

## **Influence of Ghee (Samm Barri's) on Hepatic Fatty Acid Profile and Cholesterol Levels of Plasma, Lipoproteins and Organs in Rats**

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**Abstract.** This study examined the influence of ghee (*Samm Barri's*) from sheep's and cow's milk on cholesterol levels of total plasma, lipoproteins and organs as well as the fatty acid profile of hepatic lipids. Thirty male albino rats (45 g) were fed diets containing 5% (w/w) of either corn oil (CO diet), sheep ghee (SG diet) or cow ghee (CG diet) for five weeks. Plasma total cholesterol and triacylglycerol levels were significantly higher in rats fed the SG and CG diets compared to CO diet. Both saturated diets (SG and CG) caused an increase in lipoprotein fractions (VLDL, LDL and HDL) as well as organ cholesterol levels compared with CO diet feeding. Furthermore, dietary fats altered the fatty acid composition of hepatic lipids. Ghee resulted in increases of palmitic acid (16:0), palmitoleic acid (16:1 n-7) and oleic acid (18:1 n-9). In contrast, percent compositions of linoleic acid (18:2 n-6) and arachidonic acid (20:4 n-6) was greater in rats fed corn oil. This study suggests that high consumption of ghee is hypercholesterolemic and alters the hepatic fatty acid profile.

### **Introduction**

During the last four decades, several investigators have shown that the risk of cardiovascular diseases (CVD) increases with increasing plasma concentration of total or low density lipoprotein (LDL) cholesterol and decreases with increasing high density lipoprotein (HDL) cholesterol [1-3]. It has been suggested that lowering the plasma cholesterol level decreases the risk of cardiovascular diseases [4; p.99-115]. Cholesterol is derived either from the diet or synthesized in the body [5].

The typical diet comprises three groups of fatty acids, which include saturated, monounsaturated and polyunsaturated. An increase in the dietary intake of polyunsaturated fatty acids has been found to reduce plasma cholesterol concentration

whereas saturated fatty acids lead to an elevation of plasma cholesterol [6-8]. The magnitude of lowering cholesterol in the plasma was decreased when the polyunsaturated to saturated fatty acid (P/S) ratio of the experimental diets was lowered from about 4.6 [9] to about 1.5 [10;11] and when the diet was free of cholesterol [12]. Monounsaturated fatty acids appear to have no influence on the concentration of plasma cholesterol [7].

In Saudi Arabia, ghee (Samm Barri's) is widely consumed. Most of the ghee products are prepared by manual churning of fermented milk made by farmers and bedouins in villages and desert [13]. The influence of such products on plasma lipids and lipoprotein cholesterol is still not established. Therefore, the aim of this study was to investigate the influence of ghee locally produced from cow's and sheep's milk on the concentration of cholesterol in plasma, lipoproteins and organs as well as the hepatic fatty acid profile.

## Materials and Methods

### Animals

Thirty male Albino rats, three weeks old and weighing 45 g, were used in this experiment. Animals were randomly assigned to three groups each containing 10 animals. Rats were housed two per cage in stainless steel cages in a room with controlled lighting (12 hr light, 12 hr dark; light from 7 am – 7 pm), temperature  $22^{\circ}\text{C} \pm 1$  and relative humidity 55%. All groups were checked everyday and body weights were recorded weekly throughout the experiment. Diets and water were provided ad libitum.

### Diet

The basal diet (diet 1) was similar to that recommended by the American Institute of Nutrition [14,15] (Table 1). The feed was in powdered form. All animals were fed a control diet for one week and then were randomly assigned to the three dietary fat treatments: corn oil (diet 1), sheep ghee (diet 2) and cow ghee (diet 3). The fatty acid composition of each diet was determined by gas-liquid chromatography (Table 2 [13]). Diets were stored at  $4^{\circ}\text{C}$  and fresh diets were provided to animals every second day. Food intake was recorded.

### Chemical methods

After five weeks, animals were fasted overnight, anesthetized with diethyl ether and killed. Blood was collected in a syringe containing 1% EDTA by cardiac

**Table 1. Diet composition**

Ingredient	percent composition
Cascin	20.0
DL-Methionine	0.3
Sucrose	65.0
Cellulose (fiber)	5.0
Fat source <sup>1</sup>	5.0
AIN mineral mix	3.5
AIN vitamin mix	1.0
Choline Bitartrate	0.2
	100.0

<sup>1</sup>Corn oil, sheep's ghee or cow's ghee.

**Table 2. Fatty acid composition of the diets**

Fatty acid	Corn oil (diet 1)	Sheep ghee <sup>1</sup> (diet 2)	Cow ghee <sup>1</sup> (diet 3)
12:0		4.17	2.92
14:0		12.6	9.34
16:0	10.6	38.1	31.7
18:0	1.76	11.9	9.94
18:1	25.6	21.6	33.7
18:2	60.9	1.23	4.30
Others		10.4	8.0
P/S <sup>2</sup>	5.01	0.70	0.34

<sup>1</sup>Data from ref. [13]; <sup>2</sup>P/S = sum of all polyunsaturated/sum of all saturated fatty acids.

puncture between 9 am and 11 am. Plasma was isolated by centrifugation at 4°C and 3000 rpm for 20 min. The tissues were removed, weighed and immediately frozen (-20°C) until analysis.

Total lipids of liver were extracted using the method of Folch *et al.* [16]. The extraction was performed with 24 ml chloroform-methanol [2:1, v/v) using a poly-

tron homogenizer. The extraction was repeated three times. Lipid extracts were evaporated to dryness and then redissolved in chloroform and stored. Diets and hepatic lipids were converted to methyl esters (FAME) by using the method of the American Oil Chemists' Society [17]. FAME were measured by a Shimadzu gas chromatograph (GC-16A) equipped with a flame ionization detector at 260°C. A 1 µl sample was injected on a 300 cm × 3 mm column packed with diethylene glycol succinate. The injection and column temperatures were maintained at 250°C and 190°C, respectively. N<sub>2</sub> carrier gas flow rate was set at a linear velocity of 50 ml/min. Peaks were identified by comparison of retention times with those of standard fatty acids.

Triacylglycerol concentration was determined by the method of Trinder [18] using Randox kit # 210. The cholesterol levels of total plasma, liver and heart were determined by the enzymatic method of Richmond using Randox kit # 290 [19]. HDL cholesterol concentration was measured by the method of Assman [20] using kit # 203. The cholesterol concentrations of LDL and VLDL were derived from the other lipid data using the formula (VLDL cholesterol = TG/5, LDL cholesterol = total cholesterol - HDL cholesterol - VLDL cholesterol) obtained from the method of Friedwald *et al.* [21].

### Statistical analysis

The results shown represent the means ± SEM. The data were analyzed by one-way analysis of variance [22; p.431-513]. P-values less than 0.05 indicated significance.

## Results

Animals fed either sheep ghee or cow ghee did not have any significant difference on the body and organ weights as well as food intake compared with those of the control animals receiving corn oil (Table 3).

The mean plasma triacylglycerol and cholesterol concentrations of total plasma and lipoprotein fractions are shown in Table 4. Both test diets (cow and sheep ghee) led to significantly higher total plasma triacylglycerol and total cholesterol levels compared with control group. In addition, sheep ghee resulted in highest average plasma triacylglycerol levels compared with either corn oil or cow ghee. Significantly higher HDL-, LDL- and VLDL-cholesterol contents were observed with ingestion of the sheep and cow ghee diets than with the control diet.

The effect of dietary fats on hepatic and heart cholesterol levels is presented in

**Table 3. Effect of ghee on food intake, body and organ weights<sup>1</sup>**

	Diet 1	Diet 2	Diet 3
Food intake (g)	363 ± 13.5 <sup>a</sup>	395 ± 9.42 <sup>a</sup>	373 ± 9.34 <sup>a</sup>
Body wt. (g)	167 ± 4.59 <sup>a</sup>	177 ± 6.26 <sup>a</sup>	173 ± 4.62 <sup>a</sup>
Gain wt. (g)	124 ± 3.05 <sup>a</sup>	126 ± 5.25 <sup>a</sup>	128 ± 2.95 <sup>a</sup>
Liver wt (g)	6.67 ± 0.74 <sup>a</sup>	6.92 ± 0.40 <sup>a</sup>	7.10 ± 0.41 <sup>a</sup>
Heart wt (g)	0.66 ± 0.05 <sup>a</sup>	0.72 ± 0.03 <sup>a</sup>	0.75 ± 0.07 <sup>a</sup>

<sup>1</sup>Values are means ± SEM, n = 8; Values with different superscripts differ significantly (P < 0.05).

**Table 4. Effect of ghee on the concentration of plasma lipids and lipoprotein cholesterol<sup>1</sup>**

	Diet 1	Diet 2	Diet 3
Triglyceride (mg/dL)	57.2 ± 3.90 <sup>a</sup>	159 ± 9.01 <sup>b</sup>	126 ± 9.16 <sup>c</sup>
Cholesterol (mg/dL)	82.8 ± 2.15 <sup>a</sup>	137 ± 4.57 <sup>b</sup>	124 ± 3.44 <sup>b</sup>
HDL Cholesterol (mg/dL)	24.9 ± 0.94 <sup>a</sup>	36.5 ± 0.80 <sup>b</sup>	32.7 ± 1.13 <sup>c</sup>
LDL Cholesterol (mg/dL)	38.7 ± 1.04 <sup>a</sup>	68.7 ± 3.14 <sup>b</sup>	74.1 ± 3.01 <sup>c</sup>
VLDL Cholesterol (mg/dL)	11.4 ± 0.80 <sup>a</sup>	31.8 ± 1.80 <sup>b</sup>	25.3 ± 1.26 <sup>b</sup>

<sup>1</sup>Values are means ± SEM, n = 8; Values with different superscripts differ significantly (P < 0.05).

Table 5. Higher hepatic and heart cholesterol levels were associated with feeding saturated fatty acid (sheep and cow ghee) vs the corn oil diets.

To further evaluate the influence of dietary fat on hepatic fatty acid composition, the relative fatty acid profile of total hepatic lipids was determined. The fatty acid composition of liver is shown in Table 6. Many significant difference were observed among the three groups. The tested diets did not produce any significant changes in the percent composition of stearic acid (18:0). However, ghee resulted in increases of palmitic acid (16:0), palmitoleic acid (16:1 n-7) and oleic acid (18:1 n-9). In contrast, percent compositions of linoleic acid (18:2 n-6) and arachidonic acid (20:4 n-6) were greater in rats fed corn oil.

## Discussion

Diets were consumed readily by rats and the rats grew well during the study. Food intake and body weight gain and liver weight were not affected by test diets.

**Table 5. Effect of ghee on the concentration of liver and heart cholesterol<sup>1</sup>**

	Diet 1	Diet 2	Diet 3
Liver cholesterol (mg/organ)	6.95 ± 0.60 <sup>a</sup>	13.7 ± 0.77 <sup>b</sup>	12.0 ± 0.67 <sup>b</sup>
Heart cholesterol (mg/organ)	0.54 ± 0.02 <sup>a</sup>	0.82 ± 0.07 <sup>b</sup>	0.83 ± 0.09 <sup>b</sup>

<sup>1</sup>Values are means ± SEM, n = 8; Values with different superscripts differ significantly (P < 0.05).

**Table 6. Effect of ghee on hepatic fatty acid composition**

	Diet 1	Diet 2	Diet 3
C 16:0	26.2 ± 0.85 <sup>a</sup>	29.7 ± 1.36 <sup>b</sup>	32.0 ± 1.30 <sup>b</sup>
C 16:1	6.10 ± 0.95 <sup>a</sup>	11.9 ± 0.45 <sup>b</sup>	10.5 ± 0.35 <sup>b</sup>
C 18:0	12.8 ± 2.10 <sup>a</sup>	15.1 ± 0.10 <sup>b</sup>	10.5 ± 0.35 <sup>b</sup>
C 18:1	28.1 ± 0.35 <sup>a</sup>	35.3 ± 1.95 <sup>b</sup>	37.5 ± 0.05 <sup>b</sup>
C 18:2	12.2 ± 0.70 <sup>a</sup>	2.70 ± 0.01 <sup>b</sup>	2.00 ± 0.07 <sup>b</sup>
C 18:0	14.6 ± 0.30 <sup>a</sup>	5.30 ± 0.32 <sup>b</sup>	3.40 ± 0.20 <sup>b</sup>

<sup>1</sup>Values are means ± SEM, n = 8; Values with different superscripts differ significantly (P < 0.05).

Other reports [23-25] also have shown that weight gain, liver weight, and food intake were unaffected significantly by different types of dietary fat (saturated vs. unsaturated). In addition, Fernandez *et al.* [26] demonstrated that carcass and liver weights were unaffected in guinea pigs fed different types of dietary fats.

The present study demonstrated a markedly elevated plasma triacylglycerol levels as result of consumption of saturated dietary fats compared to unsaturated fats. A similar effect was found in rats fed other sources of saturated fat vs. unsaturated fat [23].

Rats had significantly lower plasma cholesterol levels when fed polyunsaturated fat (corn oil) than when fed saturated fat (sheep's and cow's ghee). There are two types of polyunsaturated fatty acids (PUFA) in the diets, namely, n-6 and n-3 polyunsaturated. The main polyunsaturated fatty acid in corn oil is linoleic acid (60.9% of total fatty acids) (Table 2). For many years, this fatty acid was classified a

cholesterol lowering agent. This idea came from early studies of Kinsell and Michael [27] and Ahrens *et al.* [28] which showed that plant oil rich in linoleic acid lowered the plasma cholesterol concentration when substituted in diets rich in saturated fatty acids. It has been suggested that linoleic acid (18:2) lowers total and LDL cholesterol levels more than oleic acid or carbohydrate [29,30].

The mechanism responsible for the hypocholesterolemic of polyunsaturated dietary fats has not been clearly defined. However, a review of the literature [12] and data from this study provide some speculation. First, the decreases in total and LDL cholesterol concentrations may be explained by an increase in hepatic LDL receptor activity that will lead to an increase in fractional catabolic rate (FCR) for LDL. Another reason for the decrease in total cholesterol could be due to a reduction in the cholesterol synthesis rate. This hypothesis is supported by the study that high intake of polyunsaturated fat diets reduced hepatic cholesterol synthesis rates in the guinea pig [26,31,32].

Groups fed fat derived from animal sources (groups 2 and 3) showed higher total plasma cholesterol than the group fed corn oil. Several previous reports indicated that total cholesterol concentration was raised by saturated fatty acids [7,33,34]. Moreover, in a seven countries study, Keys [35] found that intake of saturated fatty acids was highly correlated with serum cholesterol levels.

In regard to lipoproteins, concentrations of cholesterol in these fractions were significantly elevated by saturated dietary fats (Table 4). The findings of VLDL, LDL and HDL are consistent with previous reports [7,25,34]. In the present study, a diet higher in polyunsaturated fat lowers the LDL-C/HDL-C ratio when compared to diets richer in saturated fat, thus a more favorable lipoprotein profile was produced when polyunsaturated fat was fed to the rats. The LDL-C to HDL-C ratio is thought to be an index of atherogeneity of the lipoprotein. Therefore, the lower the ratio, the less atherogenic lipoprotein is thought to be [36,37].

The mechanisms whereby saturated fatty acids act as a lipid class to raise total cholesterol levels are not fully understood. The increase in plasma total and lipoprotein cholesterol may have resulted from an increase in cholesterol synthesis. In addition, the raising action of saturated fats for LDL cholesterol may be due to impaired uptake of LDL from the circulation by receptor-mediated pathways [38]. Moreover, the enrichment of hepatic fatty acids with saturated fatty acids may interfere with LDL receptors and reduce the binding and internalization of circulating LDL fraction. This mechanism has been proposed by Loscalzo *et al.* [39] when these workers suggested the enrichment of cell membrane phospholipids with saturated fatty acids

impair the internalization of LDL fraction and cause accumulation of LDL in the plasma.

Hepatic and cardiac cholesterol concentrations were increased in rats on the ghee diets as compared to the animals on corn oil. This hepatic cholesterol effect was not seen by Fernadez *et al.* [26], who fed guinea pigs diets high in saturated and unsaturated fat. The conflicting observations could result from the use of different animal models and types of saturated fat.

In the present study, fatty acid composition of hepatic lipids was affected by the fatty acid composition of the diets. Faidley *et al.* [24], in a study using pigs, found greater concentrations of palmitoleic (16:1) and oleic (18:1) acids and lesser concentrations of palmitoleic (16:1) and oleic (18:1) acids and lesser concentrations of linoleic (18:2) and arachidonic (20:4) acids in plasma lipids of animals fed animals fat versus animals fed plant oil. Moreover, another study [34] reported that diets rich in stearic and oleic acids increased the percent composition of oleic acid in plasma lipids in men. In the current study, the percent contents of palmitic, palmitoleic, and oleic acids in liver of rats fed sheep ghee and cow ghee were higher and linoleic and arachidonic acids were lower than rats fed corn oil.

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## تأثير السمن البري على تركيب الحموض الدهنية في الكبد ومستويات الكولسترول في البلازما والبروتينات الشحمية والأعضاء في الفئران

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ملخص البحث. هذه الدراسة بحثت تأثير السمن البري المصنع محلياً من حليب البقر والغنم على مستويات الكولسترول في البلازما والبروتينات الشحمية والأعضاء بالإضافة إلى محتوى الكبد من الحموض الدهنية، استخدم في هذه الدراسة ٣٠ فأراً ذكراً (وزن ٤٥ جم) من نوع Albino غذيت لمدة خمسة أسابيع على علائق تحتوي على ٥٪ دهن مأخوذ إما من زيت الذرة أو سمن الغنم أو سمن البقر. أوضحت النتائج أن مستوى الكولسترول الكلي والجليسيريدات الثلاثية أعلى في الفئران المغذاة على السمن البري مقارنة بالمجموعة المرجعية المغذاة على زيت الذرة. كما أن تناول السمن البري أدى إلى زيادة محتوى البروتينات الشحمية (VLDL, LDL and HDL) والأعضاء من الكولسترول مقارنة بالمجموعة التي غذيت على زيت الذرة. من ناحية أخرى، سبب تناول السمن البري زيادة في محتوى الكبد من كل من حمض البالميتيك Palmitic وحمض البالميتي أوليك Palmitoleic وحمض أوليك Oleic. في المقابل كانت النسبة المئوية لحمض اللينوليك Linoleic وحمض الأركيدونيك Arachidonic أكبر في الفئران المغذاة على زيت الذرة، هذه الدراسة تقترح أن زيادة المتناول من السمن البري يؤدي إلى الإفراط في ارتفاع الكولسترول وتغيير تركيب الحموض الدهنية في الكبد.

