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Effect of cream and yogurt butter diets on serum lipid profile in rats

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ABSTRACT

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Background and Objectives: Hypercholesterolemia is a major risk factor associated with coronary heart disease. The medical society recommends the consumption of low-fat and low-cholesterol foods. The current study aimed to explore the effect of different diets with cream or yogurt butter on plasma triglyceride and cholesterol levels in rats.

Methods: Yogurt butter and sweet cream butter were produced and their fatty acid profiles were created using gas chromatography; then, the index of atherogenicity (IA) was calculated for each butter. The rats were divided into three groups, with one group being fed with standard pellets as the control group and the other two with pellets mixed with 10% cream or yogurt butter for 4 weeks. Serum lipid profile was compared between the groups.

Results: In comparison with cream butter and control groups, yogurt butter did not change serum triglycerides. The yogurt butter diet resulted in a lower total cholesterol than the other diets ($p = 0.04$ for cream butter and $p = 0.001$ for the control group). The rats fed with the yogurt diet had higher HDL cholesterol. IA decreased ($p = 0.0009$) and conjugated linoleic acid ($p = 0.003$) increased significantly in the yogurt butter compared with the cream butter group.

Conclusion: Yogurt butter improved serum lipid profile and fatty acid composition in rats. Further human trials are needed to confirm these results.

key words:
Butter,
Cholesterol,
Plasma lipoproteins,
Triglyceride

Introduction

Cholesterol is an essential substance for the human body; however, increased levels of blood cholesterol can cause atherosclerosis and consequently turn into one of the major risk factors for developing cardiovascular diseases (CVDs). According to the World Health Organization (WHO), CVDs were responsible for 30% of deaths worldwide and are predicted to remain the leading causes of death in the coming two decades. It is estimated that by the year 2030, approximately 23.3 million people around the world will be affected by CVDs [1]. The WHO also reported that a 10% reduction in serum cholesterol in men aged 40 could decrease the incidence of heart disease within 5 years by 50%. Different strategies, including drug therapy and non-pharmacologic approaches, i.e., dietary intervention, behavior modification, and regular exercise, are all effective in lowering blood cholesterol levels. Notwithstanding the proven cholesterol-lowering effects of particular pharmacological agents, undesired side effects such as gastrointestinal discomfort can limit their consumption [2]. Therefore, the consumption of low-fat and low-cholesterol foods has been recommended by the medical society to prevent hypercholesterolemia.

Over the past decades, intake of dairy products, especially butter, has been restricted by consumers

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because of the higher proportion of saturated fatty acids (SFA) and cholesterol present in such products [3]. Moreover, the index of atherogenicity (IA), which is a precise index used to estimate the risk of arterial lesion development, is high [4]. However, it is reasonable to consider that the adverse effects of long-term limitation of dairy products in diet would have a much greater impact on public health as milk and milk fat contain noteworthy nutrients. On the other hand, scientific reports about nutritional aspects of dairy products are contradictory, with the cholesterol-lowering effects of milk, cheese, and butter being cited more frequently [5-9]. In addition to industrially produced butter, which is made by the churning of cream, locally made butter can be found in different parts of the world, such as Yayik butter in Turkey [10], Zebda beldi in Tunisia [11], Dhan in Algeria [12]. Nehre butter, made by the shaking of yogurt, is a popular type of butter in the western and northwestern parts of Iran. Unlike cream butter, Nehre is prepared by fermenting milk with lactic acid bacteria (LAB), such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus*. The consumption of Nehre butter has been recommended by older people, but no scientific evidence has so far been given for its alleged benefits. The hypocholesterolemic effects of fermented dairy products, such as probiotic yogurt [13], fermented milk [14], fermented whey beverage [15], and *S. thermophilus*– and *Lactobacillus casei*–fermented milk containing whey protein concentrate [16], have been discussed in depth. It has been suggested that LAB can contribute to cholesterol removal.

However, although numerous studies have focused on the health effects of fermented dairy products, the effects of cream butter and yogurt butter on cholesterol levels have not been investigated. Therefore, the aim of this study was to compare the IAs of cream butter and yogurt butter and to evaluate the effects of their long-term consumption on plasma lipids in rats.

Methods

Butter manufacturing

In order to have similar fat content and fatty acid profiles in the samples, the butter was made with the same milk in the laboratory as follows:

To prepare Nehre butter (yogurt butter), pasteurized milk was inoculated with a yogurt starter culture consisting of *S. thermophilus* and *L. delbrueckii* spp. *bulgaricus* (CY-121, DSM Food Specialties, Melbourne, Australia) and fermented to a pH of 4.6. The yogurt was cooled and churned in an electrical churn. After discharging the buttermilk, the butter was worked with a spatula and stored in a closed plastic container at -20°C for analysis and consumption by the rats.

Sweet cream (cream butter) butter was produced by churning 35%-fat pasteurized cream that was aged overnight at 8°C. The rest of the procedure was the same as the yogurt butter. Each treatment was made in triplicate.

Fatty acid profile analysis

The fatty acid compositions of the two types of butter were determined by the direct transesterification method as proposed by Lepage and Roy [17]. The prepared samples were analyzed by a gas chromatograph (Model 610, Buck Scientific, East Norwalk, USA) equipped with a flame ionization detector and TR-CN100 fused silica capillary column (60 m × 0.25 mm × 0.2 μm film thickness, Teknokroma, S. Coop. Ltda, Barcelona, Spain). The injection volume was 1 μL, and the injection and detection temperature was set at 260°C. Helium was used as the carrier gas with a flow rate of 20 mL · min^{-1}, and the split ratio was set at 10^{-2}. The initial temperature of the oven was 50°C for eight minutes and then increased at a rate of 10°C · min^{-1} until 190°C. Fatty acid methyl esters were identified by comparing their retention times against standards analyzed under the same conditions. Results were expressed as g/100 g total fatty acid.

Calculating the index of atherogenicity

As proposed by Ulbricht and Southgate [18], the index of atherogenicity was calculated by dividing the sum of lauric (C12:0), palmitic (C16:0), and four times myristic (C14:0) acids by the total unsaturated fatty acids [4].

Animal grouping and feeding

The study protocol was approved by the institutional ethics committee on animal care and use. Eighteen adult male Sprague-Dawley rats (215.18 ± 28.35 g) were purchased from the central animal laboratory of the Veterinary Faculty of Tabriz Islamic Azad University and housed in colony rooms at 21°C with a relative humidity of 65% and a 12-hour light-dark cycle. They were fed with laboratory pellet chow (purchased from Niroo Sahand Co., Tabriz, Iran) and water for several weeks before the experiment. After that, the rats were randomized to 3 groups of 6 rats each. One group received a normal diet, while the pellets of the other 2 groups were mixed with 10% cream or yogurt butter. The experiment continued for 8 weeks, after which blood samples were taken after a 12-hour fast to analyze serum lipids.

Sampling and determination of serum lipids

Blood samples were taken from the retro-orbital plexus under ether anesthesia and collected in tubes. The tubes were placed in a 37°C water bath for 1.5 hours and centrifuged at 365 g for 10 minutes at 4°C.

The serum was separated from the blood and stored at -20°C for the analysis.

Total plasma cholesterol (TC) and triglyceride (TG) concentrations were measured using the CHOD/PAP and GPO/PAP methods, respectively [19], using TC and TG assay kits (Ziestchem Co., Tehran, Iran). High-density lipoprotein (HDL) was measured with a colorimetric endpoint method using an HDL assay kit (Ziestchem Co., Tehran, Iran), and LDL was computed using the following equation:

$$LDL = TC - (TG / 5 + HDL).$$

Statistical analysis

One-way analysis of variance, based on a completely randomized design with 3 replications, was used for the statistical analysis. Post hoc comparisons of means were performed using the Duncan test at a significance level of $\alpha = 0.05$, except for HDL, for which the significance level was set at $\alpha = 0.001$.

Results

Comparison of serum TC, HDL, LDL, and TG concentrations between groups has been shown in Table 1. Significant differences were observed in the concentrations of serum lipids except for TG. Serum TC and LDL-C were significantly lower in the yogurt butter group compared with the cream butter ($p = 0.04$ and $p = 0.003$, respectively) and the control group ($p = 0.001$ and $p = 0.02$, respectively). In contrast, serum HDL-C, and LDL/HDL ratio significantly increased in yogurt butter compared with the cream butter and control group ($p = 0.009$ and $p = 0.01$, respectively).

We also analyzed the fatty acid (FA) profile of the butter types used in the experiment. As shown in Table 2, there were significant differences between the two butter type in most of the fatty acids except for capric (C10:0), lauric (C12:0), myristic (C14:0), pentanoic (C15:0), palmitoleic (C16:1), and stearic (C18:0) acids ($p < 0.05$). The mean levels of oleic acid (C18:1) ($p = 0.002$), linoleic acid (C18:2) ($p = 0.0003$), and conjugated linoleic acid (CLA) isomers ($p = 0.0003$) were higher in the yogurt butter than in the cream butter group. Also, yogurt butter had a significantly smaller IA compared with cream butter ($p = 0.002$) and standard chow ($p = 0.0009$) respectively.

Discussion

In this research, we worked on a traditionally fermented dairy product, Nehre butter (yogurt butter), which is popular in the western and northwestern parts of Iran. Despite the desirable nutritional properties of butter, such as being rich in CLA, its potentially hypercholesterolemic and atherogenic effects (due to the high cholesterol and SFA content) limits recommending it for daily use. We compared some nutritional properties of Nehre butter with those of industrially produced cream butter. Yogurt butter had a lower IA compared with cream butter, suggesting that IA may be decreased through fermentation. Moreover, Nehre butter had a higher CLA content and a more favorable effect on serum lipid profile compared with cream butter and standard chow.

The cholesterol-lowering effect of milk and its products has been reported frequently, but the findings regarding plasma LDL and HDL are equivocal. A non-significant reduction in LDL was followed by atherosclerosis due to butter naturally enriched in CLA plus trans vaccenic acid (VA) consumption, as deduced by Haug et al. [20]. Several studies have investigated the effects of dairy product consumption on serum lipids. It has been shown that diets high in butter are associated with higher plasma total cholesterol and LDL compared with diets high in cheese [21, 22]. According to Tholstrup et al. [8], the moderately lower LDL-C after a cheese and milk diet compared with a butter diet can be explained as a result of the removal of some milk hypocholesterolemic factors, such as calcium and phospholipids, during butter production. Kumar et al. showed that the hypocholesterolemic effect of anhydrous milk fat (ghee) in Wistar rats may be attributable to an increased biliary excretion of cholesterol and bile acids [7].

In our study, the rats receiving yogurt butter had higher HDL and lower TC and LDL levels. These findings are in line with Asadi et al. [23], who found that optional intake of yogurt butter, compared with vegetable oil, significantly increased HDL levels in rats.

The difference in the hypocholesterolemic effects between the two butter types can be discussed in two aspects. The first approach is related to the recognized health benefits of short-chain fatty acids (SCFAs) and

Table 1. Comparison of serum lipids of rats fed with different diets

Sample	TC	TG	HDL-C	LDL-C	LDL/HDL
Yogurt butter	64.33 ± 5.92a	76.16 ± 14.63	35.10 ± 5.25a	13.96 ± 6.86a	0.42 ± 0.21a
Cream butter	77.17 ± 4.95b	59.83 ± 24.58	26.43 ± 5.16b	38.7 ± 10.80b	1.56 ± 0.63b
Control	80.17 ± 7.67b	56.33 ± 10.13	23.50 ± 4.41b	45.4 ± 6.71b	1.99 ± 0.49b
p value	0.034	0.133	0.0005	0.041	0.024

Values are shown as mean ± SD from repeated experiments, mg dL-1. Different letters in the same column are significantly different data.

Table 2: Fatty acid composition of butter samples

Fatty acids (g/100 g total fatty acids)	CB Mean ± SD	YB Mean ± SD	p value
C4:0	1.13 ± 0.43	0.45 ± 0.08	0.03
C6:0	1.66 ± 0.24	0.67 ± 0.11	0.009
C8:0	1.79 ± 0.31	0.21 ± 0.08	0.008
C10:0	3.06 ± 0.15	2.89 ± 0.63	0.08
C12:0	3.36 ± 0.18	3.74 ± 0.33	0.10
C14:0	13.75 ± 0.85	14.72 ± 0.14	0.064
C15:0	0.69 ± 0.03	0.55 ± 0.04	0.072
C16:0	45.33 ± 1.20	41.80 ± 0.92	0.006
C16:1	0.76 ± 0.02	0.79 ± 0.09	0.074
C18:0	7.34 ± 0.04	7.76 ± 0.43	0.087
C18:1	20.26 ± 0.14	25.31 ± 0.81	0.002
C18:2	0.96 ± 0.11	1.52 ± 0.17	0.0003
CLA	0.12 ± 0.00	0.21 ± 0.02	0.0001
IA	4.57 ± 0.09	3.83 ± 0.15	0.009

CB = cream butter, YB = yogurt butter, CLA = conjugated linoleic acid, IA = index of atherogenicity

CLA isomers. It is reported that the presence of SCFAs in the diet reduces the synthesis of endogenous cholesterol and its intestinal absorption [24], although not all SFAs exert the same effect on plasma cholesterol. Brassard et al. [25] reported that consumption of SFAs from cheese and butter had similar effects on HDL-C but differentially modified LDL-C concentrations. Monounsaturated fatty acids, along with CLA, are known to have hypocholesterolemic effects [20]. Moreover, the higher hypocholesterolemic potential of stearic and linoleic acids have been noted in comparison to other long-chain fatty acids [26]. Despite lower SCFA (C4:0 to C12:0) content of yogurt butter, oleic acid and CLA contents were higher than in cream butter. This is likely due to fermentation, as the ability of some species of *Lactobacillus*, *Propionibacterium*, *Bifidobacterium,* and *Enterococcus* to produce CLA has been reported frequently [27, 28]. Yogurt starter cultures are more likely to produce CLA [29]. IA is an appropriate dietary risk indicator for cardiovascular disease, so the demand for the consumption of low-IA foods is growing throughout the world. Our findings showed a significant difference in IA between the two types of butter ($p < 0.05$), with yogurt butter having 16.19% lower IA value than cream butter. With this in mind, the hypothesis that the consumption of high-IA foods would increase plasma LDL is confirmed by our results.

Another aspect used to discuss the hypocholesterolemic effects of yogurt butter is fermentation. Yogurt butter is produced with LAB, for which cholesterol-lowering effects have been discussed. Removal of cholesterol by yogurt bacteria and the symbiotic relationship between S. thermophilus and L. delbrueckii spp. bulgaricus in cholesterol assimilation has been argued by Dilmi-Bouras [30]. A number of mechanisms have been proposed for the cholesterol-lowering effect of LAB: binding cholesterol in the small intestine [30], deconjugating bile acids by hydrolysis [1], converting cholesterol to coprostanol [31], and inhibiting cholesterol absorption by producing exopolysaccharides [8]. St-Onge et al. [14] stated that SCFAs, such as butyrate, acetate, and propionate, obtained from the fermentation of carbohydrates with LAB, decrease cholesterol content by inhibiting its synthesis in the liver.

The present study showed that the reduction of serum TC can be achieved by supplementing the regular diet with yogurt butter. The lower IA of yogurt butter, in combination with the presence of LAB, is the main causes of nutritional value.

Conclusions

Consuming a diet containing yogurt butter contributes to decreased total cholesterol and LDL-C concentration in rats. In general, the cholesterol-lowering effect of fermented dairy products is confirmed with our findings.

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Declaration of interest

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Ooi L-G, Liong M-T. Cholesterol-lowering effects of probiotics and prebiotics: a review of in vivo and in vitro findings. International journal of molecular sciences. 2010;11(6):2499-522.
2. Tsai C-C, Lin P-P, Hsieh Y-M, Zhang Z-y, Wu H-C, Huang C-C. Cholesterol-lowering potentials of lactic acid bacteria based on bile-salt hydrolase activity and effect of potent strains on cholesterol metabolism in vitro and in vivo. The Scientific World Journal. 2014;2014.

3. Kim J, Jung T, Ahn J, Kwak H. Properties of cholesterol-reduced butter made with β-cyclodextrin and added evening primrose oil and phytosterols. Journal of dairy science. 2006; 89(12):4503-10.
4. Bobe G, Zimmerman S, Hammond E, Freeman A, Porter P, Luhman C, et al. Butter composition and texture from cows with different milk fatty acid compositions fed fish oil or roasted soybeans. Journal of dairy science. 2007;90(6): 2596-603.
5. Alizadeh A, Ehsani MR, Ezzatpanah H, Mousavi G, Darabi M, Rofehgarinejad L. Effect of cow and sheep milk diet on plasma lipids in rats. Milchwissenschaft-Milk Science International. 2012;67(4):410-2.
6. German JB, Dillard CJ. Composition, structure and absorption of milk lipids: a source of energy, fat-soluble nutrients and bioactive molecules. Critical reviews in food science and nutrition. 2006;46(1):57-92.
7. Kumar MV, Sambaiah K, Lokesh BR. Hypocholesterolemic effect of anhydrous milk fat ghee is mediated by increasing the secretion of biliary lipids. The Journal of nutritional biochemistry. 2000;11(2):69-75.
8. Tholstrup T, Høy C-E, Andersen LN, Chistensen RD, Sandström B. Does fat in milk, butter and cheese affect blood lipids and cholesterol differently? Journal of the American College of Nutrition. 2004;23(2):169-76.
9. Thorning TK, Raziani F, Bendsen NT, Astrup A, Tholstrup T, Raben A. Diets with high-fat cheese, high-fat meat, or carbohydrate on cardiovascular risk markers in overweight postmenopausal women: a randomized crossover trial, 2. The American journal of clinical nutrition. 2015; 102(3):573-81.
10. Sağdıç O, Dönmez M, Demirci M. Comparison of characteristics and fatty acid profiles of traditional Turkish yayik butters produced from goats', ewes' or cows' milk. Food Control. 2004;15(6):485-90.
11. Samet-Bali O, Ayadi M, Attia H. Traditional Tunisian butter: Physicochemical and microbial characteristics and storage stability of the oil fraction. LWT-Food Science and Technology. 2009;42(4):899-905.
12. Idoui T, Benhamada N, Leghouchi E. Microbial quality, physicochemical characteristics and fatty acid composition of a traditional butter produced from cows' milk in East Algeria. Grasas y Aceites. 2010;61(3):232-6.
13. El-Gawad IAA, El-Sayed E, Hafez S, El-Zeini H, Saleh F. The hypocholesterolaemic effect of milk yoghurt and soy-yoghurt containing bifidobacteria in rats fed on a cholesterol-enriched diet. International Dairy Journal. 2005;15(1):37-44.
14. St-Onge M-P, Farnworth ER, Jones PJ. Consumption of fermented and nonfermented dairy products: effects on cholesterol concentrations and metabolism–. The American journal of clinical nutrition. 2000;71(3):674-81.
15. Hong S-M, Chung E-C, Kim C-H. Anti-obesity effect of fermented whey beverage using lactic acid bacteria in diet-induced obese rats. Korean journal for food science of animal resources. 2015;35(5):653.
16. Kawase M, Hashimoto H, Hosoda M, Morita H, Hosono A. Effect of administration of fermented milk containing whey protein concentrate to rats and healthy men on serum lipids and blood pressure. Journal of dairy science. 2000;83(2): 255-63.
17. Lepage G, Roy CC. Direct transesterification of all classes of lipids in a one-step reaction. Journal of lipid research. 1986;27(1):114-20.
18. Ulbricht T, Southgate D. Coronary heart disease: seven dietary factors. The Lancet. 1991; 338(8773):985-92.
19. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clinical chemistry. 1973;19(5):476-82.
20. Haug A, Sjøgren P, Hølland N, Müller H, Kjos NP, Taugbøl O, et al. Effects of butter naturally enriched with conjugated linoleic acid and vaccenic acid on blood lipids and LDL particle size in growing pigs. Lipids in health and disease. 2008;7(1):31.
21. Biong AS, Müller H, Seljeflot I, Veierød MB, Pedersen JI. A comparison of the effects of cheese and butter on serum lipids, haemostatic variables and homocysteine. British Journal of Nutrition. 2004;92(5):791-7.
22. Nestel P, Chronopulos A, Cehun M. Dairy fat in cheese raises LDL cholesterol less than that in butter in mildly hypercholesterolaemic subjects. European journal of clinical nutrition. 2005;59(9):1059.
23. Asadi F, Shahriari A, Chahardah-Cheric M. Effect of long-term optional ingestion of canola oil, grape seed oil, corn oil and yogurt butter on serum, muscle and liver cholesterol status in rats. Food and Chemical Toxicology. 2010;48(8-9):2454-7.
24. Skeaff C, Williscroft K, Mann J, Chisholm A. Replacing cows' with sheep's dairy fat lowers plasma cholesterol concentration in participants consuming dairy fat-rich diets. European journal of clinical nutrition. 2004;58(2):250.
25. Brassard D, Tessier-Grenier M, Allaire J, Rajendiran E, She Y, Ramprasath V, et al. Comparison of the impact of SFAs from cheese and butter on cardiometabolic risk factors: a randomized controlled trial–3. The American

journal of clinical nutrition. 2017;105(4):800-9.
26. Kris-Etherton P, Derr J, Mitchell DC, Mustad VA, Russell ME, McDonnell ET, et al. The role of fatty acid saturation on plasma lipids, lipoproteins, and apolipoproteins: I. Effects of whole food diets high in cocoa butter, olive oil, soybean oil, dairy butter, and milk chocolate on the plasma lipids of young men. Metabolism-Clinical and Experimental. 1993;42(1):121-9.
27. Collomb M, Schmid A, Sieber R, Wechsler D, Ryhänen E-L. Conjugated linoleic acids in milk fat: Variation and physiological effects. International dairy journal. 2006;16(11):1347-61.
28. Coakley M, Ross R, Nordgren M, Fitzgerald G, Devery R, Stanton C. Conjugated linoleic acid biosynthesis by human-derived Bifidobacterium species. Journal of Applied Microbiology. 2003;94(1):138-45.
29. Lin T, Hung T-H, Cheng T-S. Conjugated linoleic acid production by immobilized cells of Lactobacillus delbrueckii ssp. bulgaricus and Lactobacillus acidophilus. Food Chemistry. 2005;92(1):23-8.
30. Dilmi-Bouras A. Assimilation (in vitro) of cholesterol by yogurt bacteria. Annals of agricultural and environmental medicine. 2006; 13(1):49.
31. Lye H-S, Rusul G, Liong M-T. Removal of cholesterol by lactobacilli via incorporation and conversion to coprostanol. Journal of dairy science. 2010;93(4):1383-92.