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RESEARCH ARTICLE

The Composition of Milk from Bolivian Squirrel Monkeys (*Saimiri boliviensis boliviensis*)

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Squirrel monkeys (genus *Saimiri*) give birth to relatively large neonates with large, fast-growing brains. Maternal energy expenditure during gestation and infant development is argued to be high, but may be offset by the provisioning of offspring by females other than the mother (allonursing). Milk composition is an important component of maternal energy expenditure, but has been examined in only a small number of primate species. Here, we report on the milk composition from laboratory-housed Bolivian squirrel monkey (*Saimiri boliviensis boliviensis*) dams ($n = 6$) and allomothers ($n = 2$). Milk samples ($n = 16$) representing mid-lactation were assayed for fat, sugar, dry matter (DM), crude protein (CP), and fatty acids. Gross energy (GE) was calculated from these constituents (excepting fatty acids). The goals of this project were: (1) to provide descriptive data on milk composition of squirrel monkeys, including the range of intraspecific variation; (2) to determine if milk produced by allomothers differs from milk from dams; and (3) to compare squirrel monkey milk to that of other small New World monkeys, the callitrichines. Squirrel monkey samples averaged 4.56% fat, 3.59% CP, 6.98% sugar, 16.59% DM, and 0.91 kcal/g. The proportion of the medium chain fatty acids 8:0 and 10:0 was 40 times greater than that reported for human milk samples, and 18:1 and 18:2n-6 comprise more than 60% of total fatty acids. Milk from allomothers was lower than dams in fat, DM, and GE, which may relate to variation in maternal condition between these two groups. Excluding allomothers, milk from squirrel monkeys was higher in mean GE than captive common marmosets, but did not differ in the proportion of energy from fat, CP, and sugar relative to total GE. The consistency in energy from protein between species suggests this may be a shared-derived trait of New World monkeys. Am. J. Primatol. 70:35–43, 2008.

Key words: milk; lactation; New World primates; squirrel monkey; allonursing

INTRODUCTION

Lactation is a defining characteristic of mammals, and represents a vital life history stage for the developing infant and the mother, a key to the fitness (survival and reproductive success) of both. The diversity of the mammalian radiation is reflected in the diversity of evolved lactation strategies. The lactation strategy of a species comprises the composition of the milk produced, the volume of milk produced per day, the frequency at which the offspring nurse, and the duration of lactation. All these elements must work together to simultaneously deliver the necessary nutrients to the infant for its growth and development, without irreversibly compromising maternal health. Anthropoid primates appear to have evolved a lactation strategy that consists of relatively long lactations, frequent nursing of infants, and the production of large volumes of dilute milk [Oftedal & Iverson, 1995; Power et al., 2002].

Relatively little is known about milk composition in New World primate species. Callitrichid primates appear to produce typical anthropoid primate milk [Power et al., 2002], despite their small

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size and habit of producing twins which might have been expected to have selected for a more concentrated milk. In this paper, we examine the composition of milk from captive Bolivian squirrel monkeys (*Saimiri boliviensis boliviensis*).

Squirrel monkeys are only slightly larger than the largest callitrichid primates, median adult female body weight is approximately 700 g [Bernacky et al., 2002]. Squirrel monkey dams bear a higher metabolic cost compared with other primate species on a per infant basis, with maternal investment concentrated in the prenatal and perinatal periods [Garber & Leigh, 1997; Jack, 2007]. Mean neonatal body mass ($\pm$SD) in captivity is 109.2 ± 21.9 g ($n = 283$) [Bernacky et al., 2002]. Neonatal body weights are between 14 and 20% of maternal body weight, and infants undergo rapid postnatal brain growth [Garber & Leigh, 1997; Hartwig, 1996]. Bolivian squirrel monkeys usually lactate for 4–6 months, and have a relatively long interbirth interval for a primate of their size (mean of 1.38 years for this population) [Garber & Leigh, 1997; Ross, 1991]. Allometry and the large infant-to-maternal mass ratio would suggest that squirrel monkeys might produce more concentrated milk than larger anthropoids, but the example of the callitrichids indicates that there might be phylogenetic or life history trait constraints on anthropoid primates that result in substantial conservation of milk composition.

Allomaternal care, or care of an infant in a maternal way given by someone other than the birth mother, has been documented in squirrel monkeys in studies from the field [Baldwin, 1969; Dumond, 1968; Hunt et al., 1978; Ploog, 1967] and the laboratory [Williams et al., 1988, 1994]. Infant squirrel monkeys may spend as much as 30% of their time clinging on allomothers during the first 6 months of their lives [Baldwin, 1969; Williams et al., 1988, 1994]. Allomothering usually begins during the first 2 weeks of life in this species [Williams et al., 1988]. In the wild, allomothers are reported to usually be juvenile females [Dumond, 1968]. In captivity, reports have shown that the majority (53%) of the allomothering is done by young adult females 4–6 years of age while adult females 7–9 years of age provide about 20% of the allomaternal care [Williams et al., 1994]. Some allomothers even nurse the infants (allonursing). Most of the allonursing seen in captivity was performed by females who had experienced a reproductive failure during that year [Williams et al., 1988].

Allonursing behavior has been reported in other laboratory housed or wild primate species including *Cebus* [O'Brien & Robinson, 1991; Leighty et al., 2004], *Lemur catta* [Pereira & Izard, 1989], and *Varecia rubra* [Vasey, 2007]. This report is the first to compare the milk composition of allomothers to dams.

We assayed milk samples from eight lactating Bolivian squirrel monkey females, including two allomothers, for dry matter (DM), crude protein (CP), fat, and fatty acids. The primary goals of this study were: (1) to characterize the mean values and extent of variation of squirrel monkey milk among these constituents, (2) to test whether milk of allomothers will be similar in composition to milk from true mothers, and (3) to compare the composition of squirrel monkey milk with the composition of common marmoset milk, another small New World monkey.

METHODS

All animals in this study were part of a breeding colony of squirrel monkeys (*S. boliviensis boliviensis*) maintained at the University of South Alabama Center for Neotropical Primate Research and Resources. All research reported in this study adhered to protocols approved by the Institutional Animal Care and Use Committees of the University of Arizona and the University of South Alabama. Before delivery, monkeys were housed in social groups of between 15 and 35 animals containing one adult male and between 10 and 15 adult females with their offspring. Housing consisted of indoor pens measuring approximately $4.5 \times 2.5 \times 1.5$ m, connected by porthole doors. Social groups had access to two to three pens depending on their size. All animals were fed a commercial New World Primate diet that had a guaranteed analysis of crude fat $\geq 9\%$. Fat was of both animal and plant origin. The diet was supplemented three times weekly with chopped vegetables, including celery, bell peppers, squash, and beans. Grapes, peanuts, and mealworms were fed sparingly as positive reinforcers when animals presented for clinical observations. Water was available ad libitum. The light dark schedule was maintained to track the local sunrise and sunset, so animals were exposed to long and short days annually.

At term or immediately postpartum, dams and infants were moved into maternity pens with other dams and infants. When the infant was 1-month old, it was identified with a neck chain tag and the pair returned to the pen of the original social group.

Allomothers were identified using an ad libitum data collection procedure [Altmann, 1974]. Allomothers were defined as females other than the infant's dam seen carrying the infant on more than one occasion.

To collect milk samples, females and their infants and the corresponding allomother were removed from the social group and placed in an individual cage. The upper torso of each female was wrapped using a self-clinging bandage over gauze pads to prevent nursing or milk loss. Bandages were removed 4–4.5 hr after animal capture. Milk was collected into 15-ml conical tubes by manual expression from the nipple and gentle massage of the underlying mammary gland. Milk was fully

expressed from the mammary glands. Oxytocin was not administered. The sample was frozen in cryovials and stored at −80°C until analysis. Once the sample was collected the monkey was returned unbandaged to her social group.

No early lactation samples were collected. Milk collection did not begin until after allomothers had been identified with the earliest samples collected in the 2nd month of lactation. Owing to the volume requirements to perform all the assays in duplicate, only mid-to-late lactation samples were assayed.

Laboratory analyses were conducted from January 2006 to March 2006. DM, CP, fat, and sugar were measured at the Nutrition Laboratory, Smithsonian's National Zoological Park using standard methods [Oftedal & Iverson, 1995] and were the same as used by Power et al. [2002]. All assays were performed in duplicate. DM, or total solids in each sample, was measured gravimetrically after drying 17.5 μl of sample for 3 hr at 100°C in a forced-air drying oven. Total nitrogen (TN) then was assayed using a CHN elemental gas analyzer (Model 2400, Perkin-Elmer, Norwalk, CT), which provides a rapid and accurate method of assaying TN in small volumes of milk samples. This method has been standardized at the Nutrition Laboratory against the macro Kjeldahl procedure (nitrogen recovery 98–99%) and yielded comparable results for all species tested, including for milk from several primate species. CP was estimated from TN in each milk sample (TN × 6.38). Total lipid was assayed by a micro-modification of the Rose–Gottleib procedure using sequential extractions with ethanol, diethyl ether, and petroleum ether. Total sugar was assayed by the phenol-sulfuric acid method using lactose monohydrate as the standard [Dubois et al., 1956; Marier & Boulet, 1959]. Results are expressed on an anhydrous lactose basis. All results are expressed on a weight-per-weight basis, as percent.

Energy from fat, protein, and sugar are calculated using the following energy values: 9.11 kcal/g for fat, 5.86 kcal/g for CP, and 3.95 kcal/g for sugar. Total gross energy (GE) for each sample is calculated as the sum of energy from fat, protein, and sugar. This is likely an overestimate of GE because it does not account for the nitrogen from nonprotein sources (e.g., amino sugars). However, in human milk, the percent protein as nonprotein nitrogen (NPN) is between 0.038 and 0.046% [Lönnerdal & Atkinson, 1995]. Assuming that nonhuman primate milk will be similar to human milk in the percent protein as NPN, calculations of GE for samples in this study are only very small overestimates (approximately 0.01 kcal/g). Furthermore, comparisons among samples are not affected because all samples are calculated without accounting for NPN. The mean values of fat, sugar, CP, and GE in the milk for each female were used to calculate the amounts, in grams, of fat, sugar, and protein in 1 kcal of milk for each female.

Assays to determine milk fatty acid profiles were performed at the Brain Physiology and Metabolism Laboratory, National Institute of Aging, National Institutes of Health (Bethesda, MD). All chemicals, reagents, and standards used to quantify fatty acids were purchased from Sigma Chemicals (St. Louis, Missouri), unless otherwise indicated. Two internal standards (20 μl of triheptadecanoic acid [17:0] for proportional comparison with gas chromatography peak areas and 50 μl of methyl docosatriaenoic acid [22:3n-3] to verify complete methylation) were added to samples before the addition of any chemicals. Total lipids were extracted with chloroform and methanol (2:1 volume) following the method of Folch [Folch et al., 1957]. Extracted lipids were dried under nitrogen gas and then reconstituted with 2 ml of 0.1% sulfuric acid solution in methanol. Fatty acids methyl esters were formed by heating this solution at 70°C for 3 hr [Bazinet et al., 2003; Makrides et al., 1994]. Methyl esters were separated on a 30 × .25 mm internal diameter capillary column (SP-2330; Supelco, Bellefonte, PA) by gas chromatography with a flame ionization detector (Model 6890N; Agilent Technologies, Palo Alto, CA). Runs were initiated at 80°C, with a temperature gradient to 160°C (10°/min) and 230°C (3°C/min) in 31 min and held at 230°C for 10 min. Peaks were identified by comparison with retention times of fatty acid methyl ester standards: GLC Reference Standard 68 A, 68 C, 85 and 96 (Nu-Check Prep, Inc., Elysian, Mn) and pure eicosapentaenoic acid.

Absolute fatty acid concentrations (milligram per gram of milk) were calculated by proportional comparison of gas chromatography peak areas with that of the 17:0 standard, relative to the total weight of each sample (approximately 0.0100 g). Relative concentration, given as percent composition, was calculated by dividing individual fatty acid concentration by the total concentration of all reported fatty acids.

Results are expressed as mean and standard error. Data were analyzed using JMP 4.0 (SAS Institute, 2002, Cary, North Carolina) and SPSS 13.0 (SPSS Inc., 2005, Chicago, Illinois). Significant differences in means were determined using two-tailed t tests and significant relationships between milk constituents were explored with pairwise correlations. Statistical significance was set at $\alpha < 0.05$. The data for proximate composition of milk are compared with data from common marmosets (*Callithrix jacchus*) published in Power et al. [2002] using analysis of variance.

RESULTS

The proximate composition of samples and the relative contributions of fat, sugar, and protein to

GE were stable between days 101 and 183 postpartum (Fig. 1). Milk samples collected after 183 days were significantly higher in fat than samples collected after day 183 ($t = -3.57$, $P = 0.002$). Accordingly, mean percent ($\pm$SE) energy from fat increased from 45.97 ($\pm$1.38%) to 54.53% ($\pm$1.95%) ($t = -3.6$, $P < 0.01$) and mean percent energy from sugar decreased from 30.82 ($\pm$1.03%) to 23.92% ($\pm$4.90%) ($t = 3.9$, $P < 0.01$). There was no significant change in percent energy from protein between these two time periods ($t = 1.5$, $P = 0.16$).

Change in milk composition at approximately 6 months postpartum coincides with species-defined lactation patterns. Peak lactation for this population of *S. boliviensis boliviensis* is estimated at 4–6 months [Gibson et al., 1993]. Although Williams et al. [1994] showed a gradual decrease in the amount of nursing and allonursing during the infant's first 6 months of life (from 22 to 5% of their time nursing), suckling by infants has been observed through the 11th month postpartum. Following Oftedal and Iverson [1995] results from samples representing mid-lactation (101–183 days postpartum in this data set) are reported as representative of the species. One dam (973) has three samples from this time period, four dams (10, 597, 989, 1508) have two, and one dam (1360) has one sample; the two allomothers (688, 1630) each have two samples (Table I).

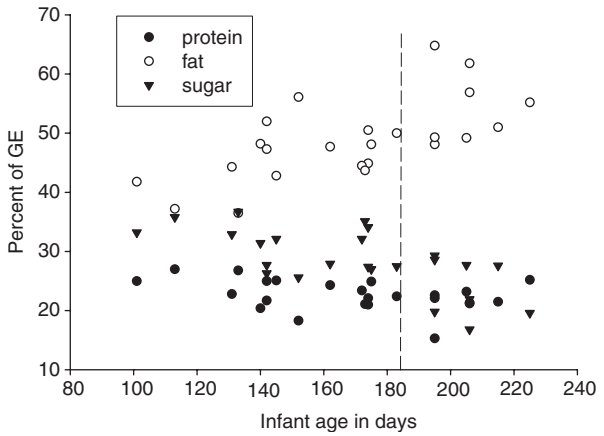

Fig. 1. After infant age of 183 days, the percentage of gross energy (GE) from fat increased and the percentage of GE from sugar decreased. The percentage of GE from protein did not vary.

Mean DM of individual mid-lactation samples ranged from 15.28 to 17.95%, mean fat from 3.48 to 5.74%, mean CP from 2.96 to 4.23%, and mean sugar from 6.63 to 7.28%. Mean values ($\pm$SE) for each dam and the mean from all dams are presented in Table II. Estimated GE ranged from 0.79 to 1.02 kcal/g, with a mean of 0.91 ± 0.03 kcal/g (Table III). Fat and DM were correlated positively with each other ($r = 0.76$, $P = 0.03$) and each was correlated positively with GE (fat: $r = 0.92$, $P = 0.001$; DM: $r = 0.89$, $P < 0.01$).

Fat was the primary energy source for all samples, ranging from 39.12 to 53.18% of total energy with a mean value of 45.97 ± 1.28%. Energy from sugar contributed a mean of $30.82\% \pm 0.93$ to GE and, like energy from fat, was quite variable (26.57 to 34.98%). The proportion of GE from protein was the least variable among individuals (20.35–25.91%) with a mean of 23.21 ± 0.60%.

Samples highest in GE were also highest in the proportion of energy from fat ($r = 0.73$, $P = 0.04$). The opposite relationship is observed for proportion of energy from sugar ($r = -0.93$, $P < 0.01$). Consequently, the proportion of energy from fat is negatively correlated with the proportion of energy from sugar ($r = -0.90$, $P < 0.01$). Females that produced high GE milk produced milk with more grams of fat and less grams of sugar per kilocalorie of milk than did females that produced lower GE milk (Fig. 2). There is no relationship between GE and the proportion of energy from protein ($r = -0.13$,

TABLE I. Maternal Age and Infant Age (Day of Lactation) for Each Milk Sample

Female ID	Source	Maternal age (est. years)[a]	Infant age sample 1 (days)[c]	Infant age sample 2 (days)	Infant age sample 3 (days)
10	Wild	>12	142	174	206
597	Wild	>9	152	183	215
688[b]	Wild	>12	101	133	195
973	Wild	>12	113	145	175
989	Wild	>8	140	172	205
1360	Lab-born	7	162	195	225
1508	Lab-born	5	142	174	206
1630[b]	Lab-born	4	131	173	195

[a]Age given is known time in captivity; wild source females were adult when captured.
[b]Allomothers.
[c]Days given for allomothers are days post-partum from the birth date of their own infant.
688 rejected her infant and it was raised in the nursery. 1630's infant died at 1 day of age.

$P = 0.76$). Importantly, the grams of protein per kilocalorie of milk did not differ.

Samples from allomothers appeared to group away from samples provided by genetic mothers. Samples from allomothers were lowest among all dams in total GE ($P = .006$) and, consequently, were highest in energy from sugar ($P = .02$) and tended to be lowest in energy from fat ($P = .06$) (Table IV). Percent energy from protein in allomother samples does not differ from that in genetic mothers, falling at both ends of the range of values ($P = .56$). When adjusted for GE, the relative proportions of fat ($P = 0.56$) and sugar ($P = 0.89$) were not different from that of genetic mothers (Fig. 2).

Twenty-three fatty acids were identified (Table IV) and internal standard concentrations and relative proportions indicated a completed methylation process. We report here on nine of these 23 fatty acids: octanoic acid (8:0), decanoic acid (10:0), palmitic acid (16:0), 18:1 (includes 18:1n-9 [oleic] and 18:1n-7 [vaccenic]), linoleic acid (18:2n-6), α-linolenic acid (18:3n-3), arachidonic acid (20:4n-6), eicosapentaenoic acid (20:5n-3), and docosahexaenoic acid (22:6n-3). These fatty acids were selected because of comparable data for human milk fatty acids. Mean percent composition of medium chain fatty acids (8:0 and 10:0) were more than 15-fold greater than mean values from great ape species [Milligan, unpublished data] and 40-fold greater than those reported from the human literature [e.g., 0.13 ± 0.06%; Gibson & Kneebone, 1981]. 16:0, 18:1n-9, 18:1n-7, and 18:2n-6 composed over 75% of total fatty acids. Polyunsaturated fatty acids (PUFAs; 18:2n-6, 18:3n-3) and long-chain PUFAs (LCPUFAs; 20:4n-6, 20:5n-3, 22:6n-3) accounted for approximately 32% of all fatty acids, of which 28.78% is 18:2n-6.

Mean percent composition of 8:0, 10:0, 18:1, 18:2n-6, 18:3n-3, 20:4n-6, and 20:5n-3 were not significantly different between dams and allomothers. Significant differences were detected in mean percent composition of 16:0 (dams: 17.82%; allomothers: 16.60%; $t = 2.23$, $P = 0.04$) and 22:6n-3 (dams: 0.42%; allomothers: 0.54%; $t = -3.97$, $P = 0.002$), although values from allomothers were within the range of dam values for both fatty acids. In fact, the highest value for percent composition of 22:6n-3 (0.68%) was provided by a dam sample.

The mean proximate composition values for the six mothers in this study were compared with the values for ten captive common marmosets from Power et al. [2002], which is the only other comparable study of proximate milk composition in New World monkeys. The milk of the squirrel monkeys was higher in GE ($P < 0.01$), fat ($P = .018$), and CP ($P < 0.01$), and lower in sugar ($P = .03$) than was the milk of the common marmosets (Table V). As would be predicted from the higher GE, the proportion of energy from sugar was lower in squirrel monkey milk ($P = .006$) and the proportion of energy

TABLE II. Mean ± SE of Proximate Values by Female and for All Females (Samples ≤183 Days)

ID	Fat (%)	CP (%)	Sugar (%)	DM (%)
10	4.81 ± 0.75	3.28 ± 0.32	6.80 ± 0.32	16.47 ± 0.09
597	5.74 ± 0.35	3.42 ± 0.33	6.63 ± 0.22	16.69 ± 0.09
688[b]	3.48 ± 0.36	3.57 ± 0.001	7.15 ± 0.10	15.34 ± 1.01
973	4.32 ± 0.59	4.01 ± 0.16	7.28 ± 0.21	16.97 ± 0.73
989	4.59 ± 0.31	3.36 ± 0.16	7.25 ± 0.13	16.18 ± 0.57
1360[a]	5.34	4.23	7.21	17.80
1508	5.14 ± 0.18	3.86 ± 0.22	6.69 ± 0.02	17.95 ± 0.09
1630[b]	3.81 ± 0.06	2.96 ± 0.14	6.79 ± 0.15	15.28 ± 0.40
All samples	4.56 ± 0.27	3.59 ± 0.15	6.98 ± 0.09	16.59 ± 0.35

[a]Only one sample met infant age criteria.
CP, crude protein; DM, dry matter.
[b]Allomothers.

TABLE III. Mean ± SE of Gross Energy and Percent Energy From Fat, Sugar and Protein by Female and For All Females (Samples ≤183 Days)

ID	GE (kcal/g)	Percentage of energy from fat	Percentage of energy from sugar	Percentage of energy from protein
10	0.90 ± 0.07	48.45 ± 3.57	30.19 ± 3.92	21.36 ± 0.35
597	0.96 ± 0.003	53.08 ± 3.05	26.57 ± 0.96	20.35 ± 2.08
688[b]	0.81 ± 0.003	39.12 ± 2.63	34.98 ± 1.77	25.91 ± 0.87
973	0.92 ± 0.06	42.69 ± 3.14	31.65 ± 2.55	25.66 ± 0.65
989	0.90 ± 0.03	46.34 ± 2.63	31.79 ± 0.33	21.87 ± 1.50
1360[a]	1.02	47.74	27.95	24.32
1508	0.96 ± 0.002	48.85 ± 1.60	27.55 ± 0.14	23.60 ± 1.46
1630[b]	0.79 ± 0.01	44.02 ± 0.27	34.02 ± 1.09	21.97 ± 0.82
All samples	0.91 ± 0.03	45.97 ± 1.28	30.82 ± 0.93	23.21 ± 0.60

[a]Only one sample met infant age criteria.
GE, gross energy.
[b]Allomothers.

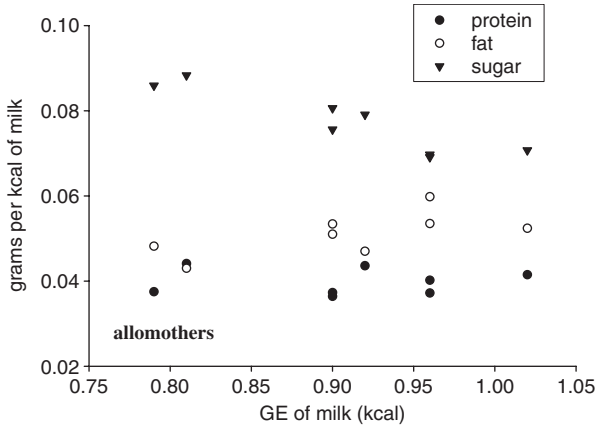

Fig. 2. The grams of crude protein, fat, and sugar in 1 kcal of milk for the eight captive squirrel monkeys in this study, plotted against the GE of the milk. Sugar is filled triangles, fat is open circles, and protein is filled circles. Grams of fat were significantly positively correlated with GE, whereas grams of sugar were significantly negatively correlated with GE. The grams of protein in 1 kcal of milk were not associated with the GE of the milk. The milk of the two allomothers are not different from the milk of the mothers after accounting for the significantly lower GE of the milk from the allomothers.

TABLE IV. Mean ± SE of the Concentrations and Percent Composition of Identified Fatty Acids From All Samples ≤183 Days

Fatty acid	Concentration (mg/g)	Percent composition
8:0	0.06 ± 0.004	5.38 ± 0.30
10:0	0.04 ± 0.003	3.80 ± 0.29
12:0	0.01 ± 0.0004	0.01 ± 0.01
14:0	1.49 ± 0.12	2.60 ± 0.21
14:1	0.07 ± 0.01	0.13 ± 0.004
16:0	0.18 ± 0.006	17.54 ± 0.26
16:1	1.32 ± 0.19	2.29 ± 0.16
18:0	2.46 ± 0.21	4.31 ± 0.18
18:1[a]	0.31 ± 0.01	29.47 ± 0.57
18:2n-6	0.30 ± 0.01	28.78 ± 0.48
18:3n-6	0.10 ± 0.01	0.19 ± 0.01
18:3n-3	0.02 ± 0.001	2.08 ± 0.07
20:0	0.07 ± 0.01	0.11 ± 0.01
20:1	0.23 ± 0.02	0.04 ± 0.01
20:2	0.19 ± 0.02	0.34 ± 0.01
20:3n-6	0.16 ± 0.02	0.30 ± 0.02
20:4n-6	0.01 ± 0.001	0.89 ± 0.03
20:5n-3	0.001 ± 0.001	0.11 ± 0.01
22:2	0.03 ± 0.003	0.05 ± 0.01
22:4n-6	0.02 ± 0.001	0.03 ± 0.01
22:5n-6	0.01 ± 0.01	0.13 ± 0.01
22:5n-3	0.12 ± 0.01	0.22 ± 0.01
22:6n-3	0.005 ± 0.01	0.45 ± 0.02

[a]Represents the sum of 18:1-9 and 18:1-7.

from fat tended to be higher ($P = .05$). Importantly, the proportion of energy from protein ($P = .13$) did not differ between the two species, and, after accounting for the GE of the milk, the proportion of energy from sugar and fat also was not significantly different between the species ($P = .24$ and .64, respectively).

TABLE V. A Comparison of the Proximate Composition of the Milks From the Six Dams in This Study With Milk From Ten Captive Common Marmosets (Data From Power et al., 2002)

	Saimiri	*Callithrix*
GE (kcal/g)	0.95 ± 0.02	0.76 ± 0.04
CP (%)	3.69 ± 0.16	2.69 ± 0.14
Fat (%)	4.99 ± 0.21	3.45 ± 0.42
Sugar (%)	6.98 ± 0.12	7.40 ± 0.11
% GE from CP	22.85 ± 0.81	20.81 ± 0.83
% GE from fat	47.98 ± 1.34	39.83 ± 2.75
% GE from sugar	29.18 ± 0.87	39.35 ± 2.31

GE, gross energy; CP, crude protein.

DISCUSSION

We report here on one aspect of the lactation strategy of the Bolivian squirrel monkey, milk composition. Bolivian squirrel monkeys appear to be able to produce relatively high-energy milk for an anthropoid primate; however, there is significant variation among individuals. Variability in the absolute amounts of fat and protein in milk has been shown in other monkeys (e.g., common marmosets; [Power et al., 2002] and rhesus macaques [Hinde, 2007]). The sources of this variation are uncertain, but may reflect differences in maternal condition. Despite the variability in absolute amounts, the proportions of fat, sugar, and protein in squirrel monkey milk are predictable relative to GE, in agreement with the findings of Power et al. [2002] for common marmosets. Indeed the proportion of protein to GE appears to be relatively constant both within and between these species.

Milk from allomothers ($n = 2$) was lower than dams ($n = 6$) in fat, DM, and total GE. Percent composition of reported milk fatty acids did not differ significantly between allomothers and dams, excepting 16:0 and 22:6n-3. However, data points from allomothers were within the wide range of values for dams for both fatty acids (16:0: 16.2–20.3%; 22:6n-3: 0.34–0.68%). This variability in percent composition may reflect differences in maternal energy stores (depot fat) or dietary intake of saturated fatty acids (16:0) and LCPUFA (22:6n-3) among all lactating females (dams and allomothers).

Williams et al. [1994] suggest allomaternal nursing and other allomaternal behaviors observed in this species (e.g., dorsal carrying of an infant by a female other than its mother) may augment infant survival; an alternative food source benefits the infant should their genetic mother die and benefits the dam by reducing the nutritional burden of lactation [Williams et al., 1994]. Determining

whether infants receive sufficient energy from the milk of allomothers is therefore critical to arguments for allomaternal nursing as an adaptive behavior in squirrel monkeys. The majority (86%) of allomaternal nursing observed by Williams et al. [1994] was performed by mothers with a reproductive failure for the year.

Both allomothers in this study had had a live birth; one had a neonatal death (1630) and the other (688) rejected a low birth weight infant, which was subsequently raised in the nursery. Reproductive failure may be a proxy for poor maternal condition. Tardif et al. [2001] report that small common marmoset mothers with twin offspring produce lower energy milk and Hinde [2007] reports rhesus macaque mothers with parasitic infection of *Balantidium coli* have significantly lower milk fat. Maternal condition may affect milk composition in Bolivian squirrel monkeys in similar ways that have been observed in other nonhuman primates. Alternatively, the fact that the allomothers went several weeks after the loss of their own infant without any physical nursing stimulation, may have resulted in an altered milk composition when compared with dams that had been nursing their infant since birth.

Our data suggest that in nursing allomothers there may be an affect on milk quality, specifically lower fat content, which in turn reduces total GE. Larger sample sizes are required to test this hypothesis, as is an understanding of energy requirements by the neonatal and infant squirrel monkey. Allomother milk may be significantly different from milk of genetic mothers on a statistical level, but this difference may not translate to significance at the biological level. Importantly, milk from allomothers provided the same amount of protein per kilocalorie of milk as did milk of mothers (Fig. 2). This result is similar to findings on protein composition in milk from human populations [Prentice, 1995]. Women in The Gambia with a low body mass index (representative of nutritional status) produced milk with similar protein concentration to European women with body mass indices in the normal range [Prentice, 1995].

The three sources for milk fatty acids are maternal depot fat, maternal diet, and de novo synthesis in the mammary gland [Iverson & Oftedal, 1995; Prentice, 1996]. Medium chain fatty acids such as 8:0 and 10:0 can be synthesized by a variety of tissues, including the mammary gland while PUFAs such as 18:2n-6 and 18:3n-3 must be obtained by the diet. 18:2n-6 is the precursor for all omega-6 (n-6) PUFAs, including 20:4n-6 and 18:3n-3 is the precursor for all omega-3 (n-3) PUFAs, including 22:6n-3. Elongation of 18:2n-6 and 18:3n-3 into 20:4n-6 and 22:6n-3 depends on a variety of physiological and dietary factors, including maternal ability to desaturate these fatty acids, their quantity, and the ratio of n-3 to n-6 fatty acids in the diet [Brenna, 2002; Carlson, 1999; Huang & Brenna, 2001; Jensen et al., 1995]. The proportion of these fatty acids in the diet also influences de novo synthesis of shorter chain fatty acids by the mammary gland [Iverson & Oftedal, 1995]. Milk fatty acids from mothers on high fat diets will reflect dietary lipids and have lower relative concentrations of fatty acids produced by mammary gland lipogenesis [Del Prado et al., 1999].

The Bolivian squirrel monkey has a high concentration and percent composition of 8:0 and 10:0 relative to humans [Gibson & Kneebone, 1981] and nonhuman apes [Milligan & Bazinof, unpublished data]. These results are similar to those from the South American squirrel monkey (*Saimiri sciureus*), where approximately 12% of fatty acids were 8:0 and 10:0 [Buss & Cooper, 1972]. The function of these fatty acids in milk is not completely understood, but may serve as energy substrates for neonates and infants [Bazinet, personal communication]. It is intriguing to hypothesize if nonhuman primates with high-energy requirements for growth and development, such as the Bolivian squirrel monkey, will produce these fatty acids in higher concentrations relative to those with slower infant growth rates.

Mean percent composition of 18:2n-6 (28.78%) and 18:3n-3 (2.08%) are notably higher than reported human values [e.g., Koletzko et al., 1988, who report percent composition of 18:2n-6 as 10.76% and 18:3n-3 as 0.81%]. The most likely explanation is dietary supply of these PUFAs is greater in the Bolivian squirrel monkey relative to humans. Both 18:3n-3 and 18:2n-6 are supplied primarily by foods of plant origin, with 18:3n-3 found in leaves and 18:2n-6 found in seed oils such as soybean oil [Sanders, 1999]. Despite having higher concentrations of LCPUFA precursors than humans, the percent composition of 20:4n-6 and 22:6n-3 in the Bolivian squirrel monkey is within the range of variation reported for humans [Boersma et al., 1991; Chulei et al., 1995; Gibson & Kneebone, 1981; Koletzko et al., 1988, 1992]. The concentration of these LCPUFA in milk depends on dietary supply (primarily animal products, such as fish and eggs) and the desaturation and elongation of their n-3 and n-6 PUFA precursors. Data from humans [Brenna, 2002; Francois et al., 2003] and baboons [Greiner et al., 1997; Su et al., 2001] indicate that 22:6n-3 levels in milk increase more with an increased source of preformed 22:6n-3 rather than increases in 18:3n-3. It is difficult to draw conclusions as to the source of milk 22:6n-3 without a better understanding of LCPUFA concentration of the diet and efficiency of the species in conversion to LCPUFA. The concentration of 22:6n-3 in a species that produces large-brained neonates is of particular interest [Martin, 1996], as 22:6n-3 is an important structural component of brain and central nervous system tissue and

a limiting nutrient on proper cognitive development [Agostoni & Giovannini, 2001; Carlson, 2001; Gibson, 1997; Gibson & Makrides, 1999; Uauy & De Andraca, 1995].

The milk of squirrel monkey dams (but not allomothers) was higher in mean GE than the mean values for GE from common marmoset milk reported in Power et al. [2002]. However, the proportions of fat, sugar, and protein relative to GE were not different between these species. These data support the hypothesis that milk composition is variable among small New World monkeys, with the variation possibly related to maternal condition. Within that variability, however, the proportions of fat, sugar, and protein relative to GE are predictable. It is especially interesting that the proportion of GE from the protein fraction of milk appears to be constant. Indeed, Buss and Cooper [1972] reported almost identical values for percent protein from four lactating squirrel monkeys (*S. sciureus*).

A consistency in the amount of GE contributed by the protein fraction of milk may be an important phylogenetic trait. Among anthropoid primates, protein contributes to GE the least in human [Jenness, 1979; Prentince, 1995; Stini et al., 1980] and ape milk [Milligan, unpublished data], is intermediate in its contribution among Old World monkeys (mainly represented by baboons and macaques) [Lönnerdal et al., 1984; Oftedal & Iverson, 1995], and is highest in New World monkeys [Power et al., 2002; this study].

Milk composition cannot be looked at in isolation when discussing the energetic costs of lactation for a species. Lactation as a life history trait is also described by the length of lactation, the quantity of milk produced, and the frequency of nursing. Hominoid milk is lower in energy than that of New World monkeys, which may relate to their relatively longer lactation period [Milligan, unpublished data]. Prosimians that park their infants during the day produce more concentrated milk than those who carry their infants and nurse them more frequently throughout the day [Tilden & Oftedal, 1997]. Allonursing in squirrel monkeys may influence milk quantity and nursing frequency. If this behavior is indeed an adaptation in this species to offset energetic costs of the mother, it must also be considered part of the lactation strategy of the Bolivian squirrel monkey.

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REFERENCES

Agostoni C, Giovannini M. 2001. Cognitive and visual development: influence of differences in breast and formula fed infants. Nutr Health 15:183–188.

Altmann J. 1974. Observational study of behavior: sampling methods. Behaviour 49:227–267.

Baldwin JD. 1969. The ontogeny of social behaviour of squirrel monkeys (*Saimiri sciureus*) in a seminatural environment. Folia Primatol 11:35–79.

Bazinet RP, McMillan EG, Cunnane SC. 2003. Dietary α-linolenic acid increases the n-3 PUFA content of sow's milk and the tissues of the suckling piglet. Lipids 38:1045–1049.

Bernacky BJ, Gibson SV, Keeling ME, Abee CR. 2002. Nonhuman primates. In: Fox JG, Anderson LC, Loew FM, Quimby FW, editors. Laboratory animal medicine, (2nd ed.). San Diego: Academic Press. p 675–791.

Boersma ER, Offringa PJ, Muskiet FAJ, Chase WM, Simmons IJ. 1991. Vitamin E, lipid fractions, and fatty acid composition of colostrum, transitional milk, and mature milk: an international comparative study. Am J Clin Nutr 53: 1197–1204.

Brenna JT. 2002. Efficiency of conversion of α-linolenic acid to long chain n-3 fatty acids in man. Curr Opin Clin Nutr Metabol Care 5:127–132.

Buss DH, Cooper RW. 1972. Composition of squirrel monkey milk. Folia Primatol 17:285–291.

Carlson SE. 1999. Long-chain polyunsaturated fatty acids and development of human infants. Acta Paediatr 88:72–77.

Carlson SE. 2001. Docosahexaenoic acid and arachidonic acid in infant development. Semin Neonatol 6:437–449.

Chulei R, Xiaofang L, Hongsheng M, Xiulan M, Guizheng L, Gianhong D, DeFrancesco CA, Connor WE. 1995. Milk composition in women from five different regions of China: the great diversity of milk fatty acids. J Nutr 125:2993–2998.

Del Prado M, Villalpando S, Gordillo J, Hernandez-Montes H. 1999. A high dietary lipid intake during pregnancy and lactation enhances mammary gland lipid uptake and lipoprotein lipase activity in rats. J Nutr 129:1574–1578.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. 1956. Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356.

Dumond FV. 1968. The squirrel monkey in a seminatural environment. In: Rosenblum LA, Cooper RW, editors. The squirrel monkey. New York: Academic Press. p 87–145.

Folch J, Lees M, Stanley GHS. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509.

Francois CA, Connor SL, Bolewicz LC, Connor WE. 2003. Supplementing lactating women with flaxseed oil does not increase docosahexaenoic acid in their milk. Am J Clin Nutr 77:226–233.

Garber PA, Leigh SR. 1997. Ontogenetic variation in small-bodied New World primates: implications for patterns of reproduction and infant care. Folia Primatol 68:1–22.

Gibson RA. 1997. Long-chain polyunsaturated fatty acids and infant development. Lancet 354:1919–1920.

Gibson RA, Kneebone GM. 1981. Fatty acid composition of human colostrum and mature breast milk. Am J Clin Nutr 34:252–257.

Gibson RA, Makrides M. 1999. Long-chain polyunsaturated fatty acids in breast milk: are they essential? In: Newburg-Kluwer Academic, editor. Bioactive components of human milk. New York: Plenum Publishers. p 375–383.

Gibson S, Williams L, McDaniel M, Bazzel J, Abee C. 1993. Allo-maternal nursing and lactation in Bolivian squirrel monkeys (*Saimiri boliviensis boliviensis*). Am J Primatol 30:314.

Greiner RCS, Winter J, Nathanielsz PW, Brenna JT. 1997. Brain docosahexaenoate accretion in fetal baboons: Bioequivalence of dietary alpha-linolenic and docosahexaenoic acids. Pediatr Res 42:826–834.

Hartwig WC. 1996. Perinatal life history traits in New World monkeys. Am J Primatol 40:99–130.

Hinde KJ. 2007. Milk composition varies in relation to the presence and abundance of *Balantidium coli* in the mother in captive rhesus macaques (*Macaca mulatta*). Am J Primatol 69:625–634.

Huang MC, Brenna JT. 2001. On the relative efficacy of α-linolenic acid and preformed docosahexaenoic acid as substrates for tissue docosahexaenoate during perinatal development. In: Mostofsky D, Yehuda S, Salem N, editors. Fatty acids: physiological and behavioral functions. Totowa: Humana Press Inc. p 99–113.

Hunt SM, Gamache KM, Lockard JS. 1978. Babysitting behavior by age/sex classification in squirrel monkeys (*Saimiri sciureus*). Primates 19:179–186.

Iverson SJ, Oftedal OT. 1995. Phylogenetic and ecological variation in the fatty acid composition of milks. In: Jensen RG, editor. Handbook of Milk Composition. San Diego: Academic Press. p 790–827.

Jack KM. 2007. The cebines: toward an explanation of variable social structure. In: Campbell CJ, Fuentes A, MacKinnon KC, Panger M, Bearder SK, editors. Primates in Perspective. New York: Oxford University Press. p 107–123.

Jenness R. 1979. The composition of human milk. Semin Perinatol 3:225–239.

Jensen RG, Bitman J, Carlson SE, Couch SC, Hamosh M, Newburg DS. 1995. Human milk lipids. In: Jensen RG, editor. Handbook of milk composition. San Diego: Academic Press. p 495–542.

Koletzko B, Mrotzek M, Bremer HJ. 1988. Fatty acid composition of mature human milk in Germany. Am J Clin Nutr 47:954–959.

Koletzko B, Thiel I, Abiodum PO. 1992. The fatty acid composition of human milk in Europe and Africa. J Pediatr 120:S62–S70.

Leighty KA, Byrne G, Fragaszy DM, Visalberghi E, Welker C, Lussier I. 2004. Twinning in tufted capuchins (*Cebus apella*): rate, survivorship, and weight gain. Folia Primatol 75:14–18.

Lönnerdal B, Atkinson S. 1995. Nonprotein nitrogen factors in human milk. In: Jensen RG, editor. Handbook of milk composition. San Diego: Academic Press. p 351–387.

Lönnerdal B, Keen CL, Glazier CE, Anderson J. 1984. A longitudinal study of rhesus monkey (*Macaca mulatta*) milk composition: trace elements, minerals, protein, carbohydrate, and fat. Pediatr Res 18:911–914.

Makrides M, Neumann MA, Byard RW, Simmer K, Gibson RA. 1994. Fatty acid composition of brain, retina, and erythrocytes in breast fed and formula fed Infants. Am J Clin Nutr 60:189–194.

Marier JR, Boulet M. 1959. Direct analysis of lactose in milk and serum. J Dairy Sci 42:1390–1391.

Martin RD. 1996. Scaling of the mammalian brain: the maternal energy hypothesis. News Physiol Sci 11:149–156.

O'Brien TG, Robinson JG. 1991. Allomaternal care by female wedge-capped capuchin monkeys. Effects of age, rank, and relatedness. Behavior 119:30–50.

Oftedal OT, Iverson SJ. 1995. Comparative analysis of nonhuman milks: phylogenetic variation in the gross composition of milks. In: Jensen RG, editor. Handbook of milk composition. San Diego: Academic Press. p 749–788.

Pereira ME, Izard M. 1989. Lactation and care for unrelated infants in forest-living ring-tailed lemurs. Am J Primatol 18:101–108.

Ploog DW. 1967. The behavior of squirrel monkeys (*Saimiri sciureus*) as revealed by sociometry, bioacoustics, and brain stimulations. In: Altmann SA, editor. Social communication among primates. Chicago: University of Chicago Press. p 149–184.

Power ML, Oftedal OT, Tardif SD. 2002. Does the milk of callitrichid monkeys differ from that of larger anthropoids? Am J Primatol 56:117–127.

Prentice A. 1995. Regional variations in the composition of human milk. In: Jensen RG, editor. Handbook of milk composition. San Diego: Academic Press. p 155–221.

Prentice A. 1996. Constituents of human milk. Food Nutr Bull 17:305–312.

Ross C. 1991. Life history patterns of New World monkeys. Int J Primatol 12:481–502.

Sanders TAB. 1999. Essential fatty acid requirements of vegetarians in pregnancy, lactation, and infancy. Am J Clin Nutr 70:555S–559S.

Stini WA, Weber CW, Kemberling SR, Vaughn LA. 1980. Bioavailability of nutrients in human breast milk as compared to formula. Stud Phys Anthropol 6:32–35.

Su H-M, Huang M-C, Saad NMR, Nathanielsz PW, Brenna JT. 2001. Fetal baboons convert 18:3n-3 to 22:6n-3 in vivo: a stable isotope tracer study. J Lipid Res 42:581–586.

Tardif SD, Power M, Oftedal OT, Power RA, Layne DG. 2001. Lactation, maternal behavior and infant growth in common marmoset monkeys (*Callithrix jacchus*): effects of maternal size and litter size. Behav Ecol Sociobiol 51:17–25.

Tilden CD, Oftedal OT. 1997. Milk composition reflects pattern of maternal care in prosimian primates. Am J Primatol 41:195–211.

Uauy R, De Andraca I. 1995. Human milk and breast feeding for optimal mental development. J Nutr 125: 2278S–2280S.

Vasey N. 2007. The breeding system of wild red ruffed lemurs (*Varecia rubra*): a preliminary report. Primates 48: 41–54.

Williams L, Gibson S, McDaniel M, Bazzel J, Barnes S, Abee C. 1994. Allomaternal interactions in the Bolivian squirrel monkey (*Saimiri boliviensis boliviensis)*. Am J Primatol 34:145–156.

Williams LE, Abee CR, Barnes S. 1988. Allomaternal behavior in *Saimiri boliviensis*. Am J Primatol 14:445.