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Influences of Olive Oil and Ghee (samen balady) on Serum Cholesterol of Jordanians

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Abstract: The effects of olive oil (OO) and ghee types (semen balady) on the serum lipid profile were studied in healthy volunteers (11 males, aged 36-44 year; 13 females aged 27-35 year). The 24 subjects ate their habitual diet with OO or ghee of cow milk fat (CMF), ghee of goat milk fat (GMF) or ghee of sheep milk fat (SMF) for 4 wk with 4 wk interval between the four diets. Compared with initial values, OO diet significantly ($P < 0.05$) reduced concentrations of serum total cholesterol (TC, -3.42%) and low density lipoprotein cholesterol (LDL-c, -4.31%). OO caused slight reduction in concentration of high density lipoprotein cholesterol (HDL-c, -2.86%), and ratios of TC/HDL-c and LDL-c/HDL-c. Whereas ghee types significantly ($P < 0.05$) increased these parameters and the SMF resulted in the highest rise of TC (+11.93%) and LDL-c (+16.16%). The highest rise in concentration of HDL-c (+8.81%) was shown with GMF. Ghee types slightly increased the ratios of TC/HDL-c and LDL-c/HDL-c compared with the initial values. SMF resulted in the highest rise of TC/HDL-c (+0.26) and LDL-c/HDL-c (+0.31) Serum triglycerides level increased (+3.38 %) after OO diet, whereas reduced (about -2.15%) after periods of ghee types. In general, the responses in serum lipids were greater in males than in females in all the four diets.

Key words: Olive oil, ghee, cholesterol, LDL-c, HDL-c, triglycerides

Introduction

Coronary heart disease (CHD), the common cause of heart attack, is one of the most frequent causes of death in the developed and developing countries, (AHA, 1989). Several inherited and lifestyle factors affecting the risk of heart diseases, among the latter are cigarette smoking, physical exercise, and diet habits (Elson, 1992; Macnair, 1994). Through a period of time many research workers showed a direct link between elevated level of blood cholesterol and the occurrence of CHD (John *et al.*, 1990). Cholesterol is the precursor of steroid hormones, bile acids and also required for normal cell functions. On the other hand, it is a major contributer to atherosclerosis plaques and most gallstones (Grundy, 1983). Low density lipoproteins (LDLs) and high density lipoproteins (HDLs) refer to the two types of lipoproteins, package of fat, cholesterol, and protein to transport it through the blood. LDLs are responsible for depositing cholesterol in the artery walls. These lipoproteins may be atherogenic. HDLs can acquire cholesterol from cells and transport it to the liver for reprocessing or bile acids formation. These lipoproteins are appearing to be antiatherogenic (Hui, 1992). The high blood LDL-cholesterol (LDL-c) concentration is associated with a higher risk of heart attack, whereas the high blood HDL-cholesterol (HDL-c) concentration may play a beneficial role (koo *et al.*, 1985). Among a number of possible causes related to blood cholesterol level, the one that is the type of fatty substances. Solid fats raise this level, whereas oils lower it (Macnair, 1994). There is a positive relationship between the dietary cholesterol intake and serum cholesterol (Cohen *et al.*, 1994). Dairy products make appreciable contribution to saturated fat and cholesterol intake. Consumption these products may be correlated with high blood cholesterol level (Rossouw *et al.*, 1981). However, the height monounsaturated fat diet can be an alternative to the presently recommended 30% fat diet to reduce the risk of heart disease (Kris-Etherton, 1999). The effects of cholesterol, saturated fatty acids (SFA), mono unsaturated fatty acids (MUFA), polyunsaturated fatty acids(PUFA) and total fat intakes on serum cholesterol have been studied extensively without clear cut relationship emerging (Thannoun, 1993).

Olive oil (OO) and Ghee (samen balady) are widely used in Jordanian foods. Ghee, which is rich in SFA , produce from butter of sheep milk fat (SMF) or goat milk fat (GMF) or cow milk fat (CMF). This study was interested in determining the effects of OO and ghee of mentioned three sources upon Jordanian blood lipid profile. So far none of the studies reported in Jordan to assess the effects of ghee types on serum lipid profile of human.

Materials and Methods

Subjects: Participants in the study were selected from different locations (Amman, Karak) in Jordan. Eleven males averaged 38 years old (ranged 36-44 yr), and thirteen females averaged 30 years old (ranged 27-35 yr) were involved in this study. Started from September 2000 to April 2001. Body weight and body mass index of males were 64 kg and 21.3, respectively, and of females were 57kg and 21.6, respectively. All subjects were healthy, normocholesterol level, normotensive, non diabetic, and

Table 1: Fatty acid profile of olive oil (OO), cow milk fat (CMF), goat milk fat (GMF) and sheep milk fat (SMF)

Fatty acid	OO %	CMF %	GMF %	SMF %
4,6,8:0	-	6.7	8.5	9.7
10:0	-	3.5	9.0	7.0
12:0	-	3.8	5.0	5.0
14:0	-	8.2	10.4	12.2
14:1	-	0.8	1.2	1.0
14:2	-	0.4	-	-
15:0	-	0.2	-	-
16:0	14.8	25	25.2	26.4
16:1	0.8	2.1	2.4	3.3
16:2	0.2	0.2	-	-
17:0	-	0.5	0.6	1.5
18:0	5.3	10.5	8.1	11.0
18:1	66.4	32.5	26.6	21.
18:2	11.9	4.2	2.3	1.4
18:3	0.6	1.2	0.7	0.5
20:0	-	0.2	-	-
SFA% (s) *	20.1	58.6	66.8	72.8
MUFA% (m)	67.2	35.4	30.2	25.3
PUFA% (p)	12.7	6.0	3.0	1.9
P:S	0.63	0.10	0.04	0.03
m+p/s	3.98	0.71	0.50	0.37
TC(mg/dl)	-	150	175	162

*SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TC, total cholesterol.

free from cardiac, renal, hepatic or bleeding disorders.

Diets: Bread (twice a day) and rice (four times a week) were contributing as the main source of carbohydrates. Chicken (twice a week) and eggs (three eggs a week) were contributing as the main source of protein. Milk and its products (three times a week). Meat was eaten only once a week. Vegetables were eaten four times a week, and fruits were eaten twice a week. This study depended on the habitual diets of subjects. The only variable was the type of oil and fat used in cooking or dipping. OO and ghee types were purchased from local producers and used in the preparation of participant diets. Fatty acid composition and cholesterol content of OO and ghee types used in this study are shown in Table 1. Repeated 24 hr-diet-recall for individual dietary intake was used in this study (Table 2). Macro nutrients and energy were calculated using food composition tables (Pellet and Shadarvian, 1970).

Study design: Before the beginning of the study the nutritional status of participants were studied, this work showed that the differences in food intake of individuals from week to another were negligible and they asked to maintain their traditional diets. The 24-subjects ate their food with OO or CMF or GMF or SMF as the sole source of fat type for 4wk with 4wk interval between the four diets. However, the period 4wk was choosen as it had previously been shown that plasma lipid levels stabilized within 2-3 wk after initiating a change in dietary fat (Bonamon and Grundy, 1988). Fasting venous blood samples (10 ml) were collected at the entry and the end of each dietary period for next analysis.

Chemical analysis: Fatty acid (FA) compositions: samples of OO and ghee types were saponified and the free FAs were methylated following the procedure of Morrison and Smith (1962). FA methyl esters were separated by Hamlet Packard gas chromatography model 5710a equipped with flame ionization detectors and column of 10% DEGS on chromosorb WDMCS (Supelco Inc.). Carrier gas flow rate was 24 ml of N_2/min. Flow rate of detectors were 30 ml of H_2/min and 300ml/min of air. The initial column temperature was 90 ℃ then raised to 180 ℃ at a rate of 6 ℃ /min. The injector and detector temperatures were 200 ℃ and 250 ℃, respectively. The identification of individual FA was made using FA-methyl ester standards to establish relative retention time. The relative content of each FA-methyl ester was reported as a percent area of total FA-methyl esters. Total cholesterol of ghee types was determined according to the method of Plummer (1978). Serum total cholesterol was determined using the enzymatic method from Arab Company for medical diagnostic, (Jordan). Triglycerides (TG) were determined by the enzymatic technique from Biocon, (Germany). HDL-c was analyzed by the precipitation technique using magnesium chloride and phosphotungstic acid from Biocon, (Germany). LDL-c was calculated using the formula of Friedewald *et al.* (1972).

Statistical analysis: The Completely Randomized Design (CRD) was used for each parameter. Differences between means were determined using Duncan's multiple range tests at $p < 0.05$ by SAS version (1986).

Results and Discussion

Food consumption: The fatty acid profiles and cholesterol content of OO and ghee types are presented in Table 1. SMF contained the higher SFA (72.8%), the lower MUFA (25.3%) and the lower PUFA (1.9%) than that in GMF, CMF, and OO. Whereas, OO contained the highest MUFA (67.2%) and PUFA (12.7%) and the lowest SFA (20.1%). The ratio of PUFA / SFA (p: s) and MUFA + PUFA (m+p)/s has been used by nutritionists to interpret the effect of dietary oil and fat on the level of blood cholesterol (Hodson *et al.*, 2001). The results showed that p: s and m+p/s ratios of OO, CMF, GMF, and SMF were (0.63, 3.98), (0.10, 0.71), (0.04, 0.50) and (0.03, 0.37), respectively. The results also indicated that OO was free cholesterol. CMF, GMF and SMF were contained 150, 175, and 162 mg cholesterol/dl, respectively.

Table 2: Food consumption of subjects

Dietary Fat Type*	Gender	Energy Cal	Carbohydrates		Fats		Proteins	
			g	% of Cal	g	% of Cal	g	% of Cal
OO	Males	2026±106	320±27	63.2	62±6	2.75	47±5	9.3
	Females	1821±124	296±22	65.0	53±6	26.2	40±6	8.8
	Total	1915	307	64.1	57.1	26.8	43.2	9.1
	Male	2004±100	309±32	61.7	64±4	28.7	48±9	9.6
CMF	Females	1813±131	288±41	63.5	55±8	27.3	42±7	9.3
	Total	1901	297	62.6	59.1	28	44.8	9.4
	Males	1947±98	308±33	63.3	59±7	27.2	46±6	9.5
GMF	Females	1783±119	292±36	65.6	51±6	25.7	39±4	8.7
	Total	1858	300	64.5	54.7	26.5	42.2	9.1
	Males	1964±84	313±36	63.7	60±7	2.75	43±5	8.8
SMF	Females	1710±132	278±29	65.0	50±4	26.3	37±6	8.7
	Total	1826	294	64.4	54.6	26.9	39.8	8.7
Means of Totals		1875	300	63.9	56.4	27.0	42.5	9.1

* OO, olive oil; CMF, cow milk fat; GMF, goat milk fat, SMF, sheep milk fat.

Table 3: Effects of dietary olive oil (OO), and ghee of cow milk fat (CMF), goat milk fat (GMF), and sheep milk fat (SMF) on concentrations of serum lipids of males and females

Serum [1] Lipids	O O		CMF		GMF		SMF	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
TC (mg/dl)	162±14	155±15*	160±10	170±17*	164±7	177±8*	165±12	186±14*
Males	151±15	147±18*	154±14	163±11*	161±16	172±18*	160±15	178±19*
Females	156	151	157	166	162	174	162	182
LDL-c(mg/dl)								
Male	106±13	100±8	104±9	112±7*	109±11	119±13*	109±15	128±17*
Females	95±14	92±15*	99±16	107±12*	104±13	112±16*	106±10	122±17*
Total	100	96	102	110	166	115	107	125
HDL-c(mg/dl)								
Males	35±7	34±4	35±6	37±2	33±5	36±5	36±4	38±2
Females	35±3	34±4	32±2	33±3	35±6	38±7	33±4	35±3
Total	35	34	33.4	34.8	34.1	37.1	34.4	36.4
TC/HDL-c								
Males	4.63±0.4	4.56±0.6	4.57±0.5	4.59±0.6	4.97±0.3	4.92±0.1	4.58±0.3	4.89±0.4
Females	4.31±0.3	4.32±0.4	4.81±0.3	4.94±0.5	4.60±0.1	4.53±0.7	4.85±0.6	5.09±0.8
Total	4.46	4.43	4.70	4.78	4.77	4.71	4.73	4.99
LDL-c/HDL-c								
Males	3.03±0.2	2.94±0.1	2.97±0.3	3.03 ±0.2	3.30±0.5	3.31±0.1	3.03±0.3	3.37±0.4*
Females	2.71±0.2	2.71±0.1	3.09±0.3	3.24±0.5	2.97±0.1	2.75±0.2	3.21±0.3	3.49±0.4*
Total	2.86	2.81	3.09	3.19	3.12	3.11	3.12	3.43
TG (mg/dl)								
Males	104±7	107±6	107±8	105±6	111±7	108±15	101±14	99±13
Females	105±7	109±6	115±5	113±7	110±6	108±3	107±4	104±5
Total	105	108	111	109	111	108	104	106

[1]: TC, total cholesterol; LDL-c, low density lipoprotein- cholesterol; HDL-c, high density lipoprotein- cholesterol; TG, triglycerides. * Significantly different from initial value $P < 0.05$.

As seen in Table 2, the proximate analysis for mean daily intake of energy, carbohydrates, fats and proteins, of both sexes at the four diets, was 1875Kcal, 300g, 56.4g and 42.5g, respectively. Food intake study was noted that OO consumed by volunteers more than any other oil or fat. It was used at least twice a day; consistently for breakfast and lunch, especially with chickpea and thyme, and many times used for cooking. The ghee made from SMF may be used more than other ghee types. The usage of ghee of CMF may be negligible. Food consumption of males was generally higher than that for females. Investigated oil and fats formed about 58% of the total dietary fat intake.

Table 4: Serum lipid responses (mg/dl and %) from the base line of males and females fed dietary olive oil (OO), and ghee of cow milk fat (CMF), of goat milk fat (GMF), and of sheep milk fat (SMF)

Dietary Fat Type	Gender	*TC		LDL-C		HDL-C		Triglycerides	
		mg/dl	%	mg/dl	%	mg/dl	%	mg/dl	%
	Males	-7	-4.32	-6	-5.66	-1	-2.86	+3	+2.88
OO	Females	-4	-2.65	-3	-3.16	-1	-2.86	+4	+3.81
	Table	-5.38	-3.42	-4.38	-4.31	-1	-2.86	+3.54	+3.38
	Males	+10	+6.25	+8	+7.41	+2	+5.71	-2	-1.87
CMF	Females	+9	+5.84	+8	+8.08	+1	+3.13	-2	-1.74
	Total	+9.46	+6.03	+8	+7.77	+1.88	+4.31	-2	-1.80
	Males	+13	+7.93	+10	+9.17	+3	+9.09	-3	-2.70
GMF	Females	+11	+6.83	+8	+7.69	+3	+8.57	-2	-1.82
	Total	+11.92	+7.33	+8.92	+8.37	+3	+8.81	-2.46	-2.22
	Males	+21	+12.73	+19	+17.43	+2	+5.56	-2	-1.98
SMF	Females	+18	+11.25	+16	+15.09	+2	+6.06	-3	-2.80
	Total	+19.38	+11.93	+17.38	+16.16	+2	+5.83	-2.54	-2.42

* TC, total cholesterol; LDL-c, low density lipoprotein- cholesterol; HDL-c, high density lipoprotein-cholesterol.

Serum lipid concentrations of the participants at starting and final of the 4wk feeding of OO or ghee types are shown in Table 3, and the serum lipids responses of the subjects are shown in Table 4. OO consumption was significantly lowered levels of serum total cholesterol (-3.42%), LDL-c (-4.31%) and caused slight reduction in HDL-c (-2.86%) under the initial level. On the contrary, ghee types were significantly raised serum total cholesterol level (about +8.43%), LDL-c (about +10.8%), and caused an increase in HDL-c level (about +6.32%) above the initial levels. Among the ghee types, SMF caused the highest raise in serum total cholesterol level (+11.93%) and LDL-c level (+16.16%). The highest raise in HDL-c level (+8.81%) showed with GMF. When the responses in serum cholesterol in the males and females subjects were considered separately for the four diets (Table 4), males were generally found to exhibit greater than females. Table 3 also show that the ratios of serum total cholesterol (TC) /HDL-c and LDL-c /HDL were slightly decreased with OO, whereas slightly increased with ghee types, but the LDL-c/HDL-c ratio after GMT was sustained. SMF resulted in higher raising effect of TC/HDL-c ratio (+0.26) and LDL-c/HDL-c ratio (+0.31) as compared to the other ghee types. Serum TG level was increased in both sexes after OO period (+3.38%), whereas reduced in both sexes after periods of ghee types (about –2.15%).

In general, serum cholesterol levels of volunteers tended to be low (average value < 200mg/dl). Several contributing factor may be affected this level. Many nutrients other than dietary fat influence cholesterol concentrations such as low cholesterol concentration diet, dietary fiber, complex carbohydrate intake and protein source (Grundy, 1986; Cohen *et al*, 1994). The results showed that OO brought about a dramatic reduction in the concentrations of serum total cholesterol, LDL-c and HDL-c and these reductions may be due to high n-9 MUFA (66.4% oleic acid) content in OO.

There has been much interest regarding the components that contribute to the beneficial health effects of the Mediterranean diet. Olive oil is the fat of choice in the Mediterranean area. Polyphenolic compounds found in olive oil may be contribute to the lower incidence of coronary heart disease in this area. Recent findings demonstrate that olive oil phenolics inhibit oxidation of low-density lipoproteins (Visioli and Galli, 1999). Moreover, OO can be advised as an alternative to high-carbohydrate diets in diabetic and carbohydrate-sensitive patients (Garg, 1994), and for routine frying or cooking practices, that is it did not produce toxic aldehydes (Grootvelt, 1998). The results were in agreement with observations of Perez-Jimenez (1995) and Kris-Etherton (1999), whereas in disagreement with results of Spiller *et al.* (1998) who mentioned that OO diet resulted in no significant change in men and women plasma total cholesterol, LDL-c and HDL-c. The hypercholesterolemic effect of ghee types, especially SMF may be related to high SFA content and low p: s and m+p/s ratios (Lee *et al.*, 1989). Besides that the ghee types contained appreciable amount of short and medium chain FAs. These FAs may be work as an activator for hepatic hydroxyl-methyl-glutarate-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis. Short chain FAs with two other FAs namely, lauric and myristic acids were thought as hypercholesterolemic agents (Elson, 1992) and may be cause high rate of cholesterol absorption (Ide *et al.*, 1979). Myristic acid appeared to be the most potent cholesterol raising SFA in human (Hajri *et al.*, 1998). Other reason, but less effect, that is the cholesterol content of ghee types may be increased blood cholesterol concentration (Gurr, 1989). Among the ghee types, SMF came the first serum cholesterol raising fat and may be due to its relatively high myristic acid content (12.2%), low p:s ratio (0.03) and m+p/s ratio (0.37), (Table 1). The results were in agreement with those results showed in

human (Barr *et al.*, 1992), in rats (Thannoun, 1993), and hamster (Cohen *et al.*, 1994).

The effect of dietary fat type on serum TG was reversed its effect on serum cholesterol, that is the blood cholesterol lowering effect of OO accompanied with slight increase in serum TG compared with the vice versa effect of ghee types. These effects imply that the differences in FA compositions of OO and ghee types may be resulted in differences in their effects on secreting and degrading of serum TG. OO period resulted in slight increase in serum TG concentration of both sexes and may be due to the increased secretion of very low density lipoprotein (VLDL)-TG (Heimberg and Wilcox, 1972). Hence, the TG reducing effect showed in both sexes after periods of ghee types may be attributed to the decreased TG secretion (Jackson *et al.*, 1977). Potenger and Getz (1971) stated that the orotic acid blocked the final step of VLDL secretion by inhibiting the linkage of carbohydrate to the apoprotein. This observation indicates the role of orotate, which occurred in milk fats, in reducing the TG transportation from liver to the blood (Nair and Mann, 1977).

High blood cholesterol is the major risk factor for cerebrovascular disease (CVD), (NCEP, 1993). Several studies confirmed that the high blood cholesterol in young adults is a predict of CVD risk in later life (Myers *et al.*, 1995). Present results showed that the replacing ghee by OO lowered the predicted risk of CVD due to the decline in serum total cholesterol. The ratio of TC/HDL-c was mentioned as indicator of CVD risk (Kinosian *et al.*, 1994). Results of this study showed a slight reduction in TC/HDL-c ratio after OO periods and slight raising in this ratio after periods of ghee types. The results also showed that the LDL-c/HDL-c ratio was in accordance with the former ratio.

Finally, ghee types showed raising effects of blood cholesterol. These effects may be due to their low p:s and m+p/s ratios. The differences between effects of ghee types on blood cholesterol level mainly attributed to these differences in their fatty acid compositions. However, the cholesterol content of ghee types had no effect on these differences. OO, which is high oleic acid, may be a good alternative of dietary fat for reducing blood cholesterol level.

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