Milk Composition Varies in Relation to the Presence and Abundance of *Balantidium coli* in the Mother in Captive Rhesus Macaques (*Macaca mulatta*)

KATHERINE HINDE*
Center for Behavior, Evolution, and Culture, Department of Anthropology, University of California–Los Angeles, Los Angeles, California

Primate infants require extensive maternal investment, and lactation is the most expensive aspect of this investment. However, the relationship between maternal condition and milk composition has been largely uninvestigated in primates. To better understand this relationship, I collected mid-lactation milk samples from 46 captive multiparous rhesus macaques (*Macaca mulatta*) at the Caribbean Primate Research Center, Sabana Seca Field Station, Puerto Rico. The maternal variables assessed were age, weight, weight for crown–rump length (CRL), and presence of parasites. Additionally the analysis included infant age, weight, and sex. Protein concentration in milk showed little interindividual variation, whereas fat had a high variance. Mothers without the lower intestinal parasite *Balantidium coli* had a significantly higher fat concentration in milk than mothers with *B. coli*, but other parasite species (*Trichuris trichiura* and *Strongyloides fulleborni*) were not associated with milk fat concentration. Females with younger infants had a higher fat concentration in their milk than mothers with older infants; however, the association between *B. coli* and milk fat remained significant after controlling for infant age. These results, obtained from a well fed captive population, indicate that even small differences among mothers are associated with milk composition. Am. J. Primatol. 69:625–634, 2007.

Key words: lactation; milk composition; maternal condition; *Macaca mulatta*; rhesus; *Balantidium coli*

INTRODUCTION

Female mammals, particularly primates, invest heavily in each infant they produce. Gestation and lactation are both energetically and physiologically...
expensive, but the greatest costs are imposed by lactation, when mothers provide
the nutrients to support infant growth, development, and activity. Mothers meet
the demands of lactation by mobilizing bodily reserves [Pond, 1984], increasing
caloric intake [Altmann, 1983; Dunbar & Dunbar, 1988; Koenig et al., 1997;
Sauther, 1994], and restricting energy expenditure [Roberts et al., 1985].
Presumably, females that are in good physical condition will be able to meet
the costs of reproduction better than females that are in poor condition.
Numerous studies have demonstrated that maternal condition can influence
aspects of lactation, including frequency and duration of nursing bouts, infant
growth and development, and length of lactation until weaning [Fairbanks &
McGuire, 1995; Gomendio, 1989, 1991; Johnson et al., 1998; Lee, 1987; Tanaka,
1997; Wilson et al., 1978]. Here I examine the effect of maternal age, weight, and
parasite infection on another important and little-studied element of maternal
investment: milk composition.
Milk composition has been studied in a number of mammalian taxa, but
research on primate milk composition has generally been restricted to species-
level descriptions. In nonprimate mammals, small species often produce high-
calorie milk due to increased metabolic demands and reduced gastrointestinal
capacity of offspring [Blaxter, 1961; Oftedal & Iverson, 1995]. However, this
relationship has not been found in primates; for example, the milk of callitrichids
is similar to that of much larger-bodied anthropoids [Power et al., 2002].
Thermoregulation pressures can lead to concentrated milks in marine mammals,
and dilute milks are found in species that live in arid climates, due to high water
turnover [Oftedal & Iverson, 1995; Oftedal et al., 1993]. Although primates live in
a variety of climates and in a range of latitudes, they generally produce quite
dilute milks, which reduces the daily cost of lactation but extends the period of
lactation over time [Oftedal & Iverson, 1995]. There is some evidence that milk
composition is linked to patterns of maternal behavior. Prosimians that “park”
their infants and have long periods of time between nursing bouts produce more
concentrated milk than prosimian species that carry their infants [Tilden &
Oftedal, 1997].
Limited evidence suggests that there may also be intraspecific variation
across individuals in milk composition. Published studies of individual values
and/or reported means with standard errors (SEs)/standard deviations (SDs)
indicate that fat is the milk constituent with the most variance among individuals
(common marmosets (Callithrix jacchus) [Power et al., 2002]; Japanese macaques
(Macaca fuscata) [Ota et al., 1991]; rhesus macaques (Macaca mulatta)
[L önnerdal et al., 1984]; and ruffed lemur (Varecia variegata), mongoose, black,
and brown lemurs (Eulemur mongoz, macaco, and fulvus), bush baby (Otolemur
garnettii and crassicaudatus), and slow loris (Nycticebus coucang) [Tilden &
Oftedal, 1997]), but the source of this variation is unknown. Two studies have
demonstrated that maternal condition affects milk production in primates. When
food was restricted by 40% in three captive baboon mothers, they produced less
milk and their infants stopped growing, but the composition of the milk did not
change because mothers quadrupled mobilization of their maternal reserves
[Roberts et al., 1985]. In a study of captive common marmosets, small mothers
rearing twins lost more weight and had lower fat concentration in their milk than
large mothers producing twins [Tardif et al., 2001]. These data indicate that
maternal condition can influence both milk yield and milk composition.
The aim of this study was to measure the extent and source of individual
variation in milk composition in a population of captive rhesus macaques (Macaca
mulatta) as a function of maternal age, weight, and presence and abundance of
Maternal Condition and Milk Composition / 627

lower-intestinal parasites. Forbes [1993] posited that mothers with parasites should restrict investment in current offspring if parasites impact the resources available to the mother for reproduction, and if the potential exists for the mothers to later recover. Mothers of prime age and weight and without intestinal parasites were predicted to produce more concentrated milk compared to mothers that were of marginal age and weight and had a parasitic infection. Additionally, infant weight was expected to be positively associated with the milk energy content that the infant received from the mother, as has been observed in a number of other species (Iberian deer [Gomez et al., 2002], grey seals [Mellish et al., 1999], marsupials [Trott et al., 2003], common marmosets [Tardif et al., 2001], and baboons [Roberts et al., 1985]).

MATERIALS AND METHODS

Subjects

Milk samples were collected once from 46 multiparous rhesus macaques (Macaca mulatta) from the Caribbean Primate Research Center, Sabana Seca Field Station, Puerto Rico. In 2003 21 mother-infant pairs were included in the study, and in 2004 25 different mother-infant pairs were sampled. The mean (±SD) infant age at sample collection was 44 (±) 19 days, with a range of 8–86 days. The subjects were socially housed in outdoor enclosures in uni-male or multi-male/multi-female groups. All enclosures had perches, climbing structures, and areas protected from the weather. Monkeys were fed commercial monkey chow once daily and water was available ad libitum. The standard diet was supplemented with fresh fruit, vegetables, and browse.

Maternal and Infant Measures

Maternal measures included weight, crown–rump length (CRL), presence of parasites, and complete blood count (CBC). Archival records were screened for maternal age, parity, and parturition date for the current parity. Maternal weight was recorded both as an absolute and corrected for CRL. A fecal smear analysis for parasites was conducted for each mother. A fecal sample was mixed with saline solution, the mixture was spread on a slide, and then examined under 10× and 40× magnification. All parasites on the entire slide were counted. However, since direct mounting in saline is designed to confirm the presence and abundance of certain protozoa and helminthes, a slight variation in stool specimen weight can influence results, and therefore individual counts are intended as indicators of abundance, rather than absolute measurements [Garcia et al., 2003]. For 23 of 46 subjects, fecal flotation was performed to assess presence of parasite ova using standardized methods [Garcia, 2003]. Immediately before milking, blood samples were drawn from the saphenous vein of the mother for a hematological profile analysis (Hemavet® 850 [Drew Scientific, Inc., Oxford, CT]) of leukocytes (neutrophils, lymphocytes, monocytes, basophils, and eosinophils). One sample was removed from the analysis because of laboratory error (n = 45). White blood cell counts were reported as K/μL. Infants were weighed, and sex was determined prior to maternal sample collection.

Milking Procedure

To obtain milk samples the females were sedated with ketamine hydrochloride (10mg/kg body weight) injected intramuscularly. The mothers were placed in mesh jackets that prevented nursing for 3–4 hr while milk accumulated.
During this period the mothers and infants were housed together. For milk sample collection, the mothers were sedated and removed from the holding cage and the infant was provided with a polar-fleece surrogate toy. The nipple areas were cleaned and chest hair was trimmed. The females were injected with exogenous oxytocin intramuscularly (0.1 ml/kg) to stimulate muscle contraction and milk let-down. The females were held upright in a sitting position, and their nipples were gently hand-stripped to mimic infant nursing behavior with periodic rests and simulated nuzzling. The mammary glands were fully evacuated to avoid sampling bias from partial collection [Oftedal, 1984]. For eight of 46 females (17%), sufficient milk for sample analysis was not available from one mammary, so both mammary glands were fully evacuated and the samples were pooled. Milk was collected once from each mother in sterile sample tubes and frozen at −20°C until a proximate analysis was performed 10–14 days later.

Milk Composition Analysis

Milk composition analysis was conducted by a private company (Sani-Pure Food Laboratories; Saddlebrook, NJ) using official methods of analysis [Association of Official Analytical Chemists, 1990] adapted for sample sizes <10 ml. Although these methods were developed for bovine milk analysis, these are the primary methods used for milk analysis for most mammals [reviewed in Oftedal & Iverson, 1995] and they have often been used for primates [Power et al., 2002; Tilden & Oftedal, 1997; reviewed in Oftedal & Iverson, 1995]. Total fat was determined by the Roese-Gottlieb ether extraction method. Protein was calculated as N/C26.38 according to the Kjeldahl procedure. Dry matter (DM) was measured gravimetrically after oven drying at 98–100°C. Ash, a proximate of mineral content, was similarly determined after the sample was heated in a furnace to 550°C. Carbohydrates were calculated by [(DM–(P+L+A))/C], where P is protein, L is fat/lipids, A is ash, and C is carbohydrates (all expressed as a percentage of the total milk). However, since this measure of carbohydrates is merely an approximation and necessarily includes the collective errors from the assays performed for the other milk constituents, carbohydrates were not included in the statistical analyses.

Data Analysis

Data analysis was conducted using linear and multiple regression for continuous-outcome variables, such as milk fat concentration and infant weight, using both continuous and categorical predictor variables. All analyses were performed using STATA 14. Statistical significance was set at α ≤ 0.05.

RESULTS

Maternal Characteristics

The 46 mothers in this study had a mean (±SD) weight of 7.7 (±2) kg and age of 8.2 (±3.3) years. The mean parity was 3.7, and was highly correlated with maternal age (correlation = 0.855, n = 46). Fecal smear analysis indicated that 59% of the mothers (27/46) had the lower-intestinal parasite Balantidium coli on the day of milk collection. The mothers varied in the number of B. coli parasites present in the feces: 10 mothers had a low abundance of B. coli (defined as ≤3 B. coli present at screening), and 17 mothers were more abundantly infected (≥5 B. coli). Fecal flotation was performed on samples from 23 of 46 mothers, and 26% of these mothers (6/23) tested positive for hookworm (Trichurus trichiura), while 17% (4/23) were positive for Strongyloides fulleborni.
## Milk Composition

Milk composition showed the typical dilute pattern of nonhuman primates (Table I). The total mean DM content was 15%, and was comprised of protein (1.8%), carbohydrates (~7.5%), fat (5.4%), and ash (0.4%). The mean water content was ~85%. Protein and carbohydrates showed low variability across individuals, but milk fat concentration had a high individual variance.

### Maternal and Infant Characteristics and Milk Composition

Mothers without *B. coli* had a 50% higher mean fat concentration than mothers infected with *B. coli* (6.7% vs. 4.5%, respectively, $F = 5.94$, d.f. = 44, $P = .019$, $R^2 = 0.12$). Moreover, abundance of the parasite was associated with milk fat concentration, as mothers without *B. coli* had a 30% higher mean fat in their milk than mothers with a low abundance of *B. coli* (5.1%), and a 60% higher mean fat concentration than mothers with a high abundance of *B. coli* (4.2%) ($F = 6.44$, d.f. = 44, $P = .015$, $R^2 = 0.13$; Fig. 1). No other maternal variables (weight, weight for trunk length, or age) were significantly associated with fat content in milk. The presence of *B. coli* was not associated with protein or carbohydrate content of milk: mothers without *B. coli* compared to mothers with

### TABLE I. Mean Values for Milk Constituents in 46 Multiparous Rhesus Macaques Each Sampled Once During Mid-Lactation (*Macaca mulatta*)

<table>
<thead>
<tr>
<th>Milk constituent</th>
<th>Mean (%)</th>
<th>SD</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>15.2</td>
<td>2.9</td>
<td>0.43</td>
</tr>
<tr>
<td>Fat</td>
<td>5.4</td>
<td>3.1</td>
<td>0.45</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>7.5</td>
<td>1.3</td>
<td>0.20</td>
</tr>
<tr>
<td>Protein</td>
<td>1.8</td>
<td>0.5</td>
<td>0.07</td>
</tr>
<tr>
<td>Ash</td>
<td>0.4</td>
<td>0.4</td>
<td>0.05</td>
</tr>
<tr>
<td>Water</td>
<td>84.8</td>
<td>2.9</td>
<td>0.43</td>
</tr>
</tbody>
</table>

![Graph of Milk Fat Concentration](image)

Fig. 1. Mothers that showed no evidence of *Balantidium coli* ($n = 19$) had a higher fat concentration in their milk than mothers that tested positive for *B. coli*, mothers with a low abundance of *B. coli* ($n = 10$) had a 5.1% concentration, and mothers with abundant *B. coli* ($n = 17$) had a 4.2% concentration ($F = 6.44$, d.f. = 44, $P = .015$, $R^2 = 0.13$, linear regression).

*Am. J. Primatol. DOI 10.1002/ajp*
**TABLE II. Mean Values for White Blood Cell Counts (K/μL) for Multiparous Rhesus Macaques Each Sampled Once During Mid-Lactation (n = 45), Categorized by Abundance of *Balantidium coli*: Zero Observed *B. coli* (n = 18), ≤3 *B. coli* (n = 10) and ≥5 *B. coli* (n = 17)**

<table>
<thead>
<tr>
<th>White blood cells</th>
<th>No <em>B. coli</em></th>
<th>Low <em>B. coli</em></th>
<th>Abundant <em>B. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>4.4</td>
<td>5.1</td>
<td>6.1</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>2.3</td>
<td>2.3</td>
<td>2.7</td>
</tr>
<tr>
<td>Monocytes</td>
<td>1.0</td>
<td>0.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.8</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Total count</td>
<td>8.5</td>
<td>9.0</td>
<td>10.8</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The finding that mothers without *B. coli* had significantly more fat in their milk than mothers with *B. coli* is the first evidence that certain aspects regarding maternal parasite load are associated with milk composition in primates. The relationship between parasite load and reproduction has been little studied in...
nonhuman primates. It has been shown that severe illness adversely affects pregnancy outcome [reviewed in Hindreckx & Binkard, 1980; Kraemer & Vera Cruz, 1971; Sackett & Holm, 1980]. However, the data presented here suggest that much less severe parasitic infection, including subclinical/asymptomatic levels, may also impact lactation. *Balantidium coli* can cause bloody diarrhea and colitis in humans [Farthing, 2003] and ulcerative colitis in primates [Toft, 1986], but is not associated with chronic enterocolitis in macaques [Sestak et al., 2003]. The association with milk fat concentration appears to be specific to *B. coli*, since other parasites (*Trichuris trichiura* and *Strongyloides fulleborni*) were not associated with milk fat. However, *T. trichuris* and *S. fulleborni* were much less prevalent in the population, and low sample sizes limit the interpretation of the results. Further investigation of *S. fulleborni* is warranted.

The precise mechanism by which *B. coli* affects milk fat concentration is unknown. It may be that *B. coli* directly competes for resources from dietary intake, causing malabsorption in the mother. Additionally, low-level infection may reduce activity levels and impair foraging or suppress appetite and decrease caloric intake [Coop & Kyriazakis, 1999]. After dairy cattle are treated for low parasite loads, their milk yield significantly increases; however, the effect on milk composition is unknown [Nødtvedt et al., 2002]. Additionally, experimental work in rodents has demonstrated that parasites do not impact pregnancy outcome but do affect postnatal maternal investment [Berdoj et al., 1995; Weatherly, 1971; Willis & Poulin, 1999]. White blood cell counts were similar between the groups, suggesting that the health consequences for *Balantidium* infection are limited. However, parasites often counter host immune defenses via antigenic responses, causing down-regulation of white blood cells following an initial response to the parasite [Gendrel et al., 2003]. For this reason, white blood cell counts do not perfectly reflect the presence or abundance of parasites.

Other maternal variables (weight, weight for CRL, and age) were not associated with milk composition. This may be because the well-fed multiparous females in this study had completed their skeletal growth and attained threshold weights above which milk composition is affected. Although maternal weight is associated with milk fat content in captive common marmosets, reproduction may be particularly costly for small-bodied marmosets that regularly produce twins [Tardif et al., 2001].

It is not clear why fat concentration varies more than protein and carbohydrates. It is possible that protein and carbohydrates are not sensitive to maternal condition, perhaps because these constituents are so important for infants that selection has minimized variation. Manipulating the energetic contribution from different milk constituents may be a way to limit maternal depletion during lactation. In a study of Iberian deer, hinds that produced calves during the normal birthing season had a higher fat content in milk, but lost weight during lactation [Gomez et al., 2002]. Hinds that calved before the normal birthing season had a high carbohydrate content in milk and gained weight during lactation. Additionally, infants of early-producing hinds were bigger and matured earlier than the infants in the normal group.

In a study of grey seals, the best predictor for pup size at weaning was total milk energy output, to which fat is the greatest contributor [Mellish et al., 1999]. In marsupials, an analysis of milk protein and carbohydrates content showed that energy content in milk was positively correlated with infant growth and development [Trott et al., 2003]. In common marmosets, small mothers rearing twins had a lower fat concentration in milk, and infant growth was compromised compared to singleton infants and infants being reared by larger mothers [Tardif.
et al., 2001]. This variation suggests that the relationship among maternal condition, milk content, and infant development may be complex and vary across taxa with different life-history trajectories, reproductive systems, and activity patterns.

In the present study neither fat concentration in milk nor maternal presence of parasites was associated with infant weight. Mothers with B. coli that have a low fat concentration in their milk may compensate by nursing more frequently than mothers that produce more concentrated milk. However, this explanation is not entirely satisfactory because if the infants were compensating by increasing their milk intake, they would also have received more protein and carbohydrates, because these constituents did not vary between parasitized and nonparasitized groups. Protein and carbohydrate concentration in milk are associated with infant weight gain in other species of mammals [Mellish et al., 1999, Gomez et al., 2002], and higher milk consumption to increase fat intake would necessarily increase protein and carbohydrate intake compared to the infants of nonparasitized mothers.

In this study the mothers of older infants had a lower fat concentration in their milk than mothers with younger infants. This cross-sectional study cannot distinguish between two possible explanations. It may be that fat concentration in milk declines as infants age. Alternatively, it is also possible that mothers that produce infants early in the birth season have a lower fat content in milk than mothers that give birth later in the birth season, as has been found in Iberian deer [Gomez et al., 2002].

The results presented here are intriguing because they suggest that a relatively subtle difference among mothers—the presence and abundance of B. coli is significantly associated with milk composition. To better understand the causes and consequences of such differences in milk composition, further data are needed. In particular, we need more information about the relationship between milk composition and milk volume, the sources and magnitude of variation in milk composition over the course of lactation, whether mothers and infants compensate for variation in the quality of milk by adjusting the timing or length of nursing bouts, and how variation in milk quality influences infant growth, development, and health.

ACKNOWLEDGMENTS

I thank Drs. Joan Silk, Melissa Gerald, Janis Gonzalez, Hector Perez, Susan Perry, Joe Manson, and H. Clark Barrett, and the research staff at the Caribbean Primate Research Center and Washington National Primate Research Center (WaNPC) for their contributions to this research project. In addition, I thank Dr. Linda Fedigan and two anonymous reviewers for insightful comments that improved the quality of the paper. Research at the Caribbean Primate Research Center, University of Puerto Rico Medical Sciences Campus, is made possible by NIH CORE grant CM-5-P40-RR03640.

REFERENCES


Sauther ML. 1994. Wild plant use by pregnant and lactating lemurs, with implications for Maternal Condition and Milk Composition / 633

Am. J. Primatol. DOI 10.1002/ajp


