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Comparative Biochemistry and Physiology, Part B 149 (2008) 74–82
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Fatty acid composition of wild anthropoid primate milks

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Received 31 May 2007; received in revised form 21 August 2007; accepted 22 August 2007
Available online 30 August 2007

Abstract

Fatty acids in milk reflect the interplay between species-specific physiological mechanisms and maternal diet. Anthropoid primates (apes, Old and New World monkeys) vary in patterns of growth and development and dietary strategies. Milk fatty acid profiles also are predicted to vary widely. This study investigates milk fatty acid composition of five wild anthropoids (*Alouatta palliata*, *Callithrix jacchus*, *Gorilla beringei beringei*, *Leontopithecus rosalia*, *Macaca sinica*) to test the null hypothesis of a generalized anthropoid milk fatty acid composition. Milk from New and Old World monkeys had significantly more 8:0 and 10:0 than milk from apes. The leaf eating species *G. b. beringei* and *A. paliatta* had a significantly higher proportion of milk 18:3n-3, a fatty acid found primarily in plant lipids. Mean percent composition of 22:6n-3 was significantly different among monkeys and apes, but was similar to the lowest reported values for human milk. Mountain gorillas were unique among anthropoids in the high proportion of milk 20:4n-6. This seems to be unrelated to requirements of a larger brain and may instead reflect species-specific metabolic processes or an unknown source of this fatty acid in the mountain gorilla diet.

Keywords: Anthropoid; Fatty acid; Milk composition; Primate

1. Introduction

Fatty acids in milk have three sources: (1) maternal depot fat; (2) maternal diet; and (3) *de novo* synthesis in the mammary gland (Stini et al., 1980; Iverson and Oftedal, 1995; Prentice, 1996; Del Prado et al., 1999; Sauerwald et al., 2001). Medium-chain fatty acids such as octanoic acid (8:0) and decanoic acid (10:0) can be synthesized *de novo* by a variety of tissues, including the mammary gland while polyunsaturated fatty acids (PUFA) such as linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) must be obtained by the diet. Fatty acid synthesis by the mammary gland may be a species-specific trait (Dils et al., 1977; Iverson and Oftedal, 1995), with certain species having higher levels of mammary gland lipogenesis. However, the proportion of fatty acids synthesized by the mammary gland depends on the amount of fatty acids from dietary or depot fat stores (Iverson and Oftedal, 1995). For example, Del Prado et al. (1999) report that rats fed a high fat diet extracted more lipid from maternal plasma, showing up to a 75% decrease in lipogenesis in the mammary gland. In this respect, milk fatty acid composition reflects the interplay between species-specific physiological mechanisms and maternal diet.

This interplay also may influence the composition of long-chain polyunsaturated fatty acids (LCPUFA) in milk. LCPUFA are supplied preformed from the maternal plasma or as

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doi:10.1016/j.cbpb.2007.08.006

metabolites of precursor PUFA in maternal plasma. 18:2n-6 is the precursor for all omega-6 (n-6) PUFA, including arachidonic acid (AA, 20:4n-6) and 18:3n-3 is the precursor for all omega-3 (n-3) PUFA including docosahexaenoic acid (DHA, 22:6n-3). Biosynthesis of LCPUFA from PUFA precursors will depend on both the quantity of 18:2n-6 and 18:3n-3 in the diet (Jensen et al., 1995; Carlson, 1999; Brenna, 2005), the ratio of n-3 to n-6 PUFA in the diet (Huang and Brenna, 2001; Brenna, 2005) and the ability to convert n-3 and n-6 PUFA into their longer chain metabolites (Agostoni et al., 2001; Carlson, 2001; Brenna, 2005). Variation in conversion efficiency among species may produce different LCPUFA milk fatty acid composition despite similar dietary intakes of n-3 and n-6 PUFA precursors.

In this study, we investigate the influence of phylogeny (genetics) and ecology (diet) on the fatty acid composition of wild anthropoid primate milks by testing the null hypothesis of a generalized fatty acid composition of anthropoid milks. This study is the first to analyze milks of wild primates from all three superfamilies of the infraorder anthropoidea: Hominoidea (apes), Ceboidea (New World monkeys), and Cercopithecoidea (Old World monkeys). Captive primates consume diets that can differ significantly from their wild counterparts, particularly in lipid type and quantity, making it difficult to tease apart phylogenetic patterns from the effect of diet. Additionally, information on the natural composition of wild primate milks is important knowledge for the formulation of appropriate milk replacers for primates living in captivity (Kirkwood and Stathatos, 1992).

Primate species included in this study vary in dietary strategy. Leaves can comprise over 68% of the diet of Costa Rican *A. palliata* (Glander, 1978) and up to 99% of the diet of *G. b. beringei* (Robbins, 2007). The *G. b. beringei* population included in this study has been observed to eat between 200–300 different plants along with wood and insects, with less than 10 food items making up the majority of their diet (Whittier, personal observation). These include celery, goose grass, droquettia vines, thistle, and bamboo. Bamboo shoots, which are highly coveted, are seasonal and tend to coincide with the rainy seasons. *C. jacchus* and *L. rosalia* exploit a wide variety of foods but generally avoid leaves and bark (Digby et al., 2007). *C. jacchus* rely heavily on plant gums and sap and are morphologically specialized to harvest and digest these plant foods (Ferrari, 1993; Power and Oftedal, 1996). *L. rosalia* lack these specializations and consume gums opportunistically, generally consuming more fruit than common marmosets (Dietz et al., 1997; Digby et al., 2007). Both species also consume arthropods (Ferrari, 1993). *M. sinica* diets are more similar to the callitrichines than to gorillas and mantled howlers, with approximately 70% of the diet by dry weight consisting of ripe fruit, and the balance a mix of immature leaves, flowers, seeds, herbs, tuburs, and mushrooms (Dittus, 1974; Hladik and Hladik, 1977).

2. Materials and methods

Milk samples from populations of five species of anthropoid primate, representing each of the three superfamilies, were analyzed for fatty acid composition (Table 1). Length of lactation is highly variable among the species included in this study, ranging from several months in *C. jacchus* to several years in *G. b. beringei* (Ross, 2003). To permit interspecific comparisons, day of lactation was corrected for by length of lactation for the species (Ross 1998, 2003; Hartwig, 1996; Kappeler and Pereira, 2003). Following Oftedal and Iverson (1995), samples from mid-lactation were considered representative for the species, and all other samples (e.g., colostrum) were excluded from analyses. Samples were collected from several research projects and individual project protocols are provided.

Table 1
Sample information

Species	Superfamily	Number of samples analyzed	Source of milk samples
Callithrix jacchus (Common Marmoset)	Ceboidea	4	ML Power
Leontopithecus rosalia (Golden Lion Tamarin)	Ceboidea	4	ML Power
Alouatta palliata (Mantled Howler)	Ceboidea	7	KE Glander OT Oftedal
Gorilla beringei (Mountain Gorilla)	Hominoidea	5	Mountain Gorilla Veterinary Project
Macaca sinica (Toque Macaque)	Cercopithecoidea	8	W Dittus

2.1. Gorilla beringei beringei

Mountain gorilla samples and data were collected from the 160 km^2 Volcanoes National Park in northeastern Rwanda (1°35′ to 1°65′ S, 29°35′ to 29°75′ E) in different vegetation zones at elevations ranging from 2500–3500 m. Gorilla milk samples were collected opportunistically from identified, habituated individuals during emergency procedures conducted by the Mountain Gorilla Veterinary Project with their conservation partners. Gorilla samples included in this study were collected between November 2002 and January 2005. Immobilization was performed by C. Whittier or F. Nutter using the Telinject remote injection system (Telinject U.S.A., Inc., Agua Dulce, CA) and all procedures were conducted at the site of immobilization. This system employs reusable plastic darts, metal needles, and CO_2 pressurized air pistols. Gorillas in this study were immobilized using 3–6 mL darts with 1.1–1.2 × 30–38 mm non-barbed needles loaded with either ketamine hydrochloride (5 mg/kg) or a combination of ketamine hydrochloride (3 mg/kg) and medetomidine hydrochloride (30 μg/kg). Some gorillas required additional anesthesia during procedures and some were reversed with 150 μg/kg of atipamezole. No gorillas were given oxytocin and all successfully recovered and returned to their family groups.

Milk was collected manually by applying pressure around the nipple area of one or both breasts and squeezing to express milk from the nipple. Milk samples were collected directly into clean 5–100 mL plastic containers, aliquotted into sterile 5 mL

cryotubes, and frozen within 6 h at −20 °C before and after cold shipment (dry ice or ice packs) to the United States (Maryland Zoo in Baltimore, Baltimore, MD). Four of the five samples were pooled from both mammary glands. Samples were transported on dry ice from the Maryland Zoo in Baltimore to the Nutrition Laboratory, Species Conservation Center, Smithsonian's National Zoological Park, Washington, D.C. (NZP), where they remained frozen until the time of analysis.

2.2. Alouatta palliata

Mantled howler samples were collected at La Pacifica, Costa Rica. La Pacifica is 20 km^2 with 6 km^2 of low-land dry and riparian forest (10° 28′ N and 85° 07′ W). Capture of mantled howler females at La Pacifica was performed by K. Glander using the Pneu-Dart™ system (Pneu-Dart, Inc., HC 31, Williamsport, PA 17701). This system employs disposable non-barbed darts with a 9 mm needle that are delivered by a CO_2 powered gun. The darts were loaded with Telazol® which is a non-narcotic, non-barbiturate, injectable anesthetic (Telazol® is a Schedule IIIN drug; DEA Registration Number 0138619). It is a combination of equal parts by weight of tiletamine hydrochloride (an arylaminocycloalkanone dissociative anesthetic) and zolazepam hydrochloride, a nonphenothiazine diazepinone with tranquilizing properties (Fort Dodge, Fort Dodge, IA 50501-0518). The dosage used was 25 mg/kg, shown to be safe and effective in more than 2600 primate captures (Glander et al., 1991). Individuals were darted at distances up to 20 m. Darted individuals falling from a tree are caught in a nylon mesh net (camper's hammock). When the darted animal does not fall, the branch on which the animal is hanging is either shaken or cut down with a saw attached to the end of an aluminum pole. Animals that recover from the capture dosage before the procedures are complete were given injections of 1–3 mg/kg of Telazol®, repeated as often as needed. After all procedures were complete, animals are placed in burlap bags (to reduce visual stimulus) until they recovered enough to walk or climb unaided.

Nipple areas were washed with distilled water and dried with a clean cloth. Immediately before milk collection, oxytocin (approximately 0.20 mL) was administered via an intramuscular route. Milk was collected via manual expression, applying pressure to the area surrounding the nipple and firmly squeezing in toward the nipple. In all instances, efforts were made to fully evacuate each gland. Only one of the seven samples was pooled from both mammary glands. Milk was collected directly into sample tube, capped, and placed into a −20 °C freezer. Samples were shipped on dry ice to the Nutrition Laboratory, NZP.

2.3. Callithrix jacchus and Leontopithecus rosalia

Milk samples from each lactating female were collected from golden lion tamarins (*L. rosalia*) at the Poço das Antas Biological Reserve, reintroduced golden lion tamarins from the Rio Vermelho farm, and common marmosets (*C. jacchus*) from the same farm. All captures of golden lion tamarins were conducted under the supervision of Golden Lion Tamarin Association (GLTA) field staff and used its procedures. The common marmoset captures were conducted using the same golden lion tamarin capture protocol. Animals were captured in Tomahawk live traps placed on platforms within the groups' normal range. Captured animals were anesthetized in the field laboratory with ketamine hydrochloride (10 mg/kg body weight). All the animals used in this research were tattooed, so that individuals could be recognized upon recapture. A radio collar was placed on one animal from each group to facilitate tracking and recapture. After the processing time with sample collections, the animals were released in the early morning, at their capture site. Once anesthetized at the field lab, the nipples were cleaned and the milk samples were collected manually by gently pressing both mammary glands and milk was expressed into a vial. Efforts were made to completely evacuate both mammary glands and no oxytocin was used. Samples represent milk collected from one mammary gland. The milk samples were frozen in a −20 °C freezer at Environmental Sciences Laboratory from Universidade Estadual do Norte Fluminense (UENF), in Campos dos Goytacazes, Brazil, and then shipped on dry ice to the Nutrition Laboratory, NZP. Samples remained frozen at −20 °C until time of analysis.

2.4. Macaca sinica

Milk samples were collected from wild toque macaques in the dry evergreen forests at Polonnaruwa, Sri Lanka, where these macaques have been investigated continuously since 1969. The trapping, handling and release of macaques were supervised by Wolfgang Dittus, Species Conservation Center, Smithsonian National Zoological Park. Individuals were identified by their natural markings and tattoos. The macaques were baited into large-sized steel box traps (1 × 1 × 1.5 m). The trap door was operated manually as a precaution against injury to the trapped animals. Macaque mothers were tranquilized by intramuscular injection of ketamine hydrochloride at a dose of 10 mg/kg (Ketalar, Park Davis Co., UK), and moved to a protected field laboratory. The anesthetic has been used safely on more than 1000 toque macaques (Dittus, personal communication). To aid milk release, a dose of 0.20–0.25 mL oxytocin (Phoenix Pharmaceuticals Inc., MI) was administered intramuscularly 20 min prior to milking. Hair was shaved off around the nipples and the skin was cleaned with distilled water. Milk was collected by evacuation of each mammary into 1.8 mL airtight cryotubes with screw caps. Samples were not pooled. Each female was kept in a holding cage until she recovered completely from the anesthetic (usually at least 3 h) and then released back into her social group. All samples were kept in a cooler box immediately after collection, but then submerged in liquid nitrogen for long term storage in Sri Lanka and international transport to the Nutrition Laboratory, NZP. In the United States, the samples were stored at −20 °C.

All samples were maintained in a −20 °C freezer at the Nutrition Laboratory until removed for subsampling or analysis. Samples that were less than 1 mL in volume were allowed to thaw at room temperature and those greater than 1 mL were placed in a warm water bath, maintained at 50 °C, per Nutrition

Laboratory (Smithsonian National Zoological Park) standard protocol, in order to ensure rapid and uniform thawing. Samples were vortexed prior to dispensing approximately 100 µl into 20 mL Kimex glass centrifuge tubes using a 250 µL positive displacement pipette. Sample weights were recorded to 0.0001 g. Samples were capped and immediately frozen.

Known amounts of glyceryl triheptadecanoate (Sigma) and 13,16,19-docosatrienoic acid methyl ester (Sigma) were added as internal standards to milk prior to extraction. Total lipids were extracted from approximately 100 µg of milk according to the method of Folch (Folch et al., 1957). Fatty acid methyl esters were formed by heating the total lipid extract in 1% H_2SO_4 in methanol at 70 °C for 3 h (Makrides et al., 1994). The methyl esters were separated on a 30 m × 0.25 mm i.d. capillary column (SP-2330, Supelco; Bellefonte, PA), using gas chromatography with a flame ionization detector (Model 6890N, Agilent Technologies; Palo Alto, CA, USA). Runs were initiated at 80 °C, with a temperature gradient to 160 °C (10 °C/min) and 230 °C (3 °C/min) in 31 min, and held at 230 °C for 10 min. Peaks were identified by retention times of fatty acid methyl ester standards (Nu-Chek-Prep, Elysian, MN). Fatty acid concentrations (mg/g of fresh milk) were calculated by proportional comparison of gas chromatography peak areas to that of the heptadecanoate methyl ester peak. The concentration of the heptadecanoate methyl ester internal standard was validated for each sample used in this study by comparison to the concentration of the second internal standard (13,16,19-docosatrienoic acid methyl ester). Relative concentration, given as percent composition, was calculated by dividing individual fatty acid concentration by the total concentration of all detected fatty acids.

Results are presented as total concentration and percent composition (the individual fatty acid concentration as a proportion of the total concentration of all detected fatty acids). Results are expressed as mean and standard error. Data were analyzed using SPSS 13.0 (SPSS, Inc., Chicago, IL, USA). Significant differences in means among species and superfamilies were determined using ANOVA and multiple pairwise comparisons between species using the Tukey–Kramer test. Statistical significance for all tests was set at $\alpha < 0.05$.

3. Results

The mean percent composition of fatty acids for each species is provided in Table 2. We report only fatty acids with eight or more carbons because the Folch method does not effectively extract short-chain fatty acids (two to six carbons). There was a significant difference in the mean mass (mg/g) of milk for total fatty acids for mountain gorillas (19.82±4.34), toque macaques (39.32±3.75), mantled howlers (25.45±3.75), common marmosets (14.01±5.30), and golden lion tamarins (12.73±5.31) ($F=6.40$, $df=4$, $p=0.001$). Therefore, differences among and between species were analyzed using percent composition to identify differences in proportions of milk fatty acids.

Table 2
Mean (±SE) fatty acid percent composition and total fatty acids (mg/g) by species

Fatty acid	*Callithrix jacchus* (*n*=4)	*Leontopithecus rosalia* (*n*=4)	*Alouatta paliatta* (*n*=7)	*Gorilla beringei* (*n*=5)	*Macaca sinica* (*n*=8)
8:0	16.61±2.27	13.79±1.65	12.33±1.76	0.03±0.01	8.87±0.96
10:0	16.71±2.20	16.18±2.28	4.88±0.78	0.36±0.09	6.79±1.47
12:0	0.02±0.01	0.02±0.005	0.01±0.002	0.13±0.01	0.01±0.001
14:0	10.37±1.91	13.55±1.98	2.71±0.11	3.77±0.53	4.12±2.13
16:0	19.86±0.80	20.58±1.49	24.97±1.19	27.10±1.14	26.79±1.31
18:0	3.68±0.63	3.52±0.36	5.04±0.33	5.67±0.31	5.21±0.65
20:0	0.11±0.02	0.09±0.01	0.15±0.13	0.19±0.04	0.11±0.01
22:0	0.03±0.01	0.05±0.004	0.05±0.03	0.07±0.02	0.04±0.003
Sum of saturates	67.40±4.63	67.78±4.82	50.14±1.95	37.94±1.47	51.93±1.96
14:1	0.11±0.01	0.07±0.01	0.10±0.01	0.54±0.004	0.29±0.03
16:1	2.41±0.35	1.72±0.38	2.77±0.27	2.23±0.52	5.29±0.57
18:1 (sum)[a]	22.71±3.44	20.81±3.73	17.57±1.28	27.14±1.97	30.47±1.69
20:1	0.28±0.09	0.41±0.09	0.28±0.07	1.04±0.08	0.31±0.07
22:1	0.03±0.01	0.04±0.01	0.02±0.01	0.13±0.01	0.03±0.01
Sum of monounsaturates	25.55±2.96	23.16±2.96	20.87±2.09	30.61±2.70	36.29±2.09
18:3n-3	2.63±0.43	1.94±0.43	15.06±0.75	16.31±1.19	2.55±0.32
20:5n-3	0.08±0.02	0.11±0.01	0.06±0.01	0.22±0.04	0.11±0.01
22:5n-3	0.21±0.10	0.24±0.10	0.16±0.07	0.69±0.08	0.19±0.07
22:6n-3	0.14±0.06	0.24±0.06	0.03±0.003	0.09±0.04	0.12±0.01
Sum n-3s	3.05±0.51	2.52±0.36	15.31±0.77	17.13±1.19	2.96±0.32
18:2n-6	3.29±0.56	5.37±1.02	12.59±0.60	10.56±0.54	8.03±0.98
18:3n-6	ND	ND	0.02±0.004	0.004±0.01	0.04±0.004
20:3n-6	0.23±0.02	0.34±0.02	0.06±0.02	0.13±0.02	0.16±0.02
20:4n-6	0.30±0.03	0.48±0.05	0.68±0.09	2.08±0.15	0.32±0.02
22:5n-6	0.01±0.02	0.04±0.02	0.04±0.01	0.12±0.01	0.05±0.01
Sum n-6s	3.88±0.61	6.31±1.03	13.43±0.57	12.90±0.46	8.64±0.99
Sum of PUFAs	6.94±1.01	8.83±1.35	28.75±0.90	30.03±1.51	11.61±0.99
Total fatty acids (mg/g)	14.01±5.30	12.73±5.31	25.45±3.75	19.82±4.34	39.92±3.75

ND=Not detected.
[a] 18:1(sum)=18:1n-9+18:1n-7 (these fatty acids did not always separate on the GC column).

To determine the appropriate level of taxonomic analysis, percent composition was compared among and between species and among and between superfamilies. The superfamilies Hominoidea and Cercopithecoidea were each represented by only one species, the mountain gorilla and toque macaque, respectively. Therefore, comparisons among the anthropoid superfamilies actually represented comparisons of milks from mountain gorillas, toque macaques, and ceboid monkeys (mantled howlers, common marmosets, and golden lion tamarins). Among ceboid monkeys, common marmosets and golden lion tamarins (subfamily Callitrichinae) were significantly different from mantled howlers in the percent composition of several fatty acids. Thus, the superfamily Ceboidea was separated into two groups, mantled howlers and callitrichines.

Mean percent composition of 8:0 and 10:0 were significantly different among the four groups (8:0: $F=20.83$, $df=3$, $p<0.001$; 10:0: $F=28.02$, $df=3$, $p<0.001$). 8:0 and 10:0 were highest in milk from the callitrichines (15.20±1.40, 16.45±1.47), followed by mantled howlers (12.33±1.76; 4.88±0.78) and toque macaques (8.87±1.68, 6.79±1.89), and were virtually undetectable in mountain gorilla milk (0.03±0.01, 0.36±0.09) (Fig. 1a and b). Callitrichine milk also was significantly higher than each of the other three groups in mean percent composition of 14:0 (11.96±1.41; results not shown).

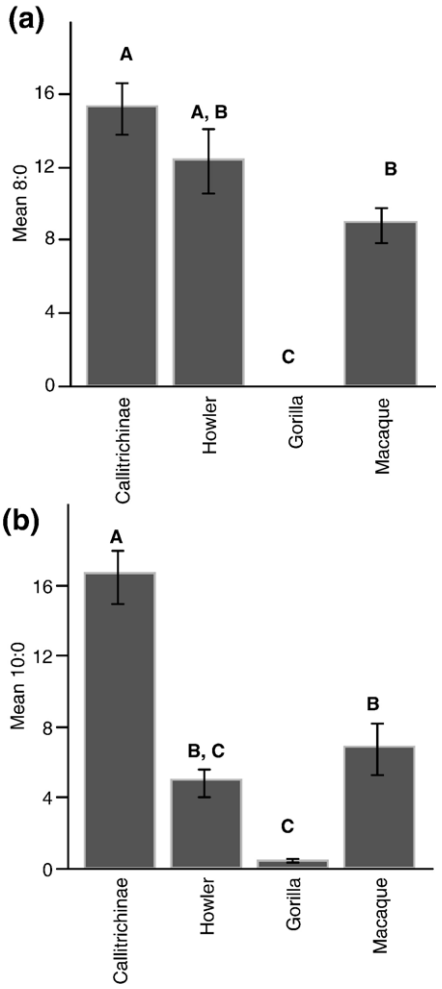

Fig. 1. Mean percent composition of (a) 8:0 and (b) 10:0 of callitrichinae (common marmosets and golden lion tamarins), mantled howlers, mountain gorillas, and toque macaques. Bars not sharing a common superscript are statistically different ($p<0.05$).

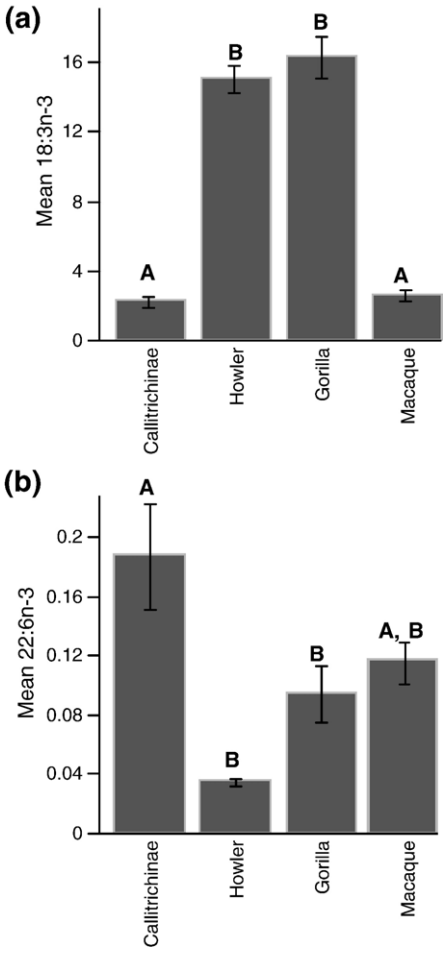

Fig. 2. Mean percent composition of (a) 18:3n-3, and (b) 22:6n-3 of callitrichinae (common marmosets and golden lion tamarins), mantled howlers, mountain gorillas, and toque macaques. Bars not sharing a common superscript are statistically different ($p<0.05$).

Mean percent composition of 18:3n-3 was significantly different among callitrichines, mantled howlers, mountain gorillas, and toque macaques ($F=150.18$, $df=3$, $p<0.001$; Fig. 2a). Mean percent composition of 18:3n-3 in mountain gorilla (16.31±1.19) and mantled howler milk (15.06±0.75) was more than six times that of toque macaque (2.55±0.32) and callitrichine milk (2.28±0.31). When analyzed at the level of the species, no significant difference was identified between mountain gorilla milk and howler monkey milk ($p=0.11$) but each was significantly different ($p<0.01$) in pairwise comparisons with milk from all other species.

18:3n-3 is a precursor for 22:6n-3, however the percent composition of 18:3n-3 and 22:6n-3 was negatively correlated among anthropoid milks (Spearman's rho=−0.61, $p<0.001$); species with the highest proportion of milk 18:3n-3 had the lowest proportion of milk 22:6n-3. Mean percent composition of milk 22:6n-3 was highest in callitrichines (0.19±0.03), followed by toque macaques (0.12±0.01), mountain gorillas (0.09±0.04), and mantled howlers (0.03±0.003) and these differences were significant among the four groups ($F=8.16$, $df=3$,

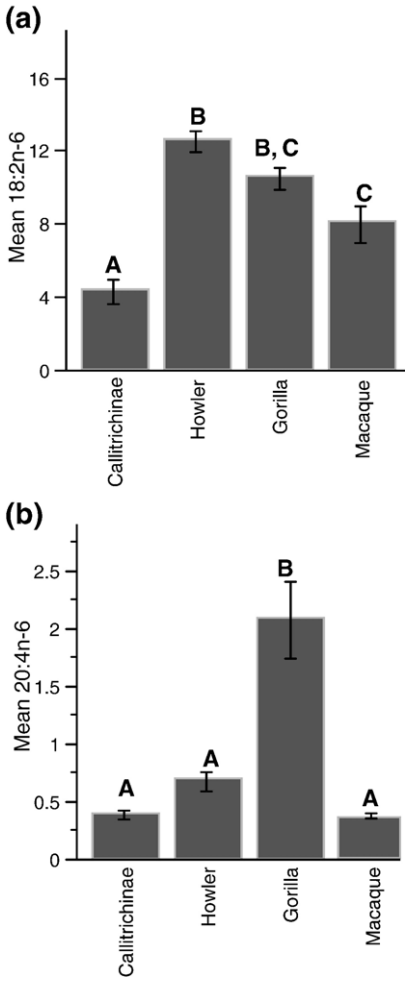

Fig. 3. Mean percent composition of (a) 18:2n-6 and (b) 20:4n-6. of callitrichinae (common marmosets and golden lion tamarins), mantled howlers, mountain gorillas, and toque macaques. Bars not sharing a common superscript are statistically different ($p<0.05$).

$p<0.001$; Fig. 2b). The correlation between percent composition of the n-6 precursor 18:2n-6 and its long-chain derivative 20:4n-6 was positive and significant ($r=0.54$, $p=0.003$). The proportion of milk 18:2n-6 was significantly different among the four groups ($F=21.70$, $df=3$, $p<0.001$; Fig. 3a). Although there was a significant difference among groups in mean percent composition of 20:4n-6 ($F=34.78$, $df=3$, $p<0.001$), only mountain gorilla milk was significantly different from any other group. Mean percent composition of 20:4n-6 in mountain gorilla milk (2.08±0.15) was three times that of mantled howler milk (0.68±0.09) and almost six times that of callitrichine (0.39±0.04) and toque macaque milk (0.32±0.02; Fig. 3b).

Saturated fatty acids make up almost 70% of the milk of callitrichines (67.59±3.10), more than 50% of the milk of mantled howlers (50.14±1.95) and toque macaques (51.93±1.96), and less than 40% of the milk of mountain gorillas (37.94±1.47; Fig. 4a). Mountain gorilla milk had the highest percent composition of n-3 PUFA (17.13±1.19) and was only slightly less than mantled howlers (13.43±0.58) in percent composition of n-6 PUFA (12.90±0.46; Fig. 4b and c). Subsequently, mountain gorillas had the highest percent composition of the sum of n-3 and n-6 PUFA (30.03±1.51), almost three times that of toque macaque milk (11.61±0.99) and almost four times that of callitrichine milk (7.88±0.86; Fig. 4d).

4. Discussion

Our findings do not support the null hypothesis of a generalized fatty acid composition for anthropoid primates. Significant variation was detected among and between species for total milk fatty acids and among and between callitrichines, mantled howlers, mountain gorillas, and toque macaques in percent composition of individual fatty acids (e.g., 8:0, 18:3n-3) and types of fatty acids (e.g., saturated, n-3 PUFA).

Percent composition of 8:0 and 10:0 were significantly higher in monkeys (Ceboidea and Cercopithecoidea) relative to the mountain gorilla (Hominoidea). Indeed, these fatty acids were almost undetectable in the mountain gorilla. Medium-chain fatty acids such as 8:0 and 10:0 are synthesized by the mammary gland and are not reflective of supply in the maternal diet. However, their concentration may be related to the amount of fiber in the diet. Popovich et al. (1997) report that diets high in fiber may provide a substrate for the bacterial fermentation of short-chain fatty acids in the colon. Short-chain fatty acids can then be converted into medium-chain fatty acids. While this may explain the high proportion of 8:0 and 10:0 in the primarily folivorous mantled howler, the virtual lack of these fatty acids in the mountain gorilla and the higher proportion in the frugivore/insectivore golden lion tamarin suggests that the ability to make 8:0 and 10:0 is not solely related to fermentation of fiber. Data from captive species support the prediction that decreased production of 8:0 and 10:0 may be decoupled from diet and reflect a derived trait in hominoids. Milligan and Bazinet (unpublished data) identified an identical pattern in percent composition of 8:0 and 10:0 in captive populations of ceboids, cercopithecoids, and hominoids, despite dietary variation between captive and wild populations within the same superfamily.

Mantled howlers and mountain gorillas had a significantly higher proportion of milk 18:3n-3 than callitrichines or toque macaques. The separation of mountain gorillas and mantled howlers from the other anthropoid species parallels their ecological separation; mantled howler and mountain gorilla diets consist primarily of leaves, which are high in 18:3n-3. High levels of milk 18:3n-3 in these species were consistent with data from other mammalian species that consume diets high in 18:3n-3 and have high milk levels of 18:3n-3, such as the koala (*Phascolarctos cinereus*) where 18:3n-3 is 32.5% of milk fatty acids (Iverson and Oftedal, 1995).

The diets of all species in this study are believed to be devoid of a preformed source of 22:6n-3, a LCPUFA primarily found in fish. Thus, we hypothesize that all milk 22:6n-3 was derived from the elongation and desaturation of 18:3n-3 from maternal diet or body stores. Despite high levels of the n-3 precursor 18:3n-3 in the diet, mountain gorillas and mantled howlers secreted milks with the lowest proportion of 22:6n-3. The highest proportion of milk 22:6n-3 among the study groups (0.19%) was found in the milks of the fruit, gum, and insect

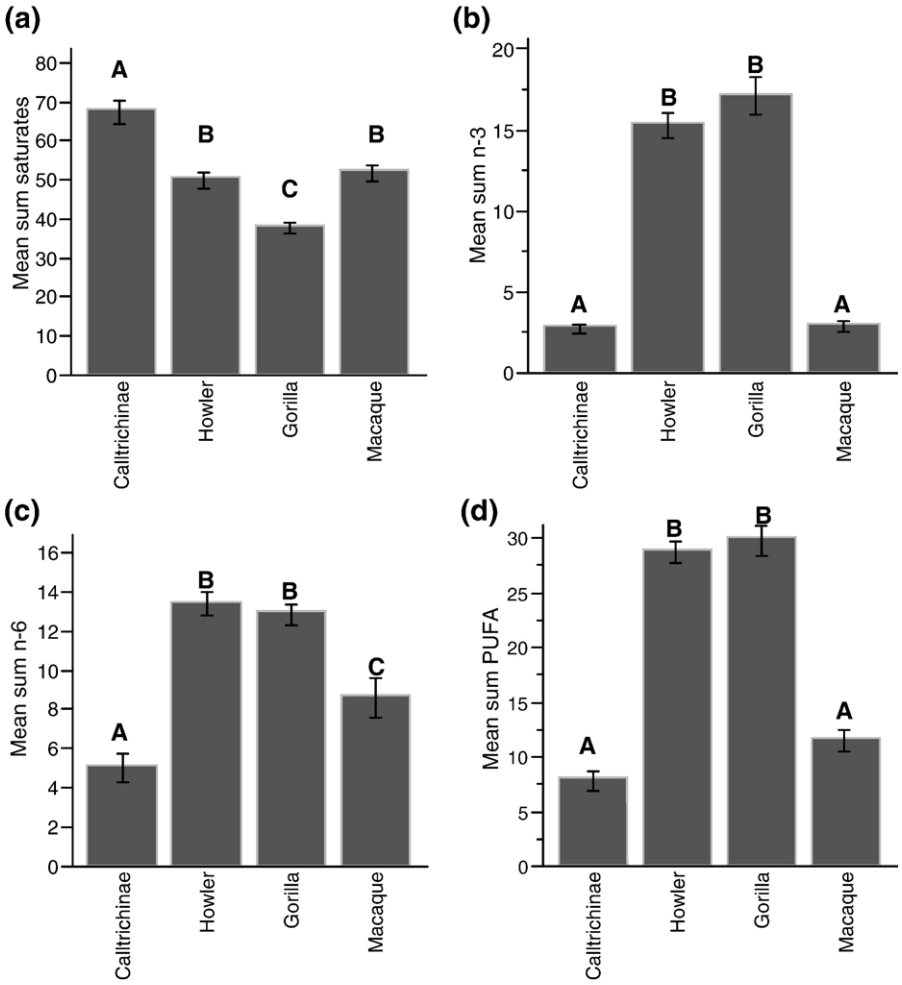

Fig. 4. Mean percent composition of (a) the sum of saturated fats, (b) the sum of n-3 PUFA, (c) sum of n-6 PUFA, and (d) Sum of PUFA. of callitrichinae (common marmosets and golden lion tamarins), mantled howlers, mountain gorillas, and toque macaques. Bars not sharing a common superscript are statistically different ($p<0.05$).

eating callitrichines. We hypothesized that the percent composition of 22:6n-3 in the wild anthropoid milks represented the maximum amount of 22:6n-3 that anthropoid primate species were able to synthesize from dietary 18:3n-3 rather than the maximum amount of 22:6n-3 they were capable of secreting into milk. To test this, we examined the milk of captive anthropoids fed chow that includes fish meal, a source of preformed 22:6n-3 (Milligan and Bazinet, unpublished data). The levels of 22:6n-3 in captive-living anthropoid populations were significantly higher than those of their wild-living superfamily counterparts. Data from humans (Brenna, 2005; Francois et al., 2003) and baboons (Greiner et al., 1997; Su et al., 2005) also support the hypothesis that 22:6n-3 levels in milk increase with an increased source of preformed 22:6n-3.

The percent composition of the 18:2n-6 metabolite 20:4n-6 was more than four times higher in mountain gorilla milk compared to milk from any monkey species. One possible explanation for this pattern could be phylogenetic; mountain gorillas, or possibly all hominoids, may be better at desaturating and elongating 18:2n-6 into 20:4n-6 relative to monkeys. Although there are comparative data from humans on 18:2n-6 and 20:4n-6 in milk, it is difficult to draw any conclusions from this data because the human diet has much more 18:2n-6 than 18:3n-3 (Burdge and Calder, 2005). It may appear that humans convert more 18:2n-6 to 20:4n-6 (Agostoni et al., 2001), but in reality the effect might be the result of significantly more 18:2n-6 relative to 18:3n-3 in the diet (Burdge and Calder, 2005). As is the case of 22:6n-3, the most efficient method for secreting 20:4n-6 into milk is to have a source of 20:4n-6 in the maternal diet. Thus, an alternative explanation to differences in metabolism could be that mountain gorillas have a source of preformed 20:4n-6 in their diet that we have yet to identify.

Mountain gorillas had higher amounts of PUFA in their milk than callitrichines, mantled howlers, or toque macaques. Day of lactation was unlikely to be a confounding variable. In a study on human milk fatty acids, Agostoni et al. (2001) report stability of PUFA throughout lactation and Makrides et al. (1995) found little to no changes in PUFA composition in mature milk, excepting 22:6n-3 which decreased slightly from week six to week 16 post-partum ($0.26\pm0.13\%$ to 0.21 ± 0.12), but then remained stable after this time.

Diet may explain higher amounts of 18:3n-3, and possibly even 20:4n-6, in mountain gorillas. However, these results may also reflect a phylogenetic difference in synthesis of LCPUFA

between hominoids and monkeys. The brain rapidly accumulates n-3 and n-6 PUFA, particularly 20:4n-6 and 22:6n-3, for structure and multiple functions during the last trimester of gestation and infancy (Burdge, 2004; Carlson, 2001; Innis, 2003). Indeed, Kothapalli et al. (2007) demonstrate that supplementation of neonatal baboons with modest amounts of 20:4n-6 (0.64% of total fatty acids) and 22:6n-3 (0.32% or 0.96% of total fatty acids) altered the expression of over 1100 genes in the brain. The dietary supply of these fatty acids must be sufficient to allow and support brain growth. Further, the dietary input of the 18-carbon precursors, their conversion by the liver to LCPUFA, and their incorporation into the brain may be quantitatively related to the ultimate size and function of the brain. Mountain gorillas have a larger relative brain size than the monkey species included in this study. It is possible that mountain gorillas may allocate more energy to the metabolic pathway necessary for LCPUFA synthesis, particularly 20:4n-6, as a result of increased requirements for LCPUFA.

To further explore this prediction, results of this study were compared to another large-brained hominoid species, *Homo sapiens*. Percent composition of LCPUFA (and their n-6/n-3 precursors) varies widely in human milk as a result of dietary differences within and among populations (Brenna et al., 2007; Yuhas et al., 2006). In a meta-analysis of 65 studies including a total of 2474 women, Brenna et al. (2007) found that the percent composition of 22:6n-3 in milk ranged from 0.06–1.4%, the highest values from coastal populations with a marine-based diet. The percent composition of 20:4n-6 was less variable (0.24 to 1.0%), despite wide variation in dietary intake of 18:2n-6 and 20:4n-6 among the study populations. Brenna et al. (2007) suggest the relationship between milk 20:4n-6 concentrations and dietary 20:4n-6 is less predictable than that of 22:6n-3.

The percent composition of 22:6n-3 in the milk of mountain gorillas (0.09%) was similar to the lowest reported human values (Brenna et al., 2007). As previously discussed, low values of milk 22:6n-3 are best explained by the lack of 22:6n-3 in the diet rather than the inability of primates to secrete more 22:6n-3 into milk. Percent composition of milk 20:4n-6 in mountain gorillas (2.08%) was nearly five times the mean of published human populations (Brenna et al., 2007; Gibson and Kneebone, 1981; Koletzko et al., 1992; Yuhas et al., 2006), despite similar values for percent composition of 18:2n-6 in milk. As compared to humans and other nonhuman primates, mountain gorilla milk appears to be unique in its 20:4n-6 composition. This trait is unrelated to increased 20:4n-6 requirements of a larger brain; human brains are relatively and absolutely larger than brains of mountain gorillas but human milk 20:4n-6 is one fifth than that identified for mountain gorillas.

Results of this study represent milk fatty acid profiles from wild-living anthropoid primates consuming natural diets and contribute to our understanding of the nutrition of the individual species presented. In addition, they provide a comparative framework for understanding human milk fatty acid profiles and possible links between milk LCPUFA composition and brain ontogeny. A limitation of this study for understanding the relationship among milk LCPUFA and brain growth and development was that it could only speak to what anthropoid primate mothers were secreting into milk. To fully understand the relationship between milk fatty acids and brain growth and development requires data on the quantity of fatty acids consumed in milk, maternal and infant plasma levels of LCPUFA, and physiological mechanisms of PUFA metabolism.

Acknowledgements

LAM: This study was funded by Wenner Gren Foundation Dissertation Fieldwork Grant 7360 and LSB Leakey Foundation Grant 1965. RPB and SIR were supported by the Intramural Research Program of the National Institute on Aging, National Institutes of Health and RPB was also received a grant from the Natural Sciences and Engineering Research Council of Canada. MRC/CAW: Office Rwandais Du Tourisme Et Des Parcs Nationaux (ORTPN) and Karisoke Research Center of Dian Fossey Gorilla Fund International. The study was also partly funded by the Morris Animal Foundation. WD: Field research was supported by the National Science Foundation (BNS 9510894), the Harry Frank Guggenheim Foundation, and Earthwatch. We thank Sunil Gunatilake and Lalith Jayawickrama for technical assistance. KEG: This research has been funded in part by NSF grants GS-31733 and BNS 8819733 plus an REU Supplement, Duke University Research Council grants, Duke University Biomedical Research Support grants, and COSHEN-Pew grants. He thanks Stephan Schmidheiny and the Board of Directors of La Pacifica for their permission to keep the La Pacifica Howler Project going on La Pacifica for the past 36 years. OTO: Laboratory supplies and equipment maintenance at the NZP Nutrition Lab were supported by the Smithsonian Institution. We thank Michael Jakubasz for assistance in sample shipping and laboratory procedures. MLP: Field and laboratory research were supported by PHS grant RR2022 and grants from PROBIO (Edital 01/1997 with funds from MMA/GEF/CNPq) to the Golden Lion Tamarin Association and from CNPq and FAPERJ.

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