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Original Article

Effect of Corn Oil, Olive Oil, Sheep's and Cow's Ghee on the Expression of apoB Protein in Syrian Mice's Intestine and Liver

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A B S T R A C T

Background and Aims: Type and saturation of fatty acids can have an important impact on the level of triglyceride, cholesterol, very low and low-density lipoproteins in the blood and thus affect the development of atherosclerosis. Saturated fatty acids have an additive effect on blood cholesterol while for unsaturated fatty acids, a lowering effect has been reported. Fatty acids can have different effects on lipoproteins metabolism and apolipoproteins expression because oils used by humans have different compositions. One of the important apolipoproteins is apolipoprotein B (apoB). This study was conducted to compare the effect of different nutritious fats on expression of apoB protein.

Materials and Methods: For this purpose, 48 Syrian male mice were selected and randomly divided into six groups of eight: chow, diet with 10% corn oil, diet with 10% olive oil, diet with 10% cow ghee, diet with 10% sheep ghee, and diet with 2% cholesterol. After two months, liver and intestine were removed and transferred into liquid nitrogen at -70ºC. Protein was extracted and the expression of apoB was studied by western blotting.

Results: An increase in the expression of intestinal apoB48 was identified in olive oil and cow ghee groups. Hepatic apoB100 expression was increased in the cholesterol group compared with the corn oil group.

Conclusion: This study indicates that olive oil and cow ghee consumption increase intestinal apoB48 expression.

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Introduction

Cardiovascular diseases cause mortality and morbidity in the developed countries and increase rapidly in the developing countries. Estimates in 2020 regarding cardiovascular diseases outbalanced from infectious diseases, and became the main cause of mortality in the world [1]. In Iran, about 50 percent of the annual deaths result from coronary artery disease (CAD). CAD is characterized by the presence of atherosclerosis in the coronary arteries of the heart [2]. Atherosclerosis is a pathological condition of the arteries and the leading cause of death in the developed countries and an important cause of myocardial infarction, stroke and peripheral artery disease. Fatty streaks are detected early in atherosclerotic lesions [3]. Several factors are involved in atherosclerosis development e.g. increase of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) in plasma, decrease of high-density lipoprotein cholesterol (HDL) in blood, hypertension, increase of homocysteine and hemostatic factors such as fibrinogen, family history and environmental factors [4]. Therefore, nutrition can have a significant role in the development of atherosclerosis by increasing or decreasing LDL and VLDL levels. Atherosclerosis does not occur under a specific level of cholesterol (150 mg/dL), and the risk gradually increases with increase in cholesterol level [5]. Because the immune response against LDL is described in atherosclerosis, LDL leakage into subendothelial space is dependent on cholesterol level.

There is evidence that increase in the concentration of apolipoproteins B (apoB) is one of the major risk factors for coronary heart disease (CHD). ApoB is a component of all atherogenic or potentially atherogenic particles, including VLDL, IDL and Lp(a), and each particle contains one apoB molecule. Particles of LDL, but not LDL-C alone, play a central role in atherogenesis. This process begins with subendothelial retention of intact apoB-containing particles. Moving LDL particles into arterial intima is a gradient–dependent process and passive diffusion rate increases with increase in circulating LDL concentration [6]. Inside the intima, LDL particles directly bind to glucosaminoglycans via apoB100, and begin a series of events in which LDL is oxidized or, in other words, modified. The modified LDL is taken up by macrophages or monocytes to create foam cells. The findings show that defect in binding LDL to proteoglycan largely reduces the atherogenic potential of LDL [7]. This indicates that LDL binding to arterial wall occurs in early stages of atherogenesis. Therefore apoB has a critical role in the development of atherogenesis and atherosclerosis. Zhi-Hong Yang's study showed that the expression of genes involved in cholesterol metabolism such as apoB is reduced with the consumption of pollock oil-containing food. Monounsaturated fatty acids-rich media compared with polyunsaturated fatty acids

(PUFAs) increase the secretion of apoB100 in CACO-2 cells [8]. Mohammadifar and colleagues observed that liquid oil, significantly reduces apoB when compared with hydrogenated oil, while ghee increases apoA and decreases triglyceride [9]. Knock down expression of liver apoB by siRNA decreases LDL cholesterol in a mouse model with human-like serum lipids; the apoB mRNA and protein expression were reduced by more than 95%. Knock down of apoB mRNA reduces specific triglycerides and total lipids in plasma levels by 70%, while the overall distribution of lipids is unchanged [10]. Ko et al. showed that diets containing fish oil exert inhibition of apoB and triglycerides secretion in strains of human apoB-transgenic mice. Myristic acid increases secretion of dense lipoprotein via inhibition of apoB degradation and recruitment of triglyceride. In this study, triglycerides synthesis was equally stimulated by oleic acid, myristic acid and docosahexaenoic acid, but its secretion was relatively decreased by myristic acid and docosahexaenoic acid [11].

Type of dietary fatty acids plays a more important role compared with dietary total fat in determining CHD risk [12]. In metabolic studies, different classes of saturated fatty acids have shown different effects on plasma lipid and lipoprotein levels [13]. Saturated fatty acids with 16-12 carbon atoms tend to increase cholesterol and LDL cholesterol level. Stearic acid (18:0) has no increasing effect on blood cholesterol in comparison with oleic acid (18:1). Among the saturated fatty acids which raise blood cholesterol, myristic acid (14:0) has more potential than lauric acid (12:0) or palmitic acid (16:0). Several studies have shown that replacing dietary saturated fat with vegetable oils rich in linoleic acid has a strong potential for lowering cholesterol level. Animal and metabolic studies indicate that increase of n-6PUFAs absorption improves insulin sensitivity [12]. In Nurses' Health Study, too much intake of n-6 PUFAs significantly lowered the incidence of type 2 diabetes [13]. In another study, even for sunflower oil (rich in linoleic acid) anti-arrhythmic effect was identified [12].

Animal ghee produced by traditional methods is still used in Iran. The traditional methods of ghee and butter preparation are different from the industrial methods. In the traditional method, types of fatty acids are quantitatively changed, which increase the short- and medium-chain fatty acids and reduce long-chain fatty acids. These changes, in addition to increasing the nutritional value, also reduce the side effects. Because animal ghees are mostly used in rural, tribal areas and some of the cities, and compounds of this ghee have been detected, identifying the effects of ghee on expression of apoB48 and apoB100 can help to better understand atherosclerosis and CHD development. On the other hand, vegetable oils such as olive have an anti-atherosclerotic effect. Therefore, comparing the effects of these two types of oil on expression of apoB can be of

great help to better understanding of the formation of atherosclerosis.

Materials and Methods

This animal experimental study was conducted on 48 N-Mary male Syrian mice with an average weight of 25-30 g. After two weeks of adaptation with animal room conditions (22±1ºĊ temperature and 12h light), the mice were randomly assigned into six groups: chow , diet with 10% corn oil, diet with 10% olive oil, diet with 10% cow ghee, diet with 10% sheep ghee, and diet with 2% cholesterol. The food eaten by mice was not measured because there is no diet limit for animals. After two months, the mice were kept overnight starved and then anesthetized by diethyl ether (MERCK, Germany). Liver and intestine (jejunum) were removed and immediately frozen in liquid nitrogen at –70ºC. Protein was extracted using 50 mg of tissue with 700 ml RIPA buffer, and was homogenized using ultrasound. Samples were centrifuged in 1.5 mL microtube with the speed of 14000 rpm at 4ºC. Electrophoresis (Clever, Taiwan) was conducted by 6% polyacrylamide gel and western blot by anti-apoB (goat anti mouse polyclonal AB) and conjugated secondary antibody. The film was scanned by Hp Scanject (HP, USA), and band densities were measured by image j software. This study was conducted after the approval by the Ethics Committee of Kerman University of Medical Sciences.

Statistical Analysis

Data for weight analysis were analyzed by paired t-test and Kruskal-Wallis test. For comparison of apoB expression, Kruskal-Wallis test was used. In this study, all groups were compared with the control group and with each other by Post Hoc Tukey test.

Results

Weight was increased significantly in all groups compared to before treatment (Significant difference: control p=0.006, corn p=0.001, olive p=0.000, cow p=0.000, sheep p=0.007, and cholesterol p=0.001) (Fig. 1). Intestinal apoB48 expression in olive oil and cow ghee groups showed a significant (p=0.031 and p=0.016, respectively) increase ratio compared with the control group. In the cholesterol group, intestinal apoB48 expression slightly increased compared with that of the control group (p=0.07). Intestinal apoB48 expression in olive oil and cow ghee groups showed a significant (P=0.034 and P=0.017, respectively) increase ratio compared to the sheep groups (Fig. 2). No significant change in expression of hepatic apoB48 was seen in the groups. The results are presented in Fig. 3. Hepatic apoB100 expression in all groups was not significantly different compared with that in the control group, but apoB100 expression was significantly higher in the cholesterol group compared with the corn oil group (P=0.044) (Fig. 4).

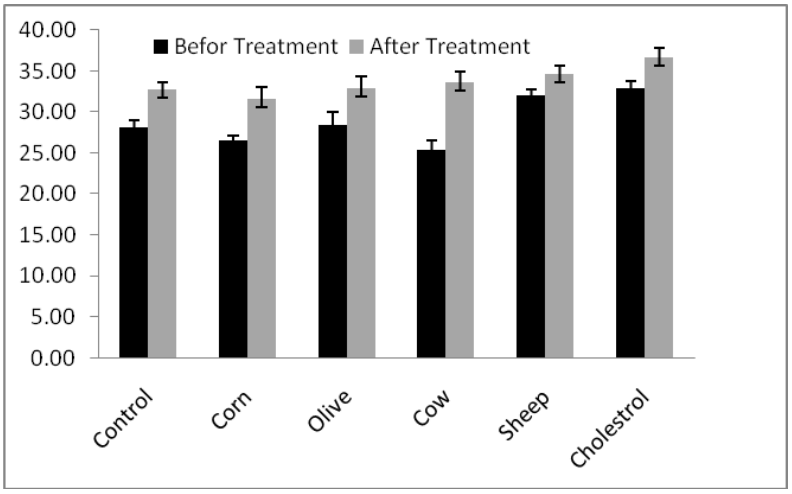

Fig. 1. Comparison of groups' weight before and after treatment. All groups show significant weight gain ratio to before of treatment (Control p=0.006, Corn p=0.001, Olive p=0.000, Cow p=0.000, Sheep p=0.007, and Cholesterol p=0.001)

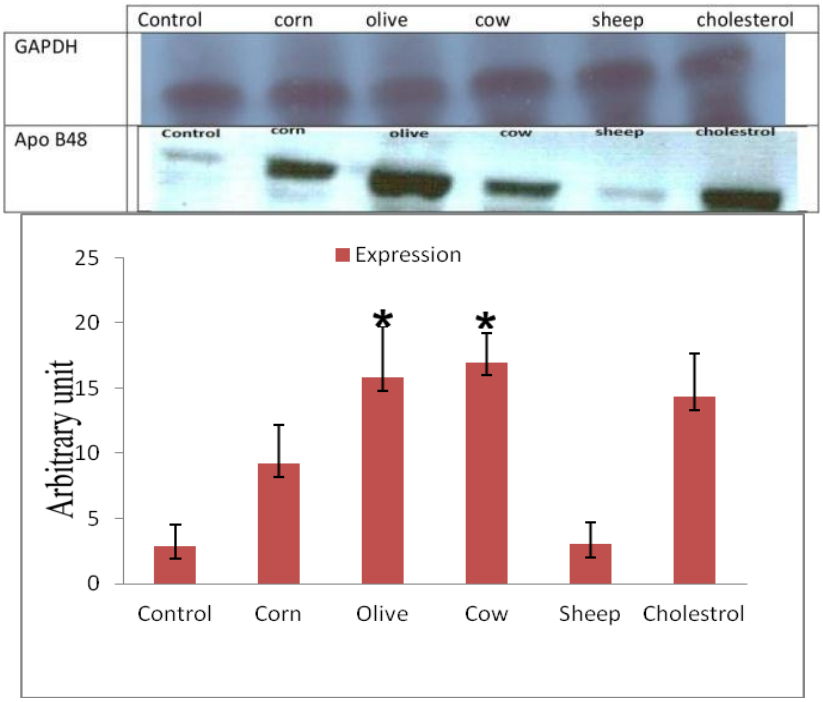

Fig. 2. Eeffect of oil consumption on intestinal apoB48 expression in mice. Data are reported as mean ± SEM. Apo B48 expression of olive oil and cow ghee were significantly increased compared with the control (p=0.031 and p=0.016, respectively) and the sheep groups (p=0.034 and p=0.017, respectively).

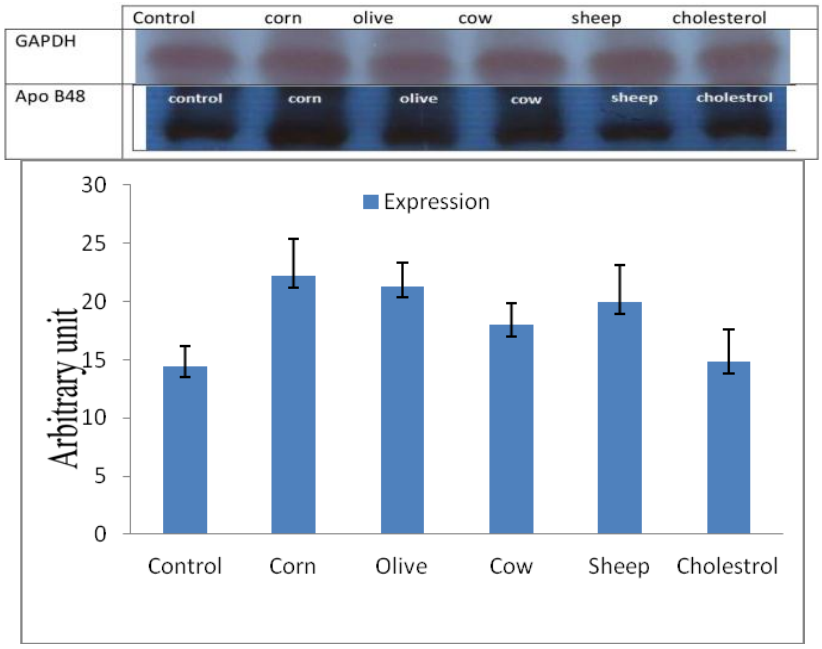

Fig. 3. Effect of oil consumption on hepatic apoB48 expression in mice. Data are reported as mean ± SEM. There were no significant differences in hepatic apoB48 expression compared with the control and other groups.

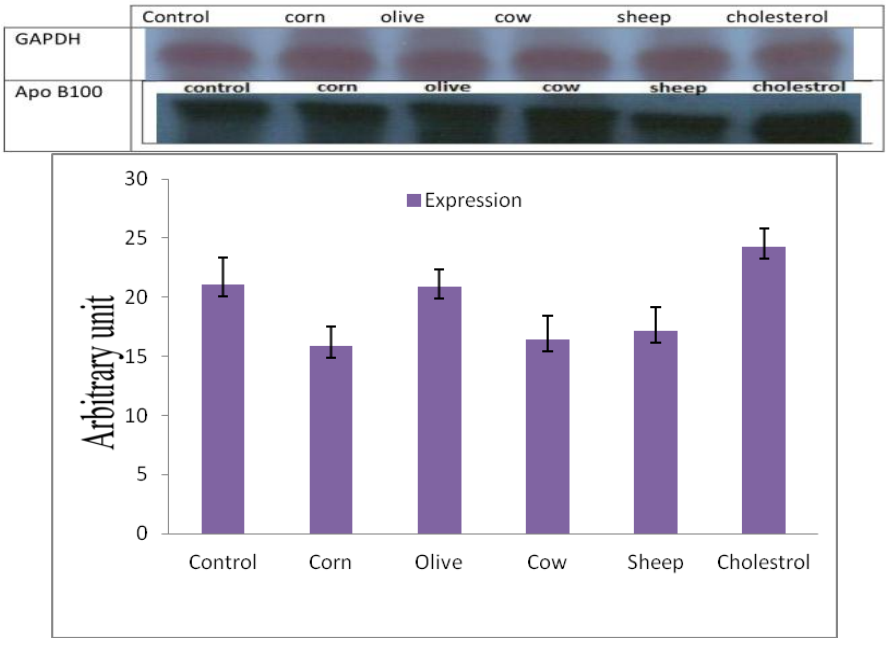

Fig. 4. Effect of oil consumption on expression of hepatic apoB100. Data are reported as mean ± SEM. ApoB100 expression in all groups is not significantly different from that of the control group, but in cholesterol group compared with corn oil group this is significantly higher (p=0.044).

Discussion

In this study, weight of mice was significantly increased after treatment compared to the initial weight. Similar results were found in Dalfardi et al. (2012) study on cholesterol, corn and olive oil, cow and sheep ghee. In our study weight gain in cow oil group was significantly increased in comparison to the control, which disagrees with some other studies (14). Our results showed high expression of intestinal apoB48 in olive oil group compared with the control group. A study demonstrated that unsaturated fatty acids, especially oleic acid, effectively join with lipoprotein by microsomal transfer protein (MTP) and believed that MTP involves only in co-translation lipidation of apoB, and protects apoB from early intracellular degradation [15]. However in Caco-2 cells, it has been indicated that MTP plays a role in higher lipidation of early particles in secretory pathway. These results indicate that short-term ingestion of olive oil may increase the number of apoB48-containing particles by activity modulation or expression of MTP [16]. In our study, increased intestinal expression of apoB48 in olive oil group is along with a small increase in hepatic apoB48 (not significant), indicating that olive oil has an additive effect on the expression of apoB48 probably via affecting on mRNA editing enzyme. In another study on Caco-2 cells, oleate led to 2 to 4 fold increase in apoB production [17]. Another study indicated that oleic acid which is found abundantly in olive oil increases triglyceride biosynthesis and secretion of triglycerides containing lipoproteins in Caco-2 cells [18], hence being in line with the result of our study which demonstrates increased intestinal apoB48 expression by olive oil. In our study, a significant increase in intestinal apoB48 expression was seen in cow ghee compared with the control, corn oil, and also sheep ghee groups. Dalfardi et al. showed that cow ghee significantly increases total cholesterol, triglycerides and LDL [14] probably due to increased chylomicrons secretion and so apoB48 intestinal expression, thus being in line with our results. Magun et al. (1988) showed that intake of high fat diet shifts intracellular apolipoproteins to lipoproteins but many apolipoproteins remain unbound to lipoprotein. Therefore, there is a constant large reservoir of unbound apolipoprotein suggesting that apolipoprotein synthesis is not the rate-limiting step in assembling and secreting lipoproteins [19]. Hence, post-translational apolipoprotein regulation is more involved, and the type and amount of fatty acids may also be involved by a mechanism in this regulatory process. As have been shown, cells which are incubated with palmitate, show reduced rate of lipoprotein secretion. Because of the cell toxicity of tripalmitoylglycerol (crystal at 37ºC), palmitate enters in phospholipids structure and is stored in the membrane. Regarding apoB constant reservoir, it is suggested that low apoB consumed for apoB containing lipoprotein assembles and remains in cells thereby being in

agreement with our result in apoB high expression in cow ghee group [15].

The length and saturation of fatty acids differentially affects the level of plasma apolipoprotein B100. Many studies on humans and animals have proven that the amount and type of dietary fat can affect the levels of apoB100-containing lipoproteins and related cholesterol and so contribute to atherosclerosis risk. López-Soldado et al. (2009) observed that, after 16h incubation of rat hepatocytes with olive oil chylomicron remnant–like particles, the amount of apoB mRNA is reduced [20]. Although, no significant changes were observed in the expression of apoB100 in all groups in comparison with the control in our study, the expression of hepatic apoB100 was significantly increased in cholesterol group, compared with corn oil group. A study done by Dashti on HepG2 cells showed that pure cholesterol does not significantly affect the intracellular cholesterol ester and accumulation of neutral lipids or apoB100 in culture media. In that research it was claimed that pure cholesterol, in addition to the absence of any effect on intracellular cholesterol ester, fails in modulation of production rate of apoB [21].

Conclusion

This study showed that expression of intestinal apoB48 in olive oil and cow ghee groups increases compared to that of the control group. Various oils in this study had a pleiotropic effect on lipid and lipoprotein metabolism; such an effect cannot be found by studying a few protein expressions. Fatty acid oils (an example of which is oleic acid) have an identified effect on lipoprotein metabolism, but it gets more complicated when the effect of more than one fatty acid is considered. Minor compounds may be present in oils that can have different effects on fatty acids function.

Conflict of Interest

There is no conflict of interests.

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