Comparison of effects of soft margarine, blended, ghee, and unhydrogenated oil with hydrogenated oil on serum lipids: A randomized clinical trail

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Abstract

BACKGROUND: Trans fatty acids (TFAs) are known as the most harmful type of dietary fats. Therefore, this study was done to compare the effects of some different oils including unhydrogenated, blended, ghee, and soft margarine with hydrogenated oil on serum lipid profile of healthy adults.

METHODS: This study was a randomized clinical trial conducted on 206 healthy participants of 20 to 60 years of age. Subjects were randomly divided into 5 groups and each of them was treated with a diet containing unhydrogenated oil, ghee, blended oil, soft margarine, or hydrogenated oil for 40 days. Fasting serum lipids were measured before and after the study.

RESULTS: Compared to hydrogenated oil, total cholesterol (TC) and triglyceride (TG) had a significant reduction in all groups, LDL-C declined in unhydrogenated oil and soft margarine groups, and apolipoprotein (Apo) B only in unhydrogenated oil group (all P < 0.05). However, there was a significant enhancement in ApoA of ghee oil (P < 0.001).

CONCLUSION: Consuming unhydrogenated oil, ghee, soft margarine, and blended oil had some beneficial effects on serum lipids.

Keywords: Clinical Trial, Dietary Fat, Commercial Oil, Lipid
from animal fat and contains high amounts of SFAs and cholesterol, a few studies reported it was useful for decreasing LDL-C and increasing HDL-C.\textsuperscript{14-18} In an animal study which was conducted in Iran, it has been observed that ghee oil consumption significantly increased HDL-C level, but did not have any significant effect on other serum lipids.\textsuperscript{17} However, there is no precise scientific information on this issue in human subjects. Thus, there are some controversies about how ghee consumption and serum lipid profile are linked.\textsuperscript{18,19} As TFAs are considered as the most harmful dietary fats, this study was conducted aiming to compare the effects of soft margarine, unhydrogenated, blended, and ghee oils with hydrogenated oil as a main source of TFA on serum lipids of Iranian adults.

**Materials and Methods**

**Study design and sampling**

This randomized clinical trial has been conducted on 249 healthy subjects aged 20-60 years in 2009. They were chosen from the Emam-Zaman Beneficiary Organization and consumed only hydrogenated oil in their diet. Normotensive, non-diabetic participants without cardiovascular diseases were invited to the study center. According to the sample size which was calculated 40 in each group and considering the dropout rate, at the beginning we invited 265 volunteers. After overnight fasting, venous blood samples were drawn at 7:00 to 10:00 am. Subjects with TC ≥ 240 mg/dl, triglyceride (TG) ≥ 400 mg/dl, LDL-C ≥ 160 mg/dl, or HDL-C ≤ 40 mg/dl, who also had body mass index (BMI) ≥ 35 were excluded. However, 16 subjects were excluded because of not meeting the inclusion criteria. Then, the remaining participants were divided into five groups of soft margarine, hydrogenated, unhydrogenated, ghee, and blended oils by simple randomization. Moreover, 43 participants were excluded from the study due to traveling, sickness, unwillingness to participate in the next sampling, or not complying with dietary recommendations. Therefore, 206 healthy subjects were included in the study. The flow chart of the study is presented in figure 1.

**Figure 1.** Flow chart showing number of eligible and excluded participants, number of participants allocated to intervention and control groups, and number of participants lost to post-text, as well as reasons for loss to follow-up
After signing informed written consents, the subjects were referred to the trained nutritionist to obtain socio-demographic characteristics, past medical history, and food habits by 24-hour recall questionnaire. Anthropometrical measurements were taken with shoes removed and the participants wearing light clothing. BMI was calculated by dividing the weight in kilograms to the square of height in meters. Eligible subjects who consumed hydrogenated oil, based on obtained food habits, were randomly divided into 5 groups. They took a diet consultation with the same amount of oil containing soft margarine, cooking and frying unhydrogenated oil, ghee, and blended or hydrogenated oils for 40 days. In order to keep the type of oils similar among each group, oils were given to the subjects (every 10 days) by the project conductor. Ghee was provided from Bakhtiari nomads. Soft margarine and blended oil were provided from companies which were their only producers in Iran at that time. The commonest brands of unhydrogenated and hydrogenated oils were bought from the supermarket. Dietary recommendations were given to the subjects by the same dietitian, so that the only difference among the 5 groups was the kind of dietary oil. Oils were provided for all family members, about 20-30 gr per person, even if one of them was chosen in the study. Subjects were followed by phone every two weeks or during their referral to the study center for taking their oil.

**Biochemical measurements**

Blood samples were taken after the subject had been fasting for 14 hours. Serum lipid levels, including TC, TG, and HDL-C levels, were measured. TC and TG were determined by standard enzymatic method using special kits in Hitachi 902 autoanalyzer and using special kits (Diasys Diagnosis Inc., Holzheim, Germany) performed by Pars-Azmun (Tehran, Iran). HDL-C was measured enzymatically after precipitating the other lipoproteins with dextran sulphate magnesium chloride. LDL-C was calculated by using the Friedewald formula. ApoA1 and ApoB100 were assayed by immunoturbidimetric methods (Diasys Diagnosis Inc., Holzheim, Germany) performed by Pars-Azmun. Direct measurement of LDL-C was performed with a turbidimetric method for those with TG $\geq$ 400 mg/dl. Apolipoproteins A and B levels were determined by Merk kits. Blood samples were collected before and after the study, at Isfahan Cardiovascular Research Center laboratory, a WHO-collaborating center which meets the criteria of the National Reference Laboratory. The lipid profile changes were the primary endpoint of the study.

**Ethics**

This study was approved by the Research Council of Isfahan Cardiovascular Research Center and registered in the Iranian Randomized Clinical Trial Center by ID number of IRCT138905124497N1.

**Statistical analysis**

In the beginning of the study, the mean of age, BMI and serum lipids levels among the three groups were compared by one-way analysis of variance (ANOVA). Comparison of the frequency distribution was conducted using chi-square test based on gender and education level. Mean of serum lipid levels before and after the study were compared by paired t-test in each group. The comparison of changes in serum lipids and ApoA and B levels between 5 groups was done with two-way ANOVA test by adjusting for age, gender, and education level. The post-study mean serum lipid was compared with analysis of co-variances test by adjusting with age, gender, education, and before study serum lipids. P value less than 0.05 was considered significant.

**Results**

The study was performed on 206 subjects including 41, 43, 39, 42, and 41 subjects in hydrogenated, unhydrogenated, ghee, blended oil, and soft margarine groups, respectively. However, 43 subjects were excluded from the study due to traveling, sickness, unwillingness to participate in the next sampling, or not complying with dietary recommendations. Therefore, 206 healthy subjects were included in the study. Thus, the participation rate was about 82.7%. They included 88 men and 118 women with the mean age of 34.8 $\pm$ 11.4 years. As shown in table 1, there is no significant difference in mean of age, serum lipids, including TC, TG, LDL-C, HDL-C, ApoA, and ApoB levels, and also gender, educational, and marital status distribution between 5 groups in the beginning of the study. Table 2 demonstrates the comparison between mean of serum lipids and ApoA and ApoB levels before and after the study in each group.

In the hydrogenated oil group, TC increased and ApoA decreased significantly ($P = 0.039$ and $P = 0.031$, respectively). Unhydrogenated oil group had a significant reduction in TC, TG ($P < 0.001$), and ApoB ($P = 0.003$) and in the ghee group, ApoA significantly increased ($P < 0.001$). Blended and soft margarine groups had a significant decline in TG ($P = 0.010$ and $P < 0.001$, respectively).

Except for LDL-C, and ApoA and ApoB levels, the comparison of the mean and percentage of
### Table 1. Basic characteristics and serum lipids in the beginning of the study

| Oil | Hydrogenated | Unhydrogenated | Ghee | Soft margarine | Blended | P    |
|-----|--------------|----------------|------|---------------|---------|------|
|     | Mean ± SD    | Mean ± SD      | Mean ± SD | Mean ± SD   | Mean ± SD |     |
| Age (year) | 32.8 ± 11.1 | 33.9 ± 11.6 | 35.4 ± 10.9 | 36.5 ± 12.2 | 36.6 ± 11.6 | 0.203 |
| Body mass index (kg/m²) | 26.5 ± 4.4 | 26.1 ± 5.3 | 25.7 ± 4.6 | 26.4 ± 4.13 | 26.8 ± 6.8 | 0.821 |
| Total cholesterol (mg/dl) | 174.9 ± 23.4 | 176.7 ± 25.4 | 183.8 ± 30.9 | 174.0 ± 31.5 | 175.8 ± 29.4 | 0.734 |
| Triglyceride (mg/dl) | 134.1 ± 52.8 | 127.4 ± 42.1 | 125.0 ± 52.0 | 114.9 ± 33.7 | 109.5 ± 26.1 | 0.268 |
| HDL-C (mg/dl) | 43.3 ± 7.5 | 45.3 ± 6.9 | 44.0 ± 5.6 | 45.3 ± 6.7 | 47.2 ± 6.8 | 0.510 |
| LDL-C (mg/dl) | 104.7 ± 19.5 | 105.9 ± 22.6 | 114.8 ± 27.2 | 105.8 ± 28.7 | 108.6 ± 23.8 | 0.392 |
| Apolipoprotein A (mg/dl) | 129.9 ± 17.3 | 126.5 ± 17.4 | 122.0 ± 14.0 | 111.1 ± 150.0 | 123.4 ± 12.9 | 0.009 |
| Apolipoprotein B (mg/dl) | 92.3 ± 17.9 | 99.4 ± 20.2 | 98.4 ± 20.1 | 94.2 ± 19.9 | 91.5 ± 18.6 | 0.605 |
| Gender (male) [n (%)] | 17.0 ± 41.5 | 18.0 ± 41.9 | 16.0 ± 41.0 | 19.0 ± 46.0 | 18.0 ± 42.9 | 0.677 |
| Illiterate [n (%)] | 17.0 ± 41.4 | 15.0 ± 34.9 | 14.0 ± 35.9 | 16.0 ± 39.0 | 16.0 ± 38.2 | 0.042 |

HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol

### Table 2. Comparison of serum lipid before and after the study

| Oil | Before | After | P    |
|-----|--------|-------|------|
|     | Mean ± SD | Mean ± SD |     |
| Hydrogenated | Total cholesterol (mg/dl) | 174.9 ± 23.4 | 178.6 ± 25.3 | 0.039 |
|       | Triglyceride (mg/dl) | 134.1 ± 52.8 | 137.5 ± 51.8 | 0.124 |
|       | HDL-C (mg/dl) | 43.3 ± 7.5 | 44.1 ± 7.0 | 0.233 |
|       | LDL-C (mg/dl) | 104.7 ± 19.5 | 107.1 ± 21.2 | 0.141 |
|       | Apolipoprotein A (mg/dl) | 129.9 ± 17.3 | 125.8 ± 13.3 | 0.031 |
|       | Apolipoprotein B (mg/dl) | 92.3 ± 17.9 | 95.7 ± 21.8 | 0.222 |
| Unhydrogenated | Total cholesterol (mg/dl) | 176.7 ± 25.4 | 173.1 ± 26.2 | < 0.001 |
|       | Triglyceride (mg/dl) | 127.4 ± 42.1 | 122.8 ± 41.6 | < 0.001 |
|       | HDL-C (mg/dl) | 45.3 ± 6.9 | 44.3 ± 6.8 | 0.324 |
|       | LDL-C (mg/dl) | 105.9 ± 22.6 | 104.3 ± 24.4 | 0.387 |
|       | Apolipoprotein A (mg/dl) | 126.5 ± 17.4 | 127.8 ± 14.8 | 0.352 |
|       | Apolipoprotein B (mg/dl) | 99.4 ± 20.2 | 93.3 ± 20.5 | 0.003 |
| Ghee | Total cholesterol (mg/dl) | 183.8 ± 30.9 | 183.5 ± 28.6 | 0.703 |
|      | Triglyceride (mg/dl) | 125.0 ± 52.0 | 122.6 ± 49.3 | 0.512 |
|      | HDL-C (mg/dl) | 44.0 ± 5.6 | 45.3 ± 7.6 | 0.244 |
|      | LDL-C (mg/dl) | 114.8 ± 27.2 | 113.7 ± 23.9 | 0.126 |
|      | Apolipoprotein A (mg/dl) | 122.0 ± 14.0 | 125.4 ± 13.0 | < 0.001 |
|      | Apolipoprotein B (mg/dl) | 98.4 ± 20.1 | 98.7 ± 17.3 | 0.788 |
| Blended | Total cholesterol (mg/dl) | 175.8 ± 29.4 | 173.5 ± 27.6 | 0.225 |
|       | Triglyceride (mg/dl) | 109.5 ± 26.1 | 95.5 ± 24.9 | 0.010 |
|       | HDL-C (mg/dl) | 47.2 ± 6.8 | 47.4 ± 7.3 | 0.438 |
|       | LDL-C (mg/dl) | 108.6 ± 23.8 | 107.1 ± 23.5 | 0.451 |
|       | Apolipoprotein A (mg/dl) | 123.4 ± 12.9 | 120 ± 13.4 | 0.237 |
|       | Apolipoprotein B (mg/dl) | 91.5 ± 18.6 | 92.5 ± 15.4 | 0.507 |
| Soft margarine | Total cholesterol (mg/dl) | 174 ± 31.5 | 169.4 ± 25.6 | 0.128 |
|       | Triglyceride (mg/dl) | 114.9 ± 33.7 | 108.2 ± 31.1 | < 0.001 |
|       | HDL-C (mg/dl) | 45.3 ± 6.8 | 46.0 ± 6.0 | 0.345 |
|       | LDL-C (mg/dl) | 105.8 ± 28.7 | 101.8 ± 25.4 | 0.109 |
|       | Apolipoprotein A (mg/dl) | 111.1 ± 14.6 | 115.8 ± 21.2 | 0.099 |
|       | Apolipoprotein B (mg/dl) | 94.2 ± 20.0 | 94.1 ± 22.4 | 0.723 |

HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol

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serum lipids, changes with age and gender adjustment, revealed a significant difference among the three studied groups (Figures 2-3). TC, TG, and ApoB levels had a significant reduction in the unhydrogenated oil group when compared with the hydrogenated oil group (P < 0.001). In the ghee oil group, TG was significantly decreased, while ApoA had a significant increase (P < 0.001). Comparing with the ghee group, the unhydrogenated oil group had a significant reduction in HDL-C (P < 0.05).

Figure 2. Comparison of the mean of serum lipid differences between unhydrogenated, ghee, soft margarine, blended, and hydrogenated oil groups
TG: Triglyceride; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol

Figure 3. Comparison of the mean of apolipoprotein differences between unhydrogenated, ghee, soft margarine, blended, and hydrogenated oil groups
APPOA: Apolipoprotein A; APPOB: Apolipoprotein B
Effect of oils on serum lipids

Table 3. Comparison of the adjusted mean of serum lipids and changes in intervention groups vs reference group after the intervention

| Group                   | Post-study Mean ± SE | Change β* | 95% (CI)** |
|-------------------------|----------------------|-----------|------------|
| **Total cholesterol (mg/dl)** |                      |           |            |
| Unhydrogenated          | 173.9 ± 1.2          | -7.1c     | -10.8(-3.5) |
| Blended                 | 175.2 ± 1.6          | -5.9b     | -10.1(-1.7) |
| Ghee                    | 177.8 ± 1.3          | -3.3c     | -7.1-0.5   |
| Soft margarine          | 172.7 ± 1.6          | -8.4c     | -12.7(-4.1) |
| Hydrogenated            | 181.1 ± 1.5          | R†        | -          |
| **Triglyceride (mg/dl)** |                      |           |            |
| Unhydrogenated          | 117.7 ± 1.3          | -8.4c     | -12.6(-4.1) |
| Blended                 | 116.5 ± 1.8          | -9.6b     | -14.6(-4.6) |
| Ghee                    | 119.8 ± 1.5          | -6.3b     | -10.7(-2.0) |
| Soft margarine          | 114.8 ± 1.9          | -11.2c    | -16.2(-6.3) |
| Hydrogenated            | 126.1 ± 1.7          | R†        | -          |
| **LDL-Cholesterol (mg/dl)** |                      |           |            |
| Unhydrogenated          | 166.4 ± 1.3          | -3.8 (2.0)c | -7.8(-0.1) |
| Blended                 | 106.7 ± 1.7          | -3.5 (2.3) | -8.1-1.1   |
| Ghee                    | 107.7 ± 1.4          | -2.5 (2.1) | -6.6-1.7   |
| Soft margarine          | 104.0 ± 1.8          | -2.5 (2.3)c | -10.8(-1.5) |
| Hydrogenated            | 110.2 ± 1.6          | 0         | -          |
| **HDL-Cholesterol (mg/dl)** |                      |           |            |
| Unhydrogenated          | 43.6 ± 0.5           | -1.5 (0.9) | -3.2-0.1   |
| Blended                 | 45.4 ± 0.7           | -0.07 (0.9) | -2.1-0.2   |
| Ghee                    | 46.1 ± 0.6           | 0.6 (0.8) | -1.1-2.3   |
| Soft margarine          | 45.7 ± 0.7           | 0.2 (1)   | -1.7-2.2   |
| Hydrogenated            | 45.5 ± 0.7           | 0         | -          |
| **Apolipoprotein A (mg/dl)** |                      |           |            |
| Unhydrogenated          | 126.3 ± 1.8          | 3.7 (2.9) | -1.9-9.4   |
| Blended                 | 120 ± 2.4            | -2.6 (3.3) | -9.1-4     |
| Ghee                    | 120.1 ± 1.9          | 3.5 (2.9)c | 2.4-9.4    |
| Soft margarine          | 121.8 ± 2.6          | -0.8 (3.5) | -7.8-6.2   |
| Hydrogenated            | 122.6 ± 2.3          | 0         | -          |
| **Apolipoprotein B (mg/dl)** |                      |           |            |
| Unhydrogenated          | 90.5 ± 1.6           | -8.2 (2.3)b | -13.2(-3.2) |
| Blended                 | 96.2 ± 2.1           | -2.5 (2.9) | -8.2-3.3   |
| Ghee                    | 96.7 ± 1.7           | -1.9 (2.6) | -7.1-3.2   |
| Soft margarine          | 95.5 ± 2.2           | -3.2 (2.9) | -8.9-2.7   |
| Hydrogenated            | 98.6 ± 2.0           | 0         | -          |

* β: Regression coefficient of baseline serum lipids; ** 95% confidence interval; † Hydrogenated was considered as reference group; a: P < 0.05; b: P < 0.01; c: P < 0.001

SE: Standard Error; CI: Confidence interval; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol

However TC and TG declined in blended oil and soft margarine groups significantly (P < 0.001 and P < 0.05, respectively).

The adjusted mean level of serum lipids after the intervention and the mean changes of serum lipids in each intervention group versus hydrogenated oil group are presented in Table 3. As it shows the soft margarine group had the most significant reduction.
in TC and TG (β ± SE: -8.4 ± 2.2; β ± SE: -11.2 ± 2.5, respectively) (P < 0.001). LDL-C had a significant reduction in unhydrogenated oil and soft margarine (β ± SE: -3.8 ± 2, P < 0.001; β ± SE: -2.5 ± 2.3, P < 0.05, respectively). ApoA had a significant increase only in the ghee group and ApoB declined significantly only in the unhydrogenated oil group (β ± SE: 3.5 ± 2.9, P < 0.001; β ± SE: -8.2 ± 2.3, P < 0.001, respectively).

**Discussion**

This study indicated that unhydrogenated oil can generally reduce serum lipid levels when compared with hydrogenated oil. However, changes in serum lipids, except for TG reduction and ApoA enhancement, were not significant when the ghee oil group was compared with the hydrogenated oil group (serum HDL-C levels had an insignificant increase). Moreover, blended oil and soft margarine as 2 new products of oil in Iran could reduce TC and TG.

Several studies have indicated that hydrogenated fat and/or TFAs could increase TC, TG, and LDL-C, decrease HDL-C, and enhance the LDL-C: HDL-C ratio. However, the responsible mechanisms for these changes are complicated. It has been proposed that the serum lipid-raising effect of hydrogenated fat is due to either delayed LDL-C clearance or enhanced LDL-C production. Matthan et al. have reported that hydrogenated oil decreased HDL-C, and raised LDL-C by increasing ApoA-I and decreased LDL ApoB-100 catabolism. Thus, it was indicated that damaging the cholesterol catabolism is responsible to a greater degree than decreasing its synthesis for the higher serum TC seen by intake of high hydrogenated and saturated fat diets. However, Kelley et al. showed that a diet containing cotton seed oil could not modify serum lipids including TC, TG, LDL-C, HDL-C, ApoA and ApoB in comparison with a normal diet.

The study by Al-Amoudi and Abu Araki indicated that a blend of the various specific vegetable oils improved serum lipid profiles due to a synergistic effect of various blending oils. Enhancement oxidative stability and the synergistic effect of different vegetable oils might cause the serum lipid improvement in the blended oil group of the current study.

According to the study by Asgary et al. the average TFA contents in hydrogenated oils, and unhydrogenated cooking and frying oils produced in Iran were 35.2 ± 4.8%, 0.9 ± 0.3%, and 772.6 ± 0.8%, respectively. Therefore, serum lipid modification by unhydrogenated oils seems reasonable in this study.

Ghee oil is an important dietary fat used in India and other South Asian countries, which contains high amounts of SFAs (about 59% of its whole fatty acids). SFAs, except for stearic acid, increase serum TC. Therefore, ghee oil, that is high in cholesterol and SFAs, is considered as harmful. On the other hand, ghee is a good source of oleic acid which is capable of protecting LDL-C particles from oxidation and prevents atherosclerosis. Furthermore, according to Asgary et al. the average TFA content in ghee produced by Bakhtiari nomads (the kind of ghee that was used in this study) is 8.3 ± 0.7 which is 1.4 times less than the amount of existing TFA in hydrogenated oils.

Kumar et al. study indicated that consumption of ghee in the diet, even with high intakes, does not increase serum lipids. This animal study did not show any linking between ghee consumption and hypercholesterolemia and hyperlipidemia, which are considered to be risk factors for heart diseases. Interestingly, consuming increased levels of ghee reduced serum TC and TG levels. Another idea was that there is a link between the consumption of anhydrous milk fat, such as ghee, and increased risk of heart diseases. However, use of excess intake of ghee as a means for lowering serum TC is not recommended, but the study indicates that there is no reason for apprehension for consuming ghee in the diet, which is an age-old practice that is relished in Indian cuisine. Mozaffarian et al. stated that substituting 8% of energy intake from TFA with SFA cause a decrease in CVD by modifying TC:HDL-C ratio. Therefore, it confirms the suitable effect of ghee on serum lipid profile.

**Limitation**

As the subjects used the oils for cooking at home, blinding was not applicable. Thus, it was the limitation of this study.

**Conclusion**

Blended and soft margarine as two new kinds of oils in Iran had some beneficial effects on serum lipids. Furthermore, ghee was useful in modifying serum, including TG and HDL-C, and unhydrogenated oil and frying oil consumption resulted in a general reduction in serum lipids. Therefore, it can be said that ghee might be effective on serum lipid modification in metabolic syndrome, but it should not be forgotten that ghee, which is traditionally made from milk fat,
has high amounts of SFAs, and also its production method should be carefully supervised.

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**Conflict of Interests**

Authors have no conflict of interests.

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