

**NONHUMAN PRIMATE MILK COMPOSITION: RELATIONSHIP
TO PHYLOGENY, ONTOGENY, AND ECOLOGY**

by

Lauren Anne Milligan

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DEDICATION

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ABSTRACT

This dissertation provides a comprehensive and systematic examination of anthropoid primate milk composition and its relationship to a species' evolutionary history, ecological context, and life history strategy. Milk samples from 14 species of anthropoid primate (*Alouatta paliatta*, *Callithrix jacchus*, *Cebus apella*, *Gorilla beringei beringei*, *Gorilla gorilla gorilla*, *Hylobates lar*, *Leontopithecus rosalia*, *Macaca mulatta*, *Macaca sinica*, *Pan paniscus*, *Pan troglodytes*, *Pongo pygmaeus*, *Saimiri boliviensis boliviensis*, and *Symphalangus syndactylus*) were analyzed for proximate composition (fat, protein, lactose, dry matter, and minerals) and milk fatty acid composition. The objectives of this study were identification of primitive features in anthropoid milks, shared-derived features of anthropoid families or superfamilies, and unique-derived features of species, including *Homo sapiens*.

Results did not support the null hypothesis of a generalized anthropoid milk composition. Variation among anthropoids in milk fatty acid profiles and proximate milk composition was influenced by phylogeny and the life history strategy of the species, as well as the diet and environment (captive or wild living) of the mother.

Maternal diet had a direct influence on fatty acid profiles and created distinct groupings of wild and captive living individuals. Phylogenetic patterns were identified within captive and wild groups, particularly a distinction between milk fatty acid profiles of hominoids (including humans) and monkeys.

Significant variation in proximate milk composition was identified at the level of the superfamily. Cercopithecoid milk was highest in mean fat, dry matter, the proportion

of energy from fat, and total gross energy. Ceboid milk was highest in mean protein and the proportion of energy from protein. Hominoid milks were lowest in mean fat, protein, dry matter, the proportion of energy from fat, and total gross energy.

Hominoid milk also was lowest in the degree of plasticity in milk composition. Milk of captive living monkeys was higher than milk of wild living monkeys in mean fat, percent energy from fat, and total gross energy. Milk fat and energy also were highly variable within captive living monkeys. In contrast, fat and total gross energy were not significantly different between captive and wild living hominoids and were less variable among captive living hominoids as compared to monkeys. The lack of variability and the relatively low energy values in hominoid milk suggest that it may be buffered against environmental fluctuations. Larger body size and a longer duration of lactation may permit hominoids, including humans, to decouple maternal condition from milk energy and instead relying on energy storage.

CHAPTER 1: INTRODUCTION

Introduction

Continuation of the foetal pattern of brain and body growth into the first year of postnatal life in human beings...poses special problems with respect to lactation, since it is quite clear that a pattern of early postnatal brain growth that is unique among mammals must require a unique milk to supply the needs of the developing human infant. It is, of course, possible that the great degree of postnatal brain development in human beings merely requires the provision of standard nutrients in greater quantities. However, it is also possible that human breast milk contains components which are not normally present in significant quantities in the milk of other precocial mammals with a normal pattern of postnatal brain growth (Martin, 1983: 41).

In 1983, Robert Martin proposed that human milk composition should be species-specific due to the unique ontogenetic priorities of the human neonate. As compared to other mammals, human infants show a greater degree of postnatal brain growth which Martin (1981) models as a continuation of the fetal brain to body growth relationship for at least the first year after birth. It is more energetically efficient for brain growth to occur during fetal life because the metabolic capacity of the mother exceeds that of the infant (Martin, 1981, 1983) However, the size of the female pelvic inlet imposes a limit on fetal brain growth (Foley and Lee, 1991; Martin, 1981; Shipman and Walker, 1989; Vasey and Walker, 2001). With selection for increased brain size over the course of human evolution, there came a point at which the pattern of brain growth shifted from rapid fetal growth and development to rapid fetal and postnatal growth and development. Martin (1981) argues that an apelike trajectory of brain growth would not have been possible for adult cranial capacities at or greater than 850 cm^3 , the threshold at which an apelike trajectory of brain growth would be constrained by pelvic inlet size, and when the postnatal continuation of the fetal growth pattern becomes necessary (Martin, 1981).

Testing for a unique human milk composition necessitates comparison to the milks of our closest living relatives, the nonhuman primates. However, despite numerous arguments for a unique human milk composition in the anthropological literature (e.g. Foley and Lee, 1991; Martin, 1983; Vasey and Walker, 2001), the composition of human milk has never been evaluated in a comparative context with multiple species of nonhuman primate. Indeed, little is known about primate milk, particularly the milk of great apes.

This dissertation provides a comprehensive and systematic examination of anthropoid primate milk composition and its relationship to a species' evolutionary history, ecological context, and life history strategy. By providing quantifiable data on proximate and milk fatty acid composition from multiple species of anthropoid primate, this study identifies primitive features in anthropoid milks, shared-derived features of anthropoid families or superfamilies, and unique-derived features of species, including *Homo sapiens*.

Research Questions

This study is the first to examine anthropoid primate milk composition from an evolutionary perspective. The lack of previous research on the nature of variation of milk composition among anthropoids necessitated very broad research questions. Variation in milk composition (includes data on milk fatty acids, as well as the concentration of total fat, protein, lactose, dry matter, calcium and phosphorus) is investigated within species

(intraspecific) and between species (interspecific) with respect to a species' evolutionary history, ecology, and life history. The null hypothesis states that there is no variation in anthropoid primate milk composition, or rather, that milk composition has been conserved across the suborder Anthroidea. The following research questions are addressed:

Question 1: Does milk composition follow a phylogenetic pattern? If milk composition is influenced by a species' evolutionary history, then milk should be more similar between closely related species than between distantly related species. Related questions include: (a) Are there any aspects of milk composition that are shared by all anthropoid primates? Primitive features of anthropoid milk represent a remote (older) evolutionary pattern. (b) Are there any aspects of milk composition that are shared by all cercopithecoids, all ceboids, or all hominoids? These shared-derived features of superfamilies indicate evolutionary trajectories since the time that this superfamily last shared a common ancestor with other anthropoid superfamilies. (c) Are there any aspects of milk composition that are unique to a particular species? Unique-derived features of milk composition represent evolutionary modifications during the evolution of the species.

Question 2: Does milk composition vary with respect to ecological factors? Ecological factors investigated in this study include a species' dietary strategy (e.g., folivore, frugivore) and living conditions (wild living or captive-housed). Species included in this

study vary widely in dietary niches in the wild, but many milk samples were provided only by captive living females. Related questions include: (a) How does a species' dietary strategy influence the composition of milk? (b) Is there a relationship between the "quality" of a species' diet and the energy available in the milk produced? (c) What is the influence of a captive living diet on milk composition? (d) What is the influence of a captive living lifestyle (e.g. fed *ad libitum*, reduced energy expenditure) on milk composition? Although the diet and lifestyle of a captive living female primate may differ from wild conspecifics, samples from both groups has the potential to inform on the capabilities of the mother in milk production under a wide range of environments.

Question 3: Does milk composition vary with respect to life history traits? Species included in this study vary widely with respect to adult female body size, neonatal body size, adult brain size, neonatal brain size, age at first reproduction, and duration of lactation. Related questions include: (a) Is there a relationship between the duration of a species' lactation and the composition of the milk produced, such that species with similar lactation periods produce more similar milks? (b) Is there a relationship between maternal body mass and the energy of the milk produced? (c) Does milk from primates that grow at a relatively faster rate differ in regard to nutrients that are related to growth? (d) Do primates with relatively larger brains than predicted for their body size produce milks of higher energy and/or with higher concentrations of brain specific nutrients?

Question 4: Is the composition of human milk species-specific? Martin (1981,1983) hypothesizes that encephalization in genus *Homo* must have been accompanied by selection for milk constituents that could support rapid brain growth in the neonate. He argues that human milk composition is species-specific due to the unique ontogenetic priorities of human neonates compared to nonhuman primates.

Organization of the Dissertation

The first five chapters of this dissertation provide a theoretical and comparative framework for research questions and the subsequent interpretation of the anthropoid milk data. Chapter 2 outlines the theoretical framework adopted in this study and presents specific predictions derived from theory about anthropoid milk composition. Chapter 3 situates milk composition in an evolutionary context and presents a comprehensive review of the biological role and function of individual milk components, as well as data on human milk composition, nonhuman primate milk composition and the composition of other mammalian milks. In chapter 4, I provide a brief summary of fatty acid biochemistry and a review of the literature on the relationship between fatty acids and brain growth and development. I also present data tables on human milk fatty acid composition from multiple human populations that will be used as a comparative sample for data on anthropoid milk fatty acids. Chapter 5, the last of the background chapters, summarizes the evolutionary history, diet, and life history strategy for all species included in this study. This chapter includes data tables on dietary strategy and life

history traits and will be used to test research questions. Chapter 6 describes the milk samples included in this analysis and chapter 7 details the methods of milk analysis used to determine milk composition. Chapter 8 presents results and a discussion of milk fatty acid profiles of anthropoid primates and chapter 9 presents results and a discussion of proximate milk composition (fat, protein, sugar, dry matter, and minerals) of anthropoid primates. Both chapters 8 and 9 conclude with a discussion comparing human milk composition (fatty acid or proximate) to data from anthropoid primates. Finally, chapter 10 presents conclusions and their relevance to phenotypic plasticity in milk production by primate species and remote versus recent patterns in milk composition among anthropoid lineages.

**CHAPTER 2: THEORETICAL FRAMEWORKS AND PREDICTIONS FOR
ANTHROPOID MILK COMPOSITION**

Introduction

This dissertation examines variation in anthropoid milk composition using an evolutionary framework. Hypotheses about milk composition are modeled as if a product of natural selection. It is assumed, therefore, that variation exists in milk composition, some of this variability is inheritable (have a genetic component), and some compositions associate with a higher fitness, or reproductive success, than others (following Endler, 1986). Milk composition satisfies these three tenets of natural selection. Variation in milk composition has been demonstrated between and within mammalian orders, families, and species (Ofstedal and Iverson, 1995). The lack of extensive cross-cultural variation in human milk composition suggests that milk composition is highly heritable at the level of our species (Jenness, 1979; Prentice, 1995, 1996; Prentice and Prentice, 1995; Stini et al., 1980). Finally, as milk is the only source of nutrition for neonates and developing infants, selection should favor the production of a milk composition that would somehow balance the interests of maternal and offspring survival.

As a product of natural selection, milk composition is predicted to be more similar among species that share a more recent evolutionary history. This observation is the basis for research questions relating milk composition to phylogeny. Questions regarding the relationship between milk composition and ecology (diet, captivity) and life history come from theoretical derivations of evolutionary theory, including the maternal energy hypothesis (Martin, 1981, 1996), parent-offspring conflict theory (Trivers, 1974), and life history theory (Charnov, 1993; Cole, 1954; Medawar, 1952; Promislow and Harvey,

1990; Read and Harvey, 1989; Roff, 1992; Stearns, 1992; Williams, 1957). The combination of theoretical considerations and empirical data on primate biology and ontogeny contributed to the development of predictions – both direct and indirect - concerning the relationship between milk composition and a species' ecology and life history. This chapter summarizes the specific aspects of these theories that are most germane to hypotheses on milk composition and the predictions from them that can be tested with a data set on anthropoid milk composition.

Maternal Energy Hypothesis

Theoretical framework

The maternal energy hypothesis (Martin, 1981, 1996) suggests that neonatal brain size is determined by the energy available to the mother (maternal metabolic turnover) and should therefore increase with maternal body size raised to the 0.75 power. Martin's hypothesis was derived from allometric equations. Allometry is the relationship between the overall size of an individual organism and the size of one part of the individual (e.g., brain size, neocortex size). Allometric analyses express the bivariate regression of a biological variable on the y axis and an estimate of size on the x axis (Fleagle, 1985), and are used as a statistical concept in evolutionary studies (Lande, 1985). There are three types of allometric relationships: ontogenetic, intraspecific, and interspecific (Fleagle, 1985). This project is concerned with differences among individuals of different species and will therefore focus on interspecific allometry – variation in milk composition among

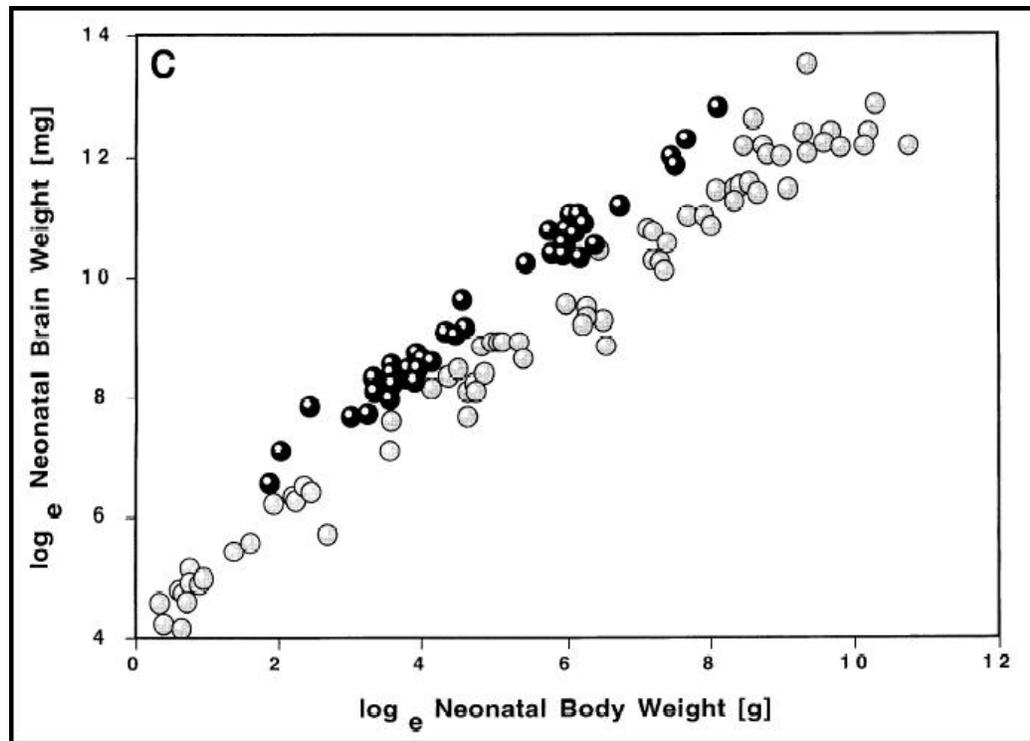
species that differ in body size (Fleagle, 1985; Lande, 1985). Two concepts integral to interspecific allometric analyses are scaling principles and grade shifts (Martin and Harvey, 1985). The underlying assumption of interspecific allometric relationships is that individual traits usually change in a predictable manner with respect to body size according to a scaling principle (Lande, 1985). An exception is when there is a significant reorganization due to an adaptive change, or grade shift (Martin and Harvey, 1985). If an allometric relationship is expressed as $y = bx^k$, the scaling principle is the exponent or slope (k) and a grade shift would be indicated by a change in the intercept (b) (Martin, 1996; Martin and Harvey, 1985).

One of the best known scaling relationships is that between body size and basal metabolic rate (BMR), also known as Kleiber's law (Kleiber, 1932). The interspecific scaling exponent for this relationship is 0.75 and the correlation between variables is 0.98, indicating that deviations from the line described by this equation are limited, especially in placental mammals (Martin, 1982, 1996). This scaling exponent is equivalent to that of brain size and body size in placental mammals, suggesting that the relationship between brain size and BMR is isometric with (or directly proportional to) that of body size and BMR (Martin, 1983, 1996). Further, adult brain weight is isometric with neonatal body size, providing a link between maternal BMR and neonatal brain size.

Martin (1981, 1983, 1996) proposes that the brain size of any mammal is the largest size compatible with the metabolic resources available to the mother during gestation and lactation. The allometric relationships between brain size, body size, and BMR are similar among adult primates (including humans) and nonprimate mammals,

but differ when the same relationships are explored in fetuses and neonates. The “grade shift” shown in Figure 2.1 is the result of the larger size of the neonatal brain relative to neonatal body mass across the primate order (Martin, 1996; Sacher, 1982). Compared to other placental mammals, primates have larger brains at birth because of decreased investment in fetal body development (Martin, 1983; Vasey and Walker, 2001).

Figure 2.1. Neonatal brain weight (mg) by body weight (g), from Martin (1996). Black circles represent nonhuman primates, gray circles represent nonprimate placental mammals.



Neonatal brain and body size are large in humans relative to nonhuman apes (Martin, 1983) and human mothers must therefore make a greater energetic investment

during the prenatal period compared to nonhuman apes (and other primates) (Vasey and Walker, 2001). Humans also are unique in the rate of postnatal brain growth. The human brain growth spurt is perinatal (Brenna, 2002) as humans extend the rapid rate of prenatal brain growth through the first year of extrauterine life (Martin, 1983, 1996).

Chimpanzees show decelerated brain growth during the sixth month of postnatal life and it is this difference that is believed to account for the differences in adult brain sizes between chimpanzees and humans (Robson, 2004; Uauy et al., 1996). The continuation of the fetal pattern of brain growth in humans – a unique pattern among mammals - would therefore require, among other things, a greater energetic investment by human mothers during the postnatal period as well (Martin, 1983; Vasey and Walker, 2001). Martin argues that explanations for increases in brain size should be sought in changes in the allocation of maternal resources to fetal and postnatal development.

Theoretical predictions

The underlying factor for both prenatal and postnatal investments in human and nonhuman primates is the metabolic turnover and reserves of the mother, which is expected to be a function of maternal mass. The cost of growing a larger newborn with a larger relative brain is borne solely by the mother (Martin, 1981, 1983). Human mothers may meet these costs in the prenatal period through a slight extension of the gestation period or small increases in BMR throughout the gestation period (Vasey and Walker, 2001). As it relates to postnatal investment, determining milk composition is a direct

method of measuring maternal energy investment (Vasey and Walker, 2001) as mothers must ensure brain growth through lactation.

Martin (1981, 1996) hypothesizes that encephalization in the genus *Homo* must have been accompanied by selection for milk constituents that could support rapid brain growth in the neonate. Human milk composition is argued to be species-specific due to the unique ontogenetic priorities of human neonates compared to nonhuman primates (Foley and Lee, 1991, Martin, 1981, 1983; Vasey and Walker, 2001). The unique human pattern of brain growth necessitated “a unique milk to supply the needs of the developing human infant” (Martin, 1983: 41). Martin (1995) suggests that a test of this hypothesis would be accomplished through a comparative study of nonhuman primate milk. An extension of Martin’s predictions, Vasey and Walker (2001: 342) propose that the “critical detailed comparison... would be between human milk and that of African great apes.” This suggestion – that human milk was unique among primates because of unique ontogenetic priorities of the human neonate - was the foundation for this dissertation project.

Implicit in Martin’s hypothesis is the idea that biological components that facilitate and support brain growth should be found in human milk in higher concentrations than they are found in other nonhuman primate milks and/or found only in human milk. Robson (2004) tests Martin’s prediction regarding the relationship between human brain size and the unique nature of human milk. She focuses on milk fatty acids, specifically the LCPUFA docosahexaenoic acid (DHA) and arachidonic acid (AA) because of their prevalence (over 50%) in neural lipids. Robson (2004) concludes that

human milk fatty acid composition is not unique, but her nonhuman primate comparative sample consists of only two species, both of the genus *Macaca* (rhesus macaques, *M. mulatta*, and Japanese macaques, *M. fuscata*). Further, Japanese macaque samples were colostrum samples, which do not represent true milk and are likely to have higher fatty acid concentration than samples collected from the midlactation phase (Iverson and Oftedal, 1995). Lastly, because her reference samples were from captive living individuals, milk fatty acid profiles may have been influenced by the captive diet. Robson (2004) laments the lack of comparative data from nonhuman primate milks, especially from great ape species, but hypothesizes that even if such data are presented, nonhuman primate milk composition is unlikely to differ from human milk composition.

This study tests Martin's and Robson's alternative predictions through a comparative analysis of both milk fatty acid composition and proximate milk composition in a much larger sample of anthropoid primates, including wild living individuals, as well as seven species of hominoids. Most important for Martin's predictions may be the composition of *Cebus apella* samples. *Cebus* monkeys are unusual in being highly encephalized, and they exhibit brain growth both pre- and postnatally. If specific constituents in milk, including LCPUFA, are necessary for the growth of a larger brain, they should be found in higher concentrations in the milk of *Cebus* relative to other anthropoid primates.

The relationship between maternal body size (metabolic capacity) and milk composition also will be explored in relation to somatic growth. Following Lee (1999), while maternal body size and energy balance determine the mother's ability to provide

the necessary milk resources for brain growth during gestation and lactation, the growth requirements of infants also includes somatic growth to a metabolic weaning mass. This growth requirement may be separate from brain growth and may be reflected in the energetics of lactation, measured directly through milk composition (Lee, 1999).

Parent-Offspring Conflict Theory

Theoretical Framework

Trivers (1972) defines parental investment (PI) as any investment made by the parent in an individual offspring that increases the offspring's chances of surviving, and thus reproductive success, at some cost to the parent's ability to invest in other offspring. An extension of PI theory is parent-offspring conflict (POC) theory (Trivers, 1974). Natural selection acts in slightly different ways on genes expressed in the parent that influence parental care and on the same genes in offspring (Godfray, 1995). Because the parents are equally related to each offspring, parental investment should be divided equally among offspring. The offspring, however, should try to obtain a disproportionate amount of resources from parents. Offspring share only half of their genetic material with siblings and should therefore demand investment from their parents at the expense of future (or current) siblings (Maestriperi, 2002). Although the two sets of genes have a common evolutionary interest, POC is predicted to occur during the period of parental care (Godfray, 1995). In fact, Trivers sees conflict of interest as "an expected feature of such relations" (Trivers, 1974:249).

Assuming conflict of interest is inevitable, resolution models focus on how parents and offspring address the conflict. Alexander (1974) argues that the conflict would be one-sided as the parent would always be in a better position for resolution in their favor. Indeed, Trivers (1974) acknowledges that maternal interests were likely to be favored because mothers were in control of both the rate of investment and the amount of investment (Lee, 1996). Parker and Macnair (1978, 1979; Macnair and Parker, 1978, 1979) disagree and propose that an intermediate distribution of resources between parent and offspring (i.e., a compromise) would be the most likely resolution and could be an evolutionary stable system. If a parent-offspring compromise is indeed the solution, evolutionary models of parental investment should include both the parental and infant perspective.

Theoretical Predictions

Trivers (1974) hypothesizes that parents and offspring would disagree over the quantity and quality of parental investment. To increase either the duration or amount of parental investment, offspring are expected to manipulate parents psychologically. Offspring tantrums during weaning are argued to be an example; by behaving younger, infants manipulate the parents into providing investment past the period at which the parent would normally do so.

Lee (1996) argues that as a result of Trivers' POC theory (1974), weaning (the cessation of lactation) became the central focus of evolutionary models of lactation and reproductive strategies. Duration of lactation is only one aspect of parental investment

through lactation. Milk composition, another component of a species' lactation strategy, also represents investment by the mother. Trivers' theory focuses on behaviors (e.g., tantrums by the offspring, refusal to nurse by the mother) that represent the conflict over resources, or its resolution. Predictions of the theory can be extended to maternal or offspring physiology, including milk composition. Indeed, Lee (1996) predicts that milk quality (composition) and quantity (yield) will reflect both the needs of the mother and the infant. She hypothesizes that infants drive the production of milk at a cost to the mother. Constraints on the mother's capacity to supply those needs will place limitations on infant growth. Thus, the pattern and rate of growth and development of the offspring establishes a necessity for production of sufficient milk quality and quantity, while maternal energy balance affects the pattern and rate of offspring growth and development (Lee, 1996). Infants may grow faster if mothers produce higher energy milks or milks with higher protein concentrations, but mothers may be limited by their physiological energy stores.

This dissertation will investigate to what extent milk composition reflects a compromise between mother and infant. Based on Trivers' POC theory (1974) and Lee's (1996) predictions regarding the relationship between maternal energy stores and milk production, milk composition is predicted to vary with respect to maternal energy balance. Mothers will increase investment via milk quality, but only when they are physiologically capable of doing so.

Life History Theory

Theoretical framework

Life history theory, in the broadest sense, is concerned with how natural selection has molded the way that organisms grow, develop, reproduce, and die (Stearns, 1992). Organisms are predicted to behave in a way that maximizes their fitness at each age through the optimal allocation of resources to maintenance, growth, and reproduction (Partridge and Harvey, 1988). It is a theory about fitness (Nylin and Gotthard, 1998), because natural selection is assumed to be the primary force shaping life history variation (Roff, 1992), but it is also a theory about life ways (Morbeck, 1997a).

Fitness is a measure of reproductive success (Dobzhansky, 1970). The primary “goal” of all organisms is reproduction, but when and at what rate organisms reproduce varies considerably. Charnov’s (1993) life history model proposes that individuals have a finite amount of energy that can be spent on either growth or reproduction. Before reproductive maturity, all available energy is devoted to growth, and at maturity, all energy is devoted to reproduction and maintenance. Charnov’s model implies that there is a trade-off between fecundity, or potential reproductive capacity (measured by age at maturity, gestation length, litter size, age at weaning, and interbirth interval; Harvey, 1990), and survival to maturity (Purvis et al, 2003); delaying reproduction to increase body size may increase fecundity, but also increases the chances of dying before reproducing.

The 'speed' of a species' life history is a central concept for models of mammalian life history (Charnov, 1993; Cole, 1954; Roff, 1992; Stearns, 1992). As originally formulated, the life history of a mammal lay along a slow-fast continuum (Cole, 1954; Medawar, 1952; Promislow and Harvey, 1990; Read and Harvey, 1989; Williams, 1957). At one end of the continuum are organisms that reproduce at an early age, produce large litters, and have short lives. On the other end are organisms that delay reproduction, produce a small number of offspring, and lead long lives. Among mammals, the general rule is that smaller species lead faster lives and larger species lead slower lives (Pagel and Harvey, 1993; Purvis et al., 2003). Larger species reach reproductive maturity at a later age, have a longer gestation, produce smaller litters, have a longer duration of lactation, wean infants at a larger body size, and have longer interbirth intervals (Millar, 1977; Purvis et al., 2003; Stearns, 1983). Extrinsic mortality schedules or "unavoidable mortality in the natural environment" (Kappeler et al., 2003) are cited as an explanation for variation among species of similar body size (Charnov, 1993; Promislow and Harvey, 1990; Roff, 1992; Stearns, 1992). However, not all life history variation is explained by body size.

The life history of primates is considered one of the slowest among mammals (Charnov and Berrigan, 1993; Harvey et al., 1987). After controlling for body size, primates have relatively late ages at maturity, long gestations, small litters, large neonates, long interbirth intervals, slow postnatal growth rates, and long life spans (Charnov, 1991; Kappeler et al., 2003; Ulijaszek, 2002). A continuum of life history paces exists within the primate order as well; prosimian primates "live faster and die

younger” than anthropoid primates (Purvis et al., 2003; Martin and MacLarnon, 1985). The slow-fast continuum within primates is classically represented in Adolph Schultz’s (1969) diagram of the length of life stages (e.g., gestation, infancy, adult) in primates, including humans. Leigh and Blomquist (2007), among others (Bogin, 1997, 2001; Gould, 1977; Leigh, 2001), critique this model as orthogenetic as it implies that there was a direction or goal for the changes in patterns of growth and development over the course of primate evolution.

An alternative view to modeling primate life histories on the slow-fast continuum is Leigh’s (Leigh and Blomquist, 2007; Pereira and Leigh, 2003) modes of ontogeny, or what Morbeck (1997b: 124) refers to as “a mosaic of temporal trajectories in different body systems” (Grand, 1983; Tanner, 1978). Modes of growth and development are alternative forms and ways of operating and are the result of modularity. Leigh and Blomquist (2007, following Raff, 1996) define modularity as variation in the degree to which morphological structures or organ systems are interrelated during development. Different organ systems and/or morphological structures may be disassociated during growth and development because of differences in pattern (timing) and rate of growth (Leigh and Blomquist, 2007; Pereira and Leigh, 2003). For example, adult body mass is variable among primates as is the mode of development to attainment of adult body mass (Pereira and Leigh, 2003). Some primates reach adult body size through a consistent decrease in the rate of body mass growth (e.g., marmosets) while others (e.g., gorillas) have periods of rapid growth (Leigh and Blomquist, 2007). For the latter group, postnatal development is both slow and fast, depending on the time period when growth is

measured. Further, growth spurts – both the timing and rate – differ with respect to sex, leading to divergent modes of ontogeny between males and females of the same species (Pereira and Leigh, 2003).

Slow life-history traits are posited to be directly related to encephalization in the primate order in general, and great apes in particular (Lee, 1996). That is, brain size has been argued to be the “pacesetter” for life history as larger brains require more time for development (Harvey and Clutton-Brock, 1985; Harvey et al., 1987; Ross, 2003, 2004). Brain modularity argues against the role of the brain as the pacesetter. Leigh and Blomquist (2007) compared age at brain growth cessation, adult brain size, and age at first reproduction. They found that brain growth curves did not correlate with age at first reproduction--the time it takes to grow a brain and the length of the juvenile period were unrelated. Adult brain size and age at first reproduction were strongly correlated, even after adjusting for the effects of phylogeny, suggesting an indirect relationship between brain ontogeny, body size, and age at first reproduction (Leigh and Blomquist, 2007).

Theoretical predictions

There is a diversity of life history features among primates, including differences in body size, brain size, metabolic rate, mode of infant care, habitat use, and diet (Kappeler et al., 2003; Ross, 1998, 2003; Ross and MacLarnon, 1995). Although these features unfold based on species-defined patterns, flexibility is inherent because they center on individual life stories (Morbeck, 1997a). With so many ways of being an anthropoid primate, milk composition is predicted to vary with respect to other aspects of

a species life history. A broad life history framework provides the theoretical and empirical foundation to develop predictions on the nature of this variation. A whole organism-whole lives approach (Morbeck, 1997a) provides an additional dimension through which to address how individual females negotiate the process of lactation.

Life history theory is concerned with energy devoted to growth, maintenance, and reproduction. Lactation is one cost of reproduction. Like gestation, the cost of lactation is argued to be related to maternal body size and to the evolutionary history of the species (Martin and MacLarnon, 1985). Lee (1996) argues that the energy cost of lactation is inescapable; mothers must convert maternal nutrients or body reserves to milk. Although the conversion of maternal stores or dietary intake to lactation is relatively efficient, most mammalian mothers still need to increase their non-reproductive energy intake above normal levels to sustain infant growth (Lee, 1996). For example, baboon mothers are estimated to require 1.5 times their normal energy intake and human females require 1.3 times the energy as compared to when they are not pregnant (Lee, 1996, 1999).

Lee (1996) proposes that the differences among species in the costs of energy transfer (maternal energy to milk energy) are related to the maternal ability and capacity for energy storage prior to lactation, which in turn relates to dietary quality and other ecological factors such as seasonality and group size. If this is true, milk energy (determined primarily by fat) will vary with respect to maternal energy stores and Lee (1996) predicts that the energy content of milk will scale with maternal mass.

Body mass is a basic life history variable with tangible links to every morphological, physiological, and behavioral trait (Pereira and Leigh, 2003: 152).

Lee (1996) looks at lactation from the perspective of the mother, and what the mother is able to provide. Oftedal (1984) looks at lactation from the perspective of the infant, and what the infant needs for optimal growth and development. He proposes a positive correlation between the protein concentration of a species' milk and the rate of infant growth in that species. Anthropoids with more rapid infant growth rates should have milk with higher protein concentration than those that grow more slowly. Within anthropoids, growth rates are highest among New World monkeys, followed by Old World monkeys, apes and, finally, humans (Ulijaszek, 2002). Within each anthropoid superfamily, folivorous primates generally have more rapid rates of growth than frugivores or omnivores of similar body size (Leigh, 1994b). The juvenile period of frugivores and omnivores is characterized by increased risk of predation and feeding competition with adult conspecifics, favoring a slow growth rate (Janson and van Schaik, 1993). The juvenile risk hypothesis argues that the juvenile period in folivorous primates is associated with reduced intraspecific feeding competition which would relax constraints on delayed maturation and instead favor more rapid growth and truncated growth periods (Leigh, 1994b). It assumes that rates of growth are higher in folivorous primates than non-folivorous but closely related species. Leigh (2004) predicts high protein levels in the milk of folivorous primates. Power et al. (2002) propose that it is the energy provided by protein in milk, rather than the absolute concentration of protein, that varies with respect to growth rates and should therefore be highest among New World monkeys, followed by Old World monkeys and apes.

Modularity in patterns and rates of growth and development presents a problem for this predicted pattern. The juvenile risk model is not able to explain growth spurts in otherwise 'slow-growing' species nor does it explain variability in growth rates within species (Leigh and Blomquist, 2007). Fluctuations in growth rates lead to both 'fast' and 'slow' phases in the ontogeny of a given species, but milk composition is argued to be relatively consistent during the period when the infant is nutritionally dependent on the mother (midlactation, Oftedal and Iverson, 1995). Further, sex differences in modes of ontogeny suggest that male and female offspring may differ with respect to energy requirements at certain points in their development. While it does not make direct predictions for milk composition, Leigh and Blomquist's (2007) modes of ontogeny model suggests a cautious interpretation of the relationship, or lack thereof, between milk composition (protein or percent energy from protein) and growth rates. It also suggests that a more appropriate test of this relationship would integrate data on milk composition with data on growth rates at various stages of postnatal development.

Environmental heterogeneity is likely the rule, rather than the exception, during the evolutionary history of most species (Lee and Kappeler, 2003). Pereira and Leigh (2003) argue that plasticity must have been a target of selection within the primate order because of the length of developmental phases in primates relative to the chances for change in living conditions. Within primates, hominoid species have the slowest life history pace, or the longest developmental phases, and thus the greatest chances of encountering variation in the environment. If plasticity is indeed favored with the lengthening of developmental phases, then more flexibility in life history traits are

predicted among hominoid species. As it relates to milk composition, lactation is absolutely longer in hominoid species. Changes in living conditions would be more likely during the lactation period of hominoids relative to monkeys, and may have selected for more flexibility of the lactation strategy. It will therefore be important to consider both mean values for a species or higher taxonomic level and individual values within that taxonomic level for particular milk components.

A phenotype is considered plastic if it demonstrates environmental responsiveness (West-Eberhard, 2003). Ranges of possible phenotypic expression, or possible responses to the environment, are referred to as reaction norms (Pereira and Leigh, 2003; Stearns, 1992).

The reaction norm perspective maintains that ontogenies themselves are principal targets of selection, which results in functional changes in traits' developmental sequences, patterns of allometry, and degrees of plasticity (Pereira and Leigh, 2003: 152).

This dissertation will incorporate a reaction norm perspective and consider compositional differences and differences in the variation in composition among species and higher taxa as a possible link between life history, diet, and socioecology. The reaction norm perspective is especially influential for research questions considering the effect of captivity on milk composition. Lee and Kappeler (2003) argue that the behavior and biology of captive primates is at the maximum of their reaction norm. If a norm of reaction exists for milk composition, with different milks as a result of different environments, then captive living individuals are predicted to produce milk that represents the maximum of this range of possible expression.

Overlap between captive and wild living individuals is to be expected however. In comparing body size of wild and captive living olive baboons, Strum (1991: 229) argues that “a dichotomy between captive and noncaptive is overly simplistic [because] conditions for growth in wild can overlap those in the captivity.” Indeed, Leigh (1994a) reports that differences between captive and wild living primates in body weight are in degree, rather than categorical. If maternal body size and milk composition are correlated, differences in degree, rather than kind, are expected between captive- and wild living primates in milk composition. If milk quality is not related to body size, differences may still persist as a result of differences in energy intake and expenditure in captivity, which may influence maternal energy balance.

Summary

Evolutionary theory is the foundation for research questions in this study, and milk composition is modeled as a product of natural selection. The maternal energy hypothesis, parent-offspring conflict theory, and life history theory all share an evolutionary perspective and provide a framework for the development of more specific questions about variation in milk composition among anthropoids. Specific predictions that will be addressed in this dissertation include:

- (1) Milk energy will vary with respect to maternal energy stores. Milk energy will scale with maternal mass (Lee, 1996).
- (2) Milk protein (or percent energy from protein) will positively correlate with species growth rates, and follow the order (from highest milk protein to lowest)

New World monkeys, Old World monkeys, and apes (Oftedal, 1984; Power et al., 2002). Growth spurts and intraspecific variation in growth rates may confound this relationship (Leigh and Blomquist, 2007).

- (3) Protein concentration will be highest in milks from folivorous primates (Leigh, 2004).
- (4) More chances for changes in living conditions favors phenotypic plasticity (Pereira and Leigh, 2003). A longer duration of lactation may select for increased variability (plasticity) in milk composition.
- (5) Captive living primates are at the maximum of many life history norms of reaction (Lee and Kappeler, 2003). If milk composition is plastic and has ranges of possible phenotypic expression, captive living primates may produce milk at the maximum end of the range.
- (6) Milk composition is a compromise between mother and infant (Lee, 1996; Trivers, 1974). Milk composition will not reflect only the needs of the infant, but the physiological ability of the mother to meet those needs. Milk composition will vary with respect to maternal energy balance.

Human neonates have a unique pattern of ontogeny and selection would have favored a milk composition that could facilitate and support rapid postnatal brain growth (Martin, 1981, 1983). Fatty acids that are important for brain growth and development (DHA and AA) will be found in higher concentrations in the milk of humans as compared to nonhuman primates. These fatty acids should also be higher in *Cebus* and *Saimiri* milks, as they are the most highly encephalized primate following humans.

CHAPTER 3: LACTATION AND MILK COMPOSITION

Introduction

Milk production represents the singly most influential and unique feature of mammalian reproduction (Gittelman and Thompson, 1988: 868).

Lactation is a defining characteristic of mammals; all extant mammals possess mammary glands and provision their young with milk. The diversity of mammalian taxa is reflected in the diversity of milk composition, volume of milk produced, nursing behavior and length of lactation among mammalian species (Oftedal and Iverson, 1995). For example, hooded seals (*Cystophora cristata*) lactate almost continuously for four days, producing milks high in fat and low in sugar while humans lactate for up to four years, producing milks low in fat and high in sugar (Oftedal, 2000; Oftedal et al., 1988). These differences reflect variation in ecology, patterns and rate of growth and development of offspring, and the evolutionary history of each species.

Lactation represents an energetically expensive phase of reproduction for all mammalian mothers (Gittelman and Thompson, 1988; Oftedal and Iverson, 1995). It requires mothers to mobilize and transfer large quantities of nutrients in milk, placing nutrient demands on the mother (Oftedal, 2000). Mothers meet these demands by increasing the energy in their diet (Altmann, 1983; Oftedal, 2000; Sauther, 1994), reducing energy output (Roberts et al., 1985), or by storing nutrients during periods of abundance (Oftedal, 2000; Pond, 1984). Here, again, lies a source of variation in mammalian reproduction. The demands of lactation are met in a variety of ways depending on factors such as the condition of the environment, the species' ontogenetic

priorities, and genetically programmed physiological traits, such as fat storage and metabolism (Oftedal, 1984, 2000).

In this chapter, I present an overview of mammalian lactation, with a focus on milk composition. I begin by situating lactation and milk composition in an evolutionary and life history framework. The discussion then focuses on milk as a complex biological fluid, with a presentation of data on individual milk components, including nutritive and non-nutritive factors. Finally, I present a comparative context for the data collected in this dissertation by discussing the composition of human milk, nonhuman primate milk, and that from well-studied mammalian taxa.

An Evolutionary Perspective on Lactation

Origins of lactation

Is it conceivable that the young of any animal was ever saved from destruction by accidentally suckling a drop of scarcely nutritious fluid from an accidentally hypertrophied cutaneous gland of its mother? And if one was so, what chance was there for the perpetuation of such a variation? (Mivart, 1871, quoted from Blackburn et al., 1989: 3).

Studies of living mammalian orders can inform on the origins of lactation. Among extant mammals, there are many similarities in both mammary gland structure (Blackburn et al., 1989) and in milk composition (Oftedal and Iverson, 1995). These shared traits suggest that the complex form of lactation that is currently present was established before extant lineages of mammals diverged (Blackburn et al., 1989). Pond (1977, 1984) estimated lactation was present in mammals of the Late Triassic

(approximately 210 mya), and Goldman et al. (1998) believe it developed in reptilian insectivores 190 mya. Monotremes lactate but are oviparous, providing strong evidence that the evolution of lactation must have preceded the practice of vivipary in mammals (Blackburn et al., 1989; Pond, 1977, 1984). Additionally, provisioning of young with milk must also have preceded the practice of giving birth to highly altricial offspring, as is found in species of monotremes, marsupials, and some eutherian mammals (Blackburn et al., 1989).

It has been argued (Pond 1977, 1984) that lactation is responsible for the evolution of several important mammalian characteristics, such as diphyodonty and ability to live in impoverished and/or quickly changing environments. The change in parental feeding strategy meant that mothers were no longer required to forage for food for their offspring. This was important for several evolutionary adaptations. Although production and secretion of milk by the mother for the young and foraging for food to bring to the young are similar in energy expenditure (Pond 1977), suckling of milk is less energetically expensive than foraging. For the offspring this provides an obvious advantage in that more calories can be invested in growth rather than food procurement, or what Pond (1977) refers to as maintenance. Pond (1984) also contends that lactation as a parental feeding strategy permits mammals to reproduce successfully in many types of environments. This is advantageous for the mother because offspring could survive in any environment that was able to support the mother. There would also be an advantage in impoverished environments because mammalian mothers can store fat and necessary minerals that can be transferred to offspring at a later time in milk (Ofstedal, 1984; Pond

1977, 1984). Pond (1977) believes the ability to reproduce successfully in many types of environments allowed mammals to undergo adaptive radiation in the Cenozoic, when many other classes of animals, such as reptiles, were in decline.

Blackburn et al. (1989) and Oftedal (2002) propose that both physiological and phylogenetic evidence indicate that the mammary gland is most closely related to epitrichial glands, a type of sweat gland located in the dermis and associated with a hair follicle. As a result, the mammary gland most likely resulted from an evolutionary precursor of extant epitrichial glands, which include sebaceous and apocrine glands (Blackburn et al., 1989). The evolutionary link between sebaceous and apocrine glands possibly provides a basis for understanding the nature of the composition and selective advantage of the earliest lactational secretions. Homologies between secretions of these sweat glands and the mammary gland include both functional and structural (secondary and tertiary) similarity between lysozyme and milk protein α -lactalbumin (Blackburn et al., 1989), which have identical amino acids in 49 positions (Jenness, 1979). Lysozyme is found in the small intestine, most bodily secretions (including mammalian milk), and in egg whites of most birds. The protein α -lactalbumin is an important part of the lactose-synthetase system and allows the mammary gland to synthesize lactose and its derivatives (Blackburn et al., 1989). Blackburn et al. (1989) suggest that lysozyme, an integument-derived secretion, may be ancestral to the mammary gland secretion α -lactalbumin.

As the function of lysozyme is antimicrobial, it follows that the original function of proto-lacteal secretions may have been antimicrobial rather than nutritional. Further support for this hypothesis is found in evolutionary conserved traits in milks of extant

mammals. All mammalian milks that have been studied (Ofstedal and Iverson, 1995) contain antimicrobial factors, of which the highest concentration is found in colostrum, secretions produced by the mammary glands during the first days after parturition (Emmett and Rogers, 1997; Goldman et al., 1998). The universal presence of antimicrobial factors, such as lysozyme and secretory immunoglobulin A (sIgA), suggest that they are plesiomorphies in mammalian milk.

This evolutionary scenario identifies the original function of lactational secretions as protection of the infant from infection. This was followed by the gradual production of nutritious factors in the secretions, and further selection based on differences in environmental pressures, ecological niches, and reproductive life histories among mammals to produce the variation in composition found among extant mammals.

Lactation as a strategy

Lactation is the most energetically expensive component of a mammalian female's reproductive strategy (Gittelman and Thompson, 1988; Pond, 1984, 1997). The lactation strategy can be divided into four, interrelated traits: the composition of the milk produced, the volume of milk produced, the frequency at which the mother feeds the infant throughout the day, and the total duration of lactation per reproductive cycle. All these elements must work together to deliver the necessary nutrients to the infant for its growth and development without irreversibly compromising maternal health. The duration of lactation is considered a life history trait of the species. I argue that milk composition, volume of milk produced, and nursing frequency can also be modeled as

life history traits. As such, each of the four components of the lactation strategy is subject to natural selection, as each interacts with the environment and other life history traits to maximize the fitness of the individual.

Life history traits are products of natural selection and therefore a species' life history strategy is intimately tied to their evolutionary history. Natural selection elaborates, rather than innovates (Chamberlain, 1996), leading to similarities in life history traits among closely related species. The wide range in the age at weaning among primates (e.g., Ross, 2003) suggests that duration or length of lactation has been under selection over the course of primate evolution, and similarities within genera, families, and even superfamilies indicate a phylogenetic ordering to such variation. A species' lactation strategy therefore may carry phylogenetic baggage, both in regard to the genetic makeup of inherited trait and the range of variation (phenotypic plasticity) in this expression.

Like other life history traits, the lactation strategy also may be an adaptive response to ecological variation (Hill and Kaplan, 1999; Kaplan et al. 2000; Morbeck 1997a, b). Morbeck (1997b) argues that external factors such as climate, the quality and quantity of available food, disease ecology, and group structure can affect the timing, duration, and energetic effort of an individual's life history. As an example, primate mothers may choose to lengthen lactation if ecological conditions are poor, or shorten lactation when conditions are good (Lee, 1996). The extent to which they are able to do this is part of their evolutionary history, however, and the degree of plasticity is itself a product of natural selection (Morbeck, 1997a),

The plasticity of life history traits is also affected by the expression of other life history traits. Among seasonally breeding Old World monkeys, such as rhesus macaques, the length of lactation is related to the ability to conceive; mothers that fail to conceive during the breeding season will continue to lactate into the second year of an infant's life (Gomendio, 1989). Selection on other aspects of female's reproductive strategy, such as age at first reproduction or adult body mass, may require modifications to one or more components of the lactation strategy.

The reproductive rate of a mammal can be accelerated by reducing the age at maturity, shortening the gestation length or interbirth interval, or by increasing litter size (Purvis et al., 2003). Among primates, reproductive rates were increased employing each of these tactics, demonstrating that natural selection did not act on the reproductive strategy of all primate species in the same manner. The same scenario is likely for the lactation strategy. Further, it is unlikely that there is such a thing as *the* lactation strategy of a species. Because environmental heterogeneity is the rule, rather than the exception, one phenotype would not confer a high fitness in all environments (Lee and Kappeler, 2003), resulting in a highly plastic lactation strategy, especially for species with large geographic ranges, highly seasonal environments, or a long duration of lactation.

Milk as a compromise

Life history theory asserts that life history traits have evolved as a suite, and that the target of selection is fitness over an individual's lifetime rather than fitness at one point in time or the maximization of one particular life history trait (Alberts and Altmann,

2003). Using this theoretical framework, a species' lactation strategy – the composition and quantity of milk produced, the frequency by which the mother feeds, and the age at which the infant is nutritionally independent – should maximize mother's fitness, or reproductive success, over her lifetime. However, lactation also affects the fitness of the offspring. The offspring seeks to maximize its own lifetime fitness which may or may not be at odds with that of the mother (Trivers, 1974). Therefore, when making predictions about milk composition or other aspects of a species' lactation strategy the interests of both the mother and her offspring must be addressed.

Parent-offspring conflict theory (Trivers, 1974) predicts that mothers and infants should have *behavioral* conflicts over the allocation of parental investment, such as length of lactation. Much empirical work has been done on the issue of weaning conflict, that is, the conflict between mother and infant on the scheduling of when the infant must become nutritionally independent of the mother (see Maestripieri, 2002, for review of literature). Both conflict and cooperation have been demonstrated in primate species with regard to cessation of lactation, suggesting that the needs of both mother and the infant are important for selection.

Because milk composition represents a large investment by the mother, an extension of Trivers' theory would predict that milk composition would reflect the *physiological* conflict, or compromise, between what the infant wants and what the mother is able to give. Infants may grow faster if mothers produce milk with higher fat concentrations, but mothers may be limited by their physiological energy stores. The amount of fat in the milk of extant mammals is thus a compromise between infant energy

needs for growth and development and maternal abilities to access fat in the diet, or store fat on her body and mobilize those fat stores during lactation (Oftedal, 2000).

Milk composition, like other traits of a species, is not an innovation, but rather an elaboration on ancestral milks, or, before that, ancestral secretions. All extant mammals share a common lactating ancestor, and thus, all extant milks were developed from a common milk foundation. Regardless of whether selection favored the mother or the infant, natural selection can only work on existing variation. Milk is a biological fluid with physiological limits (Oftedal, 1984). Changing one aspect of milk composition can have profound effects on other aspects, arguing against saltational changes in milk composition without concomitant changes in other aspects of a species life history.

What is milk?

At parturition, the breast replaces the placenta as the neonate's primary source of nutrition and passive immunity (McDade and Worthman, 1999: 713).

Mammalian milks are composed of organic and inorganic materials that serve nutritional functions, immune functions, or both. The major nutritional components of mammalian milk are fat, protein, sugar, ash, and water (Oftedal, 1984). In the first section, I discuss the origins and significance of each of these constituents in milk as well as their relationship with one another. In the following section, I will discuss immune or non-nutritive factors in milk. Finally, I discuss how nutritional and non-nutritional constituents in milk vary in concentration over the course of lactation

Nutritional components in milk

Milk fat (also referred to as the milk lipid globule membrane) comes from specialized regions of the apical plasma membrane of the mammary epithelial cells, from endoplasmic reticulum, and may also come from other intracellular components (Keenan and Patton, 1995). Fat in milk is made up of primarily triglycerols (approximately 98%), which are three fatty acids, usually of 16 – 20 carbons, on glycerol backbones (Jensen et al., 1995). The remaining milk fats are in the form of free fatty acids, phospholipids and cholesterol (Jensen et al., 1995). Phospholipids are similar to triglycerols in structure, but can have a different chemical backbone (sphingomyelin rather than glycerol), and instead of a third fatty acid, have a phosphate group attached to an alcohol in the third position (Jensen et al., 1995). Fat contains twice the energy of protein or sugar (Ofstedal, 1984, 2000) and is therefore an important energy source in milk. Additionally, milk fats are used by the developing infant specifically for brain growth and many other functions within the developing central nervous system (see Chapter 4).

Human milk proteins are synthesized inside of the cell by the Golgi apparatus, but also come from cell products and from maternal serum (Lönnerdal and Atkinson, 1995). Most mammalian species have caseins as the major category of proteins (the other being whey proteins) (Lönnerdal and Atkinson, 1995; Ofstedal, 1984). Casein proteins are found in the pellet after milk has been centrifuged and are derived from the Golgi. Serum proteins are more soluble and are, generally, the whey fraction of the milk (Lönnerdal and Atkinson, 1995; Ofstedal, 1984). Casein proteins are easier for the infant to digest and provide an energy substrate and amino acids (Lönnerdal and Atkinson, 1995). Whey

proteins include antibodies, enzymes, anti-microbial factors, and α -lactalbumin, the latter of which is of high nutritional quality and, in humans, supplies many of the amino acids required by infants (Lönnerdal and Atkinson, 1995; Kunz and Lönnerdal, 1993).

Colostrum, milk produced during the first several days of lactation, is highest in protein concentration due to the high secretion of immune factors that are believed to be under the regulation of pregnancy hormones, including prolactin and estrogen (Goldman et al., 1998). Protein concentration decreases over the first several weeks of lactation as a result of the decrease in immune factors, and then remains relatively stable thereafter (Kunz and Lönnerdal, 1993; Lönnerdal and Atkinson, 1995; Lönnerdal et al., 1984).

Lactose is the predominant milk carbohydrate of most mammalian milks, including human and nonhuman primates (Ofstedal and Iverson, 1995). Lactose, composed of one galactose and one glucose molecule, is the most consistent macronutrient in human milk (Newberg and Neubauer, 1995). Lactose is believed to have multiple functions within milk. First, as a disaccharide, the osmolarity of lactose is less than that of two monosaccharides and would be less likely to cause osmotic stress in infants after consumption of milk (Newberg and Neubauer, 1995). Second, the individual molecules – glucose and galactose - that make up lactose are argued to have important roles in energy supply to the growing infant body and brain (Newberg and Neubauer, 1995). Other carbohydrates in milk include oligosaccharides, nucleotide sugars, glycolipids, glycoproteins, and mucins. These are not as well understood as lactose as a result of their complexity and the difficult nature of isolation (Newberg and Neubauer, 1995). The amounts of fat, sugar, and water in a species' milk are interdependent, and

their contribution to milk composition must be considered together (Oftedal, 1984).

Milks higher in fat must be lower in water, and less dilute milks are lower in sugar.

Ash is another term for the mineral component of milk. In human milk, the primary minerals are calcium, phosphorus, sodium, magnesium, and potassium (Oftedal, 1984). Minerals in milk are used for skeletal growth and maintenance, muscle contractions, membrane fluidity, protein synthesis, and as electrolytes (Atkinson et al., 1995; Neville et al., 1995). The concentration of phosphorus and calcium are positively correlated, and both are proportional to the amount of protein (casein) in milk, which binds these minerals (Atkinson et al., 1995; Prentice and Prentice, 1995). The ash component also includes iron, which is available in only trace amounts to human infants (0.2 to 0.8 mg/L; Casey et al., 1995). Human infants have a compensatory mechanism for the low iron content of milk and are born with large iron stores in the liver and hemoglobin (Casey et al., 1995; Stini et al., 1980). The bioavailability of iron in human milk suggests that it may play an important role for the infant, but its low, narrowly constrained concentration suggests that other mechanisms, perhaps competition with pathogens, may have selected for a conservative pattern of iron concentration (Casey et al., 1995; Stini et al., 1980).

The concentration of fat, sugar (lactose), and water (dry matter) are interrelated; increasing the aqueous portion of milk (water and sugar) means that the lipid portion must decrease. If selection favors dilute milk in a mammalian population, perhaps due to aridity (Oftedal, 1984), the energy content of the milk will be reduced as a result of the decreasing lipid portion. Because protein binds many of the minerals in milk, milks with

lower protein concentration will have correspondingly lower concentrations of calcium and phosphorus (Prentice and Prentice, 1995).

Non-nutritional components in milk

Milk provides more than nutrition to the developing infant. Non-nutritive immune factors that are passed from mother to infant (passive immunity) are necessary both for immediate immunocompetence of the infant, and for enhancement and development of the infant's immune system (Goldman et al., 1982, 1998). Additionally, breast milk contains immune factors that stimulate the development of the neonate's intestine and play a critical role in the neonate's development of intestinal host immune defenses (Bines and Walker, 1991).

The defense factors in mammalian milk consist of antimicrobial, anti-inflammatory, and immunomodulating agents and leukocytes (Goldman et al., 1998; Table 3.1). Many of these factors are multi-functional, such as lactoferrin which is antimicrobial *and* anti-inflammatory. Thus, these categories should not be seen as mutually exclusive. In addition, several factors that have nutritive significance also have immune functions, such as fatty acids and oligosaccharides (Kunz and Rudloff, 1993; Newberg et al., 1999). Individual defense factors, like individual types of cells in the immune system, do not work alone but act synergistically and with immune agents that the infant produces to combat antigens (Goldman and Goldblum, 1995). Innate and adaptive immune cells are necessary for a competent immune system from an early age - interdependence among defense agents is a requirement for immunity.

The immune factors present in milk (summarized in Table 3.2) are well adapted to the environment in which they are needed. SIgA, lactoferrin, and lysozyme are resistant to digestive enzymes and can persist in the gut without degradation (Goldman and Goldblum, 1995; Lönnerdal, 1996). Most microorganisms enter the neonate through the mucosal tissues, and antibodies present in milk are thus able to react with and provide immunity against pathogens of mucosal surfaces, especially enteric bacteria (Hoshower, 1992). Indeed, the majority of sIgA antibodies are directed against enteric pathogens. What is especially important about these transformed maternal antibodies is that they are specific to antigens that would be recognized by IgM antibodies on B cells in the Peyer's patches of the mother (Goldman, 2001). In this respect, sIgA antibodies ingested and utilized by the infant through the breast milk will be directed against pathogens encountered by the mother (they are her memory B cells) and therefore, pathogens the infant is likely to encounter in the environment. Passive immunity conferred through breast milk is an example of inheritance of an acquired characteristic, maintained by a genetic component subject to natural (Darwinian) selection.

Table 3.1. Categories of defense agents in milk

TYPE OF DEFENSE FACTOR	SPECIFIC FACTORS
Antimicrobial	SIgA, lactoferrin, lysozyme, glycoconjugates, oligosaccharides, digestive products of milk lipids
Anti-inflammatory	Antioxidants, epithelial growth factors, cellular protective agents
Immunomodulating	Nucleotides, cytokines, anti-idiotypic antibodies
Leukocytes	Neutrophils, macrophages, and lymphocytes (most are T cells)

From Goldman et al. (1998)

Table 3.2. Specific defense agents in milk and their function

AGENT	FUNCTION
Oligosaccharides	Inhibits binding of bacterial pathogens and toxins to epithelium
Lactoferrin	Decreases multiplication of bacteria and fungi by binding iron/ Attacks certain retroviruses
Lysozyme	Disrupts peptidoglycans on the cell walls of bacteria
SIgA	Antibodies inhibit adherence of pathogens to epithelium; neutralize toxins/ Resistant to proteolytic enzymes that are in the stomach so it can persist in the gut more so than other Ig types
alpha-lactalbumin	Possible role in inducing apoptosis in tumor cells
Interleukin 10	Anti-inflammatory. Promotes T helper II cells and B cell development/activity
Interleukin 6	Aids terminal differentiation of IgA-producing cells
Fatty acids (linoleic/lauric acid)	Anti-infective properties by disrupting enveloped viruses

From Goldman (1993), Goldman et al. (1998) and Slade and Schwartz (1987)

Stage of Lactation

The relationship between milk composition and stage of lactation has only been systematically investigated in one species of primate, *Homo sapiens*. Human milk composition changes over the course of lactation (summarized in Prentice, 1996) and is divided into four stages: colostrum (one to five days after delivery), transitional milk (five to 14 days after delivery), mature milk (greater than 14 days after delivery), and involutinal milk (milk at the end of lactation). The transition between each stage is a matter of degree, with the most rapid changes occurring the first week of lactation (Emmett and Rogers, 1997; Prentice, 1996; Stini et al., 1980) with the transition from colostrum to "milk" (colostrum and transitional milk are not considered "true milk") (Prentice, 1995). In humans, colostrum has the highest concentration of immune factors and therefore total protein (Goldman et al., 1982; Prentice, 1996). Protein concentration gradually declines, mostly as a result of the decrease of secretory immunoglobulin A (sIgA) and lactoferrin (Neville et al., 1995). After one or two weeks, human milk composition stabilizes, with less marked changes occurring over a much longer time frame (Prentice, 1995, 1996).

Identifying human milk as colostrum, transitional, mature or involutinal requires analysis of composition as it cannot be determined from the day of lactation. Females vary in milk composition due to both cultural (frequency of nursing, duration of lactation) and biological factors (Prentice, 1995; Prentice and Prentice, 1995). Day 90 of lactation for one mother may represent involutinal milk because of infrequent nursing and the desire to wean, while day 90 may represent mature milk for a mother who nurses

frequently and continues to do so for several years. Even when the composition of a sample is determined, assignment to a specific stage is subjective, particularly the transition from mature to involutinal types wherein changes are small and gradual (weeks, rather than days) (Prentice, 1995, 1996).

Human Milk Composition

Compared with other mammals, human milk is low in protein and has a low energy density, excepting lactose (Table 3.3; Jenness, 1979; Lönnerdal and Atkinson, 1995; Oftedal and Iverson, 1995; Prentice, 1996). Fat is the most variable component in human milk (Prentice, 1995, 1996). Fat concentration changes throughout the course of a day, and even within an individual feed (Bitman et al., 1986; Emmett and Rogers, 1997; Jenness, 1979; Prentice, 1996; Stini et al., 1980). The highest fat content occurs at the midmorning nursing and the lowest occurs in the later hours of the night (Jenness, 1979). This diurnal change in fat can be significant, varying from 3 to 5 % (Jenness, 1979). Over the course of a feed the concentration of fat increases from “foremilk” to “hindmilk” (Jenness, 1979; Prentice, 1996; Stini et al., 1980). Prentice (1996) reported that the fat content of human milk can change as much as five-fold during the course of a feeding. Highest milk yield, like highest fat concentration, appears to occur in the morning (Stini et al., 1980). An additional factor affecting diurnal variation is the frequency at which the mother eats (Prentice, 1996). Mothers who eat more often will have more diluted milk than those who eat with less frequency.

Human milk is regarded as having a very high concentration of immune factors, particularly during the colostrum phase (Goldman et al., 1998; Slade and Schwartz, 1987). Of the factors listed in Table 3.2, sIgA and lactoferrin are present in the highest concentrations in human colostrum. The concentrations of these factors, and therefore total protein concentration, decrease over the first three months of lactation, but appear to remain stable throughout the total duration of lactation (Goldman et al., 1982). Research on rhesus macaque milk (Milligan, 2005) suggests that human milk may be unique among nonhuman primates in its high concentration of sIgA (Figure 3.1). Human evolution was marked by a unique disease ecology: the first epidemiological transition. The cultural changes associated with agriculture, including increased population density and a more sedentary lifestyle, promoted an increase in infectious diseases, thereby creating a novel ecological setting for human populations (Barrett et al., 1998). Increases in number of pathogens and pathogen virulence would have placed strong selective pressure on the human immune system, particularly the immune system of infants and children and may have selected for increased immune factors in milk to increase neonatal and infant survival (Milligan, 2005).

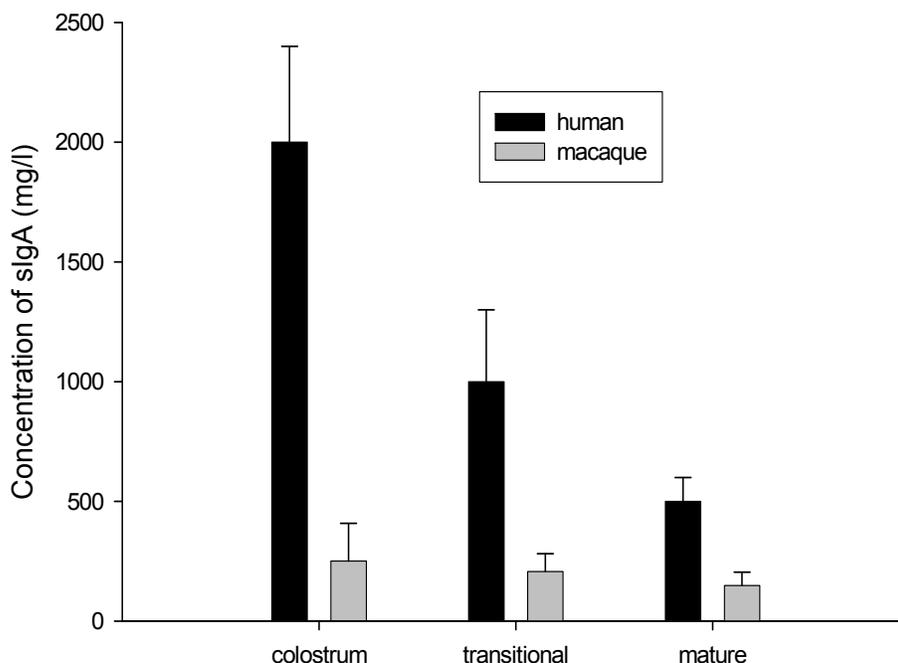
Human mothers have a relatively low stress of lactation and do not expend as much energy per day in milk production as many other mammals (Prentice et al., 1996; Vasey and Walker, 2002). However, human lactation is quantitatively longer in total duration per offspring relative to body size (Prentice and Prentice, 1995). Each of these characteristics has been modeled as an adaptation, as human infants have relatively slower rates of postnatal growth for body size and a longer period of dependency

(Ulijaszek, 2002). Comparative data from nonhuman primates, particularly closely-related hominoid species, will indicate if reduced stress of lactation or lengthening of the lactation period are in fact adaptations to human ontogeny, or relate to more remote patterns in our hominoid, catarrhine or anthropoid ancestry.

Table 3.3. The composition of human breast milk. All values given are for mature milk.

COMPONENT	COMPOSITION AND REFERENCES
Lactose	7.0 g/100ml (Prentice, 1996) 6.8 g/100ml (Stini et al., 1980)
Protein	1.1 g/100ml (Prentice, 1996; Stini et al., 1980) 0.8 – 1.0 g/100ml (Jenness, 1979)
Lipid/Fat	4.0 g/100ml (Gibson and Makrides, 1999) 4.2 g/100ml (Ofstedal, 1984; Prentice, 1996) 4.5 g/100ml (Stini et al., 1980)
Dry Matter	12.8 g/100 ml (Ofstedal, 1984; Stini et al., 1980)
Calcium	0.033 g/100 ml (Ofstedal, 1984)
Phosphorous	0.015 g/100 ml (Ofstedal, 1984)
Energy	60 – 75 kcal/100ml (Jenness, 1979) 75 kcal/100ml (Stini et al., 1980)

Figure 3.1. The concentration of sIgA in the milk of humans (Goldman et al., 1982) and rhesus macaques (Milligan, 2005) from three lactation stages.



Maternal diet, maternal energy balance, and milk composition

The composition of human breast milk is highly resistant to changes arising from deterioration of the nutritional status of the mother (Stini et al., 1980: 6).

Supplementation experiments are the method used to determine the effect of diet on milk composition (see Prentice, 1995, for review of literature). Providing malnourished women (determined by a body mass index, BMI, < 18.5) in The Gambia with biscuits containing protein, calcium, and/or carbohydrate resulted in little to no change in both milk composition and milk volume (Prentice, 1995). Nutritionally compromised women show similar concentrations of milk protein to well nourished woman (Prentice, 1995; Prentice and Prentice, 1995), suggesting that even low levels of

dietary protein intake allow for production of milk with the necessary concentration of protein for optimal human infant (somatic) growth. Indeed, Prentice and Prentice (1995) found the macronutrient content of human breast milk (protein, fat, lactose) to be surprisingly insensitive to maternal dietary differences.

Changes were observed when supplementation involved dietary fat, fatty acids, and vitamin B12 (Prentice and Prentice, 1995); supplementation of the maternal diet with these factors increases their composition in milk. Milk fatty acid profiles are most affected by maternal diet, as dietary fat is one source for milk fat. The other two sources of milk fat are *de novo* synthesis within the mammary gland and depot fat transferred through the maternal bloodstream (Prentice, 1996; Stini et al., 1980). The profile of long-chain polyunsaturated fatty acids (LCPUFA) in breast milk is responsive to fatty acids in the mother's diet and in stored depot fat. Vegetarian mothers, for example, produced milk that contained little to no fatty acids derived from animal fat and more fatty acids derived from dietary vegetable fat (Dettwyler and Fishman, 1992).

Alternative sources of fat in milk production allows for contingencies or compensatory actions when environmental conditions do not permit adequate dietary intake of fat. Most important for humans may be the use of depot fat stores during lactation. Human females gain weight during pregnancy by way of fat deposition (McFarland, 1997). Fat that is stored in the hip and the thigh is more metabolically active during lactation than fat in other depots in the body and is also highly resistant to weight loss (McFarland, 1997). When dietary fat is low, lactating women are able to use fat from their depot fat stores as a source for milk fat. Indeed, lactating women on low fat diets

produced more milk fat from subcutaneous depot fat rather than from dietary fat (Emmett and Rogers, 1997).

Fat content in milk is of particular importance because the human brain is approximately one-third lipid, all of which must be supplied to fetuses and young infants by the mother *in utero* and in milk, respectively. The importance of maternal transfer of stored fat to the infant during lactation therefore is an extension of the pattern observed during gestation. Placental transfer of fat includes the mobilization of up to 3 – 5 % of the maternal brain volume during the last trimester (Holdcroft et al., 1997), probably because of the high concentration of essential fatty acids in this tissue (Vasey and Walker, 2002). After birth, fat stored in the mother's hips and thighs can be mobilized as fuel for the metabolic requirements of the growing infant brain.

Investigations on the relationship between maternal body composition and milk composition indicate that there is not a strong relationship between energy balance and milk quality or quantity. Women who have large stores of depot fat (BMI > 26) were found to produce milk with higher fat concentration than women with low BMIs (<18). However, the higher BMI group produced less milk than the lower BMI group (Barbosa et al., 1997). As a result of this inverse relationship between milk fat concentration and milk volume, the total amount of fat secreted into milk had no significant association with maternal body fat (Barbosa et al., 1997; Butte et al., 1984). Even when milk volume is ignored, the difference in milk fat production between these two groups was small ($2.73 \pm 0.37\%$ vs. $2.89 \pm 0.35\%$) and well within the range of variation reported for populations of well-nourished women (Jensen et al., 1995). Consequently, these authors

(Barbosa et al., 1997) also reported that infant growth velocities were not significantly different between the low and high BMI groups. Because milk fat is the main energy source for human infants, supplying more than 50% of the calories (Jenness, 1979; Jensen et al., 1995), consistent production of essential milk fat from diverse sources may have been critical to fitness of the infant and the mother.

The ability to produce milk of a consistent quality over an extended period of lactation in environments with patchy resources would have offered a large advantage. It has been suggested that despite evidence that diet and nutritional status have only a small effect on milk composition and yield, there may be a threshold below which the quality and quantity of milk may be compromised (Emmett and Rogers, 1997; Lönnerdal, 1986). Lönnerdal (1986) argues that there must be a lower limit of dietary nutrient intake that would be inadequate for milk production. However, of the human populations investigated thus far (Prentice, 1995), breast milk composition and volume seem to be very “well-buffered” (Jensen et al., 1995) against ecological variability (Prentice et al., 1994). Butte et al. (1984) found no relationship between milk quantity or milk quality and maternal anthropometric indices such as maternal weight, height, and body fat. The lack of cross-cultural variation in human milk composition and the relatively small role played by the environment in altering this composition seem to support the view that milk composition in humans is heritable and highly conserved.

Milks of Nonhuman Primates and Mammals

Nonhuman Primate Milk Composition

This study is not the first inter-specific comparison of primate milks. It is the first, however, to investigate the nature of variation among nutritional components in anthropoid primate milks from an evolutionary perspective. Previous research on nonhuman primate milk proximate composition (fat, sugar, protein, minerals) has been limited to single-species descriptions (Ben Shaul, 1962; Buss, 1968a, 1968b; Buss and Cooper, 1972; Kanazawa et al., 1991; Lönnerdal et al., 1984; Nishikawa et al., 1976; Ota et al., 1991; Power et al., 2002; Taylor and Tomkinson, 1975; Tilden and Oftedal, 1997; Urashima et al., 1999) or has focused on comparing primate milks to those of other mammalian orders (Oftedal, 1984; Oftedal and Iverson, 1995). One notable exception is work by Davis et al. (1994) on amino acids in primate milks. They demonstrate phylogenetic similarities with regard to amino acid composition of nonhuman primate milks. The amino acid pattern of human milk is most similar to that of the great apes (represented by chimpanzees and gorillas), followed by Old World monkeys (represented by rhesus macaques and baboons). Davis et al. (1994) also report a pattern of low amino acid concentration in primates compared to non-primate mammals, indicating that there may be some factors in milks that tend to be shared among closely-related species.

Descriptive studies provide the foundation for the current state of knowledge of nonhuman primate milk composition. This foundation, however, is weak and uneven. Milk collection from nonhuman primates has been opportunistic by necessity and the

number of samples from each species is highly variable. Rhesus macaque (*Macaca mulatta*) milk is the most thoroughly investigated due to their ubiquity in captive lab populations. Little is known, however, about the milk of nonhuman apes, and what is known comes from only a few ape milk samples. Taylor and Tomkinson (1975) report on milk composition for one female gorilla (*Gorilla gorilla*) and Ben Shaul (1962) reports on one orangutan (*Pongo pygmaeus*) and one chimpanzee (*Pan troglodytes*). As a result of the small sample sizes, no ape species were included in Oftedal and Iverson's (1995) report on gross composition of mammalian milks. Oftedal and Iverson's (1995) report also excluded many other studies because of methodological issues. Results acquired through the use of assays identical to those applied to bovine milk, for example, were excluded because these assays are inappropriate without modifications that accommodate the nature of primate milk. In light of these problems, the dissertation study used the methods standardized by Oftedal (and summarized in Oftedal and Iverson, 1995) in the Nutrition Laboratory, Department of Conservation Biology, Smithsonian's National Zoological Park, Washington, D.C. (described in detail in Chapter 7).

Power et al. also used these methods in their analysis of captive (2002) and wild (in press) callitrichine primates, as did Oftedal and Iverson (1995) on wild red and mantled howler monkeys. Power et al. (2002) tested the hypothesis that body size and reproductive rate may have an effect on milk composition. Specifically, smaller-bodied monkeys, such as common marmosets and golden lion tamarins (= callitrichines), should produce milks with higher protein and energy content than larger-bodied monkeys such as howlers (Oftedal and Iverson, 1995), rhesus macaques (Lönnderdal et al., 1984), or

humans (data compared to Oftedal, 1984). Among nonprimate mammals, body size is correlated with energy in milk, where smaller-bodied mammals produce higher energy (kilocalories per gram of milk) milks due to increased metabolic demands and infant digestive abilities (Oftedal and Iverson, 1995). Despite large differences in body size, the total energy of the milk produced by the callitrichines was within the range of values reported for howlers, macaques, and humans. However, milk samples from the callitrichines were higher in the amount of energy provided by protein (see Chapter 7 for calculation).

It is significant that body size does not explain variation in milk energy among the primates. Variation may instead relate to the rate of somatic growth, with faster-growing primate species producing milks with more energy from protein (Oftedal, 1984; Power et al., 2002). Growth rates decrease across the major primate taxonomic groups, from prosimians to New World monkeys to Old World monkeys to apes and humans (Ulijaszek, 2002). Power et al. (2002) predict that percent energy from protein would follow the same ordering. This dissertation will test Power et al.'s (2002) hypothesis through a phylogenetic comparison of percent energy from protein.

Power et al. (in press) also provide testable hypotheses regarding the effect of captivity on milk composition of nonhuman primates. Milk samples from wild living common marmosets were similar in percent energy from protein to those from captive living common marmosets (Power et al., 2002), but were lower in fat, total protein, and gross energy. The consistency in percent energy from protein, regardless of diet and environment, was hypothesized to be an adaptation of marmosets that relates to

requirements for infant growth and development. Milks from both populations were variable in fat and total gross energy, which the authors argue is part of the species' lactation strategy. The smaller range of variation among the wild living marmosets was not explained by the authors. Similar body weights between the two populations seemed to argue against the possibility of use of maternal body stores. I would hypothesize, however, that, like many traditional human populations, wild living populations of marmosets would have a greater reliance on body stores because less fat is supplied directly in the diet. Further, marmosets females, weighing between 350 – 400 grams, do not have the storage capacity of humans. Digestive capacity and storage capacity scale to body mass (Calder, 1984; Kleiber, 1961). That is, there is an allometric relationship between these physiological properties and body size (Gittleman and Thompson, 1988). These allometric relationships further affect the relative abilities of different sized primates (mammals) to respond to periods of energy deprivation or abundance such that a larger primate is able to store proportionally more energy and to draw upon proportionally more reserves than a smaller primate (Gittleman and Thompson, 1988). Therefore, the difference between wild- and captive living populations of small bodied primates in milk fat concentration is predicted to be greater than that of larger bodied primates because of differences in ability to store energy.

Several studies have investigated the relationships among maternal condition and nursing frequency and duration of lactation among nonhuman primates (Fairbanks and McGuire, 1995; Gomendio, 1989; Johnson et al., 1998; Lee, 1987). The relationship between maternal condition and milk composition has been examined in baboons

(Roberts et al., 1985), common marmosets (Tardif et al., 2001), and rhesus macaques (Hinde, 2007). Restriction of food in captive baboon mothers affected the quantity, but not the quality, of milk produced (Roberts et al., 1985). The authors attributed the lack of effect on milk quality to the mobilization of maternal fat reserves. Tardif et al. (2001) report that small common marmoset mothers with twin offspring produced lower fat, and subsequently lower energy milks, than larger mothers with twins. Finally, Hinde (2007) reports rhesus macaque mothers with parasitic infection of *Balantidium coli* have significantly lower milk fat than those free of parasites. As is true in humans, milk fat is the most variable component of nonhuman primate milk composition and the most malleable with respect to ecological variation. Variation in body size and hence, metabolic rates, among nonhuman primate mothers may lead to variation in the relationship between maternal energy balance and milk composition. Larger body size may permit certain species, such as baboons, to cope with changes in energy balance through mobilization of body stores better than smaller species, such as common marmosets (cf. Gittleman and Thompson, 1988).

In summary, the generalization that nonhuman primate milk is dilute and low in energy is overly simplistic (Oftedal and Iverson, 1995). Nonhuman primate milks exhibit several axes of variability (Power et al., 2002) including the concentration of fat, total gross energy, the energy provided by fat, and the energy provided by protein. Some of this variability may be explained by captivity, but much of it may be related to a species' evolutionary history or overall life history pattern.

Table 3.4. Results (mean percent) from previous research on nonhuman anthropoid milk

Species	Reference	Fat	Protein	Lactose	Dry Matter	Ash
<i>Macaca mulatta</i>	Lönnerdal (1996); Lönnerdal et al., (1984); Kunz and Lönnerdal (1993, 1994)	4.6%	2.3%	7.9%	NR	NR
<i>Macaca fuscata</i>	Ota et al., 1991	4.2%	1.6%	6.2%	NR	NR
<i>Macaca fascicularis</i>	Nishikawa et al. (1976)	5.2%	1.6%	NR	12.2%	0.40%
<i>Papio sp.</i>	Buss (1968a, 1968b); Roberts et al. (1985)	4.5%	1.5%	7.8%	14.0%	0.30%
<i>Cercopithecus talapoin</i>	Buss and Cooper (1970)	3.0%	2.1%	7.2%	12.3%	0.30%
<i>Pongo pygmaeus</i> *	Ben Shaul (1962)	3.5%	1.43%	6.02%	NR	NR
<i>Pan troglodytes</i> *	Ben Shaul (1962)	3.7%	1.2%	7.0%	NR	NR
<i>Gorilla gorilla</i> *	Taylor and Tomkinson (1975)	2.5%	3.0%	3.6%	NR	NR
<i>Saimiri sciureus</i>	Buss and Cooper (1972)	5.1%	3.5%	6.3%	NR	0.30%
<i>Alouatta seniculus</i> ^a	Oftedal et al. (unpublished data)	1.1%	1.9%	6.6%	11.3%	NR
<i>Alouatta palliata</i> ^a	Oftedal and Glander (unpublished data)	1.6%	2.2%	7.8%	11.7%	NR
<i>Callithrix jacchus</i> ^a	Power et al. (unpublished data)	2.3%	2.2%	8.0%	12.7%	NR
<i>Callithrix jacchus</i>	Power et al. (2002)	3.6%	2.7%	7.4%	14.0%	NR
<i>Leontopithecus rosalia</i> *	Power et al. (2002)	5.2%	2.6%	7.2%	16.1%	NR

*Sample sizes were $n \leq 3$

^aMilk collected from wild individuals

NR = Not reported

Milks of nonprimate mammals

Oftedal and Iverson (1995) provide a comprehensive list of mammalian milk composition (fat, crude protein, lactose, dry matter and ash). From this list, milks from three species of equid, four species of carnivore, three species of pinnipid and two cetaceans were selected for comparison with human and nonhuman primate milk. These species were selected to demonstrate (1) similarities in milk composition among closely related species (species within the same genera and/or order) and (2) the diversity of milk composition among mammalian species.

Equid milks are surprisingly “primate-like” in composition – dilute (almost 90% water) with the majority of energy coming from lactose (Oftedal and Jenness, 1988) – despite disparate infant growth trajectories. However, it is not only the percent of each of these components but the actual composition of fat (types and concentrations of fatty acids) and protein (casein:whey) that are under selection. Regardless, the similarity in overall composition suggests that selection may produce similar milk composition (phenotypes) in different phylogenetic lines. Milk composition may be homoplastic, the result of convergent evolution, rather than common ancestry. Oftedal and Iverson (1995) speculate that the high water content of equid milks may be related to high water requirements of infants due to evaporative cooling in hot environments. Dilute milks of nonhuman primates have been argued to serve a similar function.

Among carnivores (brown and black bear, domestic dog, and African lion), milk of canids and felids is, in general, quite similar. Bear milk is quite different, being higher in fat (and subsequently dry matter) and having a smaller proportion of dry matter

represented by protein (Oftedal and Iverson, 1995). Oftedal (1993, 2000) attributes these differences to the reproductive strategy of the bear. Bears lactate during hibernation, and therefore must rely on body stores to supply energy and nutrients for milk production, secretion, and composition over a long period without eating. Bear milk is lower in sugar than that of other carnivores because, while fasting, females must minimize glucose use (Oftedal, 2000). Indeed, any female that lactates while fasting is predicted to have milk with a lower lactose concentration, and Oftedal (2000) argues that mammals that produce high sugar milks (e.g., nonhuman primates or equids) would only be able to fast for a brief period of time while maintaining lactation. High fat and dry matter and low protein in the milks of bears are hypothesized to be related to the need to conserve water and protein while fasting. Thus, *Ursus* species illustrate the strong influence that the reproductive strategy can have on milk composition.

Like brown and black bears, seals of the family Phocidae (Order Pinnipedia, includes hooded, grey, and northern elephant seals) lactate during a time of fasting. Their milk, as predicted, is low in sugar, higher in fat and dry matter, and lower in protein in order to conserve glucose, water, and protein, respectively. That their milk is significantly higher in fat than that of bears is attributed to the relatively shorter period of lactation (4 – 45 days) and the need to provide pups with fat stores for insulation and the postweaning fast (Oftedal, 2000). Hooded seal pups gain almost 300 grams of fat per kilogram of body weight over the four day lactation period (Oftedal, 2000).

Baleen whales (includes fin and humpback whales) are similar to phocid seals in their lack of feeding during the lactation period (Oftedal, 2000). Although lactation is

absolutely longer (approximately 6 months), this lactation period is considered “brief” relative to body size and therefore is like that of seals (Oftedal and Iverson, 1995). The majority of the energy transferred to calves in milk is in the form of lipids. Oftedal (2000) estimates that a blue whale (*Balaenoptera muscalus*) will transfer up to 16,000 kg of fat (or 33% of maternal body composition of fat) to the calf over the 6 months of lactation. Baleen whales are physiologically capable of this large mobilization of maternal fat stores, depositing up to 45,000 kg of blubber during pregnancy (Oftedal, 2000).

Table 3.5 Milk composition (means) of equids, carnivores, pinnipeds, and cetaceans. All data from Oftedal and Iverson (1995).

Species	Fat	Protein	Lactose	Dry Matter	Ash
<i>Equus burchelli</i> (Plains zebra)	2.2%	1.6%	7.0%	11.3%	0.4
<i>Equus asinus</i> (Ass)	1.8%	1.7%	5.9%	10.8%	0.4
<i>Equus przewalskii</i> (Przewalski horse)	1.5%	1.6%	6.7%	10.5%	0.3
<i>Ursus arctos</i> (Brown bear)	17.1%	9.2%	2.2%	31.9%	1.5%
<i>Ursus americanus</i> (Black bear)	25.1%	7.0%	3.0%	37.6%	NR
<i>Canis familiaris</i> (Domestic dog)	9.5%	7.5%	3.8%	22.7%	1.1%
<i>Panthera leo</i> (African lion)	8.7%	11.8%	3.2%	26.8%	NR
<i>Cystophora cristata</i> (Hooded seal)	61.1%	4.9%	1.0%	69.8%	NR
<i>Halichoerus grypus</i> (Grey seal)	59.8%	9.2%	NR	71.1%	NR
<i>Mirounga angustirostris</i> (Northern elephant seal)	51.9%	10.2%	< 0.025%	65.8%	NR
<i>Balaenoptera physalus</i> (Fin whale)	33.2%	10.5%	2.3%	46.5%	1.1%
<i>Megaptera novaengliae</i> (Humpback whale)	33.0%	12.5%	NR	48.4%	1.6%

Summary

Milk is a complex biological fluid of organic and inorganic components secreted by the mammary gland. Lactation is a defining feature of all mammals, and is believed to predate the evolution of live birth and diphyodont dentition. The original function of milk, or lactational secretions, is hypothesized to have been for transfer of immune factors from mother to offspring. Extant functions of milk constituents include nutrition, energy, and immunity, with many components demonstrating multiple functions. Milk composition is just one component of a species' lactation strategy. To understand the energetics of lactation, the volume of milk produced, nursing frequency and length of lactation must also be considered. Milk is the product of natural selection, and is probable that milk composition has been fine-tuned over the course of mammalian evolution. However, it is unlikely that milk represents the perfect or ideal food for each species, as natural selection can only work with existing variation. Human milk, relative to other mammals, is described as being dilute, low in fat and protein, and high in sugar. Although this generalization has been applied to all nonhuman primates, it is likely to be overly simplistic, with possible variation within the Order Primates. Human milk composition varies little with respect to maternal diet or maternal energy balance. Exceptions include fatty acid composition, which is strongly influenced by the fatty acid profile of the maternal diet, and total fat content, which is the most variable component in human milk. Milk composition of equids, canids, pinnipeds and cetaceans demonstrate the range of

variability among mammalian milks and the similarities in partitioning of major milk constituents among closely related species.

CHAPTER 4: FATTY ACIDS

Introduction

The goals of this chapter are (1) to provide the necessary background for research questions regarding fatty acid profiles of anthropoid primates, and (2) to provide comparative data from the literature on human milk fatty acids. The chapter begins with an overview of lipid biochemistry, including information on fatty acid nomenclature, structure, function, and source of milk fatty acids. Then, more detail is provided on long chain polyunsaturated fatty acids (LCPUFA) and their role in brain growth and development. As part of this discussion, I include data from studies comparing breast-fed to formula-fed infants. Finally, data on human milk fatty acids from the literature are presented, as these data are important for the comparison to milk fatty acid profiles of anthropoid primates in Chapter 8.

Fatty Acid Biochemistry

Fatty acid structure and nomenclature

Fatty acids are molecules composed of a hydrocarbon chain with a methyl group at one end and an acid group on the opposite end (Figure 4.1). This structure is the template for fatty acids from two to 80 carbons in length, although fatty acids over 26 carbons in length are rare. The most common fatty acids in human tissues are between eight and 22 carbons long (Cunnane, 2005) and the most common milk fatty acids are between 16 and 20 carbons long (Jensen et al., 1995).

Saturated fatty acids lack double (covalent) bonds between carbons (Figure 4.2). Short-hand biochemical nomenclature uses the total number of carbons followed by colon and a zero, the latter of which indicates the lack of double bonds (e.g., 6:0, 8:0, 10:0, or 22:0). The term “saturated” refers to the chemical structure of the carbons – they are bonded to two hydrogens. Unsaturated fatty acids are fatty acids with one or more double bonds between the carbons (Figure 4.2). The carbons with double bonds are bonded to only one hydrogen, hence the term “unsaturated.” If there is only one double bond, a fatty acid is referred to as monounsaturated and if there are more than one double bond, polyunsaturated (PUFA). The short hand nomenclature for unsaturated fatty acids is as follows: the number of carbons, followed by a colon, followed by the number of double-bonds, and, lastly, notation for where the first double bond is located (number of carbons from the methyl, or omega, “n” end). For example, 18:1n-9 (oleic acid) is a fatty acid with 18 carbons and one double bond at the ninth carbon from the omega end. 22:6n-3 has 22 carbons, six double bonds, with the first double bond at the third carbon from the omega end.

In addition to being grouped by the number of double bonds, fatty acids are also grouped by the number of carbons. Short chain fatty acids contain between two to six carbons, medium chain fatty acids between eight and 12 carbons, and long chain fatty acids are those with more than 12 carbons. LCPUFA are polyunsaturated fatty acids with 18 or more carbons. In addition to describing fatty acids using their biochemical short hand (e.g., 8:0 or 18:3n-3), fatty acids also will be referenced as medium or long chain, and the acronyms PUFA and LCPUFA are used exclusively in the remainder of this

dissertation. Table 4.1 provides a summary of adopted fatty acid nomenclature and shorthand.

Figure 4.1 Structure of a fatty acid

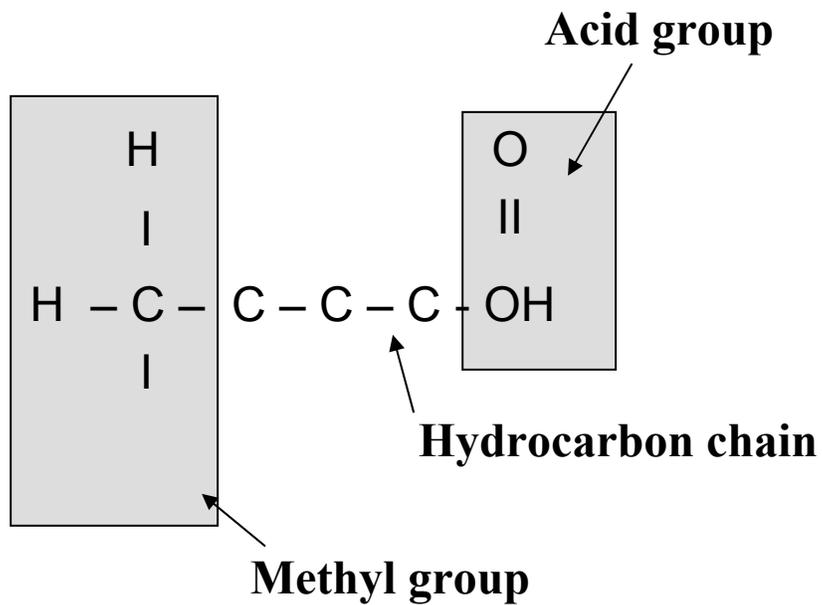


Figure 4.2. Diagram of a saturated and monounsaturated fatty acid (From biology.clc.uc.edu).

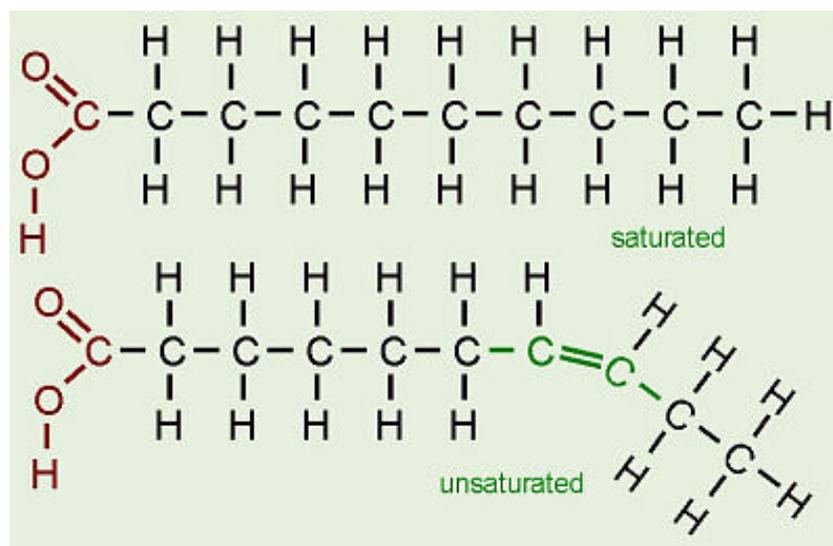


Table 4.1. Summary of fatty acid nomenclature adopted in this study.

Short chain fatty acids	4:0 – 6:0
Medium chain fatty acids	8:0 – 12:0
Long chain fatty acids	> 12 carbons
PUFA	Polyunsaturated fatty acid(s)
n-3 PUFA	18:3n-3 (alpha-linolenic acid, ALA)
n-6 PUFA	18:2n-6 (linoleic acid, LA)
LCPUFA	Long chain polyunsaturated fatty acid(s)
n-3 LCPUFA	20:5n-3 (eicosapentaenoic acid, EPA) 22:6n-3 (docohexaenoic acid, DHA)
n-6 LCPUFA	20:4n-6 (arachidonic acid, AA)

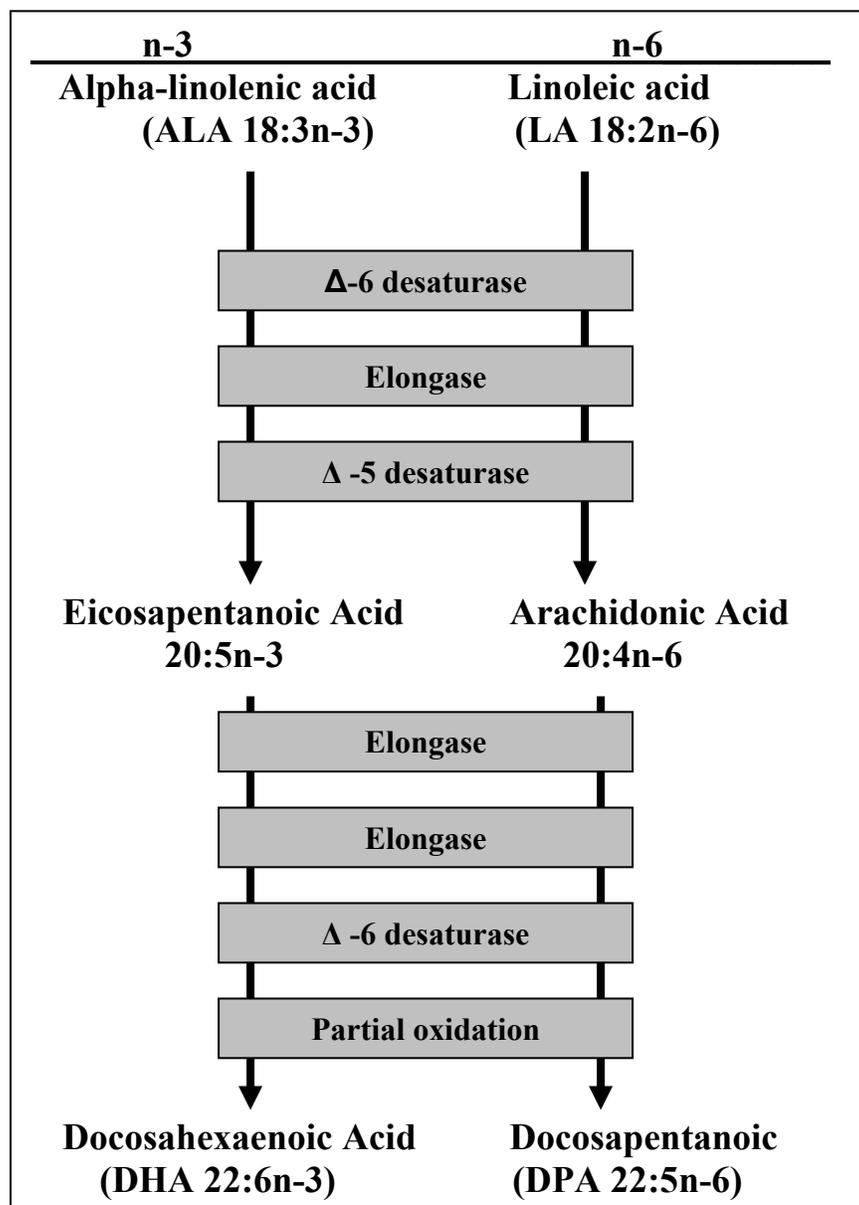
Omega-3 and omega-6 fatty acids

There are two classes of PUFA fatty acids, omega-3 (=n-3) and omega-6 (=n-6). These fatty acids can not be synthesized from shorter chain fatty acids or by the mammary gland, and are instead obtained through the diet (Huang and Brenna, 2001; Lands, 1992; Simopoulos, 1991). For this reason they are referred to as essential fatty acids. N-3 PUFA important to the human diet include 18:3n-3 (alpha-linolenic acid), 20:5n-3 (eicosapentaenoic acid), and 22:6n-3 (docosahexaenoic acid). N-6 PUFA important to the human diet include 18:2n-6 (linoleic acid) and 20:4n-6 (arachidonic acid). Of these, only 18:3n-3 and 18:2n-6 are actually essential, as 20:5n-3, 22:6n-3, and 20:4n-6 can be synthesized from their n-3 and n-6 PUFA precursors (Huang and Brenna, 2001). Indeed, the primary metabolic role of 18:2n-6 and 18:3n-3 are as essential precursors for 20:4n-6 and 22:6n-3, respectively (Huang and Brenna, 2001). These fatty acids are often referred to as the parent fatty acids. 20:4n-6 and 22:6n-3 are synthesized by a series of reactions in the liver's endoplasmic reticulum where two carbon units (elongation) and double bonds (desaturation) are added. Figure 4.3 describes the pathway from the n-3 and n-6 parent fatty acids to their longer chain derivatives, including the enzymes necessary for each step. The efficacy of desaturation and elongation of the precursors will be discussed later in this chapter.

The mammary gland does not synthesize n-3 or n-6 PUFA and a dietary supply of the fatty acids is required. 18:3n-3 comprises approximately 60% of the lipids found in plants, especially leaves, and is also present in flaxseed and soybean oils (Cunnane, 2005; Sanders, 1999). 18:2n-6 is found in sunflower and corn oils, and is the most

prevalent PUFA in Western diets (Brenna, 2002). N-3 LCPUFA variants (20:5n-3 and 22:6n-3) are found in fish and products containing fish lipids or oils. Not all fish contain the same amounts of 22:6n-3. Higher concentrations of 22:6n-3 (and 20:5n-3) are found in cold water marine fish compared to freshwater or tropical fish (Cunnane, 2005). 20:4n-6 is found in terrestrial and aquatic animal fats as well as eggs (Chulei et al., 1995; Yuhas et al., 2006).

Figure 4.3 Metabolic pathway from 18:2n-6 to 20:4n-6 and 18:3n-3 to 22:6n-3 (adapted from Carlson and Kingston, 2007).



Functions of fatty acids

Fatty acids are found in triglycerides and phospholipids, and are present as free fatty acids (Jensen et al., 1995). Triglycerides (Figure 4.4) are macro-molecules of three

fatty acids anchored to a glycerol backbone. They are storage fats that are tapped for energy, and the fatty acids attached to the glycerol backbone are usually composed of saturated and monounsaturated fatty acids (Cunnane, 2005). Phospholipids (Figure 4.5) are made up of two fatty acids (usually PUFA or LCPUFA) anchored to either a glycerol backbone or a sphingosine backbone. They also contain a phosphate group, which is attached to several possible nitrogen-containing “head groups” (Cunnane, 2005).

Phospholipids are structural fats found in cell membranes and are particularly rich in the membranes of the brain, heart and liver (Cunnane, 2005). The fatty acid composition of the phospholipids (e.g., PUFA or LCPUFA) influences the fluidity and permeability of the cell membrane, which in turn determines the function of the cell. Phospholipids are the most significant component, quantitatively, of membrane lipids (Connor et al., 2001). In addition to being a part of triglycerides and phospholipids, fatty acids are also found as unique molecules, circulating in the plasma. Called free fatty acids, they are oxidized by the body and used as energy substrates (Jump and Clark, 1999).

Fatty acids have multiple functions within the body. Fatty acids from body fat are the main alternative fuel to glucose (Cunnane, 2005). Mitochondria convert fatty acids to fuel in a process called beta-oxidation. Fatty acids are broken down, two carbons at a time, to make acetyl CoA which is then used to make ATP (Cunnane, 2005). Both 18:2n-6 and 18:3n-3 are extensively oxidized for fuel (Brenna, 2002; Cunnane, 2005; Huang and Brenna, 2001). Fatty acids between 16 and 24 carbons in length are important as structure in membranes (Cunnane, 2005). The lipid composition of membranes determines their shape, fluidity, elasticity, permeability and bilayer stability (Carlson and

Kingston, 2007). Palmitic acid (16:0) and oleic acid (18:1n-9) are important components of brain phospholipids (Cunnane, 2005) and the LCPUFA 22:6n-3 has a key role in the structural development of retinal, neural and synaptic membranes (Gibson, 1997; Gibson and Makrides, 1999, 2000).

The 20 carbon fatty acids 20:4n-6 and 20:5n-3 are precursors to metabolic products known collectively as eicosanoids. Eicosanoids play a role in the function of almost all organs, tissues, and cells in our body (Funk, 2001; Huang and Brenna, 2001), and are likened to hormones because of their involvement as “mediators” of inflammation and immune function (Fritsche, 2006). Other eicosanoid functions include central nervous function, regulation of blood pressure, platelet aggregation, vasodilation, and the regulation of other hormones (Gibson and Makrides, 2000; Mostofsky et al., 2001). Additionally, 20:4n-6 is believed to have neurotransmitter capabilities (Gibson, 1997). Competition between the two different classes of PUFA occurs in eicosanoid formation (Simopoulos, 1991). Increased consumption of the n-3 LCPUFA 20:5n-3 or 22:6n-3 affects the n-6 prostaglandin production pathway, decreasing the production of eicosanoids derived from 20:4n-6 (Simopoulos, 1991).

Fatty acids have also been shown to be involved in gene expression. Research on unicellular and multicellular organisms, including humans, suggests that fatty acids, including 18:1n-9, 18:2n-6, 18:3n-3, and 20:4n-6, can rapidly modulate the transcription of genes involved in their own metabolism (Jump and Clark, 1999; Kliewer et al., 1997). Additionally, some fatty acids or their longer chain metabolites may act like hormones and control the activity or amount of specific transcription factors (Jump and Clark,

1999). LCPUFA may play an important role in gene expression in the brain and central nervous system. Kothapalli et al. (2006, in press) demonstrated that supplementation of infant baboons with 22:6n-3 and 20:4n-6 at levels normally found in human breast milk influenced the expression (either up- or down-regulation) of over 1100 genes in the brain. They hypothesized that LCPUFA may play a particularly important role in regulation of fatty acid metabolism in the brain of the baboon, and presumably all primates (Kothapalli et al., in press).

Figure 4.4. Diagram of a triglyceride (From biology.lsu.edu)

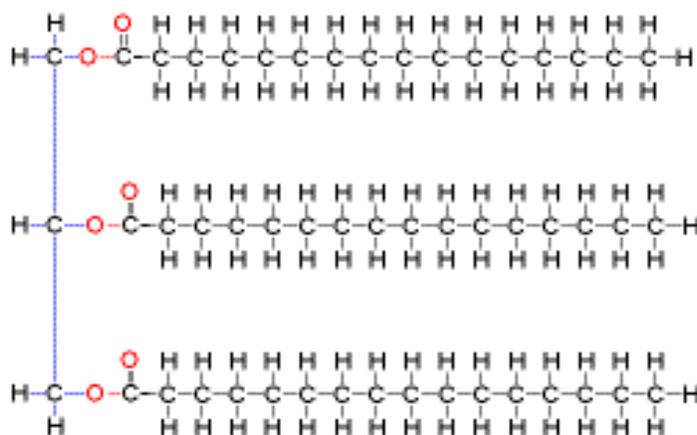
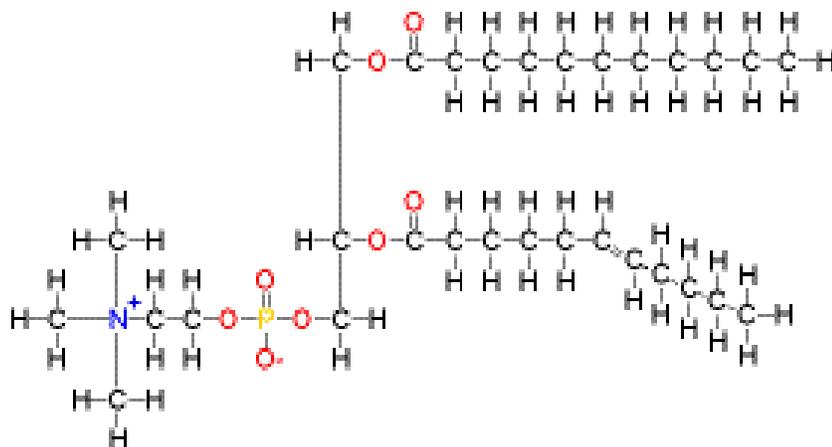


Figure 4.5. Diagram of a phospholipid (From biology.lsu.edu)



Fatty acids in milk

Fatty acids in milk are found in triglycerides and phospholipids, and are present as free fatty acids (Jensen et al., 1995). Milk fatty acids are taken up from maternal plasma or are synthesized *de novo* by the mammary gland (Del Prado et al., 1999; Iverson and Oftedal, 1995; Prentice, 1996; Sauerwald et al., 2001; Stini et al., 1980). Fatty acids are transported to the mammary gland from the diet as chylomicrons, from the liver as very low density lipoproteins (referred to in the literature as VLDL), and from the adipose tissue attached to albumin (Agostoni et al., 2001).

Short and medium chain fatty acids are synthesized by the mammary gland, or by other tissues in the body. The mammary gland can also elongate (addition of carbons) short and medium chain fatty acids (e.g., the gland synthesizes 8:0 and then elongates it to 16:0 prior to secretion). Fatty acids shorter than 16 carbons in length are generally uncommon in the diet, and their concentration in milk is directly associated with

manufacture by the mammary gland. As discussed previously, the mammary gland can not manufacture PUFA, such as 18:2n-6 and 18:3n-3. The presence of these fatty acids in milk reflects their consumption in the maternal diet, either past (maternal fat stores) or present.

Lipogenesis (synthesis of fatty acids) by the mammary gland is influenced by the proportion and types of fatty acids in the maternal diet and maternal depot fat (Iverson and Oftedal, 1995; Jensen et al., 1995; Koletzko et al., 1992). Rats fed high fat diets decrease mammary gland synthesis of short and medium chain fatty acids (Del Prado et al., 1999). Human diets low in fat and high in carbohydrates were associated with increased *de novo* synthesis of fatty acids with 12 – 14 carbons (Koletzko et al., 1992). Lipogenesis is also believed to be under genetic control and may be a species-specific trait (Dils et al., 1977; Iverson and Oftedal, 1995). Fatty acids in milk are thus expected to vary depending on the types and proportions of fats in the maternal diet and maternal body stores, and the evolutionary history of the species. Of the fatty acids that are not synthesized by the mammary, roughly 30% are estimated to be derived directly from the maternal diet and the remaining 70% from maternal body stores (Koletzko et al., 2001a).

LCPUFA are supplied directly from the maternal plasma (from the diet or depot fat stores) or as metabolites of precursor fatty acids in maternal plasma. Biosynthesis of LCPUFA from PUFA precursors takes place in the liver and depends on the quantity of 18:2n-6 and 18:3n-3 in the diet (Brenna, 2002; Carlson, 1999; Jensen et al., 1995), the ratio of n-3 to n-6 PUFA in the diet (Brenna, 2002; Huang and Brenna, 2001), and the ability to convert n-3 and n-6 PUFA into their longer chain metabolites (Agostoni et al.,

2001; Brenna, 2002; Carlson, 2001). Thus, if species differ with respect to conversion efficiency of n-3 or n-6 PUFA, milk LCPUFA proportions may differ, despite similar dietary intakes of n-3 and n-6 precursors.

Conversion efficacy in humans is relatively low (Brenna, 2002; Burdge, 2004; Huang and Brenna, 2001). Based on research with labeled [¹³C] 18:3n-3 and 22:6n-3, Huang and Brenna (2001) estimate the bioequivalence (defined as the relative efficacy of brain accretion between the 2 molecules) of 18:3n-3 and 22:6n-3 to be approximately 7:1. It appears that more 18:3n-3 is used for energy (beta-oxidation) than conversion to longer chain n-3 PUFA. Scheaff-Greiner et al. (1996) identified the primary metabolic function of 18:3n-3 to be carbon recycling—the use of carbons for synthesis of saturated and monounsaturated fatty acids. It appears that the most efficient method for increasing the proportion of 22:6n-3 in milk is by direct transfer of these fatty acids from circulating lipids from the diet or depot stores.

The Brain and LCPUFA

More has been written about the possible role of docosahexaenoic acid in human brain evolution than for any other brain selective nutrient (Cunnane, 2005: 157).

The LCPUFA 20:4n-6 and 22:6n-3 are the n-6 and n-3 fatty acids found in the highest concentrations in cell membranes (Carlson, 2001). Together, they make up a third of all lipids in the brain's grey matter (Gibson, 1997), with 22:6n-3 in particularly high concentrations in membranes surrounding neural synapses (Carlson, 2001) and retinal phospholipids (Scheaff-Greiner et al., 1997) and photoreceptors (Carnielli and Sauer,

1996). The brain appears to be selective in the incorporation of fatty acids, preferring those with 20 and 22 fatty acids rather than their 18 carbon precursors (Carlson, 1999, 2001; Carnielli and Sauer, 1996; Innis, 2003; Koletzko et al., 2001b). The brain and the retina have relatively lower proportions of 18:2n-6 and 18:3n-3 as fatty acids than other organs in the body and relatively higher proportions of 20:4n-6 and 22:6n-3, suggesting that specific mechanisms allow the brain and retina to accumulate such large amounts of these fatty acids (Innis, 2003). Further, this dichotomy indicates an important, possibly critical, role for LCPUFA in neural and visual processes (Carlson, 1999; Gibson and Makrides, 1999; Innis, 2003). Once the brain has incorporated 22:6n-3, it is highly resistant to change (Carlson, 2001). One exception may be during the last weeks of human pregnancy when mothers lose approximately 3% brain volume as lipids, much of which is 22:6n-3, to the developing fetus (Holdcroft et al., 1997).

Brain growth in mammals is associated with increased incorporation of LCPUFA in phospholipids located, primarily, in the cerebral cortex (Farquharson et al., 1992). The period of maximum accumulation is during the brain growth spurt, which among mammals occurs *in utero* (Huang and Brenna, 2001). Thus, most LCPUFA are transferred by the placenta to the developing fetal brain. Humans are unique in that their brain growth spurt is perinatal (Huang and Brenna, 2001), selectively incorporating 22:6n-3 from the third trimester (approximately 26 weeks of gestation) through the second year of postnatal life (Scheaff-Greiner et al., 1997). A larger relative brain size means that humans may also be unique in their nutritional requirements for 22:6n-3. In

humans therefore, LCPUFA transfer and the time period of its greatest nutritional demand may be important during both gestation and lactation.

Identical enzymes are used to desaturate both 18:2n-6 and 18:3n-3 (Figure 4.3), leading to competition between these fatty acids, most especially over access to Δ -6 desaturase (Innis, 2003). The result is a very intimate and complex relationship between dietary supply of 18:2n-6 and 18:3n-3 and synthesis of 20:4n-6 and 22:6n-3. Among mammals, including humans, diets deficient in 18:3n-3 are associated with decreased production of 22:6n-3 and the reciprocal increase of 18:2n-6 and 20:4n-6 (Brenna, 2002; Huang and Brenna, 2001; Innis, 2003). On the other hand, higher concentration of 18:2n-6 in the diet inhibits synthesis of 22:6n-3, presumably by out-competing 18:3n-3 for access to Δ -6 desaturase (Brenna, 2002; Carlson, 2001; Innis, 2003). Thus, the absolute concentrations of n-3 and n-6 PUFA *and* their ratio determine conversion to LCPUFA. In addition, 22:6n-3 is believed to be a minor quantitative fate of 18:3n-3 as it is the least efficiently formed of the n-3 LCPUFA (Brenna, 2002). A large endogenous pool of 18:3n-3 is not associated with increased conversion of 22:6n-3 in infant or adult humans (Brenna, 2002; Francois et al., 2003) or infant baboons (Scheaff-Greiner et al., 1997; Su et al., 2005).

The human fetus obtains the majority of the LCPUFA from placental transfer of preformed LCPUFA rather than from the conversion of 18 carbon precursors (Brenna, 2002; Carlson, 1999; Carnielli and Sauer, 1996; Koletzko et al., 2001b). Lauritzen et al. (2001) estimate that the developing human fetus assimilates at least 400 mg of 22:6n-3 per week during the last trimester, with little 18:3n-3 sequestered by the brain for

production of 22:6n-3 (Huang and Brenna, 2001). However, the human fetal liver does have the ability to convert 18:3n-3 (and 18:2n-6) into LCPUFA. Desaturase activities have been detected in aborted fetuses as young as 17 weeks gestational age (Brenna, 2002). Like infants and adults, however, human fetuses appear to be relatively inefficient at this process, and total whole body conversion is highly dependent upon the supply of PUFA and LCPUFA in the maternal diet (Brenna, 2002; Burdge, 2004; Carlson, 2001).

With inefficient conversion of 18:3n-3 by the offspring, the demand for 22:6n-3 therefore is mainly on the mother. Consequently, maternal plasma concentrations of 22:6n-3 must increase during pregnancy by either increased dietary consumption of 22:6n-3, increased mobilization of 22:6n-3 from body stores, or increased maternal abilities to convert 18:3n-3 to 22:6n-3 (Burdge, 2004). Maternal plasma concentrations of 22:6n-3 have been shown to increase throughout the course of gestation without increasing dietary intake of 22:6n-3, possibly due to the effect of increased estrogen (Burdge, 2004; Giltay et al., 2004). Higher levels of circulating estrogen are associated with upregulation of 22:6n-3 synthesis from 18:3n-3 (Giltay et al., 2004).

The importance of placental transfer of 22:6n-3 (and 20:4n-6) in humans is demonstrated by the fact that neonatal body fat has higher concentrations (three to four times) of these two fatty acids than the mother's body fat (Cunnane, 2005). Cunnane (2005) estimates that the reserve 22:6n-3 in the newborn human could provide all the 22:6n-3 needed for growth and development of the brain and body for 50 days. He refers to this supply as "insurance". Human infants are able to synthesize 20:4n-6 and 22:6n-3 from their precursors (Carnielli and Sauer, 1996; Salem et al., 1996; Sauerwald et al.

1996), but higher 22:6n-3 concentrations are found in the brains of infants who consume more 22:6n-3 (Carlson, 2001). Because the pathway from 18:3n-3 to 22:6n-3 is inefficient and susceptible to inhibition (and to some extent 18:2n-6 to 20:4n-6, but see below), a supply of preformed LCPUFA – either across the placenta or in milk - reduces the possibility of compromises in early brain development (Cunnane, 2005).

The first evidence in humans that higher dietary intake of 20:4n-6 and 22:6n-3 led to higher membrane concentrations of these fatty acids, relative to diets containing only 18:2n-6 and 18:3n-3, came from studies comparing formula-fed and breast-fed infants (Carlson, 1999). The most consistent finding of these studies has been the lower level of cerebral 22:6n-3 in the brains of infants fed formula with no 22:6n-3 compared to those receiving various levels of 22:6n-3 in breast milk (Gibson and Makrides, 1999). Additionally, among the latter group, the proportion of milk 22:6n-3 was positively correlated with visual and language development among breast-fed infants (Innis, 2003). Based on the composition of human breast milk, it is recommended that infant formulas contain 22:6n-3 as at least 0.2% of total fatty acids and 20:4n-6 as at least 0.35% of total fatty acids (Koletkzo et al., 2001a).

The n-6 LCPUFA 20:4n-6 is not considered a brain selective fatty acid because it is “easier” to make brain 20:4n-6 than brain 22:6n-3 (Cunnane, 2005). Su et al. (1999) found that over half of the 20:4n-6 required by developing fetal baboons came from 18:2n-6 supplied by the mother. They concluded that even small amounts of dietary 18:2n-6 may be able to supply the fetal brain’s requirement for 20:4n-6. During lactation, however, little milk 20:4n-6 is derived from circulating 18:2n-6 in maternal plasma (Del

Prado et al., 2001). Instead, the majority of milk 20:4n-6 in humans came from maternal depot stores of 20:4n-6, presumably consumed in the diet at some point or converted from dietary 18:2n-6. In developing countries, undernourished women with little 20:4n-6 in their diet had similar proportions of milk 20:4n-6 to well-nourished women who had access to dietary sources of 20:4n-6 (Del Prado et al., 2001). Infants fed formula lacking 20:4n-6 (the norm until 2002) had no significant difference in brain 20:4n-6 concentration from infants receiving 20:4n-6 via breast milk (Farquharson et al., 1992; Makrides et al., 1994). This finding suggests that human infants have a compensatory mechanism for 20:4n-6 and are not reliant on the dietary (milk) supply of this fatty acid. Placental transfer of 20:4n-6 may be more critical for levels of 20:4n-6 in the developing infant brain than transfer during lactation. While 20:4n-6 may be converted in sufficient quantities by 18:2n-6, it is still unclear if the rate of 22:6n-3 synthesis by the maternal and fetal/infant liver is sufficient to support optimal brain and retinal development in the developing human fetus and infant (Innis, 2003).

Human Milk Fatty Acids

Fatty acids in human milk have been investigated in mothers under various dietary habits and maternal conditions, leading to the conclusion that there is no such thing as *the* human milk fatty acid profile. However, some consistency has been noted across populations who exhibit variation in diet and body composition, suggesting that some aspects of milk fatty acids may be decoupled from maternal diet. Here, I review

data from several human populations and several dietary strategies in order to highlight consistencies and important differences in milk fatty acids.

In a study of nine populations (Australia, n = 48; Canada, n = 48; Chile, n = 50; China, n = 50; Japan, n = 51; Mexico, n = 46; the Phillipines, n = 54; the United Kingdom, n = 44; and the United States, n = 49), Yuhas et al. (2006) found the percent composition of 8:0 and 10:0 to be quite consistent, varying from 0.16 to 0.28% for 8:0 and 1.46 to 2.35% for 10:0. The predominant saturated fatty acid in all populations was 16:0. The mean percent composition of 18:2n-6 was 12.93%, but the range was quite large (7.90% in the Phillipines and 17.75% in Chile). The extremely high value in Chile was attributed to a high dietary intake of maize and the low value in the Phillipines to a diet low in essential fatty acids and total fat. Despite this variation in the PUFA precursor, the proportion of 20:4n-6 was consistent among populations (mean = 0.41%). This supports Koletzko et al.'s (1992, 2001b) argument that 20:4n-6 composition in human milk is not dependent on dietary supply of 18:2n-6 or 20:4n-6 but, rather, is conserved. Regardless of the mother's intake of 20:4n-6, the proportion of milk 20:4n-6 was similar across populations.

The percent composition of 18:3n-3 was consistent among seven of the nine populations at approximately 1% of total fatty acids (the exceptions were 2.02% in China and 0.43% in the Phillipines). The most variable component among the human populations investigated by Yuhas et al. (2006) was the proportion of 22:6n-3 in milk, which ranged from 0.17 to 0.99%. This finding is similar to reported ranges of 22:6n-3 in milk of women from populations within Africa (0.1 – 0.9%) and Europe (0.1 – 0.6%)

(Koletzko et al., 1992). The authors of both studies attribute the variation in milk 22:6n-3 to differences in fish consumption among populations.

Both Yuhas et al. (2006) and Koletzko et al. (1992) conclude that the proportion of saturated and monounsaturated fatty acids are consistent within and among human populations, as is the proportion of 20:4n-6. The most variable components appear to be among and within populations appear to be n-3 PUFA and LCPUFA, particularly 18:3n-3 and 22:6n-3. Del Prado et al. (2001) and Mitoulas et al. (2003) suggest that the consistent finding of variability in n-3 PUFA and LCPUFA may indicate a dependence on these fatty acids from the immediate diet rather than body depot stores.

To further explore the relationship between maternal diet and fatty acid composition, Table 4.2 presents data from human populations consuming four different diets: a typical western diet (higher in fat, more fat from animals, less fish), a non-Western diet (low animal fat and total fat, high consumption of fish), vegetarians (no meat or fish, but including dairy and eggs), and vegans (no fats originating from animals). The mean percent 8:0 is reported only for two of the four populations but falls within the range of values reported by Yuhas et al. (2006). Vegetarian and vegan women consume less fat in general, and obtain more dietary fat from vegetable sources than omnivorous women (Finley and Lönnerdal, 1985; Jensen et al., 1995; Sanders, 1999; Sanders and Reddy, 1992). Milk from vegetarian and vegan mothers was lower in 16:0, 18:0 (data not shown), and 22:6n-3 than milk from women on a Western diet, but higher in 18:2n-6 and 18:3n-3. Nigerian women consuming a non-Western diet had similar

Table 4.2. Fatty Acid composition for human females from four different dietary strategies: Western, Non-western, Vegetarian, and Vegan.

Fatty acid	Australian (Western)¹	Nigerian (Non-western)²	Vegetarian³	Vegan⁴
8:0	0.13 ± 0.06	Not detected	0.16 ± 0.03	NR
16:0	22.44 ± 1.82	23.35 (16.1 – 30.4)	15.31 ± 0.73	18.1 ± 1.34
18:2n-6	10.75 ± 4.22	11.06 (5.4-13.8)	28.82 ± 1.39	23.8 ± 1.40
18:3n-3	0.59 ± 0.16	1.41 (0.64 – 5.45)	2.76 ± 0.16	1.36 ± 0.18
20:4n-6	0.40 ± 0.10	0.82 (0.38 – 1.48)	0.68 ± 0.03	0.32 ± 0.02
20:5n-3	0.16 ± 0.07	0.48 (0.17 – 1.57)	NR	NR
22:6n-3	0.32 ± 0.17	0.93 (0.70 – 2.16)	0.22 ± 0.08	0.14 ± 0.06

¹Means and standard errors from Gibson and Kneebone's (1981) data on mature milk from Australian women (n = 120)

²Median values and ranges adapted from Koletzko et al. (1991) from women from Udo, Bendel State, Nigeria (n = 10). Reported in Jensen et al. (1995)

³Means and standard errors adapted from Specker et al.'s (1987) data on vegetarian mothers from New England (n =12). Reported in Jensen et al. (1995)

⁴Means and standard errors adapted from Sander and Reddy's (1992) data on vegan mothers from England (n = 19). Reported in Jensen et al. (1995)

NR = not reported

18:3n-3 values, suggesting that their intake of green leafy vegetables was comparable to that of vegetarian and vegan women (Sanders, 1999). Not surprisingly, 22:6n-3 varied widely and was lowest in vegans, who lacked any preformed source of this fatty acid and highest in Nigerian women, who had access to both dried and fresh fish. This table also illustrates that the proportion of 22:6n-3 in the milk can not be predicted from levels of milk 18:3n-3; Australian women on a Western diet had the lowest proportion of 18:3n-3 but did not have the lowest proportion of 22:6n-3.

Summary

Research on the functions of fatty acids suggests that they play a critical role in several physiological systems, including immune function, inflammation, and neurological development. PUFA and LCPUFA are essential fatty acids, universally required in the diet because they can not be synthesized by the mammary gland. As such, infants are reliant on the supply of these fatty acids. The proportion of n-3 PUFA and LCPUFA is more strongly correlated with dietary supply than that of n-6 PUFA and LCPUFA in humans. This suggests that the elongation to 20:4n-6 may be a more efficient pathway in humans, and that inclusion of 20:4n-6 may be unnecessary in infant formulas. The “necessary” proportion of 22:6n-3 in milk for developing human infants is hotly debated, as both too much and too little can be detrimental to the development of the infant. Data on human milk from several populations with distinct diets suggests that maternal diet is an important consideration when interpreting laboratory results on milk fatty acids. Despite differences in the nutritional consumption of vegetables, mammals and fish, milk fatty acid composition indicates many shared attributes across human mothers.

**CHAPTER 5: NATURAL HISTORY OF STUDY SPECIES:
BACKGROUND ON PHYLOGENY, ECOLOGY, AND LIFE HISTORY**

Introduction

In this dissertation, I argue that milk composition can be modeled as a life history trait. Specifically, milk composition is predicted to vary with respect to phylogeny, ecology, and other life history traits (Harvey et al., 1987; Kappeler et al., 2003; Leigh, 1994b; Leigh and Blomquist, 2007; Pereira and Leigh, 2003; Ross, 1995, 1998, 2003). This chapter reviews background data on primate classification, diet, and life history traits that are needed to test this prediction. I begin with a brief summary of the classification of anthropoid primates considered by this study. This summary includes information on genera, subfamily, family, and superfamily for each species as well as a review of molecular and fossil evidence in support of these classifications. Next, I discuss why the concept of grades might be as important as that of phylogenetic clades in interpretation of milk composition data. Milk composition may be more similar among species with similar ecological niches than among species that share a recent common ancestor. Finally, I summarize the dietary and life history strategies of species in the study and provide categorical variables that will be applied to research questions in subsequent chapters.

Taxonomy of anthropoid primates

Phylogeny is an important component of life history evolution (see Stearns, 1983, 1992; Harvey and Clutton-Brock, 1985; Gittleman and Thompson, 1988). Recent

common ancestry is expected to bring about greater similarity in life history traits among taxa. Milk composition will be analyzed with respect to species' evolutionary history. The evolutionary relationships among study species therefore provides a phylogenetic framework for analyzing milk composition.

All of the species included in the study are members of the suborder Anthropoidea (Mivart, 1864). Anthropoid primates are divided into two infraorders, catarrhini (Geoffroy, 1812) and platyrrhini (Geoffroy, 1812). Catarrhines (= Old World anthropoids) are further divided into Superfamily Cercopithecoidea (Gray, 1821) and Superfamily Hominoidea (Gray, 1825), while there is only a single superfamily of platyrrhines (= New World anthropoids), Superfamily Ceboidea (Gray, 1825). Sarich and Wilson (1967a, b) estimated a last common ancestor between Old and New World anthropoids at approximately 45 million years ago (mya) and between cercopithecoids and hominoids at approximately 30 mya. Estimated dates have oscillated over the last forty years due to the application of different statistical analyses, calibration points (which fossil find is used to “zero” the molecular clock), and types of molecular data (e.g. mtDNA vs. nuclear, coding vs. non-coding DNA) (Glazko and Nei, 2003). For example, Goodman et al. (1998) and Steiper and Young (2006) propose divergence dates for Old and New World anthropoids quite close to those of Sarich and Wilson. However, Glazko and Nei (2003) estimate that Old and New World anthropoids last shared a common ancestor much more recently, between 32 – 36 mya, and cercopithecoids and hominoids diverged between 21 – 25 mya. This project follows Glazko and Nei's (2003) dates for major branching events of anthropoid infraorders and superfamilies because of their

agreement with the fossil record in both the New and Old World. The adopted classification (Table 4.1) and divergence dates at levels below the superfamily are described in more detail below.

This study compares milk samples from five hominoid genera: *Gorilla*, *Hylobates*, *Pan*, *Pongo*, and *Symphalangus*. Classifications of hominoids based on morphological features placed *Gorilla*, *Pan*, and *Pongo* in the family Pongidae, leaving the family Hominidae only for humans and their bipedal ancestors (Marks, 2005; Wood and Richmond, 2000). Molecular analyses of ape genomes indicate that *Homo*, *Gorilla*, and *Pan* are a monophyletic group (containing all descendents of a common ancestor) and *Pongo*, *Gorilla* and *Pan* are paraphyletic (containing some descendents of a common ancestor but not those of *Homo*) (Bailey, 1993; Begun, 1994; King and Wilson, 1975; Ruvolo, 1997). This study follows Groves' (2001) grouping of *Gorilla*, *Pan*, and *Pongo* within the family Hominidae, *Gorilla* and *Pan* (with *Homo*) in Subfamily Hominidae and *Pongo* in Subfamily Ponginae. Wood and Richmond (2000) propose the subfamily Gorillinae for all gorilla species, but many classifications based on molecular data (e.g. Wildman et al., 2003) separate *Gorilla* from *Pan* and *Homo* only at the level of the subtribe. Groves' (2001) classification was adopted because it was the most conservative classification that maintained a monophyletic group.

Gorilla classification follows Groves (1986, 2001), recognizing two species of *Gorilla* (*Gorilla gorilla* and *G. beringei*) as well as subspecies within each. Western lowland gorillas are classified as *Gorilla gorilla gorilla* and wild populations of Rwandan mountain gorillas are classified as *Gorilla beringei beringei*. Although researchers have

suggested that chimpanzees and bonobos be placed within the genus *Homo* (Horai et al., 1995; Gagneux et al., 1999; Ruvolo, 1995; Wildman et al., 2003), they are classified here, respectively, as *Pan troglodytes* and *Pan paniscus*. Dates of divergence between *Gorilla* and *Pan* and *Gorilla/Pan* and *Pongo* are estimated at 6 – 8 mya and 12 – 15 mya, respectively (Glazko and Nei, 2003). Estimates of divergence between *Gorilla gorilla* and *Gorilla beringei* are more recent, approximately 1 – 2 mya (Yu et al., 2004). Sequencing of the X chromosome suggests that *Pan troglodytes* and *Pan paniscus* split between 0.69 to 1.22 mya (Kaessmann et al., 1999), while β -globin (Horai et al., 1992; Pesole et al., 1992) and mtDNA (Bailey et al., 1992) sequence analyses suggest an earlier split between 2.5 and 2.78 mya. In this study, the evolutionary distance between chimpanzees and bonobos is assumed to be similar to that between western lowland gorillas and mountain gorillas.

Members of the family Hylobatidae are often referred to as lesser apes, due to their relatively smaller body size. This family branched off from other hominoids between 16 and 23 mya (Roos and Geissman, 2001; Sibley and Alhquist, 1987). Evolutionary relationships among extant taxa within the Hylobatidae are debated (Roos and Geissmann, 2001; Takacs et al., 2005). This study follows the Takacs et al. (2005) designation of four monophyletic clades or genera within the family Hylobatidae: *Nomascus*, *Bunopithecus*, *Symphalangus*, and *Hylobates*. Siamangs are placed into the genus *Symphalangus* rather than with gibbons in the genus *Hylobates*.

Two cercopithecoid species are considered in this study: *Macaca mulatta* and *M. sinica*. The genus *Macaca*, which includes 21 recognized extant species, can be divided

into three phyletic lineages: *silenus-sylvanus*, *sinica-arctoides* (which includes *M. sinica*), and *fascicularis* (which includes *M. mulatta*) (Thierry, 2007). Morphological and molecular data suggest the divergence of these lineages approximately 5.5 mya (Fooden, 1976; Delson, 1980; Hoelzer and Melnick, 1996), the maximum estimate for divergence of *M. sinica* and *M. mulatta*.

Also included in this study are five ceboid genera from two families, the Atelidae and the Cebidae. Although the number of families within the Superfamily Ceboidea is under debate (Groves, 2001; Hershkovitz, 1977; Schneider et al., 1996, 2001; Schneider and Rosenberger, 1996), atelids and cebids are always grouped as separate families and are thought to have last shared a common ancestor 26 mya (Schneider et al., 2001). The Atelidae are represented by only one species, *Alouatta palliata*. The Family Cebidae is represented by two subfamilies, the Cebinae (which includes *Cebus* and *Saimiri*) and Callitrichinae (*Callithrix* and *Leontopithecus*). These subfamilies last shared a common ancestor about 16 mya, and genera within both subfamilies are assumed to have diverged 12 – 6.5 mya (Schneider et al., 2001). Therefore, this study assumes *Cebus* and *Saimiri* are as closely related to one another as are *Callithrix* and *Leontopithecus*.

Table 5.1. Classification used in this dissertation for study species

Species (or Subspecies)*	Classification
<i>Pan troglodytes</i> (Blumenbach, 1799) Chimpanzee	Superfamily: Hominoidea (Gray, 1825) Family: Hominidae (Gray, 1825) Subfamily: Hominidae (Gray, 1825) Genus: <i>Pan</i> (Oken, 1816)
<i>Pan paniscus</i> (Schwartz, 1929) Bonobo	Superfamily: Hominoidea (Gray, 1825) Family: Hominidae (Gray, 1825) Subfamily: Hominidae (Gray, 1825) Genus: <i>Pan</i> (Oken, 1816)
<i>Gorilla beringei beringei</i> (Matschie, 1903) Mountain Gorilla	Superfamily: Hominoidea (Gray, 1825) Family: Hominidae (Gray, 1825) Subfamily: Hominidae (Gray, 1825) Genus: <i>Gorilla</i> (Geoffroy, 1853)
<i>Gorilla gorilla gorilla</i> (Savage, 1847) Western Lowland Gorilla	Superfamily: Hominoidea (Gray, 1825) Family: Hominidae (Gray, 1825) Subfamily: Hominidae (Gray, 1825) Genus: <i>Gorilla</i> (Geoffroy, 1853)
<i>Pongo pygmaeus</i> (Linnaeus, 1760) Bornean Orangutan	Superfamily: Hominoidea (Gray, 1825) Family: Hominidae (Gray, 1825) Subfamily: Ponginae (Elliot, 1912) Genus: <i>Pongo</i> (Lacépède, 1799)
<i>Hylobates lar</i> (Linnaeus, 1771) Lar Gibbon or White-handed Gibbon	Superfamily: Hominoidea (Gray, 1825) Family: Hylobatidae (Gray, 1870) Subfamily: Hylobatinae (Gray, 1870) Genus: <i>Hylobates</i> (Illiger, 1811)
<i>Symphalangus syndactylus</i> (Raffles, 1821) Siamang	Superfamily: Hominoidea (Gray, 1825) Family: Hylobatidae (Gray, 1870) Subfamily: Hylobatinae (Gray, 1870) Genus: <i>Symphalangus</i> (Gloger, 1841)

*Species and subspecies designations used in this study follow those used by researchers who provided milk samples

Compiled from Groves (1986, 2001, 2003) and Hershkovitz (1984)

Table 5.1 (continued)

Species (or Subspecies)*	Classification
<i>Macaca mulatta</i> (Zimmerman, 1780) Rhesus Macaque	Superfamily: Cercopithecoidea (Gray, 1821) Family: Cercopithecidae (Gray, 1821) Subfamily: Cercopithecinae (Gray, 1821) Genus: <i>Macaca</i> (Lacépède, 1799)
<i>Macaca sinica</i> (Linnaeus, 1771) Toque Macaque	Superfamily: Cercopithecoidea (Gray, 1821) Family: Cercopithecidae (Gray, 1821) Subfamily: Cercopithecinae (Gray, 1821) Genus: <i>Macaca</i> (Lacépède, 1799)
<i>Alouatta palliata</i> (Gray, 1849) Mantled Howler	Superfamily: Ceboidea (Gray, 1825) Family: Atelidae (Gray, 1825) Subfamily: Atelinae (Gray, 1825) Genus: <i>Alouatta</i> (Lacepede, 1799)
<i>Cebus apella</i> (Linnaeus, 1758) Tufted Capuchin	Superfamily: Ceboidea (Gray, 1825) Family: Cebidae (Bonaparte, 1831) Subfamily: Cebinae (Bonaparte, 1831) Genus: <i>Cebus</i> (Erxleben, 1777)
<i>Saimiri boliviensis boliviensis</i> (Geoffroy and De Blainville, 1834) Bolivian Squirrel Monkey	Superfamily: Ceboidea (Gray, 1825) Family: Cebidae Subfamily: Cebinae Genus: <i>Saimiri</i> (Voight, 1831)
<i>Callithrix jacchus</i> (Linnaeus, 1758) Common Marmoset	Superfamily: Ceboidea (Gray, 1825) Family: Cebidae (Bonaparte, 1831) Subfamily: Callitrichinae (Thomas, 1903) Genus: <i>Callithrix</i> (Erxleben, 1777)
<i>Leontopithecus rosalia</i> (Linnaeus, 1766) Golden Lion Tamarin	Superfamily: Ceboidea (Gray, 1825) Family: Cebidae (Bonaparte, 1831) Subfamily: Callitrichinae (Thomas, 1903) Genus: <i>Leontopithecus</i> (Lesson, 1840)

*Species and subspecies designations used in this study follow those used by researchers who provided milk samples

Compiled from Groves (1986, 2001, 2003) and Hershkovitz (1984)

Clades and Grades

This project models milk composition as a biological trait that has been subject to natural selection. Like other biological traits, a species' milk composition is the result of evolutionary tinkering - all extant anthropoid primates share a common ancestor, therefore all anthropoid primate milks have been modified from this ancestral composition. Previous research (Ofstedal and Iverson, 1995; Power et al., 2002) suggests that relative to other mammalian orders, anthropoid milk is dilute and low in protein and energy. These are assumed to be primitive traits of anthropoid milk. The goal of this project is to identify shared-derived or unique-derived traits within, between or among superfamilies, families, genera, or species. Shared-derived properties are assumed to testify principally to phylogenetic heritage, whereas unique-derived properties are assumed to result from the processes of adaptation.

Do anthropoid primates that are more closely related have milk that is more similar in composition? An alternative to this hypothesis, and implicit in the adoption of an evolutionary perspective, is that milk composition may instead vary with respect to grade and reflect a shared adaptive complex (Simpson, 1949) in addition to shared genetic ancestry. For example, apes and Old World monkeys share a more recent common ancestor with one another than either does with New World monkeys. However, similarities in diet, body size and other life history traits – factors that are predicted to have a strong influence on milk composition (Lee, 1996) - among both monkey superfamilies may lead to convergence in milk composition. Although strongly linked to

phylogeny, diet and life history do show variability among closely related anthropoid primates, suggesting milk composition may also be variable within phyletic lineages.

Ecology and Life History of Study Species

Ecological and ontogenetic variation in extant primates can not be fully explained by shared evolutionary history. That is, more closely related species (or genera, families) do not always have similar dietary niches or rates and/or patterns of growth and development. Mountain gorillas and howler monkeys are folivorous but are classified as different superfamilies. *Cebus* (capuchin) monkeys are more similar to *Pan*, *Gorilla*, and *Pongo* in relative brain size (Fragazy et al., 2004) than to other monkeys in the family Cebidae. Milk composition may vary with respect to phylogeny, but like other life history traits, may also be an adaptive response to ecological variation (Hill and Kaplan, 1999; Kaplan et al., 2000; Morbeck, 1997a). In this section, I present data on dietary and life history strategies of the study species. When possible, data from the study groups (diet and life history) were included rather than data from the literature more generally (see notations in subsequent Tables).

Diet

It is difficult to quantify the diet of species included in this study as all involve some level of flexibility. Therefore, a dietary category, or strategy, was assigned to each species in this study (Table 5.2). Even when diet is converted to a quantity (e.g., percent

fruit and seeds or percent leaves), there remain issues of seasonality and food preference. The 14 nonhuman primate species were placed into one of five dietary categories: frugivore, folivore, gumnivore, frugivore-folivore, and frugivore-insectivore. Placement into each category was based upon data on the percentages of fruit/seeds or percent leaves in the diet, categories assigned in the literature, or both. Table 5.2 summarizes the diet of each species included in this study and the assigned dietary category used in subsequent analyses.

Frugivorous primates included both species of *Pan*, orangutans, gibbons, and toque macaques. The two folivorous primates are the mountain gorilla and the mantled howler. Only one species, the common marmoset, is a gumnivore. Species with a more omnivorous diet were grouped in the mixed categories of frugivore-folivore and frugivore-insectivore. The former category includes lowland gorillas, siamangs, and rhesus macaques, and the latter includes tufted capuchins, squirrel monkeys, and golden lion tamarins.

Table 5.2. Wild diet of study species and dietary categories used in analyses

Species	Description of diet	Reference(s)	Dietary category
<i>Pan troglodytes</i>	Primarily frugivores, but quite omnivorous. Amount of leaves in diet depends on field site. Vertebrates, insects and eggs can contribute up to 10% to diet.	Goodall (1986), McGrew (1992), Stumpf (2007), Wrangham et al. (1994, 1998)	Frugivore
<i>Pan paniscus</i>	Primarily frugivores. Have a heavy reliance on THV (terrestrial herbaceous vegetation), particularly those lower in fiber and higher in carbohydrates.	Kano (1992), Stumpf (2007)	Frugivore
<i>Gorilla beringei beringei</i>	Primarily folivores, less than 3% fruits and seeds in diet. Seasonal use of bamboo.	Fossey and Harcourt (1977), Goldsmith (2003), Remis (2003),	Folivore
<i>Gorilla gorilla gorilla</i>	Consume both fruits and leaves. Consumption of fruit is greater than mountain gorillas but varies seasonably.	Goldsmith (1999, 2003), Robbins, (2007), Rowe (1996)	Frugivore-Folivore
<i>Pongo pygmaeus</i>	Primarily Frugivores. Eat leaves and bark for fallback foods during times of low fruit availability. Can spend up to 100% of time foraging consuming fruit.	Knott (1998, 1999), Knott and Kahlenberg (2007)	Frugivore
<i>Symphalangus syndactylus</i>	Consume both fruit and leaves. 32 – 61% of diet is fruit, generally more folivorous than Lar gibbons. Insects can comprise up to 15% of diet.	Bartlett (2007), Chivers (1974), MacKinnon and MacKinnon (1980), Palombit (1997)	Frugivore-Folivore
<i>Hylobates lar</i>	Primarily frugivores. Between 50 – 67% of diet	Bartlett (2007), Ellefson (1974),	Frugivore

	is fruit. Certain populations rely more heavily on leaves and insects.	MacKinnon and MacKinnon (1980), Raemaekers (1979)	
<i>Macaca mulatta</i>	Omnivores. Mainly folivorous but can also be highly frugivorous.	Ménard (2004), Thierry (2007),	Frugivore-Folivore
<i>Macaca sinica</i>	Primarily frugivores, but diet is variable. This group is often provisioned by humans.	Dittus (personal communication), Thierry (2007)	Frugivore
<i>Alouatta palliata</i>	Primarily folivores. Costa Rican populations reported to consume between 9 – 16% fruit in diet.	Glander (1978), Rosenberger and Strier (1989)	Folivore
<i>Cebus apella</i>	Highly omnivorous, frugivore-insectivore, occasionally hunt for vertebrates, eat nuts.	Jack (2007), Robinson and Janson (1987)	Frugivore-Insectivore
<i>Saimiri boliviensis boliviensis</i>	Highly omnivorous, frugivore-insectivore, can spend up to 80% of time foraging for insects.	Jack (2007), Janson and Boinski (1992)	Frugivore-Insectivore
<i>Callitrix jacchus</i>	Primarily gummivores. Spend between 68 – 76.4% of time feeding on gums/sap, remainder is on fruit/seeds. Avoid leaves and bark.	Digby et al. (2007), Ferarri (1993), Power and Oftedal (1996).	Gummivore
<i>Leontopithecus rosalia</i>	Rely primarily on fruit (83 % of time) and also forage for insects (15% of time). Opportunistic gummivore.	Dietz et al. (1997), Digby et al. (2007)	Frugivore-Insectivore

Life history traits and quantitative issues

The relationship between milk composition and other life history traits has not been systematically investigated among primates. Thus, isolation of life history traits in this study was based on a number of assumptions and predictions from the nonhuman primate life history literature. The majority of published life history data come from captive living nonhuman primates and therefore represent a species' life history strategy under unnatural conditions. Generally, primates in captivity give birth at an earlier age, have longer life spans, and are larger (mass and height) than their wild counterparts (Leigh, 1994a; Watts, 1990; Zihlman et al., 1990, 2004). Behavioral and physiological data from captivity are assumed to be at the maximum of a species' reaction norm (Lee and Kappeler, 2003). Tables listing life history information (Table 5.3 – 5.8) report longest life span, largest body mass, longest lactation period, and earliest age at first reproduction for all species. Reporting these for all species permits interspecific comparisons of reproductive and life history strategies under optimal conditions.

As one of four components of a species' lactation strategy, volume of milk produced, nursing frequency, and total duration of lactation were expected to influence milk composition. Of these three, only the length of lactation had been extensively reported in the life history literature and was reported as number of days. This gives the impression that age at weaning is a measurable and predictable unit of time, such as length of gestation. In general, weaning occurs at the time when infants have reached approximately one third of their adult body mass (Lee, 1996; Lee et al., 1991). For reasons relating to climate, social status, group size, or maternal nutrition, weaning does

not always coincide with this threshold mass and infants do not reach this threshold mass in the same amount of time (Lee and Bowman, 1995). Therefore, the total length of lactation is best conceived of as a range. A mean value is provided for study species in Table 5.3 – 5.5 more as a matter of convention than an ideal description. Data were taken from Kappeler and Pereira (2003), who provide several mean values in their data set. In all cases, the longest lactation period was included in the data set.

Next, as lactation is part of the larger reproductive strategy of the female, life history traits related to female reproduction were also considered. These were length of gestation, female age at first reproduction, and neonatal body mass (adult and neonatal brain size are discussed separately below). Gestation length, age at first reproduction, and neonatal body mass were taken primarily from Ross (2003), but other sources also were consulted (Garber and Leigh, 1997; Hartwig, 1996; Leigh and Shea, 1996; Kappeler and Pereira, 2003; Ross, 1991) to confirm Ross' data or to provide data for species not included in Ross' (2003) data set.

Like other life history traits (Deaner et al., 2003; Harvey et al., 1987; Leigh, 2004; Martin, 1995; Martin and Harvey, 1985; Martin and MacLarnon, 1990; Ross 1998, 2003), milk composition may correlate with adult body mass. This dissertation predicts that absolute body mass is related to a species' lactation strategy and therefore may be related to milk composition. Smaller primates have lower absolute energy needs than larger primates as a general rule, but energy demands of smaller primates per unit mass are higher than those of larger primates (Leonard et al., 2003). Increased body size also brings with it a suite of physiological and behavioral features that allow individuals to

meet the unique demands for fueling a large body. A larger body size permits individuals to store more energy, and energy storage may be particularly important during lactation, affecting milk production and possibly even milk composition.

However, body mass does not explain all the variation among primates, nor are the allometric relationships between body mass and other life history traits identical among species (Leigh and Blomquist, 2007; Pereira and Leigh, 2003). For example, age at first reproduction, absolute and relative brain size, and adult body size are positively correlated in some but not all primate species (e.g., squirrel monkeys) (Pereira and Leigh, 2003). Leigh and Blomquist (2007) argue that many life history traits are quasi-independent because of disassociation (or modulation) of developing morphological structures. This analysis of milk composition is mindful of modularity in life history traits, and no statistical tests will be performed with milk composition data and body size data. Additionally, because body mass has a much wider range of variation (orders of magnitude) than other life history traits, adult female and neonatal body mass were log transformed to normalize the distribution.

Another way to control for variation among rates and patterns of growth and development is to convert life history traits to relative quantities. Length of lactation can be corrected by total life span to produce a percentage of the life span spent on each lactation event. Relative length of lactation was calculated by dividing the length of lactation (days) by the total life span (days) and is reported in Table 5.6. Age at first reproduction is important as an absolute term, indicating the amount of time each species dedicates to growth and development prior to reproduction. Relative to life span,

however, age at first reproduction indicates how much of the species' life is spent in the pre-reproductive stages. Age at first reproduction (years) was divided by the total life span (years), and is reported in Table 5.6.

Data on adult female brain size and neonatal brain size were taken primarily from Harvey et al. (1987). This was the best available data set for nonhuman primates included in this study because of overlapping research interests (e.g., the relationship between life history variables) and a focus on life history traits as a method for looking for patterns within the primate order. However, Harvey et al.'s (1987) data were not without problems. First, body and brain mass values did not come from the same individuals. Although the influence of body size and brain size is not equivalent for all nonhuman primates (Pereira and Leigh, 2003), larger bodied animals tend to have larger brain masses (Harvey and Clutton-Brock, 1985). Second, sample sizes of many species were small and much of the female data was extrapolated from male data, assuming a predetermined amount of sexual dimorphism in body and brain size. Thus, results obtained by comparing these data to milk composition are treated with extreme caution.

Because brain size is integral to research questions, adult and neonatal brain mass data were manipulated in several ways. First, brain masses were log transformed to normalize the data set, then divided by log (body mass) to determine how much of the body mass is accounted for by brain mass (Table 5.6). Second, brain size and body size were used to calculate encephalization quotients (EQ) for each species if available (Table 5.7, 5.8). The EQ value describes how much the actual brain size differs from the brain size predicted by body size and permits the comparison of brain sizes among species with

different body sizes (Jerison, 1955, 1977). EQ expresses the relative size of the brain using an equation that incorporates the exponent and the coefficient from the regression of the log of brain mass on body mass. Following Marino (1998, adapted from Jerison), the following equation was applied to brain and body mass data presented in Table 5.3 – 5.5:

$$\frac{\text{Brain mass}}{0.17 (\text{body mass})^{0.72}} = \text{EQ}$$

The exponent of 0.72 is a more specific coefficient from a regression analysis including only anthropoid primates (Marino, 1998). EQ results for adult and neonatal study species are presented in Table 5.7 and Table 5.8.

To be conservative, adult EQ values were also used to group study species into three EQ categories, least encephalized (EQ 1), moderately encephalized (EQ 2), and highly encephalized (EQ 3). EQ values were not available for adult *Pan paniscus*, *Gorilla beringei*, or *Saimiri boliviensis*. These species were grouped with data available on taxa that share a recent common ancestry (*Pan troglodytes*, *Gorilla gorilla*, and *Saimiri sciureus*, respectively). EQ values and categories will be compared to proximate milk composition in Chapter 9.

EQ 1: *Alouatta palliata*, *Gorilla gorilla*, *Gorilla beringei*, *Pongo pygmaeus*, and *Macaca sinica* (EQ < 0.75).

EQ 2: *Callithrix jacchus*, *Leontopithecus rosalia* and *Macaca mulatta* (EQ 0.75 – 0.90)

EQ 3: *Cebus apella*, *Saimiri boliviensis*, *Hylobates lar*, *Pan troglodytes*, *Pan paniscus*, and *Symphalangus syndactylus* (EQ > 0.90).

Table 5.3. Life history traits of hominoid species included in study

Species	Adult female body mass (g)	Adult female brain mass (g)	Neonatal body mass (g)	Neonatal brain mass (g)	Female age at 1 st reprod. (yrs) ^b	Length of gestation (days)	Length of lactation (days) ^a	Female Lifespan (yrs) ^a
<i>Pan troglodytes</i>	45000	380	1742	128.0	13	235	1825	55
<i>Pan paniscus</i>	33200	NA	1400	NA	13	228	1080	55
<i>Gorilla gorilla gorilla</i>	82745	457	2122	227.0	10	260	1000	55
<i>Gorilla beringei beringei</i>	70000 ^c	NA	NA	NA	10 ^d	260 ^d	1000 ^d	55 ^d
<i>Pongo pygmaeus</i>	37078	302	1728	170.3	9.68	250	1280	58.7
<i>Symphalangus syndactylus</i>	10700	121.7	517	NA	NA	231	639	38.0
<i>Hylobates lar</i>	5900	115.2	410	50.1	9.3	205	730	35.6

^a If a range was reported in the literature, analyses used the maximum value

^b If a range was reported in the literature, analyses used the minimum value

^c Data is specific to study populations. Provided by institutions/individuals that supplied milk samples

^d Values reported are from *Gorilla gorilla gorilla* (no available data on *Gorilla beringei beringei*).

Other life history data compiled from Bauchot and Stephan (1969), Fossey (1979), Harvey et al. (1987), Judge and Carey, 2000, Kappeler and Pereira (2003), Leigh and Shea (1996), Marino (1998), Ross (2003), Smith and Jungers (1997), Smith and Leigh (1998), and Stumpf (2007)

NA = data not available

Table 5.4. Life history traits of cercopithecoid species included in study.

Species	Adult female body mass (g)	Adult female brain mass (g)	Neonatal body mass (g)	Neonatal brain mass (g)	Female age at 1 st reprod. (yrs) ^a	Length of gestation (days)	Length of lactation (days) ^b	Female Lifespan (yrs)
<i>Macaca mulatta</i>	8800	95.1	475	54.5	4	167	210	25
<i>Macaca sinica</i>	3200	69.9	NA	NA	5	168	NA	25

^a If a range was reported in the literature, analyses used the minimum age (youngest reported age at first reproduction)

^b If a range was reported in the literature, analyses used the maximum value (latest reported day for nursing/weaning)

^c Data is specific to study populations. Provided by institutions/individuals that supplied milk samples

Life history data compiled from Bercovitch and Harvey (2004), Dittus (1975), Harvey et al (1987), Judge and Carey, 2000, Kappeler and Pereira (2003), Leigh and Shea (1996), Ross (1991, 2003), Smith and Jungers (1997), Smith and Leigh (1998), and Thierry (2007)

Table 5.5. Life history traits of ceboid species included in study

Species	Adult female body mass (g)	Adult female brain mass (g)	Neonatal body mass (g)	Neonatal brain mass (g)	Female age at 1 st reprod. (yrs) ^a	Length of gestation (days)	Length of lactation (days) ^b	Female Lifespan (yrs)
<i>Alouatta palliata</i>	5824	51.5	480	30.8	3.6	186	325	20
<i>Cebus apella</i>	2201	63.5	240	29.0	5.5	155	264	44
<i>Saimiri boliviensis boliviensis</i>	700 ^c	24.4	109 ^c	NA	3 ^c	151 ^c	240 ^c	21
<i>Callitrix jacchus</i>	352	8	30	4.4	1.5	148	90 ^c	11.7
<i>Leontopithecus rosalia</i>	659	12.9	50	NA	2.4	129	90 ^c	24.7

^a If a range was reported in the literature, analyses used the minimum age (youngest reported age at first reproduction)

^b If a range was reported in the literature, analyses used the maximum value (latest reported day for nursing/weaning)

^c Data is specific to study populations. Provided by institutions/individuals that supplied milk samples

^d Value represents mass of two neonates (twins)

Other life history data compiled from Fragaszy and Adams-Curtis (1998), Garber and Leigh (1997), Gibson et al. (1993), Hartwig (1996), Harvey et al. (1987), Judge and Carey (2000), Kappeler and Pereira (2003), Ross (1991, 2003) and Williams et al. (1994)

Table 5.6. Relative life history traits. All body and brain masses were log transformed.

Species	Neonatal brain/ body mass %	Adult female brain/ body mass %	Neonatal/ Adult female body mass %	Age at first reproduction/life span	Length of lactation/life span
<i>Pan troglodytes</i>	0.65	0.55	0.70	23	9.1
<i>Pan paniscus</i>	NA	NA	0.70	23	5.3
<i>Gorilla gorilla gorilla</i>	0.71	0.54	0.68	18	5.0
<i>Gorilla beringei beringei</i>	NA	NA	NA	18 ^a	5.0 ^a
<i>Pongo pygmaeus</i>	0.69	0.54	0.71	17	6.0
<i>Symphalangus syndactylus</i>	NA	0.41	0.54	NA	4.6
<i>Hylobates lar</i>	0.65	0.54	0.69	26	5.6
<i>Alouatta palliata</i>	0.65	0.50	0.68	18	9
<i>Cebus apella</i>	NA	0.53	NA	13	2
<i>Saimiri boliviensis boliviensis</i>	0.56	0.45	0.71	14	3
<i>Callitrix jacchus</i>	0.61 ^b	0.54	0.71 ^b	13	2
<i>Leontopithecus rosalia</i>	NA	0.49	0.72 ^b	10	1
<i>Macaca mulatta</i>	0.44	0.35	0.58	16	12
<i>Macaca sinica</i>	NA	0.39	0.60	20	NA

Brain masses and neonatal body masses from Harvey et al. (1987). NA = unavailable

^a Values are from *G. gorilla*. ^b Neonatal mass represents two infants

Table. 5.7. Adult encephalization quotients (EQ) in ascending order.

Species	Adult Brain Mass (g)	Adult Body Mass (g)	EQ
<i>Alouatta palliata</i>	52.7	6896	0.534
<i>Gorilla gorilla</i>	480.3	114654	0.643
<i>Pongo pygmaeus</i>	351	63730	0.717
<i>Macaca sinica</i>	84	8392	0.739
<i>Leontopithecus rosalia</i>	12.3	559	0.761
<i>Macaca mulatta</i>	95.1	8800	0.809
<i>Callithrix jacchus</i>	7.7	233	0.894
<i>Symphalangus syndactylus</i>	131.5	11117	0.945
<i>Pan troglodytes</i>	390	48893	0.965
<i>Hylobates lar</i>	99.7	5664	1.164
<i>Saimiri (sciureus)</i>	24.2	648	1.346
<i>Cebus apella</i>	69.5	2327	1.540

Brain and body masses from Marino (1998) excepting *Leontopithecus* (Kappeler and Pereira, 2003). EQ was calculated as $EQ = \text{brain mass} / 0.17(\text{body mass})^{0.72}$ (Marino, 1998)

Table 5.8. Neonatal encephalization quotients (EQ) in ascending order (species were excluded if no available data on neonatal body mass and/or brain size).

Species	Neonatal Brain Mass (g)	Neonatal Body Mass (g)	EQ
<i>Alouatta palliata</i>	30.8	480.0	2.126
<i>Callithrix jacchus</i>	4.4	30.0	2.236
<i>Cebus apella</i>	29.0	240.0	3.298
<i>Pan troglodytes</i>	128.0	1742.0	3.493
<i>Macaca mulatta</i>	54.5	475.0	3.791
<i>Hylobates lar</i>	50.1	410.0	3.874
<i>Pongo pygmaeus</i>	170.3	1728.0	4.674
<i>Gorilla gorilla</i>	227.0	2122.0	5.374

Brain and body masses from Harvey et al. (1987).

EQ was calculated as $EQ = \text{brain mass} / 0.17(\text{body mass})^{0.72}$ (Marino, 1998)

Summary

This dissertation asks if milk composition is related to a species' evolutionary history, diet, and/or life history strategy. The classification of species within the Suborder Anthropoidea will be the primary framework for comparisons of milk samples. After exploring variation at the level of the superfamily, family, or genera, milk composition will also be compared among species with similar dietary strategies. Captive and wild diets can be very different and highly variable in that latter case, making categorical descriptions necessary. These categories describe the species' dietary strategy rather than quantifying their actual diet, and the categories take into account possible variation among populations in dietary consumption of fruit, seeds, leaves, insects, or gums. Efforts to quantify the life history strategy of a species were more problematic. Life history traits are quite plastic and are expected to vary between captive and wild populations, within wild populations, and among wild populations living in different environments. Mean values reported are those that best describe the maximum of a species' reaction norm for each life history trait. The maximum of the range of values is the target because it describes the potential of the species under optimal conditions. Absolute and relative life history traits are important considerations, including the total duration or length of lactation, age at first reproduction relative to life span, and the encephalization quotient, which is the relationship between a species' actual brain size compared to the brain size predicted for body size.

CHAPTER 6: MATERIALS

Introduction

This study analyzed 107 milk samples from 14 species of anthropoid primates, including both wild living and captive-housed individuals. Nine species (*Cebus apella*, *Gorilla gorilla gorilla*, *Hylobates lar*, *Macaca mulatta*, *Pan paniscus*, *Pan troglodytes*, *Pongo pygmaeus*, *Saimiri boliviensis boliviensis*, and *Symphalangus syndactylus*) are represented by only captive living individuals (Table 6.1). Three species (*Alouatta palliata*, *Gorilla beringei beringei*, and *Macaca sinica*) are represented by only wild living individuals (Table 6.2). Two species (*Callithrix jacchus*, *Leontopithecus rosalia*) are represented by both wild and captive living individuals (Table 6.1, 6.2).

Milk collections of all species were opportunistic. Most samples collections were conducted by other researchers prior to the development of this project. Exceptions include *Cebus apella* (tufted capuchins) and one sample from *Gorilla gorilla gorilla*. As a result, available details on milk donors and methods of milk collection for study species vary. More detailed information were available for the five species of anthropoid primate living in the wild. Protocols for animal capture and milk collection were provided by individuals directly involved with each study.

In this chapter, I summarize information on milk samples included in this study. This includes information on study populations, the number of samples collected from each species, and inclusion criteria for statistical analyses. Appendix 1 includes additional information on populations (including housing conditions, habitat, diet, and social group) and methods of sample collection.

Captive populations included in study

Callithrix jacchus: Four common marmoset females (n = 4) provided one milk sample each (n = 4). Females were part of a breeding colony at the Southwest National Primate Research Center (San Antonio, TX). Females were housed in family groups, with a breeding male and female and their immature offspring. All females were fed *ad libitum* one of two, isocaloric, homogenous, gelled, purified diets containing lactalbumin, dextrose, sucrose, soybean oil, cellulose, agar, and vitamin and mineral premixes for the duration of the study period (the onset of lactation)(Power et al., 2002).

Cebus apella: Eleven tufted capuchins (n = 11) provided one milk sample each (n = 11). Females were part of a breeding colony maintained at Alpha Genesis, Inc., Yemassee, SC. The animals were housed in a large group, including males, females, and their offspring. Females carried their young within the larger group. Females were fed Harlan-Teklad New World Primate Monkey Chow.

Gorilla gorilla gorilla: Three Western lowland gorillas (n = 3) provided five milk samples (n = 5). Samples were part of the mammalian milk collection at the Nutritional Laboratory. Samples are from gorillas in three zoos: Zoo Atlanta (n = 1), San Diego Wild Animal Park (n = 3), and the Philadelphia Zoo (n = 1). Permission to use samples from the San Diego Wild Animal Park (SDWAP) and the Philadelphia Zoo was provided by Olav Oftedal (Director, Nutrition Laboratory). Samples from SDWAP were from days 3,

242, and 690 of lactation. Samples from day 3 and 242 were from the same mother, but different offspring (day 3 from her second offspring, and day 242 from her first). The sample from the Philadelphia Zoo is from day 108 of lactation, provided by a wild caught female. The sample from Zoo Atlanta was provided to this project by T Stoinski (Veterinarian, Zoo Atlanta, Dian Fossey Gorilla Fund International). This sample is from day 1105 of lactation. The Zoo Atlanta female gorilla was fed primarily with Monkey Chow and supplemented with fruit and vegetables. She also browsed her enclosure for enrichments (leaves, insects). During the day, she was housed with one adult male (silverback), her most recent offspring (a male born in 2002), two young males (both born 1998), and one other adult female. At night, she was housed inside with other individuals of the same species in visual, olfactory, and auditory contact.

Hylobates lar: One white-handed gibbon female ($n = 1$) provided one milk sample ($n = 1$). This female was housed at the Minnesota Zoo (Jim Rasmusen, Veterinarian) on June 6, 1996. The Minnesota Zoo provided four samples to the Nutrition Laboratory, but only one was available for analysis for this project. The exact date of lactation for this sample is unknown because cryovials containing milk samples were not labeled with date of collection. Date of parturition was given as April 27, 1995 and dates of sample collection were May 15, 17, or 19. Therefore, this sample is from day 18, 20, or 22 of lactation. No information was available on housing conditions or diet.

Leontopithecus rosalia: The single captive living golden lion tamarin female ($n = 1$) provided one milk sample ($n = 1$). She was housed at the Smithsonian's National Zoological Park. Although captive by definition, this female was free-ranging in the zoo's golden lion tamarin habitat, and has been observed to supplement her zoo diet of monkey chow with insects and fruits when available.

Macaca mulatta: Rhesus macaque females ($n = 22$) provided one sample each ($n = 22$). Females were part of a breeding population at the California National Primate Research Center (Davis, CA). $N=21$ (right?) sampled once between 3 and 4 months post-partum. All subjects were housed in outdoor 1/2 acre corrals with 20 feet with chain-link fencing on the sides and top. The corrals were a mix of grass, dirt, and gravel substrate. The subjects were housed in stable social groups of intact matrilineal and social groups that vary between approximately 50 to 150 individuals. The social groups had about a 1:4 male to female sex ratio among adults, but this ratio can vary from corral to corral. The monkeys lived full time in these enclosures and are the breeding colony of the California National Primate Research Center. The monkeys were fed commercially available Purina Monkey Chow (outdoor Old World monkey chow) twice daily and two to three times a week the group was provided with fruit, vegetables, and browse in addition to the monkey chow. All milk analyses were performed under the direction of KJ Hinde, excepting fatty acid analysis.

Pan paniscus: One bonobo female ($n = 1$) provided two milk samples ($n = 2$) which were a part of the Nutrition Laboratory's mammalian milk collection. The female was housed at the Milwaukee Zoo (Milwaukee, WI) and milked several times between September and November, 1995. Samples included in this study were collected at days 46 (09/09/95) and 76 (10/09/95) of lactation. No information was available on housing conditions or diet.

Pan troglodytes: Female chimpanzees ($n = 4$) provided one milk sample each. Three samples ($n = 3$) were obtained from three females housed at the Southwest National Primate Research Center (SNPRC). The following information on SNPRC samples was provided by KM Brasky (Veterinarian, SNPRC). Female chimpanzees were part of a research colony at SNPRC. There is a moratorium on the breeding of chimpanzees in the United States, and all males in the colony had received a vasectomy. However, one male in the colony remained fertile, leading to three pregnancies. Samples were collected from females during annual physical exams from June 25 – 30, 2005. Females were housed together in a large indoor/outdoor enclosure. They were fed Purina monkey chow once a day and seasonal fruits and vegetables twice a day. They also received for food enrichment such as frozen fruit juice, yogurt, milk bones, sugar free cereal, and nuts.

The St. Louis Zoological Park (St. Louis, MO) donated one chimpanzee milk ($n = 1$) sample to the Nutrition Laboratory's mammalian milk collection. The sample was collected on November 3, 1992 (day 97 of lactation). No information was available on housing conditions or diet of this chimpanzee.

Pongo pygmaeus: One Bornean orangutan ($n = 1$) provided one sample ($n = 1$). The female orangutan was housed at Zoo Atlanta. The sample was collected on February 3, 2005 and was received on February 5, 2005 by the Nutrition Laboratory for use in this project. The sample is from day 430 of lactation (approximately 1.75 years). Zoo veterinarians reported that this offspring was still nursing regularly as of June 2006 despite eating solid foods since age two. The mother's diet consisted of primarily fruits and vegetables, supplemented with Monkey Chow. She was housed in a group that consists of herself, her offspring, and the offspring's sire. At night, she was housed inside with other individuals of the same species in olfactory and auditory contact.

Saimiri boliviensis boliviensis: Eight female Bolivian squirrel monkeys ($n = 8$) provided three milk samples each ($n = 24$). All females were part of a breeding colony of squirrel monkeys maintained at the University of South Alabama Center for Neotropical Primate Breeding and Research Resource (CNPRR). Four of the eight females included in this study were feral-born and four were born into the colony at CNPRR. This study was approved by the University of South Alabama Institutional Animal Care and Use Committee. Animals were housed in social groups of between 15 - 35 animals. Each group contained one adult male and between 10 - 15 adult females with their offspring. Animal housing consisted of indoor pens measuring approximately 4.5 m X 2.5 m X 1.5 m, connected by port hole doors. Social groups had access to two to three pens depending on size. All animals were fed a New World monkey chow daily and supplemented with insects and fresh fruits and vegetables.

Symphalangus syndactylus: One siamang ($n = 1$) provided one milk sample ($n = 1$). The siamang milk sample was part of the Nutrition Laboratory's mammalian milk collection, donated in 1987 by the Riverbanks Zoological Park (Columbia, SC). No information was available on housing conditions or diet.

Table 6.1. Milk sample information on captive living anthropoid primates

Species	Institution or Organization that provided samples	Num. of Individuals	Num. of Samples	Infant Age Range (days)
<i>Pan troglodytes</i>	Southwest National Primate Research Center (San Antonio, TX) ^{b, c}	3	3	451 - 550
	St. Louis Zoo (St. Louis, MO) ^{a, b}	1	1	97
<i>Pan paniscus</i>	Milwaukee Zoo (Milwaukee, WI) ^b	1	2	46 - 76
<i>Pongo pygmaeus</i>	Zoo Atlanta (Atlanta, GA) ^{a, c}	1	1	430
<i>Gorilla gorilla gorilla</i>	Zoo Atlanta (Atlanta, GA) ^{a, c}	1	1	1105
	Philadelphia Zoo (Philadelphia, PA) ^b	1	1	108
	San Diego Wild Animal Park (San Diego, CA) ^b	3	3	3 - 242
<i>Hylobates lar</i>	Minnesota Zoo (Apple Valley, MN) ^b	1	1	18 - 22
<i>Symphalangus syndactylus</i>	Riverbanks Zoo (Columbia, SC) ^b	1	1	54
<i>Macaca mulatta</i>	California National Primate Research Center (Katherine Hinde, Davis, CA) ^{a, b, c}	22	22	91 - 123
<i>Callithrix jacchus</i>	Southwest National Primate Research Center (ML Power, San Antonio, TX) ^{a, b}	4	4	26 - 32
<i>Leontopithecus rosalia</i>	Southwest National Primate Research Center (ML Power, San Antonio, TX) ^{a, b}	1	1	50
<i>Cebus apella</i>	Alpha Genesis, Inc., (Yemassee, SC)	11	11	1 - 402
<i>Saimiri boliviensis</i>	University of Southern Alabama (Mobile, AL) ^c	8	24	101 - 225

^aOxytocin was used in collection of samples, ^b Samples were donated to NZP from these institutions prior to start date of this project. Dr. Olav Oftedal, Director, Nutrition Lab, permitted use of these samples for this project. ^c Samples were donated to NZP from these institutions/individuals for specific use in this project

Wild populations included in study

Alouatta palliata: Eight wild living mantled howler females ($n = 8$) from La Pacifica, Costa Rica provided one milk sample each ($n = 8$). La Pacifica is 2000 ha with 600 ha of low-land dry and riparian forest ($10^{\circ} 28' N$ and $85^{\circ} 07' W$). The average group size for mantled howlers in Costa Rica is between 12 to 16 individuals, approximately 40% of which are adult females, 25% are adult males, and the remaining amount are juveniles and infants (Di Fiore and Campbell, 2007). Leaves are believed to comprise at least 68% of the diet (Glander, 1978). Samples were provided to this project by KE Glander and OT Oftedal.

Callithrix jacchus and *Leontopithecus rosalia*: One milk sample from each lactating female were collected from golden lion tamarins at the Poço das Antas Biological Reserve, reintroduced golden lion tamarins from the Rio Vermelho farm, and common marmosets from the same farm. There were a total of four females from each species ($n = 4$) for a total of four milk samples each ($n = 4$). Four samples from each species (*C. jacchus*, $n = 4$; *L. rosalia*, $n = 4$) were donated to this project by ML Power. *Callithrix jacchus* and *Leontopithecus 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 (Ferarri, 1993). *C. jacchus* generally live in groups from 3 – 16 individuals consisting of 2 – 7 adult males and 2 – 6 adult females while *L. rosalia* groups are usually smaller (2 – 11 individuals), with anywhere from 0 - 4 adult males and 0 – 5 adult females (Digby et al., 2007).

Gorilla beringei beringei: Six wild living mountain gorillas ($n = 6$) provided one milk sample each ($n = 6$). Mountain gorilla samples and data were collected from the 160 km² 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 samples included in this study were collected between November 2002 and January 2005.

Mountain gorillas live in groups consisting of several adult females, immature offspring, and at least one silverback male (Robbins, 2007). The *G. 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 (C Whittier, personal observation). These include celery, goose grass, droquettia vines, thistle, and bamboo. Bamboo shoots, which are highly coveted, are seasonal and tended to coincide with the rainy seasons.

Macaca sinica: Eight wild living female wild toque macaques ($n = 8$) provided one milk sample each ($n = 8$). Females were part of a population living in the dry evergreen forests at Polonnaruwa, Sri Lanka. These macaques have been studied continuously at this location since 1969. *Macaca sinica* diets are more similar to the callitrichines than to

gorillas and mantled howlers, with approximately 75% of the diet consisting of fruit and/or seeds (Rowe, 1996). This population of toque macaques lived commensally with humans in multi-male, multi-female groups (approximately a 3:1 female to male ratio). Samples were donated to this project by W Dittus and OT Oftedal.

Table 6.2. Milk sample information on wild living anthropoid primates

Species	Institution or Organization that provided samples	Num. of Individuals	Num. of Samples	Infant Age Range
<i>Gorilla beringei</i>	Morris Animal Foundation Mountain Gorilla Veterinary Project (MR Cranfield and CA Whittier), Rwanda	6	6	7 - 878
<i>Macaca sinica</i> ^a	W Dittus, Sri Lanka	9	9	26 - 233
<i>Alouatta palliata</i> ^a	KE Glander and OT Oftedal, Costa Rica	8	8	7 – 365 ^b
<i>Callithrix jacchus</i>	ML Power, Brazil	4	4	25 - 35 ^b
<i>Leontopithecus rosalia</i>	ML Power, Brazil	4	4	30 – 60 ^b

^a Oxytocin was used in collection of samples,

^b Infant ages estimated by field researchers.

Stage of Lactation

All mammalian milks experience a colostrum and transitional stage (Oftedal and Iverson, 1995). The length of each stage is highly variable, influenced by the developmental stage of the neonate (altricial or precocial), length of lactation, and weaning rates (Oftedal, 1984; Oftedal and Iverson, 1995). For example, the hooded seal (*Cystophora cristata*) lactates almost continuously for four days. Thus, colostrum is measured in minutes, rather than in days as is the case for humans (Oftedal et al., 1988).

Length of lactation varies widely among nonhuman primate species included in this study. Lactation in callitrichines (*Callithrix* and *Leontopithecus*) is approximately three months (Garber and Leigh, 1997; ML Power, personal communication; Ross, 2003), while that of *Pan troglodytes* is approximately five years (Goodall, 1986; Kappeler and Pereira, 2003; Leigh and Shea, 1996; Ross, 2003). It is assumed that the length of each stage (colostrum through involutorial) also varies. To permit interspecific comparisons, this study followed Oftedal and Iverson's (1995) emphasis on nutritional, rather than developmental, stages to describe milk samples. Lactation was divided into three stages: early lactation, midlactation, and late lactation. Samples from early and late lactation were excluded from analyses (Table 6.3). Criteria for exclusion are described in detail below.

Rather than described in days, early lactation is defined as the period of changing composition prior to midlactation (Oftedal and Iverson, 1995). There are no comparative data available on length of early lactation in nonhuman primates. For the purposes of this

study, early lactation in humans (days 1 – 7) is regarded as representative for the Primate order. This assumption is likely to overestimate early lactation in most species included in this study, but more importantly, will exclude all questionable samples. Based on these criteria, five samples were excluded from statistical analyses (Table 6.3).

Table 6.3. Samples excluded from analyses

Species	Sample ID	Reason for Exclusion
<i>Alouatta palliata</i>	76	Early lactation (day 7 of lactation)
<i>Cebus apella</i>	26	Early lactation (day 1 of lactation)
<i>Cebus apella</i>	D63	Early lactation (day 1 of lactation)
<i>Gorilla gorilla</i>	2003-393	Early lactation (day 3 of lactation)
<i>Gorilla berengei</i>	BKA	Early lactation (day 7 of lactation)
<i>Saimiri boliviensis boliviensis</i>	10 Dec	> 183 days
<i>Saimiri boliviensis boliviensis</i>	597 Nov	> 183 days
<i>Saimiri boliviensis boliviensis</i>	688 Dec	> 183 days
<i>Saimiri boliviensis boliviensis</i>	989 Nov	> 183 days
<i>Saimiri boliviensis boliviensis</i>	1360 Nov	> 183 days
<i>Saimiri boliviensis boliviensis</i>	1360 Dec	> 183 days
<i>Saimiri boliviensis boliviensis</i>	1508 Dec	> 183 days
<i>Saimiri boliviensis boliviensis</i>	1630 Dec	> 183 days

Midlactation is defined as the period of maximum lactation performance, when infants are completely dependent on mothers for absolute nutrition requirements (Ofstedal and Iverson, 1995). Late lactation is the period of declining yields and mixed feeding (solid foods and milk consumption). Following Ofstedal and Iverson (1995), only samples

representing midlactation are included in this study's analyses. Correct categorization of midlactation and late lactation milk samples requires accompanying data on milk yield and feeding behavior of infants, available for some, but not all species in this study. Species for which information on milk yield and/or total length of lactation were available include: *Alouatta palliata*, *Callithrix jacchus*, *Gorilla beringei beringei*, *Leontopithecus rosalia*, *Macaca mulatta*, *Macaca sinica*, and *Pongo pygmaeus*.

For the remaining samples, inclusion in analyses depended on whether or not the sample was representative of the species' defined length of lactation, as provided in the life history literature (see references, Table 5.3 – 5.5), or population defined length of lactation, as provided by the primate facility or observations in the field (Table 6.4). The day of lactation for each sample was divided by the species' defined length of lactation (see references, Table 6.4), resulting in a percentage of the total length of lactation. Samples collected from *Hylobates lar*, *Pan paniscus*, *Pan troglodytes*, *Symphalangus syndactylus* fell between 5 – 30% of species' defined lactation periods and were included in analyses as midlactation. Only one sample from *Pan paniscus* was included in analyses because samples collected from the same female can not be treated as independent data points. The mean concentration was calculated for all components from day 46 and day 76, excluding fat for which there was only data from the day 76 sample.

The range of corrected stage of lactation for samples from *Cebus apella*, *Gorilla gorilla gorilla*, and *Saimiri boliviensis boliviensis* exceeded 100%. This means that sample collection dates exceeded the species' defined length of lactation. These samples were not immediately excluded from the study, however. Length of lactation (or age of

weaning) is not a clearly defined transition but rather a process. The mean value reported in the literature represents an average, indicating that there are individuals who weaned earlier and those who weaned later. Observational data for Bolivian squirrel monkeys, lowland gorillas, and tufted capuchins from the providing institutions suggest that females were nursing frequently, and milk sample volumes did not appear compromised (reduced) by day of lactation. Despite being collected after the species' mean length of lactation, these samples may be similar in composition to midlactation samples. To test this hypothesis, I plotted day of lactation against percent energy from fat, sugar and protein for *Saimiri boliviensis* (Figure 6.1), *Gorilla gorilla gorilla* (Figure 6.2), and *Cebus apella* (Figure 6.3).

The 24 total *Saimiri* samples were provided by eight lactating females. Each female contributed three samples from different days of lactation. Each sample was considered a separate data point in Figure 6.1. The proximate composition of *Saimiri* samples and the relative contributions of fat, sugar and protein to GE were stable between days 101 and 183 post partum. Milk samples collected after 183 days were significantly different from samples collected on or before 183 days in mean percent energy from fat and sugar (two-tailed t-test, $p < 0.05$). Mean percent (\pm SE) energy from fat increased from 45.97 % (\pm 1.38 %) to 54.53 % (\pm 1.95 %) ($t = -3.6$, $p = 0.002$) and mean percent energy from sugar decreased from 30.82 % (\pm 1.03 %) to 23.92 % (\pm 4.90 %) ($t = 3.9$, $p = 0.001$).

Table 6.4. Samples included in statistical analyses

Species	Num. of samples	Day(s) of lactation	Species defined lactation period (days)	Corrected stage of lactation (%)
<i>Pan troglodytes</i>	4	97 – 550	1825	5 – 30
<i>Pan paniscus</i>	1	46/76	1080	~ 7
<i>Gorilla gorilla gorilla</i>	4	108 – 1105	1000	11 – 111
<i>Gorilla beringei beringei</i>	5 (4)*	39 – 440	1000	4 – 25
<i>Pongo pygmaeus</i>	1	430	1280	34
<i>Symphalangus syndactylus</i>	1	119	639	19
<i>Hylobates lar</i>	1	54	730	7
<i>Macaca mulatta</i>	22	91 - 123	210 ^a	43 – 59
<i>Macaca sinica</i>	8	26 – 233	320	8 – 73
<i>Alouatta palliata</i>	7	39 – 180	325	12 – 55
<i>Cebus apella</i>	9	37 – 384	264	14 – 152
<i>Saimiri boliviensis boliviensis</i>	8	≤ 182	240 ^b	75
<i>Callithrix jacchus</i>	8	26-32	90 ^c	29 – 35
<i>Leontopithecus rosalia</i>	5	30-60	90 ^c	33 – 67

*Five samples were used for fatty acid analysis and four were included in proximate analysis

^a Personal communication KJ Hinde

^b Personal communication S Gibson

^c Personal communication ML Power

Compiled from Bauchot and Stephan (1969), Dittus (1975), Fossey (1979), Fragaszy and Adams-Curtis (1998), Garber and Leigh (1997), Gibson et al. (1993), Hartwig (1996), Kappeler and Pereira (2002), Leigh and Shea (1996), Ross (1991, 2002), Smith and Jungers (1997), Smith and Leigh (1998), Thierry (2007) and Williams et al (1994)

There was no significant change in percent energy from protein between these two time periods ($t = 1.5$, $p = 0.16$). Samples were excluded after day 183 of lactation. Means by individual mother were calculated for samples collected on or before day 183, resulting in eight data points for Bolivian squirrel monkeys. Change in milk composition at approximately six months postpartum coincides with species-defined lactation patterns. Peak lactation for this group of *Saimiri boliviensis boliviensis* is estimated at four to six months, although many females were observed to nurse for up to 9 months [CNPRR

unpublished data]. Changes in *Saimiri* samples after peak lactation suggests that samples later in lactation may differ from those representing midlactation, particularly in percent energy from fat.

For lowland gorillas (Figure 6.2), increasing infant age is associated with an increase in percent energy from protein and a decrease in percent energy from sugar. Sample sizes were too small to permit statistical tests of these differences, but the stability of percent energy from fat and the small changes in contribution of energy from sugar and protein suggests that the sample from day 1105 (provided by Zoo Atlanta) can be considered midlactation (Oftedal, personal communication), and were included in analyses.

Cebus samples collected prior to day 264 are lower in mean percent energy from fat and higher in mean percent energy from protein than samples collected after day 264 (Figure 6.3). However, a sample from day 348 of lactation is more similar to samples collected prior to day 264 than it is to those collected after day 264, suggesting that changes in milk composition may not be related to day of lactation and may instead approximate the range of intraspecific variation found in tufted capuchins. The decision was made to include all *Cebus* samples, except those that represent early lactation, based on the observation that the relative contributions of percent energy from fat, protein, and sugar were consistent over time – approximately 50% of energy came from fat, 30% from sugar, and the remaining 20% from protein.

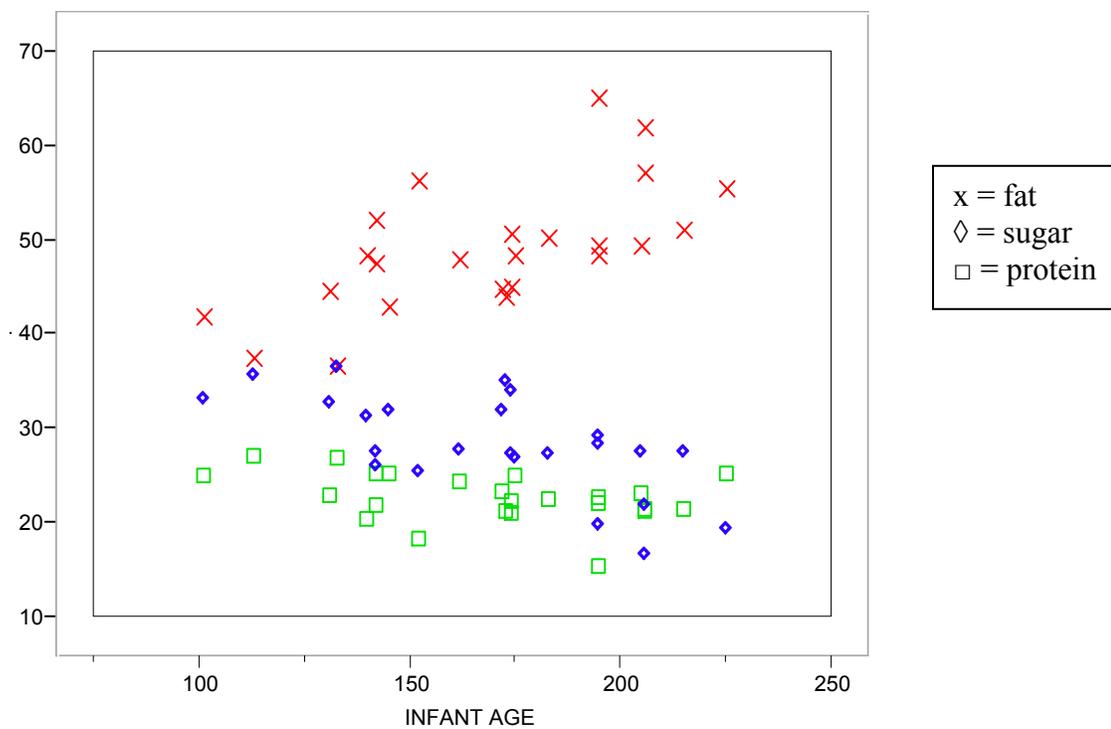
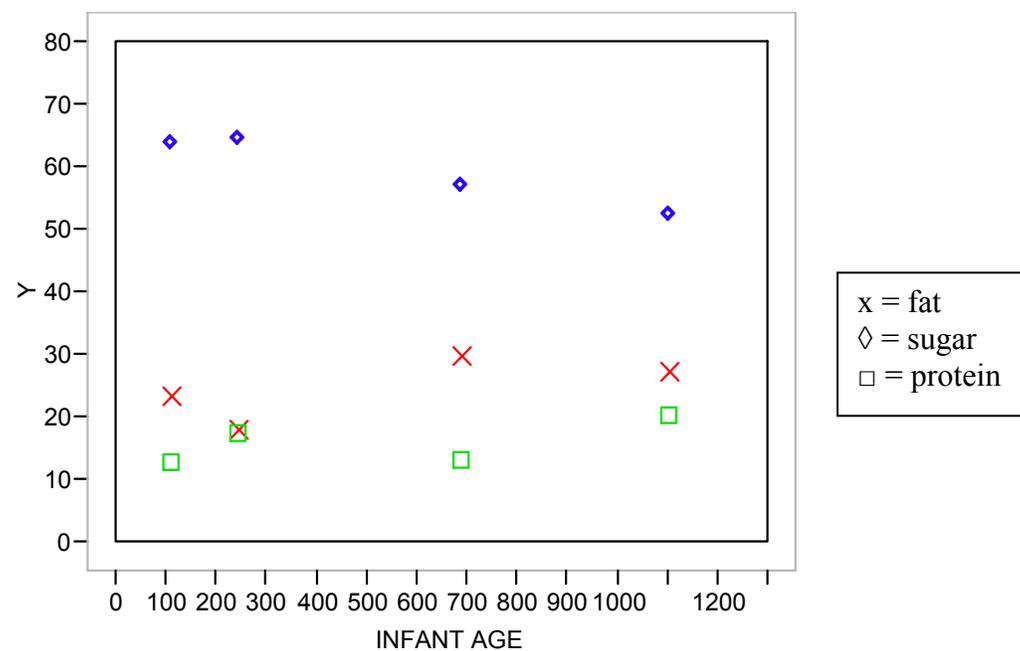
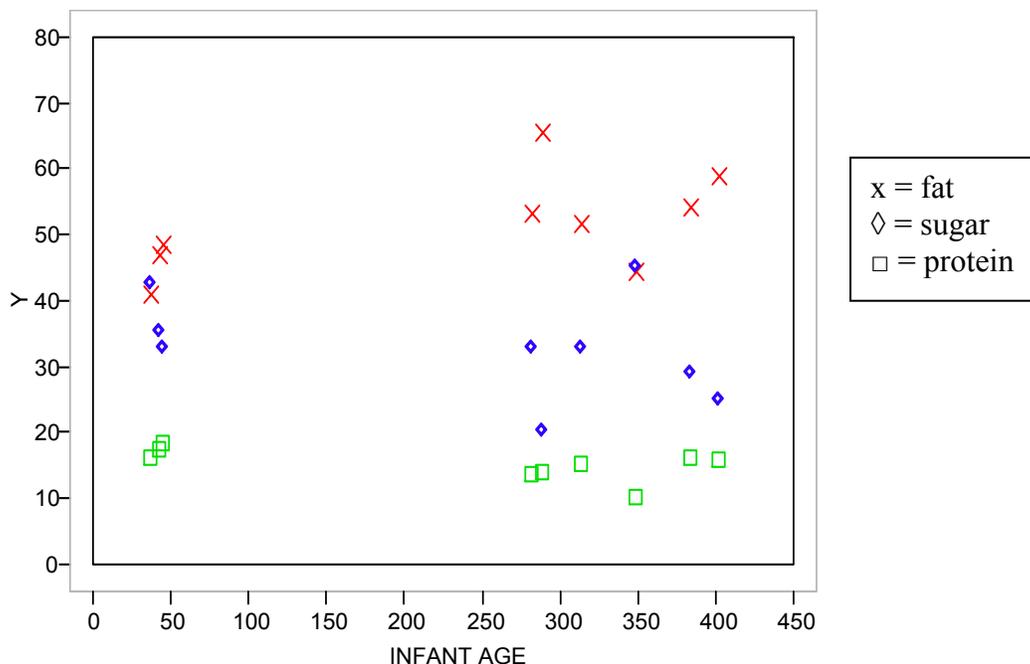
Figure 6.1. *Saimiri*: Percent Composition of Fat, Sugar and Protein by Infant Age (days)Figure 6.2. *Gorilla*: Percent Composition of Fat, Sugar and Protein by Infant Age (days)

Figure 6.3: *Cebus*: Percent Composition of Fat, Sugar and Protein by Infant Age (days)
 X = Fat, \diamond = Sugar, \square = Protein



Sample Volumes

The volume of individual milk samples varied widely within and between species. Samples from *Gorilla beringei beringei*, *Pan paniscus*, *Saimiri boliviensis*, and wild *Callithrix jacchus*) were less than or equal to 1 ml, while the *Symphalangus syndactylus* sample, one *Gorilla gorilla gorilla* sample (day 1105), and several *Cebus apella* samples were over 10 ml in volume. The majority of samples were between 1 and 3 ml in volume. Assays for proximate (fat, sugar, protein, dry matter, and minerals) and fatty acid composition could be performed with as little as 0.5 ml of milk. Sample size only affected the analysis of one *Gorilla beringei beringei* sample which was approximately

0.25 ml in volume. This sample was assayed for fat and fatty acid composition only (Table 6.4) and only appears in comparative analysis for fatty acids.

Summary

This dissertation investigates the nature of variation in milk composition among anthropoid primates. Milk samples were collected from 14 species of anthropoid primate representing each of the three anthropoid superfamilies. Three species were represented by wild living populations, two species were represented by both wild- and captive living individuals, and nine species were represented by captive living populations. There were a total of 84 samples, 17 from hominoids, 30 from cercopithecoids, and 37 from ceboids. These samples represent midlactation (Ofstedal and Iverson, 1995) for each species, allowing for interspecific comparisons. All samples (except for one sample from *Gorilla beringei beringei*) were of sufficient volume for all necessary milk assays.

CHAPTER 7: METHODS OF ANALYSIS

Introduction

The methods for subsampling and proximate (fat, sugar, crude protein, dry matter and minerals) analyses followed standardized methods from the Nutrition Laboratory, Department of Conservation Biology, Smithsonian's National Zoological Park, Washington, D.C. These methods had been previously applied to nonhuman primate milk samples (Ofstedal and Iverson, 1995; Power et al., 2002; Tilden and Ofstedal, 1997), providing this study with important information regarding appropriate volumes of sample required for each assay. Adoption of these methods also permitted direct comparison to published results on nonhuman primate milk. Methods to determine fatty acid composition followed standardized methods used by RP Bazinet and SI Rapoport at the Brain Physiology and Metabolism Section, National Institute of Aging, National Institutes of Health, Bethesda, MD (cf. Bazinet et al., 2003).

Each assay is a micro-procedure, developed to extract nutritional information from small volumes of milk. This was particularly important for this study because many samples were less than 1.0 ml in volume. Subsampling was performed in the following order: fat, fatty acid, dry matter (crude protein), sugar, mineral digest, residual. Priority was given to fat and fatty acid for two reasons. First, these components of milk composition were most applicable to this project's research questions. Second, these data would make the largest contribution to the scientific community. This study was the first to systematically investigate fatty acid composition from wild- and captive living anthropoid primates.

Methods of Milk Analysis

Subsampling

All samples were maintained in a -20° C freezer until removed for subsampling or analysis. To minimize the number of times a sample was defrosted, each sample was thawed and simultaneously subsampled for all milk assays. Samples that were less than 1 ml in volume were allowed to thaw at room temperature and those greater than 1ml were placed in a warm water bath, maintained at 50° C, in order to ensure rapid and uniform thawing. Samples were vortexed prior to each subsampling procedure. Subsamples were done in duplicate, except those for fatty acids. In each procedure, weights (g) of samples were recorded. This is an especially important step for milk analyses because percent fat and other properties of the milk sample can lead to significant variation in the relationship between dispensed volume of sample and sample weight. All results were reported on a weight-for-weight basis, but will be presented as percent of total composition (100 x weight in grams). Figure 7.1 summarizes the order of subsampling and volume of milk required for each procedure.

(1) Fat (Total Lipids): For fat analysis, approximately 125 µl of milk was delivered into 2 ml centrifuge tubes using a 250 µl positive displacement pipetter (PDP) and the weight of the sample was recorded to 0.0001 g. Tubes were sealed with parafilm and placed immediately into the freezer.

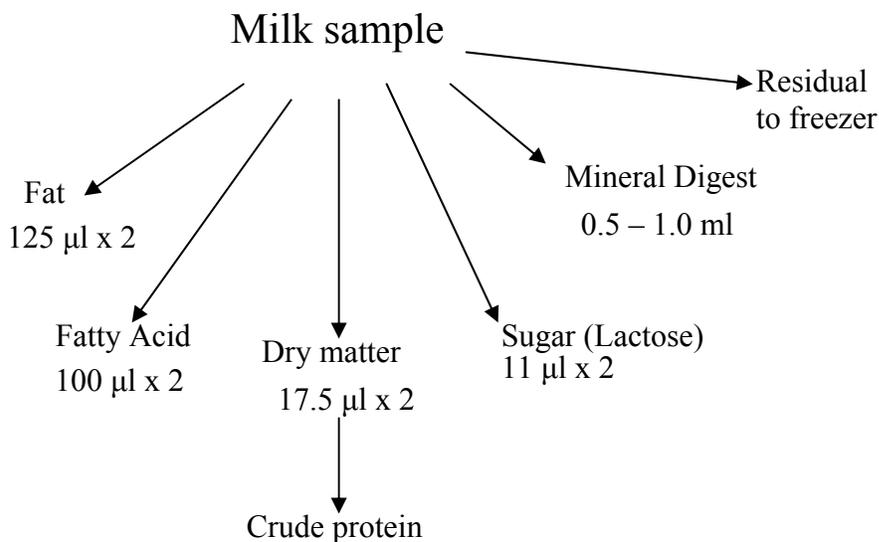
(2) Fatty Acids : Fatty acid analysis requires approximately 100 μ l of sample, dispensed into 20 ml Kimex glass centrifuge tubes using a 250 μ l PDP. Sample weights were recorded to 0.0001 g. Samples were capped and immediately frozen.

(3) Dry Matter and Crude Protein: The procedure for determining crude protein also determines the amount of total solids, or dry matter, in each sample. The 25 μ l PDP was used to dispense approximately 17.5 μ l into pre-weighed tin foil capsules. Total weight was recorded to 0.001 mg. Capsules were placed directly into a forced-air drying oven at 100° C for three hours, allowed to cool to room temperature in a dessicator, and then reweighed to 0.001 mg. Remaining samples were pipetted into cryovials ranging from 1.0 – 5.0 ml in volume.

(4) Sugar: Sub-sampling for sugar requires approximately 11 μ l of sample be delivered by a 25 μ l PDP into a tared 30 ml Nalgene bottle. Sample weight was recorded to 0.0001 g and distilled water was added until the total weight reached approximately 25 g. Final weights were recorded to 0.0001 g, bottles were capped and placed into freezer.

(5) Mineral Digest and Residuals: Due to variation in original sample size, residual milk volumes varied from less than 1 ml to 12 ml. For each milk sample with sufficient residual volume, a 1 ml cryovial was labeled and stored for mineral digest, with remaining vials labeled for additional potential assays, *e.g.*, secretory Immunoglobulin A (sIgA) and oligosaccharides. Residuals were placed immediately into a -20 °C freezer.

Figure 7.1. Subsampling protocol for each milk sample. Subsampling proceeded in order from left to right.



Fat

Total lipid was assayed by a micro-modification of the Rose-Gottlieb procedure (AOAC, 1975). Milk samples were treated with ammonium hydroxide (NH_4OH) to dissolve proteins and ethyl alcohol to assist in precipitation of proteins. This was followed by the addition of diethyl and petroleum ether to extract fats. Samples were centrifuged for five minutes. The top layer was transferred via pipette into an aluminum pan. Fats were extracted a total of three times, the second time with alcohol and ethers and the last time with only ethers. After sequential extractions, aluminum pans were placed in a 100°C forced-air drying oven for 20 minutes and then cooled in a desiccator for at least 25 minutes before recording their weights to 0.0001 g.

Extracted lipids were removed from each pan by dispensing heated petroleum ether into the pan, gently swirling the pan, and then pouring off all liquids. Care was taken not to remove any undissolved material from the pan. This sequence was completed three times for each sample to ensure that all fats had been removed. Pans were placed into the 100° C forced-air drying oven for 20 minutes, allowed to cool in a dessicator for at least 25 minutes, and then weighed to 0.0001 g. The weight of the fat in the sample was calculated by the difference in weights of the pan before and after washing with petroleum ether, and the percent fat as the fat weight divided by the total weight of milk sample used.

The concentration of fat in human breast milk is highly variable. Fat increases in concentration from foremilk to hindmilk (Jenness, 1979; Prentice, 1996; Stini et al., 1980) as well as over the course of the day (Jenness, 1979). Previous research on nonhuman primates and other mammals show similar patterns of variation. Although efforts were made to homogenize each sample prior to subsampling for the microfat assay, this project could not control for the time of day of sample collection nor complete evacuation of each mammary.

Fatty Acids

Two internal standards [20 µl of triheptadecanoic acid (17:0) and 50 µl of methyl docosatriaenoic acid (22:3n-3)] were added to samples prior to the addition of any chemicals. Human milk samples were run with and without internal standards to validate

that neither of these fatty acids is present in human milk¹. The addition of internal standards as the initial step in the protocol serves several purposes. First, the addition of 17:0 (a triglyceride, or three fatty acids on a glycerol backbone) provides a check that the methylation process was completed. Second, the amount of internal standard in each sample is proportional to the total fatty acids in the sample. The concentration of 17:0 was a known value used to calculate the concentration of individual fatty acids. Should any of the samples be spilled during preparation, or should all the lipid layers not be fully extracted, future calculations remain unaffected because a proportional amount of the internal standard was also lost with milk fatty acids. The 22:3n-3 internal standard was used as an additional check for the concentration of 17:0. The concentration of 22:3n-3 in each sample should be approximately equal to that of 17:0. Discrepancy between the internal standards would indicate a problem with the extraction process, methylation process, or reading by the gas chromatographer. Although the use of a second internal standard is rare, the 22:3n-3 standard was utilized in this project to ensure accuracy of fatty acid data for each sample.

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 (H₂SO₄) solution in methanol (MeOH). Fatty acids methyl esters were formed by heating this solution at 70° C for 3 hours (Bazinet et al., 2003; Makrides et al., 1994). One milliliter of water and 3 ml of

¹ Concentration of fatty acids were not determined for human milk samples. Human milk samples were not used to validate any other assay because all proximate assays had been previously applied to nonhuman primate milk.

heptane were added and samples were centrifuged. Heptane is fat soluble, and forms the top layer in the centrifuged solution. This layer was pipetted into a glass centrifuge tube and placed into a speed vacuum (AE S1010, Savant Instruments Inc., Farmingdale, New York) until solution had completely dried. Each sample was then reconstituted with 1.5 ml trimethylpentane (isooctane).

Methyl esters were separated on a 30 m x .25 mm internal diameter capillary column (SP-2330; Supelco, Bellefonte, Pennsylvania) by gas chromatography with a flame ionization detector (Model 6890N; Agilent Technologies, Palo Alto, California). Runs were initiated at 80° C, with a temperature gradient to 160° C (10°/min) and 230° C (3° C/min) in 31 minutes and held at 230° C for 10 minutes. 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, Minnesota) and pure eicosapentaenoic acid. Absolute fatty acid concentrations (mg/g 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 standard (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.

Dry Matter

Dry Matter (DM), or total solids in each sample, was measured gravimetrically after drying 17.5 µl of sample for three hours at 100° C in a forced-air drying oven. Samples were weighed to 0.001 mg both before and after drying to determine percent dry

matter. Dried samples were placed in a sealed plastic tray inside of a dessicator for use in crude protein analysis.

Crude protein

Crude protein was estimated from total nitrogen (TN) in each milk sample. Nitrogen was assayed using a Carbon-Hydrogen-Nitrogen (CHN) elemental gas analyzer (Model 2400, Perkin-Elmer, Norwalk, CT), which provides a rapid and accurate method of assaying TN in small volume 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 cow's milk. Samples were prepared for analysis on the CHN following the protocol for determination of dry matter in each sample. After removing from the oven and recording dry weights, samples were placed in a sealed plastic container inside of a dessicator to prevent any change in sample weight. Samples remained in the dessicator until CHN analysis. The percent of crude protein (CP) in each sample is calculated as: $6.38 \times \text{percent total nitrogen} \times \text{percent dry matter}$. Cow milk samples (17.5 μl) and National Institute of Standards and Technology (NIST) standard milk powders were used as check standards and were analyzed at specific intervals during runs of primate milk samples to ensure consistency of the nitrogen reading.

Sugar (Lactose)

Total sugar was assayed by the phenol-sulfuric acid method, using lactose monohydrate as the standard (Dubois et al., 1956; Marier and Boulet, 1959). Sulfuric acid

hydrolyzes mono-, di- and oligosaccharides. When these hydrolyzed sugars are hot, they react with phenol and form a stable yellow-orange chromogen. After this solution has cooled, the concentration of sugar is measured using a ultra-violet (UV) spectrophotometer (Beckmann DU 640 Spectrophotometer).

A standard curve was created through use of a blank and five standard solutions made from a lactose stock solution (Table 7.1) of known lactose concentration. Standards consist of between 1 and 5 ml of 1,000 ppm lactose stock solution, increasing by 1 ml increments for each standard that have been diluted with approximately 100 g of distilled water. Prior to the addition of stock solution, the 100 g Nalgene bottle was tared. Desired volume of stock solution was added with a 1 ml digital pipette, and the weight was recorded (0.0001 g). Distilled water was added until final solution weight reached approximately 100 g. Final standard solution weight was recorded (0.0001 g) and concentration of each standard was calculated as the product of the concentration of the stock solution and the weight of the stock solution added (between approximately 1 to 5 g,) divided by the total weight of the standard solution (approximately 100 g). The range of standard concentrations used in this project was 10.394 $\mu\text{g/ml}$ to 51.805 $\mu\text{g/ml}$.

One milliliter of heated diluted phenol (11%) and 7.4 ml of sulfuric acid were added to 1.6 ml of each standard, for a total solution volume of 10 ml. Blanks were made by substituting 1.6 ml of distilled water for standard solution. After the addition of phenol and sulfuric acid, the solution was vortexed and permitted to cool at room temperature for 10 minutes. Each tube was then placed into a room temperature water bath until they were read on the UV spectrophotometer. The blank and five standards were aspirated and

the absorbance was measured at 490 nm. Based on known concentrations of standards and assumed concentration of 0 mg/g for the blank, a standard curve was calculated.

Sample solutions were created with 1.6 ml of each sample (approximately 11 μg of milk diluted with distilled water to a total of 25 g), and the same volumes of phenol and sulfuric acid as were the standards. Each sample was aspirated three times and mean percent absorbance was measured directly and converted to a concentration value through the use of the standard curve. Results were expressed on an anhydrous lactose base (calculated concentration $\times .95$) because standards were made from lactose α -monohydrate. Percent sugar in a sample was calculated as the mean concentration given by the UV spectrophotometer divided by the concentration of milk (μg milk/mg of H_2O) in each sample.

Mineral Digest

Prior to analyzing samples for mineral content, all organic material must be removed. This was accomplished by digesting 0.5 – 1.0 ml of sample in 20 ml of 70% nitric acid and 5 ml of 70% perchloric acid. Samples were pipetted into a pre-weighed 125 ml flask and weighed to 0.0001 g. Nitric and perchloric acids were added, flasks were covered with watch glasses and immediately placed on hotplates inside a perchloric acid fume hood. Samples then were subjected to several digestion stages. During the first stage, the sample turns burnt orange and emits orange puffs of smoke, signifying the oxidation of organic material by the nitric acid. This stage was followed by the digestion of the majority of the organic material. The nitric acid was boiled off, and the final stages

involved digestion of sample by perchloric acid. Flasks were removed from the hotplates once the sample volume reached approximately 1 ml. A small amount of Millipore water was added immediately from a squirt bottle (approximately 1 – 2 ml), and when the flask cooled (approximately 2 – 3 minutes), an additional 5 ml of Millipore water was added. Cooled flasks were weighed to 0.0001g and, subtracting flask weight, digested weight was brought up to approximately 25 g through the addition of Millipore water. Digested solutions were transferred into labeled 30 ml Nalgene bottles and kept at room temperature for use in phosphorus and calcium procedures. Both the original weight of the sample (between 0.5 – 1.0 g) and the final weight of the digested sample are used in calculations to determine the percentage of minerals in each sample.

(a) Phosphorus

The percent phosphorus in each sample is determined by a colorimetric procedure similar to that used to determine percent lactose. Standards and samples were aspirated through an ultra-violet (UV) spectrophotometer (Beckmann DU 640 Spectrophotometer) to determine absorbance, which was then converted to a concentration ($\mu\text{g/ml}$) from use of a standard curve (seven standards, the first of which is a blank, each run in duplicate). The standards were created by weighing 1 ml aliquots (volume dispensed determined by desired concentration of standard) of a stock solution (Table 7.1) with known phosphorus concentration to 0.001 g. Each standard was brought to a final weight of approximately 100 g by the addition of Millipore water. Final standard concentrations prepared for this project ranged from 1.01 to 9.98 ppm (parts per million). Standards (1 ml) and samples (1

– 4 ml)² were pipetted into tared glass tubes, and weights were recorded (0.0001 g). Millipore water and molybdovanadate (MV) reagent (Table 7.1) were added to each sample to reach a final solution volume of 10 ml. Each solution is 20% (2 ml) MV solution, with the amount of Millipore varying relative to the aliquot volume of each sample. Each sample tube was aspirated three times and the concentration was calculated from the mean absorbance reading.

Concentration of phosphorus (ppm) in each aliquot of digest was calculated by multiplying the concentration value provided by the UV Spectrophotometer by the proportion of aliquot relative to total solution volume. Concentration of phosphorus in each milk sample (ppm) was then determined by multiplying this value by the dilution factor of the original milk sample, prior to perchloric digestion. The dilution factor is simply the total solution weight after sample digest (approximately 25.000 g) divided by amount of milk sample added to flask before digest (between 0.500 – 1.000 g).

(b) Calcium

The percent calcium in each milk sample was determined by analysis of digested samples on the Atomic Absorption (AA) Spectrophotometer (Perkin Elmer Life and Analytical Sciences, AAnalyst 800 flame/furnace, 2001). The AA Spectrophotometer measures concentration of calcium in each sample through the use of a lamp set to a wavelength that will excite only calcium atoms. The autosampler aspirates a small

² Sample volume varied based on the estimated concentration of phosphorus in each sample. Both *Macaca* and several New World monkey species had been previously run, giving approximate values for phosphorus. Ape digest samples were run at various volumes to determine the smallest amount necessary that would give concentrations at the middle of the standard curve.

amount of each sample, which is then sprayed into the mixing chamber. Here, gases – acetylene and nitrous oxide - carry the sample into the flame. The amount of light that passes through the flame at that time will reflect the number of calcium atoms in the light path. Samples with a low calcium concentration have fewer calcium atoms, therefore absorbing less light than those with a high concentration. The AA spectrophotometer determined the level of absorption for each sample, which was then converted to a concentration (ppm) based on known values from the standard curve.

Six ‘normal’ standards and one high purity standard were used to create a standard curve. The desired range for a standard curve at the Nutrition Laboratory for determining calcium in primate milk samples is 0.500 to 2.500 ppm (M Jackubasz, personal communication). A calcium stock solution of known calcium concentration was created (Table 7.1). To make each standard, the desired volume of stock solution was added to a tared 50 ml Nalgene bottle, weight was recorded (0.0001 g), and final weight was brought to approximately 125 g by the addition of Millipore water. The concentration of calcium in each standard solution (ppm) was calculated by multiplying the concentration of the stock solution by the weight of the stock solution added and dividing this product by the total volume of the standard. The high purity (HP) standard used for primate milk assays was a duplicate of standard 3 (approximately 1.5 ppm). The AA Spectrophotometer requires a HP standard as an internal check to ensure continuity throughout the entirety of the run. Concentrations of Standards 1 – 6 and the HP standard were entered into the AA Spectrophotometer software, and a standard curve was created.

Samples for calcium analysis were made with aliquots of digested milk samples and Millipore water. The volume of digested sample added varied between approximately 0.3 and 2.0 g. To estimate the calcium concentration in each digested sample necessitated data on concentration of phosphorus in each sample, as the relative concentration of these two minerals in milk is correlated. Concentration of each sample also needed to take into account the small range of standard concentrations range permitted by the AA Spectrophotometer. Each pre-determined sample volume was added to a tared 15 ml Nalgene tube and weight was recorded to 0.0001 g. Final volume of all samples was brought to 10 ml through the addition of Millipore water and final weight was recorded. Duplicates for each sample were made, using identical weights of digest diluted to 10 ml with Millipore water.

Samples were read by the AA Spectrophotometer and a printout was generated providing the mean concentration ($\mu\text{g/g}$) of the three readings by the autosampler. The percent Ca in each sample was calculated by first multiplying the concentration of Ca (AA spectrophotometer reading) by the percent of digested solution in each sample (total volume/aliquot of digest added). This product was then multiplied by the percent of original milk sample in the total digest solution. A final multiplication by 10^5 was required to go from ppm to percent calcium in each milk sample.

Table 7.1. Stock solutions and reagents used in assays

Solution or Reagent	Protocol for preparation
Lactose stock solution (1 mg lactose/g)	Weigh approximately 1 g of α -monohydrate powdered lactose (0.001 g) in a tared weigh boat. Add to a tared 1000 ml Nalgene bottle, and bring final weight to approximately 1000 g through the addition of distilled water. Record final weight (0.001 g). Concentration of lactose in the stock solution calculated as the weight of the lactose powder divided by the total solution weight.
Phosphorus Stock Solution (0.1 mg P/ml)	<p>Dry KH_2PO_4 overnight at 60°C. Weigh 8.788 g dried KH_2PO_4 on a weigh boat and record weight. Add to a tared 1 L volumetric flask and fill to volume with Millipore water. Record final weight (0.001 g). To further dilute to 0.1 mg P/ml, tare an additional 1 L volumetric flask. Add approximately 50 g of solution and record the weight (0.001 g). Fill to volume and record the final weight. Concentration of phosphorus in the first solution (P_c1) is calculated as:</p> $\frac{[\text{Weight of dried } \text{KH}_2\text{PO}_4 \times 0.2276 \text{ (known value)}]}{\text{Total solution weight}} \times 1000$ <p>Concentration of phosphorus in the final solution (P_c2) is calculated as:</p> $\frac{\text{Weight of original solution (1)} \times P_c1}{\text{Total solution weight (2)}}$
MV reagent	Weigh out approximately 40 g ammonium molybdate and 2 g of ammonium metavanadate and dry overnight at 60°C . In a 1000 ml beaker, dissolve 40 g ammonium molybdate in 400 ml hot distilled water and cool. In a 500 ml beaker, dissolve 2 g ammonium metavanadate in 250 ml hot distilled water and cool. Add dissolved ammonium metavanadate to a 2 L volumetric flask. Add 250 ml perchloric acid, mix and allow solution to cool again. Add ammonium molybdate solution to flask while stirring, and bring volume up to 2 ml with cold distilled water.
Calcium Stock Solution (1000 μg Ca/ml)	Dissolve approximately 2.5 g of dried calcium carbonate (CaCO_3) in 50 ml of Millipore water. Add drop-by-drop a minimum volume of 6N HCl (approximately 10 ml) to effect complete solution of the CaCO_3 . Dilute the resulting solution quantitatively to a volume of 1 L with Millipore water. Final concentration is approximately 1000 $\mu\text{g}/\text{ml}$ Ca.

Calculations: Energy Content of Milk

Energy from fat, protein, and sugar were calculated using the following energy value (following Oftedal, 1984; Oftedal and Iverson, 1995; Power et al., 2002): 9.11 kcal/g for fat; 5.86 kcal/g for crude protein; and 3.95 kcal/g for sugar. Total gross energy for each sample was calculated as the sum of energy from fat, protein, and sugar. It should be noted that calculations of energy from crude protein, and therefore total gross energy and percent energy from fat, protein, and sugar, may be overestimated in this study. Crude protein was estimated from total nitrogen in each sample but does not account for nitrogen from nonprotein sources (e.g., free amino acids, nucleotides, uric acids). In human milk, 0.038% to 0.046% of protein is nonprotein nitrogen (NPN) (Lönnerdal and Atkinson, 1995). It is assumed that nonhuman primate milk will be similar to human milk in the percent protein as NPN. Therefore, calculations of energy from protein for samples in this study were only very small overestimates. Comparisons among samples were not affected because all samples were calculated without accounting for NPN. Methods for estimation of crude protein also were unable to distinguish between nutritional and non-nutritional protein sources. Non-nutritional sources of protein in milk include antibodies (e.g., sIgA) and other immune components (e.g. neutrophils, lysozyme, lactoferrin). These nitrogenous proteins are part of the whey protein fraction of milk and are not a source of energy to the developing neonate and infant (Lönnerdal and Atkinson, 1995). Their contribution to the total crude protein in primate milk is unknown (but see Lönnerdal et al., 1984; Milligan, 2005), but is assumed

to be similar among primate species included in this study. Although individual values for GE and percent energy from protein may be overestimated (which in turn affects percent energy from fat and sugar), there is internal consistency within the data set and with other datasets that employed identical methods to determine CP in milk (Power et al., 2002; Tilden and Oftedal, 1997), permitting comparisons among and between species' values.

Methods of Quantitative Analysis

Previous analyses of nonhuman primate milk composition have been limited to intraspecific comparisons (e.g., Power et al., 2002, in press; Tilden and Oftedal, 1997) or descriptions of the milk of primates as a whole (e.g. Oftedal, 1984; Oftedal and Iverson, 1995), and statistics were primarily descriptive, including means and standard deviations (or standard errors) of individual milk components (e.g., fat, protein, lactose). Descriptive statistics are a necessary first step in research on nonhuman primate milk composition because they provide a foundation for future research questions and statistical inquiries. Descriptive statistics on proximate milk composition follow Power et al. (2002) and Oftedal and Iverson (1995) in presenting means and standard deviations of species and means and standard errors of groupings above the species level for fat, crude protein, lactose, dry matter, calcium and phosphorus. Descriptive statistics on fatty acid composition of nonhuman primate milk follow the human literature (Gibson and Kneebone, 1981; Koletzko et al., 1992, 2001b; Jensen et al., 1995; Specker et al., 1987;

Yuhas et al., 2006) and Iverson and Oftedal (1995) by reporting mean concentration (mg of fatty acid per g of milk) and mean percent composition (percent of total fatty acids), (plus or minus standard errors) for individual fatty acids. Absolute values are the raw data calculated by the proportional comparison of gas chromatography peak areas with the internal standard fatty acid. Percent composition of fatty acids is a measure of relative concentration and is the most widely used method for reporting fatty acids in the literature (both human and nonhuman) because it controls for variation in total fat in milk samples.

Choices for inferential statistical tests were guided by research questions and the nature of the data set. Principal components analysis (PCA) was employed to identify patterns in milk composition, an extremely useful first step when few *a priori* predictions about the data set (particularly fatty acid profiles) had been formulated. Differences in means among species, families, and superfamilies were compared using analysis of variance (ANOVA). Pairwise comparisons (differences between species, families, and superfamilies) were performed with either two-tailed t-tests or Tukey-Kramer tests depending on the size of the comparative samples. Correlation between milk constituents was determined through both parametric and nonparametric methods. Pearson's product moment correlation was used when both variables were normally distributed and Spearman's rank correlation coefficient was employed when the relationship between two variables was not assumed to be linear.

Methods of quantitative analyses for milk fatty acid profiles are described in detail in Chapter 8 and those for proximate milk composition in Chapter 9. Statistical

significance for all tests was set at < 0.05 . Analyses were performed in JMP 5.1.2 (SAS Institute, 2004) and SPSS 13.0 (SPSS, 2004).

CHAPTER 8: FATTY ACIDS IN ANTHROPOID PRIMATE MILK

Introduction

Fatty acids in milk are supplied from maternal plasma (maternal diet or maternal depot fat) or are manufactured (via *de novo* synthesis) in the mammary gland (Del Prado et al., 1999; Iverson and Oftedal, 1995; Prentice, 1996; Sauerwald et al., 2001; Stini et al., 1980). Medium chain fatty acids such as octanoic acid (8:0) and decanoic acid (10:0) can be synthesized 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 through dietary sources. Fatty acid synthesis by the mammary gland may vary by species (Dils et al., 1977; Iverson and Oftedal, 1995) and certain mammalian species may have higher levels of mammary gland lipogenesis. However, the proportion of fatty acids synthesized by the mammary gland depends on the proportion of fatty acids from dietary or depot fat stores (Iverson and Oftedal, 1995; Jensen et al, 1995; Koletzko et al., 1992). 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. Human diets low in fat and high in carbohydrates lead to increased *de novo* synthesis of fatty acids with 12 – 14 carbons (Koletzko et al., 1992). Thus, milk composition is highly plastic and 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 directly from the maternal plasma (from the diet or depot fat stores) or as metabolites of precursor fatty acids in maternal plasma. 18:2n-6 is the precursor for all omega-6 (n-6) PUFA, including arachidonic acid

(20:4n-6) and 18:3n-3 is the precursor for all omega-3 (n-3) PUFA, including docosahexaenoic acid (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 (Brenna, 2002; Carlson, 1999; Jensen et al., 1995), the ratio of n-3 to n-6 PUFA in the diet (Brenna, 2002; Huang and Brenna, 2001) and the ability to convert n-3 and n-6 PUFA into their longer chain metabolites (Agostoni et al., 2001; Brenna, 2002; Carlson, 2001). Variation in conversion efficiency among species may produce different LCPUFA milk fatty acid profiles despite similar dietary intakes of n-3 and n-6 precursors.

It follows that fatty acid profiles are not simply a reflection of the supply of fatty acids in the maternal diet but instead reflect the interaction of maternal abilities to synthesize and elongate fatty acids with the concentration and proportion of fatty acids in the maternal diet and depot fat stores.

Research Questions

In this chapter I investigate how the relationship between diet and physiology is negotiated among anthropoid primates. Specifically, I address the nature of variation of milk fatty acids from the perspective of diet, phylogeny and ontogeny to identify primitive, shared-derived and unique-derived features among anthropoid primates. The following questions are addressed:

(1) What is the effect of diet on fatty acid profiles of anthropoid primates? Is there a relationship between fatty acids in the maternal diet and fatty acids in milk?

- (a) How does captivity affect *de novo* synthesis of medium chain fatty acids and the concentration of PUFA and LCPUFA? Does a diet higher in saturated fats affect the concentration of medium chain fatty acids? Does the inclusion of certain PUFA and LCPUFA in captive diet formulas affect the milk fatty acid profiles of captive living individuals?
- (b) How does the percent of leaves in the diet (degree of folivory) affect milk fatty acid profiles among wild groups of anthropoid primates? Do high amounts of 18:3n-3 found in leaves translate to high proportions of 18:3n-3 in the diet?
- (2) Do milk fatty acid profiles show any phylogenetic patterns? Is milk composition constrained by evolutionary history?
- (a) Do anthropoid primates that are more closely related show more similar milk fatty acid profiles, regardless of differences in dietary strategy? Is the milk of chimpanzees more similar to that of other hominoids than it is to other omnivorous primates, such as tufted capuchins and squirrel monkeys?
- (b) Are there patterns in milk composition that are shared between wild and captive individuals of the same species (or genera, family, superfamily)? Are there aspects of milk composition that are under genetic control and are unrelated to dietary intake?
- (3) Do milk fatty acid profiles vary with respect to life history traits?
- (a) Is there a relationship between growth rates/patterns and milk fatty acid composition? Do anthropoid primates that grow and develop at a faster rate (age

at first reproduction relative to life span) share any similarities in milk fatty acid profiles?

(b) Is there a relationship between relative neonatal and/or adult brain size and milk fatty acid composition? Do anthropoid primates with larger relative brain size (represented by encephalization quotients) have higher concentrations/proportions of fatty acids found in high quantities in lipid membranes, such as 20:4n-6 and 22:6n-3?

(4) Do human milk fatty acid profiles share patterns with other anthropoid primates? Are values reported for human milk fatty acids within the range of variation identified for anthropoid values?

Materials and Methods

Milk samples

Milk samples from 14 species representing each of the three anthropoid superfamilies were analyzed for fatty acid composition. Samples were collected from wild groups of *Alouatta palliata*, *Callithrix jacchus*, *Gorilla beringei*, *Leontopithecus rosalia*, and *Macaca sinica* and captive groups of *Callithrix jacchus*, *Cebus apella*, *Gorilla gorilla*, *Hylobates lar*, *Leontopithecus rosalia*, *Macaca mulatta*, *Pan troglodytes*, *Pan paniscus*, *Pongo pygmaeus*, *Saimiri boliviensis boliviensis*, and *Symphalangus syndactylus*.

Methods of milk analysis

Fatty acids were determined by GC with a flame ionization detector (Chapter 7). This method allows for determination of the types of fatty acids present in a sample and the concentrations of each. The output from a GC is a figure of peaks, with time (minutes in columns) on the X axis and units of peak height (pA) on the Y axis. The amount of time that each fatty acid spends in the column of a gas chromatographer is dependent on the number of carbons and double bonds in each fatty acid. That is, samples with more carbons and more double bonds will be released by the columns later in time. The area under each peak (= area under the curve, calculated as an integral) corresponds to the concentration of each fatty acid in the sample. A high concentration could be represented by tall, thin peaks as well as short, wide peaks. Peaks were identified by comparison with retention times of fatty acid methyl ester standards and pure eicosapentaenoic acid.

Absolute fatty acid concentrations (mg/g 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 standard (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. The application of these methods is summarized in Dodds et al. (2005).

A total of 27 fatty acids were identified (Table 8.1). Separate peaks (two peaks without a distinct zero point on the X axis in between) did not always form for 18:1n-9 (oleic acid) and 18:1n-7 (vaccenic acid). Because they are so similar in structure, these fatty acids are released from the GC columns almost instantaneously. The area under the

whole curve was used to calculate 18:1(sum). For those samples that formed two peaks, areas under the curve were summed, and concentration and percent composition were calculated as 18:1(sum). Concentrations and percent compositions for these fatty acids are represented by the notation 18:1(sum), thus reducing the total number of fatty acids included in analysis to 26.

Methods for quantitative analysis

To minimize the number of variables and to detect structure in the relationship among variables, I performed principal components analysis (PCA) on covariances of concentration and percent composition of individual fatty acids. PCA combines two or more correlated variables into a single factor or principal component. The number of principal components is dependent upon the number of variables used in the analysis and the relationship (covariance) among variables. The first principal component explains the largest percent of variation. The second principal component explains the next most variation and is orthogonal to the first, and so on until 100% of the variation in the data set has been explained. The output of PCA on covariances (in a multivariate analysis platform) is a table with eigenvectors for each variable used in analysis. Eigenvectors are coefficients (from 0 to 1) that when applied to original variable values, produce the principal component (PC) variable. They indicate magnitude and direction, and can be positive or negative.

The advantage of using PCA in this study was the ability to detect patterns in milk fatty acid composition. It is unlikely that there was significant variation in all 26 fatty

acids identified. Plots of principle components can help to determine which fatty acids might be most important in explaining variation between samples. A disadvantage of PCA is that it can not be used to determine statistical significance and can only be used for data mining, rather than testing hypotheses.

Fatty acids included in PCA were 8:0, 10:0, 16:0, 18:0, 18:1(n-9 and n-7), 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3, and 22:6n-3. These fatty acids were selected because of their wide distribution of values and/or their applicability to research questions (diet, life history, and physiological traits). For example, medium chain fatty acids such as 8:0 and 10:0 are synthesized by the mammary gland. Differences between species in concentration and relative proportion of these fatty acids may reflect selection on mammary gland lipogenesis. 18:2n-6 and 18:3n-3 vary in the composition of wild and captive diets, but are also important for their role as precursors to the LCPUFA 20:4n-6, 20:5n-3, and 22:6n-3. These three fatty acids, particularly 22:6n-3, play an integral role in brain growth, development, and multiple functions within the central nervous system (Brenna, 2002; Carlson, 1999, 2001; Gibson and Makrides, 1999)

JMP In 5.1.2. software automatically calculates PC variables for each data point (milk sample) in the database, but does not determine how many principal components to extract. Following the Kaiser criterion (Kaiser, 1960), I included principal components with an associated eigenvalue greater than 1.0. PC variables were placed into the original database as columns, with each milk sample having a unique PC value. These values became another data point, and were plotted against one another in all possible combinations (e.g., PC 1 by PC 2, PC 1 by PC 3, PC 2 by PC 3) to detect patterns in

sample distributions. PCA was not used to determine statistical significance. Instead, results guided choice of tests for differences in means between and among groups.

Distributions of all fatty acids (concentrations and percent compositions) were plotted to check for normality. The majority of these distributions were normal except for a small number of outliers with high values. All outliers were identified and closely investigated. Outliers were maintained in the group if there were no comparative data points from conspecifics. For example, with only one *Pongo* sample, I was unable to determine if the individual value was representative of this group or aberrant. Fatty acids that lacked a normal distribution for concentration *and* percent composition were not included in statistical analyses (14:1, 16:1, 20:3n-6, and 22:5n-3). These fatty acids were maintained in calculations of total monounsaturated fatty acids (14:1, 16:1, 18:1, etc), total n-3 PUFA, total n-6 PUFA, and total PUFA as these distributions were normal.

Significant differences in mean concentration and percent composition among species and superfamilies were determined using analysis of variance (ANOVA). A non-significant p-value from an ANOVA test ($\alpha \geq 0.05$) indicates that the means of all groups are not significantly different from each other, but this is not a test for significant differences between groups. Tukey–Kramer tests were used to perform multiple pairwise comparisons between groups (species, genera and families). This test is preferred over t-tests when dealing with unequal group sizes (number of individuals of each species, genera, family and superfamily). T-tests (two tailed because the direction of the difference was unspecified) were used when differences between size of groups were

smaller. Statistical significance for all tests was set at $\alpha < 0.05$. All data were analyzed using JMP 5.1.2 (SAS Institute).

Results

Effects of captivity

PCA analysis on concentration (Table 8.2a, 8.2b; Figure 8.1) and percent composition of individual fatty acids (Table 8.3a, 8.3b; Figure 8.2, 8.3) separate samples into wild and captive groups. Eigenvector values for PCA on concentration (Table 8.3b) suggest that 16:0, 18:1 (n-9 and n-7), 18:2n-6, and 18:3n-3 may either be significantly different between captive and wild samples or vary within each group. Mean values for concentration of 16:0, 18:1(sum), 18:2n-6, and 18:3n-3 were compared between captive and wild groups using Tukey-Kramer tests. Mean concentrations (mg/g) of 16:0, 18:1(sum), and 18:2n-6 were significantly higher in captive (11.36; 15.99; 14.00) versus wild samples (6.29; 6.32; 2.25) while the concentration of 18:3n-3 was higher in wild samples (1.92 vs. 1.13) ($p < 0.05$ for all values).

Table 8.1. Fatty acids identified by gas chromatography

Type of Fatty Acid	Chemical Formula and Name
Saturated Fatty Acids (no double bonds)	8:0; Octanoic acid
	10:0; Decanoic acid
	12:0; Lauric acid
	14:0; Myristic acid
	16:0; Palmitic acid
	18:0; Stearic acid
	20:0; Eicosanoic acid
	22:0; Docosanoic acid
Monounsaturated (one double bond)	14:1; Tetradecenoic acid
	16:1; Hexadecenoic acid
	18:1n-9; Oleic acid
	18:1n-7; Vaccenic acid
	20:1; Eicosenoic acid
	22:1; Docosenoic acid
	24:1; Tetracosenoic acid
Polyunsaturated (more than one double bond)	20:2; Eicosadienoic acid
	22:2; Docosadienoic acid
Polyunsaturated Omega-3 (More than one double bond; First double bond located at the third carbon from the omega end)	18:3n-3; Alpha-linolenic acid
	20:5n-3; Eicosapentaenoic acid
	22:5n-3; Docosapentaenoic acid
	22:6n-3; Docosahexaenoic acid
Polyunsaturated Omega-6 (More than one double bond; First double bond located at the sixth carbon from the omega end)	18:2n-6; Linoleic acid
	20:3n-6; Dihomo-gamma linolenic acid
	20:4n-6; Arachidonic acid
	22:4n-6; Docosatetraenoic acid
	22:5n-6; Docosapentaenoic acid

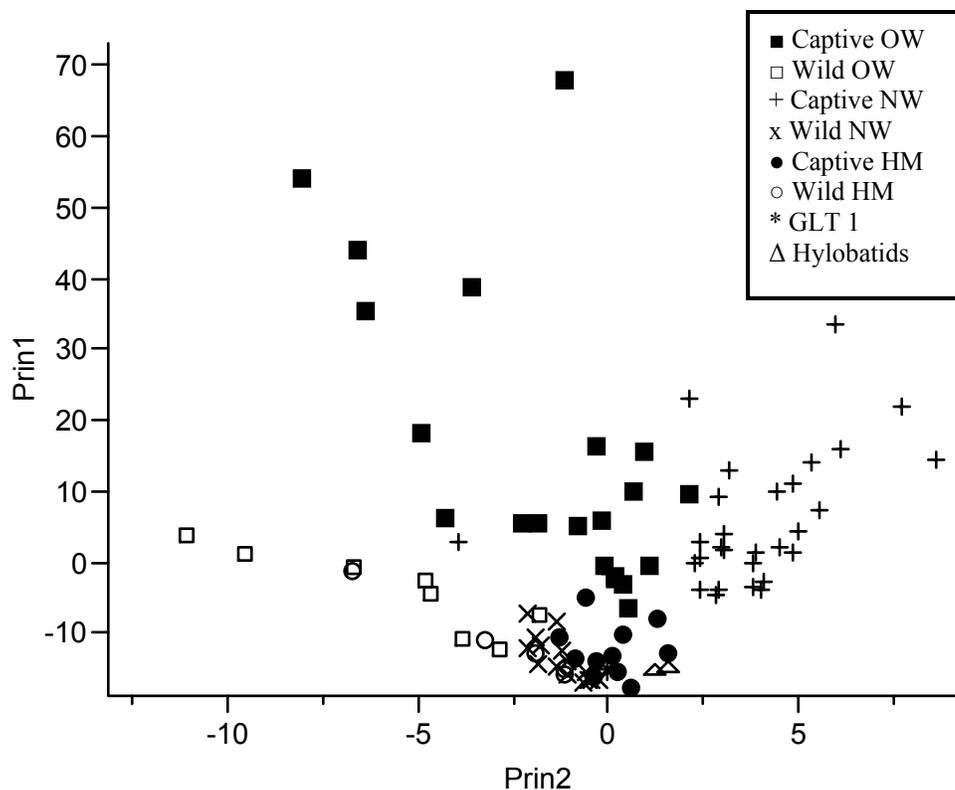
Table 8.2a. Principal Eigenvalues and associated variance (concentration, mg/g)

	Eigenvalue	% Variance	Cum % variance
PC 1	255.45	92.64	92.64
PC 2	12.98	4.71	97.35
PC 3	3.38	1.23	98.58
PC 4	2.01	0.73	99.30
PC 5	1.03	0.36	99.68

Table 8.2b. Eigenvectors for Principal Components 1 -5 (concentration, mg/g)

Fatty Acid	PC 1	PC 2	PC 3	PC 4	PC 5
8:0	0.0646	-0.0163	0.7470	-0.1954	0.2814
10:0	0.0289	0.1397	0.6333	-0.0258	-0.4029
16:0	0.4563	-0.5312	0.0849	0.5442	-0.3712
18:0	0.1117	-0.0365	-0.0129	0.0845	-0.1043
18:1(sum)	0.6889	-0.2719	-0.0996	-0.5362	0.2764
18:2n-6	0.5470	0.7881	-0.0684	0.2110	-0.0524
18:3n-3	0.0167	-0.0345	0.1369	0.5705	0.7257
20:4n-6	0.0098	0.0222	-0.0041	0.0106	0.0631
20:5n-3	0.0029	-0.0023	-0.0030	-0.0032	0.0033
22:6n-3	0.0087	0.0043	-0.0081	-0.0114	-0.0055

Figure 8.1. Bivariate plot of PC 1 by PC 2 (concentration, mg/g)



Results from PCA on percent composition indicated that 8:0, 10:0, 18:1(sum), and 18:2n-6 may differ significantly between captive and wild groups (variation along the x axis) or may explain variation within each group (variation along the y axis). All captive samples had positive PC 1 values (Figure 8.2, 8.3) and all wild PC 1 values were negative, but both had similar distributions along the y axis (PC 2 and PC 3). Interesting exceptions to this pattern are the data points representing two hylobatids (*Hylobates lar*, *Symphalangus syndactylus*) and one golden lion tamarin (*Leontopithecus rosalia*). These captive samples do not cluster with other captive samples but instead cluster with wild samples (Figures 8.2, 8.3). The golden lion tamarin sample clusters with other wild

ceboids in both bivariate plots while the hylobatid samples group with wild hominoids only in the bivariate plot of PC 3 by PC 2 (Figure 8.1).

These three samples were removed from analysis, and mean percent composition of 8:0, 10:0, 18:1(sum) 18:2n-6, and 18:3n-3 from captive and wild samples were compared using two-tailed t-tests, with significance validated by Tukey-Kramer tests (Table 4). Percent composition of 8:0 and 10:0 in wild samples was more than twice that in captive samples. The mean percent composition of 18:0 was only slightly higher in captive groups, but this difference was still significant. Similar to results from mean concentration, mean percent composition of 18:1(sum) and 18:2n-6 were significantly higher in captive groups, while 18:3n-3 was significantly lower.

Values for the golden lion tamarin and hylobatid samples are included in Table 8.4 for comparison to wild and captive samples. Percent composition of 8:0 and 10:0 for the golden lion tamarin sample are higher than mean values for wild and captive samples, but are within the range of values for wild groups (20.64% and 22.21%, respectively, both from wild *Callithrix jacchus*). The golden lion tamarin sample provides the lowest value for percent composition of 18:2n-6 among captive samples, but fits well within the range of values among wild samples. These comparisons suggest that the golden lion tamarin sample clustered with wild, rather than captive, samples as a result of similar values for percent composition of 8:0, 10:0 and 18:2n-6. Hylobatid samples are distinct from other captive samples in their high percent composition of 8:0 and 10:0, and have the lowest values among captive samples for percent composition of 18:1(n-7 and n-9).

Bivariate plots of principal components (Figure 8.1 – 8.3) indicate that within wild and captive groupings, samples appear to cluster by superfamily. This was further explored by analyzing differences between mean concentration and mean percent composition of milk fatty acids from wild groups and captive groups separately.

Table 8.3a. Principal Eigenvalues and associated variance (percent composition)

	Eigenvalue	% Variance	Cum % variance
PC 1	114.54	55.55	55.55
PC 2	50.55	24.51	80.06
PC 3	26.44	12.82	92.88
PC 4	8.56	4.15	97.03
PC 5	4.96	2.40	99.43

Table 8.3b. Eigenvectors for Principal Components 1 -5 (percent composition)

Fatty Acid	PC 1	PC 2	PC 3	PC 4	PC 5
8:0	-0.3642	-0.4311	-0.0945	0.1919	0.7786
10:0	-0.3022	-0.4471	-0.1795	-0.1296	-0.5003
16:0	-0.1204	0.544	0.0268	-0.6049	0.3014
18:0	0.0279	0.0846	0.0169	-0.079	0.0740
Sum 18:1	0.2954	0.3467	-0.6186	0.5265	0.0560
18:2n-6	0.8006	-0.3576	0.2788	-0.1262	0.1769
18:3n-3	-0.1800	0.2504	0.7032	0.5252	-0.0495
20:4n-6	0.0046	0.0195	0.0539	0.0711	-0.1007
20:5n-3	0.0004	0.0047	-0.0007	-0.0026	0.0013
22:6n-3	0.0102	-0.0032	-0.008	-0.0136	0.0085

Figure 8.2. Bivariate plot of PC 2 by PC 1 (percent composition)

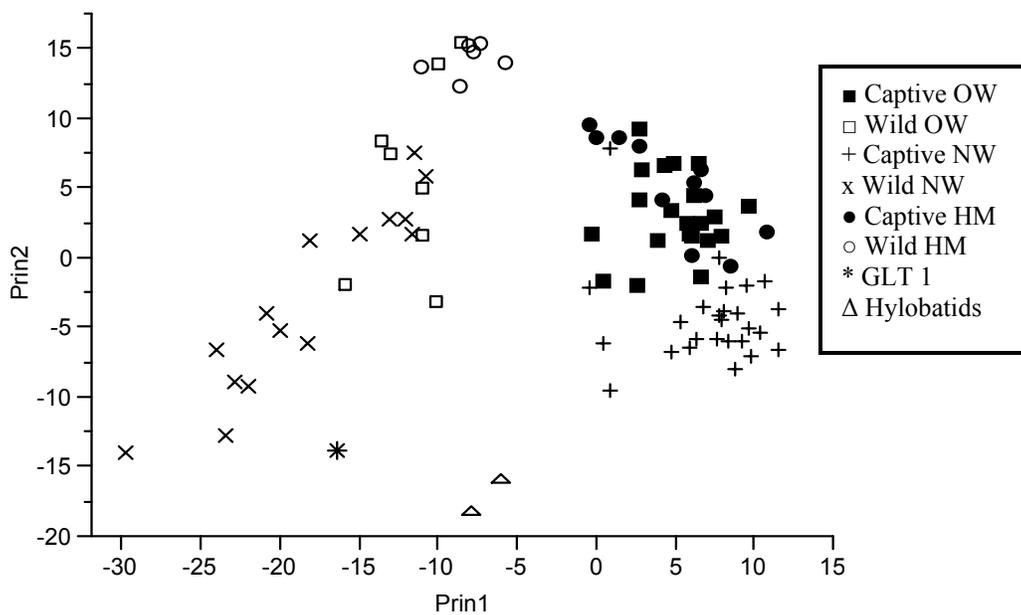


Figure 8.3. Bivariate plot of PC 3 by PC 1 (percent composition)

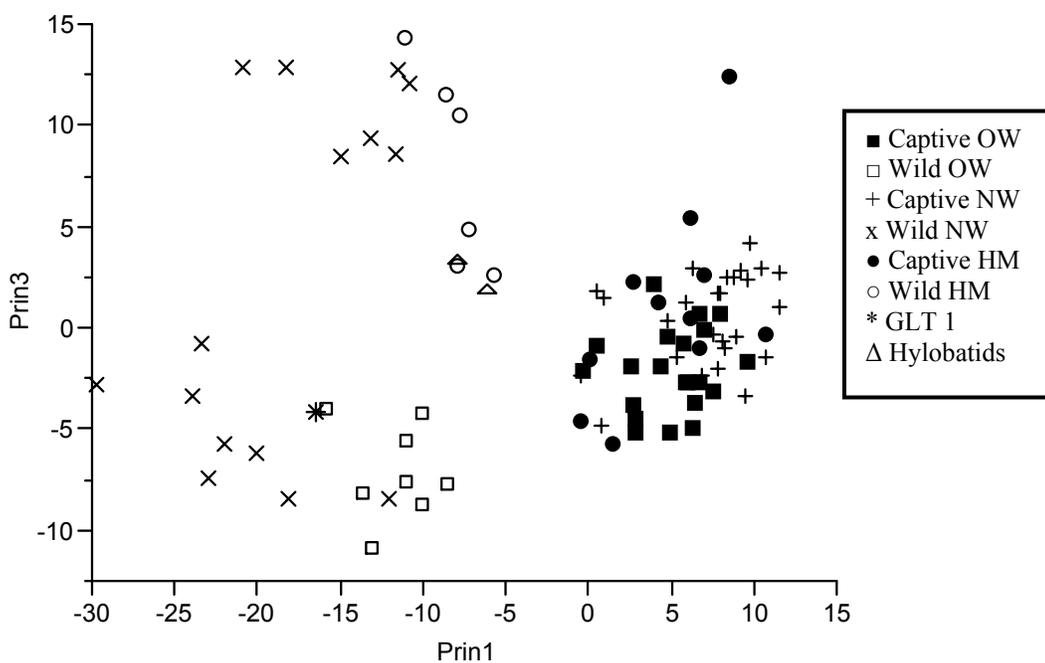


Table 8.4. Mean \pm SE percent composition of selected fatty acids for wild and captive groups.

Fatty Acid	Captive	Wild	t statistic	p value	Lion Tamarin	Hylobatids (mean)
8:0	3.56 \pm 0.27	9.99 \pm 1.21	-7.30	< 0.0001	18.60	16.64
10:0	3.02 \pm 0.25	7.83 \pm 1.22	-5.50	< 0.0001	15.56	13.33
16:0	20.96 \pm 0.53	24.60 \pm 0.79	- 3.83	0.0002	15.54	14.03
18:0	5.30 \pm 0.12	4.77 \pm 0.21	2.29	0.024	3.37	3.96
Sum 18:1	29.15 \pm 0.39	24.07 \pm 1.28	4.99	< 0.0001	20.43	17.87
18:2n-6	26.41 \pm 0.51	8.56 \pm 0.67	19.88	< 0.0001	11.50	22.72
18:3n-3	2.21 \pm 0.13	8.46 \pm 1.26	-7.46	< 0.0001	0.61	2.66
22:6n-3	0.37 \pm 0.02	0.11 \pm 0.01	7.46	< 0.0001	0.12	0.21

Samples from wild living groups

Variation among wild samples was first explored at the level of species.

Comparisons among species were performed with ANOVA and between species comparisons were performed with Tukey-Kramer tests. Mean concentration of all but two fatty acids (12:0 and 14:0) was significantly different among species (Table 8.5). Several pairwise comparisons show significant differences between toque macaques (cercopithecoidea) or mountain gorillas (hominoidea) and all other species. Among ceboids (mantled howlers, common marmosets and golden lion tamarins), no significant differences were found between marmosets and golden lion tamarin (subfamily

Callitrichinae) and the mean concentration of only two fatty acids (18:3n-3 and 18:2n-6) were significantly different between mantled howlers and each of the callitrichines. Tests for significant differences between species indicate that variation might be better explained by analysis at the level of superfamily.

Significant differences among superfamilies in mean concentration were identified in 8:0, 10:0, 16:0, 18:0, 18:1(sum), 20:4n-6, 20:5n-3, and 22:6n-3 (Table 8.6). Between superfamilies, the mean concentrations of 8:0, 10:0, and 20:4n-6 were significantly different between hominoids and both monkey superfamilies. Cercopithecoids were different from hominoids and ceboids in concentration of 16:0, 18:0, 18:1(sum), and 22:6n-3. Ceboids were significantly different from hominoids and cercopithecoids in mean concentration of 20:5n-3. No significant differences were identified among or between superfamilies in mean concentration of 18:3n-3 or 18:2n-6. When analyzed at the level of the species, *Callithrix* and *Leontopithecus* were significantly different from *Alouatta* in the mean concentration of 18:2n-6 and 18:3n-3. When grouped together for superfamily comparisons, this variation is masked and no significant differences were found between groups. Removal of callitrichines does not affect ANOVA results for 18:2n-6 but does indicate significant differences among these three species for concentration of 18:3n-3 ($F = 8.94$, $df = 2$, $p = 0.002$). This finding cautions against grouping these three species without first investigating the nature of variation among ceboids.

Table 8.5. Mean (\pm SE) Fatty Acid Concentration (mg/g) by species and tests for significance among and between species

Fatty Acid	Mountain Gorilla	Mantled Howler	Common Marmoset	Golden Lion Tamarin	Toque Macaque	F Ratio	p Value	Sig. between species (Tukey-Kramer)
8:0	0.005 \pm 0.57	3.56 \pm 0.49	2.41 \pm 0.70	1.67 \pm 0.70	3.35 \pm 0.49	6.94	< 0.001	GB/AP;GB/MS
10:0	0.07 \pm 0.50	1.60 \pm 0.43	2.35 \pm 0.61	1.92 \pm 0.61	2.57 \pm 0.43	3.97	0.01	GB/MS
12:0	0.002 \pm 0.001	0.002 \pm 0.001	0.005 \pm 0.001	0.002 \pm 0.001	0.003 \pm 0.001	1.43	0.25	None
14:0	0.77 \pm 0.30	0.82 \pm 0.26	1.45 \pm 0.37	1.60 \pm 0.37	1.67 \pm 0.26	2.21	0.09	None
16:0	5.49 \pm 1.28	6.06 \pm 1.11	2.79 \pm 1.57	2.66 \pm 1.57	10.64 \pm 1.11	6.50	0.001	MS/LR; MS/CJ; MS/GB
18:0	1.06 \pm 0.18	1.17 \pm 0.15	0.50 \pm 0.22	0.46 \pm 0.22	1.99 \pm 0.15	12.45	< 0.001	MS and all species
20:0	0.03 \pm 0.005	0.03 \pm 0.004	0.02 \pm 0.006	0.01 \pm 0.006	0.04 \pm 0.004	5.69	0.002	MS/CJ; MS/LR
22:0	0.01 \pm 0.001	0.01 \pm 0.001	0.004 \pm 0.002	0.01 \pm 0.002	0.014 \pm 0.001	6.39	0.001	MS/CJ; MS/LR
Sum Saturates	7.44 \pm 2.16	13.25 \pm 1.87	9.52 \pm 2.64	8.33 \pm 2.64	20.27 \pm 1.87	6.66	< 0.001	MS/CJ; MS/LR; MS/GB
14:1	0.01 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01	0.01 \pm 0.01	0.08 \pm 0.01	8.74	< 0.001	MS and all species
16:1	0.48 \pm 0.28	0.71 \pm 0.24	0.35 \pm 0.34	0.22 \pm 0.33	2.16 \pm 0.24	9.38	< 0.001	MS and all species
18:1(sum)	5.58 \pm 1.38	4.49 \pm 1.19	3.13 \pm 1.69	2.86 \pm 1.69	11.95 \pm 1.19	8.14	< 0.001	MS and all species
20:1	0.20 \pm 0.03	0.07 \pm 0.03	0.04 \pm 0.04	0.06 \pm 0.04	0.12 \pm 0.03	4.53	0.007	GB/AP; GB/LR; GB/CJ
22:1	0.02 \pm 0.002	0.01 \pm 0.002	0.01 \pm 0.002	0.01 \pm 0.003	0.01 \pm 0.002	9.25	< 0.001	GB and all species
Sum Monounsaturates	6.30 \pm 1.67	5.31 \pm 1.44	3.54 \pm 2.04	3.16 \pm 2.04	14.32 \pm 1.44	8.35	< 0.001	MS and all species
18:3n-3	3.19 \pm 0.46	3.53 \pm 0.39	0.36 \pm 0.56	0.26 \pm 0.58	1.03 \pm 0.39	11.55	< 0.001	AP/MS; AP/CJ; AP/LR; GB/MS; GB/CJ; GB/LR
20:5n-3	0.04 \pm 0.006	0.02 \pm 0.005	0.01 \pm 0.007	0.01 \pm 0.007	0.05 \pm 0.005	7.09	< 0.001	MS/AP; MS/CJ; MS/LR
22:5n-3	0.11 \pm 0.01	0.04 \pm 0.01	0.03 \pm 0.01	0.03 \pm 0.01	0.07 \pm 0.01	10.24	< 0.001	GB and all species
22:6n-3	0.02 \pm 0.004	0.01 \pm 0.004	0.02 \pm 0.005	0.03 \pm 0.006	0.04 \pm 0.004	9.47	< 0.001	MS/CJ; MS/GB; MS/AP
Sum n-3s	3.36 \pm 0.46	3.60 \pm 0.40	0.41 \pm 0.57	0.33 \pm 0.57	1.19 \pm 0.40	11.18	< 0.001	AP/CJ; AP/LR; AP/MS GB/CJ; GB/LR; GB/MS
18:2n-6	2.08 \pm 0.51	3.01 \pm 0.44	0.44 \pm 0.62	0.74 \pm 0.62	3.24 \pm 0.44	5.62	0.002	MS/LR; MS/CJ AP/LR; AP/CJ
18:3n-6	0.001 \pm 0.002	0.004 \pm 0.002	undetectable	undetectable	0.014 \pm 0.002	13.08	< 0.001	MS and all species

Fatty Acid	Mountain Gorilla	Mantled Howler	Common Marmoset	Golden Lion Tamarin	Toque Macaque	F Ratio	p Value	Sig. between species (Tukey-Kramer)
20:3n-6	0.02 ± 0.005	0.01 ± 0.004	0.03 ± 0.006	0.04 ± 0.006	0.06 ± 0.004	15.20	< 0.001	MS/CJ; MS/GB; MS/AP LR/AP
20:4n-6	0.40 ± 0.04	0.16 ± 0.03	0.04 ± 0.04	0.06 ± 0.04	0.12 ± 0.03	15.03	< 0.001	GB and all species
22:5n-6	0.02 ± 0.003	0.01 ± 0.003	0.001 ± 0.004	0.005 ± 0.004	0.02 ± 0.003	7.03	< 0.001	GB/LR; GB/CJ; MS/CJ
Sum n-6s	2.54 ± 0.53	3.21 ± 0.46	0.52 ± 0.65	0.87 ± 0.65	3.47 ± 0.46	5.58	0.002	MS/LR; MS/CJ; AP/LR; AP/CJ
Sum of PUFA	5.90 ± 0.95	6.81 ± 0.82	0.94 ± 1.16	1.20 ± 1.16	4.66 ± 0.82	6.92	< 0.001	AP/LR; AP/CJ; GB/LR; GB/CJ

GB = Gorilla (n = 6), AP = Howler (n = 8), CJ = Marmoset (n = 4), LR = Tamarin (n = 4), MS = Macaque (n = 8)

A confounding variable in the comparison of concentration of fatty acids is the variation among samples (inter- and intra-specific) in total fat (grams of fat per gram of milk). Samples that are 6% fat by weight will undoubtedly have higher total fatty acid weights (concentration) than samples that are 2% fat by weight. There are two methods to correct for the influence of total fat on fatty acids in samples. The first method is to correct all fatty acid concentrations by the total fat in each sample. Instead of comparing mg/g of 8:0 between samples, the comparison now becomes mg/g of 8:0 per mg of fat in each sample. The second method is to compare the proportion, or relative contribution, of each fatty acid to the total fatty acids in the milk sample, expressed as percent composition. Both methods of correction were explored.

Significant differences among and between superfamilies (for samples from wild living anthropoids) in concentrations corrected by total fat in each sample are presented in Table 8.7. Similar to results from absolute concentration (Table 8.6), 8:0, 10:0, and 20:4n-6 are significantly different among superfamilies and between hominoids and both monkey superfamilies (8:0 and 10:0 being significantly lower in hominoids and 20:4n-6 being significantly higher). Also similar was the lack of significant difference among mean concentration of 18:2n-6. Whereas concentration of 16:0, 18:0, 18:1(sum) varied significantly among and between superfamilies without correcting for fat, differences were not significant using corrected values. Importantly, correcting for total fat also negated significant differences among and between superfamilies in the concentration of 22:6n-3.

Table 8.6. Variation in fatty acid concentration among (ANOVA) and between (Tukey-Kramer) superfamilies (wild samples only).

Fatty Acid	F Ratio	p value	Between superfamilies: Significant differences
8:0	11.326	< 0.001	HM/OW; HM/NW
10:0	8.103	0.0018	HM/OW; HM/NW
16:0	9.609	< 0.001	OW/HM; OW/NW
18:0	14.719	< 0.001	OW/HM; OW/NW
18:1(sum)	16.311	< 0.001	OW/HM; OW/NW
18:2n-6	2.293	0.121	
18:3n-3	2.879	0.0748	
20:4n-6	22.805	< 0.001	HM/OW; HM/NW
20:5n-3	15.561	< 0.001	NW/OW; NW/HM
22:6n-3	11.850	< 0.001	OW/HM; OW/NW

HM = hominoids (= mountain gorillas)

NW= ceboids (= howlers, golden lion tamarins, common marmosets)

OW = cercopithecoids (= toque macaques)

Table 8.7. Variation in fatty acid concentration corrected by total fat in each sample among (ANOVA) and between (Tukey-Kramer) superfamilies (wild samples only).

Fatty Acid	F Ratio	p value	Between superfamilies: Significant differences
8:0	7.4296	0.0031	HM/NW; HM/OW
10:0	3.981	0.032	HM/NW; HM/OW
16:0	0.052	0.946	
18:0	0.2395	0.7888	
18:1(sum)	1.03	0.3722	
18:2n-6	0.259	0.774	
18:3n-3	6.38	0.006	HM/OW
20:4n-6	45.692	< 0.0001	HM/NW; HM/OW
20:5n-3	6.959	0.0041	HM/NW; HM/OW
22:6n-3	0.0322	0.9644	

Differences among and between superfamilies also were analyzed using percent composition. Mean percent composition of 8:0 and 10:0 are significantly different at the level of superfamily (8:0: $F = 12.28$, $df = 2$, $p = 0.0001$; 10:0: $F = 7.02$, $df = 2$, $p = 0.003$). They were highest in ceboids (11.83 ± 1.09 , 10.28 ± 1.23), followed by cercopithecoids (8.87 ± 1.68 , 6.79 ± 1.89), and were virtually undetectable in hominoids (0.025 ± 2.12 , 0.36 ± 2.39) (Figure 8.4a, 8.4b). No significant differences among or between superfamilies were found in mean percent composition of other medium chain

saturated fatty acids (12:0 and 14:0) (Figure 8.4c, 8.4d). These fatty acids also were not significantly different when analyzed by mean concentration at the species level.

Mean values for percent composition of 16:0 and 18:0 were significantly different among superfamilies (16:0: $F = 6.827$, $df = 2$, $p = 0.004$; 18:0: $F = 6.013$, $df = 2$, $p = 0.006$), with the lowest values for both found in ceboids (22.24%, 4.22%). However, the proportion of 16:0 (palmitic acid) in milks from all three superfamilies is large, ranging from 22.24% in ceboids to 27.10% in hominoids. The contribution of 18:0 is less than that of 16:0, but variation among superfamilies is also small (4.21% to 5.67%).

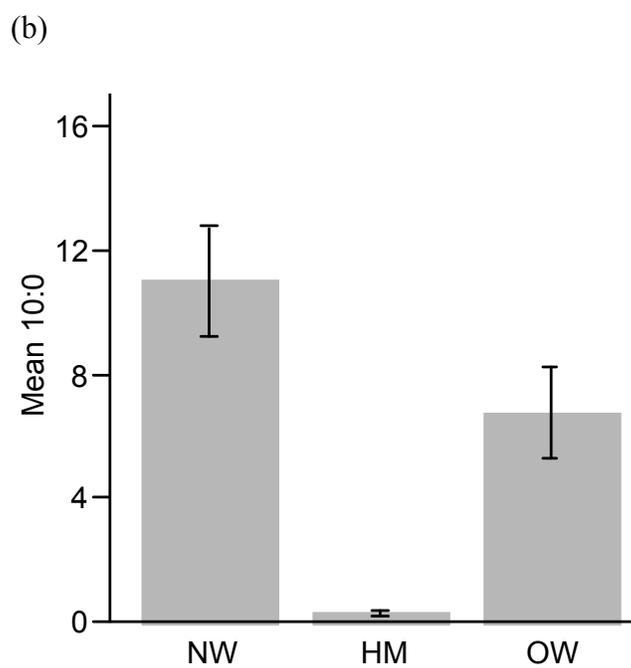
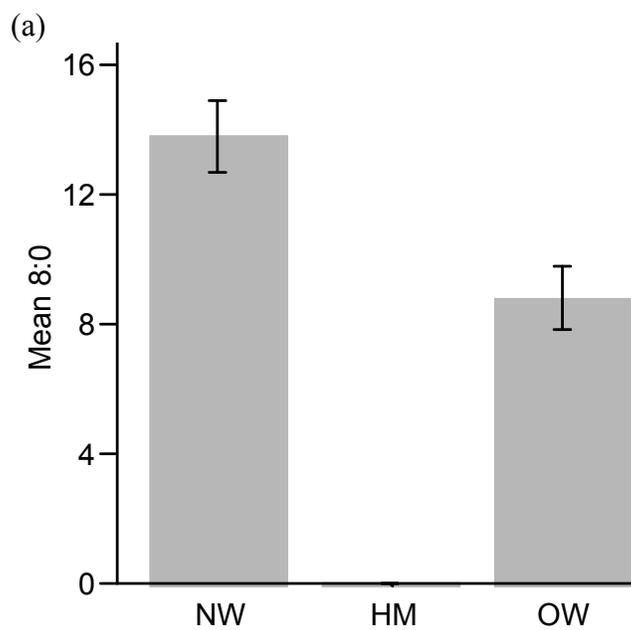
Percent composition of 18:3n-3 was significantly different among superfamilies ($F = 10.81$, $df = 2$, $p = 0.0003$) (Figure 8.5a). Percent composition of 18:3n-3 in hominoids (16.31 ± 2.33) was more than twice that of ceboids (6.98 ± 1.20) and more than six times that of cercopithecoids (2.55 ± 1.84). 18:3n-3 is a precursor for 22:6n-3, however no significant differences were found among or between superfamilies in mean percent composition of 22:6n-3 (hominoids: 0.094 ± 0.035 ; ceboids: 0.123 ± 0.018 ; cercopithecoids: 0.115 ± 0.028) (Figure 8.5b). Indeed, there is a negative correlation ($r = -0.496$, $p = 0.006$) between percent composition of 18:3n-3 and 22:6n-3.

The opposite trend was identified with the n-6 precursor 18:2n-6 and its long chain derivative 20:4n-6. 18:2n-6 is not significantly different among superfamilies ($F = 0.765$, $df = 2$, $p = 0.47$) (Figure 8.6a) but mean percent composition of 20:4n-6 is significantly higher in hominoids (2.08 ± 0.14) than ceboids (0.52 ± 0.072) and cercopithecoids (0.32 ± 0.11) (Figure 8.6b). The correlation between the precursor and long chain metabolite is weak and non-significant (0.3166 , $p = 0.09$). Removing

mountain gorillas increases the correlation coefficient to $r = 0.5306$ ($p = 0.009$), suggesting mountain gorillas may differ from monkeys in conversion of 18:2n-6 to 20:4n-6.

Saturated fatty acids make up more than 50% of the milk of ceboids (59.62 ± 2.21) and cercopithecoids (51.93 ± 3.13), and less than 40% of the milk of hominoids (37.19 ± 3.62) (Figure 8.7a). The difference between monkeys and hominoids is almost entirely due to the larger contribution (up to 11%) of 8:0 and 10:0 to the milks of monkeys. The composition of n-3 and n-6 PUFA is significantly different among the three superfamilies ($F = 15.52$, $df = 2$, $p < 0.001$; $F = 3.64$, $df = 2$, $p < 0.001$). Milk from hominoids had the highest percent composition of both n-3s (17.89 ± 2.03) and n-6s (13.35 ± 1.52) (Figure 8.7b, 8.7c) driven by significantly higher levels of 18:3n-3 and 20:4n-6. Subsequently, hominoids have the highest percent composition of PUFA (31.24 ± 3.38), almost twice that of ceboids (17.51 ± 2.07) and almost three times that of cercopithecoids (11.61 ± 2.93) (Figure 8.7d).

Figure 8.4. Mean percent composition by superfamily of (a) 8:0, (b) 10:0, (c) 12:0, (d) 14:0. NW = Ceboidea, HM = Hominoidea, OW = Cercopithecoidea.



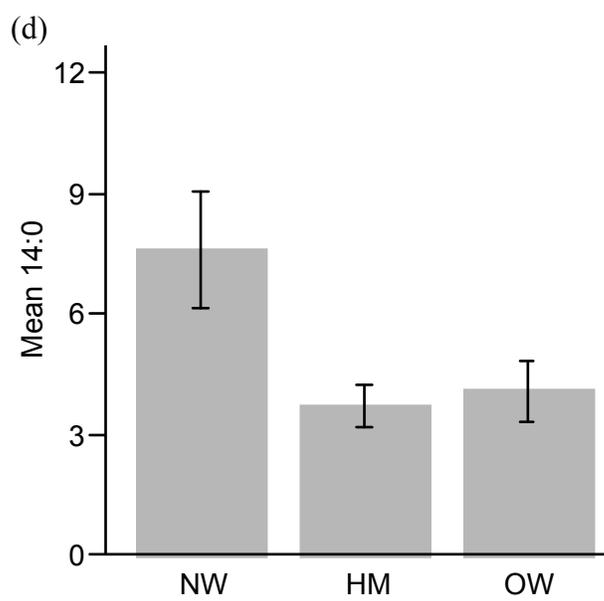
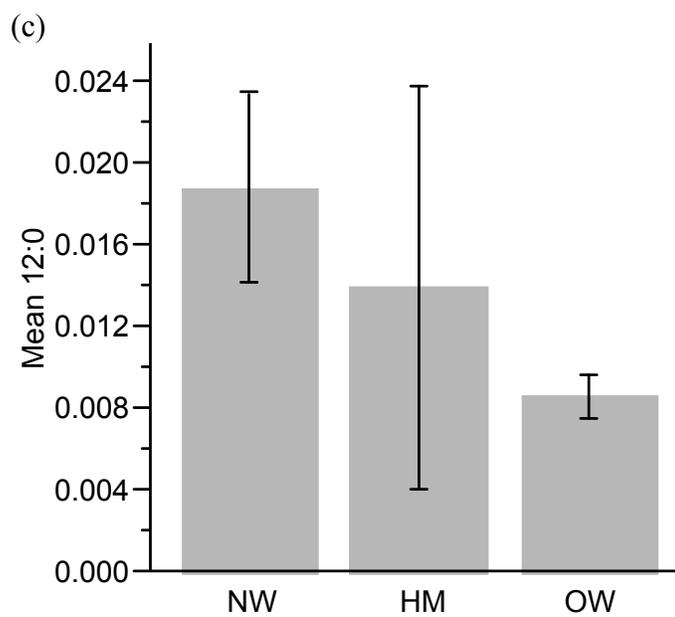


Figure 8.5. Mean Percent Composition by Superfamily of (a) 18:3n-3 (ALA), and (b) 22:6n-3 (DHA).

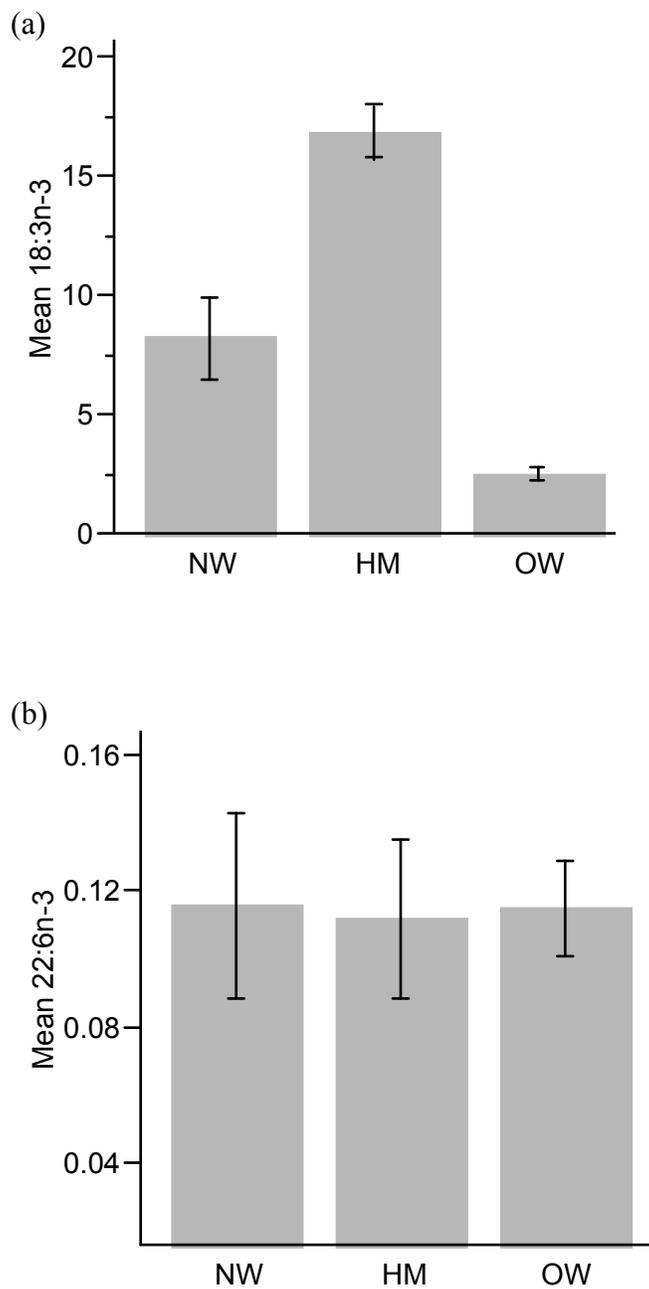


Figure 8.6. Mean percent composition by Superfamily for a) 18:2n-6 (LA) and b) 20:4n-6 (AA).

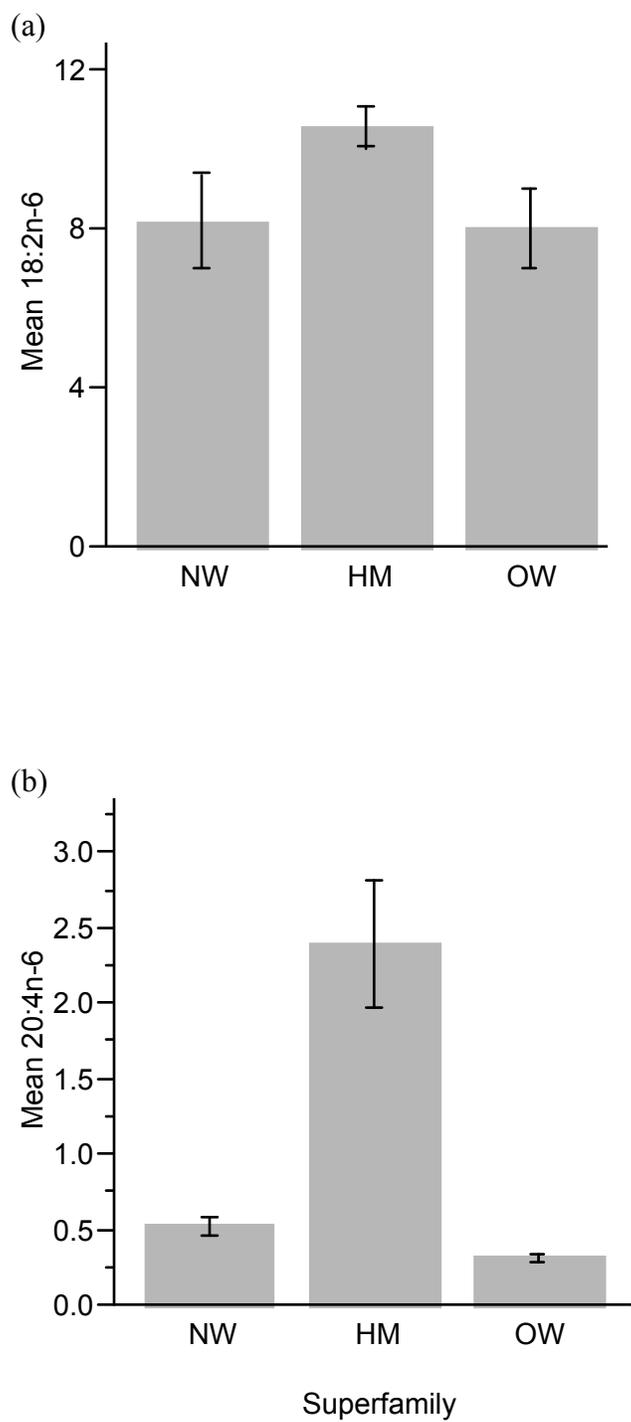
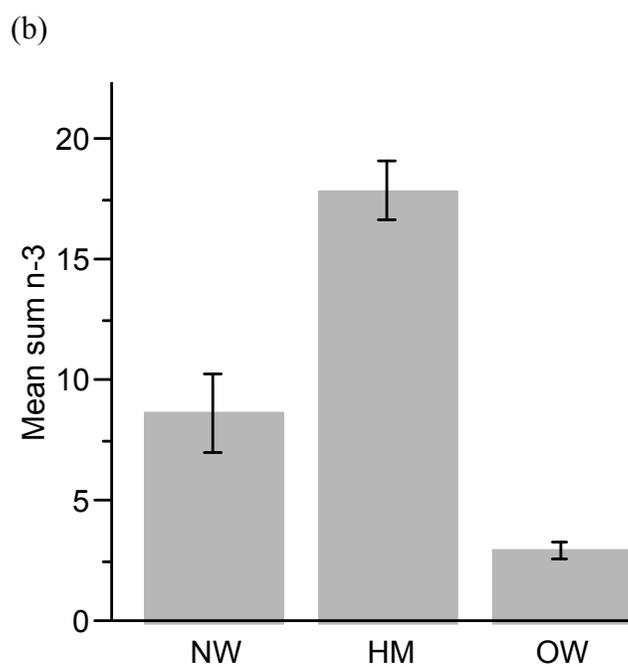
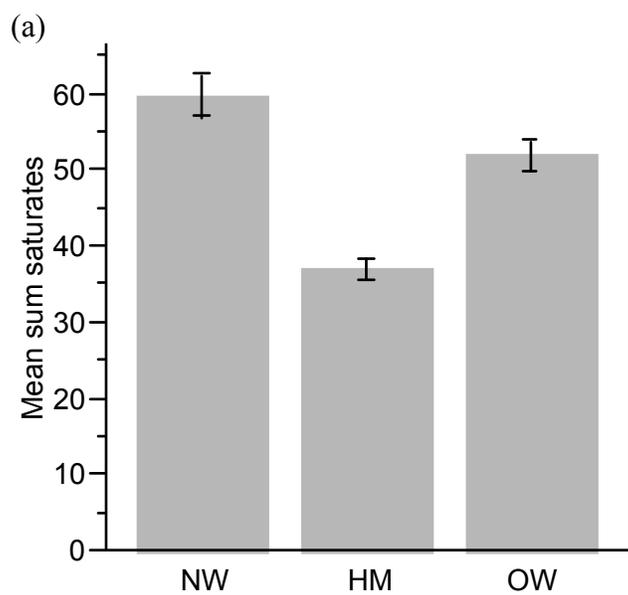
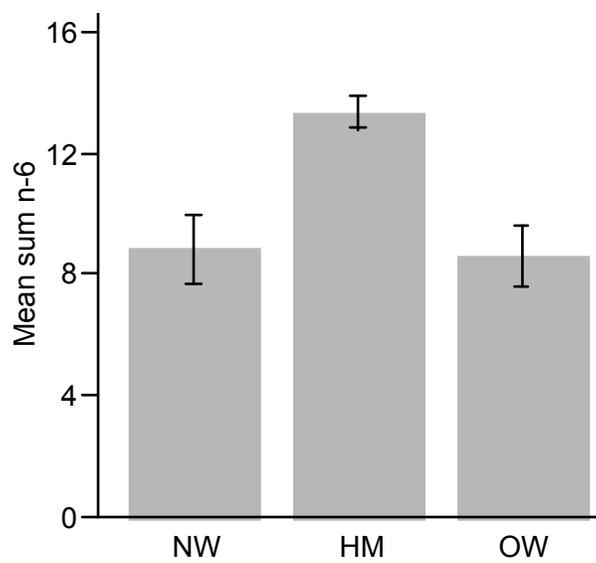


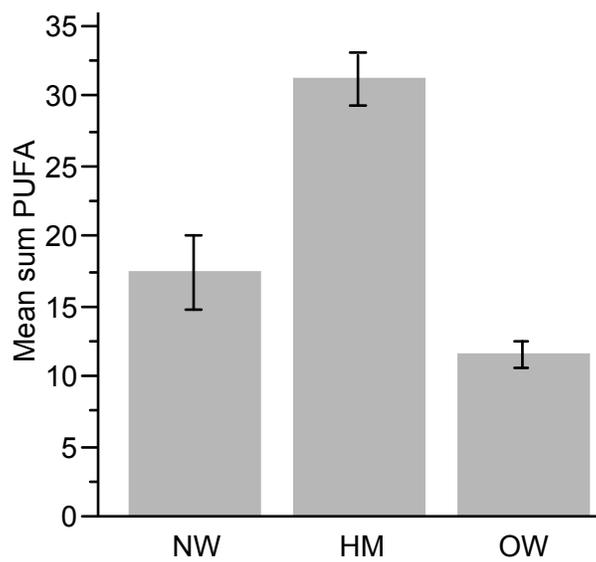
Figure 8.7. Mean percent composition by superfamily of (a) the sum of saturated fats, (b) the sum of n-3 PUFA, (c) sum of n-6 PUFA, and d) Sum of PUFA



(c)



(d)



Samples from captive living groups

PCA analysis on all species indicated that captive samples represented a distinct group. Within this group, phylogenetic patterns may also exist. Patterns that mimic those of wild samples are of particular interest, as they would suggest phylogenetic (conserved) rather than ecological influences. Using results from wild groups as a guide, I investigated variation in mean percent composition at the level of superfamily for 8:0, 10:0, 16:0, 18:0, 18:1(sum), 18:2n-6, 18:3n-3, 20:4n-6 and 22:6n-3. Variation also was investigated in types of fatty acids (saturates, n-3, n-6, and total PUFA). Outliers exposed during PCA (one golden lion tamarin and two hylobatids) were excluded from analyses.

Mean percent composition of 8:0 and 10:0 were significantly lower in captive hominoids compared to both ceboids and cercopithecoids (Figure 8.8a, 8.8b), confirming the pattern identified among wild samples. The contribution of 8:0 and 10:0 to milks of wild monkeys was significantly higher than that of captive monkeys (Figure 8.4a, 8.4b, 8). Another important difference is that in wild groups, the proportions of 8:0 and 10:0 in hominoids were close or equal to zero. There was a measurable contribution of 8:0 and 10:0, albeit small (0.5 – 1.0%), to captive hominoid milks.

Percent composition of 16:0 was highest among hominoids (25.60%) followed by cercopithecoids (23.97%) and ceboids (17.87%). This is the same ordering as percent composition of 16:0 from wild samples, and values indicating the relative contribution of this fatty acid to total fatty acids are also quite similar, except for ceboids (approximately 5% lower than wild samples). Percent composition of 18:0 was almost equal in captive

samples from cercopithecoids and hominoids (6.08%, 5.91%) and both were significantly different from ceboids (4.72%).

Captive samples were significantly lower than wild samples in percent composition of 18:3n-3 both as a whole (Table 8.4) and by superfamily ($p < 0.05$) (Figure 8.9). Samples from hominoids, captive and wild, were higher than both monkey superfamilies, but only significantly different from cercopithecoids as a result of high values in *Alouatta*. Despite large differences in percent composition of its n- fatty acid precursor, mean percent composition of 22:6n-3 from wild samples was not different among or between superfamilies. Percent composition of 22:6n-3 of captive samples is also not significantly different among ($F = 2.86$, $df = 2$, $p = 0.06$) or between superfamilies, although 22:6n-3 does contribute a significantly higher proportion to the milks of all captive anthropoids ($t = 7.88$, $p < 0.001$) (Figure 8.10). The correlation between percent composition of 18:3n-3 and percent composition of 22:6n-3 is almost identical to that of wild samples ($r = -0.493$, $p < 0.001$).

The relationship between 18:2n-6 and 20:4n-6 identified in wild samples was not seen in captive samples. Rather, variation was identified between (ceboids with cercopithecoids/hominoids) and among superfamilies in 18:2n-6 ($F = 12.998$, $df = 2$, $p < 0.0001$) and 20:4n-6 ($F = 36.67$, $df = 2$, $p < 0.0001$) (Figure 8.11, 8.12). Percent composition of 18:2n-6 was positively correlated with percent composition of 20:4n-6 (0.723, $p < 0.001$). This relationship between precursor and long-chain metabolite is much stronger than was seen for wild samples, even when mountain gorilla samples were removed from consideration.

Figure 8.8. Mean percent composition of (a) 8:0 and (b) 10:0 of captive and wild samples by superfamily.

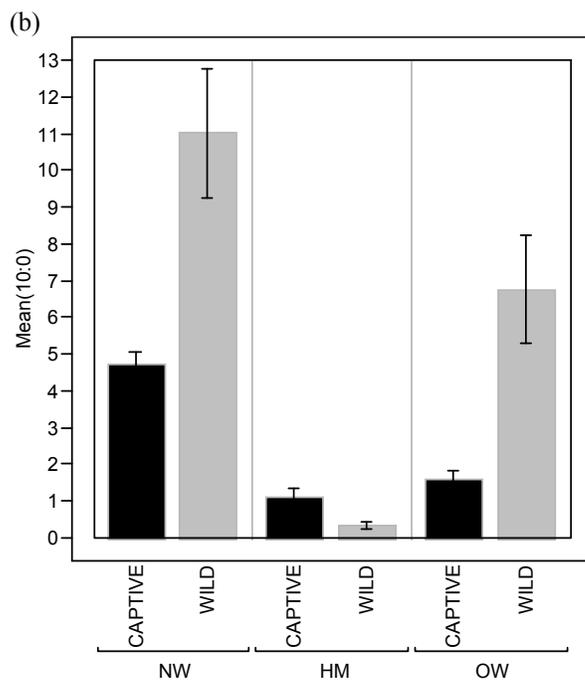
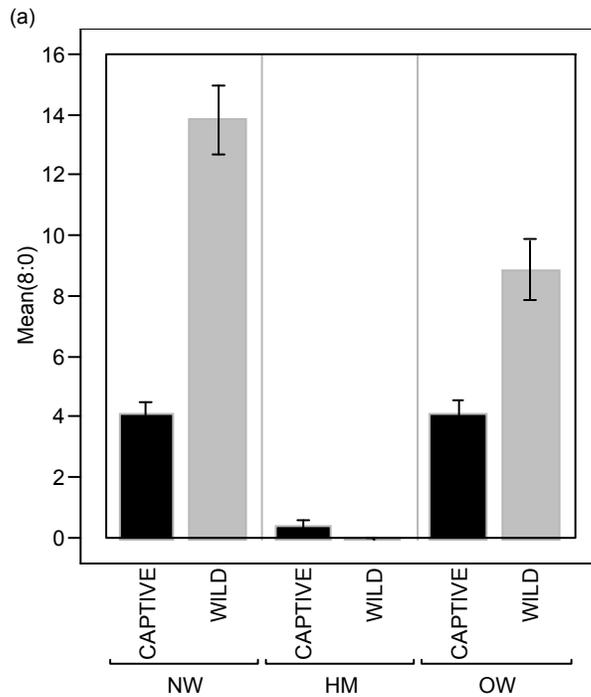


Figure 8.9. Mean percent composition of 18:3n-3 in wild and captive samples by superfamily.

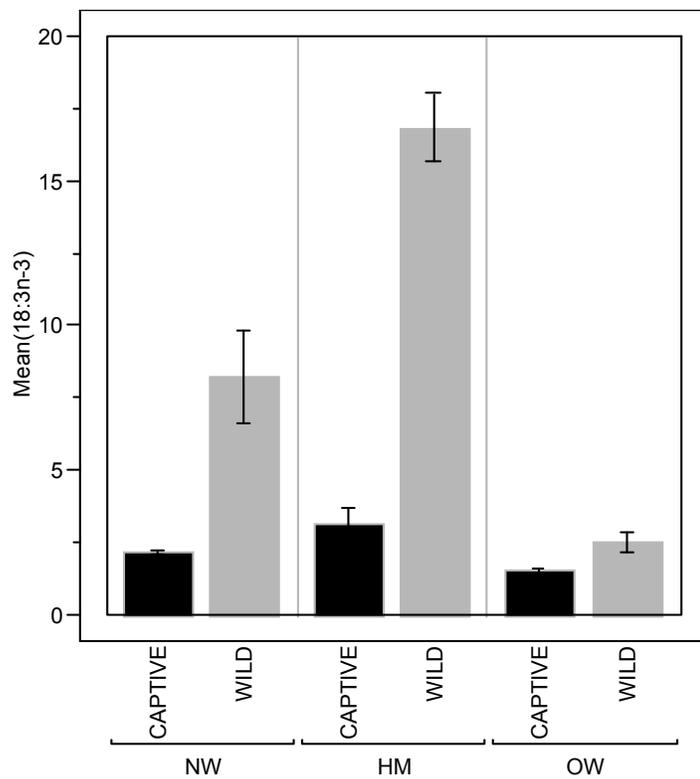


Figure 8.10. Mean percent composition of 22:6n-3 in wild and captive samples by superfamily.

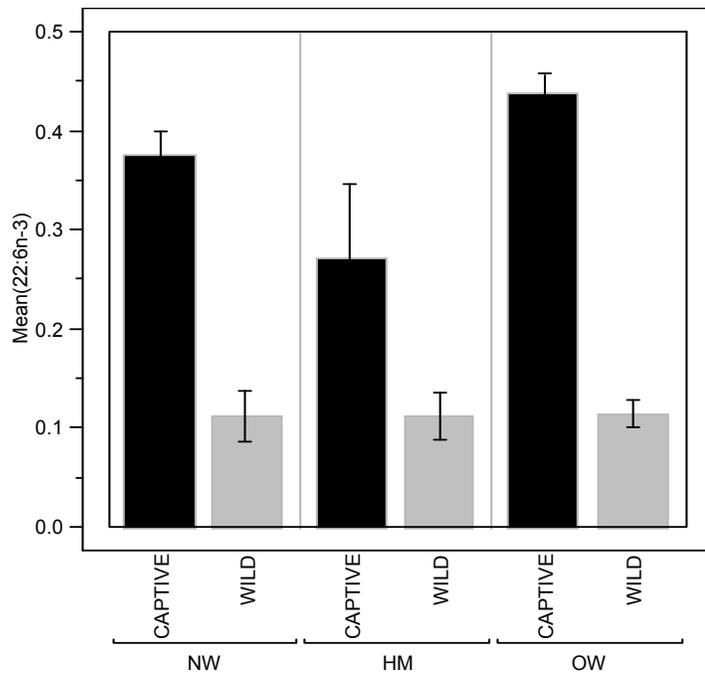


Figure 8.11. Mean percent composition of 18:2n-6 in captive and wild samples by superfamily.

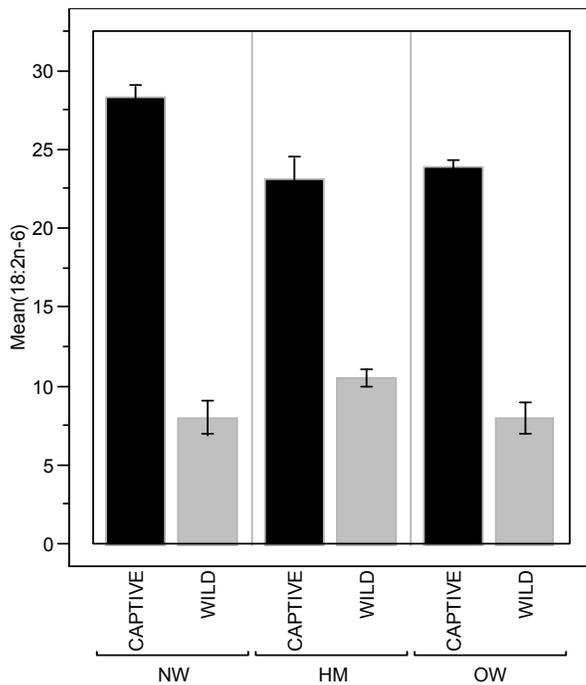
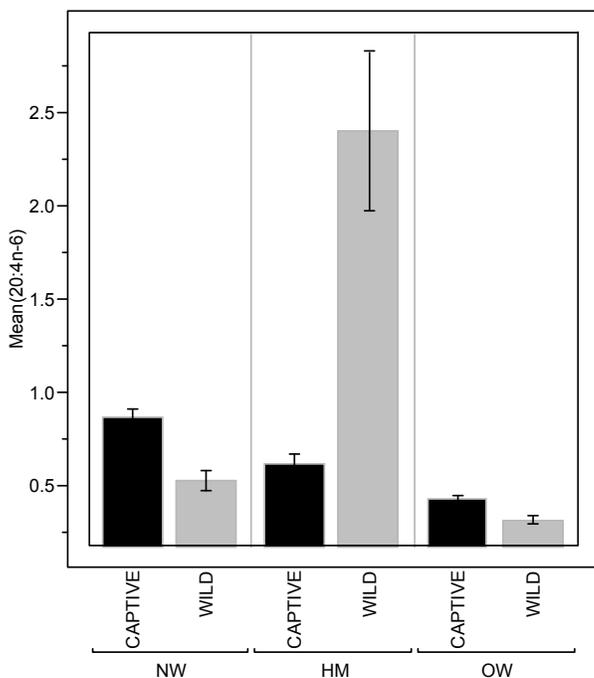


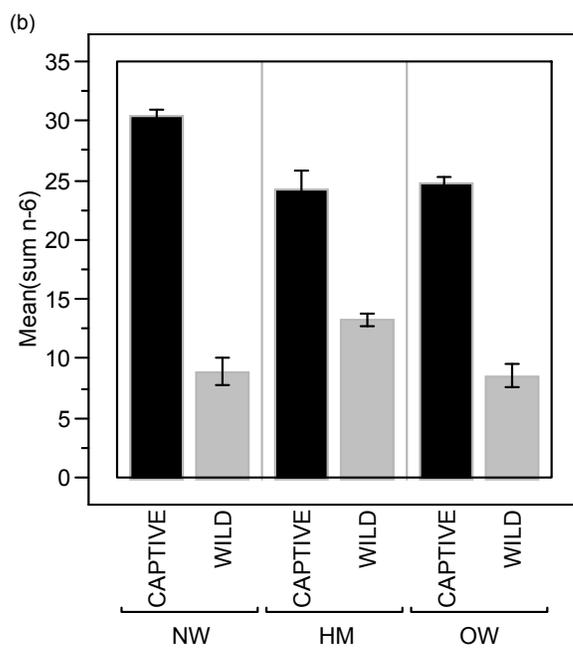
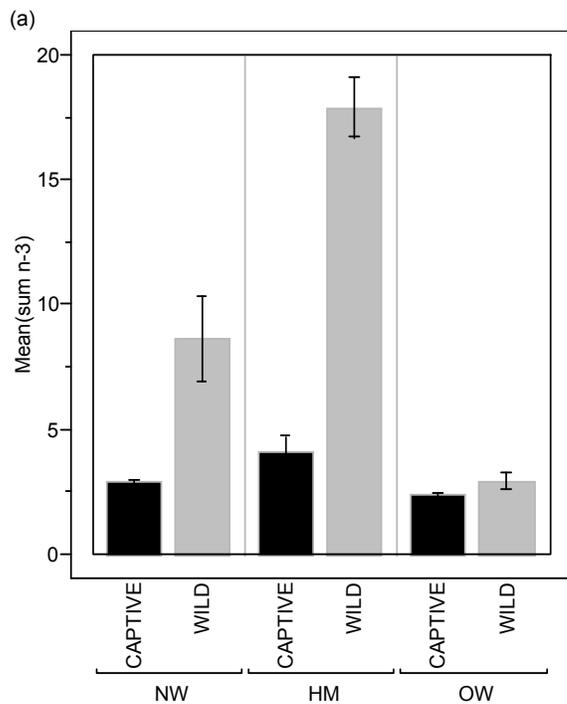
Figure 8.12. Mean percent composition of 20:4n-6 in captive and wild samples by superfamily.



The mean percent composition of saturated fatty acids for captive hominoid samples was just slightly higher (38.61 ± 1.15) than that from wild hominoid samples, but was the highest value among superfamilies, and significantly higher than ceboids (35.13 ± 0.71) but not cercopithecoids (37.38 ± 0.87). This was the opposite pattern observed among wild samples. The contribution of saturated fatty acids to total fatty acids, although significantly different among superfamilies ($F = 4.051$, $df = 2$, $p = 0.022$), is also less variable (approximately 3% in captive samples compared to over 20% in wild samples). The difference between wild and captive samples can be explained by the decreased contribution of 8:0 and 10:0 to the milk of monkeys and a slightly increased contribution of 16:0 and 18:0 to the milks of hominoids.

High values for percent composition of 18:3n-3 and 20:4n-6 among wild hominoid (mountain gorilla) samples resulted in significantly higher percent composition of n-3, n-6 and PUFA fatty acids among hominoids. Captive hominoids are higher than and significantly different from both monkey superfamilies only in the proportion of n-3 PUFA (Figure 8.13a). N-3 PUFA comprised $4.13 \pm 0.29\%$ of the milk of hominoids, $2.90 \pm 0.18\%$ of the milk of ceboids and $2.40 \pm 0.21\%$ of the milk of cercopithecoids. Ceboids are highest in the contribution of n-6 PUFA to milk ($30.44 \pm 0.63\%$) followed by cercopithecoids ($24.75 \pm 0.76\%$) and hominoids ($24.27 \pm 1.01\%$) (Figure 8.13b). Consequently, ceboids are also highest in total percent PUFA ($33.34 \pm 0.73\%$), followed by hominoids (28.41 ± 1.18) and cercopithecoids (27.15 ± 0.89).

Figure 8.13. Mean percent composition of (a) n-3 and (b) n-6 PUFA in captive and wild samples by superfamily.



Discussion

Effects of captivity: diet and maternal energy balance

Fatty acids in milk come from *de novo* synthesis by the mammary gland (lipogenesis) or are provided from circulating lipids in maternal plasma (reflecting maternal diet, past or present). These are not mutually exclusive pathways and mammary gland lipogenesis is strongly influenced by fatty acids in the maternal diet. Diets high in carbohydrates and low in fat are associated with increased production of medium chain fatty acids (4 – 14 carbons in length) by the mammary gland while those higher in fat are associated with decreased lipogenesis (Agostoni et al., 1999; Del Prado et al., 1999; Jensen et al., 1995; Koletzko et al., 1992). There is also a relationship between maternal energy balance (net residual of energy intake minus energy expenditure, Ellison, 2003) and the source of circulating fatty acids (Del Prado et al., 1999). Females in positive energy balance have milk fatty acid profiles that reflect maternal diet while the milk fatty acid profiles of mothers in negative energy balance reflect maternal fat stores.

The primary influence of captivity on milk fatty acids was diet. Captive individuals were likely consuming different fatty acids or fatty acids in different proportions than their wild counterparts. Research on human milk suggests that the main dietary factors affecting milk fatty acid profiles are the amount of carbohydrates and PUFA in the diet (Agostoni et al., 1999), two factors that are likely to differ between captive and wild individuals.

Captivity also may affect maternal depot fat, an alternative source for milk fatty acids. Captive individuals are generally larger (stature, body mass) and grow faster than their wild counterparts (Leigh, 1994a; Zihlman et al., 1990, 2004). As a result of increased energy intake and decreased energy expenditure, captive individuals are more likely to be in positive energy balance than wild individuals. Increased consumption of fatty acids combined with decreased expenditure of these fatty acids for energy suggests that captivity may allow mothers to transfer fatty acids directly into milk rather than depot stores. Milk fatty acids from captive living female anthropoids represent the capabilities of anthropoid mothers under conditions of positive energy balance, and can be modeled as the upper limits of the reaction norm for milk composition.

Wild and captive samples were separated into two distinct groups by PCA analysis. This was the result of large (and significant) differences in concentration and percent composition of 8:0, 10:0, 18:2n-6, 18:3n-3, and 22:6n-3 (Table 8.4). Milk from wild living groups had a larger contribution from the medium chain fatty acids 8:0 and 10:0. Wild and captive hominoid values (excepting hylobatids) were close to zero, and the difference between groups is better expressed as a comparison between wild and captive monkeys. Both fatty acids are synthesized by the mammary gland and act as precursors to other medium chain fatty acids (including 16:0 and 18:0). Higher values of 8:0 and 10:0 in wild monkey samples may reflect the increased synthesis of these fatty acids by the mammary gland and/or the decreased elongation of these fatty acids into longer-chained fatty acids as a result of relatively lower total lipids in the diet.

Wild samples were higher in mean percent composition of 18:3n-3. This difference between wild and captive samples is explained by higher values in mountain gorillas and mantled howlers. When these species are removed from the analysis, wild and captive samples are not significantly different in mean percent composition of 18:3n-3. This finding indicates that the proportion of 18:3n-3 in milk is not related to captivity and may be better explained by the dietary specialties of these species.

Like 18:3n-3, 18:2n-6 must be obtained from dietary sources. The highest value for wild samples was approximately 2% lower than the lowest value for captive samples. The lack of overlap suggests that captive individuals are consuming a source of this fatty acid not available to individuals living in the wild. Monkey chow, a common food item at both zoos and primate research facilities, provides 18:2n-6 in soy, corn and sunflower oils. Indeed, 18:2n-6 is the most prominent n-6 PUFA in western human diets due to high consumption of foods containing these oils (Brenna, 2002).

Monkey chow also usually contains fish meal as a protein source (Scheaff-Greiner et al., 1997). Fish meal (which contains fish oils) is high in 22:6n-3, providing captive individuals with a preformed source of this essential fatty acid. It is unlikely that 22:6n-3 in milks of captive individuals resulted from elongation (addition of carbons) and desaturation (addition of double bonds) of the 18:3n-3 molecule; there was a negative correlation between percent composition of 18:3n-3 and 22:6n-3 for wild and captive samples.

Diets were known for all captive living individuals in this study except the hylobatids, bonobo, and one chimpanzee. All individuals were provided with monkey

chow, but their overall diets varied in the amount of fruits, vegetables, insects and nuts used to supplement the monkey chow. That the hylobatids, bonobo and chimp cluster with their captive counterparts for percent composition of 18:2n-6 and 22:6n-3 suggests that the diets of these four females also provided these fatty acids.

One golden lion tamarin and two hylobatid samples from captive living individuals were excluded from analyses of the effect of captivity on milk fatty acid profiles because they clustered with samples from wild living females. There are no data on diets of the captive hylobatid samples but the captive golden lion tamarin's diet was well-documented by zoo attendants at the National Zoological Park. This female was free-ranging in the zoo's golden lion tamarin habitat. "Sienna" was a perfect case study for the effect of captivity on milk fatty acid profiles. Her foraging activity was more similar to her wild living tamarin counterparts, as was her diet which included insects, leaves, and fruit but very little monkey chow (Power, personal communication). Subsequently, milk fatty acid composition was more similar to wild than captive callitrichines, particularly in percent composition of 8:0, 10:0, 18:2n-6, and 22:6n-3.

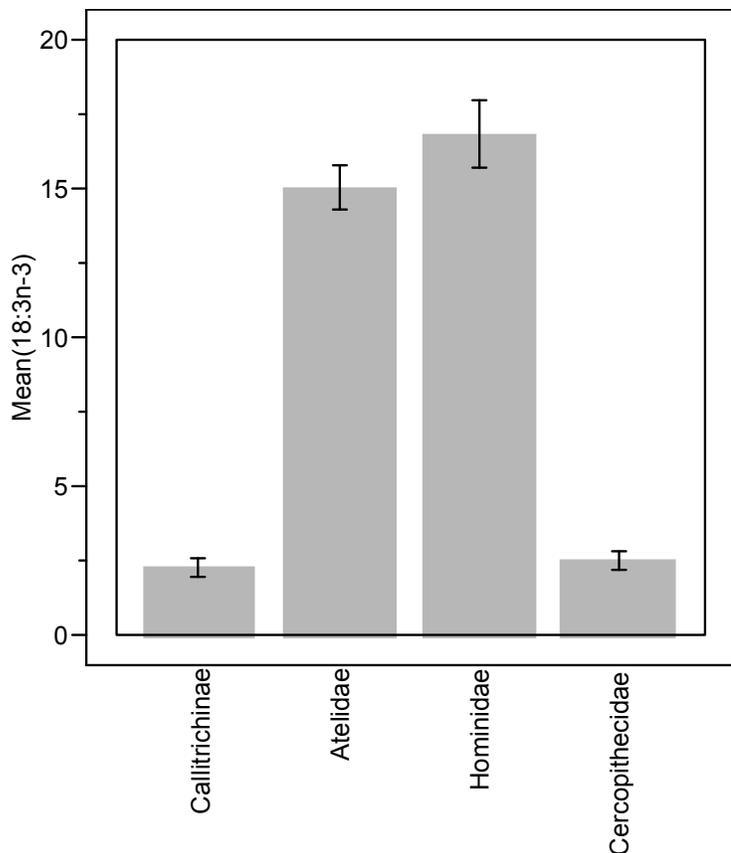
Dietary patterns among wild living anthropoids

Differences due to dietary specializations were explored only in samples from wild groups. Mountain gorillas and howlers were significantly different from all other species in 18:3n-3 concentration (Table 8.5). When analyzed as percent composition at the level of superfamily, hominoids appear to have significantly more 18:3n-3 in their milk than either monkey superfamily. However, reporting the mean value for ceboids

masked variation among individual species. The mean percent composition for mantled howlers was 15.06%, which is not significantly different from the hominoids, represented by mountain gorillas ($t = 1.71$, $p = 0.11$).

The separation of mountain gorillas and mantled howlers from the all other anthropoid species (Figure 8.14) reflects their dietary specialization in leaves (Fossey and Harcourt 1977; Glander, 1978; Goldsmith, 2003; Remis, 2003; Rosenberger and Strier, 1989), which are high in 18:3n-3 (Sanders, 1999). High levels in these species are 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 comprises 32.5% of milk fatty acids (Iverson and Oftedal, 1995).

Figure 8.14. Mean percent composition of 18:3n-3 by family (wild samples only)



Phylogenetic patterns

Captivity had a strong effect on milk fatty acid profiles. However, many patterns that were identified among wild living groups also were demonstrated among captive living groups. These patterns might therefore reflect the evolutionary history of species (or genera, family, or superfamily) rather than the response to ecological variation.

Significant variation was detected at the level of the species and superfamily for individual fatty acids (e.g., 8:0, 18:3n-3) and type of fatty acids (e.g., n-3s, PUFA). Concentration and percent composition of 8:0 and 10:0 were significantly higher in wild monkeys (Ceboidea and Cercopithecoidea) relative to mountain gorillas (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 circulating maternal lipids. 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 (three – six carbons in length) from fiber in the colon. Short chain fatty acids can then be converted into medium chain fatty acids. While this may explain the high concentration of 8:0 and 10:0 in the primarily folivorous mantled howler, the virtual lack of these fatty acids in the mountain gorilla suggests that the ability to make 8:0 and 10:0 is not related to fermentation of fiber.

Data from captive species supports the prediction that decreased production of 8:0 and 10:0 may be decoupled from diet and reflect a hominoid trait. An identical pattern in percent composition of 8:0 and 10:0 was found in captive groups of ceboids, cercopithecoids and hominoids, despite variation in diets between captive and wild groups, even of the same species. Iverson and Oftedal (1995) offer a possible mechanism. In a phylogenetic study of milk fatty acids across several orders within the Class Mammalia, they suggest the production of large amounts of medium chain fatty acids, including 8:0 and 10:0, among primates (as well as rabbits and rodents) may be due to the presence of an enzyme specific to mammary tissue. Their study did not include members of the superfamily Hominoidea. This dissertation is the first to report on the fatty acid composition of hominoid milk. Results seem to contradict the concept of a generalized

pattern for primate medium chain fatty acids. Rather, selection on activity of this enzyme may have been relaxed in hominoids, leading to reduced (or no) activity.

Monkeys and hominoids may also differ with respect to the elongation of 8:0 and 10:0 into longer-chained fatty acids, such as 16:0 or 18:0. Palmitic acid (16:0) is the first fatty acid formed by lipogenesis from shorter-chained precursors (Jensen et al., 1995). The percent composition of 16:0 was higher in milks of hominoids than either monkey superfamily; this pattern holds for both wild- and captive living groups. Hominoid metabolism may be derived from that of monkeys, favoring the elongation of 8:0 or 10:0 into 16:0. This explanation is not mutually exclusive from differences in enzyme activity between hominoids and monkeys, and the two could be operating in concert to produce increased levels of 8:0 and 10:0 in the milks of monkeys and higher levels of 16:0 in the milks of hominoids.

Interestingly, the two hylobatid samples (one each from *Hylobates lar* and *Symphalangus syndactylus*) have the highest values for 8:0 and 10:0 and the lowest values for 16:0 among the hominoids. Their medium chain fatty acid profiles match those of monkeys much better than other apes, suggesting that the difference in 8:0 and 10:0 percent composition is between great apes and all other anthropoid species investigated. The function of 8:0 and 10:0 in milk is hypothesized to be an energy substrate (Iverson and Oftedal, 1995). Relative to other apes, hylobatids are smaller-bodied and grow and develop on a more monkey-like trajectory (Leigh and Shea, 1996). It is interesting to hypothesize that enzyme activity controlling synthesis of 8:0 and 10:0 may be reduced in species with slower rates of somatic growth, such as the great apes.

Another pattern that was identified in both wild and captive milk samples was the negative correlation between the percent composition of 18:3n-3 and 22:6n-3 in milk. The correlation among wild living groups ($r = -0.496$, $p < 0.001$) was almost identical to that of captive living groups ($r = -0.493$, $p = 0.006$), despite a significant difference between these groups in the percent composition of 18:3n-3. Nonhuman primate females appear to be limited in the amount of 18:3n-3 in maternal plasma (diet, depot fat) that they can elongate and desaturate into 22:6n-3. One possible explanation is that metabolically, nonhuman primate mothers may be constrained in the energy available to elongate and desaturate 18:3n-3. The 18:3n-3 that was not synthesized into 22:6n-3 would then accumulate in milk.

Humans (Agostoni et al., 2001; Farquharson et al., 1992; Huang and Brenna, 2001) and baboons (Brenna, 2002; Scheaff-Greiner et al., 1997) have demonstrated inefficiency in conversion of 18:3n-3 to 22:6n-3. Experiments in both species with [^{13}C]-labeled 18:3n-3 and 22:6n-3 (reviewed in Brenna, 2002) indicated low conversion rates of 18:3n-3 to 22:6n-3, and the majority of milk 22:6n-3 came directly from [^{13}C]-labeled dietary 22:6n-3. When combined with data from this dissertation, it could be suggested that anthropoid primates, as a whole, are inefficient in conversion of 18:3n-3 to 22:6n-3.

Data suggest that the most efficient way to increase the proportion of milk 22:6n-3 in nonhuman anthropoids is to increase 22:6n-3 in the maternal diet. Despite the wide range of variation in 18:3n-3 by superfamily among wild living anthropoids (Figure 8.5a), the mean proportion of 22:6n-3 was nearly identical among superfamilies (Figure 8.5b). The percent composition of 22:6n-3 in the milk of wild anthropoids may thus

represent the maximum amount of 22:6n-3 that anthropoid primates are able to synthesize from 18:3n-3, but does not represent the maximum amount that anthropoid primates can put into milk. Captive groups, with a source of preformed 22:6n-3 in monkey chow, had significantly higher values of 22:6n-3 (Figure 8.10). This demonstrates that 22:6n-3 levels are not conserved, but rather, are highly sensitive to dietary intakes of 22:6n-3.

Synthesis of 22:6n-3 from 18:3n-3 can not be explored without consideration of the proportion of 18:2n-6 and 20:4n-6 in maternal plasma. The same enzymes (e.g., $\Delta 5$ - and $\Delta 6$ -desaturase) are used to add double bonds in the pathway from 18:3n-3 to 22:6n-3 and the pathway from 18:2n-6 to 20:4n-6 (Burdge, 2004; Innis, 2003). In this regard, the PUFA precursors 18:3n-3 and 18:2n-6 compete with each other for access to these enzymes, especially $\Delta 6$ -desaturase (Burdge, 2004; Huang and Brenna, 2001; Innis, 2003). Thus, levels of each PUFA precursor in maternal plasma will influence enzymatic activity, and LCPUFA synthesis.

Brenna (2002) reports that the rate of conversion of 18:3n-3 to 22:6n-3 in humans is highly dependent on the concentration of 18:2n-6 and 20:4n-6 in the maternal diet. High levels of n-6 PUFA (or, a high n-6 to n-3 ratio) reduce the concentration of n-3 PUFA in tissues, and in milk (Huang and Brenna, 2001). The reverse is also true; high concentration of n-3 PUFA in maternal plasma can reduce the conversion of 18:2n-6 to 20:4n-6. Diets of captive anthropoids were significantly higher than wild living anthropoids in the proportion of 18:2n-6, due to the inclusion of oils high in this n-6 fatty acid in monkey chows. An increased proportion of 18:2n-6 in captive diets may have limited the conversion of 18:3n-3 to 22:6n-3, but such a hypothesis is impossible to test

with the data at hand. Captive groups received a pre-formed source of 22:6n-3, and had correspondingly high values of this fatty acid. The diet of captive primates created noise, likely obscuring any nuanced relationships between n-3 and n-6 PUFA.

However, it is unlikely that the diets of wild living groups had “excessive” concentrations of n-6 PUFA. Instead, among wild living anthropoids, especially mantled howlers and mountain gorillas, high concentrations of n-3 PUFA may have affected the synthesis of n-6 PUFA. If high concentrations of 18:3n-3 in the diet (or maternal plasma) affected the synthesis of n-6 PUFA, one would predict that mountain gorillas, with over 16% of milk fatty acids as 18:3n-3, might have a reduced proportion of 20:4n-6 in their milk. Interestingly, the percent composition of 20:4n-6 was more than four times higher in mountain gorillas compared to wild- and captive living monkeys. Mountain gorillas may be better at desaturating and elongating 18:2n-6 into 20:4n-6 relative to monkeys and other hominoids (Figure 8.12). The correlation between 18:2n-6 and 20:4n-6 for captive living anthropoids was $r = 0.723$ ($p < 0.001$) and for wild living anthropoids, $r = 0.3166$ ($p = 0.09$). Removal of mountain gorillas from the latter calculation produced a correlation of $r = 0.5306$ ($p = 0.009$). This suggests that mountain gorilla physiology is metabolically distinct from other anthropoid primates, including closely related lowland gorillas (*Gorilla gorilla*). A high proportion of 18:3n-3 in the diet seems to be an insufficient explanation, as mantled howler milk has nearly identical values for percent composition of 18:3n-3 (15.06%) but had approximately one fourth of the 20:4n-6 found in mountain gorilla milk. As is the case of 22:6n-3, the most efficient method for putting 20:4n-6 into milk is to have a source of 20:4n-6 in the maternal diet. An alternative

explanation to a metabolic difference in mountain gorillas could be that they have a source of preformed 20:4n-6 in their diet that has not yet been identified.

Milk fatty acids and life history traits

Research questions regarding life history traits and fatty acid composition of milk focused on correlations between rates of somatic growth and relative brain size and the percent composition of particular fatty acids. However, no statistical analyses were performed between milk fatty acid data and available data on nonhuman primate life history traits. First, key components of the primate life history data set were unreliable, particularly measures of neonatal and adult brain size. Second, data were not available for many of the species included in this study. Finally, captivity produced more noise in the results than was predicted, particularly for PUFA and LCPUFA, and would confound any statistical tests for significance.

Some tentative conclusions can be drawn based on patterns identified in both captive- and wild living anthropoid groups. The milk of New World monkeys is highest in percent composition of 8:0 and 10:0, followed by Old World monkeys, and then the great apes (including humans). This order is the same as that of growth rates among anthropoid primates, suggesting that selection may have favored a higher proportion of these fatty acids, which are used as energy substrates, in faster growing primates.

The percent composition of the LCPUFA 22:6n-3 and 20:4n-6 from wild- and captive living anthropoids, with the exception of mountain gorillas, were not significantly different at the level of the superfamily. LCPUFA, particularly 20:4n-6 and 22:6n-3, are

necessary for proper retinal and neural development, including myelination of the brain and central nervous system (Carlson, 2001). 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). The dietary supply of these fatty acids must be sufficient to allow and support brain growth. The dietary input of the 18-carbon precursors, their conversion by the liver to LCPUFA, and their incorporation into the brain should be related quantitatively to the ultimate size and function of the brain. This dissertation identified no differences in the proportion of 22:6n-3 in the milks of wild anthropoid primates, despite variation in relative brain size among species. Although the proportion of 20:4n-6 was highest among the larger-brained mountain gorilla, the similarity among other apes (humans, captive lowland gorillas and chimps) and all other anthropoid primates in this study suggest that high levels in the mountain gorilla are not related to larger brain size. A higher proportion of milk 22:6n-3 seems to be the result of dietary, rather than phylogenetic or life history, variation.

Human milk fatty acids in an evolutionary perspective

Human milk fatty acid profiles have been well-studied, particularly LCPUFA. The relationship between maternal diet and milk fatty acids prohibits the description of *the* human milk fatty acid profile. However, cross-cultural investigations of human milk fatty acid composition indicate that some fatty acid proportions are consistent despite dietary variation (Jensen et al., 1995; Koletzko et al., 1992; Yuhas et al., 2006). In this

section, I discuss results on human milk fatty acids (presented in Chapter 4) with respect to findings on anthropoid milk fatty acid profiles (summarized in Table 8.8) to address questions of primitive, shared-derived, and unique-derived traits of human milk fatty acid composition.

Anthropoid traits

The predominant saturated fatty acid in human and nonhuman anthropoid primate milk was 16:0 (palmitic acid). 16:0 in milk can come from the diet; palmitic acid makes up between 20 to 30% of animal and vegetable fats, and up to 40% of palm oil (Jensen et al., 1995). It can also be made within the body by elongating shorter-chain fatty acids. 16:0 is the first fatty acid in the 8:0 lipogenesis pathway (Jensen et al., 1995). It may be possible that high levels of 16:0 in anthropoids, including humans, are the result of the ubiquitous nature of this fat in animals and vegetables, or of the compensatory elongation of shorter-chain fatty acids. Whatever the source, the high proportion of 16:0 in milk appears to be a primitive trait of anthropoid milk (cf. Iverson and Oftedal, 1995).

The proportion of 18:3n-3 in milk was strongly influenced by the degree of folivory, or amount of leaves, in the diets of wild anthropoid primates. Among humans, vegetarians and vegans are likely to be more “folivorous” than women on typical Western diets. Indeed, the lowest 18:3n-3 values were identified in women on a Western diet. This pattern moves beyond the primate order. Mammalian species that consume more leaves and/or grasses, such as the koala or equids (Iverson and Oftedal, 1995), have larger quantities of milk 18:3n-3. Human milk is also not unique in the proportion of

18:2n-6. Diets high in this n-6 PUFA (e.g., nonhuman anthropoids consuming monkey chow or Chilean women consuming a diet of primarily maize) were associated with higher milk 18:2n-6. Values from vegetarian and vegan women were similar to captive living anthropoids, probably due to the fact that these groups consumed higher quantities of soy and canola oils than their omnivorous or captive living counterparts.

Table 8.8. Fatty Acid composition (Mean percent composition \pm SE) for captive and wild hominoids (HM), ceboids (NWM), and cercopithecoids (OWM).

Fatty acid	Wild HM	Captive HM	Wild NWM	Captive NWM	Wild OWM	Captive OWM
8:0	0.03 \pm 2.12	0.50 \pm 0.56	11.83 \pm 1.09	4.20 \pm 0.34	8.86 \pm 1.68	4.13 \pm 0.403
16:0	27.10 \pm 1.41	25.55 \pm 0.69	22.24 \pm 0.87	17.53 \pm 0.43	26.79 \pm 1.22	23.97 \pm 0.50
18:2n-6	10.56 \pm 1.49	23.61 \pm 1.16	8.06 \pm 0.91	28.94 \pm 0.69	8.03 \pm 1.29	23.88 \pm 0.81
18:3n-3	16.31 \pm 2.33	3.27 \pm 0.34	6.98 \pm 1.20	2.29 \pm 0.21	2.55 \pm 1.84	1.55 \pm 0.25
20:4n-6	2.08 \pm 0.14	0.63 \pm 0.04	0.52 \pm 0.07	0.79 \pm 0.03	0.32 \pm 0.11	0.44 \pm 0.03
20:5n-3	0.22 \pm 6.02	0.18 \pm 0.03	0.08 \pm 0.01	0.08 \pm 0.02	0.11 \pm 0.03	0.16 \pm 0.02
22:6n-3	0.09 \pm 0.04	0.30 \pm 0.05	0.12 \pm 0.02	0.34 \pm 0.03	0.16 \pm 0.03	0.44 \pm 0.03

Human and nonhuman anthropoid milk n-3 and n-6 PUFA appear to be intimately tied to dietary supply of these fatty acids. Further, human milk does not appear to be distinct from that of anthropoids in the proportion of these fatty acids; all anthropoids may have similar metabolic capabilities in transferring dietary n-3 and n-6 PUFA to milk. Results from this project suggest that conversion of 18:3n-3 to 22:6n-3 by human

mothers and levels of both 20:4n-6 and 22:6n-3 in human milk also may be traits shared by anthropoids.

Studies on both human and baboons have demonstrated that these species are inefficient in the conversion of 18:3n-3 to 22:6n-3 (reviewed in Burdge, 2006; Scheaff-Greiner et al., 1997; Su et al., 1999, 2005). Among adult humans, increased consumption of 18:3n-3 was not associated with increased 22:6n-3 in plasma lipids (Burdge and Calder, 2005), and in one study (Finnegan et al., 2003), was associated with decreased plasma lipid concentrations of 22:6n-3. Human neonates and infants may be less efficient than adults in conversion to 22:6n-3. Without a preformed source of 22:6n-3 in their diet, the rate of 22:6n-3 formation from 18:3n-3 (measured with deuterated 18:3n-3 ethyl esters) in human infants may be inadequate in meeting neural requirements, especially in preterm infants who have an increased requirement for 22:6n-3 (Salem et al., 1996). Findings are similar in baboons. In a study using [¹³C]-labeled 18:3n-3 and 22:6n-3 (Su et al., 1999), the relative accretion of labeled 22:6n-3 as a percentage of the dose received was seven times greater in baboons receiving 22:6n-3 than those receiving 18:3n-3. Scheaff-Greiner et al. (1997) found that preformed 22:6n-3 was incorporated into fetal baboon tissues at least one order of magnitude higher than was 22:6n-3 derived from 18:3n-3. Su et al. (1999) concluded that lipid plasma responses to preformed n-3 PUFA and LCPUFA supplementation were similar between baboons and humans. The negative correlation between mean percent composition of 18:3n-3 and 22:6n-3 in milk of wild- and captive living anthropoids suggests that metabolic synthesis of 22:6n-3 may be a shared physiological trait of all anthropoids. Mountain gorillas and mantled howlers

consume diets containing large amounts of 18:3n-3, which was reflected in a significantly higher proportion of milk 18:3n-3. No significant differences were identified in milk 22:6n-3 between these species and other wild living anthropoids. Indeed, mean percent composition of 22:6n-3 was lowest in mountain gorillas (although not significantly different), despite having the highest proportion of milk 18:3n-3. This data set can only speak to the relationship between maternal diet and milk fatty acids, and it is possible that compensatory mechanisms are present in neonates and infants to convert milk 18:3n-3 into longer chain n-3 fatty acids. The inefficiency of among adults and infants in both the human and baboon does not support this position, however. Among anthropoids, the most efficient way to increase 22:6n-3 in milk and in tissues, including neural tissues, seems to be with a preformed source in the diet.

The strong relationship between maternal dietary intake of 22:6n-3 and the proportion of 22:6n-3 in the milk has been well documented in the human (Agostoni et al., 2001; Brenna, 2002; Koletzko et al., 1992, 2001; Yuhas et al., 2006) and nonhuman primate (Iverson and Oftedal, 1995) literature. 22:6n-3 is the most variable milk fatty acid among human populations. The same degree of variability identified in human milk (0.1 – 0.99%: Gibson and Makrides, 1999; Koletzko et al., 1992; Yuhas et al., 2006) was identified in samples from wild and captive anthropoids (Figure 8.15).

Nonhuman anthropoids have the capacity to produce milk with comparable 22:6n-3 levels to humans, if given a dietary supply of this fatty acid. Selection does not seem to have provided humans with a unique mechanism for supplying 22:6n-3 during lactation, a finding that seems surprising in light of the increased requirements for 22:6n-3 in brain

and central nervous system tissues among human infants relative to nonhuman primates (Agostoni et al., 2001; Carlson, 2001; Gibson and Kneebone, 1981; Gibson and Makrides, 1999, 2000).

Yuhas et al. (2006) reported little variation among nine human populations in the proportion of 20:4n-6 in milk. However, as illustrated in Table 4.1, there may be a relationship with the amount of animal products (primarily meat and eggs) in the diet and levels of milk 20:4n-6. Vegetarians have lower levels of 20:4n-6 than women on Western and non-Western diets that consume animal products, despite significantly higher levels of milk 18:2n-6. Although the pathway from 18:2n-6 to 20:4n-6 requires less energy (fewer steps for elongation and desaturation) than that from 18:3n-3 to 22:6n-3 (Agostoni et al., 2001), a high proportion of 18:2n-6 in the diet does not equate to high levels of milk 20:4n-6. In a study of labeled 18:2n-6, Del Prado et al. (2001) found that little human milk 20:4n-6 originates from conversion of 18:2n-6, but instead comes from maternal depot stores of 20:4n-6. It appears, therefore, that the picture is more complex, and that the inclusion of 20:4n-6 in the diet (past or present) does increase milk 20:4n-6 among humans.

How, then, to explain the high levels of 20:4n-6 in the milk of vegetarian, and primarily leaf-eating, mountain gorillas? Percent composition of 20:4n-6 for mountain gorillas (2.08%) were nearly five times those of the mean reported by Yuhas et al. (2006) despite almost identical values for percent composition of milk 18:2n-6. As compared to humans and other nonhuman primates, including lowland gorillas, mountain gorilla milk appears to be unique in its 20:4n-6 composition. Further investigation into mountain

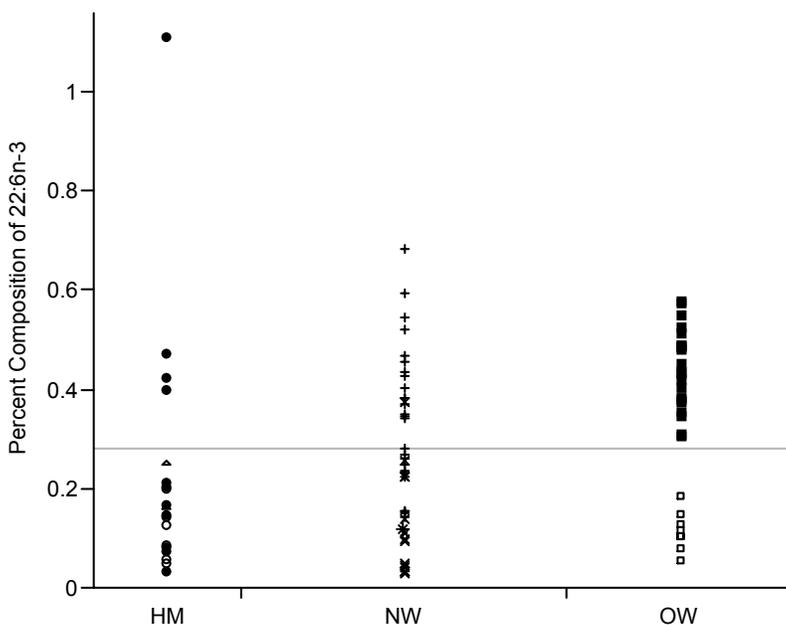
gorilla diet and metabolism of 18:2n-6 is required to understand the possible evolutionary significance of this finding. Excluding this species, nonhuman anthropoids, both captive and wild, produced milks with similar proportions of 20:4n-6 to published human values and higher than milks from vegetarian and vegan mothers. Thus, human milk does not appear to have a special claim on high values of 20:4n-6 for growth and maintenance of a relatively larger brain.

Human milk 20:4n-6, however, may be unrelated to 20:4n-6 levels in the infant brain. Until recently (2002), human infant formulas lacked any preformed source of 20:4n-6. Therefore, any 20:4n-6 in the formula-fed infant brain was presumed to come from 18:2n-6 that the infant synthesized *in vivo*. Comparing post-mortem brain tissue of formula-fed and breast-fed infants revealed no significant difference in the concentration of 20:4n-6 (Farquharson et al., 1992; Makrides et al., 1994). This finding is critical to issues surrounding infant formula supplementation as it suggests that infants have a compensatory mechanism for 20:4n-6 and are not reliant on the dietary (milk) supply of this fatty acid.

This finding may also speak to issues in human evolution. 20:4n-6 is an essential component of neural tissues, and natural selection may have acted on infant metabolism in order to regulate the concentration of this fatty acid in the developing infant brain, regardless of dietary supply. This suggests that 20:4n-6, found mainly in animal tissue and eggs, may not have been a consistent part of the hominid diet. Sarkadi-Nagy et al. (2003, 2004) report similar findings in baboon neonates fed formula without 20:4n-6, formula with 20:4n-6 and breast milk (with 0.68% 20:4n-6). No significant differences

were found in the concentration of 20:4n-6 in the brain among these groups, suggesting that the compensatory mechanism for 20:4n-6 synthesis in brain tissues may be part of our anthropoid, or at least Old World Anthropoid, legacy.

Figure 8.15. Percent composition of 22:6n-3 by superfamily.



Hominoid traits

Data on human fatty acid composition supports the hypothesis that a low proportion of 8:0 and 10:0 in milk is a derived trait of great apes (hominoids, excluding hylobatids). The highest reported value in Yuhas et al.'s (2006) cross-cultural study for 8:0 was 0.28%, which is within the range identified for captive hominoids and is far below that of either captive or wild living monkeys. Their highest reported value for 10:0 was 2.35%; this value (from the Philippines) is higher than captive and wild hominoids,

and similar to captive Old World monkeys, but is one third of that of wild Old World monkeys and one fifth that of wild New World monkeys. (Figure 8.8b). Similar proportions (between 2 – 3%) of 10:0 were identified in the milk of women from Tanzania and Surinam (Muskiel et al., 1987), supporting the finding among nonhuman mammals that higher values of 10:0 come from females with low fat, high carbohydrate diets (Del Prado et al., 1999; Iverson and Oftedal, 1995). Higher proportions of 10:0 reflect increased lipogenesis by the mammary gland to compensate for the lack of lipid in the diet. However, human values never approach those identified from wild living monkeys, suggesting a phylogenetic difference in lipogenesis between great apes and monkeys, and possibly, lesser apes. With only two hylobatid samples, I am cautious about making conclusions about the placement of lesser apes into the overall pattern. More samples from great and lesser apes will be necessary to test the hypothesis that a lower proportion of energy from 8:0 and 10:0 in milk is a great ape (Hominidae, Pongidae) pattern, possibly related to reduced energy requirements of somatic growth that accompanied the phylogenetic split from the Hylobatidae.

Are there any unique human traits?

A pattern of early postnatal brain growth that is unique among mammals must require a unique milk to supply the needs of the developing human infant (Martin 1983: 41).

Martin (1995) predicted that an investigation of primate milks would reveal the biochemical requirements necessary for human brain growth, emphasizing LCPUFA such as 20:4n-6 and 22:6n-3. His prediction was based on the larger relative brain size of

humans and the unique trajectory of postnatal brain growth that involved increased requirements for 20:4n-6 and 22:6n-3 (Innis, 2003). This comparative study of anthropoid milk fatty acid composition suggests that human milk fatty acid profiles fit well within the larger hominoid or anthropoid pattern. Wild living anthropoid primates produced the lowest proportion of 22:6n-3 in their milk, but similar values have been reported for human mothers. Captive living anthropoids, when provided with a dietary source of 22:6n-3, were able to produce milks with similar proportions and concentrations of this n-3 LCPUFA.

Although seemingly “a nail in the coffin” for Martin’s hypothesis (1983, 1995), results from this dissertation do not speak to *how much* LCPUFA the infant was consuming nor do they indicate how LCPUFA are utilized by the developing anthropoid infants. Data on milk composition reflect what the mother is physiologically capable of manufacturing, which is not equivalent to what the infant is able to extract over the course of lactation. For example, human mothers with a high BMI produced milks of higher fat concentration than women of low BMI, but produced less milk (Barbosa et al., 1997). The overall fat consumption by infants of both populations was, thus, not significantly different. Conversely, if human infants ingest more milk than nonhuman primates, their intake of 22:6n-3 will be higher despite similar proportions of this fatty acid in milk. Humans may also differ from nonhuman primates in fatty acid metabolism. Selection may have acted on human infant metabolism, such that any 22:6n-3 that was ingested was preferentially used for brain growth and development. One possible mechanism was suggested by Chamberlain (1996). He argued that the enzyme

ethanolamine phosphotransferase, which sequesters n-3 LCPUFA, might have been under selection over the course of human evolution.

It is also possible that selection operated on maternal physiology, to ensure the transfer of necessary building blocks for fetal and infant brain growth and development. In humans, the amounts of 22:6n-3 and 20:4n-6 increase markedly in the central nervous system during the last trimester the first year of postnatal life (Carlson, 2001). This pattern is different than that observed in rats, who obtain most of the 22:6n-3 after birth (Carlson, 2001). It may be possible that larger brain size, and the pattern of rapid postnatal brain growth, selected for preferential placental transfer of LCPUFA among humans (and their ancestors who shared this brain growth trajectory). Maternal plasma concentrations of 22:6n-3 have been demonstrated to increase over the course of pregnancy, and the placenta is believed to selectively incorporate 22:6n-3 and 20:4n-6 rather than elongate and desaturate their n-3 and n-6 precursors, respectively. Tentative support for preferential transfer of LCPUFA comes from examination of body fat composition of human neonates. At birth, human baby fat contains very low amounts of 18:2n-6 and 18:3n-3, despite their inclusion in maternal fat stores and higher than maternal fat levels of 20:4n-6 and 22:6n-3 (Cunnane, 2005).

Higher concentrations of 22:6n-3 in pregnant humans has been partially explained by an increase in the concentration of circulating estrogen, which enhances the synthesis of 22:6n-3 from 18:3n-3 (Burdge, 2004; Giltay et al., 2004). To my knowledge, no research has been conducted on the relationship between estrogen and 22:6n-3 synthesis

in nonhuman primates. Increased concentrations of circulating estrogen in humans may result in increased placental transfer of 22:6n-3, regardless of dietary intake of 22:6n-3.

Agostoni (2005) offers an interesting perspective on LCPUFA fatty acids in milk. He proposes that fetuses may become accustomed to the supply of fatty acids from their mother during intrauterine life. Infants are “imprinted” with a specific fatty acid pattern during gestation, and their fatty acid metabolism may be “programmed” to the maternal environment and the infant’s genetic background. Based on these assumptions, Agostoni (2005) believes that the fatty acid composition of his/her own mother’s milk is the best for any infant and that he/she has been adapted during fetal life to manage (and grow and survive with) a specific fatty acid pattern.

Conclusions

(1) What is the effect of diet on fatty acid profiles of anthropoid primates? Is there a relationship between fatty acids in the maternal diet and fatty acids in milk? There is a strong influence of maternal diet on anthropoid fatty acid profiles, particularly 18:3n-3, 18:2n-6, and 22:6n-3. A higher mean percent composition for 18:3n-3 was identified in the folivorous species *Gorilla beringei* and *Alouatta palliata*. Captive living individuals had significantly higher proportions of milk 18:2n-6 and 22:6n-3 due to the inclusion of monkey chow in their diet. Subsequently, the contribution of medium chain fatty acids to the milks of captive living females was reduced. Higher dietary lipid intakes reduced lipogenesis by the mammary gland. Captivity also had an effect on maternal energy

balance. Milk fatty acids from captive living female anthropoids represent the capabilities of anthropoid mothers under conditions of positive energy balance, and can be modeled as the upper limits of the reaction norm for milk composition.

(2) Do milk fatty acid profiles show any phylogenetic patterns? Is milk composition constrained by evolutionary history? After controlling for diet, medium chain fatty acid profiles varied with respect to phylogeny. Milk from monkeys (ceboids and cercopithecoids) was significantly higher than apes in the proportion of 8:0 and 10:0. The derived nature of ape milk may be the result of differences in lipogenesis of these fatty acids by the mammary gland and/or increased elongation of these fatty acids into 16:0 by apes, relative to monkeys. Anthropoids, as a whole, appear to be inefficient in conversion of 18:3n-3 into 22:6n-3. Without a preformed source of 22:6n-3 in the diet (as was the case for wild living individuals), no significant differences were identified in milk 22:6n-3 across the three superfamilies. Mountain gorillas appear to be unique among anthropoids in the production of milk with a higher proportion of 20:4n-6. It is unclear whether the mechanism for this increased production is dietary or metabolic.

(3) Do milk fatty acid profiles vary with respect to life history traits? The phylogenetic difference in percent composition of 8:0 and 10:0 may relate to differences in somatic growth rate between monkeys and apes. A proposed function for 8:0 and 10:0 in milk is as an energy substrate. Monkeys may have an increased need for these fatty acids due to more rapid periods of growth and development, and shorter (relatively and absolutely) lengths of lactation. No relationship was identified between relative brain size and LCPUFA composition among wild living anthropoids. Captive diets created too

much “noise” in the data to be able to test for significance. Lack of significant variation among superfamilies suggested that dietary intake of 22:6n-3 was more important in determining milk 22:6n-3 levels than was relative brain size.

(4) Do human milk fatty acid profiles share patterns with other anthropoid primates? Are values reported for human milk fatty acids within the range of variation identified for anthropoid values? Human milk fatty acid composition has primitive traits shared with all anthropoids in this study and derived traits shared with hominoid species in this study. Palmitic acid was the predominant saturated fatty acid in milks of human and nonhuman anthropoids. Reported values for percent composition of 18:3n-3, 18:2n-6, 22:6n-3 and 20:4n-6 in humans do not appear to be unique, and overlapped or were identical to those identified in wild- and captive living anthropoids. Additionally, all anthropoids appear to be inefficient in conversion of 18:3n-3 to 22:6n-3. 8:0 and 10:0 make a very small contribution to the milk of humans, following a great ape pattern.

**CHAPTER 9: PROXIMATE MILK COMPOSITION
OF ANTHROPOID PRIMATES**

Introduction

Milk is a complex biological fluid composed of organic and inorganic materials that serve nutritional functions, immune functions, or both. The major nutritional components of mammalian milk are fat, protein, sugar, minerals, and water (Ofstedal, 1984). The concentration and composition of these nutrients vary among species, within species, and even within individuals (Emmett and Rogers, 1997; Jensen et al., 1995; Prentice, 1996; Stini et al., 1980). Variation in milk composition among mammals demonstrates that milk composition has been modified by the forces of evolution since the emergence of the class Mammalia. Variation in milk composition within species and individuals further demonstrates that milk composition is highly plastic and influenced by both genetic instructions and environmental variation.

Milk is also a compromise. Milk composition reflects the requirements of the infant (nutritional and immune) *and* the physiological ability of the mother to meet those requirements. Interspecific comparisons of milk composition are therefore comparisons of how this compromise between mother and infant has been negotiated across species. Data on milk yield and nursing frequency which are not yet available would provide a more complete picture of exactly what the infant is receiving. This dissertation provides an understanding of what mothers are physiologically capable of producing under various environmental conditions and a framework for identifying evolutionary modifications on lactation strategies.

Research Questions

Anthropoid milk, relative to that of other mammalian orders, has been described as dilute, low in total energy, and low in energy from protein (Ofstedal and Iverson, 1995). Such a generalization masks possible variation in milk composition within the anthropoid suborder. Variation in the lactation and reproductive strategies of anthropoids (e.g., female body mass, age at first reproduction, duration of lactation, reproductive life span) indicate that there is more than one way to be an anthropoid primate. It follows that there may be more than one type of anthropoid primate milk. In this chapter I investigate the nature of variation in anthropoid proximate milk composition (fat, protein, lactose, dry matter, and minerals) from the perspective of diet, phylogeny and ontogeny to identify primitive, shared-derived and unique-derived features. I test the null hypothesis of a generalized anthropoid milk composition and address the following research questions:

- (1) What are the relationships among milk components, milk energy, and the proportion of energy in milk provided by sugar, protein, and fat? Do milks with higher energy values have higher fat content? Is protein concentration positively correlated with the concentration of phosphorus or calcium?
- (2) Does proximate milk composition show any phylogenetic patterns? Is proximate milk composition constrained by evolutionary history?
 - (a) Is the milk of closely-related species more similar in proximate composition than that of distantly-related species?

- (b) Is there such a thing as ape milk or New World monkey milk, or are such generalizations prohibited due to differences in composition at the level of the species?
- (3) What is the effect of captivity on milk composition?
- (a) Which components of milk are most sensitive to captivity? Which components of milk appear to be insensitive to captivity?
- (b) Does milk from captive living individuals differ from that of wild living individuals of the same, or closely-related, species?
- (4) Does a species dietary strategy influence milk composition?
- (a) Do folivorous primates have higher concentrations of protein or higher proportion of milk energy provided by protein relative to other dietary strategies?
- (b) Is there a relationship between dietary quality (folivores being the lowest quality diet, frugivore/insectivore being the highest quality) and milk composition?
- (5) Does milk composition show a relationship to other aspects of a species lactation strategy or overall life history strategy?
- (a) What is the relationship between milk composition and maternal body size?
- (b) What is the relationship between milk composition and length of lactation, both absolute and relative (as a percentage of the life span)?
- (c) Do primates with larger EQ values produce higher energy milks? Is there a relationship between EQ and percent energy from fat or sugar?
- (6) Does human milk share similarities with anthropoid primate milk?

- (a) Are similarities in life history traits between humans and apes (similar gestation length, longer duration of lactation relative to monkeys, larger body size relative to monkeys) reflected in similar milk composition?
- (b) Does human milk composition differ from that of other anthropoid primates because of unique ontogenetic requirements of human neonates and infants?

Materials and Methods

Milk samples

Milk samples from 14 species representing each of the three anthropoid superfamilies were analyzed for total fat, crude protein, lactose (sugar), dry matter, phosphorus, and calcium. Samples were collected from wild populations of *Alouatta palliata*, *Callithrix jacchus*, *Gorilla beringei*, *Leontopithecus rosalia*, and *Macaca sinica* and captive populations of *Callithrix jacchus*, *Cebus apella*, *Gorilla gorilla*, *Hylobates lar*, *Leontopithecus rosalia*, *Macaca mulatta*, *Pan troglodytes*, *Pan paniscus*, *Pongo pygmaeus*, *Saimiri boliviensis boliviensis*, and *Symphalangus syndactylus*.

Methods of milk analysis

All samples were maintained in a -20° C freezer until removed for subsampling or analysis. Samples were vortexed prior to each subsampling procedure. Subsamples were done in duplicate. In each procedure, weights (g) of samples were recorded and final composition of fat, crude protein, lactose, and dry matter (DM) were recorded as a

weight-per-weight basis. Results for fat, crude protein, lactose, and DM are expressed as both weights and percent of total weight. Methods are based on previous research on nonhuman primate milk composition (Ofstedal and Iverson, 1995; Power et al., 2002, in press; Tilden and Ofstedal, 1997).

For microfat analysis, approximately 125 μ l of milk was delivered into 2 ml centrifuge tubes using a 250 μ l positive displacement pipetter (PDP) and the weight of the sample was recorded to 0.0001 g. Tubes were sealed with parafilm and placed immediately into the freezer until time of analysis. Total lipid was assayed by a modification of the Rose-Gottleib procedure (AOAC, 1975).

Dry matter was measured gravimetrically after drying 17.5 μ l of sample for three hours at 100° C in a forced-air drying oven. Samples were weighed to 0.001 mg both before and after drying to determine percent dry matter. Dried samples were placed in a sealed plastic tray inside of a dessicator for use in crude protein analysis. Crude protein was estimated from total nitrogen in each milk sample. Nitrogen was assayed using a CHN elemental gas analyzer. The percent of crude protein in each sample was calculated as: $6.38 \times \text{percent total nitrogen} \times \text{percent dry matter}$.

Sub-sampling for sugar requires approximately 11 μ l of sample be delivered by a 25 μ l PDP into a tared 30 ml Nalgene bottle. Sample weight was recorded to 0.0001 g and distilled water was added until the total weight reached approximately 25 g. Final weights were recorded to 0.0001 g, bottles were capped and placed into freezer until time of analysis. Total sugar was assayed by the phenol-sulfuric acid method, using lactose monohydrate as the standard (Dubois et al., 1956; Marier and Boulet, 1959). Sulfuric acid

hydrolyzes mono-, di- and oligosaccharides. When these hydrolyzed sugars are hot, they react with phenol and form a stable yellow-orange chromogen. After this solution has cooled, the concentration of sugar is measured using a ultra-violet spectrophotometer.

Mineral analyses required removing all organic material from each milk sample by digesting 0.5 – 1.0 ml of sample in 20 ml of 70% nitric acid and 5 ml of 70% perchloric acid over a heat source (hot plates). After a series of digestions, the sample was removed from the hot plate when it was approximately 1 ml in volume. A small amount (approximately 1 – 2 ml) of Millipore water was immediately added and when the flask cooled (approximately 2 – 3 minutes), an additional 5 ml of Millipore water was added to the digested sample. Cooled flasks were weighed to 0.0001g and, subtracting flask weight, digested weight was brought up to approximately 25 g through the addition of Millipore water. Digested solutions were transferred into labeled 30 ml Nalgene bottles and kept at room temperature for use in phosphorus and calcium procedures. The percent phosphorus in each sample was determined by a colorimetric procedure similar to that used to determine percent lactose. Standards and samples were aspirated through a UV spectrophotometer to determine absorbance, which was then converted to a concentration ($\mu\text{g/ml}$) from use of a standard curve (seven standards, the first of which is a blank, each run in duplicate). The percent calcium in each milk sample was determined by analysis of digested samples on the AA Spectrophotometer.

In addition to gross composition data, energy values for fat, protein, and sugar, as well as total gross energy were calculated for each milk sample. Energy from fat, protein, and sugar were calculated using the following energy values (following Oftedal, 1984;

Oftedal and Iverson, 1995; Power et al., 2002): 9.11 kcal/g for fat; 5.86 kcal/g for crude protein; and 3.95 kcal/g for sugar. As described in Chapter 7, total gross energy for each sample was calculated as the sum of energy from fat, protein, and sugar and the percent of energy from fat, protein, and sugar were calculated by dividing energy values for each component by total gross energy.

Methods of quantitative analysis

Statistical methods employed in this chapter are modeled after published studies of intra- and interspecific comparisons on nonhuman primate milk (cf., Power et al., 2002, in press; Oftedal and Iverson, 1995). Data on proximate composition were normally distributed and permitted the use of parametric statistics. The relationships between milk constituents were analyzed using linear regression and pairwise correlation analysis (Pearson product-moment correlations). Significant differences in mean concentration among families and superfamilies and among species within the superfamily Ceboidea were determined using analysis of variance (ANOVA). A non-significant p-value from an ANOVA test ($\alpha \geq 0.05$) indicates that the means of all groups are not significantly different from each other, but this is not a test for significant differences between groups. Tukey-Kramer, a post-hoc method of analyzing differences between groups of unequal sizes, was used to perform multiple pairwise comparisons between groups. Two-tailed t-tests also were used to compare differences between superfamilies, where group sizes were larger and differences between numbers of samples in each group were reduced.

In addition to testing between differences in means between groups, t-tests and ANOVAs also were employed to determine differences in range of values by superfamily. One assumption made in performing t-tests and ANOVA is that there is equal variance between each group. SPSS 13.0 performs the Levene test for equality of variances and determines the Levene statistic. If this statistic is significant, the variance between groups is equal. An insignificant Levene statistic therefore indicates that the data are not of equal variance between groups.

Finally, factor analysis (using PCA as the method of factor extraction) was used to combine individual components of milk into a single data point. Milk data included in factor analysis were percent energy from fat, protein, and sugar, and total gross energy. Extracted factors, or principle components, were then plotted against life history variables, including female body mass and length of lactation to look for patterns both along the x axis (variation with respect to the life history trait) and y axis (intra- and interspecific variation).

Statistical significance for all tests was set at $\alpha < 0.05$. Data were analyzed using JMP 5.1.2 (SAS Institute, 2004) and SPSS 13.0 for Windows (SPSS, 2004).

Results

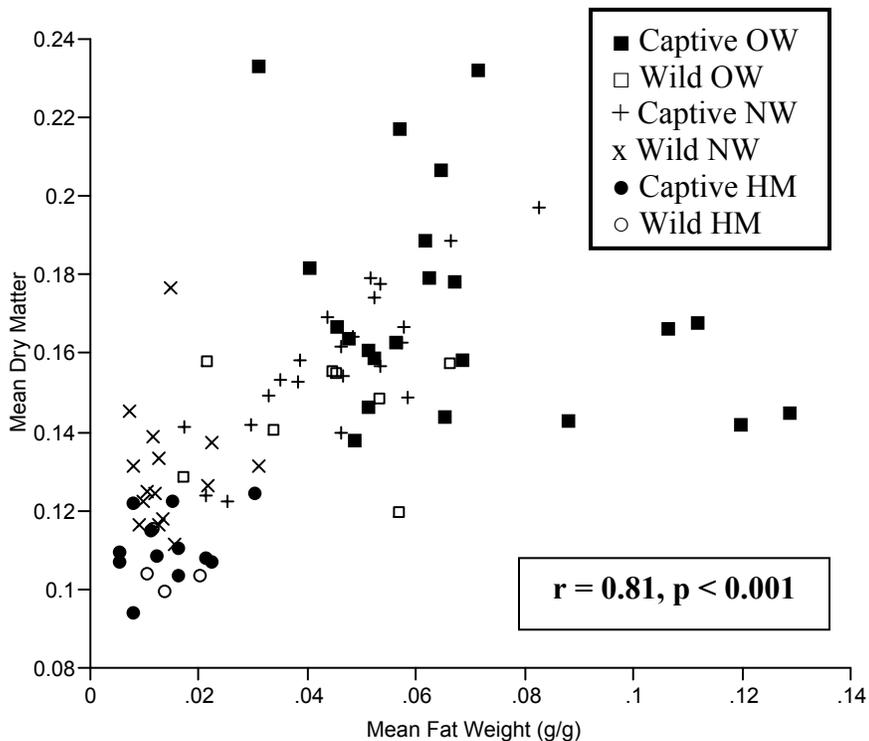
Relationships among milk constituents

Previous analyses on milk composition from humans, nonhuman primates, and other mammals (Power et al., 2002, in press; Oftedal, 1984, 1993, 2000; Oftedal and

Iverson, 1995;) identified significant correlations among milk constituents. These include a positive correlation between fat and dry matter, protein and dry matter, phosphorus and protein, calcium and protein, calcium and phosphorus, and fat and total gross energy. Correlation analysis was used to test for significance between these milk constituents among anthropoid primates in this study. The following shorthand was used in figures: NW = New World monkeys (ceboids), OW = Old World monkeys (cercopithecoids), and HM = apes (hominoids).

As predicted based on previous literature, mean fat and dry matter were positively and significantly correlated ($r = 0.81$, $p < 0.0001$; Figure 9.1). Increases in milk fat decreases the aqueous portion of milk and increases the solid fraction of milk. Protein also was positively correlated with dry matter, and although the relationship was significant ($r = 0.57$, $p < 0.0001$), the strength of the correlation was less than that between fat and dry matter.

Figure 9.1. Dry Matter by Fat

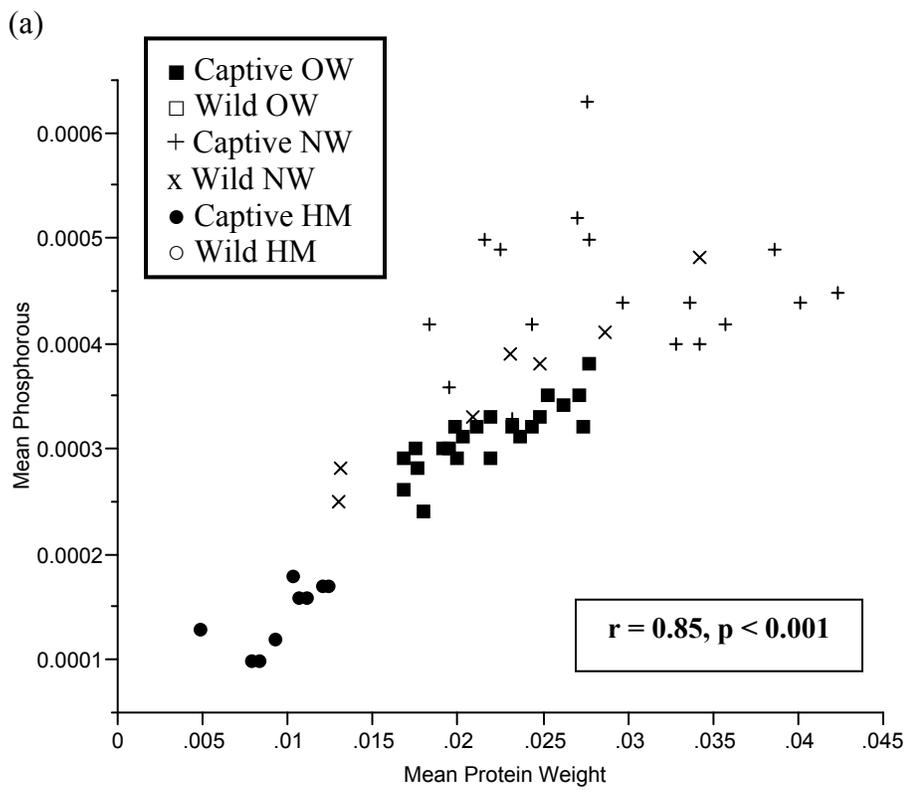


Both calcium and phosphorus were predicted to have a positive correlation with crude protein because these minerals are bound by the casein proteins in milk (Atkinson et al., 1995; Prentice and Prentice, 1995). Phosphorus was positively correlated with protein ($r = 0.48, p = 0.02$; Figure 9.2a) but calcium was not ($r = 0.14, p = 0.6$; Figure 9.2b). The distribution of data points in Figure 9.2b suggests that there may be phylogenetic differences in the relationship between calcium and protein.

The relationship between calcium and phosphorus is shown in Figure 9.3a. These minerals are not significantly correlated ($r = 0.33, p = 0.11$) and when the residuals were plotted (Figure 9.3.b), a phylogenetic pattern was detected. Residuals of almost all ceboid samples were positive while those from cercopithecoids and hominoids were negative.

This indicates that ceboid milks, in general, had higher phosphorus than would be predicted for the amount of calcium and cercopithecoids and hominoid milk, in general, had less phosphorus than would be predicted for the amount of calcium. As will be discussed below, higher than expected phosphorus relates to overall higher mean protein in ceboids compared to cercopithecoids and hominoids.

Figure 9.2. (a) Phosphorus by protein and (b) calcium by protein



(b)

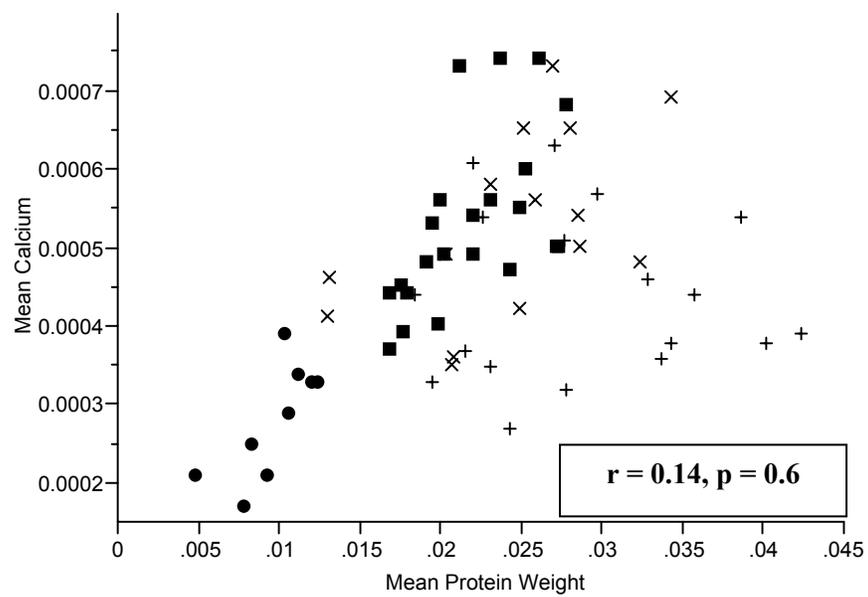
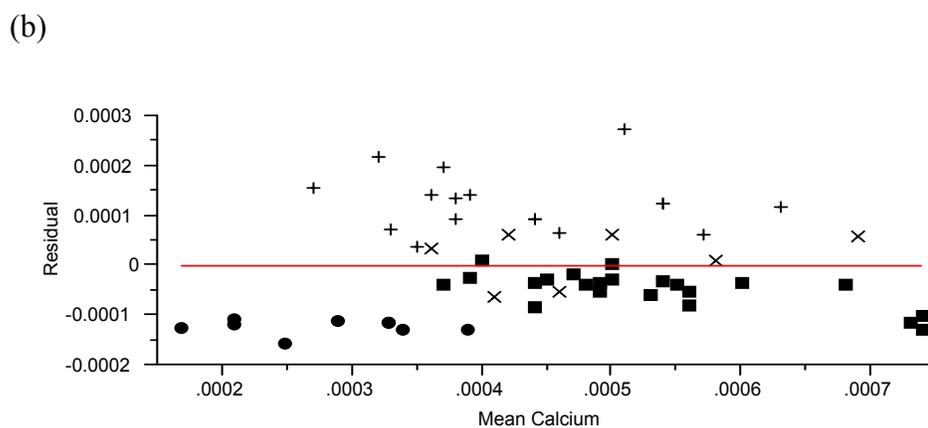
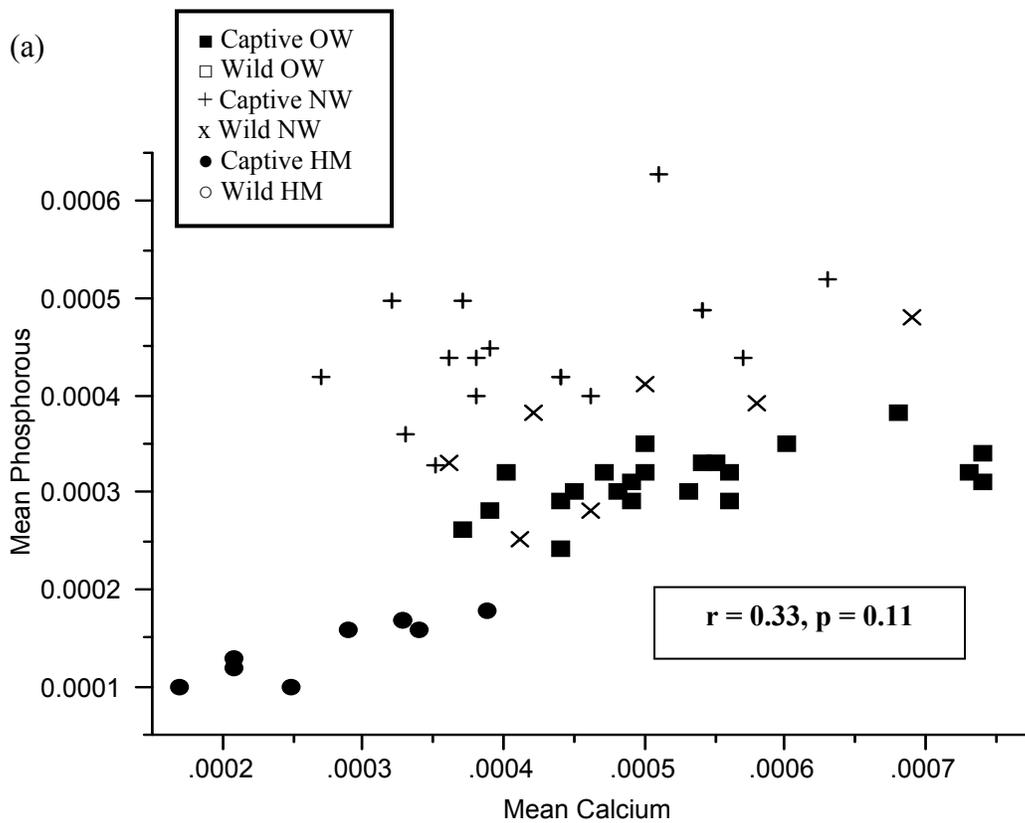


Figure 9.3. (a) Phosphorus by calcium and (b) plotted residuals from this bivariate plot



The prediction that samples higher in mean fat would be higher in total gross energy was confirmed. The correlation between fat and total gross energy was strong and significant ($r = 0.95$, $p < 0.0001$) and is described by the equation: $\text{fat} = -0.041 + 0.1404(\text{gross energy})$ (Figure 9.4). Deviations from this line were small. In general, samples from New World monkeys (captive and wild) had lower fat content than predicted by their total gross energy while those of captive hominoids and captive and wild Old World monkeys tended to have higher fat content than predicted by their total gross energy.

The relationship between the proportion of energy provided by fat and total gross energy also was positive and significant ($r = 0.88$, $p < 0.0001$; Figure 9.5). An identical, but inverse relationship was identified for percent energy from sugar and total gross energy ($r = -0.88$, $p < 0.0001$; Figure 9.6) indicating that samples highest in total gross energy were highest in the proportion of energy provided by fat and lowest in the proportion of energy provided by sugar. The correlation between percent energy from protein and total gross energy was negative and significant, although not as strong as that between fat or sugar and total gross energy ($r = -0.52$, $p = 0.001$; Figure 9.7).

These figures also were used to identify outliers that were excluded from subsequent quantitative analyses. In Figure 9.6, one captive living *C. apella* sample with over 12% lactose had a higher than predicted percent energy from sugar and a wild living *G. beringei* sample had a lower than expected percent energy from sugar value (5.13%). This same *G. beringei* sample had a higher than predicted percent energy from protein value, the result of higher mean percent protein than any conspecific or individual in

superfamily Hominoidea (3.16%). These samples were included in descriptive statistics for each species, but excluded from ANOVA and pairwise comparisons at the level of family or superfamily.

The distribution of data points in figures 9.5 – 9.7 suggests phylogenetic patterns in the relationship between total gross energy and the proportion of energy from protein, sugar, and fat as well as differences in the range of values for these four milk data points. Phylogenetic patterns in milk composition are addressed in the following results section.

Figure 9.4. Fat Weight (g/g of milk) by GE (kcal/g of milk)

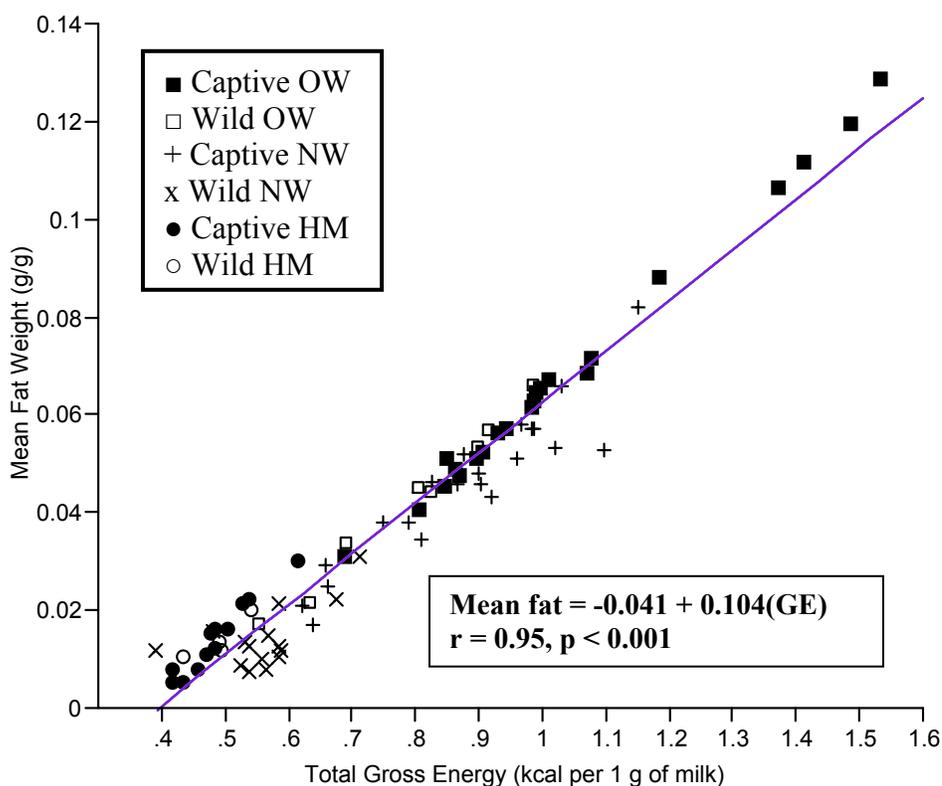


Figure 9.5. Percent energy from fat by GE

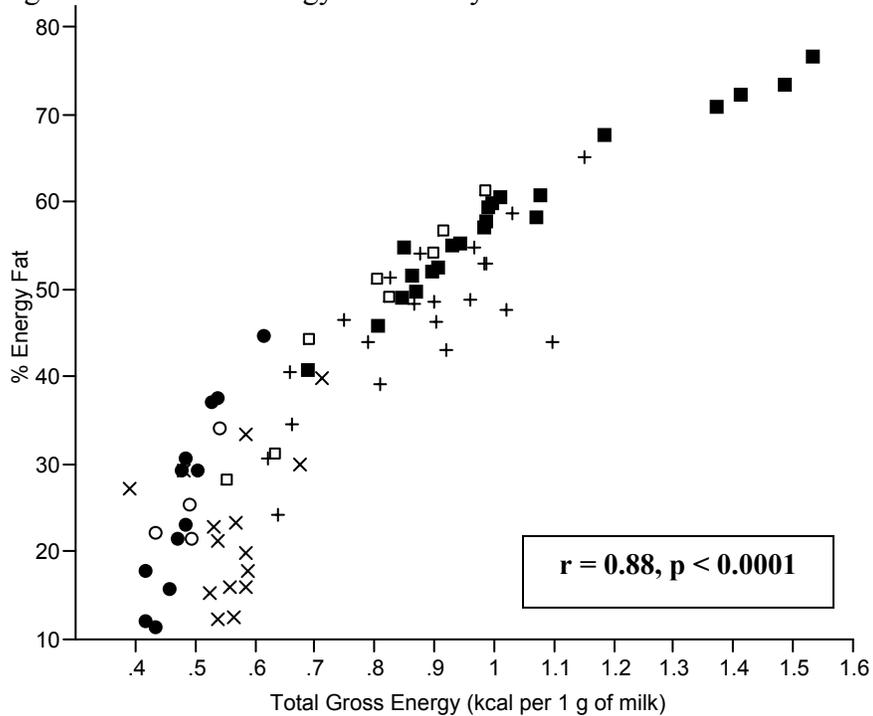


Figure 9.6. Percent energy from sugar by GE

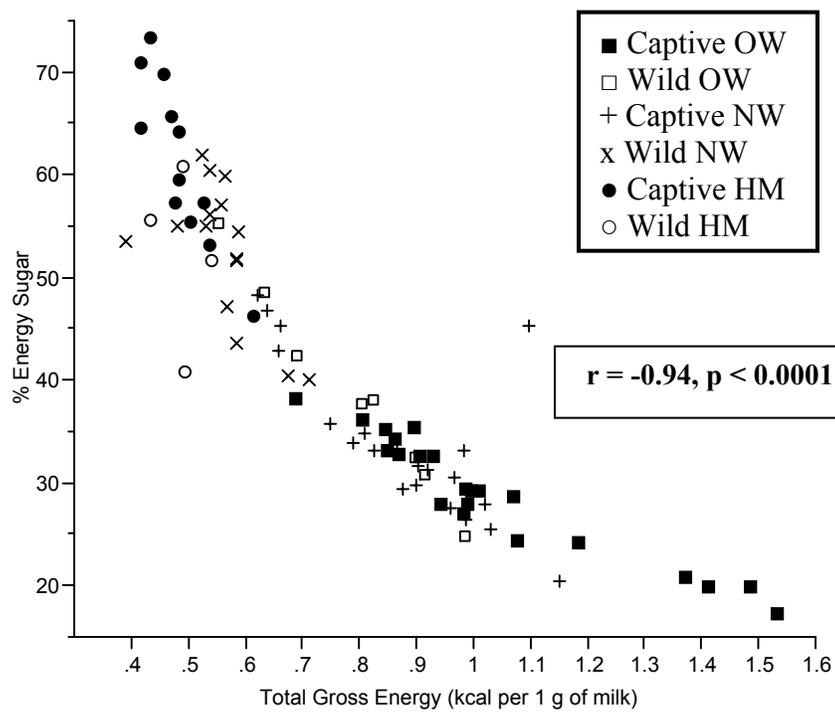
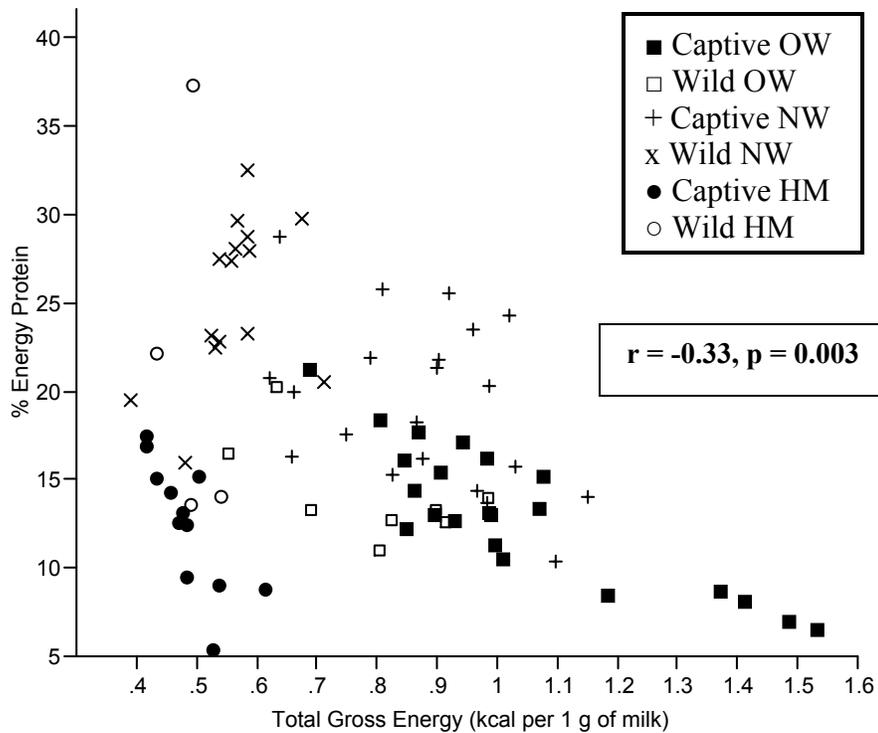


Figure 9.7. Percent energy from protein by GE



Phylogenetic patterns in milk composition

Descriptive statistics (mean \pm SD) were calculated for each species on the percent of fat, crude protein, lactose, dry matter, calcium, phosphorus, as well as total gross energy, and percent energy from fat, protein, and sugar. Tables 9.1 and 9.2 describe samples from captive living individuals and Tables 9.3 and 9.4 describe samples from wild living individuals. Results from fatty acid data analyses suggested that captivity may affect proximate milk composition, possibly through fat concentration. Therefore, at the level of the species, descriptive statistics were computed separately for these groups. Two species – *Callithrix jacchus* and *Leontopithecus rosalia* – were represented by individuals

living in captivity and the wild. Fat and dry matter were lower and sugar was higher in wild living individuals of *C. jacchus* and *L. rosalia*. Significance could only be determined for *C. jacchus* because captive living only one sample represented captive living *L. rosalia*. Mean fat and sugar were significantly different in wild living individuals ($t = 2.71, -5.84$, respectively, $p < 0.01$). Although based on a small sample size ($n = 4$ for each group), these results suggest fat, and therefore dry matter, and total gross energy are also likely to vary between milk samples from captive and wild populations. ANOVA and pairwise comparisons were not performed at the level of the species because many species were represented by only one sample (*Hylobates lar*, captive *Leontopithecus rosalia*, *Pan troglodytes*, *Pongo pygmaeus*, and *Symphalangus syndactylus*). Significant differences in means were investigated between and among families (Tables 9.5 and 9.6) and superfamilies (Tables 9.7 and 9.8).

Table 9.1. Mean percent \pm SD for proximate composition by species from samples from captive living individuals

Species	<i>n</i>	Fat	Crude Protein	Lactose	Dry Matter	Calcium	Phosphorus
<i>Callithrix jacchus</i>	4	3.32 \pm 1.77	2.65 \pm 0.40	7.55 \pm 0.05	14.06 \pm 1.26	ND	ND
<i>Cebus apella</i>	9	5.23 \pm 1.55	2.35 \pm 0.35	7.57 \pm 1.99	16.40 \pm 1.95	0.042 \pm 0.001	0.046 \pm 0.009
<i>Leontopithecus rosalia</i> ^a	1	2.10	2.20	7.60	12.40	0.061	ND
<i>Saimiri boliviensis</i>	8	4.66 \pm 0.77	3.59 \pm 0.42	6.98 \pm 0.27	16.59 \pm 0.99	0.044 \pm 0.007	0.044 \pm 0.003
<i>Macaca mulatta</i>	22	6.79 \pm 2.65	2.18 \pm 0.36	7.21 \pm 0.38	17.15 \pm 2.83	0.053 \pm 0.001	0.031 \pm 0.003
<i>Gorilla gorilla</i>	4	1.31 \pm 0.37	1.17 \pm 0.13	7.22 \pm 0.49	10.73 \pm 1.18	0.034 \pm 0.005	0.017 \pm 0.001
<i>Hylobates lar</i> ^a	1	0.80	1.12	8.14	12.20	0.034	0.016
<i>Pan paniscus</i> ^a	1	0.55	1.13	8.12	10.73	ND	ND
<i>Pan troglodytes</i>	4	2.01 \pm 0.83	0.90 \pm 0.10	7.43 \pm 0.30	11.45 \pm 0.75	0.021 \pm 0.004	0.011 \pm 0.001
<i>Pongo pygmaeus</i> ^a	1	2.16	0.49	7.66	10.81	0.021	0.013
<i>Symphalangus syndactylus</i> ^a	1	0.56	1.21	7.53	10.95	0.033	0.017

ND = No data available

^a Values are from one sample, no SD was calculated

Table 9.2. Mean total GE and percent energy (\pm SD) from fat, protein, and sugar by species for captive living individuals

Species	<i>n</i>	Total GE (g/kcal)	% Energy Fat	% Energy Protein	% Energy Sugar
<i>Callithrix jacchus</i>	4	0.75 \pm 0.18	37.98 \pm 15.54	21.08 \pm 7.20	40.94 \pm 8.97
<i>Cebus apella</i>	9	0.75 \pm 0.18	51.41 \pm 7.53	15.32 \pm 2.37	33.27 \pm 7.77
<i>Leontopithecus rosalia</i> ^a	1	0.62	30.83	20.78	48.39
<i>Saimiri boliviensis</i>	8	0.91 \pm 0.08	46.38 \pm 4.25	23.12 \pm 2.04	30.50 \pm 3.05
<i>Macaca mulatta</i>	22	1.03 \pm 0.23	58.09 \pm 9.21	13.09 \pm 3.90	28.81 \pm 5.84
<i>Gorilla gorilla</i>	4	0.47 \pm 0.04	24.96 \pm 5.60	14.56 \pm 2.25	60.47 \pm 4.73
<i>Hylobates lar</i> ^a	1	0.46	15.84	14.26	69.89
<i>Pan paniscus</i> ^a	1	0.44	11.46	15.15	73.39
<i>Pan troglodytes</i>	4	0.53 \pm 0.07	33.74 \pm 9.93	10.03 \pm 1.76	56.23 \pm 8.38
<i>Pongo pygmaeus</i> ^a	1	0.53	37.26	5.43	57.29
<i>Symphalangus syndactylus</i> ^a	1	0.42	12.17	16.91	70.92

ND = No data available

^aValues are from one sample, no SD was calculated

Table 9.3. Mean percent \pm SD for proximate composition by species from samples from wild living individuals

Species	<i>n</i>	Fat	Crude Protein	Lactose	Dry Matter	Calcium	Phosphorus
<i>Alouatta palliata</i>	7	1.83 \pm 0.68	2.25 \pm 0.78	6.67 \pm 0.73	13.17 \pm 2.14	0.049 \pm 0.001	0.036 \pm 0.008
<i>Callithrix jacchus</i>	4	0.90 \pm 0.19	2.64 \pm 0.13	8.20 \pm 0.22	13.42 \pm 0.98	0.065 \pm 0.006	ND
<i>Leontopithecus rosalia</i>	4	1.11 \pm 0.21	2.54 \pm 0.60	7.70 \pm 0.33	12.28 \pm 0.70	ND	ND
<i>Macaca sinica</i>	8	4.23 \pm 1.71	1.87 \pm 0.31	7.42 \pm 0.56	14.54 \pm 1.46	ND	ND
<i>Gorilla beringei</i>	4	1.42 \pm 0.43	1.82 \pm 0.92	6.50 \pm 1.09	10.59 \pm 0.67	ND	ND

Table 9.4. Mean total GE and percent energy (\pm SD) from fat, protein, and sugar by species for wild living individuals

Species	<i>n</i>	Total GE	% Energy Fat	% Energy Protein	% Energy Sugar
<i>Alouatta palliata</i>	7	0.56 \pm 0.11	29.10 \pm 6.20	23.02 \pm 5.13	47.88 \pm 6.91
<i>Callithrix jacchus</i>	4	0.56 \pm 0.02	14.49 \pm 2.68	27.65 \pm 0.37	57.86 \pm 2.72
<i>Leontopithecus rosalia</i>	4	0.56 \pm 0.03	18.36 \pm 3.49	26.65 \pm 4.76	54.99 \pm 4.77
<i>Macaca sinica</i>	8	0.79 \pm 0.15	47.03 \pm 11.83	14.18 \pm 2.89	38.79 \pm 9.86
<i>Gorilla beringei</i>	4	0.49 \pm 0.04	25.92 \pm 5.76	21.82 \pm 11.07	52.26 \pm 8.44

Significant differences were identified among families (ANOVA) in fat, protein, dry matter, calcium, and phosphorus (Table 9.5). Lactose was not significantly different among or between families. Mean fat was highest in cercopithecoid milks (6.11%) and significantly different between cercopithecoids and each of the four other families. Mean fat was also significantly higher in cebids (3.59%) compared to both ape families (hominids and hylobatids).

Mean protein was highest among cebid milks (2.78%), followed by the other ceboid family, the atelids (2.25%). Cebid samples were significantly higher in mean protein than cercopithecoids, hominids, and hylobatids. Hominid milks also were significantly different in mean protein from atelids and cercopithecoids. Dry matter is a measure of solids in milk, of which fat and protein are the largest contributors. Cercopithecoid milk was highest in mean dry matter, followed by cebids. Significant differences in dry matter were identified between cercopithecoids and atelids, hylobatids, and hominids, as well as between cebids and hominids.

Not all samples were analyzed for calcium and phosphorus because of limitations of the original sample size. Therefore, mean values for minerals were calculated using smaller values of n than reported in Table 9.5 (and subsequently, in Table 9.7). As previously presented, the correlation between calcium and phosphorus, albeit positive, was not significant. Cercopithecoids produced milk with the highest mean calcium (0.053%) but had only the third highest mean phosphorus values (0.031%). Conversely, milks of cebids were highest in mean phosphorus (0.045%) but only third highest in mean calcium (0.048%). Significant differences in mean calcium were identified between

hominids and cercopithecids, atelids, and cebids. For mean phosphorus, cebids were significantly higher from all other families and cercopithecids and atelids were significantly higher than both ape families.

Cercopithecoid milks were highest in mean fat, and consequently, were significantly higher in mean total gross energy (0.97 kcal/g) and percent energy from fat (55.14%) from the other four families. Cercopithecoid milks were almost twice as high in energy and percent energy from fat as hominid milks, and over twice as high in energy and almost four times higher in percent energy from fat as hylobatid milks. Cebid milks were significantly higher in total energy (0.79 kcal/g) than milks from atelids (0.56 kcal/g), hominids (0.50 kcal/g), and hylobatids (0.44 kcal/g), but were not significantly different from these families in percent energy from fat in milk (29.10%, 27.66%, and 14.00%, respectively).

Atelid milks were highest in the percent energy from protein (23.02%), but were only significantly different from cercopithecoid (13.38%) and hominid (14.73%) milks. These two families also were significantly different from cebid milk (21.52%), which was only slightly lower than atelids in percent energy from protein.

Samples lowest in total gross energy (hylobatids and hominids) were highest in percent energy from sugar (Figure 9.6). Hylobatid milks (70.41%) were significantly higher in percent energy from sugar than atelids (47.88%), cebids (40.21%), and cercopithecoids (31.47%). Hominid milks (57.61%) were significantly higher in percent energy from sugar than cebids and cercopithecoids, and significant differences also were detected between cercopithecoids and atelids and cebids.

Table 9.5. Mean percent \pm SE for proximate composition by family (includes captive and wild living individuals)

Family	Fat	Crude Protein	Lactose	Dry Matter	Calcium	Phosphorus
Atelidae (n = 7)	1.83 \pm 0.70	2.25 \pm 0.21	6.67 \pm 0.31	13.17 \pm 0.76	0.049 \pm 0.004	0.036 \pm 0.002
Cebidae (n = 16)	3.59 \pm 0.38	2.78 \pm 0.10	7.51 \pm 0.16	15.05 \pm 0.41	0.048 \pm 0.002	0.045 \pm 0.001
Cercopithecidae (n = 30)	6.11 \pm 0.34	2.10 \pm 0.10	7.26 \pm 0.15	16.45 \pm 0.37	0.053 \pm 0.002 ^a	0.031 \pm 0.001 ^a
Hominidae (n = 13)	1.55 \pm 0.49	1.22 \pm 0.15	7.17 \pm 0.22	10.90 \pm 0.54	0.026 \pm 0.004 ^b	0.025 \pm 0.014 ^b
Hylobatidae (n = 2)	0.68 \pm 1.31	1.17 \pm 0.39	7.84 \pm 0.58	11.58 \pm 1.43	0.033 \pm 0.007	0.017 \pm 0.007
ANOVA F-test, p value	F = 16.02 p < 0.001	F = 21.04 p < 0.001	F = 1.75 p = 0.15	F = 16.47 p < 0.0001	F = 8.28 P < 0.0001	F = 51.08 p < 0.0001
Tukey-Kramer pairwise Signif. differences	CR/CB CR/AT CR/HN CR/HL CB/HN CB/HL	CB/CR CB/HN CB/HL AT/HN CR/HN CR/HL	None	CR/AT CR/HL CR/HN CB/HN	CR/HN AT/HN CB/HN	CB/AT CB/CR CB/HL CB/HN AT/HL AT/HN CR/HL CR/HN

AT = Atelidae; CB = Cebidae; CR = Cercopithecoidae; HN = Hominidae; HL = Hylobatidae

^aCercopithecidae sample size for calcium and phosphorus are from a population of n = 22

^bHominidae sample size for calcium and phosphorus are from a population of n = 7

Table 9.6. Mean total GE and percent energy (\pm SE) from fat, protein, and sugar by family (includes captive and wild living individuals)

Family	Total GE	% Energy Fat	% Energy Protein	% Energy Sugar
Atelidae (n = 7)	0.56 \pm 0.06	29.10 \pm 3.65	23.02 \pm 1.90	47.88 \pm 2.92
Cebidae (n = 16)	0.79 \pm 0.04	38.27 \pm 2.30	21.52 \pm 0.99	40.21 \pm 1.81
Cercopithecidae (n = 30)	0.97 \pm 0.03	55.14 \pm 1.76	13.38 \pm 0.92	31.47 \pm 1.41
Hominidae (n = 13)	0.50 \pm 0.04	27.66 \pm 2.58	14.73 \pm 1.34	57.61 \pm 2.07
Hylobatidae (n = 2)	0.44 \pm 0.12	14.00 \pm 6.83	15.59 \pm 3.56	70.41 \pm 5.47
ANOVA F-test, p value	F = 19.12 p < 0.0001	F = 18.10 p < 0.0001	F = 11.48 p < 0.0001	F = 22.69 p < 0.0001
Tukey-Kramer pairwise Signif. differences	CR/CB CR/AT CR/HN CR/HL CB/AT CB/HN CB/HL	CR/CB CR/AT CR/HN CR/HL HL/AT HL/CB HL/HM	AT/HN AT/CR CB/HN CB/CR	HL/AT HL/CB HL/CR HN/CB HN/CR AT/CR CR/CB

Analysis at the level of the superfamily combines atelids and cebids into superfamily ceboidea and hominids and hylobatids into superfamily hominoidea. Significant differences between ceboids were identified only in mean phosphorus and total gross energy. Between hominoid families, percent energy from fat was significantly different. Only one family of the superfamily cercopithecoidea was included in this study; values reported for cercopithecoids are identical to those from cercopithecids. Many of the differences that were identified between cercopithecids and other families

are echoed in results from analysis at the level of the superfamily, and suggest that superfamily may be an appropriate level of analysis for most milk constituents.

Table 9.7. Mean percent \pm SE for proximate composition by superfamily (includes captive and wild living individuals)

Superfamily	Fat	Crude Protein	Lactose	Dry Matter	Calcium^a	Phosphorus^a
Ceboidea (n = 37)	3.26 \pm 0.35	2.68 \pm 0.10	7.34 \pm 0.16	14.70 \pm 0.38	0.048 \pm 0.002	0.042 \pm 0.001
Cercopithecoidea (n = 30)	6.15 \pm 0.39	2.10 \pm 0.10	7.26 \pm 0.15	16.45 \pm 0.42	0.053 \pm 0.002	0.0031 \pm 0.001
Hominoidea (n = 15)	1.44 \pm 0.53	1.22 \pm 0.14	7.25 \pm 0.22	10.98 \pm 0.57	0.028 \pm 0.004	0.014 \pm 0.002
ANOVA F-test, p value	F = 28.97 p < 0.001	F = 37.91 p < 0.001	F = 0.11 p = 0.90	F = 30.04 p < 0.001	F = 16.56 p < 0.001	F = 76.12 p < 0.001
Tukey-Kramer pairwise Signif. Differences	OW/NW OW/HM NW/HM	OW/NW OW/HM NW/HM	None	OW/NW OW/HM NW/HM	OW/HM NW/HM	OW/NW OW/HM NW/HM

NW = Ceboidea; OW = Cercopithecoidea; HW = Hominoidea

^aCalcium and phosphorus calculations from smaller groups of hominoid (n = 9), ceboid (n = 24) and cercopithecoid (n = 22)

Table 9.8. Mean total GE and percent energy (\pm SE) from fat, protein, and sugar by superfamily (includes captive and wild living individuals)

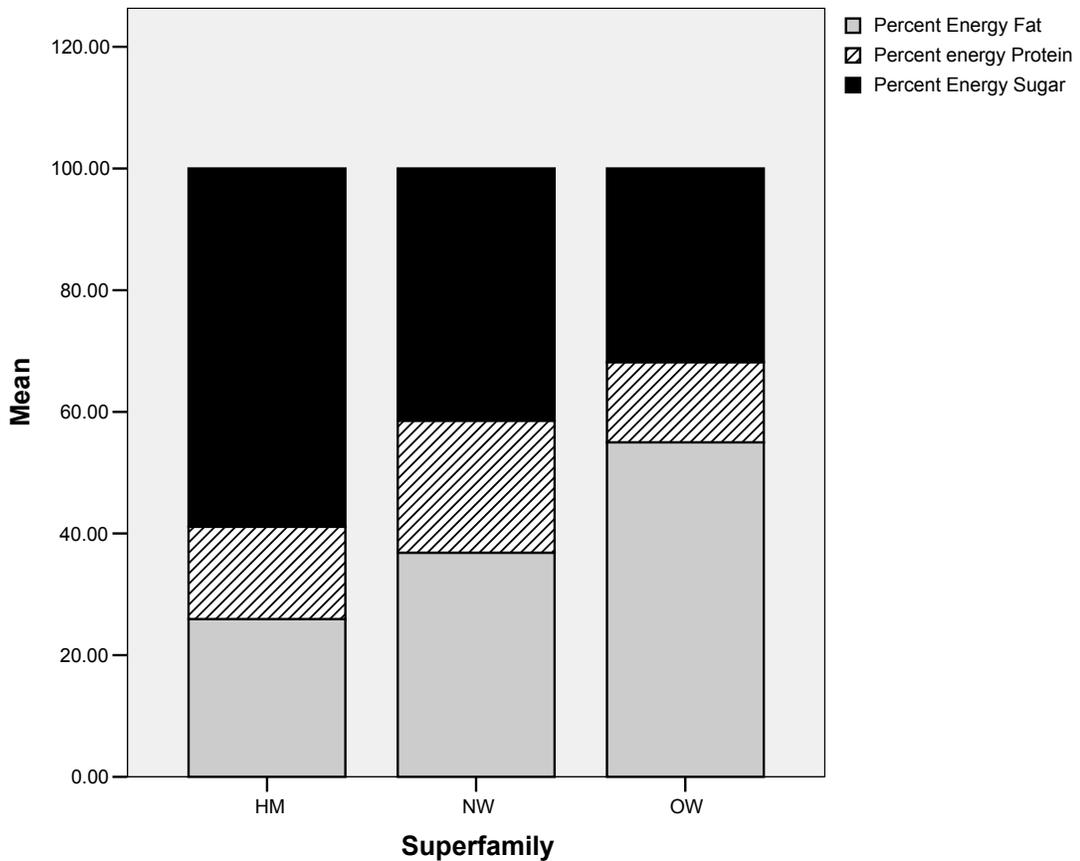
Superfamily	Total GE	% Energy Fat	% Energy Protein	% Energy Sugar
Ceboidea (n = 36)	0.74 \pm 0.03	36.49 \pm 2.11	21.81 \pm 0.88	41.70 \pm 1.67
Cercopithecoidea (n = 30)	0.97 \pm 0.04	55.14 \pm 2.31	13.38 \pm 0.96	31.47 \pm 1.83
Hominoidea (n = 15)	0.49 \pm 0.05	25.95 \pm 3.16	14.84 \pm 1.32	59.21 \pm 2.51
ANOVA F-test, p value	F = 31.64 p < 0.001	F = 32.30 p < 0.001	F = 23.16 p < 0.001	F = 39.86 p < 0.001
Tukey-Kramer pairwise Signif. Differences	OW/NW OW/HM NW/HM	OW/NW OW/HM NW/HM	OW/NW HM/NW	OW/NW OW/HM NW/HM

Means and standard errors by superfamily and results from ANOVA and pairwise comparisons are reported in Table 9.7 and Table 9.8. Significant differences were identified among and between the three superfamilies in mean fat, protein, dry matter, phosphorus, total gross energy, percent energy from fat and percent energy from sugar. Components that differed among but not between all three superfamilies in pairwise comparisons were calcium and percent energy from protein. Hominoids were significantly lower in calcium than ceboids and cercopithecoids, and ceboids were significantly higher than cercopithecoids and hominoids in percent energy from protein.

Cercopithecoidea were significantly higher in total gross energy (0.97 kcal/g) than ceboidea (0.74 kcal/g) and hominoids (0.49 kcal/g). The proportion of energy from fat,

sugar, and protein also varied among superfamilies (Figure 9.8). The majority of energy in the milk of cercopithecoids is provided by fat, followed by protein and then sugar. The milk of hominoids demonstrated the reverse trend, with more than half of the energy (59.21%) provided by sugar, followed by fat, and then protein. Although the milk of ceboids gets the majority of its energy from sugar, it had a significantly higher proportion of energy from protein (21.81%) than cercopithecoids (13.38%) and hominoids (14.84%).

Figure 9.8. Proportion of total gross energy as fat, protein, and sugar by superfamily



Cercopithecoidea included only one genus – *Macaca*- and more than half of the hominoid species were represented by only one sample each. Ceboidea, however, included five species from different genera and large enough sample sizes for each to permit statistical analysis of variation within this superfamily. Mean protein was significantly different among ceboids ($F = 9.64$, $df = 4$, $p < 0.0001$) and between *Saimiri* and all other ceboid species ($p < 0.05$; Figure 9.9a). Indeed, *Saimiri* samples produced the highest mean percent protein (3.59%) among all species included in this dissertation (Table 9.1. and 9.3).

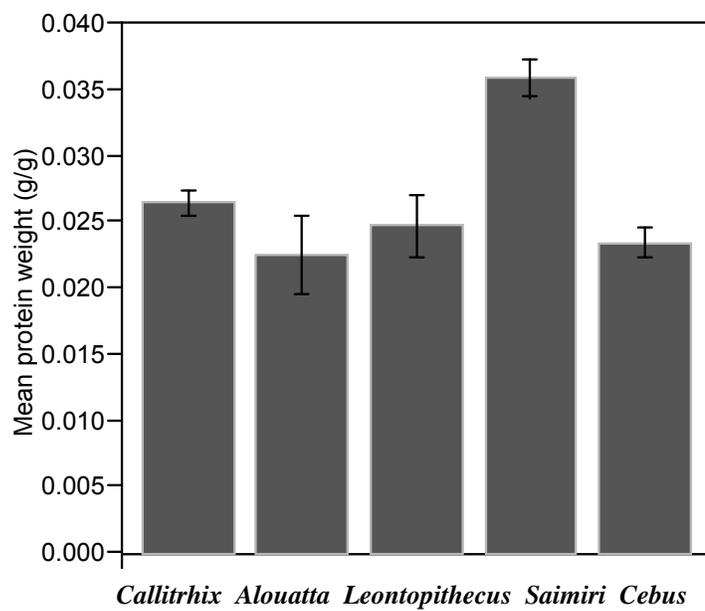
Mean percent energy from protein also was significantly different among Ceboid species ($F = 8.20$, $df = 4$, $p < 0.0001$) but did not follow the same pattern as mean percent protein. Percent energy from protein was highest in *Leontopithecus* (25.47%) and *Callithrix* (24.83%), followed by *Saimiri* (23.12%), *Alouatta* (23.02%), and finally *Cebus* (15.32%). Significant differences were identified between *Cebus* and all other species ($p < 0.0001$).

Ceboids were significantly different in mean fat ($F = 15.63$, $df = 4$, $p < 0.0001$; Figure 9.9b). Between species, significant differences in mean fat were identified between subfamily cebinae (*Cebus* and *Saimiri*) and all other species ($p < 0.05$), but not between *Cebus* and *Saimiri*. The same significant differences were identified for percent energy from fat ($F = 17.25$, $df = 4$, $p < 0.0001$) and GE ($F = 15.48$, $df = 4$, $p < 0.0001$). Percent energy from fat and percent energy from sugar are inversely correlated ($r = -0.93$, $p < 0.0001$) and the reverse pattern was identified for mean percent energy from sugar among ceboids ($F = 15.21$, $df = 4$, $p < 0.0001$). Samples highest in percent energy from

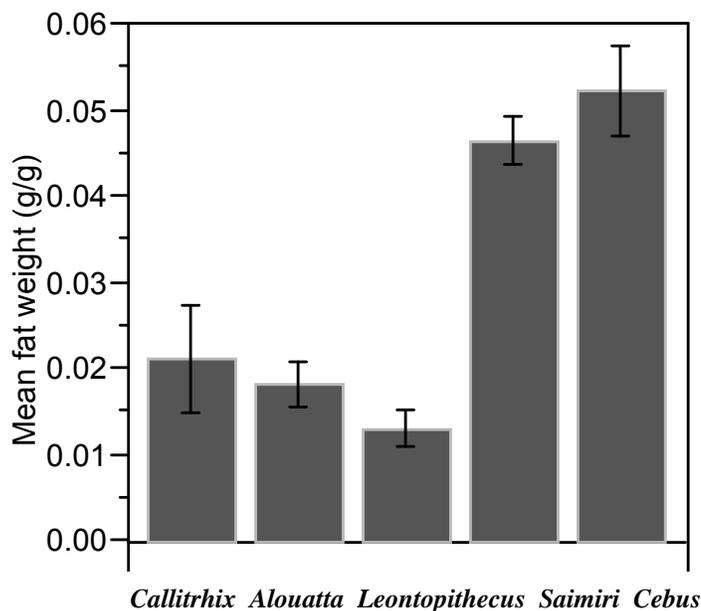
fat -*Saimiri* and *Cebus* - were lowest in percent energy from sugar, and were significantly different from all other species ($p < 0.05$), but not from each other.

Figure 9.9. Mean percent energy from (a) protein and (b) fat by species, ceboids only

(a)



(b)



Effect of captivity

The effect of captivity was investigated by separating samples into wild and captive by superfamily. Among cercopithecoids and ceboids, samples from captive living individuals were significantly higher in mean percent fat (6.78%, 4.53% respectively) than those from wild living individuals (4.23%, 1.39%). As predicted from their positive correlation with mean fat, mean percent energy from fat (Figure 9.10) and total gross energy (data not shown) also were significantly higher in captive living than wild living monkeys. Wild hominoid samples (mountain gorillas) were not significantly different from captive hominoid samples in mean fat, mean percent energy from fat (Figure 9.10a) or total gross energy. Indeed, captive- and wild living hominoids were identical in mean

fat (1.4%), mean percent energy from fat (25.96 and 25.92%), and total gross energy (0.49 kcal/g).

Mean protein was higher in captive living cercopithecoids (2.18% vs. 1.86%) and ceboids (2.85% vs. 2.43%), but the difference was significant only among cercopithecoids ($t = 2.31$, $p = 0.04$). Interestingly, percent energy from protein was not significantly different between captive and wild living cercopithecoids, but was significantly higher in wild as compared to captive living ceboids (Figure 9.10b). Wild and captive living hominoid samples were not significantly different in mean protein or mean percent energy from protein.

Mean sugar was not significantly different between captive and wild living individuals within each superfamily, but because of the relationship between energy provided by sugar and energy provided by fat, significant differences between captive- and wild living individuals were identified in ceboids and cercopithecoids (Figure 9.10c). Samples from wild living monkeys had significantly higher energy from sugar values than those from captive living monkeys.

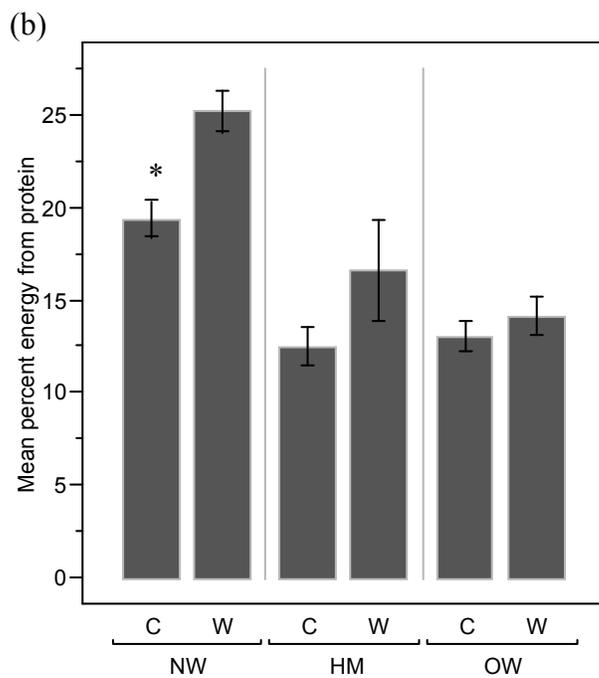
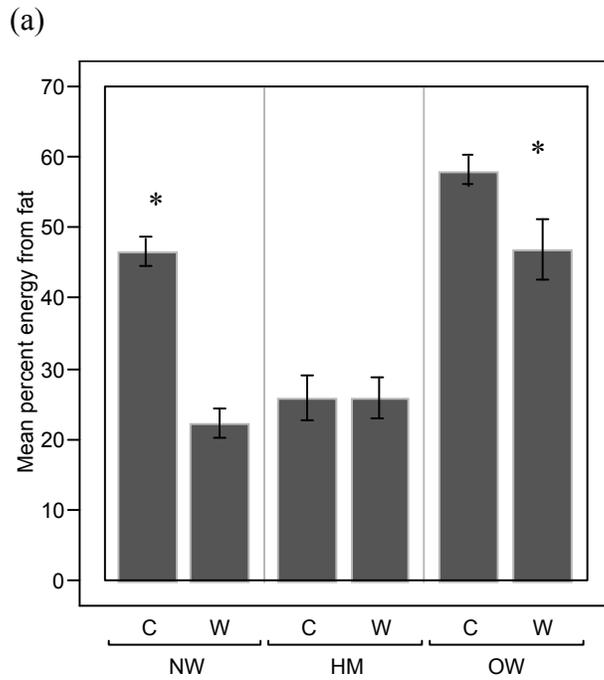
Effect of diet

Species were assigned to one of five dietary categories based on the dietary preferences and behaviors of wild living individuals (Chapter 5). These categories were: folivore (mountain gorillas and mantled howlers), frugivore (chimpanzees, bonobos, orangutans, gibbons, and toque macaques), gummivore (common marmoset), frugivore-folivore (siamangs and rhesus macaques), and frugivore-insectivore (tufted capuchins and squirrel monkeys). Differences among and between dietary categories in mean percent

fat, protein, and sugar, and total gross energy were investigated by plotting all data points by dietary category (e.g., Figure 9.11) instead of means. Having already identified phylogenetic differences, and differences due to captivity, it was necessary to investigate whether differences in dietary category were actually reflecting the inclusion of a particular species within a dietary category.

Mean fat ($F = 7.92$, $p < 0.0001$), mean protein ($F = 14.53$, $p < 0.0001$), and mean total gross energy ($F = 7.75$, $p < 0.0001$) were significantly different among dietary categories. Mean fat was highest among frugivore-folivores (5.74%), followed by frugivore-insectivores (4.13%). These categories were dominated by those species which were highest in mean fat – rhesus macaques, tufted capuchins, and squirrel monkeys (Figure 9.11). Mean energy from protein is highest among frugivore-insectivores, but Figure 9.12 indicates that mean energy from protein is simply highest among ceboids, particularly tufted capuchins and squirrel monkeys. Removing captive individuals from this analysis (Figure 9.13) suggests that phylogeny is the most influential variable on mean protein (or energy from protein).

Figure 9.10. Mean percent energy from (a) fat, (b) protein, and (c) sugar from captive- and wild living individuals, by superfamily (* indicates significant difference, $p < 0.05$).



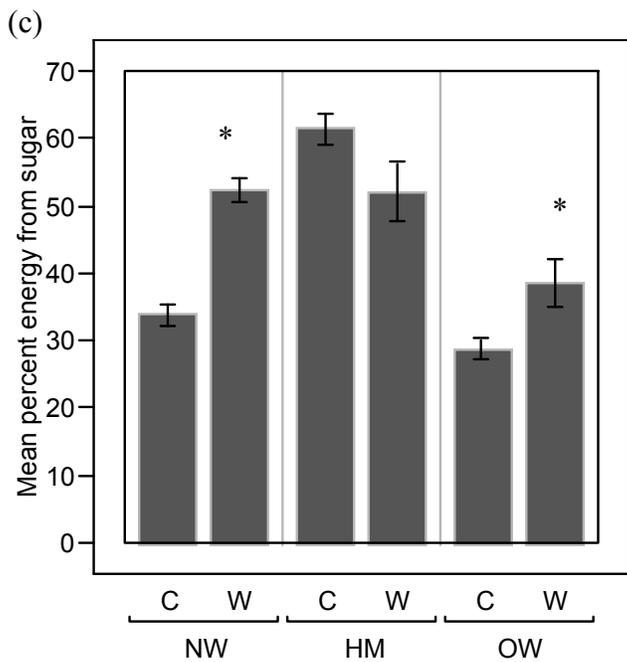


Figure 9.11. Distribution of mean fat by dietary category

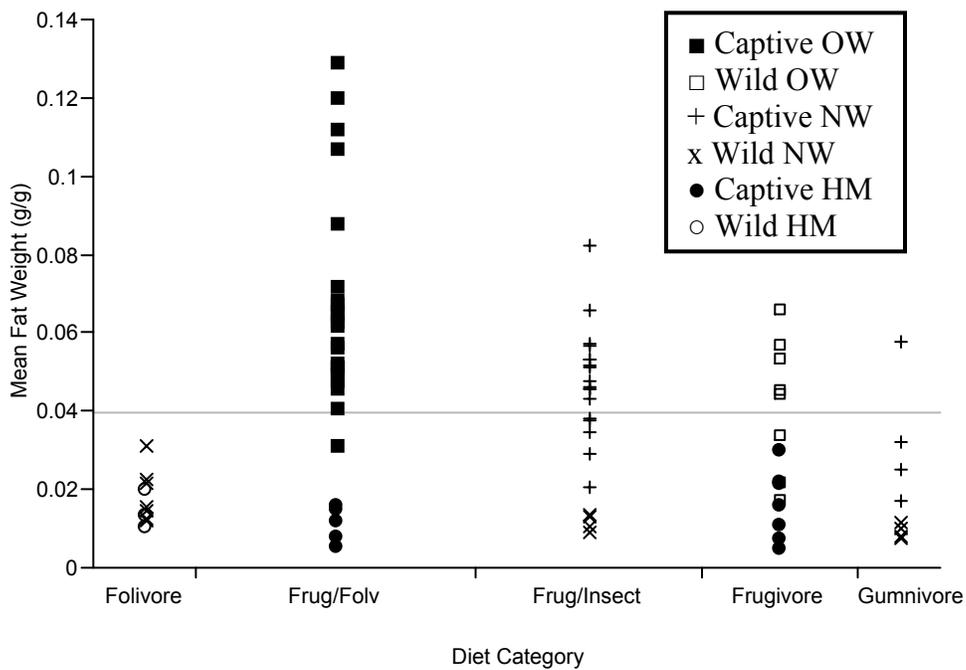


Figure 9.12. Distribution of energy from protein by dietary category

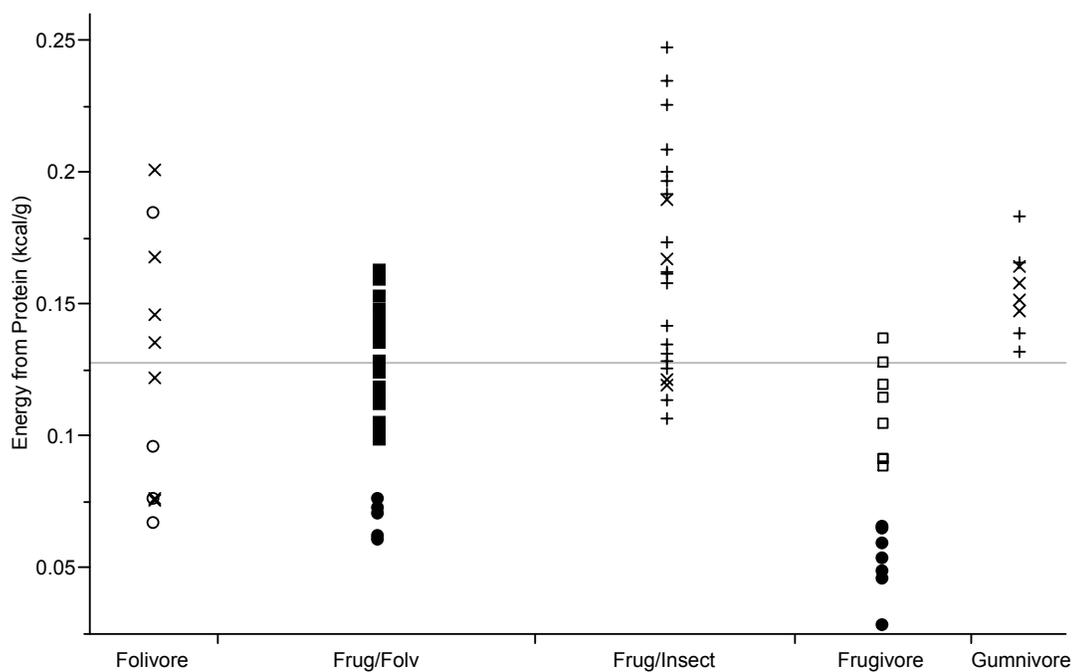
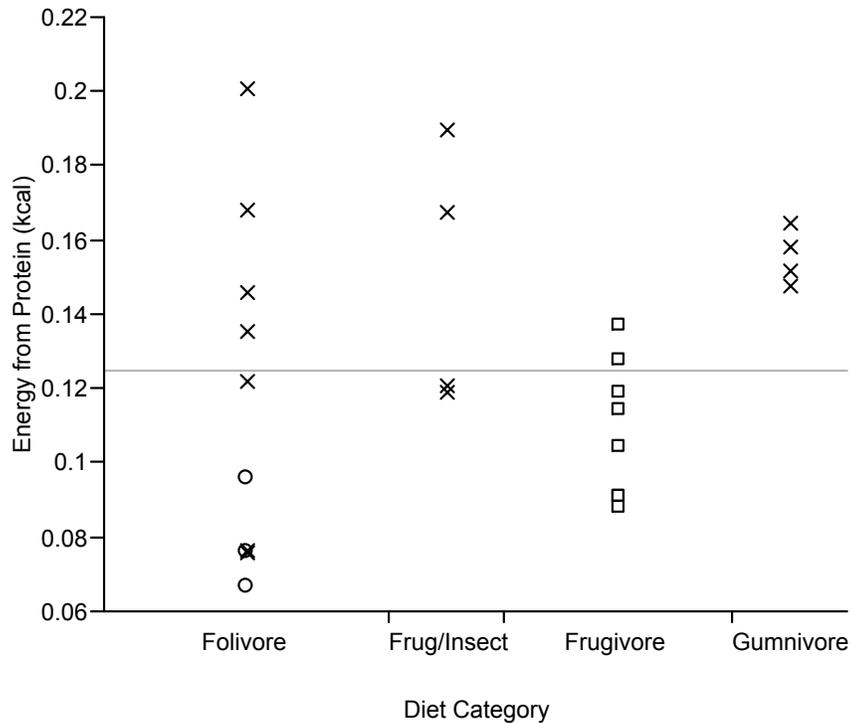


Figure 9.13. Distribution of energy from protein by dietary category, wild living individuals only.



Phylogenetic patterns in variability of milk composition

Figure 9.5, 9.6, and 9.7 describe the relationship between percent energy from fat, sugar, and protein with total gross energy, respectively. Data points were coded by superfamily and captivity in order to assess the relationship of each of these variables to milk composition. This section will focus on the distribution of data points with respect to phylogeny.

Total gross energy (kcal per gram of milk) was plotted along the x axis of all three figures. It was most variable among cercopithecoids (0.98 kcal/g), followed by ceboids (0.76 kcal/g), and hominoids (0.20 kcal/g). Only one sample from a cercopithecoid was within the range of values for hominoids (*M. sinica*, 0.55 kcal/g) but ceboid samples

overlap with both hominoids and cercopithecoids providing some of the lowest and highest energy samples.

The spread of data points along the y axis in Figure 9.5 represents the range of variation in percent energy from fat. Ceboids were the most variable of the three superfamilies with a range of 58.10%, followed by cercopithecoids (48.11%) and hominoids (33.39%). Ceboid samples overlapped with both hominoids and cercopithecoids, but only four samples from cercopithecoids were within the range of values for hominoids. The relationship between percent energy from sugar and total gross energy was the inverse of that for percent energy from fat and total gross energy. Thus, ceboids also were most variable in percent energy from sugar (48.19%), followed by cercopithecoids (38.05%) and hominoids (27.07%).

Variation in percent energy from protein was lower than that for either percent energy from fat or sugar, but was still highest among ceboids (22.05%). Interestingly, hominoid samples were the next most variable (16.69%) followed by cercopithecoids (14.74%).

Percent energy from protein was negatively correlated with total gross energy (Figure 9.7), and although significant, the correlation was weaker than percent energy from fat (sugar) and total gross energy. The distribution of points suggests that there may be differences among superfamilies in the relationship between percent energy from protein and total gross energy. Pairwise correlations were rerun by superfamily and results supported this prediction. As a group, the correlation was $r = -0.52$. The correlation was identical among only ceboids. Among cercopithecoids, the correlation

was stronger ($r = -0.78$, $p < 0.0001$), and among hominoids, it was non-significant ($r = -0.35$, $p = 0.18$).

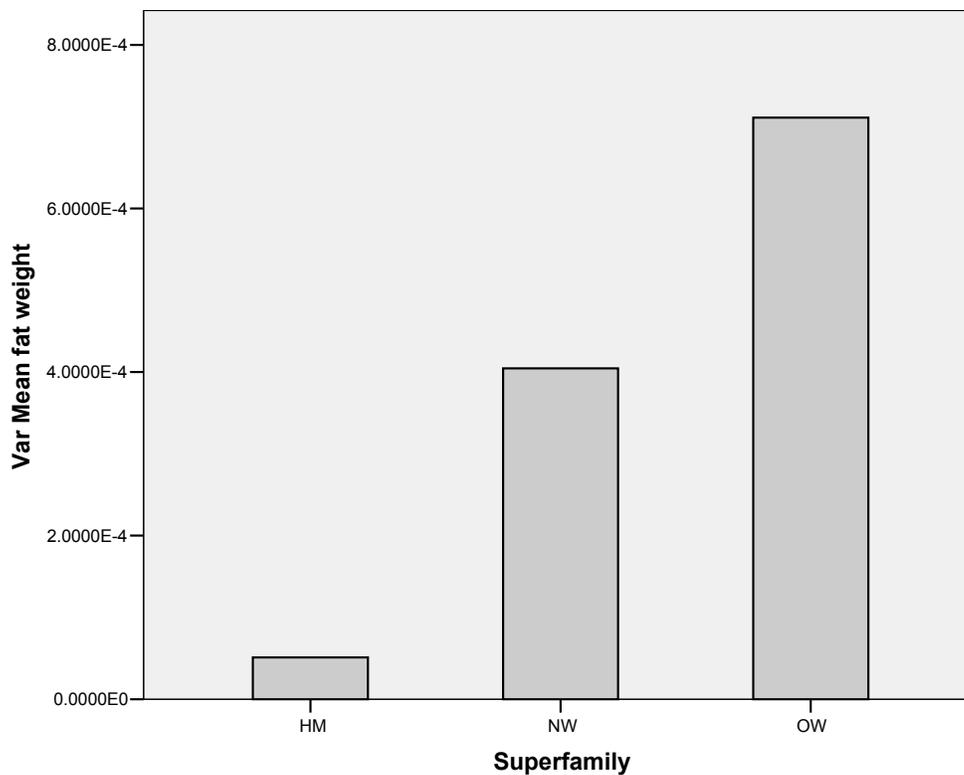
Fat is the most variable component in human milk, and was the most variable component among samples included in this study. However, fat was more variable among cercopithecoids, followed by ceboids and hominoids (Figure 9.14). Tests for equality of variances between hominoids and both monkey superfamilies were not significant (Table 9.9), indicating that the range of variation in mean fat was not equal between these superfamilies. Percent fat is positively and significantly correlated with total gross energy ($r = 0.95$) and percent of energy provided by fat ($r = 0.97$), suggesting that the variability in percent fat is driving the variability in total gross energy and percent energy from fat described by Figure 9.5. Variation in percent energy from protein also was investigated using the Levene test of equality of variances. No significant Levene statistics were identified between superfamilies, indicating that although there was a significant difference in mean percent energy from protein, the range of values of this milk component is not significantly different among or between superfamilies.

Table 9.9. Results of t-tests (mean fat weight) with Levene's test of equality of variances

Comparison	Levene Statistic	Significance
NW – HM	19.996	< 0.0001
NW – OW	0.235	0.630
OW - HM	7.775	0.008

*A significant value indicates that variances between groups are not equal

Figure 9.14. Variance in fat weight by superfamily



Relationship to life history traits

Milk composition was analyzed with respect to adult female encephalization quotients (EQ), female body mass (log transformed), and duration of lactation (absolute and relative). Specific predictions tested in this section include:

(1) Metabolically expensive neural tissue requires nutritionally dense, higher-quality foods (Walker et al., 2006). Species with larger relative brain sizes will require higher quality foods, including milk. Milk of larger-brained anthropoids will be higher in total gross energy.

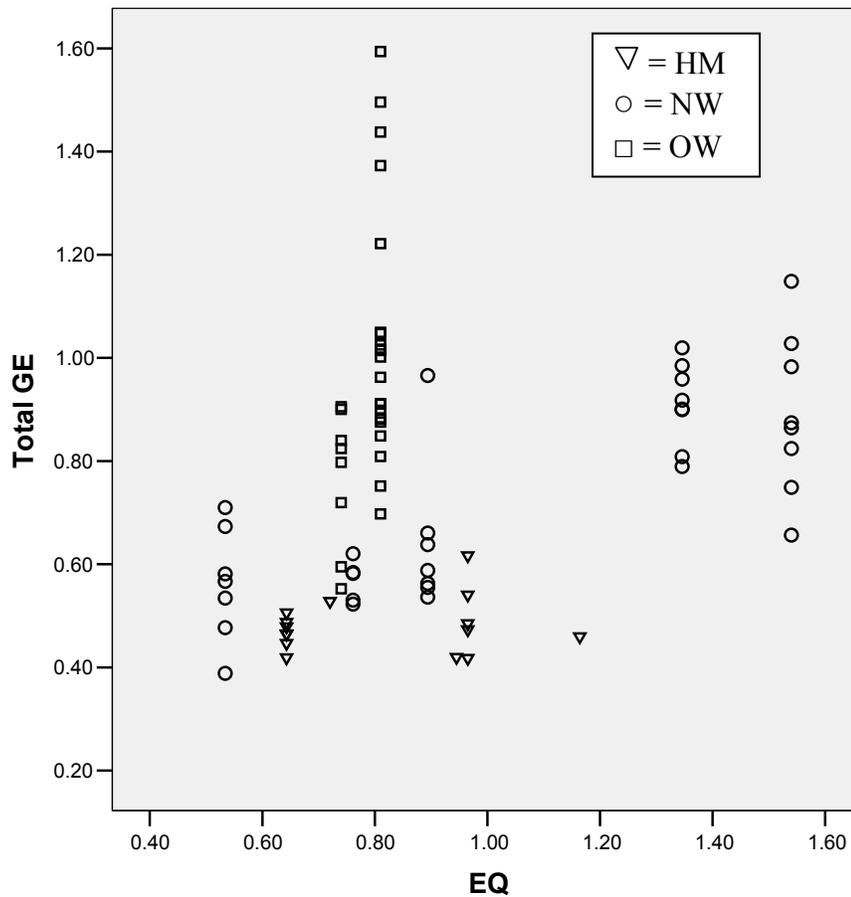
(2) Species with high growth costs need additional energetic investment (Lee, 1996; Leigh, 2004; Oftedal, 1984; Power et al., 2002). Milk of species with high growth costs will be higher in energy provided by protein.

(3) Milk energy is a function of maternal mass (Lee, 1996; Martin, 1981; 1996). Milk energy, and therefore milk composition, will correlate with adult female body mass.

(4) Milk composition will correlate with other aspects of a species' lactation strategy. Variation in duration of lactation will influence milk composition among anthropoid primates.

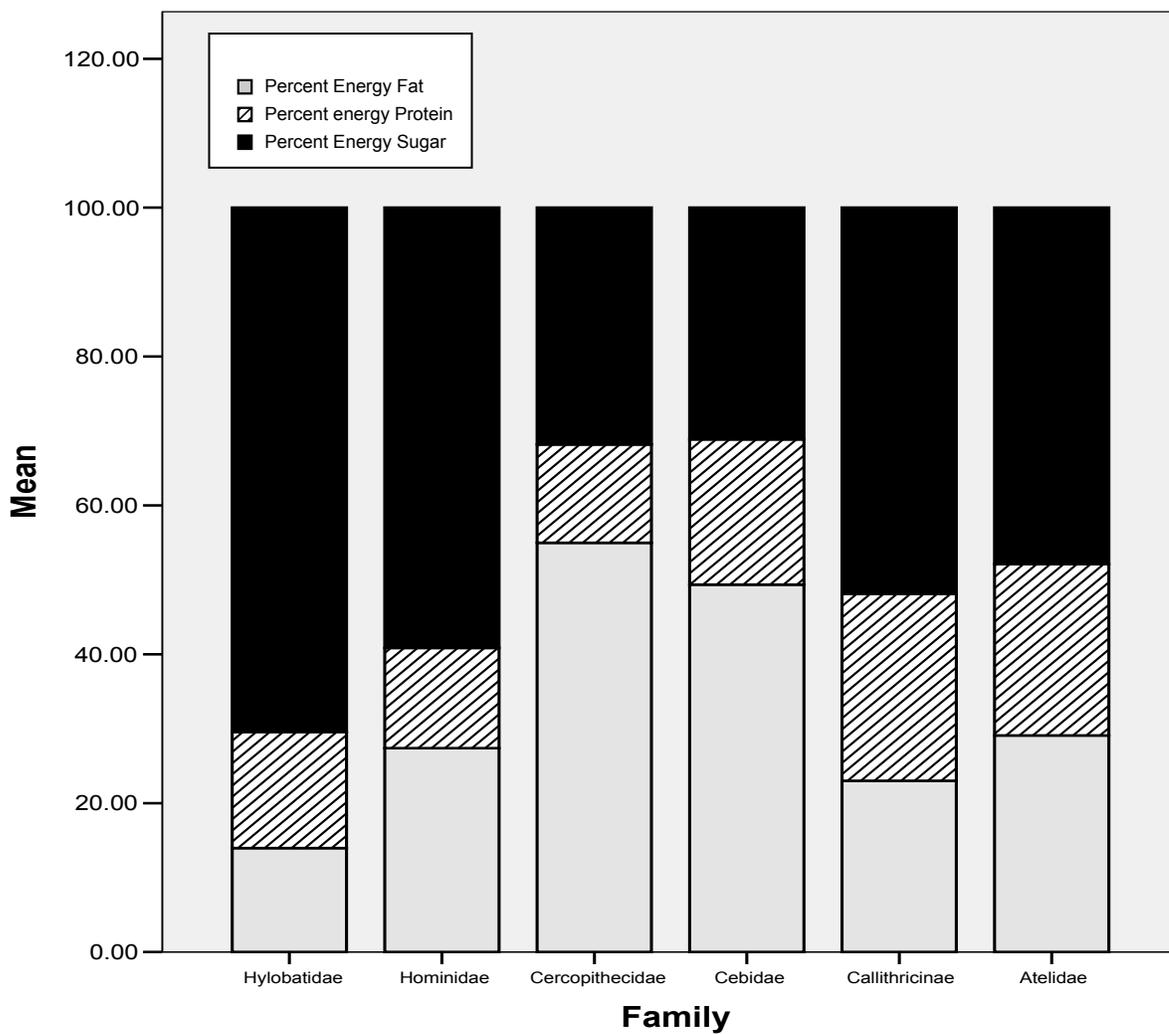
EQ and total gross energy were positively and significantly correlated ($r = 0.36$, $p = 0.001$; Figure 9.15). Although significant, this correlation indicates that EQ does not explain the majority of the variation in total gross energy. Mean total gross energy is significantly different among the three EQ groups ($F = 9.46$, $df = 2$, $p < 0.0001$) but species with the highest EQ values (*Cebus* and *Saimiri*) do not have the highest total gross energy milks.

Figure 9.15. Total gross energy by EQ values.



Percent energy from protein was highest among ceboids, followed by hominoids, and cercopithecoids (Figure 9.8). Within ceboids, milks from callitrichines (*C. jacchus* and *L. rosalia*) were highest in percent energy from protein (25.10%), followed by atelids (22.55%), and finally cebines (19.21%). Of these three ceboid groups, only callitrichines and cebines were significantly different from one another, supporting the decision to dissect the family Cebidae into subfamilies for this analysis. Percent energy from protein was almost identical between cercopithecoids milks (15.10%) and the two samples from hylobatids (15.60%), but neither were significantly different from hominoids (13.20%).

Figure 9.16. Percent energy from fat, protein, and sugar by family (Cebids were further subdivided into subfamily Callitrichinae and Cebinae).



Using factor analysis (PCA), total gross energy, percent energy from fat, percent energy from protein, and percent energy from sugar were expressed as one variable (Table 9.10). This variable (factor score 1) explained 76.83 % of the variation in the data set and was plotted against log female body weight (Figure 9.17) and length of lactation (Figure 9.18).

Factor score 1 was not significantly correlated with log (female body mass) ($r = 0.02$, $p = 0.88$). However, this diagram illustrates the range of variation in milk composition (expressed on the y axis by factor score 1) with respect to phylogeny.

Among hominoids, this factor showed little variation and had negative values for all hominoid data points. Factor scores for both monkey superfamilies were quite variable, and were both positive and negative. Investigation of individual data points reveals that among ceboids and cercopithecoids, negative factor scores represent samples from wild living individuals. All wild living *C. jacchus*, *L. rosalia*, and *A. paliatta* had factor scores less than zero. *M. sinica* presents an interesting exception; only two samples produced negative factors scores for this wild living population.

Factor score 1 was significantly correlated with absolute length of lactation ($r = -0.26$, $p = 0.02$; Figure 9.18) and relative length of lactation ($r = -0.40$, $p < 0.001$). In general, samples from species that have a longer duration of lactation (both absolute and relative) have lower factor scores. What is significant about this figure is that factor scores for samples from species that lactate longer than one year (all hominoids) are never greater than one, while samples from species that lactate for less than one year (all monkeys) can have numerous factor score 1 values, in either the positive or negative direction.

Table 9.10. Eigenvalues and associated variance for total gross energy, percent energy from fat, percent energy from protein, and percent energy from sugar.

Factor Analysis (PC) Component	Eigenvalue	% Variance	Cumulative % Variance
1	3.073	76.830	76.830
2	0.837	20.913	97.743
3	0.09	2.257	100.00

Figure 9.17. Factor score 1 by (log) female body mass

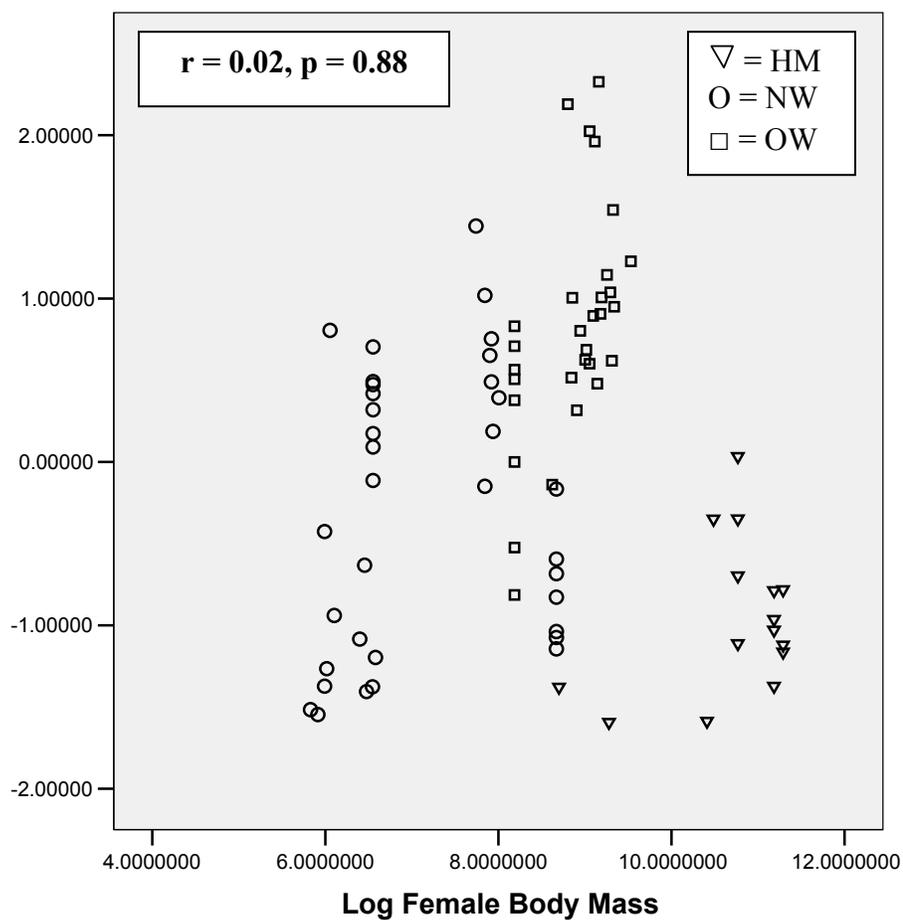
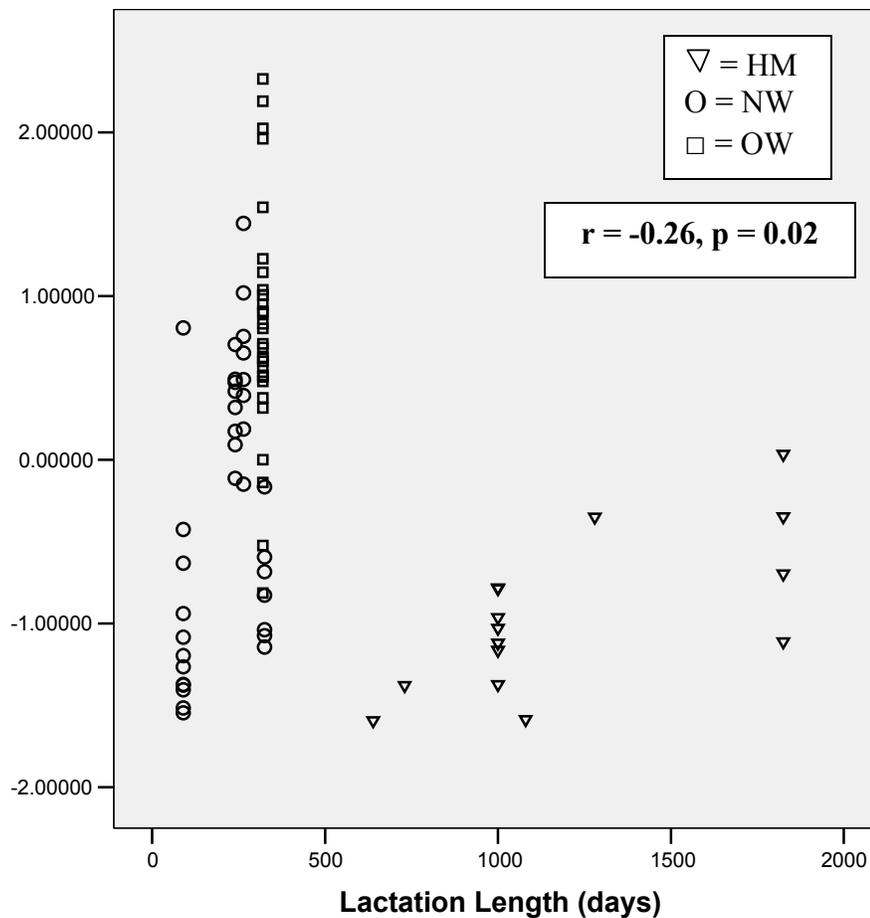


Figure 9.18. Factor Score 1 by Duration of Lactation (days)



Discussion

Relationships among milk components

Each milk sample was analyzed for fat, protein, sugar (lactose), dry matter, calcium, and phosphorus. Knowing the weight of fat, protein, and sugar in each milk sample permitted calculating the energy provided by each of these components, the total gross energy, and the proportion of that total energy from each component. The

relationship among milk constituents has been documented for human milk (Jenness, 1979; Jensen, 1995), cow's milk (Jensen, 1995), and the milk of many other mammals (e.g., Oftedal and Iverson, 1995), including nonhuman primates (e.g., Lönnerdal et al., 1984; Power et al., 2002). Results indicate that there are correlations between particular components, and these correlations appear to be consistent within and across taxa. Using results from human milk as a guide, the relationships among milk components from this study's anthropoid milk samples were investigated.

Samples higher in fat and protein were higher in dry matter but the relationship was stronger for fat than protein. Methods for protein analysis measured all nitrogenous protein, which includes both casein proteins (found in the pellet portion of milk) and whey proteins (found in the aqueous portion of milk). Dry matter does not measure all protein in milk, only that found in the pellet. Among humans, casein proteins are estimated to be approximately 40% of milk proteins (Stini et al., 1980). Lönnerdal et al. (1984) report a similar whey to casein ratio among rhesus macaques. This suggests that this ratio may be a conservative estimate for all anthropoid primates and that only approximately 40% of milk proteins contribute to dry matter.

Casein proteins bind calcium and phosphorus but only phosphorus was significantly correlated with protein. However, removal of ceboid samples from both correlation analyses (phosphorus and calcium with protein) increased the correlation coefficients and led to a significant correlation between calcium and protein ($r = 0.88$, $p < 0.0001$) among cercopithecoid and hominoid samples. Samples from ceboids also diverged from cercopithecoids and hominoids in the relationship between calcium and

phosphorus (Figure 9.3a, b). Calcium and phosphorus were positively and significantly correlated in hominoid and cercopithecoid samples ($r = 0.86$, $p < 0.001$) but were not significantly correlated in ceboid samples ($r = 0.28$, $p = 0.19$). Ceboids, in general, had higher phosphorus than would be predicted by the concentration of calcium in their milk. As will be discussed in regard to phylogenetic differences, this pattern may be explained by higher total protein in ceboid milks relative to the other two superfamilies.

The concentration of fat in a milk sample was almost isometric with total gross energy ($r = 0.95$). Per gram of milk, fat provides over 3 kcal/g (approximately 55%) more energy than protein and over 5 kcal/g (approximately 130%) more energy than sugar. Deviations from the line expressing the relationship between fat and energy (Figure 9.4) can be explained by variation in percent protein among superfamilies. Namely, higher protein in ceboid milks increases total gross energy without a concomitant increase in fat. Thus, ceboids had lower fat concentrations than predicted by total gross energy.

The proportion of milk energy provided by fat was positively and significantly correlated with total gross energy and an identical, but inverse correlation was identified for the proportion of milk energy provided by sugar and total gross energy (Figure 9.5, 9.6). Samples highest in total gross energy were highest in percent energy from fat and were, consequently lowest in percent energy from sugar. Samples highest in total gross energy were, generally, lowest in percent energy from protein. The correlation coefficient for all samples was $r = -0.33$ ($p = 0.003$). The distribution of data points in Figure 9.7 suggested the relationship between total gross energy and percent energy from protein might differ among superfamilies. When analyzed at the level of the superfamily, the

correlation was strongest for cercopithecoid samples ($r = -0.63$), followed by hominoid samples ($r = -0.58$) and ceboid samples ($r = -0.44$). These results suggest that the relationship between percent energy from protein and total gross energy is stronger in superfamilies that are less variable in percent energy from protein (cercopithecoids and hominoids).

Phylogenetic patterns in milk composition

Results do not support the null hypothesis of a generalized anthropoid milk composition. Differences in species' sample sizes prohibited statistical comparisons of means. A visual comparison of means suggested that species that were more closely related (subfamily, family, and superfamily) have more similar milk compositions. For example, in both captive and wild samples, highest mean fat was from *Macaca* samples and New World monkey milks were higher in protein than that of Old World monkeys or apes. Sugar did not follow a consistent pattern, and was equally variable within superfamilies as among superfamilies. The range of variation among anthropoid species in percent sugar was equivalent to that reported among humans (Prentice, 1995) suggesting that the concentration of milk sugar is variable both within and among anthropoids. Indeed, when mean sugar was compared among and between anthropoid families (and superfamilies), no significant differences were identified.

Cercopithecoid milk was significantly different in mean fat from cebid, atelid, hominid, or hylobatid milk. In fact, milk from cercopithecoids had 2.5% more milk fat than cebids, 4.28% more milk fat than atelids, 4.56% more milk fat than hominids, and

5.43% more milk fat than hylobatids. Variation among anthropoid families in mean percent protein was less than that of fat; highest mean percent protein came from cebid samples (2.78%) and the lowest from hylobatids (1.17%). Comparing results of this study to previous research on nonhuman primate milk (Table 3.4) indicates that protein is, generally, not as variable as fat among primates and is generally highest among the cebids. An exception is the 3.0% protein value reported by Taylor and Tomkinson (1975) for one gorilla milk sample. Samples from gorillas and other hominids included in this study suggest that, relative to other families, hominid milk is significantly lower in mean protein. This sample's aberrant lactose value (3.6%) strongly indicates that it may have been contaminated or analyzed with inappropriate methods and, at the very least, the protein value should be viewed with extreme caution.

Milk from cercopithecids was highest in total gross energy, and was significantly different from all other families. The range of variation in this value among families was quite high. Cercopithecids had 120% more energy in their milk than hylobatids and 94% more energy than hominids. Cercopithecid milks also had a significantly higher proportion of energy from fat (55.14%). Although percent energy from fat was variable among families, the only significant differences between or within ceboid or hominoid families was between hylobatids (14.00%) and cebids (38.27%), atelids (29.10%), and hominids (27.66%).

Percent energy from fat and sugar were inversely correlated. Thus, samples lowest in percent energy from fat (hylobatids, hominids, and atelids) were highest in percent energy from sugar. The range of percent energy from sugar among families was

almost 30%. Cercopithecids were significantly lower than all other families with only 31.47% of energy provided by sugar and hylobatids were significantly higher than all other families except for hominids, with 70.41% of energy provided by sugar.

Percent energy from protein was less variable than percent energy from fat or sugar, ranging by approximately 10%. Atelid and cebid milks had the highest proportion of milk energy from protein (23.02% and 21.52%, respectively), cercopithecids the lowest (13.38%), and intermediate values from hylobatid (15.59%) and hominids (14.73%).

Hominids and hylobatids were significantly different only in percent energy from fat. Cebids and atelids were significantly different in gross energy and phosphorus. With only two samples from hylobatids, it is not possible to conclude whether these samples are representative of the range of variation of this family. Therefore, they were grouped with hominids in superfamily Hominoidea, but differences in mean percent energy from fat were considered. Variation among ceboid families was also minimal. Differences between cebids and atelids in total gross energy and phosphorus may be explained by captivity (as will be discussed below) rather than phylogeny. Additionally, sample size permitted analysis of variation within the ceboid superfamily, so differences in all milk components will be explored at the level of the species. Differences between cebids, atelids, and the remaining families were predicted to persist when these families were analyzed as one unit (superfamily Ceboidea).

The milk of cercopithecoids was highest in mean fat, dry matter, calcium, total gross energy, and percent energy from fat, and intermediate in mean protein and

phosphorus. Cercopithecoid samples have almost twice the fat of ceboid samples and over four times the fat of hominoid samples. These differences translated into significant differences in total gross energy. Cercopithecoid milk had 30% more energy than ceboid milks and almost 100% more energy than milk of hominoids.

The milk of ceboids was highest in mean protein, phosphorus, and percent energy from protein, and was intermediate in mean fat, percent energy from fat, percent energy from sugar, and calcium. Protein in ceboid milk was over 25% higher than cercopithecoid milk, and 120% higher than that of hominoids. Higher mean percent protein among ceboids may explain higher than predicted percent phosphorus relative to percent calcium from both captive and wild living ceboid individuals, but particularly among captive living ceboids (Figure 9.3b). Percent energy from protein in ceboid milk was 47% higher than hominoids and 63% higher than cercopithecoids.

The milk of hominoids was lowest in mean fat, protein, dry matter, calcium, phosphorus, total gross energy, and percent energy from fat, was intermediate in percent energy from protein, and was highest in percent energy from sugar. Per gram of milk, hominoid milk had half of the energy (kcal) as that of cercopithecoid milk and one third of the energy of ceboid milk. Although intermediate in percent energy from protein, hominoids were significantly different from ceboids but not cercopithecoids.

Phylogenetic patterns within the superfamily Ceboidea

This study included five species of New World monkey: *A. palliata* (n = 7), *C. jacchus* (n = 8), *C. apella* (n = 9), *L. rosalia* (n = 5), and *S. boliviensis* (n = 8). Sufficient

and nearly equivalent sample sizes among species permitted analysis of variation within this superfamily. Analysis at the level of the family grouped *C. jacchus*, *C. apella*, *L. rosalia*, and *S. boliviensis*. Separating these species reveals significant variation in mean protein, percent energy from protein, fat, percent energy from fat, percent energy from sugar, and total gross energy.

Saimiri milks were highest in mean protein (3.59%), followed not by *Cebus*, their closest relatives, but by *Callithrix* (2.65%), *Leontopithecus* (2.47%), then *Cebus* (2.35%), and finally *Alouatta* (2.25%). *Saimiri* was significantly higher than all ceboids, as well as all cercopithecoids and hominoids. No significant differences were identified between the remaining ceboid species. *Saimiri* species are only slightly larger than the largest callitrichid primates (Table 5.x), but *Saimiri* mothers bear a higher metabolic cost compared to other primate species on a per infant basis, with maternal investment concentrated in the pre- and perinatal periods (Garber and Leigh, 1997; Jack, 2007). Neonatal body weights are between 14 to 20% of maternal body weight (the largest of any anthropoid primate), and infants undergo rapid postnatal brain growth (Garber and Leigh, 1997; Hartwig, 1996). Allometry and the large infant-to-maternal mass ratio suggests that *S. boliviensis* might produce more concentrated milks than larger anthropoids, but the example of the callitrichids (Power et al., 2002) indicates that body size is not a sufficient explanation of variation among anthropoid primate milk. It is interesting to hypothesize if increased neonate body size relative to maternal body size was accompanied by an increase in percent protein in milk.

Mean fat separated cebines (*Saimiri* and *Cebus*) from the other ceboids. Milk from *Cebus* was highest in fat (5.23%), followed by *Saimiri* (4.65%), *Callithrix* (2.11%), *Alouatta* (1.83%), and *Leontopithecus* (1.31%). This result may reflect a phylogenetic pattern. Relative to other ceboid species, *Saimiri* and *Cebus* are highly encephalized and produce large-brained neonates (Garber and Leigh, 1997; Hartwig, 1996). Higher energy requirements of the developing infant brain may be partially met through higher milk fat, which provides more energy per gram than protein or sugar and thus increases total gross energy of the milk. Mean gross energy of *Cebus* and *Saimiri* was 0.91 kcal/g, 0.27 kcal/g greater than the mean gross energy of samples from *Callithrix* and approximately 0.35 kcal/g greater than *Alouatta* or *Leontopithecus*. However, these differences may also be an artifact of the data set. That is, the samples with the highest mean percent fat and percent energy from fat were samples from only captive living individuals, while the other three species were represented by both captive- and wild living (*Callithrix* and *Leontopithecus*), or only wild living individuals (*Alouatta*).

Effect of captivity

In captivity, behavior and physiology are predicted to be at the maximum of the species' reaction norm (Bentley, 1999; Lee and Kappeler, 2003; Robson et al., 2006). Reduced energy expenditure, reduced feeding competition, and a consistent supply of food among captive living individuals can lead to larger body size (Leigh, 1992, 1994a; Strum, 1991), more body fat (Dufour and Sauter, 2002), as well as the alteration of the timing of life history traits, such as age at first reproduction (earlier) and life span

(longer) (Ross, 1998, 2003). This study tested the hypothesis that captivity would influence milk composition.

Power et al. (in press) hypothesized that the condition of reproductive females in captivity would be more variable than in the wild. At one extreme are females that are overweight due to increased energy intake and reduced energy expenditure. The accumulation of body fat by captive living female primates has been demonstrated (Dufour and Sauther, 2002; McFarland, 1997; Periera and Pond, 1995; Rutenberg et al., 1987, Zihlmann and McFarland, 2000). At the other extreme are reproductive females who are in poor condition but are able to reproduce because of the lower demands of the captive environment (Tardif et al., 2001). In the wild, neither extreme would be found. Wild living primates are not overweight and reproductive females in poor condition would not be able to reproduce.

The effect of captivity on a species' milk could only be examined in *Callithrix jacchus*, with four samples each from captive- and wild living individuals. Mean fat was significantly higher ($t = 2.71$, $p < 0.01$) and more variable among captive living *C. jacchus*. Among captive living individuals, percent fat in milk ranged from 1.71% to 5.82% with a mean of 3.32%. Among wild living *C. jacchus*, percent fat in milk ranged only from 0.72% to 1.14%, with a mean of 0.90%. Consequently, mean total gross energy and percent energy from fat also were significantly higher in captive samples. Mean protein was identical between the two groups (2.65%) but was more variable among samples from captive living (2.26 – 3.13%) than wild living (2.51 – 2.80%) individuals. Sugar was significantly higher in milk from wild living *C. jacchus* ($t = -5.84$, $p = 0.006$),

with no overlap in the ranges between the populations. The range of percent sugar values was greater among wild samples (8.0 – 8.5%) than captive samples (7.49 – 7.58%). The inverse relationship between sugar and fat suggests that both components may be related to total gross energy. Power et al. (in press) found that *C. jacchus* samples highest in total gross energy were higher in fat while those lower in total gross energy were highest in sugar for individuals living in either captivity or the wild. The important difference, therefore, appears to be the ability of captive living *C. jacchus* to produce high energy milks.

Comparing captive and wild samples within ceboidea and cercopithecoidea mirrored many of the same patterns identified in *C. jacchus*. Mean fat was more variable and significantly higher among captive living ceboids (6.52%; $t = 8.60$, $P < 0.001$) and captive living cercopithecoids (9.78%; $t = 3.08$, $p < 0.001$) compared to wild living ceboids (range: 2.37%) and cercopithecoids (range: 4.90%).

Mean protein did not differ with respect to captivity in ceboids ($t = 1.93$, $p = 0.06$), and was only slightly more variable among captive samples (1.83 - 4.23%) than wild (1.29 – 3.42%). Mean protein was significantly higher in captive living cercopithecoids ($t = 2.31$, $p = 0.03$), and like in ceboids, was only slightly more variable in captive (1.68 – 2.77%) than wild samples (1.51 – 2.34%).

Mean milk total gross energy of captive living ceboids ranged from 0.62 to 1.14 kcal/g, with a mean of 0.88 kcal/g. Mean milk total gross energy from wild living individuals was 0.56 kcal/g, which was significantly lower than that of captive living individuals ($t = 8.29$, $p < 0.0001$). Mean milk total gross energy of captive living

cercopithecoids ranged from 0.68 to 1.53 kcal/g, with a mean of 1.03 kcal/g. This was significantly higher than mean fat in wild living cercopithecoids (0.79; $t = 3.37$, $p = 0.003$). Wild living ceboids and cercopithecoids produced milks with energy values within the lower range of captive living individuals, but the highest energy milks from wild living individuals (ceboids: 0.71 kcal/g; cercopithecoids: 0.91 kcal/g) were still below the mean for captive living individuals of each superfamily.

Captive living ceboids and cercopithecoids had a significantly higher proportion of milk energy from fat than wild living individuals, although the difference was much more pronounced in ceboids (Figure 9.10a). The proportion of energy from sugar was lower in captive living monkeys, but the difference was only significant among ceboids (Figure 9.11b). This difference is explained by the inverse relationship between total gross energy and percent energy from sugar. In this study, samples lowest in total gross energy were highest in percent energy from sugar. The inverse relationship between total gross energy and percent energy from protein explains why samples from captive living individuals had a significantly lower proportion of energy from protein. These samples were higher in percent energy from fat which, in turn, reduces the amount of energy provided by protein, despite similar mean protein values between the two populations.

Captive living monkeys were able to produce higher energy milks than wild living monkeys. This difference was primarily due to the ability of captive individuals to produce milks with higher amounts of fat. Power et al. (in press) do not believe that this difference is related to body size. In their sample of *C. jacchus*, body mass was similar between captive- and wild living reproductive females. However, females of similar body

masses may differ in regard to energy balance and body composition. Captivity may allow reproductive females to be in positive energy balance, defined as a positive net residual of energy intake after accounting for energy expenditure (Ellison, 2003).

Although reproductive female primates in the wild may reduce energy expenditure (Dufour and Sauther, 2002; Miller et al., 2006; Nievergelt and Martin, 1999) or increase energy intake (Boinski, 1988; Dunbar and Dunbar, 1988; Saito, 1988; Smith, 1977), it is unlikely that these females, in general, are able to achieve a positive net energy balance (Altmann, 1983; Altmann and Alberts, 2003; Miller et al., 2006; Nievergelt and Martin, 1999).

Captivity did not influence the milk composition of hominoids. Samples from wild living hominoids (mountain gorillas) were identical to, and therefore not significantly different from, samples from captive living hominoids in percent fat (1.4%) and total gross energy (0.49 kcal/g). In percent energy from fat, samples from captive- and wild living individuals were nearly identical (25.96 vs. 25.92%). Mean fat and total gross energy were more variable among captive living hominoids. Mean fat ranged from 0.55 to 3.04% and total gross energy from 0.42 to 0.62 kcal/g, compared to ranges of 1.07 to 2.04% (fat) and 0.44 to 0.54 kcal/g (total gross energy) in wild living hominoids. However, unlike ceboids and cercopithecoids, the lowest values for mean fat and total gross energy were from one captive living female, and wild mountain gorillas were capable of producing milk with fat and total gross energy values that exceeded the captive sample mean. Hominoids also diverged from monkeys in that higher mean protein was identified among wild living individuals. Mean percent protein of mountain

gorillas was 1.81%, while that of captive living hominoids (which includes the closely related lowland gorilla) was 1.02%. When the outlier mountain gorilla sample was removed from this comparison (protein = 3.16%), the difference between the groups is reduced but still significant. Although based on a very small sample size, mountain gorillas appear to produce milks of higher protein content relative to other hominoids included in this study.

Relationship to diet

Sources of milk fat are maternal diet, maternal depot stores, and *de novo* synthesis by the mammary gland (Del Prado et al., 1999; Iverson and Oftedal, 1995; Prentice, 1996; Sauerwald et al., 2001; Stini et al., 1980). Differences in percent fat in milk can be explained by differences in diet, differences in maternal depot stores, or physiological differences in lipogenesis by the mammary gland. Captive living primates demonstrated a different pattern of milk fatty acid composition from wild living primates (Chapter 8). It was thus predicted that captive living primates would differ in mean fat, and thus, total gross energy, from wild living primates. As was discussed above, this prediction was confirmed. A species' dietary strategy appears to have little effect on milk fat (Figure 9.11). Milk fat is highest in captive living anthropoids. Regardless of captivity, milk fat is highest in cercopithecoïd milk, followed by ceboïd milk, and lastly, hominoid milk.

The juvenile risk hypothesis (Janson and van Schaik, 1993) argues that folivorous primates should grow more rapidly relative to nonfolivores, because of reduced feeding competition with adults. Based on Oftedal's (1984) prediction that milk protein and

infant growth rates will be positively correlated, Leigh (1994b) predicted that infants of folivorous species would have increased milk protein. He further argued that this increased protein would be the result of higher protein intakes in leaves, which have moderately high protein content. Leigh's (1994b) prediction was tested in study samples, which included two species of folivorous primates: mantled howlers and mountain gorillas. Energy from protein (protein weight x 5.86 kcal/g) was highest among frugivore-insectivores, followed by gummivores, and then folivores and frugivore-folivores, whose milks had identical mean percent protein (1.16%) (Figure 9.12). Energy from protein in *Alouatta* samples was actually lowest among ceboids. Captivity does not seem to be a sufficient explanation for protein values in ceboids. Milks from wild living *C. jacchus* and *L. rosalia* were higher than many samples from captive living individuals of the same species. However, folivorous hominoids (mountain gorillas) did have higher mean protein than other hominoids, but not higher than non-folivorous wild living monkeys (Figure 9.14). Further, while captivity (diet, energy expenditure) may affect the fat content of milk, it is unlikely that differences in protein are the result of differences in diet or energetics between captive and wild living individuals. Supplementation of malnourished and underweight human females (BMI < 18.5) in The Gambia with protein biscuits did not change the milk protein concentration (Prentice et al., 1983). Differences among nonhuman anthropoids in protein concentration appear to be correlated with phylogeny rather than captivity.

Phylogenetic patterns in variability of milk composition

Research questions posed at the beginning of this chapter focused on the relationship between milk composition and phylogeny, diet, captivity, and life history. It became apparent during data analysis that what may be more important than differences in individual milk components were phylogenetic differences in the degree of variation in these milk components.

Milk from monkeys was more variable in composition (percent of individual components) than milk from apes. Variance measures the spread of individual values around the mean of those values. The variance of fat weight of milk from cercopithecoids was higher than that of ceboids, and was close to zero in hominoids (Figure 9.14). The test for equality of variances among these three groups identified significant differences in variance between hominoids and each monkey superfamily in mean fat (Table 9.9) as well as total gross energy (data not shown).

Cercopithecoid milk was most variable in mean fat and total gross energy. Mean fat and total gross energy were significantly higher in captive samples. However, variability was a hallmark of both groups. Among captive samples, the range of mean fat was over 9% and that of wild cercopithecoid samples was almost 5%. The pattern for milk energy was the same; although the mean was higher among samples from captive cercopithecoids, both populations were extremely variable in milk energy (see cercopithecoid data points along the x axis, Figure 9.5). Although not as extreme, ceboid milk also varied in percent fat and total gross energy. Within *C. jacchus* (a population that includes captive- and wild living individuals) milk fat ranged from 0.72 to 5.8%.

Within *C. apella* (which includes only captive living individuals) milk fat ranged from 2.94 to 8.23%.

Ceboid milk was most variable in sources of milk energy, demonstrated in plots of the proportion of energy provided by fat, protein, and sugar (see ceboid data points along the y axis in Figure 9.5 – 9.7). In Figure 9.5 (and 9.6), samples from both captive- and wild living ceboids fell within the range of hominoid samples, and samples from captive living ceboids also were within the range of cercopithecoids. Ceboid samples were among the lowest and highest in the percent of energy from fat and sugar. Or, to put it another way, ceboid milks were hominoid-like and cercopithecoid-like.

In stark contrast to the highly variable milk of monkeys was the lack of variability among hominoid milks. The range of total gross energy among hominoid samples (0.19 kcal/g) was only 20% of the range identified among cercopithecoids. Fat ranged only by 2.5% compared to over 7% in ceboids and over 11% in cercopithecoids.

While statistical significance is important, biological implications must also be considered. How are monkeys able to produce milks with over 12% fat and twice the energy of hominoid milks, and why do they do so? Why does hominoid milk not vary with respect to captivity while captive living monkeys, particularly cercopithecoids, were producing milk with significantly more fat and energy than wild conspecifics (e.g., *C. jacchus*) or closely-related species (e.g., *M. sinica*)?

Variation in a life history character trait is not random (Kappeler et al., 2003). The small ranges for mean fat and total gross energy suggest that hominoids may be constrained in their ability to produce high energy milk. Further, that even captive

hominoids could not produce milks with the same fat or energy values as wild living monkeys suggests that hominoid milk is less responsive to environmental variability (variation within captivity or variation as a result of captivity) than is milk from monkeys.

Following Evans (1953) and Williams (1992), West-Eberhard (2003) defines plasticity as intra-individual variation. The genome of the individual is a constant and variation in the phenotype results from variation in the environment. In this definition, a phenotype is plastic if it demonstrates environmental responsiveness. Results from this study suggest that milk composition is plastic, but that the degree of plasticity or environmental responsiveness varies with respect to phylogeny. Specifically, milk from monkeys was more variable with respect to the environment than that of hominoids, and thus more plastic.

Hominoid sample size was much smaller than that of cercopithecoids or ceboids. Lack of variability may therefore be the result of sampling bias. However, as presented in Chapter 3, human milk composition appears to be quite resistant to environmental changes. Results from hominoids match previous findings on human milk composition (Emmett and Rogers, 1997; Prentice, 1995, 1996; Prentice and Prentice, 1995). Human data will be considered with respect to nonhuman anthropoids in the last section of this chapter. In the following section, I will discuss how differences in variability of milk composition may be related to life history traits that separate hominoids from monkeys.

Relationship to life history traits

The relationship between the energetic costs of lactation and life history strategies of nonhuman primates is a well-visited subject in the literature (e.g., Barrett et al., 2006; Dewey, 1997; Dufour and Sauter, 2002; Forsum et al., 1992; Hartwig, 1996; Lee 1996, 1999; Lee et al., 1991; Leigh, 2004; Martin, 1981, 1983, 1996; Ulijaszek, 2002; Vasey and Walker, 2001) and many predictions have been made about the composition of nonhuman primate milk. An underlying assumption of milk composition is that it will be a reflection of the ontogenetic priorities of the neonate and infant. However, the physiological ability of the mother to produce milk that meets these needs may be limited due to phylogeny (genetic instructions for milk production) and ecology (diet, foraging costs, seasonality). Milk is a compromise, and therefore milk composition may not track all infant requirements for growth and development. Further, milk composition may be decoupled from other life history traits. Milk composition is predicted to scale with body size or maternal metabolic rate. Maternal metabolism may instead be more dependent on factors such as milk yield, nursing frequency, and/or duration of lactation.

Four predictions from the life history literature regarding nonhuman primate milk composition were evaluated using data produced by this dissertation. These predictions are presented and discussed separately.

(1) Metabolically expensive neural tissue requires nutritionally dense, higher-quality foods (Walker et al., 2006). Species with larger relative brain sizes will require higher quality foods, including milk. Milk of larger brained anthropoids will be higher in total gross energy.

Species with the highest EQ values (*Cebus* and *Saimiri*) did not have milks with the highest total gross energy. Rather, the highest total gross energy came from *M. mulatta*. Even when the outliers (five samples with highest gross energy from *M. mulatta*) are removed from analysis the correlation coefficient increases only from 0.36 to 0.46. As Figure 9.15 demonstrates, hominoid milks are low in energy despite relatively large brain sizes. If hominoids are ignored, the relationship among milk energy and EQ appears to hold for monkey species. However, the effect of captivity on milk energy must also be considered. *Saimiri* and *Cebus* are the only ceboid species that included only captive living individuals. Data on milk composition of wild living species of these genera are necessary to evaluate whether high energy milks are a species-specific trait, or are an artifact of captivity. The ability of these species to produce such high energy milks must not be discounted, however, particularly in *S. boliviensis* females. They are comparable in body size to the larger callitrichines, including *Leontopithecus* and *Callithrix* but produce considerably higher energy milks than captive living callitrichine females.

The majority of the body's available glucose is used by the brain for energy metabolism, assuming that the body is not fasting (Kuzawa, 1998). The glucose requirements of human infants are three to four times greater than those of adult humans per unit of body weight (Jones, 1979; Kerr et al., 1978), explained by differences in relative brain size between infant and adults (Kuzawa, 1998). Thus, as opposed to high energy (= high fat) milk, low energy milks (= milk high in the proportion of energy provided by sugar) may be correlated with brain size. Indeed, Dufour and Sauter (2002:

590) assumed that the “notably high concentration of carbohydrates in the milk of lemurs and anthropoid primates, including humans, may be related to brain growth because...brain metabolism depends on glucose.” Percent energy from sugar and percent sugar were negatively correlated with EQ, and only percent energy from sugar was significantly correlated ($r = -0.33$). Again, hominoids appear to be the “spoiler” in this correlation. Although relatively high in EQ, their milk is higher than would be predicted in percent energy from sugar. Captivity could again be a mitigating factor among monkeys. Samples from wild living species were, generally, lower in energy and therefore higher in percent energy from sugar. However, it may be possible that hominoids are derived relative to monkeys in use of glucose as fuel for growth and development.

(2) Species with high growth costs need additional energetic investment (Lee, 1996; Leigh, 2004). Milk of species with high growth costs will be higher in energy provided by protein (Oftedal, 1984; Power et al., 2002).

Based on primate growth rates, Power et al. (2002) predicted that the proportion of energy from protein would be highest among ceboids, followed by cercopithecoids, and then hominoids. Ceboid milks did have the highest percent energy from protein, but milk from cercopithecoids was just slightly lower in percent energy than that of hominoids (13.38% vs. 14.84%). The high fat samples from *M. mulatta* increased the percent energy from fat, which in turn decreased the percent energy from protein. This also may have confounded potential differences between cercopithecoids and hominoids in percent milk energy from protein. Samples from the two species of hylobatid increased

the mean percent energy from protein of the superfamily. When these samples are removed, cercopithecoids have approximately 2% more energy from protein in their milk than hominids. The higher percent energy in milks from hylobatids than hominids may be significant with respect to questions about growth rates. Higher percent energy from protein would be predicted in hylobatids relative to hominids due to differences in the length of postnatal somatic growth between the two hominoid families, but the small sample size of hylobatids cautions against making such a connection.

Lastly, within the superfamily Ceboidea, percent energy from protein does follow growth rates: the callitrichines, *Leontopithecus* and *Callithrix*, the atelid *Alouatta*, and the cebines, *Saimiri* and *Cebus*. Taken together, results on percent energy from protein seem to support predictions that species with high growth costs will produce milks with a higher proportion of milk energy from protein.

(3) Milk energy is a function of maternal mass (Lee, 1996; Martin, 1981; 1996). Milk energy, and therefore milk composition, will correlate with adult female body mass (log transformed) and (4) Milk composition will correlate with other aspects of a species' lactation strategy. Variation in duration of lactation among anthropoid primates will influence milk composition.

There was no correlation between milk composition (represented by the variable factor score 1) and female body mass (Figure 9.17). The correlation between milk composition and absolute length of lactation (Figure 9.18) was significant, but weak ($r = -0.26$). Factor score 1 accounted for 75% of the variation in milk energy and percent energy from fat, protein, and sugar, suggesting that this variable is a good approximation

for milk composition across species. Figure 9.17 and 9.18 do indicate that there may be a relationship between variation in milk composition, body size and length of lactation, reinforcing the observation that milk composition is more variable among monkeys, particularly Old World monkeys, than hominoids.

The relationship among mammals between storage capacity and body mass is “nearly linear” (Gittleman and Thompson, 1988). The ability to store nutrients is primarily driven by the allometric relationship to energy expenditure (Oftedal, 2000). This means that mammals of different sizes respond to energy shortages or surpluses in different ways. Larger mammals are able to store proportionally more energy than smaller mammals (Gittleman and Thompson, 1988). Further, because smaller mammals have a higher rate of energy expenditure relative to body mass than larger mammals, they are physically incapable of storing energy for any length of time (Gittleman and Thompson, 1988; Oftedal, 2000).

As it relates to lactation, larger body size permits many large mammals to “disengage lactation from maternal food intake” (Oftedal, 2000); nutrients are stored in maternal depot fat during times when food is abundant and utilized for milk nutrients at a later point in time. For example, blue whales deposit 45,000 kg of blubber prior to the reproductive season, about a third of which is transferred directly to the infant in the form of milk fat (Oftedal, 2000).

I argue that because of their larger body size, hominoids are able to disengage lactation from maternal food intake and rely more on body stores than smaller-bodied monkeys. Smaller body size prohibits the reliance on maternal body stores in monkeys –

they are limited in the amount of energy they can store and the length of storage time. Instead, excess energy in monkeys can be transferred directly and immediately into milk energy. Variation among species in milk energy production is predicted to relate to maternal energy balance, which may be more extreme in captivity (Power et al., in press). Excess energy in hominoids may be transferred into depot stores for utilization at a later date, perhaps even another reproductive event (Oftedal, 2000). Milk energy in hominoids is therefore predicted to be decoupled from maternal energy balance.

While body size may explain how monkey species are able to produce high energy milk, duration of lactation may explain why hominoids do not. Lactation in monkeys is measured in months while lactation in hominoids is measured in years. A longer lactation period would have relaxed constraints on the daily transfer of energy. At the same time, energy storage would be emphasized as a buffer against environmental changes. Thus, while there may be a short term benefit to hominoid mothers who immediately convert their excess energy intake into milk energy, this strategy is unlikely to be beneficial over the duration of lactation and would not be favored by selection. Selection would instead favor the consistent production of low energy milk, ensuring that offspring would always be provided with nutrients. Excess energy would be converted to depot stores which could be utilized over the duration of lactation, or even a future reproductive event.

Milk from monkeys was much more responsive to variability in the environment, exhibiting greater variation within captivity, and between captivity and the wild. The smaller body size of monkeys prohibits the storage of energy over the course of lactation.

However, a shorter duration of lactation may favor the immediate conversion of any excess maternal energy into milk energy. Unlike the hominoid mother, ceboid and cercopithecoid mothers can focus on short term benefits. Monkey infants who receive higher energy milks over the short may grow faster, relative to hominoids, and be able to wean earlier.

Proximate milk composition of humans in an evolutionary perspective

The goals of this study were (1) to identify traits in milk composition shared by all anthropoids; (2) to identify traits in milk composition shared by all hominoids (including humans); and (3) to identify unique-derived traits of human milk. Specifically, this study analyzed nonhuman primate milk to determine if milk composition has been modified over the course of human evolution. Is human milk composition species-specific, and if not, how is it similar to that of other anthropoids? To address these questions, data on human proximate milk composition and the range of variation in composition (presented in Chapter 3) were compared to that of nonhuman anthropoids.

Anthropoid traits

Nonhuman primate milk, compared to other mammalian orders, was described by Oftedal (1984; Oftedal and Iverson, 1995) as dilute, low in total gross energy, and low in the percent of energy provided by protein. While admittedly over-simplistic, Oftedal's statement is justified if the comparison is made between means from primate milks and

milks from other mammalian orders. For example, compared to cetaceans primate milk is considerably lower in fat and total gross energy. Relative to carnivores, primate milk is much lower in protein and the percent of energy provided by protein. However, variation identified within anthropoids strongly argues against making generalizations about a primate species, or family, based on anthropoid means. The only generalization that can be made without qualification about anthropoid milk is that it has approximately 7% sugar. Variation was identified at the level of the family and superfamily for the majority of components measured. Anthropoid primates produced milk that ranged from approximately 0.5 and 12% fat, 0.5 to 4% protein, and 0.38 to 1.53 kcal per gram of milk. The null hypothesis of a generalized anthropoid milk composition was rejected.

Hominoid traits

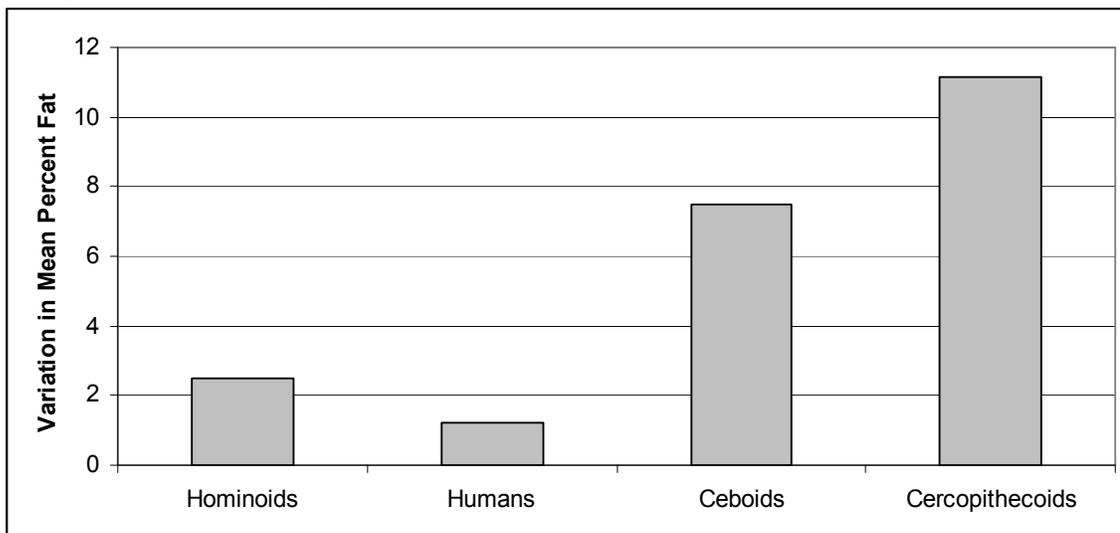
Milk composition tends to be kept within narrow limits in spite of the wide variations in kind and amount of dietary intake (Jenness, 1979, p. 226).

Milk of hominoids is lower in fat, total gross energy, and percent energy from fat than milk from cercopithecoids and ceboids. That there was overlap between hominoids and monkeys in all milk components analyzed suggests that it is not the composition of milk that separates hominoids from other anthropoids. Rather, the derived trait in hominoids is that milk composition is disengaged from environmental variability whereas the milk of monkeys was highly responsive to environmental variability. As large bodied mammals, hominoids are able to store more energy on their body for a longer period of time. Storage for lactation would be necessary when the lactation period was extended. Further, the slower postnatal growth rate of offspring would relax constraints on milk

energy. Thus, the hominoid adaptation is the production of low energy milk regardless of maternal energy balance.

The ability to produce milk of a consistent quality over an extended period of lactation in environments with patchy resources was hypothesized to have a large advantage to humans. It appears, however, that this strategy may be more remote than was originally predicted. Humans match hominoids in having a very narrow range for milk fat concentration.

Figure 9.19. Range of variation in mean percent fat in hominoids, ceboids and cercopithecoids (this study) and humans (data from Prentice, 1995).



Data are available on the relationship between energy balance and milk production in humans, permitting a test of the hypothesis that these two variables are not correlated among hominoids. Although human populations vary with respect to maternal energy balance, human milk fat (and energy) is relatively conserved and appears to be as variable within females (and populations) as between females (and populations) (Jensen

et al., 1995; Prentice, 1995). Women with high BMIs (>26) produced milks of approximately 2.89% milk fat, which was only 0.14% higher than women with low BMIs (< 18) (Barbosa et al., 1997).

In addition, neither milk quantity nor quality was found to significantly correlate with maternal weight, height, or body fat (Butte et al., 1984). The reliance of reproductive females on stored fat also is established. If dietary intake of fat during lactation is low, human females compensate by utilizing fat from their depot stores (Emmett and Rogers, 1997). Indeed, it is believed that women in undernourished populations are able to continue to lactate through reliance on fat reserves (Jelliffe and Jelliffe, 1978). The human adaptation of the production of relatively low energy milk combined with an emphasis on fat storage is a vestige of their evolutionary history. Jensen et al.'s (1995) description of human milk as “well-buffered” against ecological variability should be extended to all hominoids.

Hylobatids may present an interesting exception, however. Hylobatids are the “monkey-like ape in the middle.” In regard to adult female body size, hylobatids are considerably smaller than other apes and overlap with the body sizes of many monkey species, but are more similar to apes in length of lactation relative to body size (Table 5.3 – 5.5; Ross, 2003). Samples from captive living hylobatids were among the lowest in fat and total gross energy, which seems to suggest that length of lactation may have been an important selective constraint on milk energy. If they responded to captivity in similar ways to monkeys, higher fat and energy would be predicted, but only if maternal energy balance was positive. With only two samples and no corresponding data on maternal

condition, it is with caution that hylobatids are included as hominoids from the perspective of milk composition.

Unique-derived traits?

Modern human infants are referred to as secondarily altricial - they have several precocial traits (long gestation relative to body size, larger and fatter neonate body size, and single births) coupled with altricial features (immature motor skills and high dependence at birth relative to nonhuman primates) (Correia et al., 2004; Foley and Lee, 1991; Kuzawa, 1998; Martin, 1981; Portman, 1990; Shipman and Walker, 1989; Vasey and Walker, 2001). Compared to other hominoids, human infants show a greater degree of postnatal brain growth which Martin (1981) models as a continuation of the fetal brain to body growth relationship for at least the first year after birth. It is more energetically efficient for brain growth to occur during fetal life because the metabolic capacity of the mother exceeds that of the infant (Foley and Lee, 1991; Martin, 1981; Vasey and Walker, 2001). However, the size of the female pelvic inlet imposes a limit on fetal brain growth (Foley and Lee, 1991; Martin, 1981; Shipman and Walker, 1989; Tague and Lovejoy, 1986; Vasey and Walker, 2001). With selection for increased brain size over the course of human evolution, there came a point at which the pattern of brain growth shifted from rapid fetal growth and development to rapid fetal and postnatal growth and development.

A brain growth trajectory like that of modern humans would necessitate a change in maternal energetics, with mothers bearing a larger energetic burden during lactation. Foley and Lee (1991) calculated that human infants from birth to 18 months are almost

10% more energetically costly than chimpanzee infants as a result of increased encephalization. It was hypothesized that mothers meet these needs by increasing infant nutritional intake during lactation, presumably through production of higher energy milk (Martin, 1996).

Nutritional intake by infants could increase through the modification of any aspect of the lactation strategy: volume of milk produced, nursing frequency, duration of lactation, or milk composition. There are no comparative data on milk volume and maternal feeding practices from nonhuman primates. The duration of lactation in humans is debatable, but can be assumed to be similar to or slightly attenuated from the duration of lactation among nonhuman apes of comparable body size (Kennedy, 2005).

Human milk is similar in protein and sugar concentration, and is only slightly higher in fat than samples of milk from other hominoids. Table 3.3 provided a mean fat of 4%. However, none of the traditional societies cited in Prentice (1995) produced milk over a 24 hour period with a percent fat exceeding 4%. Four to 5% may thus be considered the maximum concentration for fat in human milk. The maximum concentration of hominoid milk is unknown, but the highest percent fat for hominoids in this study was just over 3%, which indicates that the difference in milk fat between humans and other hominoids may be only 1%. While this difference may prove to be statistically significant, a more important question is if this difference is biologically significant. Does a 1% increase in milk fat support the human pattern of postnatal brain growth?

The human brain is approximately one-third lipid, all of which must be supplied to fetuses and young infants by the mother *in utero* and in milk, respectively. A relatively larger brain with a longer period of postnatal growth may have selected for an increased ability in human females to store energy and later mobilize this stored energy for lactation. The human brain has “obligatory and inflexible requirements” (Kuzawa, 1998, citing Armstrong, 1983). Development of significant fat stores may be a physiologically-linked adaptation to changes in rate and pattern of brain growth (Ulijaszek, 2002). Although large fat stores are found in many female mammals, including nonhuman primates, human female fat stores are argued to be relatively larger than (Dufour and Sauter, 2002) and different from (Pond, 1998) the female primate pattern. Further, the degree of difference in fat mass between males and females is greater in humans than nonhuman primates. While human males are heavier than females, females are fatter, with 34% more fat mass on average than males (Wells, 2006). Wells (2006) argues that the greater fat mass of human females relative to human males and to nonhuman primate females indicates the importance of reproductive energetics in human evolution. Specifically, he proposes that the evolution of fatness in human females is at least partly the result of encephalization.

Dufour and Sauter (2002) report on four non-Western human populations, all of whom have greater than 20% body fat. There is little comparative data on nonhuman apes, but data on captive lowland gorillas (Zihlman and McFarland, 2000) show that one captive female gorilla had the potential to store similar amounts of body fat. It is unknown if wild living apes are capable of maintaining the percent body fat of human

females. This type of data would be invaluable to determine if adult female fat storage is indeed a unique-derived human adaptation to support brain growth.

Another possibility is that the cost of growing a larger brain was not met through alterations to the lactation strategy at all. Modern human infants have a precocious condition of adipose development at birth (Correia et al., 2004; Kuzawa, 1998; Ulijaszek, 2002) and are the fattest species on record at birth (Kuzawa, 1998). Fatness is argued to be an adaptation to the higher lipid needs of newborns for rapid brain growth during the first year of life (Correia et al., 2004). Like maternal depot fat stores, infant fat stores (deposited primarily during the third trimester) would act as a buffer against disruptions in energy transfer during lactation (Kuzawa, 1998). As reviewed by Kuzawa (1998), this hypothesis is indirectly supported by data. Without comparative data from nonhuman primates, it is not possible to say whether this is a unique human adaptation or a non-adaptive consequence of other ontogenetic changes (such as increased fat storage by the mother).

With the available comparative data, I would argue that human milk composition is not species-specific. This statement is not equivalent to saying that human milk is decoupled from human brain growth and development. The relationships between neonatal and infant ontogeny, maternal energy stores, and lactation are still under investigation in humans and are unknown in closely-related nonhuman primates, prohibiting such a blanket declaration. What must be considered, however, is that human reproduction is built on a hominoid foundation. To fully understand the human

reproductive strategy requires a more thorough understanding of the reproductive strategies of other hominoid and anthropoid species.

Conclusions

(1) What are the relationships among milk components, milk energy, and the proportion of energy in milk provided by sugar, protein, and fat? Milk fat was positively and significantly correlated with dry matter, percent energy from fat, and total gross energy. Samples highest in total gross energy were highest in percent energy from fat, and lowest in percent energy from sugar. Phosphorus was positively and significantly correlated with protein but calcium was not. Phosphorus and calcium were not significantly correlated. This was explained by variation in mean protein between ceboids and cercopithecoids and hominoids.

(2) Does proximate milk composition show any phylogenetic patterns? Is proximate milk composition constrained by evolutionary history? The milk of cercopithecoids is highest in fat, dry matter, calcium, percent energy from fat, and total gross energy. Cercopithecoid milks had twice the fat of cebooids and over four times that of hominoids. Cercopithecoid milk is also most variable in percent fat and total gross energy. Ceboid milks were highest in mean protein, phosphorus, and percent energy from protein. Ceboid milks were intermediate between cercopithecoid and hominoid milks in fat, dry matter, percent energy from fat, and total gross energy. They were the most variable in percent energy from fat, protein, and sugar, and variation within the ceboid superfamily was

examined. Within ceboids, fat was highest in cebines and lowest in callitrichines. Protein was highest in *Saimiri*, both within Ceboidea and across the anthropoids. Hominoid milks were lowest in fat, protein, dry matter, calcium, phosphorus, percent energy from fat, and total gross energy. Samples from hominoids had over 50% of the energy from sugar, the highest mean of any anthropoid superfamily. There was also a phylogenetic pattern in variation in milk composition. Cercopithecoid and ceboid milks had very large ranges of values for fat and total gross energy within captive living samples and between captive and wild samples. In contrast, milk from hominoids was more tightly constrained and did not appear to respond to environmental variability.

(3) What is the effect of captivity on milk composition? Captivity affected milk composition and the variation in milk composition among ceboids and cercopithecoids. Captivity does not affect the protein composition of milk, although can alter the percent energy provided by protein because of the effect of captivity on percent energy from fat. Samples from captive living monkeys were significantly higher in mean fat, percent energy from fat, and total gross energy. The range of variation in female condition may be more variable among captive living individuals than in the wild. In monkeys, this variation translated into differences in milk energy. In hominoids, it doesn't appear that maternal condition has any effect on milk energy.

(4) Does a species dietary strategy influence milk composition? There is no relationship between a species dietary strategy and proximate milk composition. Folivorous primates do not have higher protein concentrations than other dietary strategies and primates with higher dietary quality (e.g. frugivore-insectivore, frugivore)

do not produce higher energy milks. Higher energy milks were produced by cercopithecoid or ceboid species in positive energy balance (captive), regardless of dietary specialization.

(5) Does milk composition show a relationship to other aspects of a species lactation strategy or overall life history strategy? Variability in milk composition decreases with adult female body mass and duration of lactation. Larger bodied anthropoids are able to store more energy than smaller bodied anthropoids. Having a larger body also permits storage over an extended period of time. A longer duration of lactation would have favored storage over the immediate transfer of dietary energy into milk energy because of the possibility of environmental fluctuations. An extended period of lactation would have relaxed constraints on milk energy, thus favoring the consistent production of low energy milk over an extended period of time with a reliance on depot fat as a source of milk fat. Smaller bodied monkeys can not rely as extensively on storage, but a shorter lactation period may have favored the conversion of excess maternal energy into milk energy.

(6) Does human milk share similarities with anthropoid primate milk? Human milk is relatively low in fat, protein, dry matter, minerals, percent energy from fat, and total gross energy. Human milk composition is also resistant to change, and is quite similar across females with different diets, body fat, and energy expenditure. In these regards, human milk seems to fit well within the hominoid pattern. As a large bodied mammal, human females have disengaged lactation from maternal diet. Energy is stored on the body as a buffer for ecological variability. A relatively larger brain in humans may not be related to human milk composition. However, the small differences in milk fat

(presumably 1% or less) may prove to be biologically significant. Over the duration of lactation, even a small increase in milk fat (and subsequently energy) may provide the necessary energy for the rapidly growing brain. Alternatively, human adult females may diverge from other hominoids females in storage ability or in the ability to transfer significant energy to the fetus, producing the fattest neonate of any mammalian species.

CHAPTER 10: CONCLUSIONS

Introduction

This dissertation presented data on the milk composition of 14 species of anthropoid primates, including fatty acid composition and the concentrations of fat, protein, sugar, dry matter, calcium and phosphorus. The null hypothesis of a conserved milk composition across the suborder Anthroidea was rejected and variation was identified with respect to a species' evolutionary history, diet, body size, duration of lactation, and somatic growth rate. This chapter summarizes results by answering the research questions presented in Chapter one. Next, results are discussed with reference to evolutionary models linking dietary changes and increased relative brain size over the course of human evolution, including the expensive tissue and aquatic diet hypotheses. I conclude this dissertation by outlining directions for future research based on the results of this study.

Summary of Findings

Question 1: Does milk composition follow a phylogenetic pattern? If milk composition is influenced by a species' evolutionary history, then milk should be more similar between closely related species than between distantly related species. Related questions include: (a) Are there any aspects of milk composition that are shared by all anthropoid primates? (b) Are there any aspects of milk composition that are shared by all

cercopithecoids, all ceboids, or all hominoids? (c) Are there any aspects of milk composition that are unique to a particular species?

Milk composition is influenced by a species phylogenetic history. This dissertation identified aspects of milk composition that were shared by all anthropoids, as well as aspects that were unique to particular superfamilies, families, and species. Results on fatty acids and proximate milk composition are summarized separately.

The predominant saturated fatty acid in anthropoid milks was 16:0 (palmitic acid). 16:0 made up approximately 20% of total fatty acids in anthropoid milk. Among wild living anthropoids, no significant variation was identified at the level of the superfamily in the percent composition of 22:6n-3. This finding was surprising in light of the variation in 18:3n-3 and suggested that anthropoid primates may be limited in the amount of 18:3n-3 that can be elongated and desaturated into 22:6n-3. Higher levels of 22:6n-3 were identified only in the milks of individuals with a preformed source of 22:6n-3 (fish meal from Monkey Chow). Milk from monkeys (ceboids and cercopithecoids) was higher in the proportion of the medium chain fatty acids 8:0 and 10:0 than milk from hominoids, where these fatty acids were virtually undetectable. A notable exception were the hylobatid samples ($n = 2$) which were more similar to monkeys than other apes in the composition of both 8:0 and 10:0. Hominoid milks were higher than monkey milks in the percent composition of 16:0. Great ape metabolism may favor the elongation of medium chain fatty acids into longer chain fatty acids and/or monkeys may simply be producing higher levels of 8:0 and 10:0. The milk of wild living mountain gorillas was unique among anthropoids in the percent composition of 20:4n-6. Samples from mountain

gorillas had a mean percent composition of 20:4n-6 more than twice that of captive living hominoids and almost four times that of ceboids or cercopithecoids. Several explanations were offered. Mountain gorillas may be unique among anthropoids in their ability to elongate and desaturate 18:2n-6 or may have a source of 20:4n-6 in their diet that has yet to be identified.

The concentration of lactose appears to be a conserved trait among anthropoids. Although the percent lactose in milk varied within species, mean values were not significantly different when analyzed at the level of the family or superfamily. Anthropoids milks are approximately 7% lactose. Variation in fat, protein, dry matter, total gross energy, calcium, phosphorus, and the percent of energy in milk provided by fat, sugar, and protein were identified at the level of the family and/or superfamily. Milk of cercopithecoids was highest among superfamilies in mean fat, dry matter, total gross energy, and the percent energy provided by fat. Milk of ceboids was intermediate among superfamilies in mean fat, dry matter, total gross energy, and percent energy provided by fat and was highest among superfamilies in protein and the percent energy provided by protein. Hominoid milk was lowest among superfamilies in fat, dry matter, total gross energy, and percent energy from fat and highest in the percent of milk energy provided by sugar.

In addition to differences in mean concentration of individual milk components, superfamilies also differed in regard to the range of variability in the concentration of individual milk components. Milk from monkeys (ceboids and cercopithecoids) was highly variable in fat, total gross energy, and the percent energy provided by fat.

Variation was identified among captive living individuals of the same species and between captive and wild living individuals of the same superfamily. The milk of monkeys was strongly influenced by the environment. In contrast, percent fat and total gross energy were quite conserved among hominoids, both among species and between captive and wild living individuals. Hominoids were derived; their milk showed no relationship to environmental variation.

A fundamental difference between extant monkeys (both New and Old World) and hominoids is the high degree of taxonomic diversity in the former and the lack of taxonomic diversity in the latter. There are currently more family, genera, and species of monkey than of ape, primarily the result of a significant pruning of the ape family tree during the late Miocene (Fleagle, 1999). Differences in taxonomic diversity between monkeys and apes may be reflected in differences in adaptational diversity. Thus, variation in milk composition among monkey species and/or a lack of variation in milk composition among ape species could be at least partly explained by the evolutionary history of each phylogenetic lineage. Milk samples were collected opportunistically and did not evenly sample all lineages of Old and New World monkey. Indeed, samples from Old World monkeys were represented by only one genus, *Macaca*. However, results suggested that milk composition in monkeys was influenced by both phylogeny and ecology. Ape milk, in contrast, appears to be invariable with respect to both factors. Thus, while reduced taxonomic diversity among apes offers a possible explanation for similarity in milk composition among ape species, it can not fully explain the lack of

response to environmental variation that separates hominoid milk from that of other anthropoid primates.

Question 2: Does milk composition vary with respect to ecological factors? Ecological factors investigated in this study include a species' dietary strategy (e.g., folivore, frugivore) and living conditions that affect diet (wild living or captive housed). Species included in this study varied widely in dietary niches in the wild, but many milk samples were provided only by captive living females. Related questions include: (a) How does a species' dietary strategy influence the composition of their milk? (b) Is there a relationship between the quality of a species' diet and the energy available in the milk produced? (c) What is the influence of a captive living diet on milk composition? (d) What is the influence of a captive living lifestyle (e.g. fed *ad libitum*, reduced energy expenditure) on milk composition?

Both diet and captivity (e.g., the effects on activity level and competition) were responsible for variation in milk composition among anthropoid primates. Maternal diet had a strong effect on the fatty acid composition of milk. Captive living primates generally consumed diets higher in total fat than wild living primates and the two groups also differed in the types of fatty acids consumed. Milks from captive living individuals were higher in 18:2n-6 and 22:6n-3 (fatty acids supplied in Monkey Chow) than milks from wild living individuals. Higher values of 22:6n-3 among captive living anthropoids indicated that nonhuman anthropoids could increase the proportion of milk 22:6n-3 through increased maternal consumption of 22:6n-3. Milks from wild living monkeys

were higher in 8:0 and 10:0 than milks from captive living monkeys. This difference may be explained by increased lipogenesis (synthesis of fatty acids by the mammary gland) or the decreased elongation of these fatty acids into 16:0, 18:0 or longer chained fatty acids due to the lower lipid content of the wild diet.

Milk from wild living *Alouatta palliata* (mantled howlers) and *Gorilla beringei* (mountain gorillas) had a significantly higher proportion of 18:3n-3 than the milks of any other anthropoid primate. This difference was argued to be the result of the heavy reliance by both species on leaves, the primary dietary source for 18:3n-3.

The dietary strategy of a species defined in terms of the categories frugivore, gummivore, and so on seemed to have little influence on the proximate composition of milk. The milk of frugivorous hominoids (*Pan paniscus*, *P. troglodytes* and *Pongo pygmaeus*) was more similar in concentration of fat and protein and in total gross energy to other hominoids than it was to the other frugivorous anthropoid in the study, *Macaca sinica*. Within dietary categories variation was best explained by phylogeny (superfamily) and whether the sample was obtained from a captive or wild living individual.

The effect of captivity and phylogeny on milk composition made it difficult to address the relationship between milk energy and dietary quality. Among wild living anthropoids, there was no relationship between the percent of leaves in the diet and milk energy. Species with low quality diets (diets with the highest percent leaves: *A. palliata* and *G. beringei*) produced milks similar in mean total gross energy to frugivore-

insectivores (*Leontopithecus rosalia*) and gummivores (*Callithrix jacchus*) but slightly lower in mean total gross energy than frugivores (*Macaca sinica*).

Captive living monkeys (cercopithecoids and ceboids) produced milks with significantly higher mean fat, mean percent energy from fat, and mean total gross energy than wild living monkeys. Captivity did not affect the protein composition of milk. The effect of captivity on milk fat, and subsequently, milk energy was argued to be related to maternal energy balance. Among monkeys, females in positive energy balance (the result of increased energy intake and decreased energy expenditure) produced higher energy milks. Values from captive living monkeys overlapped with those from wild living monkeys, albeit on the low end of the captive range. The important difference seems to be the ability of captive living females to produce very high fat (and thus high energy) milks. Among hominoids, there was no effect of captivity on milk composition - maternal condition appears to be disengaged from milk energy.

Question 3: Does milk composition vary with respect to life history traits? Species included in this study varied widely with respect to adult female body size, neonatal body size, adult brain size, neonatal brain size, age at first reproduction, and duration of lactation. Related questions include: (a) Is there a relationship between the duration of a species' lactation and the composition of the milk produced, such that species with similar lactation periods produce more similar milks? (b) Is there a relationship between maternal body mass and the energy of the milk produced? (c) Does milk from primates that grow at a relatively faster rate differ in regard to nutrients that are related to growth?

(d) Do primates with relatively larger brains than predicted for their body size produce milks of higher energy and/or with higher concentrations of brain specific nutrients?

This dissertation identified variation in anthropoid milk fatty acid and proximate composition with respect to life history traits. Milk composition was influenced by the rate of somatic growth, duration of lactation, and adult female body mass. Milk composition did not appear to be related to relative brain size (EQ). However, the influence of captivity on milk composition may mask possible relationships between the concentration of milk fat or milk sugar and EQ. For example, hominoid milks were higher in percent energy provided by sugar than monkey milks. One possibility is that hominoids are derived relative to monkeys in use of glucose as fuel for growth and development. However, wild living monkeys were similar to hominoids in percent energy from sugar, suggesting that anthropoids that produce low energy (due to low fat) milks will produce milks with a larger proportion of energy from sugar.

An important phylogenetic pattern identified in anthropoid fatty acid profiles was the high proportion of 8:0 and 10:0 in the milk of monkeys and the almost undetectable levels of these fatty acids in the milk of the great apes (*Gorilla*, *Pan*, and *Pongo*). These fatty acids are believed to be used as energy substrates and may be found in higher proportions among anthropoid species with faster somatic growth rates. Iverson and Oftedal (1995) propose that the synthesis of these fatty acids is related to the activity of a particular enzyme in the mammary gland. The activity of this enzyme may be derived among great ape with the primitive anthropoid trait being a higher synthesis of 8:0 and 10:0. The high proportion of these fatty acids in the milks of hylobatids is particularly

intriguing in regard to their function in milk. Relative to monkeys, hylobatids have a slower rate of somatic growth but are similar in adult female body size. Confirmation of hylobatid milk as being monkey like or ape like in regard to medium chain fatty acid composition would be helpful in determining the function of these fatty acids in milk.

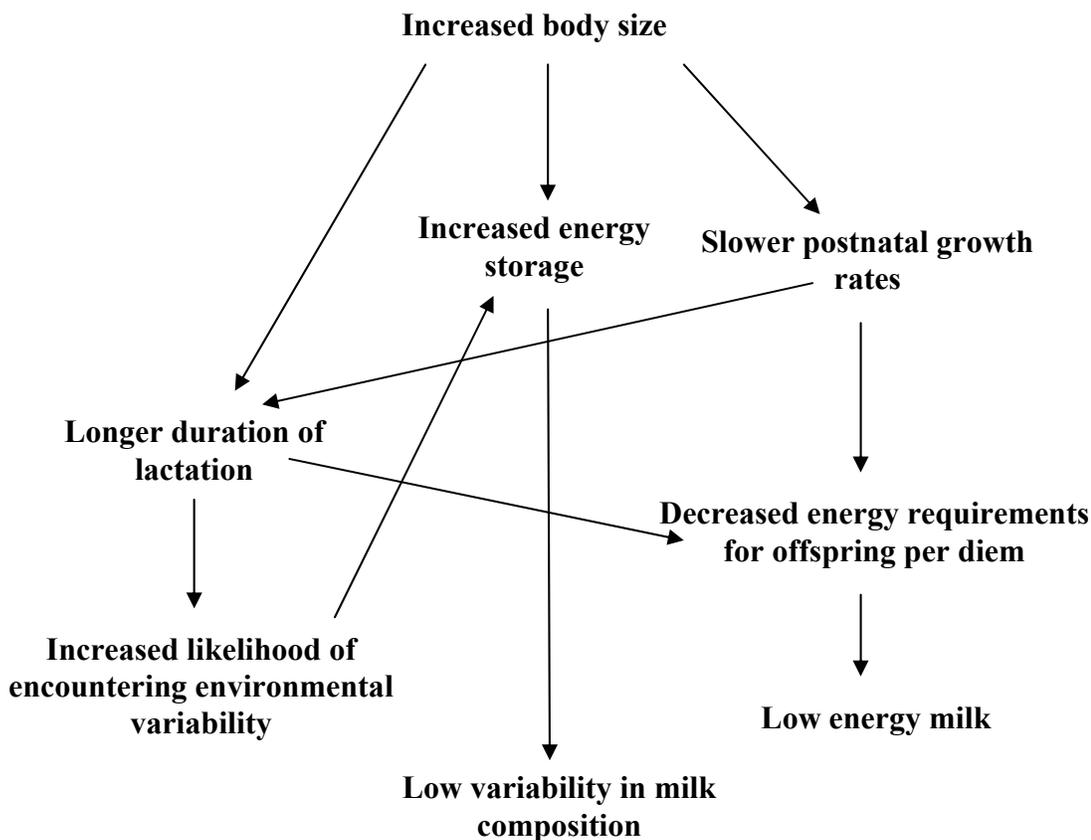
A relationship was identified between energy from protein and rate of growth. The proportion of energy from protein was highest among ceboids, the superfamily with the most rapid postnatal growth rates. Within ceboids, percent energy from protein also followed growth rates: *Leontopithecus* and *Callithrix*, *Alouatta*, and *Saimiri* and *Cebus*. Milk from cercopithecoids was just slightly lower in percent energy than that of hominoids, but this difference was best explained by the high fat in *M. mulatta* samples. Samples higher in fat are higher in percent energy from fat, which in turn reduced the percent energy from protein. If the concentration of protein is considered, the highest protein values are found among the ceboids, followed by cercopithecoids, followed by hominoids.

The relationship between duration of lactation and milk composition can not be discussed separately from the relationship between body size and milk composition. The effects of larger body size and increased duration of lactation on milk composition were summarized in Figure 10.1. Among mammals, larger body size is associated with a decrease in the postnatal growth rate and an increase in the duration of lactation. Increased body size also was associated with increased storage capacity, both in terms of absolute energy that can be stored on the body and the amount of time that energy can be stored on the body. A slower postnatal growth rate would relax selective constraints on

the amount of energy transferred via milk per day. A lengthening of the lactation period was accompanied by an increased risk from environmental variability. With no immediate advantage to producing high energy milks, selection would favor storage as a buffer against possible fluctuations in the environment. Energy would be stored by the mother rather than transferred directly to the milk, resulting in little variation in milk fat, and subsequently milk energy, among hominoids.

Results on LCPUFA composition indicated that individuals with a dietary source of 22:6n-3 produced milks with a higher percent composition of 22:6n-3. This “noise” from captive diets made it difficult to examine the relationship between relative brain size and 22:6n-3 composition among anthropoid primates. However, the percent composition of 22:6n-3 was not significantly different at the level of the superfamily among wild living anthropoids or among captive living anthropoids, despite differences in relative brain size among species within each of these groups. The highest EQ values of the study sample belong to *Cebus* and *Saimiri* but both species were fed diets with a preformed source of 22:6n-3. That the proportion of 22:6n-3 in their milks is similar to that of the relatively smaller brained rhesus macaque suggests that levels of this fatty acid in milk were reflective of dietary supply rather than relative brain size.

Figure 10.1. A schematic representation of the relationship between increased body size and the consistent production of low energy milks. Direction of arrows indicates causal relationship.



The effects of captivity also made it difficult to assess the relationship between milk energy and relative brain size; samples from captive living monkeys were significantly higher in total gross energy than those from wild living monkeys. The species with the largest relative brain sizes in the sample, *Cebus* and *Saimiri*, produced milks with higher energy than the other captive living ceboid species, *Callithrix* and *Leontopithecus*. Results from *Saimiri* were particularly interesting, as they were just slightly larger in adult body size than the callitrichine species. However, without samples

from wild living cebines or data on maternal body composition from the females who provided the milk samples from this study, it is impossible to say whether higher energy milks in this subfamily are linked to larger relative brain size among cebines, captivity, or both.

Question 4: Is the composition of human milk species-specific? Martin (1981, 1983)

hypothesizes that encephalization in genus *Homo* must have been accompanied by

selection for milk constituents that could support rapid brain growth in the neonate.

Human milk composition is argued to be species-specific due to the unique ontogenetic priorities of human neonates compared to nonhuman primates.

Results of this dissertation did not support Martin's hypothesis for a unique human milk composition. Rather, human milk composition shared many features with anthropoid milks in general and hominoid milks in particular. Like anthropoid milk, the predominant saturated fatty acid in human milk is 16:0. Reported values for percent composition of 18:3n-3, 18:2n-6, 22:6n-3 and 20:4n-6 in human milk overlapped with or were identical to those identified in wild- and captive living anthropoids. Indeed, the range of values identified for 22:6n-3 among anthropoids was the same as that reported by Yuhas et al. (2006) for nine human populations. Among humans, increased consumption of foods high in 18:3n-3 by lactating females did not result in a substantially higher proportion of milk 22:6n-3. It appeared that a relatively inefficient pathway from 18:3n-3 to 22:6n-3 was a vestige of our evolutionary history. Species with a high

proportion of 18:3n-3 in their diet (mantled howlers and mountain gorillas) had high milk 18:3n-3 values, but not high milk 22:6n-3 values.

Proximate milk composition of humans fit well within the description of the overall hominoid pattern. Like milk from nonhuman hominoids, human milk was lower in fat, protein and total gross energy than milks from cercopithecoids or ceboids. Although the concentration of fat in human milk has been described as variable, the concentration of fat in human milk actually appears quite conserved in comparison to other primate taxa. Humans, like other apes, appear to be able to disengage lactation from maternal diet as a result of larger body size because of much greater capacities for somatic energy storage. A longer duration of lactation (slower postnatal growth rates) favors the consistent production of low energy milk over the transfer of excess maternal energy directly to milk.

Diet, Brain size, and Milk Composition

The common goal will be to identify what is so special about the size of the human brain (including its ontogenetic development to reach that size) and to extract clues which indicate possible ecological factors that have promoted the emergence of a particularly large brain during human evolution...How, in energetic terms, human beings can support such an exceptionally large brain and how, in the course of human evolution, energy was made progressively available to meet the needs of an ever-increasing brain size? (Martin, 1983: 5).

Results from this study suggest that humans produce milk similar to other hominoids in mean values of milk components and the range of possible values (variability) for individual milk components. A relatively larger brain in humans may not be related to human milk composition in any direct or immediate way. In this section, I

discuss the implications of these results to hypotheses about the relationship between brain size, body size, and maternal diet over the course of human evolution.

Reconstructing hominin diets

Beginning approximately 1.9 mya, members of the tribe Hominini (following Wood and Richmond, 2000) underwent dramatic changes in body size and absolute brain size. Relative to their bipedal ancestors, early Pleistocene hominins were taller in stature, had humero-femoral ratios more similar to modern humans, and had larger brains than would be predicted for a primate of their body size (McHenry and Coffing, 2000; Wood and Richmond, 2000). These changes mark the emergence of the genus *Homo* (Wood and Collard, 1999), a transition associated with both dramatic morphological changes and a profound change in hominin behavioral ecology.

The archaeological record suggests that an increase in animal tissues in the diet coincided with the transition to *Homo*, most notably increased consumption of animal protein and fat (Bunn, 2001; Foley, 2001; Milton, 1999; Shipman and Walker, 1989). Meat is considered a high quality food because of its high nutritional returns, including an ample supply of all essential amino acids, and low digestive costs, but also was a patchy (in time and space) and unpredictable resource on the landscape (Foley, 2001). The diet of early *Homo* was not simply an australopithecine diet with more meat, however. It likely included changes in the both animal and vegetable components (Leonard and Robertson, 1992). The shift is best modeled as an improvement in dietary quality (defined in this dissertation as nutrient content and ease of digestibility) with

Homo obtaining more calories from reproductive plant parts and animal material (Leonard and Robertson, 1992, 1994) and more accurately characterized as enriched omnivory rather than simply increased carnivory.

A key difference between modern human foragers and extant nonhuman primates is the higher percentage of meat in the diet of the former (between 20 – 50%), and the means by which such meat is acquired (Bunn, 2001; Foley, 2001; Foley and Lee, 1991; Wrangham et al., 1999). Stiner (1993: 4) describes human predatory behavior as “nearly unique”. Relative to nonhuman primates that exhibit predatory behavior (e.g., chimpanzees, baboons), modern humans are more efficient in obtaining and processing game, and share a suite of behaviors that tie them to the order Carnivora, including transport of meat over long distances, caching food, and systematically processing bones for the enclosed soft tissue (Stiner, 2002). Determining where to place fossil hominin species along this spectrum between nonhuman primates and modern humans is a central focus of archaeology and paleoanthropology. Chimpanzee predation is of particular relevance as it is often modeled as the ancestral condition and likely typical of the earliest hominins (Schoeninger et al., 2001; Stanford, 2001). A shift from this ancestral pattern took place more than 2 mya, characterized by an increase in faunal remains that indicate hominin manipulation (most likely *Homo* sp.), particularly an association with hominins that possessed stone tool technology (Blumenschine et al., 1994; Brantingham, 1998; Isaac, 1978; Potts, 1988; Potts and Shipman, 1981; Stiner, 2002). Scavenging was a possible method for accessing animal material, with individuals obtaining meat from carcasses at natural death sites, or possibly through direct competition with other

predators (Bunn, 1981; Dominguez-Rodrigo, 2003; Potts, 1988; Rose and Marshall, 1996).

Of particular importance to a discussion of energetics is the question of which parts of animal carcasses hominins would have been able to access (Blumenshine et al., 1994; Bunn, 1981, 2001; Dominguez-Rodrigo and Pickering, 2003; Potts 1988).

Brantingham (1998) argues on the basis of prey body part profiles that Bed I Olduvai and FxJj 50 Koobi Fora demonstrate a hominin pattern of food transport intermediate between top predators and confrontational scavengers, creating a unique niche characterized by access to marrow of the larger limb bones, an energy rich food source. Hominin investment would have included an emphasis on early access to freshly abandoned kills, quick removal of limb elements, and subsequent transport to areas of reduced competition and predation (Brantingham, 1998; Rose and Marshall, 1996).

Models linking brain size and dietary quality

A shift to higher quality resources would be an expected correlate of larger brains and, indeed, a precondition (Foley, 2001: 313).

Increases in dietary quality are intimately tied to morphology and physiology. Evolutionary models for a possible mechanism, or “prime releaser” (Robson, 2004) for size increases argue either directly (Broadhurst et al., 1998, 2002; Crawford et al., 1999; Cunnane, 2005; Leonard and Robertson, 1992, 1994; Leonard et al., 2003; Vasey and Walker, 2001) or indirectly (Aiello and Wheeler, 1995; Aiello et al., 2001; Foley and Lee, 1991; Kennedy, 2005; Martin, 1983, 1996) for the critical role of increased dietary quality in supporting the higher energetic demands of a larger brain and body of *Homo*

relative to australopithecines. Foley and Lee (1991) estimate that modern human infants require 10% more energy than do chimpanzee infants as a direct result of increased brain size. Hominins with brain sizes exceeding extant chimpanzees at all life stages would therefore be predicted to have increased energy requirements (Foley, 2001).

Models address how relative brain size could increase without a corresponding increase in basal metabolic rate (BMR), a measure of energy expenditure. Arguments for an indirect and direct link between dietary quality and encephalization are briefly summarized. These models are not mutually exclusive from one another, nor are they mutually exclusive from models that emphasize fat storage by females. All offer an adaptive strategy to explain hominin energetics, and may have been operating in concert or in different environmental or temporal contexts over the course of human evolution.

Aiello and Wheeler's (1995) expensive tissue hypothesis proposes that enriched omnivory in hominins would have allowed for coevolution between brain and gut size, with a reduction in the size of the gut and a concomitant increase in brain size. Gut size is directly related to dietary quality, with lower quality diets, such as folivory, requiring more fermentation, and therefore longer guts (Aiello and Wheeler, 1995; Milton, 1987, 1999). Further, guts, like brains, are metabolically expensive tissues (Aiello and Wheeler, 1995). Metabolic requirements of increased brain size could be offset by the diminished requirements of a smaller gut.

Data from nonhuman primates and other mammalian orders offer support for their hypothesis by indicating a strong correlation between dietary quality and the relative sizes of the gut and the brain (Aiello and Wheeler, 1995; Milton, 1987, 1999). In a more

extensive survey of nonhuman primates (33 species), Fish and Lockwood (2003) identified a significant positive correlation between brain size and dietary quality, a proxy for gut size. Their results also suggest a relationship between evolutionary changes in diet quality and brain mass; evolutionary trajectories associated with increases in dietary quality also are associated with increases in relative brain size (Fish and Lockwood, 2003).

Further support for Aiello and Wheeler's model comes from the fossil record and a comparison of A.L. 288-1 (*Australopithecus afarensis*) with WT 15000 (*Homo erectus/ergaster*). *A. afarensis* shows similar thoracic morphology to extant nonhuman apes with an inverted funnel shape, believed to be related to a longer gastrointestinal tract, while *H. erectus/ergaster* has a more "modern," barrel-shaped rib cage (Aiello and Wheeler, 1995; Aiello and Wells, 2002). Such a change in rib cage anatomy is accompanied by a marked increase in cranial capacity from *Australopithecus*/early *Homo* to *Homo erectus/ergaster* approximately 1.8 mya (Aiello and Wheeler, 1995; Aiello and Wells, 2002; Wood and Richmond, 2000).

Aiello and Wheeler's (1995) expensive tissue hypothesis also suggests a possible feedback loop in regard to increased dietary quality and encephalization, wherein higher cognitive abilities (assumed to be associated with increased EQ values) would allow adult individuals to increase their foraging returns and increase dietary quality. This, in turn, would select for higher cognitive abilities for resource acquisition (Aiello and Wheeler, 1995).

Leonard and Robertson (1992, 1994) propose a more direct link between diet and brain size. They suggest that increased dietary quality in humans is an adaptation to fuel the high metabolic needs of a large brain. They examine energy allocated to brain metabolism in 31 primates, including humans, and found that it was positively and significantly correlated with dietary quality. In the regression model explaining the relationship between these two variables, modern humans are at the positive extreme, as they have a relatively larger brain and a relatively higher quality diet than predicted for a primate of our size.

Using data on extant humans and nonhuman primates, Leonard and Robertson develop a series of bioenergetics models to extrapolate metabolic requirements of fossil hominins. Implicit in their models is the accepted scaling relationship between resting metabolic rate (RMR) and body mass (WT), $RMR \propto WT^{0.75}$ (Kleiber, 1961). The log of both RMR and body weight for 20 anthropoid species are used to create a regression equation [$\log(RMR) = 1.839 + 0.778 \log(WT)$ (Equation 1, Leonard and Robertson, 1992)] which shows no significant deviation from the Kleiber regression. These equations illustrate that although smaller primates have lower energy needs than larger primates, their energy demands per unit mass are higher (Leonard et al., 2003). Therefore, the constraint in getting larger is the ability to obtain enough total energy. Larger primates such as *Gorilla* species solve this problem by increasing the volume of low quality food consumed, while humans, and presumably *Homo*, increased the quality of the diet, requiring less volume for the necessary energy and nutrients (Leonard et al., 2003).

Equations also are developed for RMR, brain size, and total energy expenditure (a function of dietary quality and size of day range). Application of all equations to fossil hominin species suggests a significant increase in resting energy needs in *Homo* relative to australopithecines. Specifically, *Homo erectus/ergaster* (ca. 1.6 Ma) would have resting energy requirements 35% greater than *Australopithecus africanus* (ca. 2.5 Ma) (1450 kcal/day vs. 1150 kcal/day) and total energy requirements would have increased approximately 800 kcal/day over gracile australopithecines (Leonard and Robertson, 1992). As a result of a larger relative brain size, Aiello and Wells (2002) estimate that the cost of reproduction in *Homo erectus* females would have increased by 40% from their australopithecine ancestors.

Homo was able to increase the rate of energy return through changes in foraging strategy, including increased foraging territory, consumption of food with higher caloric return (particularly animal muscle and fat), and increased efficiency in obtaining food items (Leonard and Robertson, 1992, 1994; Shipman and Walker, 1989). In this way, dietary adaptations in human evolution were guided by the energetic costs of larger brains (Leonard and Robertson 1992, 1994; Leonard et al., 2003). Meat, a high quality food, was used to fuel costs of growth and maintenance of a larger brain throughout the life cycle (Foley, 2001).

Vasey and Walker (2001) focus on how high quality foods, like meat, would be particularly important in the maternal diet during pregnancy and lactation. Prenatal growth rates are similar among humans and other anthropoids, suggesting that the emergence of the human pattern of postnatal brain growth (ca. 1.8 mya) would have

required hominin mothers to consume foods of higher nutritional value. Specifically, Vasey and Walker (2001) argue that during the evolution of the genus *Homo*, animal protein and fat would have provided mothers with the extra energy required to produce a neonate with a larger body size and relative brain size than *Pan*. Additional energy would also be required during lactation; Vasey and Walker (2001) predict a shift in lactation, possibly through compositional differences, coincident with the shift in trophic level.

Broadhurst et al. (1998, 2002) and Crawford (1992; Crawford et al., 1999) also propose that expansion in cranial capacity during human evolution necessitated increased dietary quality. However, unlike the previous models, they argue a completely different scenario for hominin dietary strategies. Rather than looking to terrestrial foods to explain changes in dietary quality, the aquatic diet hypothesis (also called the shore based scenario), proposes that growth and maintenance of a larger brain required a diet based on marine and lacustrine fish and shellfish because, unlike terrestrial plants and animals, these foods are high in the brain specific nutrients 22:6n-3, iodine, zinc, copper, iron, and selenium (Cunnane, 2005).

Of these six nutrients, 22:6n-3 (DHA) has received the most attention in the anthropological literature (Cunnane, 2005). In a criticism of the aquatic diet hypothesis, Langdon (2006) presented arguments based solely on the availability of 22:6n-3 in the terrestrial food supply and human milk. In support of the aquatic diet, Broadhurst et al. (1998: 3) boldly state “an abundant, balanced dietary intake of long chain polyunsaturated fatty acids is *an absolute requirement* for sustaining the very rapid expansion of the hominin cerebral cortex during the last one to two million years” (emphasis mine). As a

result, and because of the link between maternal dietary fatty acids and fatty acid profiles in milk, my discussion focuses on arguments for the importance of 22:6n-3 related to aquatic diet hypotheses. It must be emphasized, however, that the aquatic diet hypothesis argues that all six nutrients were important for encephalization. Therefore, any arguments for or against the essential role of 22:6n-3 in the diet must also account for the remaining five nutrients (Milligan and Bazinet, 2007).

As discussed previously (Chapter 4 and 8), 22:6n-3 has an essential role in brain growth, development, and maintenance. Indeed, 22:6n-3 has been identified as the only n-3 fatty acid of functional significance in the brain (Cunnane, 2005; Martinez, 2002). However, humans (like all other mammals) are unable to synthesize 22:6n-3 *de novo* and the elongation and desaturation of 18:3n-3 to 22:6n-3 is relatively inefficient. If 22:6n-3 is so critical for brain growth and development, why didn't natural selection operate on increased efficiency in conversion from 18:3n-3 over the course of human evolution? Proponents of the aquatic diet hypothesis argue that there was no selective pressure for elongation and desaturation of 18:3n-3 because hominins had access to a preformed source of 22:6n-3 (e.g., fresh and saltwater species of fish, shellfish and crustaceans). Although some animal tissues contain 22:6n-3, particularly brain and bone marrow, Broadhurst et al. (1998) and Cunnane (2005) argue that these sources would not have served as a consistent source of 22:6n-3 because they would be perishable and unevenly and unpredictably distributed on the landscape. Fish and shellfish, in contrast, are argued to have a more predictable distribution (Broadhurst et al., 1998; Cunnane, 2005). The aquatic diet hypothesis does not discount that hominin access to terrestrial animal and

plant sources. These food sources are argued to be an important aspect of the diet *in addition* to shoreline foods.

Model predictions and nonhuman primate milk composition

The evolution of larger brains among homini[n]s would be expected to correlate with changes in life history, and there would thus be a series of adaptive/biological changes that would occur in association with the proposed expansion of the diet to include more meat (Foley, 2001: 313).

The expensive tissue hypothesis argues for a reallocation of energy as a result of the dietary shift to enriched omnivory. Although no specific predictions about changes in milk composition over the course of human evolution are proposed, a reallocation hypothesis does not require a modified composition of modern human milk for validity. Indeed, similarity in human and nonhuman hominoid milk could be used to support the expensive tissue hypothesis. If human milk composition is not species specific but is simply that of a large bodied hominoid, energy for brain growth and development must be provided from another source, e.g., as a trade-off for gut volume. The limitation of this model is that it is unable to explain if *all* the energy required by a relatively larger brain size can be provided by this trade-off. Although a decrease in the size (volume) of the gut may free up energy that can be directed for brain growth and development, a relatively larger brain may require energy from additional sources, including energy from the diet, past (adipose tissue stores) or present, or changes in the lactation strategy. Thus, Leonard and Robertson's (1992, 1994; Leonard et al., 2003) and Vasey and Walker's (2001) arguments for increased dietary quality do not necessarily oppose the expensive tissue hypothesis and could be operating in concert, as infants would not begin consuming meat

or marrow before most brain growth would have been achieved. One of these additional sources may be increased energy stores for female hominins as a direct result of increased consumption of terrestrial animal protein and fat. Stored energy may not translate to increased protein or fat in the milk, as evidenced by modern human milk composition (Jensen et al., 1995) but may be critical in milk production, stabilizing or even increasing milk production.

A direct link between increased meat in the diet and milk composition is tenuous. Animal based fatty acids, particularly 20:4n-6, would be predicted to increase in concentration and thus, relative proportion, in milk as more animal tissues were incorporated in the diet. However, as discussed in Chapter 8, increased milk 20:4n-6 does not influence tissue concentration (including the brain) of 20:4n-6. Formula fed human and baboon infants with no source of 20:4n-6 had similar brain concentrations to those receiving 20:4n-6 through breast milk (Farquharson et al., 1992; Makrides et al., 1994; Sarkadi-Nagy et al. 2003, 2004). As argued earlier, this evidence suggests that a compensatory mechanism for 20:4n-6 synthesis in brain tissues may be part of our anthropoid, or at least Old World anthropoid, legacy. Thus, increased dietary 20:4n-6 is an unlikely prime releaser for encephalization in human evolution.

Unlike 20:4n-6, there is a strong correlation between consumption of foods rich in 22:6n-3, levels of milk 22:6n-3, and infant tissue concentrations of 22:6n-3 in humans and nonhuman primates. Brain and marrow are terrestrial sources of this fatty acid. Although Broadhurst et al. (1998) and Cunnane (2005) argue that these sources are perishable and unpredictable, storage of this fatty acid in female adipose stores suggests

that consumption by females at any point during the reproductive years, or possibly earlier, could increase the proportion of 22:6n-3 in milk.

A more consistent, albeit small, source of 22:6n-3 in a terrestrial diet would result from elongation and desaturation of 18:3n-3, found in a wide variety of foods available to hominins (Carlson and Kingston, 2007). Proponents of the aquatic diet hypothesis argue that even a modest intake of fish, shellfish, and other foods of aquatic origin (6 – 12% of total dietary energy) provides significantly more 22:6n-3 than a diet of only terrestrial plants and animals. More important than the quantity of aquatic foods consumed is the predictability of acquiring these foods in space and time.

Unlike Martin's (1983) predictions regarding human milk composition, the aquatic diet hypothesis does not predict that milk of extant humans will diverge from that of nonhuman primates. Higher levels of milk 22:6n-3 are predicted in any population that consumes foods with preformed sources of 22:6n-3. Evidence from captive living nonhuman primates and human populations with a high consumption of marine based foods supports this prediction. Rather, the aquatic diet hypothesis requires that encephalization (as well as optimal growth, development, and maintenance of a relatively larger brain) would necessitate an increase in milk 22:6n-3, made possible by a consistent, predictable, and generous supply of 22:6n-3.

Carlson and Kingston (2007) argue that although the biosynthesis of 18:3n-3 to 22:6n-3 is *inefficient*, it is *sufficient* for normal brain growth, development, and maintenance in modern humans (Carlson and Kingston's emphasis). Children who receive milk without a source of 22:6n-3 (e.g., infants fed formula that was not

supplemented with 22:6n-3) or children consuming breast milk from vegan mothers have to rely on conversion from 18:3n-3 (either by the mother via breast milk or *in vivo*), and Carlson and Kingston (2007) review a number of compensatory mechanisms that ensure a sufficient supply of 22:6n-3 in maternal depot stores. As outlined in Chapter 2, natural selection acts in such a way as to minimize energy expenditure. Energy available to each organism is finite and energy allocated to one function will ultimately limit the energy available for other functions (Charnov, 1993). Keeping this in mind, it seems that natural selection would favor reliance on a preformed source of 22:6n-3 rather than reliance on conversion of 18:3n-3 to 22:6n-3, particularly if the primary function of 18:3n-3 is to supply energy to cells via beta-oxidation (Brenna, 2002). Thus, there may be a slight energetic advantage to a diet with consistent access to preformed sources of 22:6n-3, either terrestrial *or* aquatic. However, slight energetic advantages become trivial if, in fact, 22:6n-3 levels above those achieved by elongation and desaturation of 18:3n-3 are “an absolute requirement” (Broadhurst et al., 1998) for encephalization and optimal brain growth, development, and maintenance.

Models that link expansion of cranial capacity and dietary changes either directly, such as the aquatic diet hypothesis, or indirectly, such as the expensive tissue hypothesis, were utilized as points of departure for research questions about the relationship between milk composition, maternal diet, and brain size. Results of this dissertation are consistent with both direct and indirect links, suggesting that the role of lactation in human brain ontogeny is much more complex than hypothesized by Martin (1983). There remain

many questions to address concerning the evolutionary history of human milk composition and the human lactation strategy.

Directions for Future Research

This study was the first to investigate variation in milk composition among anthropoid primates in relation to life history traits, ecological factors, and a species' evolutionary history. Research questions posed in Chapter One were intentionally broad as little was known about nonhuman primate milk composition. Results of this study indicate several patterns that were not included in *a priori* hypotheses. These include the intimate relationship between maternal condition and milk energy among monkeys and the lack of such a relationship among apes, including humans. In addition, data collected in this study do not support the prediction that human milk is species specific but such a statement could not be stated conclusively because not all aspects of milk composition were investigated. The final section of the dissertation summarizes research programs that address each of these issues.

Maternal condition and milk energy

Results from this project indicate that captive living monkeys produce milks that vary widely in concentration of fat and total gross energy. It was assumed that this variability was the result of variability in maternal condition, particularly maternal energy

balance. Poor maternal condition has demonstrable effects on milk fat concentration among several nonhuman primate species. Smaller captive living common marmoset females with twins produced milks lower in fat and energy than larger mothers of twins (Tardif et al., 2001) and free ranging rhesus macaque mothers infected with the parasite *B. coli* produced milks lower in fat in energy than uninfected mothers (Hinde, 2007).

On the other side of the energy balance continuum, it appears that extremely good maternal condition is associated with the production of high energy milks among monkeys. This hypothesis could be tested with complementary data on maternal body composition (percent body fat and percent muscle mass) and milk composition. In a captive setting, the range of variation in maternal condition could be controlled, with many subjects consuming diets similar in fat content to wild living conspecifics and others consuming a higher fat diet. Other variables, such as physical activity (energy expenditure), could also be manipulated to determine which aspect(s) of captivity exert the strongest influence on milk energy.

To argue that the production of high energy milks under good conditions is adaptive requires (1) determining the primary function of milk energy for developing monkey offspring and (2) demonstrating that this function has an effect on the fitness of the mother and/or offspring. Infant ontogeny could be tracked to determine if infants receiving higher energy milks differ with respect to rate or pattern of growth and development. Additionally, it could be possible to assess the heritability of production of high energy milks. Milk from siblings and mother/daughter pairs could be compared to determine the influence of ecological and genetic factors on milk energy. Do mothers

who produce high energy milks have female siblings who also produce high energy milks? Do daughters of mothers who produced high energy milks also produce high energy milks? Do sons of mothers who produced high energy milks have greater reproductive success? Do they grow faster? Implicit in the argument for high energy milks as an adaptation is that plasticity in milk composition is also an adaptation. What is the benefit to a monkey mother with the ability to modify milk energy density in response to environmental conditions?

A complementary study would be to assess maternal condition and milk energy in a hominoid species. Patterns identified among the small hominoid sample in this dissertation require confirmation. Is milk energy relatively conserved, despite variation in the fat content of the diet and/or physical activity of the mother? Is milk energy consistent across lactation, even when maternal condition fluctuates?

Energy storage among hominoids

Hominoid milk composition was significantly less variable in fat and total gross energy than milk from monkeys. Like other large mammals, hominoids (including humans) appear to be able to decouple lactation from maternal diet through energy storage. In chapter 9, I argued that humans may be unique among hominoids in regards to fat storage. Although Zihlman and McFarland (2000) report that captive lowland gorillas are similar in percent body fat to human females, the true test of this hypothesis will come from body composition of hominoids living in the wild, or in captive conditions that approximate the wild. Are wild living apes actually storing energy on a scale

comparable to humans? If yes, how much are they able to store, and for how long? Are nonhuman apes with low BMIs able to maintain the level of body fat seen in human females with low BMIs? How much do they rely on body stores for successful lactation?

Ellison (2003) discusses modern human energetics in regard to three factors: (1) energy status (the amount of stored energy), (2) energy balance (net residual of energy intake minus energy expenditure), and (3) energy flux (absolute level of energy turnover independent of energy balance). These three dimensions of energy are likely to be equally important and interdependent in the process of selection, as physiological or behavioral changes that affect the amount of stored energy will also alter energy balance, and energy turnover can influence the amount of energy the body is able to store. While proposed in regard to reproductive energetics, Ellison's (2003) model is a useful framework for future research on maternal condition among nonhuman primates.

Figure 10.1 summarized the proposed relationship between increased body size, increased duration of lactation, and milk composition for hominoid species, including humans. However, not all hominoids are larger in body size relative to monkeys. Hylobatid species, particularly gibbons (genus *Hylobates*), are similar in adult female body mass to many of the monkey species included in this study (e.g. *Alouatta palliata*, *Macaca mulatta*) but lactate for a considerably longer period of time. Does a longer period of lactation select for low energy milks in these smaller bodied apes? Does hylobatid milk resemble the monkey pattern of high variability or the ape pattern of conserved composition?

The milk composition of hylobatids is important for testing causal relationships hypothesized in Figure 10.1. The two hylobatid samples grouped away from wild and captive living hominoid samples in plots of principle components on percent composition of fatty acids and were similar to monkeys in high proportions of medium chain fatty acids (8:0 and 10:0). However, in regard to proximate composition, the hylobatid samples were the lowest of all species in mean fat and protein, and subsequently total gross energy. If hylobatids responded to captivity in the same way as monkey species, higher energy milks are predicted. With only two samples, it is not possible to assess whether these samples are representative of the milk of hylobatids and therefore conclude they share the lactation strategy of the great apes. A comprehensive test of the causal relationships proposed in Figure 10.1 would necessitate data on milk composition from both wild and captive living hylobatids. Are milk samples from captive living individuals higher in fat and total gross energy than those from their wild counterparts? Is the variance in fat values similar between captive and wild groups? Congruence with the ape pattern would suggest that, among anthropoids, duration of lactation has a stronger influence on milk composition than body size.

Evolutionary perspective on Immune factors in human milk

Martin's argument for a unique composition for human milk focused on components related to infant nutrition. He predicted that human milk would have either the same nutrients but in higher quantities, or different nutrients that were specific to the ontogenetic priorities of human neonates. Results from this dissertation suggest that, in

regard to nutritional components, human milk composition is not species-specific.

However, human milk may be derived from nonhuman primates in the concentration of non-nutritional components, specifically immune factors.

The transition to agriculture would have presented human infants with a novel pathogen environment. Agriculture is a recent phenomenon on the human evolutionary time scale, with origins in the Levant approximately 10,000 years ago, and only a 5000 year history for the majority of the world's populations (Armelagos and Barnes, 1999; Bar-Yosef, 1980). The first epidemiological transition refers to the increase in the incidence and prevalence of infectious diseases that accompanied the shift from foraging to agriculture (Barrett et al., 1998). The increase in infectious diseases can be related to several cultural factors of an agricultural subsistence mode, including sedentary living, increased contact with animals, increased population size and density and reduced dietary variety (Barrett et al., 1998; Larsen, 2002). Permanent settlements provided larger aggregates of potential human hosts for disease as well as an increased frequency of interpersonal contact, both of which promote the development of more virulent pathogens (Baker, 1982; Barrett et al., 1998; Ewald, 2002). Pathogens also increased in absolute numbers, which served to increase both the incidence and prevalence of infectious diseases. Immune defenses are energetically expensive, making the immune system sensitive to nutritional insult and malnourished populations more susceptible to infectious agents (Hoffman-Goetz, 1986; McDade, 2003).

The cultural changes associated with agriculture, including increased population density and a more sedentary lifestyle, promoted an increase in infectious diseases,

thereby creating a novel ecological setting for human populations. Increases in number of pathogens and pathogen virulence would have placed strong selective pressure on the human immune system. The most vulnerable members of the population would be those with immature immune systems, such as infants and children, or compromised immune systems, such as pregnant women and the very old (McDade and Worthman, 1999; Ortner, 2001). However, natural selection decreases in strength with age, particularly in post-reproductive individuals (Rose, 1991). Therefore, selection may have been strongest on genetic components associated with the development of the immune system.

If the changes in population density and pathogen load that occurred at the transition from hunting and gathering to agriculture exerted enough selective pressure on the content of human milk, it may be possible that passive immunity in humans – the transfer of immune factors from mother to infant - is species-specific among mammals. This hypothesis was tested by comparing sIgA levels in the milk of rhesus macaques to those in human milk (Milligan, 2005; Figure 3.1). Rhesus macaque milk was significantly lower in sIgA concentration at all stages of lactation, but due to methodological issues, these results were treated as preliminary until additional nonhuman primate milk samples could be analyzed.

Several milk samples from this study were large enough to allow for the storage of residuals after fatty acid and proximate analyses. These samples were stored with the specific intent of determining sIgA concentration, and sample volume permitting, lactoferrin concentration to test the hypothesis that human milk composition is unique in having a high concentration of immune factors.

Martin argued that a unique evolutionary event was responsible for modifying human milk composition. In his model, a shift in brain ontogeny approximately 1.8 mya selected for specific nutrients, including LCPUFA, to either increase in concentration in milk relative to other mammals or to be unique to human breast milk. A comparative study of immune factors in human milk may illuminate a more recent novel event in human evolution as the impetus for modification of human milk composition.

APPENDIX A: METHODS OF MILK COLLECTION

A. Captive populations included in study

Callithrix jacchus: Female common marmosets ($n = 4$) were part of a breeding colony at the Southwest National Primate Research Center (San Antonio, TX). Animals were housed in family groups, with a breeding male and female and their immature offspring. Females were separated from their infants shortly after emerging from the nest box in the morning. They were kept separated for three to four hours to allow accumulation of milk in the mammary glands. Each female was anesthetized with ketamine hydrochloride and injected intramuscularly (IM) with oxytocin³ (2 – 3 IU). Nipples were cleaned with distilled water and milk was manually expressed into a vial. Efforts were made to completely evacuate both mammary glands. Milk was stored at -20° C. Samples were shipped on dry ice to the Nutrition Laboratory, Department of Conservation Biology, Smithsonian's National Zoological Park (hereafter, Nutrition Lab) and were maintained frozen at -20° C until time of analysis. Four samples from this population were donated to this project. This information was provided by ML Power (personal communication; Power et al., 2002).

Cebus apella: Tufted capuchin females were part of a breeding colony maintained at Alpha Genesis, Inc., Yemassee, SC. Collection of milk samples was approved by the Alpha Genesis, Inc., Animal Care and Use Committee, Animal Study Proposal (ASP) #

³ Oxytocin is administered to aid in milk letdown. Oftedal (1984) found that administration of oxytocin may affect milk composition through a decline in lactose concentration. This will be discussed in more detail in subsequent chapters.

04-16-05. All milk was collected on June 2-3, 2005. Lactating female mothers with attached infants were netted from the large group and given Ketamine at 0.15ml/kg IM. Once mothers were sedated (approximately five minutes, they were given oxytocin at ~1ml IM. Infants were allowed to hang on moms during the collection. If infants jumped off, they were placed in transfer cages for holding. Fifteen to 30 minutes after oxytocin injection, milk was manually expressed into a sterile conical tube, tube labeled, volume measured, and placed into -80° C freezer until shipped on dry ice packs to the Nutrition Laboratory. When mothers recovered, infants were returned (if needed) and both were returned to the group. The group is approximately 60 monkeys of various ages, with a female to male ratio of approximately 3:7.

Gorilla gorilla gorilla: Four western lowland gorilla samples ($n = 4$) were part of the mammalian milk collection at the Nutrition Laboratory. One sample ($n = 1$) was provided by Zoo Atlanta and the Philadelphia Zoo and three were provided by the San Diego Wild Animal Park (SDWAP). Permission to use the Philadelphia Zoo and SDWAP samples was provided by Olav Oftedal (Director, Nutrition Laboratory). Samples from SDWAP were from days 3, 242, and 690 of lactation. Samples from day 3 and 242 were from the same mother, but different offspring (day 3 from her second offspring, and day 242 from her first). Mothers were reported to be in good health at the time of collection, and no oxytocin was used in the collection of samples. The sample from the Philadelphia Zoo is from day 108 of lactation, provided by a wild caught female. The sample was collected in November, 1984 and shipped shortly thereafter to the Nutrition Lab. The inventory sheet

indicated that the mother was sedated with Ketamine/atropine and no oxytocin was administered during collection of this sample. The sample was frozen on arrival to the Nutrition Laboratory on January 8, 1985 and remained frozen (- 20° C) until time of analysis. The sample from Zoo Atlanta was provided to this project by T Stoinski (Veterinarian, Zoo Atlanta, Dian Fossey Gorilla Fund International). This sample is from day 1105 of lactation and was pooled from both mammary glands. The mother was sedated with Telazol (injectable) and maintained on isoflurane. Telazol is a non-narcotic, non-barbiturate, injectable anesthetic. Oxytocin (3 ml IM) was administered prior to milk collection. The sample was collected on June 7, 2005 and shipped the next day on dry ice to the Nutrition Lab. The sample was frozen upon arrival on June 9, 2005 and was placed immediately into the laboratory's -20 °C freezer until time of analysis.

Hylobates lar: One white-handed gibbon milk sample ($n = 1$) was provided by the Minnesota Zoo (Jim Rasmusen, Veterinarian) on June 6, 1996. The Minnesota Zoo provided four samples to the Nutrition Laboratory, but only one was available for analysis for this project. The sample was obtained by anesthetizing the mother with ketamine and allowing the infant to nurse. Samples were shipped on dry ice to the Nutrition Lab and immediately placed in the -20 °C freezer until time of analysis.

Leontopithecus rosalia: The single captive living golden lion tamarin female ($n = 1$) is housed at the Smithsonian's National Zoological Park. Milk collection from this female was opportunistic. She was brought into the National Zoological Park Veterinary

Hospital for removal of her radio collar and annual physical exam. Collection of milk concurrent with this examination was approved by the attending veterinarian and golden lion tamarin curator. The female was sedated with isoflurane gas and given 4 IU oxytocin. Milk was manually expressed into two cryovials and transported to the Nutrition Lab's -20 °C freezer, where it remained frozen until time of analysis. This information was provided by ML Power (personal communication).

Macaca mulatta: Females ($n = 22$) were part of a breeding population at the California National Primate Research Center (Davis, CA). Mothers were separated from their infant as part of an assessment study under investigation by J. Capitanio. Four hours after the mother was placed in the holding cage, they were anesthetized with ketamine hydrochloride (10 mg/kg) IM. Nipple areas were cleaned and chest hair was trimmed. Females were injected with exogenous oxytocin intramuscularly (0.1ml/kg) to stimulate muscle contraction and milk let-down. This dose is likely to lead to full evacuation of the mammarys, which is necessary to avoid bias in such studies. Females were held upright, in a sitting position, as this is most common position in which mothers nurse. Nipples were gently hand stripped mimicking infant nursing behavior with periodic rests and simulated nuzzling. Milk was collected from each mammary gland separately into sterile sample tubes and frozen at -20° C. Ketamine boosts of 5 mg/kg were administered as necessary to maintain sedation. Samples were shipped on dry ice from Davis, CA to the Nutrition Lab and remained frozen at -20° C until time of analysis. All milk analyses were performed under the direction of KJ Hinde, excepting fatty acid analysis.

Information on rhesus macaque samples was provided by KJ Hinde (personal communication).

Pan paniscus: Little information is available on the donor female ($n = 1$) or method of milk collection. Milk samples ($n = 2$) were part of the Nutrition Laboratory's mammalian milk collection. The female was housed at the Milwaukee Zoo (Milwaukee, WI) and milked several times between September and November, 1995. Samples included in this study were collected at days 46 (09/09/95) and 76 (10/09/95) of lactation. Although the methods for anesthetizing the female are unknown, no oxytocin was used in collection of samples. Samples were collected into 1.0 ml cryovials and placed immediately in a -20° C freezer. Samples were shipped on dry ice packs overnight to the Nutrition Lab where they were placed immediately in a -20° C freezer until time of analysis. This information was taken from inventory sheet on file at the Nutrition Lab.

Pan troglodytes: Female chimpanzee milk samples were obtained from the Southwest National Primate Research Center (SNPRC) ($n = 3$) and the St. Louis Zoo (St. Louis, MO) ($n = 1$). The following information on SNPRC samples was provided by KM Brasky (Veterinarian, SNPRC). Female chimpanzees were part of a research colony at SNPRC. There is a moratorium on the breeding of chimpanzees in the United States, and all males in the colony had received a vasectomy. However, one male in the colony remained fertile, leading to three pregnancies. Samples were collected from females during annual physical exams from June 25 – 30, 2005. Mothers were sedated with

100mg telazol plus 100mg xylazine IM. The infants were removed for approximately 30 minutes, and were then reunited with mothers. No oxytocin was administered during collection of samples. Days of lactation for the SNPRC samples are 451, 473, and 550 and were stored at -20 °C until shipment on dry ice to the Nutrition Laboratory. Samples were frozen upon receipt, and were immediately thawed for subsampling and dry matter analysis. The St. Louis Zoological Park (St. Louis, MO) donated one chimpanzee milk ($n = 1$) sample to the Nutrition Laboratory's mammalian milk collection. The sample was collected on November 3, 1992 (day 97 of lactation) and received frozen on dry ice by the Nutrition Laboratory on January 12, 1993. Oxytocin (10 mg IM) was administered to help stimulate milk flow, but no information was available on the inventory sheet regarding methods of sedation.

Pongo pygmaeus: The single ($n = 1$) orangutan sample in this project was provided by Zoo Atlanta (T Stoinski, Veterinarian). The sample was collected on February 3, 2005 and was received on February 5, 2005 by the Nutrition Laboratory for use in this project. The sample is from day 430 of lactation (approximately 1.75 years). The mother was sedated with Telazol injectable and maintained on isoflurane. Oxytocin (10 mg IU) was administered prior to the collection of the milk sample.

Saimiri boliviensis boliviensis: All animals in this study ($n = 8$) are part of a breeding colony of squirrel monkeys maintained at the University of South Alabama Center for Neotropical Primate Breeding and Research Resource (CNPRR). Four of the eight

females included in this study were feral-born and four were born into the colony at CNPRR. This study was approved by the University of South Alabama Institutional Animal Care and Use Committee. To collect milk samples, females and their infants were removed from the social group and placed in an individual cage for four hours. The upper torso of the female was bound using a self-clinging bandage wrap to prevent nursing or milk loss. Bandages were removed four hours after animal capture. Milk was collected into storage tubes by manual expression from the nipple. Oxytocin was not administered. Once the sample was collected the female was returned to her social group. The sample was then frozen and stored at -80°C until shipment to the Nutrition Laboratory where it was maintained in a -20°C freezer until the time of analysis.

Symphalangus syndactylus: The siamang milk sample ($n = 1$) was part of the Nutrition Laboratory's mammalian milk collection, donated in 1987 by the Riverbanks Zoological Park (Columbia, SC). Milk was collected on December 17, 1986 while the female was immobilized with ketamine for tuberculosis testing and an inoculation for tetanus. No oxytocin was administered. Milk was collected from both mammarys, and samples were pooled for a total sample volume of 12 ml. The milk sample was frozen and shipped overnight on ice packs to the Nutrition Laboratory where they were placed immediately into a -20°C freezer until time of analysis. This information was taken from inventory sheet on file at the Nutrition Lab.

B. Wild populations included in study

Alouatta palliata: Capture of Costa Rican mantled howler females 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 carbon dioxide powered gun. The darts were loaded with Telazol (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 per kg, shown to be safe and effective in more than 2,600 primate captures (Glander et al., 1991). Individuals were darted at distances up to 20 meters. 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 are given injections of 1-3 mg/kg of Telazol, repeated as often as needed. After all procedures are complete the animals are placed in burlap bags until they recover enough to walk or climb unaided. The bags are kept in the shade and are the best means of holding an animal until it recovers because the bag reduces visual stimulus. 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. Milk was collected directly into clean sample tube, capped and placed into a – 20° C freezer. Samples were shipped on dry ice to the Nutrition Laboratory (NZN).

Callithrix jacchus and *Leontopithecus rosalia*: All captures of golden lion tamarins were conducted under the supervision of Golden Lion Tamarin Association field staff and used its procedures. The common marmoset captures were conducted using the same 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. Oxytocin was not used. 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 (NZN) where they were maintained in a -20° C freezer until time of analysis.

Four samples from each species (*C. jacchus*, $n = 4$; *L. rosalia*, $n = 4$) were donated to this project by ML Power.

Gorilla beringei beringei: Mountain 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 CO₂ pressurized air pistols. Gorillas in this study were immobilized using 3 - 6 ml darts with 1.1 - 1.2 x 30 - 38 mm non-barbed needles loaded with either ketamine (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 hours at -20° C before and after cold shipment (dry ice or ice packs) to the United States (Maryland Zoo, Baltimore, MD). Samples ($n = 6$) were transported on dry ice from the Maryland Zoo to the Nutrition Laboratory, Department of Conservation

Biology, Smithsonian's National Zoological Park, Washington, D.C. (NZIP), where they remained frozen at -20° C until time of analysis.

Macaca sinica: The trapping, handling and release of macaques were supervised by Wolfgang Dittus, Department of Conservation Biology, Smithsonian National Zoological Park. Individuals were identified by their natural markings and tattoos. The macaques were baited into large-sized steel box traps (1 x 1 x 1.5m). The trap door was operated manually as a precaution against injury to the trapped animals. Macaque mothers were tranquilized by IM injection of ketamine hydrochloride at a dose of 0.1 ml/kg body weight (Ketalar, Park Davis Co., UK) and moved to a protected field laboratory. The anesthetic has been used safely on more than 1,000 toque macaques (Dittus, personal communication). To aid milk release, a dose of 0.20 – 0.25 ml oxytocin (Phoenix Pharmaceuticals Inc., MI) was administered IM 20 minutes prior to milking. Hair was shaved off around the nipples and the skin was cleaned with distilled water. An effort was made to evacuate both teats as completely as possible by manual massage. Milk was collected into 1.8 ml airtight cryotubes with screw caps. 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 (NZIP). In the United States, samples were stored at – 20° C. Each female was kept in a holding cage until she recovered completely from the anesthetic (usually at least 3 hours) and then released back into her social group. Eight samples ($n = 8$) were donated to this project by W. Dittus.

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