COMMUNICATION

Fatty Acid Composition of Black Bear (*Ursus americanus*) Milk During and After the Period of Winter Dormancy[1]

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Black bears give birth and lactate during the 2-3-mon fast of winter dormancy. Thereafter the female emerges from the den with her cubs and begins to feed. We investigated fatty acid patterns of milk from native Pennsylvania black bears during the period of winter dormancy, as well as after den emergence. Throughout winter dormancy, milk fatty acid composition remained relatively constant. The principal fatty acids at all times were 14:0, 16:0, 16:1, 18:0, 18:1, 18:2n-6, 18:3n-3 and 20:4n-6. After den emergence, large changes occurred in almost all the fatty acids, particularly in 18:2n-6 and 18:3n-3. Large variability among the active free-ranging animals likely reflected differences in diet. In a carnivore, with apparently limited *de novo* synthesis of fatty acids, milk fatty acid composition may be affected by factors such as transition from reliance on stored lipids to feeding, and by temporal changes in dietary intake.
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The pattern of fatty acids in milk lipids varies considerably among mammalian species. Triglycerides (TG) account for 97-99% of milk lipid in the species that have been studied (1-3). However, among different species TG are composed to varying degrees of fatty acids originating from *de novo* synthesis within the mammary gland and/or from circulating fatty acids (4,5). The extent to which milk fatty acids reflect dietary or body storage lipids will depend on the extent of *de novo* synthesis. Other factors which may affect milk fatty acid composition include lactation stage and, in humans, gestation length (1,5-7).

Carnivores are believed to secrete milk lipids that result primarily from the uptake of circulating fatty acids and thus largely reflect dietary lipids. This is in contrast to ruminants, which secrete milk lipids high in short-chain fatty acids (4:0-6:0) of *de novo* origin, and some monogastrics (*e.g.*, rabbit, rat, mouse, elephant, and primates, including human), which secrete milk lipids high in medium-chain fatty acids (8:0-12:0) of *de novo* origin (4,5,8,9). The longer-chain fatty acids (≥ 16:0) may originate from either *de novo* synthesis or from uptake by the mammary gland. Although it is generally believed that the long-chain fatty acids are derived primarily from circulating blood lipids, the mammary gland of at least one species, the guinea pig, synthesizes primarily 16:0, as well as 18:0 and 18:1 (5,10,11). Unfortunately, fatty acid synthetase of the mammary gland has been studied in relatively few species, none of which are carnivores (5,12). Degree of plasma lipid uptake by the mammary gland also has been studied in only a few species, primarily in ruminants (9,12,13), and patterns may change with starvation (13).

Bears (family Ursidae) are of particular interest because they enter a period of winter dormancy, during which females give birth to and begin nursing their cubs (14). In the black bear *(Ursus americanus),* the first 2-3 mon of milk secretion occur while the female is fasting and mobilizing body lipid stores. Subsequently, the lactating female emerges from the den with her cubs and begins to feed on an omnivorous diet during the next months of lactation (15,16). Thus we would expect that milk fatty acids would reflect the composition of stored lipid in the denned-up period and dietary lipids thereafter. Although a number of studies on fatty acid composition of bear milks (17-20) have been undertaken, they have not been sufficiently detailed or comprehensive to permit an assessment of the effect of either winter dormancy or subsequent feeding on fatty acid composition.

The relationship of milk lipid composition to lactation stage is largely unknown for most free-ranging mammals. Prior reports on milk fatty acid composition for nondomestic species have usually been based upon analysis of only one or a few samples; information on lactation stage is often omitted, and it is sometimes unclear whether samples were taken from captive or wild individuals (*e.g.*, 18,21-23). In the present study we examined the fatty acid patterns of milk obtained from wild black bears at various stages of winter dormancy, as well as after den emergence. Our aims were to investigate i) whether changes occur in milk fatty acid composition during the prolonged fast in the den and ii) the extent to which milk fatty acid composition changes upon the transition from fasting to feeding.

MATERIALS AND METHODS

Milk samples were collected from a native population of black bears in the Poconos Mountains in eastern Pennsylvania. In Pennsylvania, as in other populations, females begin denning in late November and early December, about the same time that delayed implantation of the fertilized egg occurs (14-16). The average birth date is mid-January, and the female remains in the den with her cubs until late March or early April (15). Samples from the denned-up period were obtained from nine lactating females inhabiting natural or artificial dens in January [0-1 days *postpartum* (PP)], February (3-4 wk PP) and March (7-9 wk PP) 1984, corresponding to early (n=3), mid (n=3) and late (n=3) winter dormancy, respectively. Post-dormant samples (n=4) were collected from additional

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Abbreviations: ANOVA, analysis of variance; EFA, essential fatty acid; GC, gas chromatograph; GLC, gas-liquid chromatography; HPLC, high-performance liquid chromatography; PP, *postpartum*; PUFA, polyunsaturated fatty acid; TG, triglyceride.

free-ranging females from June to October (5–9 mon PP) of the same year. Females were immobilized with a mixture of ketamine HCl and xylazine delivered with a pole syringe (denned-up bears) or a dart (free-ranging bears). Milk was expressed manually following intramuscular injection of oxytocin. Milk samples were stored frozen at −20°C until analysis.

Total milk fat was determined gravimetrically using the standard Roese-Gottlieb procedure for milks (24). All solvents used for fatty acid analysis were high-performance liquid chromatography (HPLC) grade and all containers were composed of glass or teflon. Aliquots were extracted into chloroform using a modification (25) of the method of Folch *et al.* (26). Fatty acid methyl esters were prepared using the Hilditch reagent (0.5 N H_2SO_4 in methanol) (3) and extracted into isooctane for analysis. Methyl esters were separated by temperature-programmed gas-liquid chromatography (GLC) on a J&W DB-23 glass capillary column (30 m × 0.25 mm i.d.) (J&W, Folsom, CA) on a Shimadzu model GC-14A gas chromatograph (Shimadzu Scientific Instruments, Columbia, MD). Carrier gas was helium. Starting column temperature was 153°C and temperature was increased stepwise to 220°C over a 30 min period. Peak identities were determined from known standards, and peak areas were quantified and normalized by automatic integration using a Shimadzu C-R4A Cromatopac. Fatty acids are herein designated by shorthand nomenclature of chain length:number of double bonds, where n-x denotes the position of the last double bond relative to the terminal methyl end. Data were tested by one-way analysis of variance (ANOVA) and subsequent Fisher PLSD multiple mean comparison tests or by two-tailed *t*-tests using a statistical package (Statview 512+, Brain Power Inc., Calabasas, CA) for the Macintosh. Data are presented as mean wt % ± SEM unless otherwise indicated.

RESULTS AND DISCUSSION

During the period of winter dormancy, fat content of the milk of eastern Pennsylvania black bears increases significantly as lactation progresses (27). In the present study, milk fat content was similar to that of the larger population, averaging 13.2 ± 2.75%, 22.8 ± 2.81% and 22.2 ± 3.09% at early, mid and late winter dormancy, respectively. However, despite the doubling of total fat content, milk fatty acid composition remained relatively constant throughout the period of dormancy (Fig. 1). The principal fatty acids of bear milk were 14:0, 16:0, 16:1n-9, 16:1n-7, 18:0, 18:1n-9, 18:1n-7, 18:2n-6, 18:3n-3 and 20:4n-6. During the winter dormancy, there was generally very little variability in levels of individual fatty acids among lactating females and little change occurred over time, except in levels of 16:0, 16:1n-9 and 20:4n-6, which decreased significantly after 1 day PP (ANOVA, Fig. 1). At all three stages, 16:0, 16:1, 18:0 and 18:1 accounted for about 85% of fatty acids. The major polyunsaturated (PUFA) and essential fatty acid (EFA) was 18:2n-6; the other principal PUFA were the EFA 18:3n-3 and 20:4n-6, although absolute levels were relatively low (Fig. 1). A number of minor fatty acids were also present at trace or very small amounts (≤ 0.6%) throughout lactation (Table 1).

Following the several months of lactation during winter dormancy, the female black bear emerges from the den

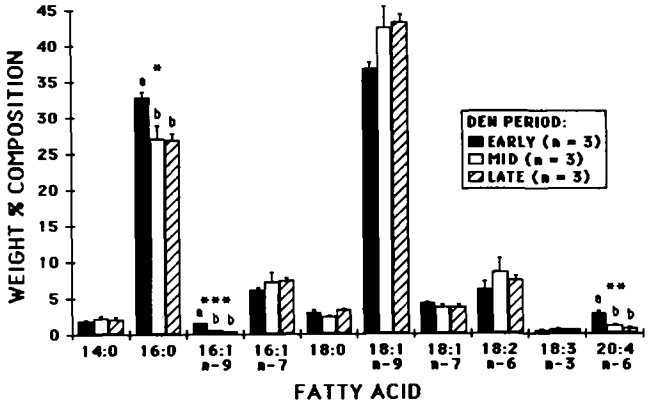

FIG. 1. Fatty acid composition of black bear milk over the period of winter dormancy. Early, 0–1 d *postpartum* (PP); mid, 3–4 wk PP; and late, 7–9 wk PP. Bars are means and vertical lines are 1 SEM. Means with different superscripts were significantly different (analysis of variance), *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

TABLE 1

Fatty Acid Composition of Black Bear Milk During and After the Period of Winter Dormancy[a]

Fatty acid	wt% Den (n=9)	wt% Post dormancy (n=4)
14:0	2.0 ± 0.16	3.2 ± 0.36[c]
16:0	28.9 ± 1.16	23.5 ± 1.74[b]
16:1n-9	0.8 ± 0.16	0.3 ± 0.04[e]
16:1n-7	6.9 ± 0.46	3.0 ± 0.38[d]
18:0	2.9 ± 0.21	5.8 ± 0.44[d]
18:1n-9	40.7 ± 1.42	37.2 ± 3.24[e]
18:1n-7	3.9 ± 0.17	1.6 ± 0.03[d]
18:2n-6	7.4 ± 0.74	15.7 ± 3.61[c]
18:3n-3	0.5 ± 0.06	5.1 ± 3.34[b]
20:4n-6	1.6 ± 0.34	0.3 ± 0.04[b]

[a] Values are means ± SEM. Minor components included 12:0 (0–0.1%), 14:1n-5 (0.2–0.3%), 17:0 (0.1–0.4%), 17:1 (0.3–0.6%), 18:1 (other isomers) (0.1–0.6%), 20:1 (0.4–0.5%), 22:1 (0.2–0.6%), 20:5n-3 (0–0.3%), 22:5n-3 (0–0.2%) and 22:6n-3 (0–0.2%). Data were tested by two-tailed *t*-test.

[b] $P < 0.05$, [c] $P < 0.01$, [d] $P < 0.001$, *t*-test.

[e] Not significant.

with her cubs and begins to roam in search of food (14,15). During this time, milk fat content was somewhat higher than it was earlier in lactation, averaging 28.0 ± 3.50%. Although there was little change or variability in milk fatty acid patterns over the period of winter dormancy, large changes occurred following emergence from the den (Table 1). Significant differences were found between the denned-up period and the active post-dormant period for every major fatty acid except 16:1n-9 and 18:1n-9. Levels of 14:0, 18:0 and 18:2n-6 approximately doubled after emergence, while levels of 16:1n-7, 18:1n-7 and 20:4n-6 were less than half those of the denned-up period. Most notable were the extremely high levels and considerable variability in the EFA 18:2n-6 and 18:3n-3 (Table 1). The levels of 18:2n-6, which averaged 7.4% during winter dormancy, were found to be as high as 21.6% after den

emergence. The levels of 18:3n-3 were even more remarkable. This fatty acid ranged between 0.2 and 0.8% during the denned-up period but was found to be as high as 15.0% during the active free-ranging period, with great variability among individual females. EFA intake may be particularly important to a bear cub because cubs are extremely small and altricial at birth [at <0.3% of maternal weight (14,15)], even for a carnivore (28). EFA appear to be adequately supplied in bear milk, with 18:2n-6 at about 6-13% of total calories over lactation, which is far in excess of human infant requirements (29,30).

In the present study, we found no evidence for *de novo* synthesis of short- or medium-chain fatty acids in the black bear; 12:0 was found in only trace amounts at a few stages (Table 1). Jenness *et al.* (31) came to the same conclusion with respect to a variety of bear milks, even though some reports from polar bears (17,19) and Yeso brown bears (20) indicate significant amounts of short-chain fatty acids. It is not clear whether the latter data may have been artifacts, as short-chain fatty acids are usually considered unique to ruminant milks (5). Whether the longer-chain fatty acids in bear milk derive from serum through uptake by the mammary gland or from *de novo* synthesis is not known. However, the long-chain n-6 and n-3 PUFA are all of dietary origin and, hence, must come from uptake.

During winter dormancy, milk lipids are secreted over an extended period of fasting. One might expect fatty acid composition to vary over this period due to changing rates of *de novo* synthesis, selective mobilization of depot fatty acids, or depletion of depot stores (*e.g.*, ref. 25), but the observed changes in fatty acid patterns were relatively minor (Fig. 1). Unfortunately, we were not able to compare milk fatty acid patterns to those of depot stores in these individuals. As such comparisons would need to be made in samples taken from the same animal (*e.g.*, ref. 25), data from other sources would not be appropriate.

The large changes that occurred after emergence (Table 1) presumably reflect dietary intake. Black bears feed primarily on a variety of fruits, nuts, acorns and succulent vegetation, as well as scavanged meat, human garbage, corn from farmed fields and, at least in Pennsylvania, handouts provided by human well-wishers (14-16). Hence the diets of individual bears may be highly variable. This may explain the high variability seen in the fatty acid patterns after emergence (Table 1). The high levels of 18:2n-6 and 18:3n-3 found in some plant materials, such as fruits, nuts, corn and leafy vegetation (*e.g.*, ref. 32), could account for the extraordinary levels found in some of the post-dormant milks. Lactating fur seals and sea lions that begin feeding after an initial fast also show substantial changes in milk fatty acid patterns with the onset of feeding (25,33). Significant increases in 18:2n-6 and 18:3n-3 have been observed in human milk lipids when subjects switch to vegetarian or corn oil-based diets (30). Although data is very limited, longer-chain PUFA have been reported in milks of the polar and grizzly bear (19). The diets of these animals are probably high in PUFA due to intake of fish and/or seal oils (*e.g.*, ref. 25).

Unfortunately, comparison of our data to those for other bears and other carnivores is difficult, given the limited nature of previous reports, as well as apparent misidentification of fatty acid peaks and/or failure to separate components with similar retention times. Species other than carnivores for which such very high levels of 18:3n-3 have been reported include the horse [n=1, (ref. 23)], koala [n=1, (ref. 23)] and red and mantled howler monkeys (wild, n=12, Iverson, S.J., and Oftedal, O.T., unpublished data), species which feed solely on vegetation.

Clearly, lactation stage and composition of the maternal diet are extremely important to the interpretation of milk fatty acid data in bears, and probably in other carnivores as well. In characterizing the fatty acid composition of any species, it is important to assay fatty acid composition on an adequate number of samples representing different lactation stages and, where known, maternal diet should be described. Results from this study also suggest that detailed data on milk fatty acid composition for free-ranging carnivores may allow inferences about the nature of their diet and thus may be useful in understanding both mammary gland physiology and the foraging ecology of a species, as has recently been demonstrated for several seal species (25,33).

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