Fecal steroids of the coprolite of a Greenland Eskimo mummy, AD 1475: a clue to dietary sterol intake^{1–3}

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ABSTRACT

Background: Sterols in feces reflect sterols in the diet. In previous analyses of the fecal steroids in 1000–2000-y-old Native American coprolites found in the dry caves of Nevada, we showed that the sterol nucleus was stable. The coprolites provided useful dietary information.

Objective: In the present study, we analyzed the fecal steroids of an Eskimo mummy buried and frozen >500 y ago in Greenland. We compared these analyses with our findings in the coprolites from Nevada and in present-day stool samples from Tarahumara Indians of Mexico and Americans consuming low- and highcholesterol diets.

Design: The fecal material from the Eskimo mummy was subjected to saponification, extraction, and digitonin precipitation. The sterols and bile acids were further analyzed by thin-layer chromatography and gas-liquid chromatography.

Results: The fecal steroids of the Greenland Eskimo mummy were remarkably similar to those of present-day stool samples. However, unlike in the stool of modern humans, a portion of the neutral steroids in the coprolite had been converted to sterol epimers. Instead of deoxycholic acid, 3α , 6β , 12α -trihydroxycholanic acid was one of the major fecal bile acids. The plant sterol output in the coprolite was only 0.4% of the output of Americans consuming 250–400 mg plant sterols/d. The ratio of bile acid to cholesterol in the coprolite was similar to that in stool from Tarahumara Indians consuming a low-cholesterol diet.

Conclusion: The sterol nucleus is stable when frozen. The analysis of coprolite showed that the young Eskimo woman had consumed a diet very low in plant sterols and moderate to low in cholesterol content. *Am J Clin Nutr* 2001;74:44–9.

KEY WORDS Cholesterol, plant sterols, fecal bile acids, fecal coprostanol, coprostanol epimers, Eskimo, Greenland, mummy, coprolite

INTRODUCTION

Steroids in feces are derived from dietary and endogenous cholesterol and from dietary plant and shellfish sterols. Accordingly, information about dietary sterol intake can be obtained through fecal steroid analysis. Fecal steroids are composed of many different compounds and are grouped into 2 categories according to their chemical structure: neutral and acidic steroids. Neutral steroids include cholesterol (both endogenous and exogenous) and its bacterially modified products (coprostanol and coprostanone), cholestanol, and plant sterols (campesterol, stigmasterol, and sitosterol) and their bacterially modified products. The acidic steroids (bile acids) include the primary bile acids (cholic acid and chenodeoxycholic acid) and their bacterially modified products, deoxycholic and lithocholic acids.

Twenty years ago, we had the rare opportunity to analyze the fecal steroid composition of 6 human coprolite samples, some >2000 y old, derived from Native American graves in the Love-lock Caves of Nevada (1, 2). Because of the dry environmental conditions in Nevada, the fecal steroid composition of the coprolites was remarkably similar to that of stool samples collected today, despite the lengthy period of storage. However, we also observed that, unlike the steroid composition of recently collected stool, 22% of the steroids in the coprolites were in the 3 α epimer form, presumably formed from the normal 3 β form during storage. From these data, we obtained some knowledge of the dietary sterol intake of these humans who lived many centuries ago. In particular, the coprolites had a high content of plant sterols. The ratio of plant sterol to cholesterol was also high, suggesting that a large proportion of the diet was derived from plant sources.

In 1985 the discovery of several well-preserved mummies on the west coast of Greenland was reported (3, 4). Carbon dating data indicated that these mummies were buried around AD 1475. Fecal material was found in one of the mummies (no. II-7), a woman in her late teens or early twenties. Because the dietary practices of Eskimos are quite different from those of the Lovelock Cave Indians of Nevada (1, 2), analysis of these prehistoric Eskimo stools was of special interest. There was also the question of how well preserved the steroid nucleus would be after \geq 500 y of storage in the dry and cold. With the cooperation of Danish pathologists, we analyzed the steroid composition of this interesting sample and compared these results with our previous analyses of the coprolites from the Lovelock Cave in Nevada and

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TABLE 1

Comparison of the composition of neutral steroids in the coprolite from a Greenland Eskimo mummy, coprolites from Nevada Indians, and presentday stool samples from Americans¹

	Greenland Eskimo (AD 1475)	Nevada Indians (AD 50) ²	Americans (AD 1983) ³	
	µg/g dried wt			
Cholesterol and metabolites				
Sterol	1020	252	2388	
Stanol	3494 ⁴	5043 ⁵	18999	
Stanone	318	380	671	
5α-Cholestanol	92	102	294	
Total	4924	5777	22352	
Plant sterols and metabolites				
Sterol	9	253	605	
Stanol	13	1904	5262	
Stanone		121	182	
Total	22^{6}	2278	6054	

 $^{1}n = 1$ sample per group.

²Data from reference 2.

³Data from WE Connor, DS Lin, MP McMurray, unpublished observations, 1983.

⁴32% in the epimer form.

⁵25% in the epimer form.

⁶In the stool of Nevada Indians and Americans, there are 3 common plant sterols (campesterol, stigmasterol, and sitosterol). Only a very small amount of sitosterol and its stanol was detected in the coprolite of the Greenland Eskimo.

of present-day stool samples of Tarahumara Indians and Americans who consumed low- and high-cholesterol diets. The studies of the Tarahumara Indians and Americans had been carried out under metabolic ward conditions (5; WE Connor, DS Lin, MP McMurray, unpublished observations, 1983). Therefore, the exact dietary composition and precise sterol intakes of these subjects were known. The cholesterol content was 50 or 1000 mg/d for the low- and high-cholesterol diets, respectively. The plant sterol intake of the Tarahumara Indians was 400–500 mg/d and that of the Americans was 250–400 mg/d. The steroid content per gram dry weight of stool for the Tarahumara Indians and Americans was calculated with the assumption that the water content of the stool was 75% (6).

MATERIALS AND METHODS

We analyzed the fecal steroids of the Greenland Eskimo mummy in comparison with reference standards and compounds known to be present in the stool of modern humans (7–9). Reference standards used for identification were 5 α -cholestane, cholesterol (5-cholesten-3 β -ol), campesterol (5-cholestane, 24 β -methyl-3 β -ol), stigmasterol (5,22-cholestadien-24 β -ethyl-3 β -ol), sitosterol (5-cholesten-24 β -ethyl-3 β -ol), coprostanol (5 β -cholestan-3 β -ol), coprostanone (5 β -cholestan-3-one), lithocholic acid (5 β -cholanic acid-3 α -ol), chenodeoxycholic acid (5 β -cholanic acid 3 α ,7 α -diol), hyodeoxycholic acid (5 β -cholanic acid 3 α ,6 α -diol), and cholic acid (5 β -cholanic acid 3 α ,7 α , 12 α -triol) from Applied Science Laboratories, Inc (State College, PA). Epicoprostanol (5 β -cholestan-3 α -ol), cholestanol (5 α -cholestan-3 β -ol), and deoxycholic acid (5 β -cholanic acid, 3 α ,12 α -diol) were obtained from Steraloids, Inc (Pawling, NY). In the present study, we used the same analytic procedures used to determine the composition of the coprolites from the Lovelock Cave in Nevada (2). These methods were developed by Miettinen et al (7) and Grundy et al (8) and adapted and modified by us. The fecal material was saponified in alcoholic sodium hydroxide. Neutral and acidic steroids were then separated by extracting the neutral steroids with petroleum ether. Neutral steroids were purified by precipitation with digitonin (10). The free sterols were recovered from the digitonide (11) and redissolved in chloroform for thin-layer chromatography (TLC). The digitonin-nonprecipitable sterols in the washings were collected and subjected to the same TLC analysis. The TLC system involved florisil plates and heptane:ethyl ether (45:55, by vol) as a solvent.

Neutral fecal steroids can be separated by TLC into 3 groups of compounds according to their structure. The cholesterol band also contains 3 plant sterols (campesterol, stigmasterol, and sitosterol) (7, 9). A small amount of ring-saturated 5α derivatives of these 4 sterols will also be found in the cholesterol band. In the coprostanol band are coprostanol and the ring-saturated homologues of the 3 plant sterols. In the coprostanone band are coprostanone and the comparable homologues of the 3 plant sterols. The sterols of each band were extracted with ethyl ether and derivatized to trimethylsilyl ethers before gas-liquid chromatography (GLC). Cholestane was used as the internal standard.

The aqueous layer left after neutral steroid extraction contained bile acids, which were saponified in a pressure cooker at ≈ 103 kPa (15 psi). The free bile acids were methylated and chromatographed with use of 2 solvent systems on the thin-layer silica gel H plate (Brinkmann Co, Burlingame, CA). The first solvent was benzene and the second was isooctane:isopropanol:acetic acid (120:40:1, by vol). The area including the bands from cholic acid to lithocholic acid was scraped off and extracted with methanol.

One-half of these bile acids were derivatized to trimethylsilyl ethers and subjected to GLC with a less polar fused silica capillary SE-30 column (silicon gum and methyl; Applied Science Laboratories, Inc, Inglewood, CA). The remaining bile acids were derivatized to trifluoroacetates and chromatographed by a more polar liquid phase QF-1 packed column (silicon gum, trifluoropropyl, and methyl; Applied Laboratories, Inc). The GLC analyses were performed on an instrument equipped with a hydrogen flame ionization detector (model 5830; Hewlett-Packard, Skokie, IL).

GLC was carried out with cholestane and hyodeoxycholic acid as internal standards (2, 8). The loss during the process was monitored by the radioactive standards of [4-¹⁴C]cholesterol (New England Nuclear Corp, Boston) for neutral steroids and [24-¹⁴C]deoxycholic acid (Tracer Lab, Waltham, MA) for bile acids. Radioactivity was measured with a liquid scintillation counter (LS 8000 series; Beckman Instruments, Inc, Fullerton, CA). Each sample was analyzed twice and the same results were obtained in repeated runs.

RESULTS

The contrast in fecal neutral steroid composition between Greenland Eskimos (AD 1475), Nevada Indians (AD 50), and modern Americans (AD 1983) is presented in **Table 1**. Although the patterns are amazingly similar, some differences exist. In both coprolites, 25–32% of the coprostanol was in the epimer form. The epimer form was not found in the stool of modern

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FIGURE 1. Gas-liquid chromatogram of the fecal neutral sterol pattern of stool from modern humans (A) and of a coprolite from a Greenland Eskimo mummy (B). Labeled peaks are as follows: 1, cholestane (internal standard); 2, cholesterol; 3, cholestanol; 4, campesterol; 5, stigmasterol; 6, sitosterol; and 7, sitostanol.

humans. The coprolite of the Greenland Eskimo had a very low plant sterol content (1/100 and 1/300 of the plant sterols of the stool from Nevada Indians and modern Americans, respectively). These vast differences in plant sterol content are also shown in **Figure 1**, in which the GLC pattern of the fecal sterols of the Greenland Eskimo coprolite is compared with that of stool from modern humans. Similar differences also existed in fecal plant stanol content (Table 1). In the coprolite, the ratio of cholesterol to plant sterols was 224. In contrast, the ratios of cholesterol to plant sterols were 2.53 and 3.69 for Nevada Indians and modern Americans, respectively.

The fecal bile acid compositions of the samples from the Greenland Eskimo, Nevada Indians, and modern Americans are listed in **Table 2**. The major fecal bile acids for modern Americans and Nevada Indians were lithocholic and deoxycholic acids. In contrast, the major fecal bile acids for the Greenland Eskimo were lithocholic and 3α , 7β , 12α -trihydroxycholanic acids. The content of deoxycholic acid was low in the Greenland Eskimo coprolite.

In projecting dietary sterol intake from the fecal steroid excretion data of the ancient stools, we compared fecal steroid excretion (cholesterol, plant sterols, and bile acids; **Table 3**) and the ratio of bile acids to cholesterol and of plant sterols to cholesterol (Table 4) between the ancient Greenland Eskimo and Nevada Indians and the modern Tarahumara Indians and Americans consuming known quantities of dietary sterols. The total fecal cholesterol excretion (cholesterol plus bile acids) in the coprolite of the Greenland mummy was comparable with that in the coprolite of the Nevada Indians (9289 compared with 8019 μ g/g stool) and lower than that in stool from either the Tarahumara Indians or Americans consuming low-cholesterol diets (16729 and 16361 µg/g, respectively). The fecal plant sterol excretion of the Greenland Eskimo was only 1/100 of the plant sterol excretion of the Nevada Indians and 1/300-400 of that of the Tarahumara Indians consuming 400-500 mg plant sterols/d and of Americans consuming 250-400 mg plant sterols/d, respectively. Furthermore, unlike in the coprolite and stool of the Nevada Indians, Tarahumara Indians, and Americans, whose fecal plant sterols included campesterol, stigmasterol, and sitosterol, only trace amounts of sitosterol and no other plant sterols were detected in the coprolite of the Greenland Eskimo (Table 1). The ratio of bile acid to cholesterol in the coprolite from the Greenland Eskimo was similar to that in stool from Tarahumara Indians consuming a low-cholesterol diet (0.89 compared with 0.72). Interestingly, this ratio was much higher than that in the Nevada Indians (0.89 compared with 0.31), despite similar total cholesterol excretion between the Nevada Indians and the Greenland Eskimo. The Nevada Indians had ratios of bile acid to cholesterol similar to that of Americans consuming low-cholesterol diets (0.31 compared with 0.36).

DISCUSSION

The most interesting result of our analysis of the coprolite from the Greenland mummy was the virtual absence of all plant sterols (Figure 1 and Table 1). Plant sterols were estimated to be 22 μ g/g dried wt of stool compared with 2417 μ g/g in the coprolites of Nevada Cave Indians, 9310 μ g/g in the stool of Tarahumara Indians who consumed 400–500 mg plant sterols/d, and 6039 μ g/g in the stool of Americans who consumed 250–400 mg

TABLE 2

Comparison of the bile acid composition of the coprolite from a Greenland Eskimo mummy, coprolites from Nevada Indians, and present-day stool samples from Americans¹

	Greenland Eskimo (AD 1475)	Nevada Indians (AD 50) ²	Americans (AD 1983) ³
	µg/g dried wt		
7β-Hydroxycholanic acid	637	_	_
Lithocholic acid	1248	467	2025
3β,12α-Dihydroxycholanic acid	140	110	479
Deoxycholic acid	240	296	2488
Chenodeoxycholic acid	135	63	68
3α , 7β , 12α -Trihydroxycholanic acid	1231		_
3β-Hydroxy-12-ketocholanic acid	_	47	42
12-Ketolithocholic acid	_	147	74
3-Keto-7α-Hydroxycholanic acid	532	31	32
Unidentified	202	304	53
Total	4365	1465	5261

 $^{1}n = 1$ sample per group.

²Data from reference 2.

³ Data from WE Connor, DS Lin, MP McMurray, unpublished observations, 1983. Comparison of total cholesterol, bile acids, and plant sterols in the coprolite from a Geenland Eskimo mummy, coprolites from Nevada Indians, and present-day stool samples from Tarahumara Indians and Americans consuming low-cholesterol (LC) and high-cholesterol (HC) diets

	Greenland Eskimo (AD 1475) ¹	Nevada Indians (AD 50) ²	Tarahumara Indians (AD 1983) ³		Americans (AD 1983) ⁴	
			LC diet	HC diet	LC diet	HC diet
	μg/g dried wt					
Cholesterol and metabolites						
Cholesterol	4924	6407 ± 2469^{5}	9589 ± 2575	19367 ± 3885	11756 ± 4287	34305 ± 15922
Bile acids	4365	1612 ± 535	6871 ± 2120	6038 ± 1279	4605 ± 2750	6846 ± 3698
Total	9289	8019 ± 2450	16729 ± 4497	25749 ± 3531	16361 ± 6742	41147 ± 18777
Plant sterols	22	2417 ± 935	9310 ± 3494	8826 ± 3445	6039 ± 3306	6219 ± 2350

 $^{1}n = 1.$

 $^{2}n = 5$ samples. Data from reference 2.

 $^{3}n = 8$ subjects. Data from reference 5. LC diet provided 50 mg cholesterol/d and 400–500 mg plant sterol/d; HC diet provided 1000 mg cholesterol/d and 400–500 mg plant sterol/d.

 ${}^{4}n$ = 6 subjects. Data from WE Connor, DS Lin, MP McMurray, unpublished observations, 1983. LC diet provided 50 mg cholesterol/d and 250–400 mg plant sterol/d; HC diet provided 1000 mg cholesterol/d and 250–400 mg plant sterol/d.

 $5\overline{x} \pm SD$.

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plant sterols/d (Table 3). However, large amounts of cholesterol were detected in the Eskimo sample. Thus, the lack of plant sterols in this sample cannot be attributed to degradation of the steroid nucleus. Consequently, the ratio of plant sterol to cholesterol was much lower in the coprolite from the Greenland mummy than in the coprolites from Nevada Indians or in the stool of Tarahumara Indians consuming 50 mg cholesterol and 400–500 mg plant sterols/d and of Americans consuming 50 mg cholesterol and 250–400 mg plant sterols/d. Therefore, the logical explanation seems to be that the plant sterol intake of these persons must have been very low because their diet lacked foods of plant origin.

Much valuable information was obtained by studying this mummy's remains; however, the cause and time of her death are still unknown (3, 4). Pollen samples from several Arctic plants such as mountain sorrel were found in the coprolite. If the Eskimo woman had picked the sorrel and eaten it directly, it would mean she died in midsummer, when the plant blooms. Or, she could have as easily ingested a flower that had been preserved in seal oil and was eaten long after the blooming season. The mummy was found near the small Inuit settlement of Qilakitsoq on the west coast of Greenland. Qilakitsoq was a principal winter camp for hunting seal, beluga, walrus, polar bear, narwhal, reindeer, and ptarmigan. It is most likely that these animals were the main food supply. In fact, hair from seals, reindeer, and arctic hare, as well as bits of feathers from ptarmigan and dovekie, were also found in the fecal material (3), an indication of the ingestion of food at the time of death. Perhaps a virtually plant-food-free diet was typical for these Eskimos during wintertime. Our finding of an extremely low plant sterol content in the stool not only indicates the lack of plant food in these Eskimos' diet but also suggests that the time of death of this young woman was during the winter, when plant food was scarce.

The fecal steroid data of this Greenland mummy also suggest a low cholesterol intake. In an attempt to shed some light on the dietary pattern of the Eskimos who lived centuries ago, we compared the steroids in the coprolite in terms of both absolute quantity (μ g/g dried wt) and relative ratios (bile acid to cholesterol and plant sterol to cholesterol) with coprolites from the Lovelock Cave in Nevada and stool from Tarahumara Indians and Americans for whom dietary sterol intakes were known (Tables 3 and 4). The cholesterol-derived content of the coprolite (cholesterol plus bile acids) of the Greenland mummy was 9289 μ g/g dried wt (Tables 1 and 2). This value was similar to the sterol excretion in coprolites from the Nevada Indians but lower than that of Tarahumara Indians and Americans consuming a low-cholesterol diet. These comparisons suggest that the cholesterol intake of Greenland Eskimos was on the low side.

TABLE 4

Comparison of ratios of bile acid to cholesterol and of plant sterols to cholesterol in the coprolite from a Geenland Eskimo mummy, coprolites from Nevada Indians, and present-day stool samples from Tarahumara Indians and Americans consuming low-cholesterol (LC) and high-cholesterol (HC) diets

	Greenland Eskimo Nevada Indians		Tarahuma (AD	Tarahumara Indians (AD 1983) ³		Americans (AD 1983) ⁴	
	(AD 1475) ¹	(AD 50) ²	LC diet	HC diet	LC diet	HC diet	
Bile acid:cholesterol	0.89	0.31 ± 0.22^{5}	0.72 ± 0.16	0.32 ± 0.09	0.36 ± 0.17	0.20 ± 0.09	
Plant sterol:cholesterol	0.004	0.47 ± 0.35	0.98 ± 0.19	0.48 ± 0.15	0.50 ± 0.20	0.20 ± 0.09	

 $^{1}n = 1.$

 $^{2}n = 5$ samples. Data from reference 2.

 $^{3}n = 8$ subjects. Data from reference 5. LC diet provided 50 mg cholesterol/d and 400–500 mg plant sterol/d; HC diet provided 1000 mg cholesterol/d and 400–500 mg plant sterol/d.

 $^{4}n = 6$ subjects. Data from WE Connor, DS Lin, MP McMurray, unpublished observations, 1983. LC diet provided 50 mg cholesterol/d and 250–400 mg plant sterol/d; HC diet provided 1000 mg cholesterol/d and 250–400 mg plant sterol/d.

On the basis of the information described above, we suggest that this female Eskimo consumed little food of plant origin. However, although her food was exclusively of animal origin, her cholesterol intake does not seem to have been high. This may have been due to the low cholesterol content of her daily food items, such as seal and fish. Other possibilities are that she died during the winter when food was scarce or that her food intake was low because of illness.

The cholesterol present in the large bowel can be hydrogenated by intestinal bacteria to form coprostanol and coprostanone (7-9). In the coprolites from Nevada Indians, we found that 78% of the neutral steroids were in the form of stanols and stanones (2). The same bacterial phenomenon occurred in the coprolite of the Greenland Eskimo. Stanols and stanones accounted for 77% of the total fecal neutral steroids (Table 1). In our previous analysis of the steroid composition of coprolites from Nevada Indians, we found that 3β stanol was modified to 3α epimers. Because the 3α epimer stanol was not detected in the modern human stool samples and the amount of the 3α epimer stanol seems proportional to the age of the coprolite, we suggested that these epimers were formed during the long period of storage (1). Interestingly, despite different geologic locations and methods of preservation, 3α stanols were also found in the coprolite of the Greenland mummy. They contributed 32% of the total fecal stanols. In comparison, for the 6 coprolites from Nevada Indians, the epimer accounted for 14%, 21%, and 50% of total stanol in 1 coprolite dated AD 1750, 3 coprolites dated AD 50, and 2 coprolites dated 100 BC, respectively. The higher conversion in the stool from the Greenland mummy (AD 1475) than in the Nevada coprolite of similar age may be attributable to the different storage conditions.

In the Greenland fecal material, cholestanol, or dihydrocholesterol, accounted for 92 µg/g dried wt, or 1.87% of the total fecal neutral steroids (Table 1). Cholestanol was also detected in the coprolites from Nevada (102 µg/g dried wt, or 1.77% of total fecal neutral steroids). In stools of present-day Americans, cholestanol constituted 294 µg/g dried wt, or 1.32% of total fecal neutral steroids. Fecal cholestanol is derived from some foods of animal origin, chiefly dairy products and eggs as well as liver. Typically, ingestion is ≈0.5 mg/d (DS Lin, WE Connor, unpublished observations, 1984). Cholestanol is also a product from cholesterol in the body (12, 13) and is excreted in the bile (14).

Similar to coprolites from Nevada Indians and the stool of modern humans, the fecal sample from the Greenland mummy contained lithocholic acid as one of the major bile acids (28.6% of total bile acids; Table 2). However, unlike in the Nevada Indian samples, deoxycholic acid was not a major bile acid in the Greenland Eskimo sample (only 5.5% of total bile acid). Instead, 3α , 7β , 12α -trihydroxycholanic acid was one of the major bile acids in this sample (28.2%). Small amounts of this bile acid were detected in human stool previously (15, 16). Other bile acids found were 7β-hydroxycholanic acid (14.6%), 3-keto,7αhydroxycholanic acid (12.2%), 3β,12α-dihydroxycholanic acid (3.2%), and chenodeoxycholic acid (3.1%). These bile acids are typically found in human stool (15-18). An important pathway for elimination of cholesterol from the body is its conversion to bile acids, which are then excreted as a complex mixture in the stool. It is well established that the complexity of this mixture is a consequence of extensive transformation of the primary bile acids induced by the intestinal flora (19). The unusual bile acid

pattern observed in this fecal sample from the Greenland Eskimo may be due to her unique dietary practice (lack of plant source food), which in turn might have affected the activity of microorganisms in the gut. Alternatively, the possibility of transformation and modification of bile acid molecules during the lengthy storage period cannot be overlooked.

Although total fecal cholesterol excretion was similar in the Greenland Eskimo and Nevada Indian samples, the excretion of bile acids was much higher in the Greenland Eskimo coprolite. Consequently, the coprolite of the Greenland Eskimo had a higher ratio of bile acid to cholesterol. Interestingly, the ratios of bile acid to cholesterol were similarly high in the Greenland Eskimo and the Tarahumara Indians (consuming a low-cholesterol diet) and similarly low in the Nevada Indians and Americans (consuming a low-cholesterol diet). In our previous sterol balance study in Tarahumara Indians, we noted that these Indians have higher bile acid synthetic rates per unit body weight than do Americans (4). Thus, in view of our finding in the present study and the definite smaller size of the Eskimo woman, she may also have had a higher bile acid synthetic rate per unit of * body weight than do Americans.

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