



### Original Article

## Comparison of the effects of clarified yogurt butter and commercial dietary oils on serum fatty acid composition in rat

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### ABSTRACT

**Objectives:** The link between dietary fat and heart disease has been established for decades. Although, the atherogenic effect of saturated fatty acids is somehow controversial, the cardioprotective effects of polyunsaturated fatty acids (PUFA) are clearly documented. This study investigated the effects of different dietary oils on serum fatty acid composition in rat.

**Methods:** Six-week male Wistar rats (n=40) were randomly assigned into 5 groups and received chow diet for 3 weeks, before blood sample collection from 4 rats of each group. Remaining rats were then received chow diet or chow diet supplemented with yogurt butter, olive oil, soybean oil, or flaxseed oil providing 53% of energy from fat for another 4 weeks. Blood samples were then collected and serum fatty acid composition analysed by gas chromatography.

**Results:** Total saturated, monounsaturated, and n-6 and n-3 PUFA were significantly higher in rats received yogurt butter, olive oil, soybean oil, and flaxseed oil supplemented diets, respectively, compare to the controls ( $P<0.05$ ). In addition, the ratio of n-6:n-3 PUFA markedly decreased by consumption of flaxseed oil compare to other diets ( $P<0.05$ ). Rats received olive, soybean, and flaxseed oils showed significantly lower serum triacylglycerol compare to the control ( $P<0.05$ ). Moreover, no significant effect observed on serum cholesterol in rats consumed yogurt butter.

**Conclusion:** We clearly showed that the composition of dietary fat impact on serum fatty acid composition and consumption of yogurt butter had no significant atherogenic effects on serum total and LDL cholesterol.

**Keywords:** Cardiovascular Diseases; Dietary Fats; Fatty Acid; Gas Chromatography; Serum

## Introduction

Epidemiological studies have confirmed a strong association between fat intake, especially saturated and *trans*-fatty acids, plasma cholesterol levels and rate of CHD mortality [1,2]. However, it is clear that early atherosclerosis is largely preventable by modifying nutritional behaviour and lifestyle [3]. While it is accepted that polyunsaturated fatty acids (PUFAs) of the n-6 and n-3 series are essential nutrients and have distinct biological effects contributing to their cardioprotective action [3], both

the absolute intakes (g/d) and the n-6/n-3 ratio should be the main consideration to achieve optimal CHD health benefits [4,5].

In addition, dietary interventional trials have confirmed that the type of fatty acid consumed is more important than the amount of total fat in determining the rate of CHD events [3], therefore, fatty acid specific identity, individual intake level and dietary origin must be considered during investigations [6]. There is a general belief in the atherogenic effect of saturated fatty acids;

however, a recent meta-analysis has shown that there is no significant evidence for concluding association of dietary saturated fat with an increased risk of CHD [7,8]. On the other hand, PUFAs display cardioprotective effects and are usually associated with reduced plasma total and low density lipoprotein-cholesterol (LDL-C) [9, 10], thereby correcting blood lipid profiles and reduce the risk of CHD mortality [3,11]. There is growing evidence indicating the impact of different dietary fats on serum and/or tissue fatty acid composition, however, mostly the effects of pure individual fatty acids, such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linoleic acid (LA), and alpha-linolenic acid (ALA), have been studied rather than commercially available dietary oils [12-15]. Furthermore, demographic and geographic variation in food habits specially consuming traditional oils in some countries may have deleterious impacts on CHD risks. This study sought to evaluate the effects of Iranian traditional saturated fatty acid-rich yogurt butter (also called clarified butter) and different commercial dietary oils including olive oil (MUFA-rich), soybean oil (n-6 PUFA-rich), and flaxseed oil (n-3 PUFA-rich) on serum fatty acid composition and blood lipid profile in rat.

## Materials and Methods

Fatty acyl methyl ester mixture (FAME) and individual fatty acid standards were purchased from Sigma-Supelco (Sigma Aldrich Company Ltd., Poole, UK). Merck Chemicals Co. (Merck Chemicals, Darmstadt, Germany) supplied chemicals and gas chromatography grade solvents. Screw-cap borosilicate glass tubes were obtained from Fisher Scientific (Fisher Scientific, Loughborough, UK) and colorimetric assay kits for determination of serum cholesterol, triacylglycerol, and HDL-C were purchased from Zist Chem Diagnostics (Zist Chem Diagnostics, Tehran, Iran).

All glassware, borosilicate glass tubes with Teflon-lined screw-caps (100×13 mm) and magnetic stirring bars (10×3 mm) were rinsed with chloroform-methanol 2:1 (v/v) and dried under nitrogen.

### Animal work

Six-week male Wistar rats (74±10 g) were obtained from Pasture Institute (Pasteur Institute, Tehran, Iran) and were studied under the protocol approved by Hamadan Universi-

ty of Medical Sciences Animal Research Committee. Rats were randomly assigned into 5 groups (n=8) and kept in individual plastic cages with constant humidity/temperature and a 12 hour dark-light cycle.

### Study design and sample collection

Rats received standard rat chow diet (CD) ad libitum for 3 weeks to stabilise blood lipid profile. At the end of 3 weeks, 4 rats from each group were sacrificed; fasting blood samples collected from inferior vena cava and serums were separated and stored at -20°C. The remaining rats received standard chow diet (group 1) or one of the isocaloric and isolipidic experimental diets (groups 2-5) containing yogurt butter (SFA-rich), olive oil (MUFA-rich), soybean oil (n-6 PUFA-rich), or flaxseed oil (n-3 PUFA-rich) providing 53% of daily energy intake from fat for 4 weeks. Rats were then sacrificed and blood samples collected.

### Fatty acid extraction and esterification

Fatty acids were extracted and esterified by a previously described direct transesterification method [16]. Briefly, 10 µl of commercial oils, 100 µl of serum samples or 50 µg of experimental diets were mixed with 2 ml of methanol-chloroform (4:1 v/v) containing 20 µg of methyl nonadecanoate (MeC19) as internal standard in a screw-cap glass tubes. 200 µl of acetyl chloride was then slowly added into tubes and tubes were placed 60 min in a water bath (100°C) for esterification. At the end of methanolysis, 5 ml of 6% potassium carbonate was slowly added to stop the reaction and to neutralize mixture. Tubes were then shaken and centrifuged at 1800×g for 10 min and an aliquots of upper phase containing fatty acid methyl esters were collected and stored at -80°C until injection into the chromatograph [16]. In addition, esterification and/or transesterification procedure was validated and the percentage of recovery was determined using a tridecaenoic acid (C13:0) and methyl nonadecanoate (MeC19:0) standard mixture [16].

### Gas chromatography analysis of fatty acids

Fatty acids were chromatographed as methyl ester on a 100-m CP-Sil88 fused silica capillary column. Analysis was performed on a Varian CP-3800 (Varian, Inc., CA, USA) gas chromatograph equipped with a flame ionization detector. Gas chromatograph was calibrated using a stand-

ard mixture of 37 fatty acyl methyl esters and the split ratio was set at 10:1. The peaks obtained were identified by comparison of their relative retention times with those of known fatty acid methyl ester standards. Quantification was accomplished by peak area comparison with the internal standard, methyl nonadecanoate (MeC19).

### **Data quantisation**

The amounts of individual fatty acids ( $C_{fa}$ ) were calculated using the expression  $C_{fa} = (A_{fa}/A_{is}) \times (C_{is}/V) \times (1/R_{rf})$ , where  $A_{fa}$  is the chromatographic area units of the fatty acids whose concentration is to be determined,  $A_{is}$  is the chromatographic area units for the internal standard,  $C_{is}$  is the concentration of the internal standard used in the reactions in terms of  $\mu\text{g/ml}$ , and  $V$  is the volume in ml of serum sample used in an experiment [17]. The relative response factor ( $R_{rf}$ ) for each peak was determined using the expression  $R_{rf} = (A_s/A_{is}) \times (C_{is}/C_s)$ , where  $A_s$  and  $A_{is}$  are the chromatographic area units of standard fatty acid methyl ester (FAME) and internal standard, whereas  $C_s$  and  $C_{is}$  are the concentration of FAME and internal standards [17]. In addition, a well-identified peak of MeC19:0 was individually detected confirming of no interference of internal standard peak and rat serum chromatogram.

### **Determination of serum lipid profile**

Serum triacylglycerol, total cholesterol and HDL-C were determined by colorimetric methods using commercial enzymatic kits as indicated in the manufacturer's instructions whereas LDL-C level was calculated according to the Friedewald's formula [18].

### **Statistical analysis**

Data was analysed by SPSS 11 and One-Sample Kolmogorov-Smirnov test was applied to determine normal distribution of data. Results were presented as mean  $\pm$  SD and independent samples T-test used to compare the mean differences. Moreover, One-Way ANOVA followed by Post Hoc, Tukey and Dunnett tests was used to analyse differences between groups and  $P < 0.05$  was considered as significant difference.

## **Results**

### **Fatty acid analysis of oils and experimental diets**

Analysis showed that clarified yogurt butter is rich (68.45%) in saturated fatty acids (Table 1), whereas olive oil, soybean oil, and flaxseed oil were highly unsaturated containing over 85%, 84%, and 89% of unsaturated fatty acids, respectively. Monounsaturated fatty acids have been found in a high content (77.73%) in olive oil and were also been detected in corresponding experimental diet (72.71%). Similarly, soybean and flaxseed oils have been found rich in n-6 PUFA (55.16%) and n-3 PUFA (55.0%), respectively (Table 1). Total polyunsaturated fatty acids were higher in both soybean (62.46%) and flaxseed oils (66.93%) and corresponding diets (61.34% and 64.21%, respectively) compare to other oils and diets. In addition, unlike yogurt butter which was more than 28% made of C6 to C15 saturated fatty acids, soybean and flaxseed oils showed no detectable amounts of C6-C15 and C17 fatty acids (Table 1). Trans C18:1 n-9 fatty acid has only been detected in yogurt butter, whereas C20:0, C20:1, and C22:0 fatty acids were detected in all commercial oils, except yogurt butter. Moreover, no measurable amount of arachidonic acid (AA), eicosapentaenoic acid (EPA), or docosahexaenoic acid (DHA) was found in any of dietary oils. Analysis also showed that fatty acid composition of experimental diets was dependent on fatty acid composition of the corresponding oil with a high SFA, MUFA, n-6 PUFA and n-3 PUFA in CD+Yogurt butter, CD+Olive oil, CD+Soybean oil, and CD+Flaxseed oil diets, respectively (Table 1).

### **Effects of dietary fat on serum fatty acid composition**

Statistical analysis of serum fatty acid composition indicated a significant increase in serum total saturated fatty acids in rats received yogurt butter, whereas consumption of olive oil caused a slight but not significant increase in serum total monounsaturated fatty acids (Table 2). Serum total n-6 PUFA has been found significantly higher after feeding of soybean oil and similarly serum n-3 PUFA has been markedly increased by the consumption of flaxseed oil. Rats consumed soybean or flaxseed oil showed a significantly higher amounts of serum total polyunsaturated fatty acids compare to other rats. A dramatic increase was also observed in the ratio of total unsaturated fatty acids (UFA) to saturated fatty acids in rats received either olive oil, soybean oil, or flaxseed oil compare to those received yogurt butter. In fact, consumption of yogurt butter sig-

nificantly reduced the ratio of unsaturated to saturated fatty acids in comparison to other diets (Table 2). In contrast to the consumption of soybean oil which caused a great elevation in the ratio of serum n-6 to n-3 polyunsaturated fatty acids, rats received flaxseed oil had the highest level of serum alpha-linolenic acid and the least amount of n-6 to n-3 ratio (Table 2). In addition, digestion of soybean oil caused a remarkable increase in serum arachidonic acid while an increment of eicosapentaenoic acid has been observed in rats treated by flaxseed oil. In the same way, consuming of soybean oil enhanced serum linoleic acid whereas alpha-linolenic acid was in-

creased by the consumption of flaxseed oil (Table 2). Analysis of serum fatty acid composition also showed that C16:0 palmitic and C18:0 stearic acids are the most abundant saturated fatty acids in the serum of all rats, regardless of the type of the oil consumed. Moreover, consumption of yogurt butter significantly increased serum myristic (C14:0) and palmitic (C16:0) acids and *trans* C18:1 n-9 was observed only in the serum of clarified yogurt butter consumed rats, whereas *cis* C18:1 n-9 isomer was markedly higher in rats received olive oil, as expected.

**Table 1.** Fatty acid composition of chow diet, commercial dietary oils, and experimental diets

Fatty acid	Fatty acid [%]								
	Oils				Experimental Diets				
	YB	OO	SO	FO	CD	CD+YB	CD+OO	CD+SO	CD+FO
C6:0	2.67					2.32			
C8:0	1.70					1.52			
C10:0	3.86					3.34			
C11:0	0.37					0.19			
C12:0	4.78					4.32			
C13:0	0.66					0.39			
C14:0	12.21				0.32	11.59	0.11	0.12	0.20
C14:1	0.86					0.66			
C15:0	1.26					1.15			
C16:0	29.38	10.92	10.53	5.70	18.8	29.96	11.6	10.87	7.36
C16:1	1.50	0.8			0.42	1.43	0.59	0.19	0.20
C17:0	0.66					0.53			
C18:0	10.9	3.17	4.27	4.50	3.61	11.35	3.28	4.03	4.36
C18:1n-9 c	19.11	76.69	21.7	21.70	34.76	19.96	71.89	22.23	22.57
C18:1n-9 t	3.90					3.17			
C18:2n-6 (LA)	5.19	7.12	55.16	11.93	37.56	6.58	10.77	54.50	14.96
C18:3n-3 (ALA)	0.99	0.59	7.30	55.00	2.91	1.08	0.91	6.86	49.25
C20:0		0.38	0.31	0.17	0.54	0.13	0.39	0.33	0.19
C20:1		0.24	0.40	0.16	0.37	0.11	0.23	0.42	0.17
C22:0		0.09	0.33	0.13	0.34	0.1	0.13	0.33	0.13
C22:1				0.69		2.32			0.59
C24:0									0.10
ΣSFA	68.45	14.56	15.44	10.50	0.37	0.11	0.10	0.12	12.34
ΣMUFA	25.37	77.73	22.1	22.57	23.98	67.0	15.61	15.80	23.54
Σn-6 PUFA	5.19	7.12	55.16	11.93	35.54	25.33	72.71	22.84	14.96
Σn-3 PUFA	0.99	0.59	7.3	55.00	37.56	6.58	10.77	54.5	49.25
Total PUFA	6.18	7.71	62.46	66.93	2.91	1.08	0.91	6.86	64.21
Total UFA	31.55	85.44	84.56	89.50	40.47	7.66	11.68	61.36	87.75
UFA:SFA	0.46	5.87	5.48	8.52	76.01	32.99	84.39	84.20	7.11
Σn-6:Σn-3	5.20	12.06	7.56	0.22	3.17	0.49	5.41	5.33	0.30

CD, chow diet; FO, flaxseed oil; OO, olive oil; SO, soybean oil; YB, yogurt butter; ALA, alpha-linolenic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids.

**Effects of dietary fat on serum lipid profile**

Triacylglycerol concentration was significantly different in the serum of test and control rats (Fig. 1A). In addition, ANOVA analysis showed a great reduction in the concentration of serum TAG in rats received unsaturated olive, soybean, or flaxseed oil. However consumption of Iranian traditional yogurt butter had no beneficial or adverse effects on serum TAG in the rats compare to the controls (Fig. 1B). Although statistical analysis showed a trend to lower levels of cholesterol in rats received soybean or flaxseed oil compare to the controls, serum total cholesterol did not significantly differ in rats consumed ei-

ther control or experimental diet, or in the rats of different experimental groups (data not shown). Serum HDL-C level was remarkably increased ( $P<0.05$ ) by consumption of experimental diets (Fig. 2A). However, the significant difference in serum HDL-C has only been observed in rats treated with yogurt butter and olive oil compared to the controls (Fig. 2B). Unlike enhancement in serum HDL-C levels, serum LDL-C has been found markedly reduced in rats received experimental diets compared to control group, although, a significant difference has not been observed between different experimental diets (data not shown).

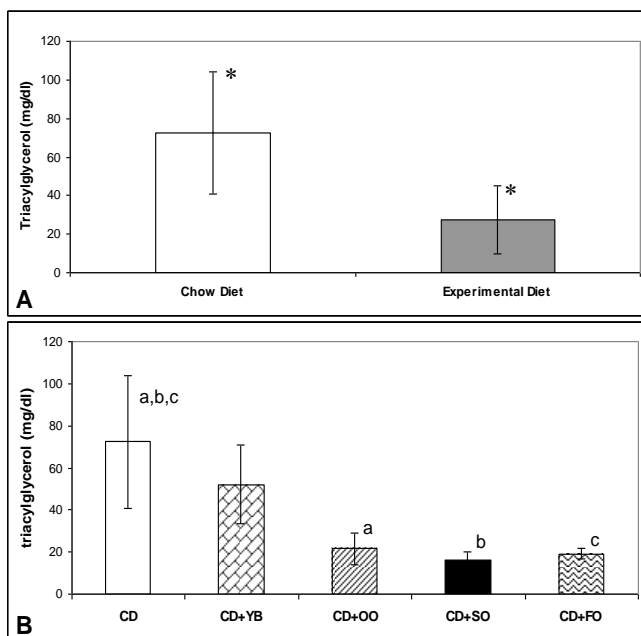
**Table 2.** Serum fatty acid composition of rats received control chow diet (CD) or experimental diet (mixture of chow diet and dietary commercial oil) (n=8)

Fatty acid	CD	CD+YB	CD+OO	CD+SO	CD+FO
C14:0	0.58±0.15 <sup>a</sup>	1.19±0.08 <sup>a-d</sup>	0.25±0.03 <sup>b</sup>	0.39±0.07 <sup>c</sup>	0.27±0.19 <sup>d</sup>
C15:0	0.63±0.18 <sup>a,b</sup>	0.52±0.07	0.28±0.05 <sup>a</sup>	0.44±0.16	0.29±0.21 <sup>b</sup>
C16:0	21.13±1.41	21.22±1.4 <sup>a-c</sup>	15.58±1.11 <sup>a</sup>	15.74±0.98 <sup>b</sup>	15.06±0.93 <sup>c</sup>
C16:1	1.79±0.77 <sup>a-c</sup>	0.9±0.18	0.32±0.54 <sup>a</sup>	0.37±0.06 <sup>b</sup>	0.61±0.19 <sup>c</sup>
C17:0	0.5±0.09 <sup>a,b</sup>	0.22±0.08 <sup>a</sup>	0.11±0.02 <sup>b</sup>		
C18:0	9.7±1.42 <sup>a</sup>	13.38±1.52 <sup>a,b</sup>	14.35±0.52	13.76±0.46	16.09±1.05 <sup>b</sup>
C18:1n-9 t		0.56±0.12			
C18:1n-9 c	16.06±3.01	14.06±2.01 <sup>a</sup>	19.68±2.06 <sup>a-c</sup>	7.88±0.97 <sup>b</sup>	10.56±1.26 <sup>c</sup>
C18:2n-6 (LA)	23.59±3.16	20.27±0.6 <sup>a</sup>	13.57±1.0 <sup>b</sup>	27.33±1.24 <sup>a,b</sup>	24.25±1.69
C18:3n-6	0.14±0.09	0.16±0.04	0.32±0.14 <sup>c</sup>		
C18:3n-3 (ALA)	0.69±0.16 <sup>a</sup>	0.42±0.15 <sup>b</sup>	0.27±0.13	0.83±0.35 <sup>d</sup>	6.44±2.11 <sup>a-d</sup>
C20:1n-9	0.09±0.08		0.11±0.13		
C20:2	0.26±0.13		1.13±0.12 <sup>b</sup>	0.2±0.16	
C20:3n-6 (DGLA)	0.85±0.12 <sup>a,b</sup>	1.41±0.34 <sup>a</sup>	26.68±2.4	0.69±0.13	0.89±0.3
C20:4n-6 (AA)	15.74±4.62 <sup>a</sup>	15.67±2.2 <sup>b</sup>	1.45±0.38 <sup>c</sup>	26.25±1.84 <sup>a-c</sup>	12.75±2.1 <sup>c</sup>
C20:5n-3 (EPA)	2.09±0.27 <sup>a</sup>	3.17±0.41 <sup>b</sup>	0.71±0.09 <sup>a</sup>	2.06±0.34 <sup>d</sup>	7.33±1.21 <sup>a-d</sup>
C22:0	0.31±0.17 <sup>a,b</sup>	0.4±0.16	3.37±0.69 <sup>a</sup>	0.64±0.12 <sup>b</sup>	0.53±0.16
C22:6n-3 (DHA)	4.9±0.74	5.5±0.27 <sup>a-c</sup>	0.54±0.08	2.26±0.15 <sup>b</sup>	2.6±0.8 <sup>c</sup>
C24:0	0.37±0.2 <sup>a</sup>	0.44±0.17 <sup>b</sup>	0.94±0.12 <sup>c</sup>	0.61±0.21	0.99±0.43 <sup>a,b</sup>
C24:1	0.5±0.13 <sup>a</sup>	0.41±0.06 <sup>b</sup>	31.99±0.77 <sup>b</sup>	0.41±0.12 <sup>d</sup>	1.27±0.17 <sup>a-d</sup>
ΣSFA	33.33±1.29 <sup>a</sup>	37.44±0.36 <sup>a-d</sup>	21.25±2.05 <sup>a,b</sup>	31.88±2.09 <sup>c</sup>	33.24±1.17 <sup>d</sup>
ΣMUFA	18.34±3.49	15.93±2.22	41.58±1.55 <sup>c</sup>	8.5±1.11 <sup>a</sup>	12.45±1.09 <sup>b</sup>
Σn-6 PUFA	40.62±3.63 <sup>a</sup>	37.52±2.32 <sup>b</sup>	5.17±0.64 <sup>c</sup>	54.46±0.91 <sup>a-d</sup>	37.91±1.98 <sup>d</sup>
Σn-3 PUFA	7.71±0.8 <sup>a</sup>	9.1±0.28 <sup>b</sup>	46.75±2.0 <sup>c</sup>	5.16±0.46 <sup>d</sup>	16.39±1.68 <sup>a-d</sup>
Total PUFA	48.33±3.53 <sup>a</sup>	46.62±2.45 <sup>b</sup>	8.11±0.87 <sup>c</sup>	59.62±1.27 <sup>a-c</sup>	54.30±0.78
Σn-6:Σn-3	5.34±0.87 <sup>a</sup>	4.12±0.24 <sup>b</sup>	2.13±0.07	10.61±0.92 <sup>a-d</sup>	2.34±0.37 <sup>d</sup>
ΣUFA:ΣSFA	2.0±0.11 <sup>a</sup>	1.67±0.02 <sup>a-d</sup>	0.32±0.14 <sup>c</sup>	2.15±0.19 <sup>c</sup>	2.01±0.10 <sup>d</sup>

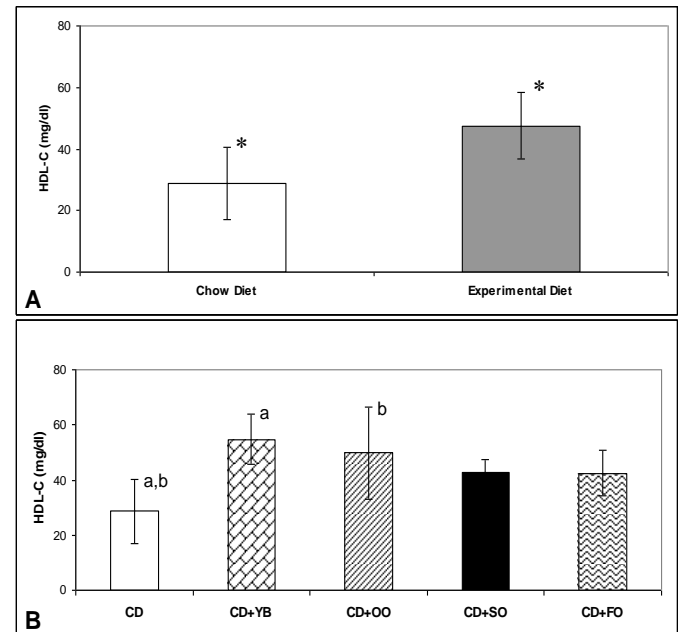
All data are mean ± SD. Similar superscripts letters in the same row indicate a significant difference among the data ( $P<0.05$ ). a-d superscript represents a, b, c, and d letters whereas a-c indicates a, b, and c. AA, arachidonic acid; ALA, alpha-linolenic acid; DGLA, di-homo- $\gamma$ -linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids.

## Discussion

The link between dietary fat and heart disease has been clearly established [11] and the results of epidemiologic investigations support the hypothesis that coronary disease risk depends on the quality rather than quantity of dietary fat [19]. Different fatty acids differ in up- or down-regulation of gene expression [20,21], thereby they differentially influence on the prevention or on the progression of diseases [14,22,23]. Although a large scale of clinical trials point to the impact of different dietary fats on serum and/or tissue fatty acid composition, mostly the effects of pure individual fatty acids, such as EPA and DHA, or their effects in the combination with a basic diet have been studied [12-15] rather than investigating the effects of commercially available dietary oils, which are mixtures of different fatty acids having nutritionally diverse effects. In this study we sought to examine the effects of dietary commercial oils including clarified yogurt butter (SFA-rich), olive oil (MUFA-rich), soybean oil (n-6 PUFA-rich), and flaxseed oil (n-3 PUFA-rich) on serum fatty acid composition in rat. This study also represents the impact of different oils on blood lipoprotein profile.



**Figure 1.** Concentration of serum triacylglycerol in (A) rats received control or experimental diets, and (B) in rats fed different experimental diets containing commercial oils. Eight Wistar rats were allocated in each group (n=8). Data is presented as Mean  $\pm$  SD and a, b, and c represent significant differences between groups designated by similar letters ( $P < 0.05$ ). Stars (\*) indicate significant difference ( $P < 0.05$ ). CD, chow diet; FO, flaxseed oil, OO, olive oil; SO, soybean oil; YB, yogurt butter.



**Figure 2.** Concentration of serum HDL-C in (A) rats received control or experimental diets, and (B) in rats fed different experimental diets containing commercial oils. Eight Wistar rats were allocated in each group (n=8). Data is presented as Mean  $\pm$  SD and a, and b represent significant differences between groups designated by similar letters ( $P < 0.05$ ). Stars (\*) indicate significant difference ( $P < 0.05$ ). CD, chow diet; FO, flaxseed oil, OO, olive oil; SO, soybean oil; YB, yogurt butter.

Our observations indicated significant increase of serum saturated fatty acids in rats received yogurt butter, which is a concomitant of high SFA content in consumed yogurt butter. Similarly, a marked increase in total n-6 and n-3 PUFA has been observed in rats received meal containing soybean oil and flaxseed oils, respectively. These observations are in accordance with a weight of evidences that indicate dietary fat modulate serum and tissue fatty acid composition [23-26].

Substitution of dietary saturated fatty acids by MUFA or PUFA to increase serum total unsaturated fatty acids, modify blood lipid profile, and to enhance the ratios of UFA:SFA and PUFA:SFA has been the main objective of different epidemiological studies and clinical trials during last few decades [19,27,28]. Accordingly, many dietary recommendations have been stated confirming the beneficial effects of the use of unsaturated fatty acids [3,4]. In the same way, our results showed that receiving a meal containing MUFA (olive oil), n-6 PUFA (soybean oil), or n-3 PUFA (flaxseed oil) significantly increases serum total UFA and markedly gives rise to UFA:SFA ratio compare to consuming of SFA (yogurt butter). Additionally, unlike the effects of saturated fatty acids, consumption of n-6 and

n-3 polyunsaturated fatty acids not only dramatically increased serum total PUFA but also enhanced the ratio of PUFA:SFA. These observations together with the results of previous works explain the benefit of substitution of dietary SFA by MUFA or PUFA in the management of coronary heart disease and in reducing the CHD risks. The results of this study showed an increment in serum n-3 PUFA and a noticeable decline in n-6:n-3 PUFA ratio in rats received flaxseed oil compare to other diets. This observation is in line with other studies that have investigated the effects of different dietary fats on n-6:n-3 PUFA ratio [23]. This finding is of great importance since the recommendation of international societies and investigators is to reduce the ratio of n-6:n-3 PUFA to a range of 3-5:1 corresponding to a lower risk of CHD [29]. It has previously been reported that flaxseed oil is richer in ALA than other dietary vegetable oils such as soybean, canola, sunflower and olive oil and its consumption increases serum and tissue content of ALA [4,30,31]. Likewise, we showed that rats fed a flaxseed oil containing meal have significantly a higher level of serum ALA and a lower ratio of LA: ALA compare to controls and other groups.

Our results clearly showed that the composition of dietary fat impact on serum fatty acid composition. Therefore, it can be concluded that daily intake of dietary commercial oils, and not necessarily pure individual fatty acids, impacts on serum fatty acid composition thereby may modify blood lipid profile in accordance to prevent or to inhibit progression of coronary heart disease.

In addition to the modulation of serum fatty acid composition, substitution of dietary saturated fatty acids by MUFA or PUFA influences on serum triacylglycerol, total cholesterol, HDL-C, and LDL-C levels [22,27,32] via up- or down-regulation of lipid metabolism-related genes expression [20,21,33] leading to the inhibition of CHD development [4,22,23,28,34]. *In vivo* human and animal studies have shown that receiving diet containing polyunsaturated fatty acids reduces serum TAG [19,22,34,35]. Similar observations made in this study confirming that rats received olive, soybean, and flaxseed oils represent a significantly lower serum TAG compare to the control group. More importantly, reduction of TAG was greater in flaxseed oil fed rats in comparison to the other groups. This ob-

ervation is in the agreement with previous studies proposing a key role for n-3 PUFA in reducing of serum triacylglycerol [19,23,27,36].

Although cholesterol lowering effects of dietary PUFA have previously been reported [23,27] and our findings also showed a trend to lower levels of cholesterol in rats received either n-6 or n-3 PUFA compared to controls, a significant difference has not been indicated between different experimental groups. The non-response of cholesterol level, as observed here, can be explained to some extent by the complex composition of oils, the presence of different saturated and unsaturated n-9, n-6, and n-3 fatty acids in oils which act differentially, and by the relatively short exposure time (4 weeks) as long-term feedings have mostly been used in other studies [26,37].

A review in literature shows contradictory effects of dietary fats on serum HDL-C indicating an increased [38], a significantly decreased [27], or unchanged remaining [36] of HDL-cholesterol level after ingestion of olive oil and corn oil. Our result obtained from olive oil feeding rats is supported by the studies that confirming a rise in HDL-C level [38]. We also showed that consumption of clarified yogurt butter increased serum HDL-C in rats, which is in line with the previous observations [39]. Similar to the effects of monounsaturated fatty acids, conflicting evidences have also been given in literature on the effects of dietary n-3 PUFA containing oils such as fish oil on serum HDL-C level [23,27]. The observation made in this study showed no significant changes in HDL-C after consumption of soybean or flaxseed oils. Our observations also showed a significantly lower LDL-C level in experimental oil feeding rats compare to controls, although One-Way ANOVA did not show notable differences between different experimental groups. These results may suggest a long-term feeding or studying tissue fatty acid composition in order to determine differential effects of different dietary oils on LDL-C level.

Unlike general belief in the atherogenic effect of saturated fatty acids a recent meta-analysis has shown that there is no significant evidence for concluding association of dietary saturated fat with an increased risk of CHD [7,8]. In the same way, the observation made in this study showed no significant atherogenic effects of dietary yogurt butter on serum total and LDL cholesterol.

## Conclusion

Since we have both undertaken serum fatty acid and lipoprotein analysis, assessing differences in their compositions and levels as they reflect dietary fat intake. Our data clearly demonstrates that dietary fat modulates serum fatty acid composition and there is a strong association between the fatty acid content of ingested oil and serum fatty acid profile in rat. Our observations also indicated comparative effects of different dietary commercial oils on serum lipoproteins.

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## Conflict of interest

The authors report no conflict of interest.

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