

## Fatty Acid Composition of Polar Lipids in Goats' Milk

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### ABSTRACT

Silicic acid column chromatography was used to separate the polar lipids of goats' milk into glycolipid, phosphatidylethanolamine, phosphatidylserine plus phosphatidylinositol, phosphatidylcholine, and sphingomyelin fractions. Each fraction was purified by column chromatography and its fatty acid profile determined by gas liquid chromatography and mass spectrometry. The glycerophospholipids each contained 18:1 as the predominant fatty acid (~45%). The sphingolipids contained a high percentage of long-chain saturated fatty acids ( $C_{22}$  to  $C_{24}$  > 45%); the glycolipid fraction also contained ca. 2% 2-hydroxy fatty acids. The data represent a comprehensive cross-sectional study of the major polar lipids found in goats' milks.  
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The fatty acid compositions of the total and neutral lipids in goats' milk have been studied (1,2); however, less is known regarding their polar lipids, which comprise about 1.6% of the total lipids (2,3). Although, as pointed out by Morrison (4), milk polar lipids do not constitute a large part of man's diet, they do represent a major fraction of the total phospholipid ingested and may be of dietary significance. For example, lecithin has recently been shown to inhibit cholesterol absorption in rats (5). The polar lipids also contain polyunsaturated acids which are essential for human nutrition and may serve as precursors for prostaglandins which regulate metabolic functions (6-8).

This paper is part of a study of the lipids of goats' milk (1) and describes the fatty acid composition of the polar lipids.

### EXPERIMENTAL

#### Materials

Raw goats milk samples were obtained from a large commercial dairy goat company, and were maintained at 5 C in transit to the laboratory. Upon receipt, the samples were lyophilized and stored at -20 C. Before lipid extraction, equal weights of 5 samples obtained during the months of April through June were mixed together to minimize nutritional, environmental, and breed differences.

#### Reagents

All solvents were of nanograde quality. Unisil silicic acid, 100-200 mesh was from Clarkson Chemical Co. (WilliamSPORT, PA). Precoated thin layer chromatographic plates SIL G-25 (Macherey-Nagel) were from Brinkmann Instruments, Inc. (Westbury, NY).

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#### Lipid Extraction

Free lipids were obtained by extracting the freeze-dried samples 4 times with petroleum ether; the bound lipids were obtained by 3 subsequent extractions with chloroform/methanol (2:1) as described previously (9). Lipid extracts and column fractions were analyzed by thin layer chromatography (TLC) (9). Developing solvents were petroleum ether/diethylether/acetic acid (90:10:1) for neutral lipids, and chloroform/methanol/water (65:25:4) for polar lipids (glycolipids and phospholipids). Iodine vapor was used to visualize the separated lipid classes. Identifications were made on the basis of migration relative to reference standards.

#### Silicic Acid Column Separations

Initial separations were carried out on Unisil columns (2.5 X 25 cm). Lipid classes were separated by modification of the procedures of Masoro et al. (10) and Rouser et al. (11). Routinely, 3.2 g of bound lipid were applied: fraction A, eluted with chloroform (575 ml), contains neutral lipids; fraction B, eluted with acetone (600 ml), contains glycolipids; fraction C, eluted with 700 ml chloroform/methanol (6:1), contains phosphatidylethanolamine; fraction D, eluted with 550 ml ethylacetate/methanol (7:4), contains phosphatidylserine and phosphatidylinositol; fraction E, eluted with 600 ml chloroform/methanol (1:1), contains most of phosphatidylcholine and sphingomyelin. Each fraction was rechromatographed on a smaller Unisil column (1 X 20 cm) using the same solvent systems to remove contaminants. Each separation step was monitored by TLC as described above. Weight distributions of the polar lipid classes were determined gravimetrically; recoveries were usually greater than 95%.

#### Preparation of Fatty Acid Methyl Esters

Glycerophospholipids (2 to 5 mg) were transesterified with 0.6 N NaOH in methanol (5 ml) for 1 hr at room temperature (12), after which 1 N HCl, chloroform, and water were added (for 10 ml total volume) and the methyl esters were recovered in the chloroform layer. If some fatty acids were not methylated, as judged by TLC, the fraction was treated with 5% HCl in methanol at 60 C for 1 hr. The methyl esters were extracted with petroleum ether.

Column fractions containing sphingomyelin and glycolipids (cerebrosides) were first purified (13) by mild alkaline hydrolysis (0.3 N NaOH, 1 hr, rt) to remove traces (if any) of glycerol containing lipids. The reaction mixture was chromatographed on a small Unisil column as described above. The purified lipids were refluxed in methanolic HCl (1 N) for 6 hr and methyl esters recovered by petroleum ether extraction.

Methyl esters of fatty acids were separated into nonhydroxylated and 2-hydroxylated esters by using a small (1 X 20 cm) Unisil column (14). Nonhydroxylated fatty acid methyl esters were eluted with petroleum ether/diethylether (96:4) and 2-hydroxylated esters with petroleum ether/diethylether (80:20).

#### Gas Liquid Chromatography (GLC) Mass Spectrometry (MS) of the 2-Hydroxy Fatty Acid Methyl Esters

A Hewlett-Packard Model 5922-B combination of GLC-low-resolution Quadropole MS interfaced to the Hewlett-Packard Model 9825-A data system was employed for GLC-MS analysis. The 70 eV electron impact mass spectra were obtained following separation on a 1.83 m X 0.64 cm (od) glass column packed with 3% OV-17 on 100/120 mesh Gas Chrom Q. The injection port was maintained at 150 C and the column temperature programmed from 140 C to 280 C at 4 C/min. Helium served as the carrier gas.

#### GLC of the Nonhydroxylated Fatty Acid Methyl Esters

GLC studies of the nonhydroxylated fatty acid methyl esters were performed on the Hewlett-Packard Model 5750 Gas Chromatograph equipped with a flame ionization detector. The methyl esters were separated isothermally at 180 C on a 1.83 m X 0.32 cm stainless steel column packed with 10% Silar 10C on 100/120 mesh Gas Chrom Q. The injection port and exit port were maintained at 200 C and 240 C, respectively, and helium served as the carrier

gas. Peak identification was attained by reference fatty acid methyl ester retention times ( $C_{12}$  -  $C_{18}$  methyl esters) and extrapolation ( $C_{19}$  -  $C_{24}$  methyl esters). Identities of the high molecular weight methyl esters in the glycolipid and sphingomyelin fractions were confirmed by GLC-MS employing the same conditions outlined above for the 2-hydroxy fatty acid methyl esters.

## RESULTS AND DISCUSSION

The milk samples in this study were obtained from a large commercial dairy which collects and processes goats' milk for human consumption. The 5 bulk milk samples are representative of commercially available goats' milk in that they were obtained from several breeds during the months of April through June when does usually are in full lactation.

The lipids were extracted first with petroleum ether (free lipid fraction) and then with  $CHCl_3$ /methanol ("bound lipid" fraction) as previously described (1). The first extract contains the majority of the neutral lipids as well as the free fatty acids of milk. The second extract consists of additional neutral lipid (46.8%) plus all of the polar lipids (53.2%). The bound lipid classes were separated by silicic acid column chromatography, and the weight distribution among the various polar lipid classes is given in Table 1.

The most notable figure in Table 1 is the relatively high glycolipid content found in this study. Early work on cows' milk (15) estimated the glycolipids to account for up to 6% of the total polar lipids. Kayser and Patton (16) subsequently estimated that the glycolipid content of milk membranes was closer to ~9% in both goats' and cows' milk. In this work, the value was found to be 16% of the total polar lipids of whole milk. The fatty acid

TABLE I  
Distribution of Polar Lipids of the  
"Bound Lipid" Fraction of Goats' Milk

	Weight (%) <sup>a</sup>
Phosphatidylethanolamine	29.7
Phosphatidylserine	2.7
Phosphatidylinositol	3.4
Phosphatidylcholine	23.7
Sphingomyelin	24.5
Glycolipids	16.0

<sup>a</sup>From chromatographic analysis of chloroform/methanol extract of goats' milk. Weight percentage was determined gravimetrically.

profiles associated with each class after purification have been determined by GC and GC-MS. The saturated and unsaturated fatty acids present in these polar lipids are shown in Table 2.

The predominant fatty acids associated with the glycerol-based phospholipids are 16:0, 18:0, 18:1, and 18:2 with the 18:1 accounting for about 50% of the fatty acids in each class. Phosphatidylcholine (PC) contained 36.9% 16:0, whereas phosphatidylethanolamine (PE) and phosphatidylserine (PS) + inositol (PI) contained only 12.9% and 7.0% of this acid. The fatty acid profiles found in this study for these glycerophospholipids are quite similar to those found for human and cows' milks (17). However, PE tended to have a lower polyunsaturated acid content, and a higher 18:0 content than reported by Moore et al. (18) for PE from goats' milk. These differences could be due to seasonal and dietary responses, but Moore's data was from a specific breed, while the data presented here may be more representative of goats' milk in general. The glycerophospholipids contained only trace amounts of hydroxy fatty acids.

The glycolipids or cerebrosides (GL) and sphingomyelin (Sph) fractions, in contrast to the phosphatidyl lipids contained significant amounts of 22:0, 23:0, and 24:0 fatty acids, indicating that these lipids are highly saturated

in goats' milk. The 23:0 chain is somewhat unusual, but these data are in good agreement with the results previously reported for the glucosyl and lactosyl cerebrosides of cows' milk fat globule membrane (16), except that the 16:0 is somewhat higher in this work. The glycolipid fraction was not separated into glucosylceramide fractions because lactosylceramide was such a minor component of the glycolipid fraction of the goats' milk. The glycolipid fraction contained 2% 2-hydroxy fatty acids and the compositional profile of these acids is given in Table 3. The 2-hydroxy fatty acid content found for goats' milk is similar to that found for cows' milk glycolipids (19) with only the C<sub>25</sub> and C<sub>26</sub> present in significantly higher amounts in goats' milk.

The sphingomyelin fraction of goats' milk (Table 2), like that of cows' milk, contains a high percentage of long-chain fatty acids (19-21), but the goats' milk sphingomyelin has 16:0 and 18:0 at higher levels. The 2-hydroxy fatty acids were not quantitated because of insufficient amount of material. Hydroxy fatty acids comprised less than 2% of the total fatty acids in the sphingomyelin fraction.

Although the fatty acid composition of cows' milk has been studied in detail, the information on cows' milk has been more fragmentary. In this study, we report the fatty acid profiles for various polar lipids of goats'

TABLE 2

Weight Percentage of the Fatty Acids in the Polar Lipids of Goats' Milk<sup>a</sup>

Fatty acid <sup>b</sup>	Glycolipids	Phosphatidyl <sup>c</sup> ethanolamine	Phosphatidyl serine plus inositol	Phosphatidyl choline	Sphingo myelin
12:0	0.8	-	-	-	-
13:0	-	-	-	-	-
14:0	2.7	0.4 (0.6)	tr	2.2	3.1
15:0	tr	-	-	-	0.1
16:0	17.4	12.9 (10.0)	7.0	36.9	26.2
17:0	0.6	-	-	-	0.5
18:0	9.5	31.6 (9.9)	26.7	9.0	10.3
18:1	2.6	46.3 (52.0)	56.1	46.3	0.6
18:2	-	5.6 (18.4)	10.2	5.6	-
18:3	-	3.4 (1.7)	-	< 0.5	-
19:0	tr	-	-	-	tr
20:0	2.6	- (2.8)	-	-	2.3
21:0	1.8	-	-	-	1.1
21:1	1.0	- (4.5)	-	-	-
22:0	22.0	-	-	-	17.9
23:0	23.0	-	-	-	20.4
24:0	13.6	-	-	-	13.3
24:1	2.4	-	-	-	4.3

<sup>a</sup>Average of duplicate determinations.

<sup>b</sup>Minor fatty acids as branched-chain were identified in milk samples but were omitted from tables.

<sup>c</sup>Values in parenthesis reported by Moore et al. (18).

TABLE 3

Composition of the 2-Hydroxy Fatty Acids in the Polar Lipids<sup>a</sup> of Goats' Milk

Carbon number	Weight (%) <sup>b</sup>	
	Glycolipids	Sphingomyelin <sup>c</sup>
16	17.0	tr
18	11.9	tr
22	14.4	tr
23	22.4	tr
24	25.4	tr
25	4.0	—
26	5.0	—

<sup>a</sup>2-Hydroxy acids were not detected in phosphatidylcholine, phosphatidylethanolamine, or phosphatidylinositol plus phosphatidylserine fractions.

<sup>b</sup>Average of duplicate determinations.

<sup>c</sup>Carbon number identified by GLC-MS but quantitation not possible because of insufficient amounts of material.

milk. The fatty acid profiles for only PE and PS from goats' milk have been previously reported (2,18); thus, those for PC, Sph, and GL are apparently reported for the first time. All of the values determined in this work are all from the same large, pooled commercial goats' milk sample. This information coupled with our report (1) on the triglyceride fatty acids from the same pooled samples present a comprehensive view of goat milk lipids.

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Reference to brand or firm name does not constitute

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