intervention group in comparison to the control group only subsequent to adjusting for the baseline value of the outcome ($P<0.001$). Regarding ALP, there was a significant decrease in the control in comparison to the intervention group (0.006) (Table 2). The intervention group demonstrated a significant reduction in grades of fatty liver in comparison to the control group ($P <0.001$). The intervention group showed a reduction in the grade of steatosis in 41.81% of patients, significantly ($P<0.001$) (Table 4). Despite adjusting for the baseline value of the outcome and mean change in weight, statistically significant differences between the intervention and control group were still observed ($P=0.03$) (Table 4).

Secondary outcomes

In regard to the impacts of the intervention on lipid profile, the intervention group compared to the control group had lower concentrations of TG ($P < 0.001$), TC ($P < 0.001$), and LDL ($P = 0.001$), even after adjusting for the initial value of the outcome and mean change in weight (Table 2). In the matter of serum concentration of HDL, the results showed a significant reduction in intervention group at the end of the trial in male patients (0.04) but not in female individuals (0.085). Also, in male patients there was a significant reduction in HDL levels after adjustment for baseline value of the outcome (0.005) (Table 3).

On the subject of glycemic variables, the intervention in contrast to the control group showed a lower serum level of FBS ($P < 0.001$), IN ($P < 0.001$), HOMA-IR ($P < 0.001$) and higher level of QUICKI ($P < 0.001$). Adjustments for the effects of baseline value of the outcome and mean change in weight did not change the results (Table 2).

In anthropometric measurements, the intervention group by comparison with the control group showed a significant decrease in weight ($P < 0.001$), BMI ($P < 0.001$), and waist-to-height ratio ($P < 0.001$). These significant differences were still observed even after adjusting for the baseline values (Table 2). About the WC, there was a significant reduction in WC in male and female patients even after adjustment for baseline value of the outcome (< 0.001) (Table 3).

Discussion

To date, no prior investigation has studied the potential impact of substituting ghee with rapeseed oil on clinical parameters of individuals diagnosed with NAFLD. Our study demonstrated that substituting ghee with rapeseed oil through 12 wk resulted in significant improvement in the severity of steatosis, some of liver enzyme levels, lipid profile, glycemic variables, and anthropometric measurements. Significant variations in liver steatosis were seen among the groups, even subsequent to adjustments made with covariates. These findings showed that rapeseed oil, by itself and independent to any associated weight loss and baseline value of outcome, contributed to the significant improvements in the outcomes as mentioned earlier. Currently, there is insufficient information available regarding the impact of rapeseed oil on hepatic steatosis in individuals with non-alcoholic fatty liver disease.

In a randomized, parallel, open-label design study by Nigam et al.⁽³¹⁾, it was found that canola oil improved hepatic steatosis significantly. Results from a study by Li et al.⁽³⁹⁾ strongly confirms the fact that consuming of sinapine, as a prebiotic agent of rapeseed oil, could prevent

insulin resistance and non-alcoholic fatty liver disease. In a separate study conducted by Li et al., consistent with our findings, the results indicated that sinapine could serve as a prebiotic, enhancing the nutritional properties of vegetable oils and potentially preventing obesity-related chronic diseases, including non-alcoholic fatty liver disease ⁽⁴⁰⁾. As previously noted, rapeseed oil is primarily comprising monosaturated fatty acids (MUFA), with reduced amounts of saturated fatty acids. Additionally, it contains higher amounts of polyunsaturated fatty acids (PUFA), which comprise alpha-linolenic acid (ALA) and linoleic acid (LA). This efficacy can at least be mainly attributed to ALA regulation of molecular mechanisms involved in the metabolism of lipids in the liver. Specifically, this can be attributed to the effects of the ALA on increasing the DNA-binding of PPAR α and reducing the DNA-binding of SREBP-1c, which are transcription factors that play a role in lipid oxidation and synthesis, respectively ^(41, 42). However, sinapine is a crucial factor in preventing the initiation of obesity and inflammation induced by a diet. This is achieved by modulation the composition of the intestinal microflora (decrease in the ratio of Firmicutes to Bacteroidetes and augmented presence of probiotics, such as Lactobacillaceae, Akkermansiaceae, and Blautia), which produces short-chain fatty acids (SCFAs), and ultimately inhibits NAFLD. Additionally, the SCFA/GPR43 (G protein-coupled receptor 43) pathway seems crucial for inhibiting inflammation and non-alcoholic fatty liver disease by gut microbes ⁽³⁹⁾. Additionally, recent research has indicated that omega-3 supplementation may lead to a reduction in serum levels of inflammatory markers such as TNF- α , IL-6, and CRP. This, in turn, has the potential to ameliorate conditions associated with chronic inflammation, including non-alcoholic fatty liver disease (NAFLD) ⁽⁴³⁾. Moreover, the results of the current research indicated that the intake of rapeseed oil improved liver enzymes such as ALT, AST, and GGT and increased ALP levels. In a report from Capanni et al. ⁽⁴⁴⁾, supplementation with n-3 PUFA (eicosapentaenoic acid and docosahexaenoic acid in the ratio of 0.9/1.5, respectively) was inversely related to AST, ALT, GGT, TG, and FBS. Consistent with our trial, contemporary studies have demonstrated that the intervention with omega-3 polyunsaturated fatty acids (PUFAs) results in the improvement of liver enzymes and reduction in liver fat ⁽⁴⁵⁾. In line with the current trial, Mahmudul Hasan et al. ⁽⁴⁶⁾ revealed that rapeseed oil could significantly increase the ALP levels. Similarly, an increase in ALP was found after supplementation with rapeseed oil in Wistar rats ⁽⁴⁷⁾. An elevation in the amount of ALP is indicative of liver cell damage, intrahepatic cholestasis, or infiltrative liver disease. It is assumed

that an increase in the mentioned enzyme may be due to the presence of erucic acid in rapeseed oil; nonetheless, further investigations are needed.

In the current study, we observed a reduction in fasting blood sugar and insulin concentration following consumption of rapeseed oil. A survey of the health benefits of MUFAs by Gillingham et al. ⁽⁴⁸⁾ disclosed that MUFAs could modulate insulin sensitivity and glycemic variables when substituted for SFA. The study accomplished by Södergren et al. ⁽⁴⁹⁾ showed that a diet based on canola oil yielded reduced levels of fasting plasma glucose as contrasted with a saturated fatty acid-rich diet. However, the levels of fasting insulin were not significantly different between the two diets. According to the findings of Gustafsson et al. ⁽⁵⁰⁾, a diet based on canola oil led to a reduction of fasting blood glucose levels by 6% when compared to diet containing more than 15% saturated fatty acids (SFA). However, previous studies have shown that replacing saturated fats with MUFA and PUFA could decrease blood lipid levels and had positive effects on glucose and insulin homeostasis ^(51, 52). The underlying mechanism for this phenomenon may involve the amelioration of postprandial triglycerides and glucagon-like peptide-1 responses in individuals with insulin resistance, as well as the up-regulation of glucose transporter-2 expression in the liver. As referred to earlier, a study has shown that the intake of MUFAs could lower blood triglyceride levels through two mechanisms; activation in PPAR α and reduction in SREBP. MUFAs can activate both PPAR α and PPAR γ , leading to an elevation in lipid oxidation and a reduction in insulin resistance, which can ultimately reduce the occurrence of hepatic steatosis ⁽⁵³⁾. An additional mechanism may be associated with the upregulation of GRP43 induced by sinapine, which leads to the production of short-chain fatty acids (SCFAs) that can inhibit inflammation in the intestines. These actions serve to prevent insulin resistance in adipose tissue. In the present clinical trial, the intake of rapeseed oil caused a significant reduction in lipid profile. Conversely, the control group displayed an increase in lipid profiles upon consumption of ghee. In line with present study, Amiri et al. ⁽⁵⁴⁾ conducted a meta-analysis demonstrated that canola oil intake was associated with improvements in total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-c), as well as a decrease in high-density lipoprotein cholesterol (HDL-c) levels. Earlier, Engel et al. ⁽⁵⁵⁾ observed that consuming of butter fat/ghee, when compared to the impacts of olive oil intake and a habitual diet, resulted in increased levels of TC, LDL-c, and HDL-c. The underlying mechanisms responsible for canola oil's ability to lower cholesterol levels, may be attributed to its high content of monounsaturated

fatty acids (MUFA), phytosterols, and stanols. Canola oil's phytosterols and stanols, have been found to reduce concentrations of LDL-c⁽⁵⁶⁾. These non-lipid constituents impede cholesterol metabolism by their structural resemblance to cholesterol, thereby curtailing cholesterol absorption in the intestine and inhibiting cholesterol esterase enzymes⁽⁵⁷⁾. Moreover, MUFA has been shown to enhance insulin and lipoprotein lipase activity, ultimately leading to decreased levels of triglycerides (TG)⁽⁵⁷⁾.

Our study revealed that the intervention group exhibited significant improvements in anthropometric measurements. Based on the findings of Dehkordi et al.'s⁽⁵⁸⁾ meta-analysis, it has been demonstrated that the intake of canola oil leads to a significant loss of weight. Furthermore, subgroup analysis disclosed that canola oil intake led to a decrease in waist circumference compared to a typical diet. Previous research has established that the storage and oxidation properties of fatty acids play a pivotal role in the controlling body weight⁽⁵⁸⁾. N-3 fatty acids are efficacious in treating obesity and have the capacity to regulate the proliferation, differentiation, and apoptosis of adipocytes⁽⁵⁹⁾. Moreover, it is suggested that PUFA may contribute to weight loss by modulating the gene expression that promotes oxidation in adipose tissue, liver, and other organs, leading to lower fat storage⁽⁶⁰⁾. Additionally, canola oil has been shown to enhance the sense of satiety and decrease hunger by stimulating the secretion of cholecystokinin, which has a satiating effect on the ileum⁽⁶¹⁾. The passage highlights the nutritional qualities of rapeseed oil, emphasizing its significance as a source of omega-3 fatty acids with a favorable omega-6 to omega-3 ratio (2:1). Additionally, it underscores the substantial polyphenol content in rapeseed oil, particularly sinapine and sinapic acid, known for their diverse physiological functions such as antioxidative, anti-tumor, and hypoglycemic properties. The suggestion is that these polyphenols, especially sinapine and sinapic acid, might contribute to improving glucose and lipid metabolism disorders as well as insulin resistance in individuals with non-alcoholic fatty liver disease (NAFLD). Furthermore, the anti-inflammatory attributes of rapeseed polyphenols are proposed to be linked to short-chain fatty acids (SCFAs) through the regulation of intestinal flora⁽³⁹⁾. In consistent with our results, Musazadeh et al. in a study indicated that adding *Camellia sativa* oil, which is abundant in omega-3 like rapeseed oil, may lead to reductions in anthropometric measurements such as weight, BMI, waist-to-hip ratio, and waist circumference, as well as ALT levels and lipid profile levels (excluding HDL levels). Additionally, in line with our results, Farhangi et al. in a trial showed that, the combination of

Camellia sativa oil with resistant dextrin and a calorie-restricted diet resulted in decreased weight, BMI, liver enzymes, and lipid profile among patients with non-alcoholic fatty liver disease^(62, 63).

The study's main advantage is that using rapeseed/canola oil as a substitute for ghee or other oils high in saturated fatty acids is an affordable approach to managing NAFLD. A limitation of the trial is that the study's diagnostic method for fatty liver grade relied on liver ultrasonography, which is low-cost, noninvasive, and widely available. Second, the study could not be blinded due to its design, and the intervention duration was short.

In conclusion, this randomized controlled trial demonstrated that replacing ghee with rapeseed oil improved NAFLD symptoms and could potentially benefit metabolic disorders. However, additional clinical trials with increased sample sizes and extended intervention periods are required. These trials would provide more accurate and reliable proof to endorse the consumption of rapeseed oil for improving health outcomes.

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Conflict of interest

There are no conflicts of interest to declare.

Author contributions

The authors' responsibilities were as follows: M. A. and F. M. S. conceived and designed the study and analysed the data; M. M. H provided material and technical support; F. M. S. wrote the manuscript; M. A. critically revised the manuscript for important intellectual content and M. A. had primary responsibility. All authors read and approved the final manuscript.

Abbreviations:

American heart association (AHA)
Alpha-linolenic acid (ALA)
Alkaline phosphatase (ALP)
Alanine amino transferase (ALT)
Aspartate amino transferase (AST)
Body mass index (BMI)
Conjugated linoleic acid (CLA)
Dietary Approaches to Stop Hypertension (DASH)
Enzyme-linked immunosorbent assay (ELISA)
Food and Agriculture Organization (FAO)
Fasting blood glucose (FBS)
gamma-glutamyl transferase (GGT)
G protein-coupled receptor 43 (GRP43)
High-density lipoprotein (HDL)
Homoeostasis model of assessment-estimated insulin resistance (HOMA-IR)
Insulin (IN)
Insulin resistance (IR)
linoleic acid (LA)
Low-density lipoprotein (LDL)
Metabolic equivalent of task (MET)
Metabolic syndrome (MS)
Mono- unsaturated fatty acids (MUFA)
Non-alcoholic fatty liver disease (NAFLD)
National Cholesterol Education Program (NCEP)
Peroxisome proliferator-activated receptors (PPAR)
Poly- unsaturated fatty acids (PUFA)
Quantitative Insulin-Sensitivity Check Index (QUICKI)
Short chain fatty acids (SCFA)
Saturated fatty acids (SFA)
Sterol regulatory element-binding protein 1 (SREBP-1c)
Total cholesterol (TC)
Trans fatty acids (TFAs)
Triglyceride (TG)
Waist circumference (WC)

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TABLE 1. Basic characteristics of individuals with non-alcoholic fatty liver disease

Variable	Intervention group (n=55)	Control group (n=55)	P value*
Age (years)	41.35 ± 9	43 ± 10.1	0.376
Education (years)	13.27 ± 2.8	12.31 ± 2.9	0.078
Monthly incomes (Million Tomans)	8.27 ± 3.8	7.52 ± 4	0.313
Gender			
Male	35 (63.6)	35 (63.6)	1.000
Female	20 (36.4)	20 (36.4)	
Liver steatosis			
Grade 2	48 (87.3)	46 (86.6)	0.589
Grade 3	7 (12.7)	9 (13.4)	
Underlying disease			

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Without	any	50 (90.9)	48 (87.27)	0.539
underlying disease				
Hypertension		1 (1.81)	3 (5.45)	
Hypothyroidism		1 (1.81)	1 (1.81)	
Gout		1 (1.81)	0(0)	
H.pylori		0(0)	2 (3.63)	
Rheumatoid arthritis		1 (1.81)	0 (0)	
Family history				
Yes		12 (21.81)	6 (10.9)	0.197
No		43 (78.18)	49 (89.09)	

Values are means \pm SDs for continuous variables and frequency (%) for categorical variables. *P values were calculated by independent sample t-test for continuous and chi-square for categorical variables.

TABLE 2. Changes in liver enzymes, lipid profiles, glycemic variables, and anthropometric measurements during the 12-week study in patients with NAFLD in the groups (n=55)

Variable	Intervention group (Mean±SD)	Control group (Mean±SD)	P ^a	P ^b	P ^c
ALT (IU/l)					
Baseline	42.7 ± 31.9	42.1 ± 22.9	0.905		
Week 12	28.3 ± 14.3	37.9 ± 19.3			
Change	-14.4 ± 25	-4.2 ± 17.2	0.014	< 0.001	0.051
P ^f	< 0.001	0.075			
AST (IU/l)					
Baseline	27.5 ± 12.1	30.8 ± 15.8	0.221		
Week 12	20.1 ± 6.2	26.7 ± 11			
Change	-7.4 ± 9.9	-4.13 ± 10.8	0.097	< 0.001	0.119
P ^f	< 0.001	0.007			
GGT (IU/l)					
Baseline	30 ± 15.7	26.6 ± 13.3	0.214		
Week 12	28.2 ± 16.6	27.8 ± 11.6			
Change	-1.8 ± 8.3	1.2 ± 5.2	0.024	< 0.001	0.6
P ^f	0.108	0.097			
ALP (IU/l)					
Baseline	167.4 ± 42.5	171.9 ± 61.7	0.658		
Week 12	173.6 ± 44.1	161.7 ± 58.1			
Change	6.2 ± 27.9	-10.21 ± 33.7	0.006	< 0.001	0.004
P ^f	0.105	0.029			
TG (mg/dl) ^e					
Baseline	178.4 ± 90.7	185.4 ± 78.5	0.667		
Week 12	138.7 ± 59.6	196.1 ± 71.5			
Change	-39.7 ± 60.9	14.6 ± 46.3	< 0.001	< 0.001	< 0.001
P ^f	< 0.001	0.024			

TC (mg/dl) ^e					
Baseline	184.4 ± 50	191.5 ± 43.7	< 0.001		
Week 12	167.2 ± 40.8	195.4 ± 41.3			
Change	- 17.2 ± 33.5	3.9 ± 25.6	< 0.001	< 0.001	0.006
P ^f	< 0.001	0.264			
LDL (mg/dl)					
Baseline	106.6 ± 36.5	109.8 ± 30.6	0.615		
Week 12	99.7 ± 28.4	116.3 ± 28.9			
Change	-7.5 ± 20	6.25 ± 21.9	0.001	< 0.001	0.012
Pf	0.008	0.032			
FBS (mg/dl) ^e					
Baseline	96.7 ± 9.6	96.7 ± 11.6	0.996		
Week 12	89.2 ± 9.3	99.5 ± 13.6			
Change	-7.5 ± 7.7	2.8 ± 7.5	< 0.001	< 0.001	<0.001
P ^f	< 0.001	0.008			
Insulin (μU/l)					
Baseline	13.2 ± 6.8	12.6 ± 4.9	0.628		
Week 12	10.1 ± 5.3	17.5 ± 5.7			
Change	-3.05 ± 7.1	4.9 ± 4.1	< 0.001	<0.001	< 0.001
P ^f	0.002	< 0.001			
HOMA-IR					
Baseline	3.2 ± 1.7	3 ± 1.3	0.622		
Week 12	2.3 ± 1.4	4.4 ± 1.7			
Change	- 0.9 ± 1.9	1.3 ± 1.2	< 0.001	< 0.001	< 0.001
P ^f	0.001	< 0.001			
QUICKI					
Baseline	0.3 ± 0.03	0.3 ± 0.04	0.645		
Week 12	0.3 ± 0.02	0.3 ± 0.02			

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Change	0.01 ± 0.03	-0.02 ± 0.03	< 0.001	< 0.001	< 0.001
P ^f	0.001	< 0.001			
Weight (kg)					
Baseline	81.1 ± 8.5	81.7 ± 7.6	0.674		
Week 12	76.8 ± 9.1	81.7 ± 7.2			
Change	-4.3 ± 3.4	0.004 ± 3.1	< 0.001	< 0.001	---
P ^f	< 0.001	0.993			
BMI (kg/m ²)					
Baseline	28.1 ± 1.7	28.23 ± 1.5	0.645		
Week 12	26.6 ± 1.8	28.3 ± 1.6			
Change	-0.04 ± 0.04	- 0.003 ± 0.03	< 0.001	< 0.001	---
P ^f	< 0.001	0.469			
WHtR					
Baseline	0.61 ± 0.07	0.63 ± 0.05	0.101		
Week 12	0.65 ± 0.05	0.63 ± 0.04			
Change	-0.04 ± 0.09	-0.003 ± 0.03	< 0.001	< 0.001	---
P ^f	< 0.001	0.434			

^a Calculated using independent sample T-test. ^b Calculated using ANCOVA, adjusted for baseline value of the outcome. ^c Calculated using ANCOVA, adjusted for baseline value of outcome and mean change in weight. ^f Calculated using paired samples T-test.

^e To change the measurement of TC in mg/dl to mmol/l, multiply the value by 0.0259. To change TG in mg/dl to mmol/l, multiply the value by 0.0113. To change FBS in mg/dl to mmol/l, multiply the value by 0.0555. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gama-glutamyltransferase; TG, triglyceride; FBS, fasting blood sugar; HOMA-IR, homoeostasis model of assessment-estimated insulin resistance; QUICKI, quantitative insulin sensitivity check index; BMI, body mass index; WHtR, waist to height ratio.

TABLE 3. Changes in HDL and WC during the 12-week study in patients (male and female) with NAFLD in the groups (n=55)

Variable		Intervention			Control		P ^a	P ^a	P ^b	P ^c	P ^f	P ^{f6}
HDL-c (mg/dl)	Baseline	Week12	Change	Baseline	Week12	Change	Baseline	Change	Change	Change	Intervention	Control
Male	41±7.3	38.8±8.3	-2.1±6.6	40.9±7.5	42.7±9.5	1.9±9.5	0.9	0.04	0.005	0.07	0.063	0.257
Female	43.7±6.4	42.6±10.4	-1.1±7.9	40.7±8.1	44±7.8	3.26±7.6	0.2	0.085	0.1	0.3	0.555	0.69
Total	42±7.1	40.2±9.2	-1.74±7	40.8±7.6	43.2±8.9	2.36±8.8	0.409	0.008	<0.001	0.03	0.071	0.051
WC (cm)												
Male	105.2±8.7	100.1±9.1	-5.1±5	108.2±6.6	108.1±6.3	-0.1±4	0.1	<0.001	<0.001	----	<0.001	0.866
Female	100.3±11.8	93.8±9.9	-6.5±3.5	105.1±8.7	104.6±8.8	-0.5±3.8	0.2	<0.001	<0.001	----	<0.001	0.559
Total	103.4±10.1	97.8±9.8	-5.6±4.6	107.1±7.5	106.8±7.4	-0.25±3.9	0.034	<0.001	<0.001	----	<0.001	0.627

^a The difference between groups calculated using independent sample T-test for. ^b The mean change difference between groups calculated using ANCOVA, adjusted for baseline value of the outcome. ^c The mean change difference between groups calculated using ANCOVA, adjusted for baseline value of outcome and mean change in weight. ^f Within group changes calculated using paired samples T-test.

WC, waist circumference.

TABLE 4. Comparison of liver steatosis grades assessed by ultrasound before and after the intervention in patients with non-alcoholic fatty liver disease in both the intervention and control groups for 12 wk.¹ (n= 55)

Group	Grade of fatty liver	Baseline n (%)	week 12 n (%)	P ₁	P ₂	P ₃	P ₄	Change ² n (%)
Intervention				< 0.001	< 0.001	0.019	< 0.001	Reduction in grade: 46 (41.81) Without change: 9 (8.18) Deterioration: 0 (0)
	Normal	0 (0)	8 (14.54)					
	Grade 1	0 (0)	31 (56.36)					
	Grade 2	48 (87.3)	16 (9.1)					
	Grade 3	7 (12.7)	0 (0)					
Control								Reduction in grade: 15 (13.63) Without change: 38 (36.54) Deterioration: 2 (1.81)
	Normal	0 (0)	0 (0)					
	Grade 1	0 (0)	10 (18)					
	Grade 2	46 (86.6)	39 (70.9)					
	Grade 3	9 (13.4)	6 (10.9)					

¹There were no significant variations between intervention and control group based on chi-square test, with regard to baseline grades of liver steatosis (p= 0.589). P₁ was calculated by chi-square test to compare fatty liver grade. P₂ was calculated by Generalized Linear Models test after adjusting for baseline value of outcome. P₃ was calculated by Generalized Linear Models test after more adjusting for mean change in weight. P₄ was calculated by Generalized Linear Models test after more adjusting for mean change in weight and baseline value of outcome. ²Based on the chi-square test significant differences were observed between groups with regard to changes in grades of fatty liver steatosis. ²On the basis of Generalized Linear Models test there were no significant differences between groups with regard to changes in grades of fatty liver steatosis after adjusting for baseline value of the outcome (P=0.057), adjusting for mean change in weight (P=0.144), but there were significant differences after adjusting for baseline value of the outcome and mean change in weight (P=0.03).

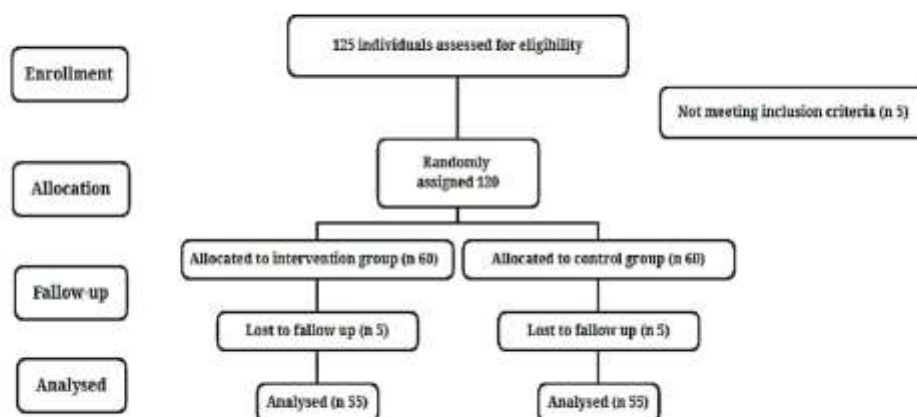


Figure 1. The flowchart of study participants based on the CONSORT guidelines.

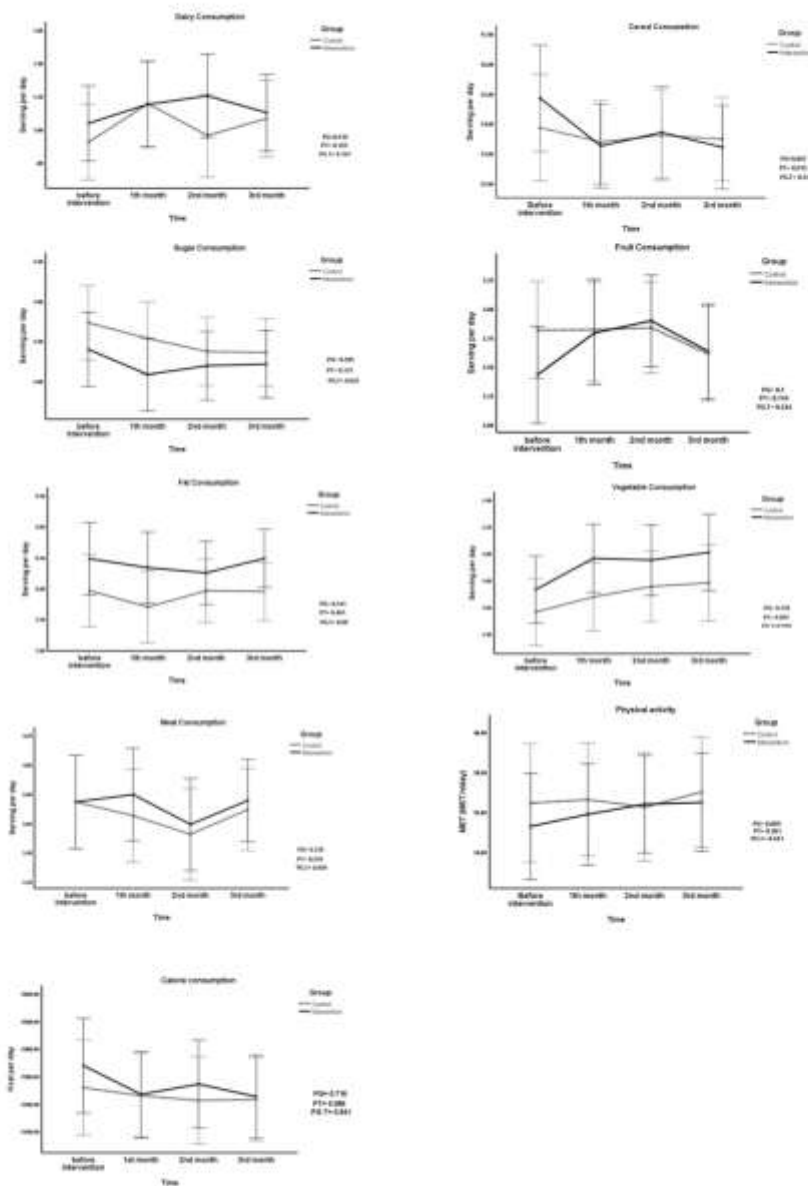


Figure 2. Changes in dietary intake and physical activity of the individuals during the 12 wk. The P values demonstrate the effect of group, time, and time \times group interaction (computed through the general linear model ANOVA for repeated measurements) MET, metabolic equivalent of task.

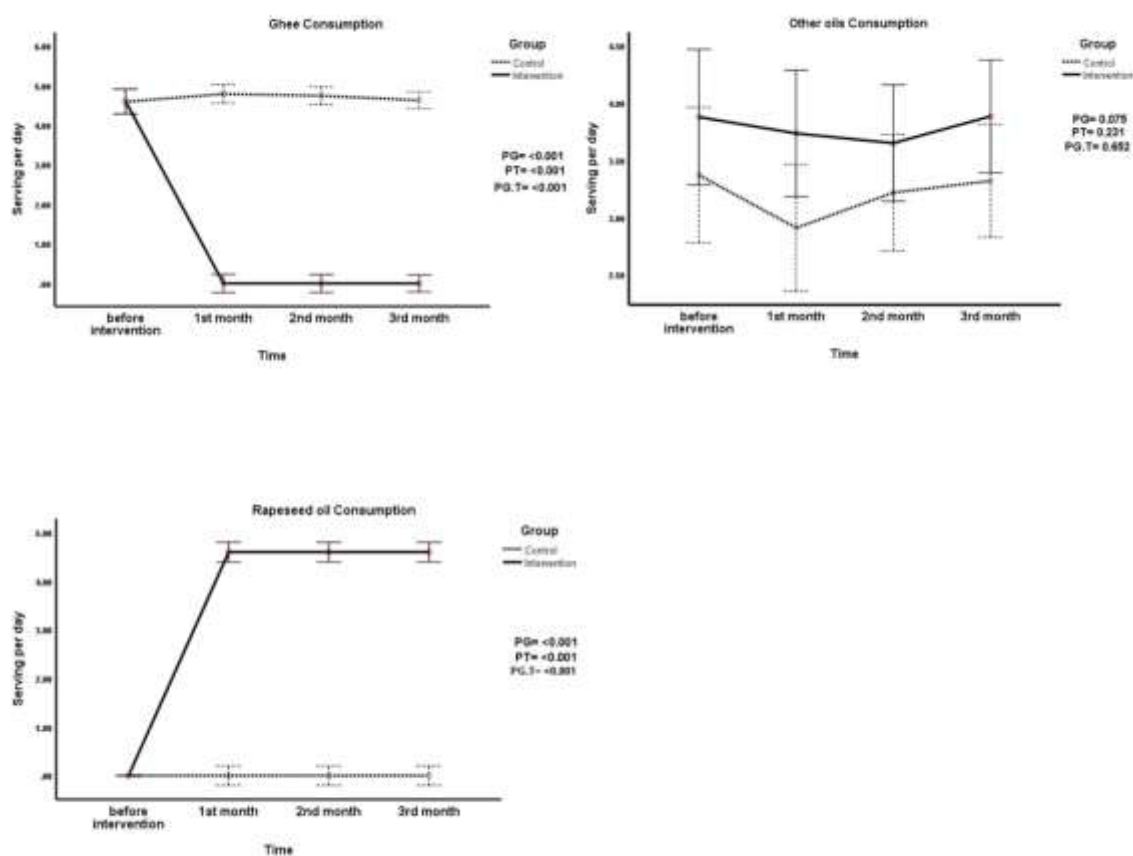


Figure 3. Changes in the content of consumed oil by the individuals during the 12 wk. The P values demonstrate the effect of group, time, and time \times group interaction (computed through the general linear model ANOVA for repeated measurements)