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A Longitudinal Study of Rhesus Monkey (*Macaca mulatta*) Milk Composition: Trace Elements, Minerals, Protein, Carbohydrate, and Fat

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Summary

The concentrations of iron, copper, zinc, calcium, magnesium, sodium, potassium, protein, carbohydrate, and fat were analyzed in milk from rhesus monkeys (*Macaca mulatta*) during the course of lactation. Concentrations of iron, copper, zinc, magnesium, sodium, potassium, and protein were higher in milk of early lactation (colostrum) than in mature milk, while concentrations of calcium increased with lactation time. Concentrations of zinc in monkey colostrum and mature milk were similar to that of human milk, while iron, copper, calcium, and protein concentrations were higher than in human milk.

There is a paucity of data in the literature regarding the effects of maternal diet on the composition of breast milk, and the resulting impact on the breast-fed infant. In addition, there is fragmentary information on the extent of nutrient utilization from milk by the breast-fed infant. While epidemiological studies can contribute to an understanding of these areas, data obtained from such studies are often difficult to interpret due to the lack of rigid definition of the maternal and infant environment, and the lack of appropriate controls. For ethical reasons, there are

several limitations in designing studies in which the investigator manipulates the maternal environment since the diet must always be kept within "normal" and "appropriate" limits; the number of blood samples taken from the infants should be few, and only rarely can justification be made for soft tissue sampling. Furthermore, if the experimental manipulation appears to be causing an insult to the infant, the study should be halted, or changed, in the interest of the child. For these reasons, it is necessary to identify and develop animal models which can be used for the study of maternal-infant nutrition. While several laboratory animals (primarily rodents) have been used to evaluate the effects of maternal dietary environment on milk composition, and the subsequent suckling outcome, the gross composition of milk from most of these species is not well matched to that of the human (12, 13, 17).

In order to evaluate the possibility of using a non-human primate as an animal model for milk composition, and metabolism studies, with special emphasis on trace elements, we have analyzed the nutrient composition of rhesus monkey milk, and noted changes throughout the course of lactation. These data are compared with data on human milk composition.

MATERIALS AND METHODS

Animals. Milk samples were obtained from healthy rhesus monkeys (*Macaca mulata*) at the California Primate Research Center. The animals were housed in five 4500-m² outdoor cor-

rals. Each corral contained approximately 25–35 adult females, their offspring from recent years and several adult males. Diet consisted of commercial monkey chow (Purina Monkey Chow, Ralston Purina Co., St. Louis, MO) plus water *ad libitum*. In addition, the monkeys were observed to feed extensively on the grass ground cover which consisted primarily of fescue and/or Bermuda grass.

Milking procedure. Lactating monkeys (*n* = 104) were immobilized with ketamine hydrochloride (Vetalar, Parke-Davis, Detroit, MI). Ocytocin (2 IU) was given intramuscularly to stimulate milk flow and the monkeys were milked by gentle hand stripping of the teats. The free-flowing milk was collected in acid-washed 5-ml plastic vials. In order not to cause undue stress, teats were not completely emptied; however, the volumes collected (5–10 ml) should represent a pool of the major part of the milk available. Collected milk was stored frozen (−20°C) until analysis. All monkeys were milked only once. The day of parturition was designated day 0 of lactation. Analyses were performed on single milk samples.

Analytical methods. Protein was determined in milk samples by a modified Lowry method (23) which minimizes interference from fat. Lactose was analyzed enzymatically using lactase and glucose oxidase according to Dahlquist (7) following precipitation of protein according to Somogyi (27). Fat was determined by a colorimetric method using the sulfuric acid-vanillin reaction (30) soon after collection to minimize lipolysis. Phosphate was analyzed according to Eibl and Lands (10). All these methods have been well established for human milk analysis and critically evaluated against other analytical methods (18). Energy content

Fig. 1. Milk iron, copper, and zinc concentrations in the monkey. Values shown are mean ± SEM.

was estimated by conventional energy factors for carbohydrate, fat, and protein.

Atomic absorption spectrophotometry. Milk samples (1 ml) were wet ashed with 16 N nitric acid (2 ml of Ultrex grade, J. T. Baker Chemical Co., San Francisco, CA), concentrated by evaporation, and diluted with distilled deionized water (5). Calcium, magnesium, iron, copper, and zinc were determined by flame atomic absorption spectrophotometry, and sodium and potassium by emission spectrophotometry (IL 551, Instrumentation Laboratories, Wilmington, MA). For calcium and magnesium analysis, the diluted ashed samples were diluted further with 0.1% lanthanum chloride in order to reduce matrix interference (4). The wet ashing method used has been documented to be superior to dry ashing methods (6). Typical recoveries of added metals range between 98 and 102%.

Statistical methods. To identify when changes in milk composition occurred, data were grouped in four time periods and analyzed by Student's *t* test. Comparisons were made between one time period and that time period immediately following.

RESULTS AND DISCUSSION

Iron concentration of monkey milk was high in early lactation, and then decreased to a value of approximately 1.2 μg/ml (Fig. 1). The absolute concentration of iron in monkey milk is considerably higher at all times than that of human milk (Table 1), though the change in milk iron concentration found during lactation for the monkey is similar to that reported for human milk (25). Despite the difference in absolute milk iron concentrations, the monkey may be an excellent model for studying milk iron metabolism in humans, since the molecular localization of iron in monkey milk appears similar to that of human milk. In particular, much of the iron in monkey milk (~50%) is bound to lactoferrin (8), an iron-binding protein present in human milk, which is absent, or found only in trace amounts, in the milk of most other species (20, 21). It has been hypothesized that it is in part the presence of lactoferrin in human milk which accounts for the high bioavailability of iron from human milk, compared to iron bioavailability from other milks such as cow's milk (11). In addition, it has been suggested that lactoferrin may have an important role as an antibacterial agent for the suckling infant (2). The observation that lactoferrin is present in monkey milk in a high concentration (>2 mg/ml) suggests that this species may be used to test the above hypotheses.

Similarly, the copper concentration was higher in monkey colostrum than in mature monkey milk. The pattern of decreasing milk copper levels was different than the iron levels which dropped during early lactation, and then remained steady; copper levels dropped throughout the lactation period with values late in lactation only 20% of initial values (Fig. 1). The pattern for
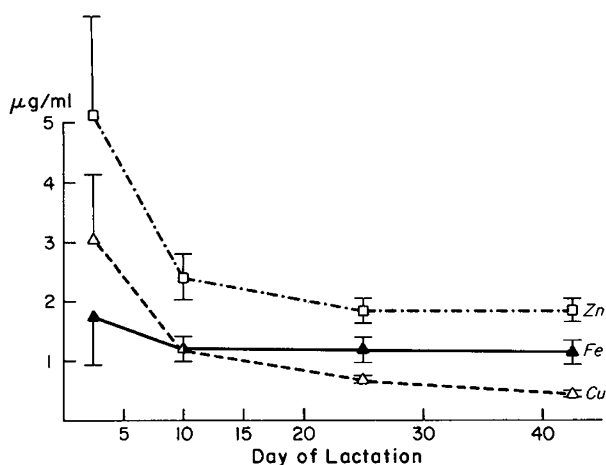
Table 1. *Composition of monkey milk during lactation**

Nutrient	Days of Lactation				
	0–5	6–15	16–35	36→	Human Mature Milk†
Iron, μg/ml	1.76 ± 0.84 (9)	1.21 ± 0.20 (15)	1.18 ± 0.22 (43)	1.15 ± 0.19 (37)	0.25 ± 0.03 (18)
Copper, μg/ml	3.04 ± 1.09 (9)	1.21 ± 0.18 (15)‡	0.60 ± 0.04 (43)‡	0.46 ± 0.04 (37)‡	0.27 ± 0.02 (18)
Zinc, μg/ml	5.15 ± 1.64 (9)	2.41 ± 0.38 (15)‡	1.84 ± 0.20 (43)‡	1.86 ± 0.18 (37)	1.35 ± 0.13 (18)
Calcium, μg/ml	350 ± 26 (9)	364 ± 27 (15)	420 ± 10 (43)‡	392 ± 14 (37)‡	270 ± 14 (18)
Magnesium, μg/ml	49.6 ± 12.1 (9)	31.5 ± 3.6 (15)‡	32.9 ± 3 (43)	30.7 ± 2.3 (37)	33.6 ± 1.1 (18)
Sodium, μg/ml	171 ± 64 (9)	95.9 ± 17.7 (15)	89.4 ± 8.6 (43)	81.8 ± 6.5 (37)	184 ± 33 (18)
Potassium, μg/ml	367 ± 33 (9)	242 ± 17 (15)‡	260 ± 8 (43)	276 ± 10 (37)	470 ± 19 (18)
Protein, %	2.49 ± 0.26 (5)	2.28 ± 0.09 (7)	2.34 ± 0.07 (13)	2.35 ± 0.09 (19)	1.32 ± 0.04 (18)
Fat, %	5.29 ± 0.97 (4)	5.42 ± 0.51 (9)	4.57 ± 0.52 (17)	4.92 ± 0.50 (21)	4.58 ± 1.39 (18)
Lactose, %	7.79 ± 0.13 (6)	8.06 ± 0.03 (8)	7.91 ± 0.06 (18)	7.92 ± 0.04 (25)	7.13 ± 0.18 (18)
kcal/100 ml	88.7	90.1	82.2	85.4	75.0

* Data are expressed as mean ± SEM. No. of samples are shown in parentheses.

† Human milk values are based on data obtained in the author's laboratory. Mature milk was collected at 3 months postpartum.

‡ Significantly different from preceding time period ($p < 0.05$).

monkey milk copper levels during lactation is different than that of human milk, where the majority of the decrease occurs during early lactation (29). As seen with milk iron concentration, the absolute concentration of copper in monkey milk is higher than that of human milk. We have not yet compared the relative molecular localization of copper in monkey milk with copper localization in human milk.

The lactation pattern of zinc concentration in monkey milk is similar to that reported for human milk (29), with high values found in colostrum, which then decreased rapidly (Fig. 1). The absolute concentration of zinc is similar between human and monkey milk (Table 1). We have previously shown that in monkey milk a significant amount of the zinc is bound to the low molecular weight ligand, citrate (16), as is true for human milk (19). The non-citrate bound zinc is also distributed similarly in the milk of the two species, but very different from most other species studied (16). As seen with iron absorption from human milk, it has been suggested that the high bioavailability of zinc from human milk relative to cow's milk may be due to a difference in molecular localization of zinc in the milks (9, 24). The monkey thus presents a model in which this hypothesis may be tested.

Similar to the lactation pattern seen in human milk, the calcium concentration of monkey milk increased slightly during lactation (Fig. 2); however, the levels of calcium are slightly higher in monkey milk (Table 1). In a limited number of samples, phosphate was analyzed and it was found that the calcium:phosphorus ratio of monkey milk is similar to that of human milk, being ~2:1.

As in human milk, magnesium concentrations were higher in monkey colostrum than in mature monkey milk (Fig.2). The absolute concentration of magnesium in human and monkey milk is similar (Table 1).

Sodium and potassium concentrations were slightly higher in monkey colostrum than in mature monkey milk (Fig. 3). The concentrations of sodium and potassium are lower in monkey milk than in human milk (Table 1). The monkey milk as a result has a similar osmolarity to that of human milk since monkey milk is higher in calcium and protein than human milk. Thus, the renal solute load from monkey milk should be very similar to that of human milk.

In contrast to human milk which has a pronounced lactation pattern with high levels of protein during early lactation, and low levels in mature milk (15), monkey milk protein concentrations decreased only slightly with lactation time (Fig. 4). As mentioned above, the protein concentration of monkey milk is higher than that of human milk (Table 1), although the casein:whey ratio of monkey milk is similar to that of human milk (1:4) (14).
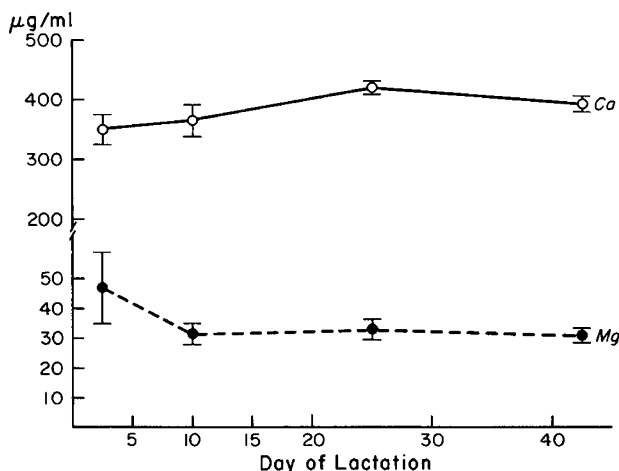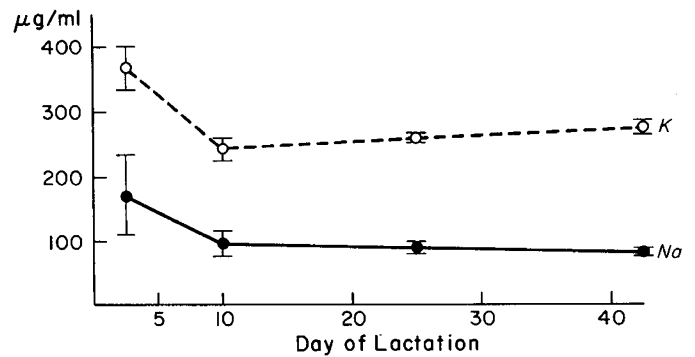

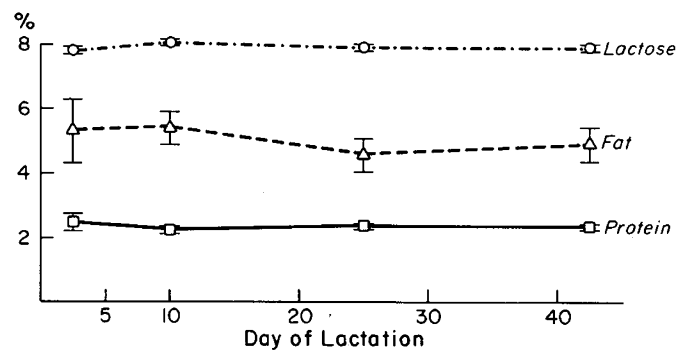Fig. 3. Milk sodium and potassium concentrations in the monkey. Values shown are mean ± SEM.

Fig. 4. Milk fat, lactose, and protein concentrations in the monkey. Values shown are mean ± SEM.

Similar to those in human milk, fat and lactose contents did not vary much with the stage of lactation (Fig. 4) and the absolute concentrations of fat and lactose are similar between the two species. As a result, caloric contents of human milk and monkey milk are similar (Table 1). While there have been studies on monkey milk composition, the milk of rhesus monkeys has not been studied in detail on a longitudinal basis and for all primates trace element information is scarce. Van Wagenen et al. (28) studied milk from rhesus monkeys and found that the concentration of protein was 2.1%, fat was 3.9%, and carbohydrates was 5.9%. Bahgat et al. (1) also analyzed protein, fat, and lactose in rhesus monkey milk. Although our values are slightly higher in general, they are in good agreement with their data. Milk of other laboratory primates, such as the baboon (3), appear to have similar gross comparison to that of the rhesus monkey. The ash content of primate milk appears higher than in human milk (3), probably reflecting the higher concentrations of calcium found in our study. The composition of both whey proteins and casein in monkey milk has a remarkable degree of similarity to those of human milk (26), and are different from virtually all other species analyzed. In addition, the ratio of casein and whey proteins in monkey (cynomolgus) milk appears similar to human milk, resulting in a similar amino acid composition (22). Fatty acid composition of monkey milk also appears similar to human milk.

Overall, nutrient composition, caloric density, and osmolarity of monkey milk and human milk are similar. Although the concentrations of protein, iron, and copper are higher in monkey milk than in human milk, the composition and/or distribution among components appears to be similar between the two species. Thus, this non-human primate should be an excellent model for studies on milk nutrient metabolism in early life when results are to be extrapolated to humans.

Fig. 2. Milk calcium and magnesium concentrations in the monkey. Values shown are mean ± SEM.

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Decreased Calcium Content and ⁴⁵Ca²⁺ Uptake in Down's Syndrome Blood Platelets

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Summary

Total intracellular Ca^{2+} and ⁴⁵Ca²⁺ uptake has been studied in blood platelets from subjects with Down's syndrome and matched controls. In Down's subjects, Ca^{2+} levels (85.5 ± 5.9 nmol/10^{-9} platelets) were significantly lower than controls, 174 ± 10.0 nmol/10^{-9} ($p < 0.0005$). A similar reduction was seen in calcium uptake (Down's platelets, 0.79 ± 0.06 nmol/10^{-9} platelets; controls, 1.17 ± 0.07 nmol/10^{-9} platelets, $p < 0.005$). The low levels of intracellular Ca^{2+} may be related to decreased granular storage of serotonin, and the decreased Ca^{2+} uptake with impaired transport by intracellular Ca^{2+}-accumulating organelles such as the dense tubular system.

Abbreviation

PRP, platelet-rich plasma

Calcium ions play an essential role in initiating or terminating cellular functions by mechanisms that either involve the influx

of Ca^{2+} or the intracellular translocation of Ca^{2+} (11). In blood platelets, Ca^{2+} content can be described as a three-compartment space, a surface component, and two intracellular components, one rapidly exchangeable with external Ca^{2+} and the other virtually nonexchangeable. The nonexchangeable space represents Ca^{2+} sequestered in subcellular storage granules, and alteration of Ca^{2+} binding and/or fluxes in the other compartments has an important role in the process of platelet aggregation and exocytosis (1). In subjects with Down's syndrome, blood platelets have decreased Na^+/K^+-ATPase activity and increased intracellular Na^+ (9). In erythrocytes, it has been reported that Na^+/K^+-ATPase is inhibited by increases in intracellular Ca^{2+} (2, 3), which may lead to raised intracellular Na^+ (13); thus, it seemed pertinent to determine the Ca^{2+} content of blood platelets from subjects with Down's syndrome as a possible factor contributing to decreased Na^+/K^+-ATPase activity.

MATERIALS AND METHODS

Materials. ⁴⁵CaCl₂ (0.6 mCi/mg) was obtained from New England Nuclear. LaCl₂ was spectrographic grade from Fisher