NUTRITENT COMPOSITION OF HUMAN MILK AND DIETARY

INFLUENCE DURING THE FIRST SIX MONTHS OF LACTATION

by

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(ABSTRACT)

Mature human milk composition was determined from monthly samples collected from five, healthy, Caucasian, lactating women. A 72-hour dietary record was kept monthly from the twelfth week of pregnancy to the sixth month postpartum. Nutrient content of milk samples was similar to values reported in the literature. Calcium and zinc concentrations of human milk decreased significantly during the five month study. Moisture, energy, total lipids, protein, and magnesium levels remained fairly constant over the course of lactation. Dietary intake during pregnancy was not significantly correlated with nutrient concentration in human milk. For dietary intake during lactation, a significant correlation was observed between caloric intake and energy content of human milk for the second month of lactation. A significant correlation was observed between protein intake and protein content in the milk for the sixth month of lactation. Zinc concentration was significantly correlated with dietary intake during the fourth month of lactation. No other significant

correlation was observed between nutrient content of human milk and dietary intake during the lactation period.

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Introduction

Since the beginning of time, human milk has been the main food source for the developing human infant. It seems apparent, therefore, that human milk, in sufficient quantities, provides adequate amounts of energy and essential nutrients for the growing infant while, at the same time, not depleting the maternal body stores. If this were not the case, either the mother or the infant would be at a nutritional disadvantage, and such a situation would not be conducive to the survival of the species (Fomon, 1986b).

If human milk is the appropriate nutriture for the growing infant, it would seem reasonable to conclude that ideal energy and essential nutrient intakes could be determined from the intake of the healthy, breast-fed infant. However, this is not to say that the concentration of any particular constituent found in human milk is always most desirable for the infant. Certain nutrients in human milk do not appear to be present in adequate concentrations to meet the infant's requirements. Such is the case for vitamin K, vitamin D, and iron, and the general recommendation is to supplement all breast-feeding infants with these nutrients (Fomon, 1986a).

How does one go about determining nutrient

concentration in human milk? One way is to conduct compositional studies. Such studies have been conducted for more than 100 years and, as of yet, no reference standard has been established for the composition of human milk. A partial explanation for this lack of data is that human milk is a dynamic and physiologically complex fluid containing a variety of biochemically and biologically active compounds (Guall & Wright, 1986). Significant compositional changes occur during the lactation period as a result of normal physiological events in the mother (Lnnerdall 1986b). Compositional changes are known to occur during a single feeding, between morning and evening feedings, among individual women, and even, within the same woman (Picciano & Guthrie, 1976). Such compositional changes have made it difficult to provide consistent and reliable data regarding the composition of human milk (Feeley et al., 1983).

Another factor which may influence the composition of human milk is maternal dietary intake. Although it is not fully established as to how maternal dietary intake affects the nutrient composition of human milk, it appears that the macronutrients of human milk, and hence its energy content, are derived primarily from endogenous synthesis. Mineral content of human milk reflects little influence from maternal diet, while the vitamin content appears to be

sensitive to maternal intake (Anderson, 1985).

While it appears difficult to provide reliable and consistent data on the composition of human milk, such information is vital when it comes to establishing infant nutrient requirements and in developing infant formulas. The present study was designed to determine the normal concentrations of moisture, total energy, total lipids, total nitrogen, calcium, copper, magnesium, and zinc in mature human milk. The investigation was conducted over a 6 month period, so concentration changes over time could be examined. The relationship of maternal dietary intake, during pregnancy and lactation, to nutrient composition of human milk was also studied.

Review of Literature

Nutrient Composition of Human Milk

Moisture

By mass and volume, the major nutrient in human milk is water, and shows very little variation throughout lactation (Vaughan et al., 1979). Water is essential to the body with regard to maintaining body temperature, as a solvent for physiological compounds, and for the transport of nutrients to and waste products from the cell (Pipes, 1985). The infant requires water to replace that lost through the skin and lungs, and excreted in the feces and urine; a small amount is necessary for growth (Pipes, 1985; Fomon, 1974; Ritchey & Taper, 1983). Requirements will vary depending on environmental temperature, activity, rate of growth, and caloric consumption (Barness, 1985). The average daily water requirement suggested for the full-term newborn infant is approximately 80 to 100 ml/kg of body weight. By about one week, the requirement has increased to 125 to 200 ml/kg, after which, it may decrease to 125 to 175 ml/kg at one year (Barness, 1985).

The moisture content of human milk is between 85%-95% of the total volume (AAP, 1981). Other data indicate moisture values to be 87.1 ml/100ml (Macy and Kelly, 1961), 88.0% for the first three months and 88.4% for 4-6 months

postpartum (Vaughan et al., 1979), and 87.5 gm/100g (USDA, 1976).

Energy

The rate of growth in the first year of life is second only to the intrauterine growth rate, and energy requirements are greatest per unit size in infancy than at any other stage of the life cycle (Pipes, 1985: Fomon, 1974). It is during this time that energy is of particular importance to the growing infant, not only for increased requirements for tissue synthesis and storage, but also due to the vulnerability to thermal stress and the energy needs associated with such trauma energy needs (Brooke, 1986).

The range of energy intake recommended for satisfactory growth during the first 6 months is 80 to 120 kcal/kg/day, decreasing to 80 to 100 kcal/kg/day for the second 6 months (Barness, 1985). More specific recommendations have been made by the Food and Nutrition Board (1980) and the Joint Expert Consultation of FAO/WHO/UNU (1984).

The Food and Nutrition Board recommendations are based on levels which reflect the general pattern of intake for thriving infants. For an infant 0.0-0.5 years, the RDA is 115 kcal/kg of body weight at birth, and this amount decreases to 105 kcal/kg by the end of the first year (FNB, 1980). In 1984, the FAO/WHO/UNU made the following

recommendations for energy intake during the first year of life. At 0-2.9 months, an intake of 116 kcal/kg/day is required, decreasing to 99 kcal per kg per day for the infant aged 3-5.9 months. The value decreases slightly to 95 kcal/kg/day at 6-8.9 months, while increasing to 101 kcal/kg/day at 9-11.9 months (FAO, 1984). All recommended intakes cover the hypothesized caloric expenditures of approximately 50% for maintenance, 25% for activity, and 25% for growth (Pipes, 1985).

The energy values which have been reported for human milk over the course of lactation are: 74.7 cal/100cc (Macy, 1949), 75 kcal/100ml (Macy and Kelly, 1961), 0.67 kcal/ml (O'Leary, 1984), and 690 kcal/l (Hambraeus, 1977). In a study conducted by Butte et al., (1984), mean monthly values of 0.68, 0.64, 0.62, and 0.64 kcal/g were reported. Also reporting monthly values, Dewey and Lonnerdal (1983) reported total energy intake and energy intake per kilogram, for a six month sequence, as 509, 564, 556, 596, 593, and 658 kcal/day; and 113, 105, 93, 93, 85, and 89 kcal/kg/day, respectively. In a study of rural Ivory Coast women, at 6 months the caloric content of milk was 71.0 kcal/100g, and the 23 month average for the study was 61.0 kcal/100g (Lauber and Reinhardt, 1979). Jelliffe and Jelliffe (1978) hypothesized that the calorie-determining

characteristics, which are the volume secreted and the fat content, may be less in human milk from undernourished mothers than in human milk from well-fed mothers.

Protein

Protein is essential, not only for its caloric value, but, also for normal growth. Essential amino acids, which are necessary for new tissue synthesis, and nitrogen, which is necessary for the maturation of existing tissue, are provided by protein (Pipes, 1985). The need for protein can be demonstrated by the increase in the protein content of the body during the first year of life, from 11 to 14.6 percent, concomitant with a 7 kg increase in body weight (FNB, 1980). Fomon (1974) states that the average increase in body protein is approximately 3.5 g/day during the first four months of life and 3.1 g/day during the next eight months.

The protein allowances established by the Food and Nutrition Board (1980) are based on the amount of protein provided by the quantity of milk necessary to ensure a satisfactory growth rate. The amount required for the first month of life is approximately 2.0-2.4 g/kg/day, gradually declining to 1.5 g/kg/day by the sixth month of life. Based on such information, the RDA for infants 0.0-0.5 years is set at 2.2 g/kg and deceases to 2.0 g/kg for an infant 0.5-1

year (FNB, 1980). The protein requirements from the FAO/WHO/UNU are as follows: 1.85 g/kg/day for 3-5.9 months, 1.65 g/kg/day for 6-8.9 months, and 1.5 g/kg/day for 9-11.9 months (WHO, 1984).

In compositional studies, protein can be measured in terms of total nitrogen available, nitrogen from non-protein sources, or nitrogen solely from protein sources (Lonnerdal et al., 1976b). It is important to keep such distinctions in mind when interpreting compositional results. When compositional studies were first conducted, the clarification of where nitrogen was found was not being made. Therefore, it is thought that some reported protein values overestimate protein content by approximately 20%, since human milk contains 20-25% non-protein nitrogen (Lonnerdal et al., 1976b; Lonnerdal et al., 1984). Currently, methods which can distinguish between non-protein nitrogen and protein nitrogen are being used.

The values which have been cited in the literature for total nitrogen are: 2.17, 1.94, 1.84, and 1.80 mg/g for a four month sequence (Butte et al., 1984), 1.1 gm/100ml (Macy and Kelly, 1961), and 1.06 g/100cc (Macy, 1949). For privileged and nonprivileged Ethiopian mothers and privileged Swedish mothers, total nitrogen values for 1.5-3.5 months postpartum for the three groups were 1.97,

1.77, and 1.61 mg/ml, respectively; while at 3.5-6.5 months postpartum, values for nonprivileged Ethiopian mothers and Swedish mothers were 1.69, and 1.48 mg/ml, respectively (Lonnerdal et al., 1976c). The total protein values which have been cited in the literature are: 1.44, 1.33, 1.32, 1.30, 1.25, and 1.27 g/100ml for a six month sequence (Dewey and Lonnerdal, 1983), and 1.1 g/100ml (Macy, 1949), over the course of lactation.

Methods currently being employed to determine protein nitrogen are amino acid analysis or the determination of total nitrogen and non-protein nitrogen values and the subtracting out of the non-protein nitrogen value. The value most widely accepted for protein nitrogen is 0.8-0.9% (Lonnerdal et al., 1976b). Values which have been reported for amino acid analysis are 0.78% and 0.88% (Lonnerdal et al., 1976a); while the value of 0.77% has been reported when non-protein nitrogen is subtracted from total nitrogen (Lonnerdal et al., 1984). In a four month study by Butte et al., (1984), sequential values of 1.61, 1.42, 1.34, and 1.31 mg/g for protein nitrogen were reported. Other values which are cited in the literature are: 1.44, 1.33, 1.32, 1.30, 1.25, and 1.27 g/100ml for the first six months postpartum (Dewey and Lonnerdal, 1983), 0.8 g/100ml (Lauber and Reinhardt, 1979), 1.4% (Finley et al., 1985b), 1.41 g/dl

(Neville et al., 1984), and 1.00 and 1.36 g/100ml, before and after a single feeding, respectively (Hall, 1979).

The concentration changes of protein over the course of lactation are inconsistent. Over one six month period, protein values of 1.44, 1.33, 1.32, 1.30, 1.25, and 1.27 g/100ml were reported, and researchers concluded that values remained constant (Dewey and Lonnerdal, 1983). However, other researchers examining protein values for the first six months reported values of 2.15, 1.55, 1.50, 1.55, 1.20 and 1.40%, and concluded that values dropped sharply initially, but did not change significantly thereafter (Finley et al., 1985b). In a study conducted with Egyptian women (Kader et al., 1972), protein values reported for the first six months were: 1.03, 1.10, 0.99, 0.88, 0.91, and 0.82 g/100ml, respectively, and tended to be fairly constant.

As for the effect of maternal dietary protein on the concentration in human milk, no pronounced effect has been noted. In studies concerned with protein supplmented to malnourished women, no significant effect on human milk protein has been observed. In a study by Forsum & Lonnerdal (1980), well-nourished women consumed a low-protein diet, a control diet, and a high-protein diet. The milk protein concentration was significantly lower when

the low-protein diet was consumed, and a significant increase was observed when women consumed the high-protein diet. Care must be taken in interpreting these results, for each diet was consumed for only four days. Other authors (Butte et al., 1984; AAP, 1981) have noted no influence from maternal dietary intake on the milk protein concentration.

Fat

In human milk, lipids provide the major fraction of the calories at 45% (AAP, 1981). Besides providing energy, lipids are also the carrier for fat-soluble vitamins and essential fatty acids, which are necessary for growth and development of the central nervous system (Jelliffe & Jelliffe, 1978).

No RDA exists for fat. In human milk, it is well documented that the fat content increases during a single feeding, but results are inconsistent as to the concentration changes over the course of lactation. During a single feeding, values of 2.42 g/100ml to 7.48 g/100ml (Hall, 1979), and 2.3% to 4.6% (Neville et al., 1984) have been reported for fore- and hind-milk samples. With regard to values during the course of lactation, Butte et al., (1984) reported values for the first four months of lactation as 36.2, 34.4, 32.2, and 34.8 mg/g, and indicated

that these values were lower than those from other studies. Dewey and Lonnerdal (1983), reported a decrease in fat concentrations during a six month study. Values for the six successive months were: 4.92, 4.58, 4.58, 4.62, 4.36, and 4.3 g/100ml. Finley et al., (1985b) reported that during the first six months of lactation, values were 4.0, 4.2, 4.0, 4.5, 3.8, and 4.0%, respectively, and the values increased during the next 14 months of lactation. In a study involving Egyptian women (Kader et al., 1972), results of 2.7, 2.5, 2.3, 2.6, 2.5, and 2.6 g/100ml were reported for the first six months, sequentially, and indicated that at the end of the first year of lactation, concentrations of fat increased. Other concentrations of fat reported in the literature are: 3.04% (Lonnerdal et al., 1976a), 4.5 g/100ml (Macy and Kelly, 1961), 2.69% (Lonnerdal t al., 1984), 4.4 g/100g fluid product (USDA, 1976), 4.54 g/100cc (Macy, 1949), 45 g/1 (Hambraeus, 1977), 3.07 g/100ml (Lauber and Reinhardt, 1979), 3.39% (Neville et al., 1984), 5.0% (Jelliffe, 1968), 5.83% (Frannson and Lonnerdal, 1982), and 4.72% (Frannson and Lonnerdal, 1980). In undernourished mothers, values range from 2.3 to 4.2 g/ml (Jelliffe and Jelliffe, 1978).

The effect of maternal intake on the lipid concentration is an interesting one. While dietary fat

intake does not affect the total amount of lipids in human milk (Anderson, 1985; Butte et al., 1984), the dietary intake does influence the fatty acid composition of human milk (Anderson, 1985; Lonnerdal, 1986a). In a study conducted by Finley et al., (1985a), no significant correlation was found between the lipid concentration of human milk and any dietary variable in generally well-nourished women. The researchers also concluded that no significant difference was noted in percentage of lipids in human milk between vegetarians and non-vegetarians.

Calcium

Calcium is found in three body systems: bone, body fluids, and striated muscle (Pipes, 1985). Calcium phosphate is deposited in the soft, fibrous, organic matrix in normal calcification; calcium located in the extracellular fluids and soft tissues controls the excitability of peripheral nerves and muscles; calcium is also required for blood coagulation, myocardial function, muscle contractibility, and for the integrity of intracellular cement substances and various membranes (FNB, 1980).

In metabolic balance studies conducted by Fomon et al., (1963), the mean calcium retention in the first four months of life was observed to be about 28 mg/kg of body

weight/day. Based on the average body weight of 5.3 kg for the reference male infant, an average retention of 148 mg/day was calculated. Using this value and assuming a retention rate of 40%, Fomon (1974) estimates the infant requirement for calcium to be 388.0 mg and sets an advisable intake, 20% above the estimated requirement, at 450.0 mg/day.

The RDA set by the Food and Nutrition Board is 360 mg for the infant 0.0-0.5 years, and 540 mg for the infant 0.5-1.0 year (FNB, 1980).

Picciano et al., (1981), reported that calcium levels are relatively constant in the first three months of lactation at 7.24, 7.31 and 7.14 mmol, respectively. Also reporting constant calcium levels were Dewey and Lonnerdal (1983), with values of 261, 275, 270, 255, 248, and 256 ug/ml for a six month sequence. Finley et al., (1985b), found that calcium levels decreased during a six month period. The values reported are: 6.0, 6.1, 5.9, 5.3, 5.6, and 5.5 mmol for the six months.

Other concentration levels defined in the literature are: 257 g/ml wet wt. at 1-3 months and 236 g/ml wet wt. at 4-6 months (Vaughan et al., 1979), 181 mg/l (Lonnerdal et al., 1984), 32 mg/100g fluid (Packard, 1982; USDA, 1976), 34.3 mg/100cc (Macy, 1949), 300 mg/l or 60 mg/kg (FNB, 1980), 297 mg/l (Hambraues, 1977), 5.5 mmol

(Finley et al., 1985b), 26.4 mg/dl (Neville et al., 1984), 28 mg/100g (Jelliffe, 1968), and 241.2 ug/ml (Frannson and Lonnerdal, 1980).

The calcium concentration of human milk shows little influence from the maternal diet (Anderson, 1985; Packard, 1982; Sims, 1978; Illingsorht & Kilpatrick; 1953). This is thought to be due to regulation by maternal body stores. If calcium intake is low in the maternal diet, it could be readily mobilized from stores in the bones, and milk concentrations would remain constant (Anderson, 1985).

Copper

Copper is necessary for normal functioning of 15 copper-containing enzymes, proteins which are required for cellular respiration, normal hematopoiesis, normal bone formation, functioning of the GI tract, and for maintenance of myelin in the nervous system (Fomon, 1974). If copper is deficient, neutropenia, leukopenia, and abnormal bone mineralization have been observed. With severe copper deficiency, anemia, which is not responsive to iron treatment, results. The anemia is a result of a reduced ceruloplasmin concentration. Ceruloplasmin is a metalloenzyme which is assumed to function as a ferroxidase. This reaction oxidizes ferrous iron to ferric iron, which is necessary in mobilizing iron from the liver

for red blood cell production. This theory is supported by increased liver iron and reduced hemoglobin formation during copper deficiency (Lonnerdal, 1986b).

No RDA has been established for copper, but safe and adequate daily dietary intakes have been set for infants 0.0-0.5 and 0.5-1.0 year, at 0.5-0.7 mg and 0.7-1.0 mg, respectively (FNB, 1980).

Again, results vary as to concentration changes during lactation. A fairly constant level was reported by Picciano et al., (1981), during a three month study, with values of 3.35, 3.24, and 3.26 umol. A significant decline between the first and second months, with a continuing decline in the following months was noted by Dewey and Lonnerdal (1983). The values for the first six months are: 0.36, 0.28, 0.27, 0.24, 0.20, and 0.21 ug/ml, respectively. The research of Finley et al., (1985b), again, demonstrates a decline in the copper levels with increasing duration of lactation. Their values are 0.75, 0.52, 0.44, 0.35, 0.36, and 0.30 mmol for a six month sequence.

Other copper levels cited in the literature are: 84.7 ug/100g (Feeley et al., 1983), 0.34 and 0.33 ug/ml wet wt. for 1-3 months and 4-6 months, respectively (Vaughan et al., 1979), 0.24 mg (Lonnerdal et al., 1984), 0.232 ug/ml (Picciano and Guthrie, 1976), 15 ug/ml (Packard, 1982), 0.15

mg/l (FNB, 1980), 400 ug/l (Macy and Kelly, 1961), 15-105 ug/100g fluid product (USDA, 1976), 0.6 ug/l (Hambraeus, 1977), 0.014 mg/100ml (Lauber and Reinhardt, 1979), 0.19 ug/ml (Neville et al., 1984), 0.27 ug/ml (Fransson and Lonnerdal, 1982), 0.2-0.3 ug/ml (Lonnerdal et al., 1981), and 0.21 mg/l (Vuori et al., 1980).

The milk concentrations of copper are affected very little by maternal diet (Packard, 1982; Sims, 1978; Kirksey et al., 1979), even when mothers are taking supplements (Anderson, 1985). However, reports indicate an excess of zinc will aggravate a marginal condition of copper deficiency (Packard, 1982).

Magnesium

Magnesium is the second most abundant intercellular cation. Approximately 50% of the magnesium is deposited along with calcium and phosphorus in bones, 25% is found in the muscles, and the remainder is located in the soft tissue (Pipes, 1985). Magnesium is an essential part of many enzyme systems and is also important in maintaining the electrical potential in nerves and muscle membranes (FNB, 1980). Wong and Teh (1968) have associated serum levels of magnesium and the occurance of tremors and convulsions in infants and children.

The RDA is set at 50 mg and 70 mg for infants 0.0-0.5

and 0.5-1.0 year (FNB, 1980), respectively. Based on a 3.3 mg tissue increment and a 20% retention rate, Fomon (1974), estimates an intake of 16.5 mg and an advisable intake of 25 mg.

Magnesium content of breast milk tends to increase during lactation, as is demonstrated in the following results. The magnesium levels reported by Picciano et al., (1981) are: 1.15, 1.27, and 1.36 mmol during the first three months of lactation. A significant increase was noted in the first six months of lactation by Dewey and Lonnerdal (1983), with magnesium levels of: 27.6, 32.4, 33.6, 35.1, 33.8, and 33.9 ug/ml for the six sequential months. In a six month study by Lauber & Reinhardt (1979), values of 1.15, 1.14, 1.28, 1.27, 1.3, and 1.25 mmol were reported. Vaughan et al., (1979) reported an increase from 31 ug/ml wet wt to 37 ug/ml wet wt for 1-3 months and 4-6 months of lactation.

Mean values reported by other researchers are: 28.3 mg/l (Lonnerdal et al., 1984), 3-4 mg/100ml (Packard, 1982), 40 mg/l (Macy and Kelly, 1961; FNB, 1980), 3.0 mg/100g fluid product (USDA, 1976), 3.5 mg/100cc (Macy, 1949), 23 mg/l (Hambraeus, 1977), 3.9 mg/dl (Neville et al., 1984), 41.40 ug/ml (Frannson and Lonnerdal, 1982), and 4.0 ug/l (Vuori et al., 1980).

As for the influence of maternal dietary intake, magnesium concentrations of human milk show no response, even if supplements are taken (Anderson, 1985).

Zinc

Zinc is distributed throughout all cells and tissues and is an essential component of many enzymes, including those which contribute to protein synthesis and bone mineralization (Pipes, 1985; Fomon, 1974). Failure to grow, decreased taste acuity, anorexia, sexual immaturity, and impaired wound healing have all been noted with zinc deficiences (Packard, 1982; Pipes, 1985). Infants are born without zinc stores, so an adequate supply of biologically-available zinc becomes essential (Pipes, 1985). As mentioned earlier, excess zinc will magnify a copper deficiency. Human milk has a low zinc:copper ratio. The RDA for zinc is 3 and 5 mg for infants 0.0-0.5 and 0.5-1.0 year, respectively (FNB, 1980). It is estimated that the fully breast-fed infant receives 0.35 mg/kg/day (Picciano and Guthrie, 1976).

The bioavailability of zinc in human milk is considerably higher than that in cow's milk or formula however, the zinc concentration in human milk declines rapidly after the first month and is usually lower then 2 mg/l after the second month of lactation

(Lonnerdal, 1986b).

Picciano et al., (1981) conducted a study over the first three months of lactation and found zinc values of: 33.8, 31.8, and 29.5 umol. Dewey and Lonnerdal (1983) reported a significant decrease between the first and second months, and then a steady decline. The actual zinc values were: 2.71, 1.67, 1.35, 0.89, 0.57, and 0.64 ug/ml. Finley et al., (1985b) reported declining values during the first six months of lactation of 5.5, 3.1, 2.0, 2.1, 1.8, and 1.4 umol.

Other zinc values cited are: 0.29 mg/100g (Feeley et al., 1983), 1.60 and 1.05 ug/ml wet wt. for 1-3 and 4-6 months, respectively (Vaughan et al., 1979), 1.38 mg/l (Lonnerdal et al., 1984), 1.59 ug/ml (Picciano and Guthrie, 1976), 0.2-0.5 mg/100ml (Packard, 1982), 0.17 mg/100g fluid product (USDA, 1976), 2 mg/l (Hambraeus, 1977), 0.23 mg/100ml (Lauber and Reinhardt, 1979), 1.07 ug/ml (Neville et al., 1984), 1.10 ug/ml (Frannson and Lonnerdal, 1982), 0.5-1.0 ug/ml (Lonnerdal et al., 1981), and 0.72 mg/l (Vuori et al., 1980).

Dietary zinc does not play a role in determining the concentration of zinc in human milk (Packard, 1982; Vuori et al., 1980), regardless of whether supplements are taken or not (Anderson, 1985).

Methodology

Subjects:

Human milk samples were collected from 5 mothers who were participating in an Anthropometric/Pregnancy Study being conducted by the Department of Human Nutrition and Foods at VPI&SU.

Mature human milk samples were analyzed, and samples were collected at 2, 3, 4, 5, and 6 months postpartum or until the mother ceased nursing. The mothers were instructed verbally and in writing (Appendixes A & B) that samples were to be collected over a three day sequence as follows: Day 1-sample from morning feeding; Day 2-sample from mid-day feeding, and Day 3-sample from evening feeding. The participants were instructed to collect one sample per day, and to keep a 72-hour food recall during the milk collection period. Milk was expressed either manually or by use of a SunMark (Style 1013) Breast Pump. Participants were provided with polyethylene storage tubes, which held approximately 30 ml, and asked to fill tubes approximately 3/4 full. The tubes were to be labeled with the date, time, and name and then frozen until samples were brought to the laboratory. Samples were stored at -20 C until analyzed.

Analysis of Samples:

All glassware and storage bottles were washed with detergent and rinsed with tap water eight times followed by rinsing eight times with deionized water. This procedure was followed by acid washing, using a 20% nitric acid (v/v) solution. Glassware was completely filled with the acid solution and allowed to stand for 15 to 20 minutes. The acid solution was transferred to another piece of glassware, being discarded after 3 uses. Glassware was then rinsed eight times with deionized water and allowed to air dry.

Each set of three samples per month was compiled as follows. The samples were brought to approximately 20 C and mixed, until homogeneous, by inverting 10 times. Equal aliquots, of approximately 25 ml, were taken from the three monthly samples and transferred to an acid-washed storage bottle. The graduated cylinder, used to measure the aliquots, was rinsed with deionized water between each sample. The samples were mixed by inverting three times, once all three aliqouts had been combined (AOAC, 1980). All analyses were conducted in duplicate, except for moisture and total energy. The percent moisture was calculated after milk samples were freeze-dried (Appendix D). Total lipid concentration was determined by the Soxhlet procedure

(AOAC, 1970) on freeze-dried samples (Appendix E).

Using a fat-free sample, total nitrogen was determined by the macro-kjeldahl method (Appendix F). Total energy concentration (Appendix H) was determined on freeze-dried samples using a Parr Adiabatic Calorimeter (Moline, Ill).

For mineral analysis, fluid milk samples were wet-ashed (Clegg et al., 1981, Appendix G) and read on a Perkin-Elmer High Convenience Model 503 Atomic Absorption Spectrphotometer (Norwalk, CT), utilizing a 1-second integration time, a Burner 040-0146, and an air-acetylene flame.

For zinc and copper, samples were read undiluted, while calcium and magnesium were diluted 1:1,000 with 0.3% lanthanum. All samples were read at the proper lamp currents and wavelengths, as recommended by the manufacturer. All standards and samples were read in the absorbance mode, with concentrations being calculated from the absorbance readings by the use of linear regression equations. Each regression equation was obtained by using at least four standard concentrations, made in either 10% HCl or 0.3% lanthanum.

Statistical Analysis

The mean and standard deviation were determined for dietary intake during pregnancy, and monthly, for each of

the nutrients analyzed, excluding copper, and for dietary intake postpartum. The Statistical Analysis System was used to determine correlation coefficients between dietary intake during pregnancy and nutrient concentration in human milk, and between dietary intake postpartum and nutrient concentration in human milk. Correlations were considered significant if the probability was less than or equal to 0.05 a priori.

Multiple linear regression tests were performed to determine if significant changes in nutrient concentration in human milk occurred over the course of lactation.

Dietary recalls collected during pregnancy and postpartum were processed by the Nutritional Analysis System at Louisiana State University. The mean calories, protein (g), fat (g), calcium (g), magnesium (g), and zinc (g) for each 72-hour period were calculated.

Results and Discussion

Subjects:

Five healthy, Caucasian, lactating females 24 to 29 years of age (mean: 26.6 +/- 1.95 years) participated in the study. The women lived in or about the town of Blacksburg, Virginia. The subjects exhibited a mild activity level and had at least a high school education. The majority of the women were employed outside of the home and the average annual gross family income was $24,373 (Downing, 1986, unpublished data).

Human Milk Analysis

The monthly mean values and standard deviations for energy, total lipids, moisture, protein, and the mineral concentrations in human milk are presented in Table 1. Analysis of copper yielded trace amounts of the element in human milk, with the actual values ranging from 0.118 to 0.433 ug/gm.

Moisture:

The mean moisture content of 88.0% was similar to the moisture value of 87.1 ml/100ml as reported by Macy and Kelly (1961). The American Academy of Pediatrics (1981) reported a moisture content range of 85-95%, and the analyzed value fell within this range.

Table 1. Mean Monthly Values for Nutrient Concentrations in Human Milk

	MONTHS				
	2	3	4	5	6
Energy (kcal/ 100ml)	74.0 (17.9;5)*	65.6 (11.3;5)	63.4 (9.0;5)	63.8 (5.4;5)	66.8 (21.0;5)
Protein (gm/ 100ml)	1.5 (0.4;5)	1.5 (0.2;5)	1.4 (0.2;5)	1.4 (0.2;5)	1.3 (0.3;5)
Lipids (%)	3.71 (1.5;5)	3.71 (1.1;5)	3.44 (0.9;5)	3.58 (0.8;5)	4.00 (2.5;5)
Moisture (%)	87.8 (1.8;5)	88.1 (1.2;5)	88.3 (1.1;5)	88.2 (0.7;5)	87.8 (2.2;5)
Calcium (ug/mg)	304 (33.8;5)	275 (21.6;5)	267 (36.2;5)	256 (37.2;5)	226 (32.2;4)
Magnesium (ug/mg)	32.1 (5.1;5)	30.9 (4.3;5)	30.5 (3.7;5)	32.1 (2.5;5)	28.8 (1.8;4)
Zinc (ug/mg)	2.72 (0.4;5)	1.83 (0.1;5)	1.49 (0.3;5)	1.35 (0.2;5)	1.18 (0.1;4)

*Standard Deviation;Number of Observations

Table 2. Correlation Coefficients Between Dietary Intake During Pregnancy and Nutrient Concentrations of Human Milk

	Month				
	2	3	4	5	6
Energy	-0.29a 0.64b 5c	-0.29 0.64 5	0.01 0.99 5	0.33 0.58 5	0.76 0.13 5
Protein	0.36 0.56 5	0.24 0.69 5	-0.33 0.59 5	0.59 0.30 5	0.85 0.07 5
Lipids	0.13 0.84 5	-0.41 0.49 5	0.12 0.85 5	0.40 0.51 5	0.74 0.16 5
Calcium	-0.47 0.42 5	-0.34 0.58 5	-0.73 0.16 5	-0.42 0.48 5	-0.52 0.48 4
Magnesium	-0.48 0.41 5	-0.05 0.94 5	-0.39 0.52 5	-0.03 0.96 5	0.09 0.91 4
Zinc	0.16 0.80 5	0.50 0.39 5	0.07 0.91 5	-0.89 0.04* 5	-0.83 0.17 4

a correlation coefficients
b Prob > ±R± under HO:RHO=0
c Number of observations
* $p < 0.05$

Table 3. Correlation Coefficients Between Dietary Intake Postpartum and Nutrient Concentrations of Human Milk

	Month				
	2	3	4	5	6
Energy	0.91a 0.03b* 5c	-0.16 0.80 5	0.25 0.69 5	0.47 0.42 5	0.48 0.41 5
Protein	0.45 0.45 5	0.85 0.07 5	0.32 0.59 5	0.53 0.36 5	0.91 0.03* 5
Lipids	0.30 0.62 5	-0.71 0.18 5	0.21 0.73 5	0.18 0.77 5	0.27 0.66 5
Calcium	-0.24 0.69 5	0.32 0.60 5	-0.68 0.21 5	0.15 0.81 5	-0.01 0.99 4
Magnesium	-0.71 0.17 5	-0.09 0.89 5	-0.07 0.91 5	-0.66 0.22 5	0.41 0.59 4
Zinc	0.27 0.67 5	0.81 0.19 5	0.97 0.03* 5	0.57 0.43 5	0.50 0.50 4

a correlation coefficients
b Prob > ±R± under HO:RHO=0
c Number of observations
* $p < 0.05$

Table 4. Linear Regression Analysis of Nutrient Concentrations on a Monthly Basis

Nutrient	Type I SS	F Value	PR>F	Parameter	Estimate of Parameter	T for HO: Parameter	PR>±T±	Standard Error of Estimate
Ca*	1316.7	14.15	0.00	A+	335.009	17.60	0.0001	19.029
				B#	-17.236	-3.76	0.0011	4.582
Mg	10.7	0.82	0.38	A	32.857	14.77	0.0001	2.225
				B	-0.483	-0.90	0.3762	0.535
Zn	6.1	62.68	0.00	A	3.169	16.53	0.0001	0.192
				B	-0.365	-7.92	0.0001	0.046
Energy	131.2	0.71	0.41	A	73.200	9.00	0.0001	8.137
				B	-1.620	-0.84	0.4070	1.918
Moisture	0.01	0.00	0.94	A	87.972	104.22	0.0001	0.844
				B	0.014	0.07	0.9445	0.199
Lipids	0.1	0.05	0.82	A	3.509	4.20	0.0003	0.835
				B	0.045	0.23	0.8220	0.197
Protein	0.07	1.15	0.29	A	1.580	10.53	0.0001	0.150
				B	-0.038	-1.07	0.2937	0.035

*For all nutrients, DF=1
+A parameter=intercept
#B parameter=month

The mean monthly moisture values of 87.8, 88.1, 88.3, 88.2, and 87.7% (Table 1) were fairly constant over the course of lactation (Table 4). These results are consistent with Vaughan et al., (1979) results of 88.0% and 88.4% for 1-3 months and 4-6 months of lactation, respectively. The small change in percentage of moisture is also consistent with reports in the literature (Vaughan et al., 1979).

Energy

The mean energy content decreased from 74 kcal/100ml to 67 kcal/100ml over the course of lactation. This decrease was not significant (Table 4). The mean energy content for the course of lactation was 67 kcal/100ml, which is consistent with the generally accepted value of 67 kcal/100ml (Butte et al., 1984).

The mean monthly energy values of 74.0, 65.6, 63.4, 63.8, and 66.8 kcal/100ml (Table 1) were slightly higher than the energy values of 0.68, 0.64, 0.62, and 0.64 kcal/g reported by Butte et al., (1984) for the first four months of lactation.

During the second and third months of lactation, a negative correlation was noted between dietary intake during pregnancy and energy content of human milk (Table 2). However, during the fifth and sixth months of lactation, a positive correlation was observed between

caloric intake during pregnancy and energy content of human milk. Although neither correlation was significant, this change in the relationship between maternal intake during pregnancy and energy content of human milk, may be a result of postpartum weight loss or a decreased caloric intake during the latter months of lactation (Table 5). Caloric intake for the postpartum period was comparable to the RDA (Table 5).

When correlation coefficients were determined for dietary intake postpartum and energy content of human milk, a significant positive correlation was noted for the second month of lactation (Table 3). Correlation coefficients for the next four months were not significant.

Protein:

The mean protein concentration in human milk of 1.4 g/100ml was slightly higher than the 1.1 g/100ml value reported by Macy (1949). The mean monthly values of 1.5, 1.5, 1.4, 1.4, and 1.3 g/100ml (Table 1) were slightly higher than Dewey and Lonnerdal (1983) values of 1.44, 1.33, 1.32, 1.30, 1.25, and 1.27 g/100ml for the first six months of lactation.

Protein values tended to by fairly constant (Table 4) during the lactation period. Such results are in agreement with results reported by Dewey and Lonnerdal (1983) and

Table 5. Mean Dietary Intake During Pregnancy and Lactation

Nutrient	RDA	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Energy (kcal)	2500	2705.6 389.3	2672.6 402.3	2512.2 506.3	2470.4 535.3	2388.0 538.5	2506.0 481.5
Fat (g)		122.5 24.3	109.2 17.8	102.3 32.3	109.0 28.9	104.6 23.0	115.8 42.3
Protein (g)	64	92.1 14.3	94.8 23.0	106.1 42.2	95.6 29.4	90.6 24.7	90.7 29.3
Calcium (mg)	1200	1343.4 470.6	1233.8 340.7	1837.2 1661.2	1138.0 482.3	1278.0 510.4	1158.3 555.6
Magnesium (mg)	450	359.0 100.0	421.8 125.0	406.2 125.0	360.4 124.3	347.0 125.4	336.4 107.1
Zinc (mg)	25	13.6 1.5	13.9 4.3	14.3 5.4	15.1 6.8	12.6 5.1	13.5 5.1

Month 1=values during pregnancy

Kader et al., (1972).

Although not significant, dietary intake during pregnancy was positively correlated with protein concentration in human milk (Table 2). This positive correlation may be explained by the present theory that the macronutrients of human milk are derived primarily from endogenous synthesis (Anderson, 1985).

The correlation between protein consumption postpartum and protein content in human milk was significant during the sixth month of lactation (Table 3). For months 2-5, no significant correlation was noted. The results agree with Butte et al., (1984) and American Academy of Pediatrics (1981), that maternal diet during lactation does not influence protein concentration in human milk.

Lipid:

The mean total lipid content for the five month period was 3.67%, which is within the range of values cited in the literature. These values are: 2.69% (Lonnerdal et al., 1984), 3.04% (Lonnerdal et al., 1976a), 3.07 g/100ml (Lauber and Reinhardt, 1979), 3.39% (Neville et al., 1984), 4.72% (Fransson and Lonnerdal, 1980), and 5.83% (Fransson and Lonnerdal, 1982).

The monthly total lipid means of 3.71, 3.71, 3.44, 3.58, and 4.00% (Table 1) were slightly lower than the

Dewey and Lonnerdal (1983) and Finley et al., (1985b) values of 4.92, 4.58, 4.58, 4.62, 4.36, and 4.30 g/10ml, and 4.0, 4.2, 4.0, 4.3, 3.8, and 4.0%, respectively, for the first six months of lactation. Although Dewey and Lonnerdal (1983) reported a decrease in the lipid concentration for the first six months of lactation, the findings of this study indicate no significant change in concentration during lactation (Table 4).

As for the dietary influence on the concentration of lipids in human milk, food intake during pregnancy was not correlated with lipid concentration for the first three months of this study. However, during the fifth and sixth months, a positive correlation was noted with dietary intake during pregnancy (Table 4). This may be explained by the current theory that the macronutrients of human milk are derived primarily from endogenous synthesis (Anderson, 1985). Therefore, by the fifth and sixth months of lactation, maternal body stores may be synthesizing the macronutrients.

The correlation between dietary intake postpartum and lipid content in human milk was negative for the third month of lactation (Table 3). During the other four months, no correlation was observed between lipid content in human milk and dietary intake. These results agree with the

literature, that dietary intake does not effect the total amount of lipids in human milk (Anderson, 1985; Butte et al., 1984).

Calcium

The mean calcium concentration of 268 mg/gm was slightly higher than the 241.2 ug/ml reported by Frannson and Lonnerdal (1980). The monthly values of 304, 275, 267, 256, and 226 mg/gm (Table 1) decreased significantly over the course of lactation (Table 4). This decrease is consistent with the decrease in calcium values reported by Finley et al., (1985b) of 6.0, 6.1, 5.9, 5.3, 5.6, and 5.5 mmol for the first six months of lactation.

Over the course of lactation, calcium concentration in human milk tended to be negatively correlated with calcium intake during pregnancy (Table 2). However the correlation was not significant. No correlation was noted between dietary intake postpartum and calcium content in human milk (Table 3), which is the same conclusion as Anderson (1985), Packard (1982), and Sims (1978) reached. A possible explanation why dietary intake does not influence the calcium content in human milk, is that the calcium may be metabolized from body stores (Anderson, 1985), and current dietary intake would have little effect on the calcium concentration.

Magnesium:

The magnesium mean value of 31 ug/gm was slightly higher than the 28.3 mg/l value reported by Lonnerdal et al., (1984). No significant change was observed in the monthly mean values of 32.1, 30.9, 30.5, 32.1, and 28.8 ug/gm (Table 1). These results differ from Dewey and Lonnerdal (1983) who reported a significant increase from 27.6 to 33.9 ug/ml for the first six months of lactation.

A significant correlation was not observed between dietary intake during pregnancy and magnesium content in human milk (Table 2). However, a negative correlation was noted between magnesium concentration in human milk and maternal intake postpartum (Table 3). Such results are consistent with Anderson's (1985) conclusion that magnesium concentration in human milk is not influenced by maternal dietary intake.

Zinc:

The overall mean zinc value of 1.74 ug/gm was similar to the values cited in the literature: 0.2-0.5 mg/100ml (Packard, 1982), 0.17 mg/100ml fluid product (USDA, 1976), and 2 mg/l (Hambraeus, 1977).

The mean monthly concentrations of 2.72, 1.83, 1.49, 1.35, and 1.18 ug/gm (Table 1) decreased significantly over

the course of lactation. Dewey and Lonnerdal (1983) and Finley et al., (1985) reported declining values of 2.71, 1.67, 1.35, 0.89, 0.59, and 0.64 ug/ml, and 5.5, 3.1, 2.0, 2.1, 1.8, and 1.4 umol, respectively, for the first six months of lactation.

Zinc intake during pregnancy was not correlated with zinc concentration in human milk (Table 2). With regard to zinc intake postpartum and zinc content in human milk, a significant positive correlation was observed in the fourth month of lactation (Table 3). A positive correlation was noted in the third month of lactation. No correlation was observed during the other months of the study. Dietary zinc does not appear to play a role in determining the concentration of zinc in human milk, a finding which is consistent with previous reports (Packard, 1982; Vuori et al., 1980).

When interpreting the results of this study, it is important to keep in mind the small sample size and the difficulty in obtaining significant results with such a sample size. Also, the significant results found may have occurred by chance.

Summary and Conclusions

Five healthy, Caucasian, lactating women collected human breast milk samples 3 consecutive days per month for months 2-6 of lactation. Monthly samples were composited and proximate analyses were conducted on the samples. Each month the subjects completed a 72-hour dietary record starting at the twelfth week of pregnancy and continuing until the sixth month postpartum. The food records completed during the lactation period coincided with the milk collection period.

The moisture content of human milk remained fairly constant over the study period and the mean moisture content was 88.0%.

The mean energy content decreased from 74 kcal/100ml to 67 kcal/100ml over the course of lactation, but the decrease was not statistically significant. The mean energy value of 67 kcal/100ml is in agreement with the generally accepted norm of 0.67 kcal/g. Dietary intake during pregnancy was negatively correlated with the energy content of human milk in the first part of lactation, while being positively correlated during the latter part of pregnancy. A significant correlation was observed during the second month of lactation between energy content in human milk and dietary intake during

lactation. No other significant correlations were noted. The protein content of human milk was fairly constant during lactation with a mean protein concentration of 1.4 g/100ml. The mean protein concentration determined in this study was slightly higher than values reported in the literature. Dietary intake during pregnancy was positively correlated with protein content in human milk. The correlation between protein intake during lactation and protein content of the milk was significant only for the sixth month of lactation.

The total lipid content increased from 3.71% to 4.00%, however, the increase was not significant. The mean total lipid concentration of 3.67% was similar to values cited in the literature. A positive correlation was noted in the fifth and sixth months of lactation with fat intake during pregnancy and lipid content of human milk.

The calcium concentration decreased significantly from 304 ug/gm to 268 ug/gm over the course of lactation. Such a decline is consistent with results from other researchers. A negative correlation was noted between calcium intake during pregnancy and calcium concentration in human milk, while no correlation was observed between intake during the lactation period and calcium content in human milk.

Magnesium concentrations showed no significant change over the course of lactation, although values decreased slightly from 32.1 ug/gm to 28.8 ug/gm. No significant correlation was noted between magnesium content in human milk and dietary intake during pregnancy. The intake during the lactation period was negatively correlated with the content in human milk.

Zinc concentration decreased significantly during the lactation period and such results are consistent with other results. Zinc intake during pregnancy was not correlated with zinc content in human milk. During the third month of lactation, a positive correlation was noted between intake during lactation and concentration in human milk. A significant positive correlation was observed during the fourth month of lactation between dietary intake during lactation and zinc content of human milk.

One possible explanation for the lack of significant changes or correlations may be the small sample size. Another explanation is that the subjects of this study were well-nourished, consuming RDA levels of all nutrients except magnesium and zinc.

In future studies, a larger sample size would be desirable. Another aspect which was not done in this study, but should be taken into consideration for future work, is

measuring total volume of milk produced in a 24-hour period. This would provide information regarding nutrient concentrations being consumed by infants in a 24-hour period. Such information would be helpful in determining infants' requirements.

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Appendix A

Breast Milk Composition Study
General Information Sheet

1. Samples are to be taken at months 1,2,3,4,5, and 6 post-partum or until the mother ceases nursing.

   Samples are to be taken on 3 consecutive days (1 sample each day) as follows:
   Day 1- morning feeding
   Day 2- mid-day feeding
   Day 3- evening feeding

2. Milk may be expressed manually or by the use of a breast pump.

3. Samples are to be stored in large plastic tubes with stoppers that are provided in the kit. Tubes are to be filled 3/4 full. Store in freezer immediately after collection.

4. Time collection of breast milk samples to coincide with 72 hour food recall.

5. Bring milk samples with you when you come to campus for regular monthly visits. Samples may be left at Solitude.

6. Keep a record of when food is introduced to your infant. We will need a food intake record for the infant (if he is on solid food) for the days that breast milk samples are collected.

7. If you have questions or problems contact:

   Dr. Janette Taper
   Leslie Reynolds
   Jim Raville

Appendix B

Instructions for Expression of Milk: Manually Expression

For purposes of this research project we will always use the fore milk (first milk obtained at the onset of feeding). The milk samples, therefore, must be collected before the baby nurses.

In your kit you have a sterile glass beaker. It is for you to collect the milk in. It has been specially cleaned so that it is free of mineral contamination. You also have 3 large plastic tubes with stoppers to store samples in, and a large container of deionized water to rinse the beaker with.

To manually express the milk:

1. Wash your hands and nipple with deionized water provided in the kit.

2. Hold the clean glass beaker under the breast.

3. Then place the thumb and the first two fingers about 1" to 1 1/2" behind the nipple. Position the thumb above and the fingers below the nipple.

4. Then push backward towards your chest.

5. Now roll the thumb and finger forward. This rolling motion is the kind you would use to make fingerprints.

6. Repeat this sequence with your right hand and then your left hand counterclockwise around your nipple. Avoid squeezing the breast, pulling out the nipple and breast, or sliding your thumb and fingers on the breast.

For each breast milk sample collect the milk into the beaker and pour into one of the tubes that is in the kit. Fill the tube 3/4 full. Stopper it with the lid that is provided with it. Date the tube and also report the time the sample was taken. (Use the Sharpie pen provided in the kit to write on the tube). Place sample in the freezer immediately.

Appendix B cont.

Rinse the beaker with the deionized water that is provided in the kit. Allow the beaker to air-dry. Do not use soap or other water.

Appendix B cont.

Expressing Milk with a Breast Pump

For the purpose of this research project we will always use the fore milk (first milk obtained at the onset of feeding). The milk sample, therefore, must be collected before the baby nurses.

In your kit you have been provided with a breast pump. It is made of plastic and has been checked in the lab and found to be free of mineral contamination. In the kit you also have 3 large plastic tubes with stoppers to store the samples in, and a large container of deionized water to rinse the breast pump with.

Use of the breast pump:

1. Wash your hands and nipple with the deionized water provided in the kit and pat dry before using the breast pump.

2. Attach bulb to plastic piece if it is not already attached.

3. Squeeze bulb to expel air and then cover nipple with the open end of plastic reservoir (bulge side down to catch the flow of milk).

4. Slowly and gradually release pressure on bulb with a gentle pulsating action, allowing bulb to expand and draw milk into reservoir. Be careful not to let milk enter bulb. (If milk enters bulb it cannot be used as part of sample-discard it).

5. Empty reservoir as necessary into one of the tubes that has been provided in the kit. Continue until you have the tube 3/4 full. Stopper the tube with the lid that is provided with it. Date the tube and also record the time that the sample was taken. (Use the Sharpie pen provided in the kit to write on the tube). Place the sample in the freezer immediately.

Rinse the plastic part of the breast pump with the deionized water that is provided in the kit. Allow to air dry. Do not use soap or other water.

Appendix C

Breast Milk Composition Study
Consent of Participation Form

During the first few months of life infants grow very rapidly and have requirements for protein, energy, and minerals that are higher per unit of body weight than at any other time in infancy or childhood. Human breast milk is able to meet these needs. The composition of human breast milk is dependent upon many factors but the most important factor is the nursing mother's food intake.

The purpose of this study is to:

1. Analyze protein and energy density of breast milk sample and relate this to mother's food recall.

2. Analyze mineral composition of breast milk by measuring concentrations of zinc, copper, calcium, and magnesium and relate this to mother's food recall.

3. In retrospect, examine changes in the composition of the breast milk over time (from months 2 to 6 post-partum).

4. In retrospect, relate changes in breast milk composition to the addition of solid foods to the infant's diet (if possible).

I have received an explanation of the Breast Milk Composition Study to be conducted at Virginia Tech in the Department of Human Nutrition and Foods. The project will be directed by Dr. Janette Taper, faculty member, in the Department of Human Nutrition and Foods.

I understand that I will be asked to give a breast milk sample each day for three consecutive days that will coincide with the 3 24-hour recalls that I am already taking. On the first of the 3 days the sample will be taken at the morning feeding; the second day at the mid-day feeding; and the third day at the evening feeding. I understand that I can bring the 3 milk samples with me when I come to campus for my regular monthly visits (months 1-6).

There are no potential risks to this study. The

Appendix C cont.

benefits of my participation are that I will be contributing to the base of information that is available on breast milk composition over time.

I understand that I am free to withdraw from the study at any time. I understand that all information will be considered private, will be treated in a confidential manner, and will not be revealed so as to cause embarrassment. Dr. Taper or one of the other members of the research staff will be free to answer any questions I may have regarding this study.

Understanding the above, I agree to participate in the Breast Milk Composition Study to be conducted at Virginia Tech.

____
Signature of Subject

____
Social Security Number

____________ ________________________
Date Signature of Interviewer

Principal Investigator: Dr. L. J. Taper

Appendix D

Moisture Determination

Approximately 20 grams of milk sample was weighed out into a rinsed, diSPo Cup. The milk samples were freeze-dried for 31 to 48 hours, using a Virtis (Model Number 10-MR-TR, Gardiner, NY) Freeze-drier.

After freeze-drying, samples were weighed, transferred to small plastic storage containers and stored in a dessicator.

The percent moisture was calculated using the following formula:

$$\% \text{ Moisture} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100$$

Appendix E

Soxhlet Fat Extraction Method for Total Fat

Approximately 1 gram of freezed-dried sample was weighed onto Whatman No.1 Filter Paper, which was then folded and paper clipped.

The principle of this procedure is to heat petroleum ether to boiling, and condense the petroleum ether vapors, which drip over the samples. After the condensed petroleum ether level raises above the level of the samples, the ether is drained off and carries the fat with it. The boiling/condensing/draining cycle is continuous.

The samples were allowed to run for 48 hours.

After this process, the petroleum ether was allowed to evaporate off, and the samples were dried in a drying oven, at 60°C, overnight.

The following formulas were used to calculate the percentage of lipid on a wet basis:

$$\%\text{ lipid dry} = \frac{\text{dry sample wt. - extracted sample wt.}}{\text{dry sample wt.}} \times 100$$

$$\%\text{ lipid wet} = \%\text{ lipid dry} \times \frac{\text{dry weight of sample}}{\text{wet weight of sample}}$$

Appendix F

Modified Kjeldahl Procedure for Nitrogen and Protein

A fat-free sample of approximately 0.5 gm was used for the determination. Approximately 10 gm of a Na_2SO_4 (anhydrous)-$CuSO_4$ mixture was added to each flask. Glass beads and 25 ml of reagent grade concentrated sulfuric acid were added.

Samples were digested on a Technicon BD-20 Heating Unit for approximately three hours. For each run, two blanks, containing the Na_2SO_4-$CuSO_4$ mixture and concentrated sulfuric, were digested.

Samples were allowed to cool before 150 ml of distilled water was added.

For the distillation procedure, approximately 1/16 tsp of granular zinc, followed by 90 ml of 50% NaOH, was added to each flask.

Receiving flasks contained 25 ml of 4% boric acid, 25 ml of water, and 3-4 drops of methyl-red/bromcresol green indicator.

Distillation was continued until approximately 225 ml had been distilled.

Each sample was then titrated with 0.1N HCl to a pale pink color.

The milligrams of nitrogen per gram of sample were calculated using the following formulas:

Normality of acid X 14.00=mg of N equivalent to 1 ml of acid
C factor=Normality of acid X 14.00
Total N=(ml HCL - ml blank) X C factor
mg N/gm=Total N $\div$ sample weight(gm, dry basis)
mg Protein/gm=mgN/gm X 6.25

All values were reported on a wet basis.

Appendix G

Mineral Determination: Wet Ash Procedure

For mineral analysis, approximately 2 ml of human milk was weighed into acid washed beakers and covered with acid washed watch covers. Milk samples were dried overnight in a drying oven at 70°C.

Samples were digested in 2 ml of redistilled HNO and 1.5 ml of 70% $HClO_4$. For each run wet ashed, two blanks containing only wet ashing agents were ashed.

All samples were digested on a Thermolyne 2200 Hot Plate (Sybron Corp.). The initial temperature setting was 250°F, slowly being increased to a maximum temperature of 325°F. Samples were digested for 10 hours, until a white ash was formed. Once samples had cooled, 5 ml of 10% HCl was added to dissolve the white ash. Samples were allowed to stand over night; after which, samples were transferred to Sarstedt Test Tubes (No. 55.518).

Appendix H

Energy Determination

Bomb Calorimeter Energy content was determined using a Parr Adiabatic Calorimeter (Parr Instrument Company, Moline Il).

Approximately 1 gm of freeze-dried sample was formed into a pellet with the Parr Pellet Maker (Moline, Il).

A crucible was weighed, and all pellets were re-weighed on a Mettler H20T (Scientific Products, Evanston, Il) balance before each bombing.

The oxygen bomb chamber was assembled by attaching a Parr 45C10 nickel-chromium fuse wire to the metal electrodes of the upper section of the bomb. The crucible was placed in the holder, such that the fuse wire was in contact with the sample and not the crucible. The upper section of the bomb was inserted into the lower section of the chamber making sure the collar is securely tightened and the release valve is closed. The chamber was filled with O_2 to 30 atomspheres of pressure.

The readied chamber was placed into the water bucket and the electrodes were attached to the chamber. Two liters of tepid water were added to the water bucket. The cover was slid into place and the thermometer was lowered into the water. The instrument was turned on and the water was allowed to equilibrate for three minutes. The temperature was recorded and this was the initial temperature reading

At this point the sample was ignited and timed for eight minutes. After this time period, the instrument was turned off and the final temperature was recorded.

To remove the chamber, the thermometer was raised and the cover removed. The electrodes were disconnected and the bomb chamber was removed from the water bucket. The release valve was opened part way to release the oxygen. The water was emptied from the water bucket and some ice was added so water would be ready for the next bombing.

Once the oxygen has been released, the chamber was disassembled. Any unburnt fuse wire was removed, measured, and length was recorded as the wire correction value.

Appendix H cont.

Using deionized water, rinse the upper section of the chamber, the crucible, and the lower section of the chamber into a glass beaker. A methyl-red indicator was added to the rinsings and titrated with 0.0709N sodium carbonate solution. This value was the acid correction value.

Concentrations were calculated according to the following equations:

Final temperature-Initial temperature=Net temperature rise
Net temperature rise X Water equivalent=Total heat liberated
Acid correction + wire correction=Total correction
Total heat liberated - Total correction=Net heat liberated
Net heat liberated - Sample weight=amount calories/gm

Concentrations were converted back to wet wt. basis, and reported as kcal/gm of wet sample.

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