Effects of dietary supplementation with ghee, hydrogenated oil, or olive oil on lipid profile and fatty streak formation in rabbits

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Abstract

BACKGROUND: Coronary heart disease is the leading cause of mortality worldwide. A high-fat diet, rich in saturated fatty acids and low in polyunsaturated fatty acids, is said to be an important cause of atherosclerosis and cardiovascular diseases.

METHODS: In this experimental study, 40 male rabbits were randomly assigned to eight groups of five to receive normal diet, hypercholesterolemic diet, normal diet plus ghee, normal diet plus olive oil, normal diet plus hydrogenated oil, hypercholesterolemic diet plus ghee, hypercholesterolemic diet plus olive oil, and hypercholesterolemic diet plus hydrogenated oil. They received rabbit chow for a period of 12 weeks. At the start and end of the study, fasting blood samples were taken from all animals to measure biochemical factors including total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride (TG), fasting blood sugar (FBS), and C-reactive protein (CRP). Moreover, aorta, left and right coronary arteries were dissected at the end of the study to investigate fatty streak formation (FSF). Data was analyzed in SPSS at a significance level of 0.05.

RESULTS: In rabbits under normal diet, ghee significantly increased TC, LDL, and HDL compared to the beginning ($P < 0.01$) and also to the other two types of fat ($P < 0.05$). Moreover, normal diet plus olive oil significantly enhanced FSF in left coronary arteries and aorta compared to normal diet plus ghee. In groups receiving hypercholesterolemic diets, ghee significantly increased HDL and CRP ($P < 0.05$) and significantly decreased FBS ($P < 0.01$). The hypecholesterolemic diet plus olive oil significantly increased HDL ($P < 0.01$). Supplementation of hypecholesterolemic diet with ghee significantly increased HDL and FBS in comparison with hydrogenated oil. Significant increase of FBS was also detected with the use of ghee compared to olive oil. Ghee also significantly reduced FSF in left and right coronary arteries compared to olive oil. FSF in left coronary arteries was significantly lower in the hypecholesterolemic diet plus ghee group compared to the hypecholesterolemic diet plus hydrogenated oil group.

CONCLUSION: According to the achieved results, future clinical trial studies and investigation of other risk factors such as inflammatory factors are required.

Keywords: Fatty Streak, Ghee, Hypercholesterolemic, Olive Oil

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Introduction

Coronary heart disease (CHD) has long been a global concern. Epidemiologic studies have shown CHD mortality rate to be 25-45% in east Mediterranean countries.[1] Clinical investigations have suggested several methods for prevention or treatment of CHD. These strategies include lifestyle modification through decreasing saturated fatty acid (SFA) intake to less than 10% of total energy intake and cholesterol to less than 300 mg daily, blood pressure and sugar control, regular exercise, and maintaining ideal body weight.[2] Using frying oil is an important part of food preparation. However, heating frying oils can produce undesirable components that affect quality of foods and pose risks on people's health and nutrition.[3] Lipid oxidation leads to decreased quality and durability of food.[4] Physical properties of natural fats and oils depend on their constitutive fatty acid type, and melting point, saturation degree, chain length, and crystal structure of their triglyceride (TG). While vegetable oils are rich in unsaturated fatty acids, solid fats consist mainly of saturated fatty acids. According to recent studies, using hydrogenated oils, which contain trans fatty acids (TFA), may increase blood cholesterol levels to higher than those caused by non-hydrogenated oils.[5] TFA intake is causally related to

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risk of coronary disease as it increases low-density lipoprotein (LDL) and decreases high-density lipoprotein (HDL). Oleic acid is an isomer of elaidic acid and the dominant fatty acid in olive oil. TFA formation during the process of partial hydrogenation produces a considerable amount of elaidic acid.[6-8] High consumption of olive oil may protect against myocardial infarction which is the hallmark of ischemic heart disease.[9] More specifically, recent reviews have summarized the effects of olive oil on cardiovascular risk factors.[10,11] Since the beneficial effects of some components of olive oil other than oleic acid have been emphasized, olive oil cannot be healthy only due to its high monounsaturated fatty acids (MUFAs) content.[12] Shankar et al. reported that serum total cholesterol (TC) levels increased significantly in the experimental group (fed with ghee provided 10% of the energy intake) at four weeks and remained persistent until the eighth week. As a similar rise was observed in HDL levels, TC to HDL ratio did not change significantly.[13]

Ghee is produced from milk by traditional methods and is usually called "yellow oil" or "Kermanshahi oil" in Iran. It consists of SFAs (65%) and MUFAs (33%).[14] While MUFAs are high in cholesterol,[15] studies have suggested the usefulness of ghee in decreasing LDL and increasing HDL.[16,17] There are some controversies about the link between ghee consumption and serum lipid profile.[18-20] In an animal study, Kumar et al. found ghee consumption to significantly increase HDL levels but not to alter other serum lipid levels.[20] This research aimed to determine the effects of diets containing olive oil, hydrogenated oil, and ghee on lipid profiles and fatty streak formation **(**FSF) in rabbits.

Materials and Methods

Grouping and treatment of rabbits

In this experimental study, 40 adult male Dutch-Polish rabbits with the mean weight of 2 kg were obtained (Pasteur Institute, Tehran, Iran). The animals were individually housed in cages with food and water available at all times. Standard diet pellets were prepared weekly (Khorak Dam Pars Co., Iran). After two weeks of acclimatization, the rabbits were randomly distributed in eight groups of five to receive normal diet, hypercholesterolemic diet, normal diet plus ghee, normal diet plus olive oil, normal diet plus hydrogenated oil, hypercholesterolemic diet plus ghee oil, hypercholesterolemic diet plus olive oil, or hypercholesterolemic diet plus hydrogenated oil. Ghee (anhydrous milk fat) was purchased from Bakhtiari nomads who prepared it by heating butter at high temperatures.

Measurement of biochemical factors

Fasting blood samples were taken to determine plasma and serum TC, HDL, LDL, and TG using a biochemistry enzymatic kit (Automatic Analyzer, Hitachi 902 set, Japan). Moreover, C-reactive protein (CRP) and fasting blood sugar (FBS) were measured through photometric method (Pars Azmoon kit, Iran) and enzymatic method using an Elan autoanalyzer (brand name of set),, respectively. All tests were performed at Isfahan Cardiovascular Research Center, Isfahan, Iran. While all factors were measured twice (in the beginning and at the end of the study), CRP was only determined at the end.

FSF assessment

At the end of the experiment, the rabbits were anesthetized with an intravenous injection of sodium pentobarbitone 5%. After dissecting the bodies, the heart, aorta, and right and left coronary arteries were removed, opened longitudinally, and prepared for accurate detection and estimation of lipid deposits in the intima. One block was prepared from the aorta, and right and left coronary arteries of each rabbit. Afterwards, three slides of each block and three sections of each slide were made. Slides were stained with hematoxylin-eosin. The prepared slides were observed using a light microscope and scored based on a scale from zero to four.[21]

Statistical analyses

Statistical evaluations were conducted in $SPSS_{10}$ (SPSS Inc., Chicago, IL, USA) and the results were expressed as mean ± standard deviation (SD). Kruskal-Wallis test was used to compare the mean measured values of biochemical and histological factors between groups. Pairwise comparisons were also made using Mann-Whitney test. McNemar's test was applied for comparisons of biochemical factors within each group.

Results

As table 1 shows, in rabbits under normal diet, ghee significantly increased TC, LDL, and HDL ($P < 0.01$). It also had non-significant effects on other factors including TG, FBS, and CRP. In contrast, hydrogenated oil and olive oil did not cause significant differences in any of the studied factors.

In rabbits receiving hypercholesterolemic diet, ghee significantly increased HDL and CRP ($P < 0.05$) and significantly decreased FBS ($P < 0.01$). However, it had no statistically significant effects on TC, LDL, and TG. In this group, hydrogenated oil significantly increased HDL and CRP ($P < 0.05$) but did not have significant effects on other factors. Using olive oil significantly increased HDL (P < 0.01). It also caused non-significant differences in TC, LDL, TG, FBS, and CRP levels (Table 2).

Among rabbits fed with normal diet, using ghee significantly increased TC, LDL, and HDL levels compared to using the other two kinds of oil. However, the three types of fat did not have significantly different effects on FBS, CRP, and TG levels (Table 3).

Adding ghee to hypercholesterolemic diets was significantly more effective than adding hydrogenated oil in enhancing HDL and FBS levels. In contrast, the two diets did not cause significantly different effects on TC, LDL, TG, and CRP levels. Moreover, while ghee could reduce FBS significantly more than olive oil, other factors were not significantly different between the two groups. There were also non-significant differences in all factors between supplementation with olive oil and hydrogenated oil (Table 4).

Table 1. Differences in blood factors between rabbits receving a normal diet (N), normal diet plus hydrogenated oil (NH), normal diet plus olive oil (NO), and normal diet plus ghee (NG)

Blood factor (mg/dl)	Diet		
	N-NH	N-NO	N-NG
TC	-13.00	-9.20	-434.00*
LDL	-9.20	0.60	-339.20*
HDL	3.44	-2.60	-88.55*
FBS	-0.45	13.80	-7.70
TG	-76.85	-54.60	-53.10
CRP	0.95	0.85	-0.41

TC: Total cholesterol; LDL: Low density lipoprotein; HDL: High density lipoprotein; FBS: Fasting blood sugar; TG: Triglyceride; CRP: C-reactive protein
* Significant difference between the N group and the NH, NO, and NG groups (P < 0.01)

Table 2. Differences in blood factors between rabbits receving a hypercholesterolemic diet (H), hypercholesterolemic diet plus hydrogenated oil (HH), hypercholesterolemic diet plus olive oil (HO), and hypercholesterolemic diet plus ghee (HG)

Blood factor (mg/dl)	Diet		
	H-HH	H-HO	H-HG
TC	26.00	-21.45	-99.80
LDL	37.20	63.15	32.06
HDL	-70.20*	-123.65**	-157.06**
FBS	18.60	-19.10	86.93**
TG	85.80	4.70	-45.80
CRP	-2.30*	-1.70	-3.70**

TC: Total cholesterol; LDL: Low density lipoprotein; HDL: High density lipoprotein; FBS: Fasting blood sugar; TG: Triglyceride; CRP: C-reactive protein
* Significant difference between the H group and the HH, HO, and HG groups (P < 0.05)
** Significant difference between the H group and the HH, HO, and HG groups (P < 0.01)

Table 3. The mean changes of measured blood factors at the end of the study (compared to baseline values) in rabbits receiving normal diets

Blood factor (mg/dl)	Diet		
	Normal + hydrogenated oil	Normal + olive oil	Normal + ghee
TC	5.00 ± 19.30*	1.20 ± 26.30**	426.00 ± 235.00
LDL	5.00 ± 4.30*	-4.80 ± 16.40**	335.00 ± 267.50
HDL	-8.20 ± 10.30*	-2.20 ± 9.00**	83.70 ± 69.00
FBS	11.20 ± 17.20	-3.00 ± 34.00	18.50 ± 10.00
TG	27.20 ± 33.60	5.00 ± 40.00	3.50 ± 59.00
CRP	-.37.00 ± .57	-0.36 ± 3.10	1.25 ± 0.80

TC: Total cholesterol; LDL: Low density lipoprotein; HDL: High density lipoprotein; FBS: Fasting blood sugar; TG: Triglyceride; CRP: C-reactive protein
* Significant difference as compared to normal diet + ghee (P < 0.05)
** Significant difference as compared to normal diet + ghee (P < 0.01)

Table 4. The mean changes of measured blood factors at the end of the study (compared to baseline values) in rabbits receiving hypercholesterolemic diets

Blood factor (mg/dl)	Diet		
	Normal + hydrogenated oil	Normal + olive oil	Normal + ghee
TC	616.80 ± 39.70	664.20 ± 27.09	742.60 ± 71.66
LDL	467.00 ± 27.10	441.25 ± 51.00	472.30 ± 18.60
HDL	139.80 ± 45.1*	193.25 ± 23.00	226.60 ± 26.27
FBS	23.80 ± 39.00*	61.50 ± 17.60**	129.30 ± 61.07
TG	52.40 ± 66.62	133.50 ± 76.00	184.00 ± 125.10
CRP	4.96 ± 1.25	3.80 ± 0.59	0.5 ± 3.10

TC: Total cholesterol; LDL: Low density lipoprotein; HDL: High density lipoprotein; FBS: Fasting blood sugar; TG: Triglyceride; CRP: C-reactive protein
* Significant difference as compared to hypercholesterolemic diet + ghee ($P < 0.05$)
** Significant difference as compared to hypercholesterolemic diet + ghee ($P < 0.01$)

Table 5. Changes in fatty streak formation (FSF) in the aorta, and right and left coronary arteries at the end of study (compared to the beginning) in rabbits fed with normal diets

FSF	Diet		
	Normal + hydrogenated oil	Normal + olive oil	Normal + ghee
Right coronary	0.33 ± 0.48*	1.10 ± 0.60	0.53 ± 0.91
Left coronary	0.06 ± 0.25*	0.88 ± 0.78	0.00 ± 0.00
Aorta	0.00 ± 0.00	0.22 ± 0.44**	0.00 ± 0.00

* Significant difference compared to normal diet + olive oil ($P < 0.05$)
** Significant difference compared to normal diet + ghee ($P < 0.05$)

Table 6. Changes in fatty streak formation (FSF) in the aorta, and right and left coronary arteries at the end of study (compared to the beginning) in rabbits fed with hypercholeterolemic diets

FSF	Diet		
	Hypercholesteromic + hydrogenated oil	Hypercholesteromic + olive oil	Hypercholesteromic + ghee oil
Right coronary	0.60 ± 1.05	1.00 ± 0.00	0.50 ± 0.90
Left coronary	1.53 ± 1.60**	1.66 ± 0.50	0.41 ± 0.51†
Aorta	0.00 ± 0.00*	1.00 ± 0.00	0.66 ± 0.57

* Significant difference compared with hypercholesteromic + olive oil ($P<0.05$)
** Significant difference compared with hypercholesteromic + ghee oil ($P<0.05$)
† Significant difference compared with hypercholesteromic + olive oil ($P<0.01$)

Among groups under normal diet, hydrogenated oil could significantly reduce FSF in the left and right coronary arteries compared to olive oil. However, the difference between groups receiving hydrogenated oil and ghee was not significant. In addition, FSF increased significantly in left coronary arteries and aorta and non-significantly in right coronary arteries in normal diet plus olive oil group compared to normal diet plus ghee group (Table 5).

FSF levels in left coronary arteries of the hypercholesterolemic plus ghee group were significantly lower than the hypercholesterolemic plus hydrogenated oil. On the other hand, there were no significant differences between the two groups in FSF in right coronary arteries and aorta. Moreover, FSF levels in hypercholesterolemic plus olive oil were not significantly different from levels in the other two groups (Table 6).

Discussion

Diet plays a major role in decreasing the incidence of CHD. Research has indicated lipid profile improvement to decrease CHD rate.[22] In our study on rabbits, ghee significantly increased TC, LDL, and HDL levels in comparison with olive and hydrogenated oils in groups under normal diet. In hypercholesterolemic groups, dietary supplementation with ghee significantly increased TC, LDL, HDL, FBS, and TG levels compared to hydrogenated oil. In a human study by Shankar et al., significant increases of TC, HDL, and very low density lipoprotein (VLDL) were observed with ghee consumption. We found TC, LDL, HDL, TG, and FBS levels to be lower (but not significantly) in the normal diet plus olive oil group compared to normal diet plus hydrogenated oil group. In hypercholesterolemic groups, there were no significant differences in LDL, HDL, TG, FBS, and CRP levels between diets containing olive oil and hydrogenated oil.[13] Navarro et al.[18] and Schick et al.[23] in animal studies and Sirtori et al.[24] in a human study declared that supplementation with olive oil did not significantly affect serum lipids levels. Our results are consistent with previous studies which suggested the existence of SFA to increase TC and LDL levels.[25]

In a clinical trial by Mohammadifard et al. ghee significantly reduced apo A levels but did not significantly change TC, TG, LDL, and HDL levels. On the contrary, in our study, normal diet containing ghee significantly increased TC, LDL, and HDL after 40 days.[26]

Scaceini et al. demonstrated that olive oil could inhibit lipid peroxidation.[27] Olive oil contains hydroxytyrosol, oleuropein, and phenols that scavenge free radicals and inhibit low density lipoprotein (LDL) oxidation.[28,29]

Hydrogenated oil contains TFA. TFA interfere in the metabolism of fatty acids and contribute to the etiology of cancer and CHD.[30]

Ghee consists of not only SFA (65%) but also MUFA (32%).[13] The positive ecological correlations between national intakes of SFA and cardiovascular mortality found in various studies.[31] The National Cholesterol Education Program in the U.S.A. permits about one third of the fat intake in the form of saturated fats.[32] MUFAs are high in cholesterol and thus increase LDL levels.[15] in one of the study, cardiovascular morbidity and mortality in India had increased after the consumption of ghee.[13]

CHD prevention requires controlling its risk factors. One strategy is to replace dietary SFA with unsaturated fatty acids. Finally, since we assessed a few number of biochemical factors and, further studies on animals as well as clinical trials are necessary to compare the effects of the three types of fat on other biochemical factors and tissues.

Conclusion

According to the effects of hydrogenated oil, ghee, and olive oil on lipid profile and FSF, other risk factors such as inflammatory factors need to be investigated.

Conflict of Interests

Authors have no conflict of interests.

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