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Goat Milk Feeding Causes an Increase in Biliary Secretion of Cholesterol and a Decrease in Plasma Cholesterol Levels in Rats

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ABSTRACT

The hypocholesterolemic effect of goat milk with respect to cow milk observed in a previous study led us to examine the influence of goat and cow milk in the diet on certain aspects of biliary physiology in normal rats. The fat content in all diets was 10% but the lipid quality was varied: the standard diet was based on virgin olive oil, and the other 2 diets included fat obtained from lyophilized cow milk and goat milk. We characterized the bile secretion, including biliary phospholipid, cholesterol, and bile acid outputs, the interrelation between bile acids and bile lipids, and the lithogenic index. The consumption of goat milk in the diet, compared with that of cow milk, caused an increase in the biliary secretion of cholesterol together with a decrease in plasma cholesterol concentration, whereas values for bile phospholipids, biliary acid concentrations, and the lithogenic index remained normal. Moreover, consumption of this type of milk decreased plasma triglyceride concentration and therefore had a positive effect, similar to that of olive oil (standard diet), on the lipid metabolism; hence, it may be recommended for consumption by the general population.
(**Key words:** goat and cow milk, dietary fat, biliary lipids, rats)

Abbreviation key: AIN = American Institute of Nutrition, **MCT** = medium-chain triglycerides, **SFA** = saturated fatty acids.

INTRODUCTION

The consumption of fat milk, from all sources, has been estimated to contribute 13.6 to 20.2% of total fat intake per person per day in Spain (MAPA, 2004). Thus, dairy products are an important source of fat in the diet. However, the consumption of such products has decreased during the last decade because of their negative image with respect to health. The association of dairy food intake with the risk of coronary heart disease has been a long-standing topic of discussion (Berner, 1993). In general, this negative effect has been attributed to high blood cholesterol concentrations arising from the saturated fatty acids (**SFA**) present in these foods. However, the type of SFA should be taken into account when considering atherogenic effects, as medium-chain triglycerides (**MCT**) do not produce this effect (During et al., 2000). In corroboration of this, in previous studies by our research groups, we have found that the consumption of goat milk, which is rich in MCT, leads to a decrease in cholesterol plasma concentrations in rats (Alférez et al., 2001).

Cholesterol from the diet is transported in chylomicrons and chylomicron remnants directly to the liver (Redgrave, 1983), where it may be excreted via the bile as unesterified cholesterol or (after conversion) as bile acids; bile acid synthesis is a major pathway for the elimination of cholesterol. Alternatively, it may be stored in the tissue as cholesteryl ester or returned to the circulation in new lipoprotein. Alterations in hepatic cholesterol metabolism in response to different types of dietary fat have been demonstrated in a number of studies. It is likely that the changes in hepatic cholesterol metabolism, which occur when animals are fed high-fat diets, alter the processing of the different types of chylomicron by the liver, and consequently the proportion of dietary cholesterol excreted in the bile (Bravo et al., 1998).

Digestion and absorption of dietary fat require the presence of an adequate concentration of bile acids in the small intestine. In different physiological and experimental situations, bile lipid secretion (cholesterol and phospholipids) seems to be determined by the secretion of bile acid (Nakano et al., 1990). Additionally, diet can induce changes in the cholesterol:phospholipid ratio (Turley and Dietschy, 1988).

For the purpose of intestinal lipid absorption, a variable flux of bile salt and cholesterol takes place from the liver into the duodenum via the biliary tract. Although most of these steroids are recovered by reabsorption in the lower intestine, by temporarily leaving the "milieu interne" of the organism, they challenge the animal's capability to maintain the homeostasis of its steroid

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pool. Hence, to understand the metabolism of cholesterol and bile salts, it is useful to perform an analysis of bile flow and steroid concentration in the bile at short intervals in vivo.

The aim of this study, thus, was to investigate the hypocholesterolemic effect of the dietary consumption of goat milk and to determine whether the biliary output of cholesterol contributed to this effect.

MATERIALS AND METHODS

Animals

We studied 28 rats (male rats, *Rattus norvegicus*, Wistar albino breed), with a mean initial body weight of 262.5 ± 5.7 g (10 to 11 wk old), purchased from the University of Granada Laboratory Animal Service. All experiments and surgical procedures were performed according to international guidelines. The experimental protocol was approved by the Ethics Committee of the University of Granada.

Diets

The diets and mineral and vitamin supplements were prepared according to the recommendations of the American Institute of Nutrition (**AIN**; Reeves et al., 1993), except that the level of fat in the diets was 10% rather than 5%. The standard diet was prepared using virgin olive oil as the source of fat (10%) and casein as the protein source (20%). The milk-based diets were created with lyophilized cow or goat milk. The analysis of milk lyophylates is shown in the footnote to Table 1. The necessary quantities of lyophilized goat or cow milk were used to obtain a diet with a 10% fat content. To obtain the 20% protein content (as recommended by the AIN; Reeves et al., 1993), the diets were supplemented with casein (Musal & Chemical, Granada, Spain), using 12.40 g of casein/100 g for the cow's milk diet and 14.50 g of casein/100 g for the goat's milk diet, as the protein provided by the lyophylate used for the milk-based diets was insufficient.

The mineral corrector was prepared according to AIN recommendations (Reeves et al., 1993) for the standard diet and to our own specifications for the milk-based diets. These specific correctors were formulated taking into account the mineral content of the lyophilized milks supplied to the rats to meet the mineral content recommendations of the AIN (Reeves et al., 1993). The lactose content of the milk diets was subtracted from the total carbohydrate content of the standard diet, and wheat starch and sucrose were added corresponding to the difference (Table 1).

The following analyses were made of the diets and lyophylates: Dry matter (AOAC, 1990), calcium, iron, copper, magnesium, and zinc (atomic absorption spectrophotometry, Perkin-Elmer 1100B, Shelton, CT), phosphorus (method of Fiske and Subbarow, 1925) and cholesterol [enzymatic kit for food analysis, Boehringer Mannheim (Grossmann et al., 1976)].

Table 1. Composition of the experimental diets.

Component [g/kg of diet (DM basis)]	Experimental diet[1]		
	Diet S	Diet C	Diet G
Protein (casein)[2]	210	204	205
Fat (olive oil; MCT[3] = 0 g)	100	0	0
Fat (cow's milk; MCT = 20.9 g)	0	99	0
Fat (goat's milk; MCT = 33.7 g)	0	0	94
Fiber (micronized cellulose)	51	52	49
Mineral specific supplement[4]	36	35	36
Vitamin specific supplement[4]	10	10	10
Choline chloride	2	2	2
L-Cysteine	1.8	1.8	1.8
Wheat starch	491	304	310
Sucrose	100	100	100
Lactose	0	195	190
kJ	17,940	18,844	18,771

[1]Diet S = Standard diet; Diet C = cow's milk diet; Diet G = goat's milk diet. Cholesterol content of the diets (in mg/kg of diet): Diet S = trace; Diet C = 23.74; Diet G = 28.53.

[2]Protein content for diets C and G comprises casein + protein from cow's or goat's milk, respectively.

[3]MCT = Medium-chain triglycerides.

[4]Mineral and vitamin supplements were formulated taking into account the mineral content of the lyophilized milks to meet the mineral requirements recommended by the American Institute of Nutrition (Reeves et al., 1993). Analysis of lyophylates used (mg/kg of diet): Fat content (cow's milk = 28.76%; goat's milk = 30.69%), protein content (cow's milk = 24.84%; goat's milk = 23.36%), lactose content (cow's milk = 40.75%; goat's milk = 39.20%), and mineral composition (in mg/100 g of lyophylate): cow's milk = Ca, 1030.5; P, 781.9; Mg, 85.25; Fe, 0.87; Cu, 0.14; and Zn, 3.51; goat's milk = Ca, 1319; P, 813.3; Mg, 89.5; Fe, 1.23; Cu, 0.25; and Zn, 4.9.

Experimental Design

Three experimental groups were established, each provided with 1 of the 3 types of diet: the standard diet (n = 8), the cow's milk diet (n = 10), and the diet based on goat's milk (n = 10) (Table 1).

During the treatments with the different types of diet, the animals were weighed and housed in individual ventilated and thermoregulated cages (22 ± 2°C) with a 12-h light:dark period. For 2 wk, diets and mineral-free water were available ad libitum to all rats. At the end of the experimental period, the rats were weighed after an overnight fast and anesthetized by intraperitoneal injection of 5 mg/100 g of BW of sodium pentobarbital (Sigma Chemical Co., St. Louis, MO). The animals were tested by tail pinch reflex until complete loss of the reflex, and body temperature was maintained at 37°C with a thermisor-controlled heated pad. To avoid the effect of fasting motor activity on biliary emp-