COMPOSITION OF HUMAN MILK AND ITS INFLUENCE ON THE GROWTH OF PREMATURE INFANTS

by

JOAN MARGARET SMITH

A THESIS
PRESENTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF FOODS AND NUTRITION
UNIVERSITY OF MANITOBA
WINNIPEG, MANITOBA

JANUARY, 1985

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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ABSTRACT

The composition of human milk fed 20 premature infants (less than 1500g), their rates of growth, incidence of hyponatremia and bone mineralization were studied during the first six postnatal weeks. Analysis of aliquots of human milk from each feeding showed that milk from mothers delivering prematurely (PT) contained significantly greater concentrations of total nitrogen (TN), nonprotein nitrogen (NPN) and sodium, and lower concentrations of calcium than mature milk donated to the nursery (D). No differences were found in fat, lactose, energy and phosphorus concentrations. The composition of PT/D mixtures was similar to the PT milk. The TN concentrations in PT milk decreased significantly from 377 mg/dL during week one to 297 mg/dL during week two to 247 mg/dL during week three and then remained stable. The six week overall mean concentration of TN was significantly greater for PT milk, 260 mg/dL than in D milk, 159 mg/dL. Mean NPN concentrations were also significantly greater in PT milk, 33.6 mg/dL than in D milk, 25.5 mg/dL. The fat concentrations in PT milk increased significantly from 1.8 g/dL during week one to 3.4 g/dL during weeks two and three to 4.3 g/dL during week six. There was no significant difference in the overall mean fat concentrations between PT milk, 3.5 g/dL and D milk, 3.9 g/dL. Lactose concentrations in PT milk increased significantly from 6.3 g/dL during week one to 7.0 g/dL during week four, but over the six week period did not differ between PT milk, 6.8 g/dL and D milk, 6.9 g/dL. Energy content reflected fat concentrations and for PT milk increased significantly from 55.2 kcal/dL during week one to between 67.0 to 75.1 kcal/dL during weeks two to six. There was no difference in mean energy content between PT milk, 68.8 kcal/dL and D milk, 69.3

kcal/dL. The PT milk contained significantly greater concentrations of sodium, 8.4 meq/L, than D milk, 4.5 meq/L. The mean calcium concentration in PT milk, 37.9 mg/dL was significantly lower than in D milk, 43.2 mg/dL but mean phosphorus concentrations in PT and D milk, 8.9 mg/dL and 8.8 mg/dL respectively, were similar. Rates of weight gain of the 20 premature infants increased rapidly during the first two weeks of life and then stabilized at 18.5 g/kg/d; with an energy intake of 141 kcal/kg/d and a protein intake of 3 g/kg/d. The positive linear correlation ($r=0.82$) between weight gain and protein intake was highly significant ($p<0.01$). Weight gain of the infants did not approach intrauterine weight gain until the sixth postnatal week. Infants fed their own mothers' milk regained their birthweight in 14.3 days while infants receiving human milk and formula mixture required 17.9 days, however six weeks mean weight gains were similar for both groups. Hyponatremia (serum sodium <130 meq/L) occurred in 8 of 19 infants in the study. The gestational age, 29.4 weeks, and sodium intake from milk alone, 0.9 meq/L, of the hyponatremic infants were significantly lower than the gestational age, 31.4 weeks, and sodium intake, 1.3 meq/L, of the normal infants. Using radiographs of the radius-ulna, 5 infants were assigned to a normal bone mineralization group, 13 infants to a delayed bone mineralization group and one infant to an overt rickets group. The mean daily intakes of energy, calcium and phosphorus for infants with normal bone mineralization were significantly greater than the intakes for infants with delayed bone mineralization or overt rickets, however intakes of calcium and phosphorus did not approach intrauterine accretion rates in any group.

Acknowledgements

I wish to thank Dr. B.M. Watts, my thesis advisor, for her guidance and encouragement throughout the course of this research project. Appreciation is also expressed to Dr. M.M.K. Seshia and Dr. G.P. Sevenhuysen for their direction and assistance and to Dr. B. Johnston for his help with the statistical analysis.

The students and staff of the department were a source of stimulating ideas and are thanked for many useful discussions. Specifically, I would like to thank Marilyn Latta and Stacy Johnson for their technical assistance with the nutrient analysis.

The cooperation of the staff in the Intensive and Intermediate Care Nurseries at the Health Sciences Centre was most appreciated, with special thanks extended to Lorna Campbell and Valerie Debooy.

Finally, I extend my sincere appreciation to Earle without whose constant encouragement and support, this thesis would never have become a reality.

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Chapter 1

INTRODUCTION

Intensive care provided by specialized neonatal units has increased survival rates for low birthweight (LBW) preterm infants. This dramatic increase in survival rates undoubtedly has occurred because of advances in knowledge and techniques of treatment of these premature infants. The advances relate to management of respiratory failure, maintenance of energy, fluid and electrolyte balance, and control of body temperature. The role of nutrition in this improved outcome has not been adequately evaluated, although it is known that early nutrition has important consequences on growth and neurological development. The Committee on Nutrition of the American Academy of Pediatrics (1977) has stated that:

> "The optimal diet for the LBW infant may be defined as one that supports a rate of growth approximating that of the third trimester of intrauterine life, without imposing stress on the developing metabolic or excretory systems."

Heird (1977) while recognizing that this may be a suitable goal, pointed out that such rates of growth are seldom achieved, and that a less rapid weight gain does not appear detrimental to the infants later development. Controversy has centered recently on the comparative benefits of human milk and formula feedings, but these discussions have been complicated by the lack of agreement on criteria to be applied to determine the adequacy of feeding regimes.

The benefits of breast milk for newborns during the first months of life have been emphasized over the past ten years. In the report on the nutritional needs of LBW infants quoted above (American Academy of

Pediatrics, 1977) the desirability of breast milk for term and larger preterm infants was stressed. For the LBW preterm infant this report concluded that:

> "with adequate intakes human milk may be the superior feeding".

However, in a review of growth data for LBW preterm infants during their first three weeks of life, Fomon et al (1977) concluded that:

> "per unit of energy, concentrations of protein, calcium and sodium in human milk are inadequate for growing premature infants".

A number of researchers over the past 36 years (Gordon et al, 1947; Davidson et al, 1967; Babson and Bramhall, 1969; Goldman et al, 1969; and Davies, 1977) have questioned the nutritional superiority of breast milk for the feeding of the small premature infant, and have shown that those fed moderately high protein formulas had greater weight gains than those fed human milk. Fleishman and Finberg (1979) concluded that:

> "careful evaluation of the data suggest that there are deficiencies in the use of human milk as the sole nutrition for rapidly growing preterm infants".

The studies discussed above all compared the growth of infants fed formula with the growth of infants fed mature human milk. However, in the late 1970's it was shown that the milk of mothers delivering preterm had significantly higher nitrogen (protein) and mineral concentrations than mature milk (Atkinson et al, 1978 and 1980; Gross et al, 1980; Schanler and Oh, 1980). Comparison of growth rates and balance studies for LBW premature infants receiving their own mothers' milk, mature donor

milk and formula have also been reported (Atkinson et al, 1981). However, the nutrient composition of the milks has not been directly related to the growth of individual infants. Since the milk of mothers giving birth prematurely may be uniquely suited to the needs of the premature infant (Atkinson et al, 1978), LBW premature infants receiving their own mother's milk may have growth rates that can be considered optimal, and the composition of this preterm milk may provide the best available standard for premature infant feeding.

The objectives of this research were:

1. To determine the influence of milk source on the total nitrogen (protein), nonprotein nitrogen, fat, lactose, energy, sodium, calcium and phosphorus contents of the milks fed to small premature infants with birthweights less than 1500 g.
2. To determine the influence of protein and energy intakes on rates of growth of small premature infants.
3. To determine the influence of sodium intakes on the incidence of hyponatremia in small premature infants.
4. To determine the influence of calcium and phosphorus intakes on the incidence of delayed bone mineralization in small premature infants.

Chapter 2

REVIEW OF LITERATURE

Since human milk uniquely satisfies the nutritional requirements of full-term infants (Brady et al, 1982) and because it offers possible immunological advantages for the immature infant, it has been preferred for feeding the low birthweight (LBW) preterm infant. How human milk is biologically adapted to an infant's growth and development has been the key question from a nutritional point of view. The least that can be said is that man has survived on human milk. Although a departure from human milk may represent nutritional improvement, establishing that this is a real improvement without a later 'price to pay' is a major problem (Gaull et al, 1982).

Much of the current interest in feeding human milk to small premature infants centres around the possibility that human milk may aid in the prevention of infection and/or necrotizing enterocolitis, a severe disease closely associated with feeding and particularly characteristic of infants <1500 g (Avery, 1979). Human milk contains macrophages, immunoglobulins, complement components and lactoferrin, factors which may impart immune benefit to the infant (Brady et al, 1982). However in view of the digestive and metabolic immaturity of LBW premature infants, such characteristics of human milk as its low renal solute load, its easily digestible fat and its specific protein composition (higher amounts of cystine and taurine, which may be important amino acids for premature infants) may also be desirable features (Fomon et al, 1977).

2.1 Composition of Human Milk

The major nutritional components, water, protein, fat, carbohydrates and minerals, of human and cows' milk are recorded in Table 1, which has been compiled from several sources. Water

Table 1

MAJOR COMPONENTS OF HUMAN MILK AND COWS' MILK

Component	Human Milk			Cows' Milk
	USDA[1]	DHSS[2]	Brostrom[3]	Brostrom[3]
Water (g/dL)	87	87	87	87
Energy (kcal/dL)	70	70	70	67
Protein (g/dL)	1.03	1.07	0.9	3.5
Fat (g/dL)	4.38	4.2	2.7-4.5	3.5
Lactose (g/dL)	6.89	7.4	7.0	5.0
Minerals				
Sodium (meq/L)	7	7	7	25
Calcium (mg/dL)	32	33	34	120
Phosphorus (mg/dL)	14	15	15	96

[1]Composition of Foods (1976)

[2]Department of Health and Social Security (1977)

[3]Brostrom (1981).

constitutes approximately 87% of the total weight of both human and cows' milk. Similar amounts of energy are supplied by human and cows' milk, 70 and 67 kcal per deciliter (dL) respectively, but the distribution of energy between protein, fat and carbohydrate differs considerably between the two milks. The concentrations of sodium, calcium and phosphorus are greater in cows' milk than human milk.

Comparison of published reports on the nutrient composition of human milk is complicated because stage of lactation, methods of milk collection and methods of biochemical analysis are different in each report and these factors affect the interpretation of the reported data. Composition has been shown to vary with stage of lactation, with time of day, and with stage during feed. Stage of lactation is an important but often overlooked variable affecting milk composition. Colostrum or 'early' milk (produced on days one to five postpartum) and transitional milk (days five to ten postpartum) are higher in antibody-rich protein and in sodium (Macy et al, 1961) but lower in fat (Macy et al, 1953) and in lactose (Lonnerdal et al, 1976b) than mature human milk (produced after 30 days postpartum). Hall (1979) reported that although the concentration of protein remained constant during the day, the amount of fat rose from early morning (6 a.m.) reaching a plateau about midday (2 p.m.). The mean rise was about 2.5 fold and it has been suggested that these observations may be significant for the collection of human milk for milk banks. During the course of one feed composition also changes. Hall (1979) reported that for one feeding there was approximately a three-fold increase in the total lipid, a 1.3 fold increase in the concentration of protein and a 1.5 fold increase in the amount of dry weight in 'hind' milk when compared to 'fore' milk.

An additional factor which may affect the composition of the milk is the nutritional status of the mother. Malnourished mothers have been reported to have approximately the same proportions of protein, fat and lactose in their milk as well-fed mothers but to produce somewhat lower volumes of milk (Jelliffe and Jelliffe, 1978b). Neither protein quantity nor quality in human milk has been correlated to maternal protein intake (Atkinson, 1979), however, results of a study by Lonnerdal et al (1976b) suggested that the protein composition of milk from well-nourished mothers may vary considerably. The cause of this variation and its possible physiological significance require further investigation. Atkinson (1979), in a discussion of factors affecting human milk composition, stated that fat, both amount and type, seems to be the most variable component in human milk. Milk fat levels differ among women and among samples from the same woman and levels do not appear to be related to the amount of fat in the diet. However the fatty acid composition of human milk has been shown to be influenced by the relative contribution of fat and carbohydrate to total milk calories, and to reflect the fatty acid composition of the maternal diet (Aitchison et al, 1977).

When most of the mothers' dietary calories were derived from carbohydrates, the content of saturated fatty acids increased, and a diet rich in polyunsaturated fats caused an increased percentage of polyunsaturated fats in the milk without altering the total fat content (Lawrence, 1980). Guthrie et al (1977) studied the fatty acid patterns of human milk to determine the impact of the modern diet, with the significant change from animal to vegetable fat, on milk composition. The results showed a change in fatty acid content to more long chain

fatty acids and an increase in linoleic acid but no change in total fat in the milk.

Many of the earlier investigators who studied human milk composition, not realizing the importance of obtaining a representative sample, took samples at the beginning, middle or end of a feeding and at various times of the day. The Department of Health and Social Security (1977) in their sampling protocol, suggested that the entire contents from one breast, obtained by hand or by an electric pump, should be obtained to provide a representative sample for nutrient analysis. Gaull et al (1982) supported this sampling protocol. However Picciano and Guthrie (1976) stressed that a complete 24 h sample must be analyzed to obtain a representative estimate of fat content.

Treatment of samples after they have been collected can affect the nutrient levels determined. Samples should be analyzed fresh or after having been stored at -20^{o}C. Once thawed, samples should be analyzed immediately because the process of freezing and thawing activates lipases in milk and can result in low fat values (Jensen et al, 1978).

2.1.1. Total Nitrogen and Protein

The major nitrogen-containing components of human milk are proteins and free amino acids, while minor components include peptides, polyamines, nucleotides, creatinine, carnitine, urea, and a component amounting to 5 to 7% of the total nitrogen content of milk which is still unidentified (Gaull et al, 1982). The proteins found in milk provide a supply of nitrogen for growth, enzymes, hormones and immunologic agents (Fomon, 1974).

Protein content of milk can be calculated using the formula: Protein = 6.38 [total nitrogen (TN) - nonprotein nitrogen (NPN)].

Nitrogen is usually determined by Kjeldahl analysis. There is some controversy about the nitrogen conversion factor for milk and reported values for protein may represent 6.38 TN or 6.25 TN. Jenness (1979) has also pointed out that since a considerable proportion of the nitrogen in human milk is in the NPN category, failure to subtract this can result in erroneously high protein contents being reported. The formerly accepted value of 1.1 to 1.2 g protein per dL human milk was established on the assumption that the TN content of human milk was in the form of protein nitrogen (Macy et al, 1953). More recently Lonnerdal and coworkers (1976b) published data on nitrogen and protein in human milk, at various stages of lactation, based on amino acid analyses. The data, at one month lactation, indicated a 'true' protein content of 0.8 to 0.9%, which was in close agreement with protein calculated using the formula 6.38 (TN-NPN).

Fomon et al (1977), while recognizing that as much as 25% of the nitrogen in human milk may be present as NPN, contended that at least some NPN, for example urea and free amino acids, may be utilized for synthesis of tissue protein. They suggested that 6.25 TN would provide an appropriate estimation of protein for comparing concentrations in human milk at various stages of lactation. Atkinson et al (1980) observed that protein nitrogen, calculated by taking the difference between TN and NPN, accounted for 82% of the TN in preterm (PT) milk over the first 29 days of lactation. G. Anderson et al (1981), in agreement with Fomon et al (1977), calculated protein as 6.25 TN. They claimed that 6.25 is a more appropriate factor than 6.38 because only 5 to 6% of the TN of human milk is nonamino acid nitrogen.

In this widely quoted paper Fomon et al (1977) suggested that human milk did not provide adequate quantities of protein and certain minerals

for the growth needs of the small, premature infant. Nutrient composition of milk of mothers giving birth prematurely had not then been investigated, but in 1978, Atkinson et al reported that the nitrogen concentration of milk from mothers giving birth prematurely was higher than that of milk from mothers giving birth at term. These results were based on 42 complete 24 hour collections of milk obtained from seven mothers giving birth at 26 to 33 weeks of gestation (PT) and 27 collections obtained from eight mothers giving birth at 38 to 40 weeks of gestation (FT). Mothers pumped their breasts by manual or mechanical methods. The PT mothers' milks had significantly higher nitrogen concentrations than the FT mothers' milks during the first month of lactation. Nitrogen concentration decreased significantly with progressing lactation at a similar rate in both groups. This study by Atkinson et al (1978) generated considerable interest and led to further studies designed to evaluate the nutritional composition of breast milk produced by mothers delivering premature infants (Table 2).

It should be emphasized that investigators of the nutritional composition of preterm, term and donor milks have used a variety of collection procedures which could have had a marked influence on the results obtained. Atkinson et al (1978, 1980) and G. Anderson et al (1981) collected complete 24 hour milk samples at specific day intervals from 2 to 29 days postpartum. Schanler and Oh (1980) analyzed aliquots of PT milk taken at each feeding from each day of collection, which had been pooled and frozen in batches corresponding to postpartum stage: 1-3, 4-6, 7-10, 11-13, 14-16, 17-21, 22-28 and 29-42 days. This milk was compared to donor milk from mothers who expressed their milk between one week and eight months postpartum. Gross et al (1980), collected morning milk samples on days 3, 7, 14, 21 and 28, while Gross et al

Table 2

PRETERM HUMAN MILK COMPOSITION STUDIES: MILK SAMPLE COLLECTION DATA

Reference	Number of Donors	Gestational Age of Infants (wk)	Collection Data: Duration of Study (d)	Collection Data: No. of Collections	Collection Data: Time of Collection
Gross et al (1980)	33	31.4	28	5	morning
Schanler and Oh (1980)	16	29.7	98	10	24 h
Atkinson et al (1980)	7	28.9	29	4	24 h
G.Anderson et al (1981)	17	28.5	29	4	24 h
Guerrini et al (1981)	25	33.3	30	7	24 h
Gross et al (1981)	12	30.0	28	5	24 h
Chan (1982)	15	35	31	3	morning
Lemons et al (1982)	20	33.0	56	6	24 h
D.Anderson et al (1983)	14	31.0	14	3	24 h

(1981) and Lemons et al (1982) collected 24 hour milk samples on specific postpartum days 3, 7, 14, 21, 28 and days 7, 14, 21, 28 and 42 respectively. D. Anderson et al (1983) obtained 24 h milk collections on postpartum days, 3, 7 and 14 from PT mothers and a single spot collection of milk from D mothers who were six to ten months postpartum at the time of milk collection.

Table 3 compares the data on the total nitrogen content of milk of mothers delivering prematurely. Gross et al (1980, 1981) found that concentrations of protein (6.38 TN) throughout the first month of lactation were significantly higher in milk from mothers delivering preterm (PT milk) than in mothers delivering at term (FT milk). Protein levels in PT milk on postpartum day 3 averaged 3.24 g/dL (508 mg/dL TN); however with progressing lactation protein concentrations decreased reaching average concentrations on day 28 of 1.81 g/dL (284 mg/dL TN). Schanler and Oh (1980) found that the TN content in the milk of mothers of premature infants was significantly higher during the initial two lactation weeks but regressed toward donor milk values, 199 mg/dL, by one month. Linear regression analysis of the pooled data for TN in the Atkinson et al (1980) study demonstrated a decrease in TN concentration at a similar rate in both the FT and PT milks over the first 29 days of lactation. Comparison of the data showed the PT milk was significantly higher in TN. In the G. Anderson et al (1981) study, protein content (6.25 TN) of both FT and PT milk decreased linearly with time. Protein concentration decreased significantly from interval to interval and was always higher in PT milk. Lemons et al (1982) documented a significant difference in the TN content of milk from mothers delivering prematurely versus mothers delivering at term. Total nitrogen and protein nitrogen were present in PT milk in higher concentrations than in FT milk. D.

Table 3

REPORTED MEAN VALUES FOR TOTAL NITROGEN CONTENT (mg/dL) OF PRETERM HUMAN MILK[1]

Reference	Days postpartum						
	1-3	4-6	7-10	14-16	17-21	22-28	29-42
Atkinson et al (1978)	359	346	331	303		259	
Atkinson et al (1980)	351	339	323	296		253	
Gross et al (1980)	508		382	340	287	284	
Schanler & Oh (1980)	470	311	281	226	219	211	211
G. Anderson et al (1981)		341	296	272		232	
Lemons et al (1982)			320	268	261	238	239
D. Anderson et al (1983)	400		320	270			

[1] Values for Atkinson et al (1978, 1980) and G. Anderson et al (1981) taken from Table 1 (MacLean and Graham, 1981). Values for Gross et al (1980) calculated from protein contents given in Table for days 3, 7, 14, 21 and 28. Values for Schanler and Oh (1980) taken from Table 2 for days listed. Data from Lemons et al (1982) are for days 7, 14, 21, 28 and 42. Data from D. Anderson et al (1983) are for days 3, 7 and 14.

Anderson et al (1983) found that the TN content in PT and FT milk varied in a similar way over the first 14 days of lactation. TN concentrations decreased significantly from 400 mg/dL at day 3 in PT milk to 270 mg/dL at day 14. These studies confirmed the earlier observations of Atkinson et al (1978) of a greater concentration of TN in the milk of mothers delivering preterm than in the milk of mothers delivering at term or mature human milk. The clinical significance of this difference has not been established.

2.1.2. Nonprotein Nitrogen

Nonprotein nitrogen (NPN) has been reported in four studies of the composition of milk of mothers of premature infants. Mean values for NPN found in three of these studies are reported in Table 4. Gross et al (1980) reported that NPN averaged between 15 and 19% of the TN concentration and remained constant during the first month of lactation. No mean values were reported. Atkinson et al (1980) found that although NPN in PT milk was on average a relatively constant 18% of the TN there was a significant decrease with time from 62 to 43 mg/dL over 4 weeks lactation. Schanler and Oh (1980) found similar NPN concentrations ranging from an initial 72.2 mg/dL at days 1 to 3 to 36.1 mg/dL at week 6 representing 15 to 18% of the TN in PT milk. Lemons et al (1982) reported much lower values of NPN in PT milk, a mean of approximately 33 mg/dL comprising approximately 13% of the TN. In three of these studies protein was precipitated by centrifugation with trichloroacetic acid and the supernatant analyzed for NPN (Gross et al, 1980; Atkinson et al, 1980; Lemons et al, 1982), while in one study NPN was determined from zinc sulfate deproteinated samples (Schanler and Oh, 1980). Concentrations of NPN were determined by the semimicro Kjeldahl method in all cases.

Table 4

REPORTED MEAN VALUES FOR NONPROTEIN NITROGEN CONTENT (mg/dL) OF PRETERM HUMAN MILK[1]

Reference	Days postpartum						
	1-3	4-6	7-10	14-16	17-21	22-28	29-42
Atkinson et al (1980)	62.0		57.1	52.2	47.3	43.0	
Schanler & Oh (1980)	72.2	50.3	49.4	38.4	38.6	32.6	36.1
Lemons et al (1982)			31.0	33.0	37.0	32.0	35.0

[1]Values for Atkinson et al (1980) calculated for days 7, 14 and 21 from regression equation. Values for Schanler and Oh (1980) taken from Table 2 for days listed. Data from Lemons et al (1982) are for days 7, 14, 21, 28 and 42.

The proportion of NPN compared to TN was higher in mature breast milk than in PT milk primarily because of the lower TN concentrations in mature milk. Lonnerdal et al (1976b) reported that NPN in milk from well-nourished mothers was present in constant concentrations throughout lactation ranging from 46 mg/dL at 0.5 to 1.5 months postpartum to 38 mg/dL at 3.5 to 6.5 months postpartum. This represented 24 to 26% of the TN concentrations, 193 to 148 mg/dL, reported for the above periods. Schanler and Oh (1980) reported a mean NPN concentration of 43 mg/dL in donated pooled milk or 23% of the TN concentration, 199 mg/dL and Atkinson et al (1980) reported that NPN concentrations were 28% of the TN in their study of mature milk.

2.1.3. Fat

Fat, which may contribute over 50% of the calories of human milk, provides essential fatty acids, the precursors of prostaglandins, fat soluble vitamins, sterols and phospholipids and probably acts as a satiety factor (Jensen et al, 1978). Ninety-eight percent of the fat in human milk is the form of triacylglycerols and the fat is more readily absorbed than that of cows' milk (Gaull et al, 1982). Because there is a lower stearic acid content in human milk than there is in cows' milk, and partly because palmitic acid is mainly esterified at the two position, fat absorption from breast milk is nearly complete (Guthrie et al, 1977; Jensen et al, 1978). Lipases, present in higher concentrations in human milk than in cows' milk, enhance the absorption of fat. Alemi et al (1981) found that infants fed a mixture of fresh human milk (40%) and formula (60%) excreted less fat than infants fed only formula. They concluded that decreased fat excretion was related to the lipases present in human milk and suggested that preterm infants

be fed their own mothers' milk if possible. Jensen et al (1982) urgently recommended that whenever possible preterm infants be fed raw breast milk in a manner that would stimulate sucking. Raw breast milk was recommended because heating milk destroys the natural lipase activity; and opportunity to suck was recommended because sucking stimulates the activity of the infants' own salivary lipases. Williamson et al (1978) reported that heat treating human milk reduced fat absorption by 33%.

Fat is a variable constituent of human milk both in its absolute quantity and in its composition. Average quantities of from 2.1 g/dL (Fomon, 1974) to 4.5 g/dL (Hambraeus 1977) have been reported. Fat content depends on stage of lactation, time of day, stage during feed and maternal dietary intake. Macy et al (1953) reported the fat content of colostrum as 2.9%, transitional milk as 3.6% and mature milk as 3.8%. Hall (1979) reported that diurnal variation influenced milk fat content which was lowest in the early morning and rose to a plateau about midday. She also reported that fat could change from 1.2% to 12.1% from the beginning to the end of one nursing. Maternal diet affected the fatty acid composition of human milk lipid but did not affect the total fat content in the study by Guthrie et al (1977).

Because of its variation in fat content, the milk used for fat analysis must be sampled carefully. Picciano and Guthrie (1976) stressed that a complete 24 h sample must be analyzed to obtain a representative estimate of fat content. In the study by Gross et al (1980) samples were collected in the morning by manual or mechanical emptying of both breasts. However, Gross et al (1981) and the other investigations on the composition of breast milk from mothers of preterm infants used 24 h expressions of milk.

Various analytical methods have been used to determine the fat

content of human milk. Because human milk contains glycerol ester hydrolase that will produce free fatty acids if the fat is not promptly removed (Jensen et al, 1978) extraction procedures to inactivate the lipase are usually employed. The fat content of milk can be estimated by the Babcock method; a wet ashing procedure using sulfuric acid as the oxidant. However, Gross et al (1980, 1981) determined fat content according to the Roese-Gottlieb technique, which uses extraction with ethyl ether-ethanol and results in values slightly higher than the Babcock method because some phospholipid is extracted. G. Anderson et al (1981) used a chloroform-methanol extraction followed by charring with sulfuric acid and colorimetric reading. The recovery of total lipids would be about the same with this and the Roese-Gottlieb method but the extraction used by G. Anderson et al (1981) would recover the phospholipids more effectively. Guerrini et al (1981) employed a turbidometric method whose reliability has been questioned by Dorea et al (1981), however, Nakai and Le (1970) found a high correlation of the turbidometric method with the Babcock method and recently Hundrieser et al (1984) found that the turbidometric method also correlated well with the Roese-Gottlieb method.

The fat concentrations in PT milk reported in the literature are extremely variable (Table 5). Milk samples collected on days 1 to 3 contained the low fat content typical of colostrum. Gross et al (1980) and D. Anderson et al (1983) reported mean fat concentrations of 1.6 g/dL for PT milk on postpartum day 3. Fat concentrations increased from these early samples to the day 7 collection when a range of 3.1 to 4.1 g/dL was reported by all researchers. G. Anderson et al (1981) reported that the fat concentration increased from an initial 3.0 g/dL at the 3 to 5 day interval to 4.3 g/dL at the 15 to 18 lactation day. Lemons et

Table 5

REPORTED MEAN VALUES FOR TOTAL FAT CONTENT (g/dL)

OF PRETERM HUMAN MILK[1]

Reference	Days postpartum						
	1-3	4-6	7-10	14-16	17-21	22-28	29-42
Gross et al (1980)	1.6		3.8	4.4	3.7	4.0	
G. Anderson et al (1981)		3.0	4.1	4.4		4.0	
Guerrini et al (1981)	2.4	2.6	3.3				3.5
Lemons et al (1982)			3.1	3.4	3.5	3.2	3.4
D. Anderson et al (1983)	1.6		3.5	3.9			

[1]Values for Gross et al (1980) taken from Table for days 3, 7, 14, 21 and 28. Data from G. Anderson et al (1981) are for days 3-5, 8-11, 15-18 and 26-29. Values for Guerrini et al (1981) were calculated from Table 1 for days 2-3, 4-6, 10 and 30. Data from Lemons et al (1982) are for days 7, 14, 21, 28 and 42. Data from D. Anderson et al (1983) are for days 3, 7 and 14.

al (1982) found that fat concentrations remained constant without any effect of duration of lactation but their first sample was taken on day 7 and was transitional milk.

G. Anderson et al (1981) reported a 20 to 30% higher concentration of fat in the PT milk when compared with FT milk at the same stage of lactation. Guerrini et al (1981) reported results in agreement with G. Anderson et al (1981) but other researchers found no effect of prematurity on fat concentrations. D. Anderson et al (1983) found the fat concentrations of spot donor milk specimens were highly variable ranging from 1.3 to 7.9 g/dL with a mean of 4.7 g/dL. This was comparable to previously reported values for mature human milk (Table 1).

2.1.4. Lactose.

There is an inverse relationship between fat and lactose content in the milk of mammals. Human milk represents one extreme with a high lactose concentration while the milk of sea mammals has almost no lactose but a very high fat content (Lonnerdal et al, 1976b). Lactose is one of the major components of human milk and by far its dominant carbohydrate, accounting for up to 90% of the 6 to 7 g carbohydrate/dL in human milk (Brostrom, 1981). Lactose, a disaccharide composed of galactose and glucose, which is synthesized by the mammary gland during lactation (Hambraeus, 1977), provides approximately 40% of the calories in human milk and ensures that there is a readily available source of galactose for the large and rapidly growing brain (Jelliffe and Jelliffe, 1978b). Lactose levels are constant throughout the day in a given mothers' milk, and even in poorly nourished mothers the levels do not vary (Lawrence 1980). Lactose digestion and absorption take place in the small intestine where hydrolysis by lactase gives readily

absorbed glucose and galactose. Lactase, an enzyme present in the gastrointestinal tract of nursing infants is specific for hydrolysis of lactose (Gaull et al, 1982) and lactase activity increases at term and decreases at weaning (Brostrom, 1981).

Gross et al (1980, 1981) found that during the first month of lactation, the concentrations of lactose were lower in milk from mothers delivering preterm (PT) than in milk from mothers delivering at term. In milk from PT mothers, lactose concentration was lowest on postpartum day 3 and increased with progressing lactation to the highest concentration on postpartum day 28 (Table 6). In the 1981 study, Gross et al found that the lactose concentration increased as milk volume increased with progressing lactation. This finding is in agreement with Lawrence (1980) who stated that lactose is influential in controlling volume. G. Anderson et al (1981) also found a significant increase in lactose concentration over the first 29 days of lactation; in PT milk the initial lactose concentration of 5.04 g/dL increased significantly to 5.97 g/dL by the 26 to 29 day stage. Lemons et al (1982) could not verify the lower concentration of lactose in PT milk during the first month of lactation, and reported that there was no significant effect of prematurity or duration of lactation. D. Anderson et al (1983) found that lactose content in PT milk remained constant and reported concentrations of 6.2 g/dL on day 3 and 7.0 g/dL on day 14.

The lactose concentrations reported for PT milk show some variability between researchers probably reflecting differences in methodology. The methods of lactose determination which have been used in the studies analyzing the nutritional composition of milk from mothers of premature infants include an automated colorimetric procedure using lactose standards (Gross et al, 1980, 1981), an enzymatic method

Table 6

REPORTED MEAN VALUES FOR LACTOSE CONTENT (g/dL) OF PRETERM HUMAN MILK[1]

Reference	Days postpartum						
	1-3	4-6	7-10	14-16	17-21	22-28	29-42
Gross et al (1980)	5.96		6.05	6.21	6.49	6.95	
G. Anderson et al (1981)	5.04	5.49	5.63			5.97	
Lemons et al (1982)			6.38	6.86	6.71	6.79	7.21
D. Anderson et al (1983)	6.20		6.90	7.00			

[1]Values for Gross et al (1980) taken from Table for days 3, 7, 14, 21 and 28. Data from G. Anderson et al (1981) are for days 3-5, 8-11,15-18 and 26-29. Data from Lemons et al (1982) are for days 7, 14, 21, 28 and 42. Data from D. Anderson et al (1983) are for days 3, 7 and 14.

(G. Anderson et al, 1981), gas chromatography (Lemons et al, 1982), and enzymatic analysis of glucose and galactose after acid hydrolysis (D. Anderson et al, 1983). G. Anderson et al (1981) suggested that the low lactose concentration in PT milk may be a consequence of the higher nitrogen. Since both are a part of the water soluble fraction, the higher nitrogen may effectively decrease the lactose concentration of the milk.

2.1.5. Energy

The average energy content of human milk varies with the stage of lactation and appears to be primarily a consequence of fat concentration. Colostrom is characterized by more protein, less fat and less carbohydrate than mature human milk and the energy content during early lactation ranges from 48 to 51 kcal/dL whereas the range reported for mature human milk is 67 to 75 kcal/dL (Brostrom, 1981). Jenness (1979) reported the energy content of mature milk ranged from 60 to 75 kcal/dL and D. Anderson et al (1983) reported that the average energy concentration of spot donor milk specimens 73.1 kcal/d, was comparable to previously reported values for mature human milk.

The majority of the studies on PT milk have also reported low total energy concentrations during the first week of lactation (Table 7), probably a reflection of the low fat content. Gross et al (1980) reported that PT milk obtained on day 3 postpartum averaged 51.4 kcal/dL but rose to 67.4 kcal/dL on day 7 and remained constant for the remainder of the first month and G. Anderson et al (1981), reported energy content of 58 kcal/dL at the 3 to 5 day stage rising to 71 kcal/dL at the 8 to 11 day stage, and then remaining stable reflecting the changes in fat concentration. D. Anderson et al (1983) reported that energy in PT milk significantly increased over the first 14 days lactation from 49 kcal/dL at day 3 to 70 kcal/dL at day 14. However,

Table 7

REPORTED MEAN VALUES FOR ENERGY CONTENT (kcal/dL) OF PRETERM HUMAN MILK[1]

Reference	Days postpartum						
	1-3	4-6	7-10	14-16	17-21	22-28	29-42
Gross et al (1980)	51.4		67.4	72.3	65.6	70.1	
G.Anderson et al (1980)		58	71	72		71	
Lemons et al (1982)			73.9	74.6	73.8	73.3	70.1
D.Anderson et al (1983)	49		67	70			

[1]Values for Gross et al (1980) taken from Table for days 3, 7, 14, 21 and 28. Data from G. Anderson et al (1981) are for days 3-5, 8-11, 15-18 and 26-29. Data from Lemons et al (1982) are for days 7, 14, 21, 28 and 42. Data from D. Anderson et al (1983) are for days 3, 7 and 14.

Lemons et al (1982) whose first samples collected on day 7 were not influenced by the low fat concentration of colostrom reported an energy value of 73.9 kcal/dL.

A precise conversion factor for available energy from a milk diet for infants is difficult to determine as it can vary under different physiological conditions such as gestational age, postnatal age and illness. Gross et al (1980) calculated caloric concentration (kcal/dL) as the sum 4.27 kcal/d protein, 3.87 kcal/g lactose and 8.87 kcal/g fat. The energy in the study by G. Anderson et al (1981) was calculated from the measured concentrations of protein (6.25 TN), fat and lactose using the formula: Energy (kcal/dL) = 5.65 kcal/g protein + 9.25 kcal/g fat + 3.95 kcal/g lactose. These factors are based on the heats of combustion for the individual nutrients (Report of the Joint FAO/WHO Ad Hoc Expert Committee, 1973) and thus are comparable to bomb calorimetry values. As a check on the accuracy of the calculated energy values, G. Anderson et al (1981) used these factors to calculate the energy from the macronutrient composition of freeze-dried milk and compared the calculated values to energy values determined by bomb calorimetry. They found that calculated energy values averaged 97% of those determined by bomb calorimetry. However, Lemons et al (1982) found the mean energy content of the milk samples as determined by bomb calorimetry to be 18.5% greater than calculated energy content, but calculations were based on the physiological energy factors of 4.27, 8.79 and 3.87 for protein, fat and lactose respectively. The lower factors used by Lemons and coworkers would account, in part, for the discrepancy between calculated values and bomb calorimetry values. The mean total energy content of PT milk as determined by Lemons et al (1982) using the bomb calorimeter was slightly higher than the energy reported by other

researchers. This is interesting because the fat content reported by them is considerably lower than that reported by Gross et al (1980) and G. Anderson et al (1981).

D. Anderson et al (1983) found good agreement between calculated energy content and energy determined with bomb calorimetry. The values obtained by them with the two methods were not different when energy was calculated assuming 4 kcal/g protein, 9 kcal/g fat and 4 kcal/g carbohydrate. The reasons for the discrepancy between the total energy content as determined by bomb calorimetry and calculated energy content reported by Lemons et al (1982) were unclear. They suggested it might reflect the inclusion of all combustible substrate in the bomb calorimetry method or cumulative error in the measurement of individual substrates.

2.1.6. Sodium

Up to 30 percent of infants weighing <1200 g at birth may experience hyponatremia (serum sodium <130 meq/L) during the first weeks of life (Roy et al, 1976), possibly related to growth requirements and excessive urinary losses. Daily sodium intakes of 3 to 6 meq or more per kilogram may be required by some infants to avoid hyponatremia. The feeding of breast milk to low birthweight (LBW) infants has been associated with serum sodium as low as 110 meq/L (Kumar and Sacks, 1978). Sodium concentrations in human milk vary dramatically with the stage of lactation. Curran (1979) reported that the sodium content of human colostrum was 23.8 meq/L, of transitional milk was 11.2 meq/L and of breast milk samples after full lactation was established ranged from <2 to >20 meq/L with a median of 7 meq/L in 124 samples. Recent investigations offer data which indicate that there is an increased level of sodium in the milk from mothers delivering prematurely when

compared to milk from mothers delivering at term, during the early weeks of lactation.

Gross et al (1980) using milk samples collected in the morning found the mean sodium concentrations were significantly higher in PT milk than FT milk. The defatted samples were analyzed by atomic emission spectrophotometry. The highest concentrations of sodium were found in PT milk produced on postpartum day 3 and averaged 26.6 meq/L. (Table 8). Sodium concentrations decreased with progressing lactation to an average of 12.6 meq/L in PT on day 28. A later study by Gross et al (1981) using 24 h sample collections confirmed these results. Atkinson et al (1980) measured sodium in complete 24 h expressions of PT human milk by emission flame spectrophotometry. They found that the sodium concentration decreased significantly over time from 23 meq/L at the 3 to 5 day stage to 13.5 meq/L by days 13 to 17 and then remained constant over the 29 days observed.

Schanler and Oh (1980) determined sodium concentration on nitric acid filtrates of individual PT milk samples and pooled donor samples expressed between one week and eight months postpartum. Using a Beckman flame photometer they found a reduction in the sodium content of PT milk throughout the 98 d study. During the first three days there was a significantly elevated sodium content, 22.2 meq/L, but after six weeks of lactation there was a significantly lower sodium content, 7.6 meq/L, compared to the donated pooled breast milk, 11.2 meq/L. Sodium was also found in significantly greater concentration in the PT milk than the FT milk during the first four weeks of lactation in a study by Lemons et al (1982) where sodium was assayed by flame emission spectrophotometry on 24 h samples collected on days 7, 14, 21, 28, 42 and 56. The concentration of sodium declined in PT milk as lactation progressed,

Table 8

REPORTED MEAN VALUES FOR SODIUM CONTENT (meq/L) OF PRETERM HUMAN MILK[1]

Reference	Days postpartum						
	1-3	4-6	7-10	14-16	17-21	22-28	29-42
Gross et al (1980)	26.2		21.8	19.7	13.4	12.6	
Atkinson et al (1980)		23.0	19.0	13.5		10.0	
Schanler & Oh (1980)	22.2	16.6	11.6	9.0	10.6	8.8	7.6
Lemons et al (1982)			17.2	12.4	10.5	9.6	8.8

[1]Values for Gross et al (1980) taken from Table for days 3, 7, 14, 21 and 28. Data from Atkinson et al (1980) are for days 3-5, 7-10, 13-17 and 27-29. Values for Schanler and Oh (1980) taken from Table 2 for days listed. Data from Lemons et al (1982) are for days 7, 14, 21, 28 and 42.

however, levels similar to pooled mature milk (7 meq/L) were not reached until approximately eight weeks of lactation.

2.1.7. Calcium

Mineral deficiency disease has frequently been observed in infants of low birthweight (LBW). Fomon et al (1977) presented data to support the belief that growing premature infants fed human milk are at risk of developing signs of inadequate skeletal mineralization and it has been pointed out that breast milk with its low concentration of calcium cannot ever meet the predicted calcium requirements of the growing premature infant (Shaw, 1976). Reported values for calcium content of mature human milk vary widely from mother to mother and study to study with values of 20 to 35 mg/dL. The relationship of maternal diet to the calcium concentration of human milk is unclear since the calcium content of milk from poorly nourished mothers has been reported to range from normal levels to somewhat lower concentrations (Jelliffe and Jelliffe 1978a). As lactation progresses, there is a gradual decrease in milk calcium concentration with a decline of 30% after nine months postpartum reported by Gurr (1981).

Although PT milk may offer some advantage over pooled mature milk in terms of higher protein and sodium, it appears to contain insufficient amounts of calcium to meet the requirements of the LBW infant. Gross et al (1980) found that concentrations of calcium remained constant throughout the first month of lactation and were similar in milk from PT and FT mothers. Breast milk samples were collected in the morning and calcium content was determined on ashed samples by atomic absorption. The concentration of calcium in PT milk ranged from 20.4 mg/dL to 24.7 mg/dL (Table 9). Lemons et al (1982) also found that calcium concentrations were the same in PT milk, ranging

Table 9

REPORTED MEAN VALUES FOR CALCIUM CONTENT (mg/dL) OF PRETERM HUMAN MILK[1]

Reference	Days postpartum						
	1-3	4-6	7-10	14-16	17-21	22-28	29-42
Gross et al (1980)	20.8		24.7	21.9	20.4	21.6	
Atkinson et al (1980)		32.2	29.8	27.5		27.2	
Schanler & Oh (1980)	27.0	35.9	31.9	29.0	29.9	28.7	24.6
Lemons et al (1982)			29.3	26.6	28.2	28.2	31.0
Chan (1982)	30.0			30.0		28.0	

[1]Values for Gross et al (1980) taken from Table for days 3, 7, 14, 21 and 28. Data from Atkinson et al (1980) are for days 3-5, 7-10, 13-17 and 27-29. Values for Schanler and Oh (1980) taken from Table 2 for days listed. Data from Lemons et al (1982) are for days 7, 14, 21, 28 and 42. Data from Chan (1982) are for days 2-4, 14-21 and 26-31.

from 26.6 to 31.0 mg/dL, and FT milk, and that these did not change over the first eight weeks of lactation. Determination of calcium concentration was by means of sodium alizarin sulfonate which may account for the somewhat higher mean values than were reported in the study by Gross et al (1980).

Atkinson et al (1980) analyzed complete 24 h collections of PT and FT milk by emission flame photometry and found that the calcium content was similar during the first month of lactation. In PT milk, the calcium concentration at the 3 to 5 day stage was 32.2 mg/dL which not decrease significantly until the 13 to 17 day stage, when the mean value was 27.5 mg/dL. Chan (1982) used atomic absorption spectrophotometry to determine the calcium content of milk from 15 PT and 10 FT mothers. Total calcium levels of PT and FT milk decreased during the first month of lactation, however, there was no significant difference between the calcium contents of milks from the two groups. Schanler and Oh (1980) found the calcium content of premature infants' mothers' milk was higher throughout the first three months of lactation than was the calcium content of donated pooled milk, but differences were statistically significant only for the 4 to 10 day and 57 to 98 day periods of lactation. The overall mean total calcium content was higher in the PT milk, 29.3 mg/dL compared to 25.6 mg/dL in the pooled donor milk and there was no decrease in the calcium content of the PT milk over the first three months of lactation.

The amount of calcium in PT milk appears to be similar to previously reported values for mature milk. Ziegler et al (1976b) have demonstrated that intrauterine accretion rates of calcium exceed 100 mg/kg/d during the last eight weeks of pregnancy. Therefore, it may be anticipated that the preterm infant's own mother's breast milk may not

provide sufficient calcium to support normal bone mineralization in the rapidly growing premature infant.

2.1.8. Phosphorus

Human milk has been considered an inadequate source of calcium and phosphorus for premature infants for many years (Von Sydow, 1946), and for the very premature infant, born before the period of rapid skeletal growth when phosphorus requirements are 60 to 70 mg/kg/day (Ziegler et al, 1976), human milk may be particularly inadequate. There have been reports of hypophosphatemic rickets occurring in premature infants fed human milk (Rowe et al, 1979). Mature human milk contains 11 to 20 mg/dL of phosphorus (Fomon, 1974). An infant fed 100 to 200 mL milk/day would receive only 25 to 75 percent of the phosphorus assimilated by a fetus of similar age in utero.

The phosphorus concentrations in PT and FT milks, determined by automated colorimetric procedures in four studies, were similar to those reported earlier for mature human milk (Table 10). Gross et al (1980) found that phosphorus concentrations remained constant throughout the first month of lactation and were similar in milk samples collected in the morning from PT and FT mothers. The average phosphorus concentration ranged from 9.5 to 14.9 mg/dL in PT milk. Lemons et al (1982) reported that the phosphorus content of PT milk, ranged from 12.9 to 13.9 mg/dL, and was significantly less than that of FT milk. The phosphorus concentrations of the complete 24 h expressions of PT milk did not change over the period of lactation studied.

Atkinson et al (1980) also analyzed complete 24 h collections of human milk and found no significant changes in phosphorus concentrations in PT milk with values ranging from 13.9 to 16.9 mg/dL. Chan (1982) found no significant differences in phosphorus concentrations between PT

Table 10

REPORTED MEAN VALUES FOR PHOSPHORUS CONTENT (mg/dL) OF PRETERM HUMAN MILK[1]

Reference	Days postpartum						
	1-3	4-6	7-10	14-16	17-21	22-28	29-42
Gross et al (1980)	9.5		14.2	14.4	14.9	14.3	
Atkinson et al (1980)		15.5	16.1	16.7		16.1	
Lemons et al (1982)			13.4	13.9	13.9	13.2	12.9
Chan (1982)	21.8			22.0		17.0	

[1]Values for Gross et al (1980) taken from Table for days 3, 7, 14, 21 and 28. Data from Atkinson et al (1980) are for days 3-5, 7-10, 13-17 and 27-29. Data from Lemons et al (1982) are for days 7, 14, 21, 28 and 42. Data from Chan (1982) are for days 2-4, 14-21 and 26-31.

and FT milk obtained at 9 a.m. during the study, however, the phosphorus concentration in PT milk on days 26 to 31, 17.0 mg/dL, was significantly lower than concentrations on days 2 to 4 of lactation, 21.8 mg/dL.

Atkinson et al (1983) stated that growing LBW infants fed preterm milk do not retain sufficient amounts of calcium and phosphorus to meet intrauterine accretion rates of these minerals. Retention of phosphorus at best averaged only 50% of the approximate intrauterine retention. Prolonged feeding of human milk without supplementation may therefore cause cumulative calcium and phosphorus deficits and that will manifest as demineralization of growing bones (Atkinson, 1983).

2.2 Milk Composition and Growth

No standards exist for preterm infant growth and this makes it difficult to establish nutritional requirements. Most feeding regimens for LBW infants aim at prompt postnatal resumption of growth approximating intrauterine growth rates (American Academy of Pediatrics Committee on Nutrition, 1977). However, ideal growth rates have not been established for the very LBW infant and using intrauterine growth rate as a goal is controversial. These rates of growth are rarely achieved and there is no evidence that failure to achieve such growth is particularly detrimental to the infant (Heird, 1977).

Controversy continues regarding the optimal protein levels in the diets of LBW premature infants. Until the late 1940's, human milk was widely used in the feeding of these infants. In 1947, Gordon et al showed that skimmed cows' milk formula, which provided infants with 4.8 to 6.0 g protein/kg of bodyweight/day, produced greater weight gains in LBW infants than did human milk, which provided 2.0 g protein/kg of bodyweight/day. Subsequently, high protein formulas became the usual feeding regime for preterm infants in most hospital nurseries. In 1967,

Davidson et al, using proprietory milk mixtures composed of cows' milk protein, concluded that the minimum intake of protein associated with consistently high weight gains was 4 g/kg/day. A study by Snyderman et al (1969), also using cows' milk, showed that premature infants fed 9 g protein/kg/day retained greater amounts of nitrogen than those fed 2 g/kg/day, but both groups had similar weight gains. In studies by Babson and Bramhill (1969) and Goldman et al (1969) LBW premature infants fed formulas containing high concentrations of protein gained weight faster than those infants fed formulas with protein concentrations similar to those of human milk.

In 1976, Raiha et al conducted a study with 106 LBW infant subjects with size appropriate for gestational age. The infants were divided into three groups based on gestational age and within each group infants were assigned one of five feedings, either banked human milk or one of four formulas. The formulas differed in quantity and quality of protein but not in mineral or fat content. The quantity of lactose was reduced in the high protein formulas in order to keep them equicaloric. The preterm infants fed pooled human milk had rates of growth (crown-rump length, femoral length, head circumference and weight), from time of regaining birthweight to time of discharge at 2400 g, which were not significantly different from those of the preterm infants fed formulas of higher protein concentrations. However Foman and Ziegler (1977) questioned the statistical analysis of the Raiha study and a two-way analysis of co-variance using birthweight as the co-variable did indicate that the groups of infants fed formula had significantly higher rates of weight gain per week than the groups fed the pooled human milk (Gaull et al, 1977). It was concluded that if weight gain is equated with growth then the formulas might offer a small advantage. It was

further contended that the measure of linear growth, which is unbiased by possible fluid retention, is the best determinate of growth. Results of other studies (Davies, 1977; Forbes, 1978) suggested that expressed, pooled mature human milk may not provide ideal nutrition for optimal growth of LBW premature infants, and that preterm infants fed donor milk demonstrated slower growth than those fed commercially prepared formulas. Foman et al (1977) estimated that the protein requirements for a hypothetical 1200 g infant were 2.54 g/100 kcal, but that banked human milk contained, only approximately 1.50 g/100 kcal. These authors therefore concluded that the concentration of protein in human milk is inadequate for growing premature infants.

In 1978, Atkinson et al reported that nitrogen concentration, over the first month of lactation, was higher in milk from mothers giving birth prematurely than in milk from mothers giving birth at term. The LBW premature infant would, they estimated, receive 20% more nitrogen than would the term infant if both were fed equal volumes of their own mothers' milk. When the nitrogen concentration of premature infants' own mothers' milk is compared to the nitrogen concentration of the same volume of mature donor breast milk, the contrast in nitrogen intake is even more dramatic. The premature infant would receive at least twice as much protein and utilizable nitrogen from an equal volume of his own mother's milk during the early stage of lactation. This study by Atkinson generated further research designed to evaluate the nutritional composition and adequacy of breast milk produced by mothers delivering preterm infants, such as the studies by Gross et al (1980) and Schanler and Oh (1980) which are discussed elsewhere in this review.

Until the study by Atkinson et al (1981) was published, no data had been reported on the growth or nutritional health of small premature

infants fed their own mothers' milk. To examine the adequacy of own mothers' milk, Atkinson et al (1981) fed either pooled breast milk (PBM), their own mothers' milk (PT) or infant formula (SMA20 or SMA24) to infants of less than 1300 g birthweight, matched for gestational age and weight. Macronutrient composition and energy of the milks are shown in Table 11. The PT milk was fed fresh within 48 hours of expression. The PBM milk, obtained from women breast-feeding term infants two to five months postpartum, was frozen four to eight weeks, thawed, pooled, sterilized by autoclaving, rapidly cooled and refrozen until used for feeding. Infants were measured for weight, crown-heel length and occipitofrontal circumference on entry to the study and biweekly thereafter. By the end of the second week of life the infants receiving PT milk or SMA had regained and surpassed their birthweights but those receiving PBM averaged only 94 percent of their initial weights. Crown-heel length and head circumference increases were similar in the three groups over the first two weeks of life. Nitrogen intake was higher for the infants receiving PT milk than for those receiving PBM or SMA, and apparent N retention was also higher in the PT group. The authors concluded that the sterilized PBM feeding regime resulted in a less desirable protein nutritional status and slower weight gain when compared to infants receiving PT milk.

Chessex et al (1983) evaluated the growth of very low birthweight (VLBW) infants fed their own mothers' milk during the first six weeks of life. Anthropometric measurements were performed in eleven growing, VLBW (<1300 g) preterm infants fed a mean milk intake of 172 mL/kg/d providing a gross protein intake of 3.0 g/kg/d and a gross energy intake of 111 kcal/kg/d. The infants gained 15.2 g/kg/d in weight, 0.98 cm/wk in length and 0.76 cm/wk in head circumference. These rates of growth

Table 11

MACRONUTRIENT COMPOSITION AND ENERGY IN MILKS[1]

	PBM[2]	PT[3] A	PT[3] B	SMA20	SMA24
Nitrogen (mg/dL)	196	328±14	267±14	246	296
Lactose (g/dL)	6.50	5.23±0.14	5.56±0.11	7.30	8.20
Fat (g/dL)	3.75	3.80±0.47	4.05±0.36	3.51	4.22
Gross Energy[4] (kcal/dL)	67	67±5	69±4	70	82

[1]Mean±SEM (n=24), adapted from Atkinson et al, 1981

[2]Pool Breast Milk.

[3]A and B represent milks fed infants at 6 to 8 and 13 to 15 days postpartum, respectively.

[4]calculated using energy conversion based on heats of combustion for milk protein, fat and lactose.

approximated reported gains for intrauterine growth of the fetus from 28 to 32 wk (Usher and McLean, 1969).

A study by Atkinson et al (1983) measured macromineral balances and growth concurrently with studies of protein and energy (Atkinson et al, 1981) in premature infants fed their own mothers' milk or modified infant formula during the first month of life. Seven pairs of infants were studied for the first two weeks and five of the pairs also at the end of the fourth week of life. Milk intakes were increased as tolerated to a maximum of 180 to 200 mL/kg/d. Anthropometric data for the infants were similar on entry to the study. There was a significant acceleration in the rate of weight gain over the four weeks in the two groups. After the first two weeks weight gain in the group fed their mothers' milk approximated intrauterine weight gain, as calculated from the growth curves of Usher and McLean (1969).However the formula-fed infants demonstrated a significantly greater weight gain, 26 g/d, than the group fed their mothers' milk, 15 g/d. Growth in length and head length and head circumference in both groups approximated intrauterine growth rates.

Rates of growth of three groups of preterm infants were compared by Gross (1983). One group was fed milk obtained early from mothers of preterm infants, one group received milk produced during the mature stage of lactation by mothers of term infants and one group received a whey-based infant formula. The infants in all the groups were comparable in birthweight, gestational age, length and head circumference at entry to the study. Rates of weight gain were greater for the groups receiving formula (27.0 g/d) and milk from mothers of preterm infants (23.7 g/d) than for the group receiving milk from mothers of term infants (15.8 g/d). The average increments in length

and in head circumference were significantly greater for the groups receiving preterm human milk and formula than for the group receiving mature human milk. Gross concluded that for the healthy preterm infant, feeding with preterm human milk or the whey-based infant formula offers a significant growth advantage over feeding with mature human milk.

Atkinson et al (1981, 1983), Chessex et al (1983) and Gross (1983) concluded that amounts of protein in early preterm milk are adequate to support rates of growth in premature infants approximating those of the third trimester of intrauterine life, and that mothers of preterm infants were therefore the best source of human milk for the infants. Anderson et al (1981) suggested that milk from the infant's own mother should be fed if available. If not, formula of known and consistent composition may be preferable to the variable nutrient composition of pooled banked milk. Of the available alternatives to the infant's own mother's milk none appear to be completely satisfactory. Much remains to be learned regarding nutritional support for premature infants during their first weeks of life.

2.3 Sodium Intake and Hyponatremia

Increased survival of smaller infants emphasizes the need for re-evaluation of the electrolyte requirements of the LBW premature infant. Sodium requirements vary greatly among infants and controversy has surrounded the feeding of the LBW infant because of the low level of sodium ($\leq$2 meq/kg/d) provided by mature human milk and formulas designed to resemble it (Roy et al, 1976). The sodium content of human milk sampled after full lactation is established has ranged from <2 to >20 meq/L with a median of 7 meq/L (Curran, 1979). A number of investigators have observed that hyponatremia occurs frequently during

the first six weeks of life in LBW infants fed 'humanized' milks that provide sodium in amounts similar to breast milk.

Hyponatremia is arbitrarily defined as serum sodium <130 meq/L. Low birthweight infants are prone to hyponatremia in the first six weeks of life because the sodium requirements of the LBW infant are higher than those of the full term infant. Reasons for the higher sodium requirement may be, first, that the rate of accumulation of sodium in the body is proportionately and absolutely higher early during the third trimester than at term (Roy et al, 1976), secondly, that sodium coprecipitates with calcium in bone when length growth is rapid, and finally, that because of renal immaturity there is a relatively high urinary sodium loss (Day et al, 1976). Balance studies suggest that LBW infants require 3 meq/kg/d to avoid hyponatremia between the age of 2 and 6 weeks.

Day et al (1976) studied 30 LBW infants fed a commercial formula and found that hyponatremia occurred in 23 infants, 14 appropriate for gestational age (AGA) and 9 small for gestational age (SGA) between the ages of 2 and 6 weeks. In 5 of the infants hyponatremia recurred after apparently adequate treatment for the deficit. Hyponatremia occurred frequently in 46 LBW infants (<1.3 kg) between 2 and 6 weeks of age studied by Roy et al (1976). A formula was reconstituted to provide two different sodium concentrations. Sodium intake in the nonsupplemented group (NS) was 2 meq sodium/kg/d and the supplemented infants (S) received 3 meq/kg/d. Infants were studied from 14 to 47 days of age. At the beginning of the study an equal number of infants (31%) in each group were hyponatremic. After supplementation only two episodes of hyponatremia occurred in the S infants (both during the first study week) but the NS infants showed a persistently high incidence of

hyponatremia until the fourth study week. Chance et al (1977) examined growth of 108 infants <1.3 kg birthweight in response to various dietary changes. Only sodium intake was shown to influence the rate of weight gain. Infants who did not receive sodium supplement gained weight at a rate less than in utero (30 g/d) whereas those given sodium supplement showed an appropriate weight gain for their postconceptual age. Kumar and Sacks (1978) found that the usual maintenance intake of 2 to 3 meq/kg/d sodium chloride was not sufficient in the extremely premature infant. LBW infants cannot conserve urinary sodium and as much as 4 to 8 meq/kg/d may be required to keep up with sodium needs in these infants. The sodium content of mature human milk is approximately 7 meq/L, while formulas usually contain 7 to 12 meq/L (Table 12). A LBW infant getting a maximum 180 to 200 mL/kg of human milk per day would fail to get the maintenance requirements of sodium.

However, milk from mothers who give birth prematurely has a higher concentration of sodium than mature milk (Atkinson et al, 1980; Lemons et al, 1982). Atkinson et al (1983) found that the amount of sodium provided by the mother's milk produced for her premature infant during the first four weeks of lactation was adequate.

In summary, balance measurements suggest that the LBW infant requires approximately 3 meq/kg/d of sodium to avoid hyponatremia between the ages of 2 and 6 weeks. Mature human milk and many formulas provide only 2 meq/kg/d. Early preterm human milk appears to provide adequate amounts of sodium. If preterm milk is not available, supplementation of formula to provide a daily total sodium intake of 3 meq/kg/d until a weight of 1.5 kg is reached was suggested (Roy et al, 1976).

Table 12

ADVISABLE NUTRIENT INTAKES AND NUTRIENT CONTENTS OF PRETERM AND MATURE HUMAN MILKS AND OF SIMILAC AND SIMILAC SPECIAL CARE FORMULAS

Nutrient	Advisable intake[1]		Preterm[2] human milk	Mature[3] human milk	Similac[4]	Similac[4] Special Care
	for 1 kg infant	for 1.5 kg infant				
Energy (kcal/dL)			73	70	68	81
Protein (g/dL)	2.4	2.1	1.5-2.2	0.9	1.6	2.2
Fat (g/dL)			3.4	2.7-3.4	3.6	4.4
Lactose (g/dL)			6.8	7.0	7.2	8.6
Sodium (meq/L)	21	18	7-18	7	10	15
Calcium (mg/dL)	123	108	31	34	52	144
Phosphorus (mg/dL)	83	73	14	15	39	72

[1]Ziegler et al (1981)

[2]Lemons et al (1982)

[3]Brostrom (1981)

[4]Ross Laboratories, Montreal, P.Q.

2.4 Calcium and Phosphorus Intake and Mineralization

The prematurely born infant is in a precarious position. If postnatal weight gain proceeds at the intrauterine rate, large amounts of calcium and phosphorus must be provided if the body is to keep pace and undermineralization or rickets is to be avoided. Because it is difficult to provide adequate amounts of calcium and phosphorus orally or intravenously, it may be fortunate that prematurely born infants usually grow slowly during the first weeks of postnatal life.

Intrauterine accretion of calcium and phosphorus increases dramatically during the last trimester of pregnancy. During that time the fetus lays down approximately 100 mg calcium per kilogram of bodyweight per day in the form of new bone (Ziegler et al, 1976; Shaw 1976). The advisable intake for calcium and phosphorus for the 1 kg body weight infant is 210 and 140 mg/kg/d respectively; for the 1.5 kg body weight infant it is 185 and 123 mg/kg/d, respectively (Ziegler et al, 1981). Preterm human milk contains approximately 30 mg calcium/dL and 14 mg phosphorus/dL and mature human milk approximately 34 mg calcium/dL and 15 mg phosphorus/dL (Table 12). Calcium and phosphorus in standard infant formulas range from 50 to 60 mg/dL and 38 to 50 mg/dL respectively (Brady et al, 1982). It is apparent that human milk and standard formulas do not provide enough calcium and phosphorus to meet normal fetal accretion rates, even if 100 percent of the calcium and phosphorus were absorbed. Thus the LBW infant may be predisposed to undermineralization when a rapid rate of growth comparable to that observed in utero is achieved.

Delayed bone mineralization, osteopenia and overt rickets have recently been reported in premature infants fed human milk. Rowe et al (1979) found that an infant of 26 weeks gestation developed hypophos-

phatemic rickets while she was being fed human milk exclusively. The infant's mother's milk contained 32 mg/dL of calcium and 11 mg/dL of phosphorus - values within the normal range. The infant had increased her birthweight (525 g) almost 3½ times by her expected date of birth. In utero her estimated accretion of calcium and phosphorus would have been 21 and 13 g respectively (Ziegler et al, 1976). The infant actually received 5.7 g of calcium and 1.9 g of phosphorus from human milk, not all of which would have been absorbed. Treatment was begun and the human milk was supplemented with 20 to 25 mg/kg/d of phosphorus as potassium phosphate. Calcium supplementation (30 mg/kg/d) was initiated after 3 days. Three months after supplementation there was no radio-graphic evidence of rickets. Sagy et al (1980) also reported radiologically proven rickets in an exclusively breast fed prematurely born infant. In contrast to the Rowe et al (1979) study examination of the mothers milk for phosphorus content repeatedly showed low values (5.8 to 6.2 mg/dL). Therapy was initiated and the infant received 20 to 25 mg/kg/d of phosphorus as potassium phosphate for 7 weeks. After 9 weeks radiologic examination revealed no evidence of rickets. These cases support the need for appropriate calcium and phosphorus supplementation of breast milk when given to the rapidly growing premature infant.

Shaw (1976) compared the rate of absorption and retention of calcium by very low birthweight infants over the first 30 to 70 days after birth to the rate of accumulation of calcium by the fetus in utero. The results showed that preterm infants fed breast milk had an absolute deficiency of calcium. Although the infants absorbed nearly two-thirds of their dietary calcium, intakes were only 56 mg/kg/d from breast milk. Infants fed other milks ingested sufficient calcium but

did not absorb enough. Calcium absorption improved with increasing postnatal age, however, intrauterine rates of calcium retention were never achieved on any milks.

It has further been speculated that standard formulas might not supply the low birthweight infant with adequate calcium, phosphorus and vitamin D for normal bone mineralization. Day et al (1975) studied 30 infants <1.3 kg from age 14 days to 1.8 kg bodyweight. Infants were pairmatched for gestational age and birthweight and randomly allocated to two treatment groups. All infants were fed SMA, 200 mL/kg/d, 160 kcal/kg/d and were given a multivitamin containing 500 IU vitamin D/dose. Infants in group A received no calcium supplement; those in group B received calcium lactate, 800 mg/kg/d. Results showed no difference in weekly length gain, weight gain or head circumference increments during the study. However, in 9 of 10 paired radiographs of the knee and tibia, group B infants had better defined bone texture and/or wider cortices than group A infants.

Implementation of serial measurements of bone mineral density which provide a more direct assessment of the mineralization process has improved the ability to measure the outcome of clinical trials. The measurement of radial bone mineral in small infants by photon absorptiometry employs a photon beam which passes through the radius (Steichen et al, 1978). Bone mineral content (BMC) is reported in grams per centimeter. Minton et al (1979) adapted photon absorptiometry to measure BMC in 30 preterm infants fed a standard commercial formula (Similac with iron, Ross Laboratories). BMC at birth correlated significantly with gestational age and birthweight. The linear rate of increase of postnatal BMC in the preterm infants was significantly less than the expected rate of increase in utero. It was speculated that the

decreased intake of calcium and phosphorus affected postnatal bone mineralization and that standard formulas failed to supply adequate minerals for mineralization in the low birthweight premature infant. Steichen et al (1980) used direct photon absorptiometry for longitudinal studies on the effect of an experimental formula. Twenty-nine premature infants received Similac (Ross Laboratories) containing 51 mg calcium/dL and 31 mg phosphorus/dL or the experimental formula containing 126 mg calcium/dL and 63 mg phosphorus/dL. The average intake of calcium and phosphorus on the experimental formula was 235 and 115 mg/kg/d, respectively, which approximated the amount required for bone mineralization in utero and was significantly greater than the standard formula. BMC was significantly greater in infants receiving the experimental formula at 2, 4 and 12 weeks postnatal age. It was concluded that undermineralization appears to be caused mainly by mineral deficiency and that calcium and phosphorus intakes of approximately 235 and 115 mg/kg/d would provide sufficient mineral to allow postnatal bone mineralization to approximate the intrauterine rate.

Very LBW infants with rickets had relatively normal serum calcium and phosphorus concentrations but elevated serum alkaline phosphatase and decreased bone mineral content in a study by Steichen et al (1981). After treatment by increasing calcium and phosphorus there was no change in serum calcium and the serum phosphorus increase was not signifiant however the alkaline phosphatase concentration decreased into the normal range. Kulkarni et al (1980) also found that serial determinations of serum calcium remained constant over weeks of treatment in 15 very LBW infants with active rickets. However, serum phosphorus was significantly lower during the phase of active rickets than during the

healing phase. Serum alkaline phosphatase activity continued to be elevated during the healing phase but gradually decreased. Shenai et al (1980) found that excluding the initial set of results obtained prior to commencement of oral feedings the mean values of serum calcium and phosphorus were in the normal range. Ten preterm infants were fed an experimental formula (Similac Special Care, Ross Laboratories) and absorbed 73% of the calcium and 81% of the phosphorus. The mean calcium and phosphorus retention rates revealed by balance studies in these infants were comparable to normal fetal accretion rates.

Direct photon absorptiometry was used by Greer et al (1982) to examine the effect of increased calcium and phosphorus on bone mineralization in premature infants also receiving this new formula (Similac Special Care, Ross Laboratories). Twelve LBW infants, gestational age 28 to 32 weeks were maintained on approximately 150 mL of formula/kg/d providing 118 kcal/kg/d, 204 mg calcium/kg/d and 116 mg phosphorus/kg/d. Average daily weight gain was 18.5 g/kg/d. Over a 3 to 5 week period, bone mineralization increased in 8 of the 12 infants and decreased in the remainder but all values remained in the range expected for BMC in utero. In 1983, Atkinson et al examined calcium and phosphorus balances in premature infants fed their own mothers' milk or formula (SMA 67 or SMA 80, Wyeth Laboratories). Calcium and phosphorus intakes from SMA were higher than from PT human milk, however retention of calcium and phosphorus did not meet intra-uterine retention rates in either feeding regimen. Radiographs of the radius-ulna taken when infants reached approximately 1.8 kg weight were assessed as to degree of mineralization. Two of three infants fed their mothers' milk and one of four infants fed SMA were "inadequately mineralized".

It appears that the problem of delayed bone mineralization in premature infants fed human milk or standard formulas is related to dietary deficiencies of calcium and phosphorus. Neither preterm nor mature human milk nor infant formula designed for full-term infants provides adequate amounts of these minerals if the infant is growing at rates that approximate intrauterine growth rates. Infants fed formulas with higher concentrations of calcium, phosphorus and other nutrients specifically developed for the premature infant have demonstrated improved mineral retentions and bone mineralization compared to unsupplemented infants. However the optimal amount of calcium and phosphorus required to support adequate mineralization of rapidly growing bone in premature infants needs to be defined.

Chapter 3

MATERIAL AND METHODS

3.1 Subjects

3.1.1. Selection and Care

Low birthweight (LBW) infants were selected from all patients admitted to the Intensive and Intermediate Care Nurseries at the Health Sciences Centre (HSC), Winnipeg, Manitoba, between May and November, 1981. The criteria for acceptance were birthweight of less than 1500 g; and freedom from congenital abnormalities. Twenty infants, five male and fifteen female with birthweights from 890 g to 1490 g met the criteria. Informed consent was obtained from the mothers of the infants prior to their inclusion in the study. Gestational age was confirmed by clinical assessment (Dubowitz et al, 1970) and ranged from 28 to 36 weeks. Eleven infants were appropriate size for gestational age and nine infants were small for gestational age, defined as below the third percentile for intrauterine growth (Usher and MacLean, 1969). Clinical details for each infant are given in Appendix A.

All infants were nursed in incubators in a thermoneutral environment with heat shields placed over the infants. When infants were able to maintain their own body temperature they were nursed in open cribs. Supportive measures or treatment were given according to the clinical condition of the infant and the nursery routine. All infants were fed intravenously a 10% glucose solution containing appropriate electrolytes during the first day of life. If an infant's condition was uncomplicated, human milk was fed by gavage on day two. The volume of human milk was increased as tolerated, with concomitant decreases in intravenous fluids. When oral feedings were not feasible

for an extended period of time, total parenteral nutrition (TPN) containing amino acids, glucose, vitamins, minerals and sometimes lipid, was administered via a peripheral vein. Ten of the twenty infants in this study received TPN and seven of these infants were also given 10% Intralipid (Pharmacia Canada Ltd., Montreal, Que.)

3.1.2. Milk Sources and Feeding Protocol

According to HSC protocol all infants less than 1500 g at birth routinely receive fresh human milk for the first 14 days of enteral feeding. If the mother is supplying milk she is encouraged to continue after this time. In cases where a mother cannot supply milk for her infant, milk is donated by nursing mothers in the community. These nursing mothers are screened for general level of health, current medications and age of infant (preferably over six weeks of age). Milk from these mothers is referred to as mature donor milk. Milk is also donated by mothers of preterm infants who are unable to receive enteral feedings. Milk from these mothers is referred to as preterm donor milk. All donor milk is screened for bacterial contamination and discarded if not fed within 48 hours. Infants in this study received their own mother's milk, donor milk. and formula. All feedings were by intermittent gavage.

3.1.3. Clinical Measurements

All growth measurements were made and recorded weekly by the nurse coordinator of the HSC study. Weights of infants were measured using an Air Shields sling scale (accuracy ±10 g) or a Toledo baby scale (accuracy ± 10g) in the Intensive Care and the Intermediate Care Nurseries respectively. Crown-heel lengths of infants were measured using a plastic coated nonstretch tape accurate to 0.25 cm. Occipiofrontal head

circumference (HC) of infants were measured using a plastic coated nonstretch tape accurate to 0.25 cm.

Radiographs of the radius-ulna of infants were obtained at three months of age for hospitalized infants or at discharge for infants released before three months of age. A pediatric radiologist assessed the radiographs and assigned the infants to one of four groups: (1) normal bone mineralization, (2) delayed bone mineralization, (3) suggestive of rickets and (4) overt rickets.

Blood was drawn biweekly for measurement of serum calcium, phosphorus, sodium and alkaline phosphatase by the HSC laboratory using the methods described below.

3.2. Milk Sampling and Storage

Samples were collected in 20 mL glass vials with screw top lids which were labelled with the infant's name and the date on which the collection was begun. The source of milk fed at each feeding was recorded on the form provided (Appendix B). Collection periods started at 0800 h each day. For each infant a one mL aliquot of each feeding was placed in the vial so that the contents were representative of the 24 hour period.

The collection period covered the first six weeks postnatal. When the samples were PT milk, then these were representative of the PT infants' mothers' milk. The D milk samples were not representative of one donor's milk, since an infant could have received milk from a number of donors during the collection period. PT milks were expressed by mothers delivering infants of 28 to 32 weeks gestational age, while D milks were supplied by mothers whose term infants were over six weeks of age. Preterm/donor (PT/D) milks were the collections which included

milk from both sources. Infants receiving their own mother's milk were supplemented with D milk if the volume of their own mother's milk was not sufficient. The mean percent of PT milk in the PT/D samples was 90, 87, 77, 66, 68 and 52 in weeks 1 to 6 respectively.

The samples were refrigerated throughout the collection period and then transported on ice to the nutrition laboratory at the University of Manitoba for analysis. Immediately upon arrival at the laboratory, the vials for each infant were combined to form samples representative of 2 or 3 day collection periods (Table 13). The analyses of TN, fat and lactose were performed on these samples. Proportional amounts of 2 and 3 day combined samples were further combined to provide milk samples representative of 7 day collection periods. The analyses of NPN, calcium, phosphorus and sodium were performed on these samples. TN, fat and NPN content were determined on fresh milk samples. Lactose, calcium, phosphorus and sodium content were determined on premeasured milk samples which were frozen and stored at -20^{o}C. Samples for mineral analysis were stored in scintillation vials.

3.3 Milk Analyses

3.3.1. Total Nitrogen and Nonprotein Nitrogen Determination

Total nitrogen (TN) content of fresh milk samples was determined by the micro-Kjeldahl method of the Association of Official Analytical Chemists (1980) with modifications indicated in Appendix C. Nonprotein nitrogen (NPN) was determined on defatted milk samples after protein had been precipitated by 12% trichlorocetic acid using the method described by Lonnerdal et al (1976a) with modifications indicated in Appendix D.

3.3.2. Fat Determination

Total fat content of fresh milk samples was measured spectrophoto-

Table 13

DESIGN FOR MILK SAMPLE COLLECTION AND ANALYSES

Nutrient	Collection Period (d)	Storage Temperature (°C)	Method of Analysis
TN[1]	2-3	4	Micro-Kjeldahl
Fat[1]	2-3	4	Turbidometric
Lactose[1]	2-3	-20	Phenol-sulfuric
NPN[1]	7	4	Acid precipitation of protein. Micro-Kjeldahl
Minerals[2]	7	-20	
Calcium			Atomic absorption
Phosphorus			Colorimetric
Sodium			Atomic absorption

[1] Values determined on duplicate samples.

[2] Values determined on triplicate samples.

metrically by the method of Nakai and Le (1970). In this procedure acetic acid was used to dissociate and solubilize the protein and fat. Turbidity, a measure of fat content, was measured by taking absorbance readings at 400 nm, after the addition of urea-imidazole solution (Appendix E).

3.3.3. Lactose Determination

Lactose content of frozen human milk samples was determined using the phenol-sulfuric acid method for total carbohydrates (Barnett and Tawab, 1957). This method is based on the principle that simple sugars, oligosaccharides, polysaccharides and their derivatives produce a stable orange color when treated with phenol and concentrated sulfuric acid. The absorbance was read spectrophotometrically at 490 nm (Appendix F).

3.3.4. Calculation of Protein and Energy Content

Protein was calculated by the following formula:

$$\text{Protein (g/dL)} = \text{TN (mg/dL)} \times 6.25/1000.$$

The 6.25 factor was considered more appropriate than the 6.38 factor because 5 to 6% of the TN in human milk is nonamino acid nitrogen (Atkinson et al, 1980). Energy content of the milk samples was calculated using caloric values of 5.65 kcal/g for protein, 9.25 kcal/g for fat and 3.95 kcal/g for lactose. These values represent the energy for combustion for milk products (Report of the Joint FAO/WHO Ad Hoc Expert Committee, 1973).

3.3.5. Mineral Determination

For mineral analyses, 7 mL of milk was dried overnight at 100°C. The dried sample was dissolved in 7 mL of acid mix (1 part 60% perchloric acid: 2 parts 30% hydrogen peroxide), heated at 70°C for 3

hours and filtered through Whatman number 4 filter paper into a 10 mL graduated cyclinder. The sample was washed three times with small aliquots deionized water and the total filtrate made back to 7 ml (Solution A).

For sodium analysis, 1 mL of Solution A was diluted with deionized water to make 500 mL. Sodium content was determined in this solution using the Perkin-Elmer atomic absorption spectrophotometer model 403 (Perkin-Elmer, Norwalk, Conn.) equipped with hollow cathode lamps. Values are means of triplicate readings and are expressed in meq/L. For calcium analysis, 1 mL of Solution A was diluted with 0.1% lanthanum oxide in 1% perchloric acid to make 100 mL. Calcium content was determined in this solution using the Perkin-Elmer atomic absorption spectrophotometer model 403 (Perkin-Elmer, Norwalk, Conn.) equipped with hollow cathode lamps. Values are means of triplicate readings and are reported in mg/dL. Phosphorus was determined as phosophate by diluting 1 mL of Solution A with deionized water to make 50 mL. A 1 mL aliquot of this solution was diluted with 2 mL reagent (3 parts 1M sulfuric acid: 1 part 2.5% ammonium molybdate: 1 part freshly prepared 10% ascorbic acid). After 1-3/4 hours incubation at 37^{o}C, triplicate samples were cooled and absorbance read on a SP6-300 Pye Unicam spectrophotometer (Canlab, Winnipeg, Manitoba) at 820 nm against a reagent blank. Phosphorus content expressed as mg/dL was determined from a potassium phosphate curve.

3.4 Blood Serum Analyses

3.4.1. Sodium Determination

Serum sodium was measured on a Beckman ASTRA 8 Analyzer (Beckman Instruments, Brea, CA) using sodium ion-selective electrodes.

3.4.2. Calcium Determination

The CALCM pack was used in the Du Pont Automatic Chemical Analyzer (Du Pont Company, Wilmington, DE) to quantitatively measure calcium in the blood serum. This method is a modification of the calcium o-cresolphthalein complexone complexometric reaction used by Sarkar and Chauhan (1967) for the measurement of serum calcium without prior protein precipitation.

3.4.3. Phosphorus Determination

The PHOS pack was used in the Du Pont Automatic Chemical Analyzer with Computer II (Dupont Company, Wilmington, DE) to quantitatively measure inorganic phosphate in the blood serum. This method is a modification of the phosphomolybdate method introduced by Fiske and Subbarow (1925).

3.4.4. Alkaline Phosphatase Determination

Alkaline phosphatase activity was measured on an ABA-100 automated analyzer (Abbott Laboratories, Irving, TEX) using p-nitrophenyl phosphate substrate. This method is an adaptation of the method introduced by Bessy et al (1946). Alkaline phosphatase catalyzes the conversion of p-nitrophenyl phosphate (colorless) to p-nitrophenol which is released as the p-nitrophenoxide ion (yellow) which has maximum light absorbance at 405 nm. The reaction is quantitated by measuring the absorbance increase over a known time. The reaction rate is proportional to the amount of alkaline phosphatase present.

3.5 Hypotheses and Statistical Analysis

The following hypotheses were formulated to carry out the objectives.

1. There will be no difference in total nitrogen (nonprotein

nitrogen, fat, lactose, energy, sodium, calcium, phosphorus) content from week to week in the milk of mothers delivering prematurely.

2. There will be no difference in total nitrogen (nonprotein nitrogen, fat, lactose, energy, sodium, calcium, phosphorus) content from week to week in the mature milk mothers donate to the hospital nurseries.

3. There will be no difference in total nitrogen (nonprotein nitrogen, fat, lactose, energy, sodium, calcium, phosphorus) content from week to week in mixtures of milk from mothers delivering prematurely and mature milk donated to the nurseries.

4. There will be no difference in total nitrogen (nonprotein nitrogen, fat, lactose, energy, sodium, calcium, phosphorus) content in the milk of mothers delivering prematurely, mature milk donated to the nurseries or mixtures of these milks.

5. There will be no relationship between the protein (energy) intakes of premature infants less than 1500 g at birth and their growth during the first six postnatal weeks.

6. There will be no relationship between the incidence of hyponatremia in premature infants less than 1500 g at birth and their sodium intakes.

7. There will be no relationship between the incidence of delayed bone mineralization in premature infants less than 1500 g at birth and their calcium (phosphorus) intakes.

8. There will be no relationship between the incidence of delayed bone mineralization in premature infants less than 1500 g at birth and their serum calcium (phosphorus, alkaline phosphatase) levels.

9. There will be no relationship between the serum calcium

(phosphorus, alkaline phosphatase) levels in premature infants less than 1500 g at birth and their calcium (phosphorus) intakes.

Data from all experiments were analyzed using the computer based Statistical Analysis System (SAS). Hypotheses 1 to 4 were tested using the General Linear Model (GLM) procedure for factorial analysis of variance for unbalanced data. Testing which means were significantly different from each other was performed using Duncan's Multiple Range test at the 0.01% level of significance. Hypothesis 5 was tested using correlation to measure the closeness of the linear relationship between weight gain and intake. The T test procedure was used to test hypotheses 6 to 9.

Chapter 4

RESULTS AND DISCUSSION

4.1. Nutrient Composition of Human Milk: A Comparison of Preterm, Mature Donor and Preterm/Donor Mixtures

Tests of the hypotheses related to the first objective of the study indicated that there were differences in total nitrogen (TN), fat, lactose and energy contents from week to week in the milk of mothers delivering prematurely (PT) but no week to week differences in mature milk donated to the nurseries (D). However, there were week to week differences in TN, fat, lactose and energy contents in mixtures of PT and D milks (PT/D). PT and PT/D milks were different from D milk in mean TN, nonprotein nitrogen (NPN), sodium and calcium contents for the six week period and PT/D milk was also different from D milk in mean fat and lactose contents.

4.1.1. Total Nitrogen and Protein Content

The mean TN content of the PT milk collected during the first week of feeding was 377 mg/dL; and for the second and third weeks was 297 mg/dL and 247 mg/dL, respectively (Table 14). TN content for weeks four to six ranged from 225 to 229 mg/dL. The TN levels corresponded to calculated protein values of 2.4, 1.9, 1.5 and 1.4 g/dL for weeks one, two, three and for weeks four to six respectively (Table 15). The TN content of the PT milk was significantly greater in week one than in week two, and in week two than in week three and subsequent weeks. For the six week period the mean TN content of PT milk was 260 mg/dL corresponding to a mean calculated protein content of 1.6 g/dL (Table 16).

Table 14

NITROGEN CONTENT[1] OF PRETERM (PT), DONOR (D) AND PRETERM/DONOR (PT/D)[2] MILK FED TWENTY PREMATURE INFANTS

Nutrient	Milk Source	Week 1	2	3	4	5	6
Total Nitrogen (mg/dL)	PT	377±26.9a[3] (12)[4]	297±11.3b (16)	247±8.8c (17)	226±4.9c (16)	225±5.8c (15)	229±10.4c (22)
	D	155±6.6 (16)	162±3.9 (21)	152±5.4 (12)	152±3.3 (3)	163±13.2 (4)	174±6.0 (6)
	PT/D	378±8.8a (3)	294±10.8b (9)	267±13.6b (16)	236±11.9bc (15)	234±12.5bc (9)	183±4.0c (2)
Nonprotein Nitrogen (mg/dL)	PT	33.8±3.3 (7)	34.7±3.3 (6)	31.2±1.0 (5)	34.1±1.7 (5)	33.7±1.8 (5)	33.6±2.3 (8)
	D	25.6±3.5 (4)	26.5±2.1 (8)	22.6±2.1 (4)	21.4 (1)	25.3±0.2 (2)	29.1±0.0 (2)
	PT/D	30.4±10.0 (2)	28.4±5.3 (3)	33.7±1.8 (6)	35.6±1.9 (5)	35.0±1.5 (4)	33.9 (1)

[1] Mean±SEM

[2] The percent of PT milk in the PT/D mixture ranged from 90% (wk 1) to 52% (wk 6)

[3] Values in the same row with different letters are significantly different $p < 0.01$)

[4] Number in brackets is the number of samples analyzed.

Table 15

ENERGY AND MACRONUTRIENT CONTENT[1] OF PRETERM (PT), DONOR (D) AND PRETERM/DONOR (PT/D)[2] MILK FED TWENTY PREMATURE INFANTS

Nutrient	Milk Source	Week 1	2	3	4	5	6
Protein (g/dL)	PT	2.4±0.2a[3] (12)[4]	1.9±0.1b (16)	1.5±0.1c (17)	1.4±0.0c (16)	1.4±0.0c (15)	1.4±0.1c (22)
	D	1.0±0.0 (16)	1.0±0.0 (21)	1.0±0.0 (12)	1.0±0.0 (3)	1.0±0.1 (4)	1.1±0.0 (6)
	PT/D	2.4±0.1a (3)	1.8±0.1b (9)	1.7±0.1b (16)	1.5±0.1bc (15)	1.5±0.1bc (9)	1.1±0.0c (2)
Fat (g/dL)	PT	1.8±0.3a (12)	3.4±0.2b (16)	3.4±0.2b (17)	3.7±0.2bc (16)	4.0±0.2bc (15)	4.3±0.2c (23)
	D	3.8±0.2 (16)	4.2±0.4 (21)	4.2±0.4 (12)	3.9±1.2 (3)	3.5±0.8 (4)	3.1±0.2 (6)
	PT/D	1.2±0.2a (4)	3.9±0.4b (9)	3.0±0.2b (16)	3.7±0.2b (15)	3.6±0.2b (9)	3.1±0.5b (2)
Lactose (g/dL)	PT	6.3±0.1a (12)	6.7±0.1ab (16)	6.7±0.1ab (17)	7.0±0.1b (16)	6.8±0.1b (15)	6.8±0.1b (23)
	D	6.6±0.2 (16)	7.0±0.1 (21)	6.9±0.1 (11)	6.9±0.1 (3)	7.4±0.1 (3)	7.1±0.1 (6)
	PT/D	5.9±0.1a (4)	6.5±0.2b (9)	6.5±0.1b (16)	6.6±0.1b (15)	6.9±0.1b (9)	6.9±0.2b (2)
Energy (kcal/dL)	PT	55.2±2.8a (12)	68.7±1.5bc (16)	67.0±1.9b (17)	69.7±2.0bc (16)	71.8±2.0bc (15)	75.1±1.6c (22)
	D	66.5±1.9 (16)	71.6±3.6 (21)	72.6±3.5 (11)	68.6±10.7 (3)	71.6±7.4 (3)	62.3±1.7 (6)
	PT/D	48.8±2.4a (3)	72.5±3.1b (9)	62.6±1.6ab (16)	68.1±2.3b (15)	68.9±2.2b (9)	62.5±5.1ab (2)

[1]Mean±SEM

[2]The percent of PT milk in the PT/D mixture ranged from 95% (wk 1) to 52% (wk 6)

[3]Values in the same row with different letters are significantly different ($p<0.01$)

[4]Number in brackets is number of samples analyzed.

Table 16

NUTRITIONAL COMPOSITION[1] OF PRETERM (PT), DONOR (D) AND PRETERM/DONOR (PT/D)[2] MILK FED TWENTY PREMATURE INFANTS

	PT	D	PT/D
Total Nitrogen (mg/dL)	260±6.9 a[3] (98)	159±2.6 b (62)	260±7.7 a (54)
Nonprotein Nitrogen (mg/dL)	33.6±1.0 a (36)	25.5±1.0 b (21)	33.4±1.3 a (21)
Protein (g/dL)	1.6±0.0 a (98)	1.0±0.0 b (62)	1.6±0.0 a (54)
Fat (g/dL)	3.5±0.1 ab (99)	3.9±0.2 a (62)	3.3±0.1 b (55)
Lactose (g/dL)	6.8±0.1 ab (99)	6.9±0.1 a (55)	6.6±0.1 b (55)
Energy (kcal/dL)	68.8±1.0 (99)	69.3±1.6 (60)	66.0±1.2 (54)
Sodium (meq/L)	8.4±0.8 a (33)	4.5±0.6 b (14)	8.0±0.7 a (20)
Calcium (mg/dL)	37.9±1.2 a (33)	43.2±1.5 b (14)	38.0±1.5 a (20)
Phosphorus (mg/dL)	8.9±0.2 (33)	8.8±0.3 (14)	8.6±0.3 (20)

[1] Mean±SEM for six weeks

[2] Percent of PT milk in PT/D mixture ranged from 90% (wk 1) to 52% (wk 6)

[3] Values in the same row with different letters are significantly different ($p < 0.01$)

[4] Number in brackets is the number of samples analyzed.

TN values ranging from 311 to 508 mg/dL have been reported for PT Milk during the first week of lactation, as was shown in Table 3. In two of the three studies which reported TN values for both days 1 to 3 and 4 to 6 (Atkinson et al, 1978 and 1980) the averaged TN values for week one were lower, 353 and 345 mg/dL, than the value in the present study, 377 mg/dL; but in the third study (Schanler and Oh, 1980) the TN value was slightly higher, 391 mg/dL. For the second week of collection TN was 297 mg/dL, a value which was in the middle of the reported range for days 7 to 16. Averaged TN values for this period, as given by Lemons et al (1982) and D. Anderson et al (1983) were almost identical to those of the present study, 294 and 295 mg/dL respectively. PT milk samples collected during the third week had a TN level of 247 mg/dL, a value in the middle of the reported range for days 17 to 21. During the fourth week, TN averaged 226 mg/dL, a value close to those reported by G. Anderson et al (1981) and Lemons et al (1982), 232 and 238 mg/dL respectively. Throughout the six weeks of the present study, the TN contents of the PT milks were consistently representative of reported values. The data confirms the high initial TN content of PT milk, and its subsequent decline with progressing lactation. By the sixth week the TN in the PT milk was 60% of the concentration during the first week of collection.

Weekly average TN content of D milk in the present study ranged from 152 to 174 mg/dL, and for the six week period the mean TN content was 159 mg/dL. This value corresponds to 1.0 protein/dL, and is similar to that found by Lonnerdal et al (1976b), who reported a mean TN concentration of 161 mg/dL in milk from mothers 1.5 to 3.5 months postpartum. The mean TN of D milk was, however lower than the 190 mg/dL

of spot donor milks as reported by D. Anderson et al (1983) and the 199 mg/dL found in milk donated by mothers who expressed their milk between one week and eight months postpartum in the Schanler and Oh (1980) study. The high variability observed by D. Anderson et al (1983) and Schanler and Oh (1980) was not evident in the present study.

PT milks were consistently much higher in TN than D milks. Preterm milks fed during the first two weeks postpartum were approximately twice as high in TN as were D milks, and even during week 6 of feeding the TN content was 30% higher. The difference between the means for the entire six weeks period, 260 mg/dL for PT milk and 159 mg/dL for D milk was highly significant. A premature infant fed his own mother's milk would receive approximately 64% more nitrogen than when fed the same volume of mature D milk during the first six postnatal weeks.

Levels of TN in the combined PT/D samples were as high or higher than those in PT milk for weeks one to five. During this period the percent of PT milk in the samples decreased from 90 to 68, yet TN concentrations remained high. It appears that the PT milk supplied by mothers unable to provide milk in sufficient volume to meet the entire requirement of their infants, contained higher concentrations of TN than PT milk supplied in adequate amounts by mothers whose infants received only PT milk. The consistently high values for TN in the PT/D milks indicates the significant contribution of even a limited supply of the mother's own milk towards provision of protein for her premature infant.

4.1.2. Nonprotein Nitrogen Content

NPN content of the PT milk ranged from 31.2 to 34.7 mg/dL during the first six weeks of lactation (Table 14) and for the entire period

the mean NPN content was 33.6 mg/dL (Table 16). Because NPN in PT milk remained relatively constant during the period of study and TN concentrations decreased with progressing lactation (Figure 1), NPN as a percent of TN rose from 9% in week one to 15% in weeks 4,5 and 6 for an overall mean of 13%.

Lemons et al (1982) also reported a narrow range of NPN concentrations 31 to 35 mg/dL, during their six week study. This range of NPN concentrations and the mean NPN, 33 mg/dL, were identical to NPN concentrations found in this study.

However, some differences between the findings of the present study and other reports are evident (Table 4). Atkinson et al (1980) reported that although NPN was on average a relatively constant 17.6% of TN, NPN in PT milk showed a significant decrease with time, from 62 mg/dL at the 3 to 5 day stage to 43 mg/dL by lactation day 28. The findings of Schanler and Oh (1980) were similar and they reported that NPN concentrations decreased from 72.2 mg/dL at days 1 to 3 to 32.6 mg/dL at days 22 to 28 and ranged from 15 to 18% of the TN. Gross et al (1980) reported no absolute values but found that percent NPN remained constant during the first month of lactation and averaged between 15 and 19% of the TN concentration.

Weekly average NPN concentrations of D milk ranged from 21.4 to 29.1 mg/dL and for the entire six week period the mean NPN concentration was 25.5 mg/dL in this study. NPN in D milk averaged between 14 and 17% of the TN concentration. D milk NPN concentrations in this study were not as high as those found in mature milk by Lonnerdal et al (1978b) who reported NPN concentrations of 46 mg/dL or 22% of the TN in milk from mothers 1.5 to 3.5 months postpartum, and by Schanler and Oh (1980)

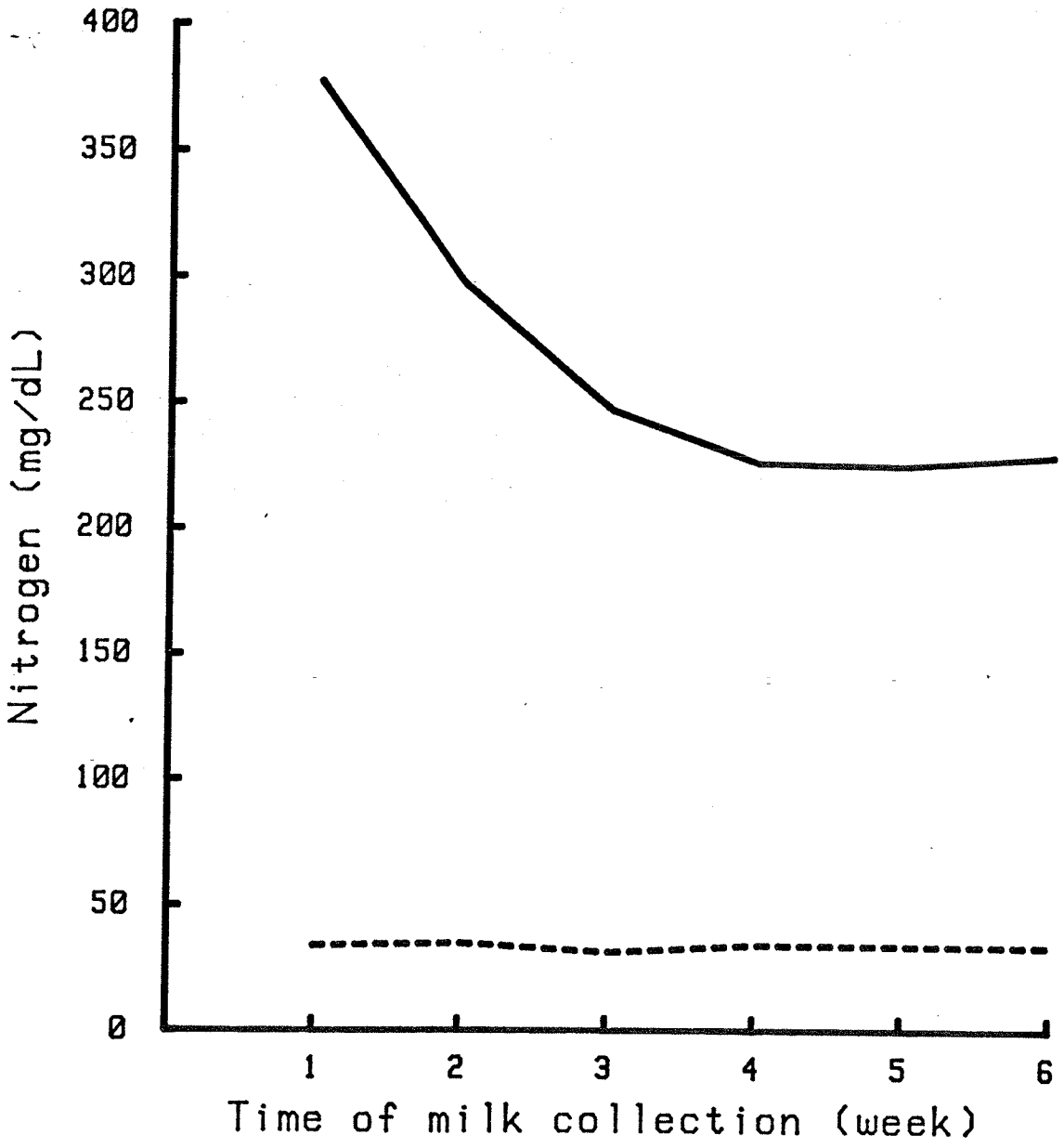

Figure 1. Comparison of total nitrogen (TN) and nonprotein nitrogen (NPN) in preterm milk.
TN ———— , NPN------ .

who reported that donated pooled milk contained 43 mg/dL NPN or 23% of the TN. Atkinson et al (1980) observed that NPN accounted for 28% of the TN in mature milk.

The range of NPN concentrations in PT/D milk, 28 to 36 mg/d, was broader than in PT milk but the mean NPN concentration 33 mg/d, was identical.

The values for NPN were lower than most reported values, possible because in this study NPN concentrations were determined on milk samples which had not been frozen. Freezing could cause disruption of the protein micelles and stimulate proteolysis of proteins producing higher concentrations of NPN. Atkinson et al (1980), Gross et al (1980), and Lonnerdal et al (1976b) determined NPN concentrations on samples frozen at -20^{o}C and the freezer temperature used by Schanler and Oh (1980) was not cited. Lemons et al (1982) whose NPN values are identical to those in this study used samples frozen at -80^{o}C. The effect of freezing human milk at various temperatures on protein stability is not known. However, research on cows' milk indicates that protein is unstable at freezer storage temperatures above -20^{o}C while at very low temperatures the protein remains stable for extended periods of time. Further study to provide information on the effect of freezing and thawing human milk is suggested.

4.1.3. Fat Content

The mean fat content of the PT milk for the first week of lactation was 1.8 g/dL, for weeks two and three was 3.4 g/dL, and for weeks four to six increased from 3.7 to 4.3 g/dL (Table 15). Fat concentrations in week one samples ranged from 0.2 to 4.4 g/dL and appeared to be dependent on the amount of colostrom in the sample. The fat content of

PT milk was significantly lower in week one than in weeks two and three and in weeks two and three than in week six. For the six week period the mean fat content of PT milk was 3.5 g/dL (Table 16).

Fat values ranging from 1.6 to 3.0 g/dL have been reported for PT milk during the first week of lactation, as was shown in Table 5. In two of three studies reporting fat values for days 1 to 3 (Gross et al, 1980 and D. Anderson et al, 1983) the fat values were slightly lower, 1.6 g/dL, than the value in the present study, 1.8 g/dL. Low fat concentrations probably reflect high colostrum content in the samples. The fat values in most reported studies fluctuated from week to week whereas in the present study fat values increased consistently and significantly over the six week period. Fat content of the combined PT and D milks did not exhibit the pattern of increasing fat content that was evident in the PT milk. Week to week variation was considerable and the difference between the means for the six weeks period, 3.3 g/dL for PT/D milk and 3.9 g/dL for D milk was significant.

Significant discrepancies between the findings of the present study and the reports of G. Anderson et al (1981) and Guerrini et al (1981) are evident in regard to fat concentrations. G. Anderson et al (1981) reported that the fat concentration increased from 3.0 g/dL at 3 to 5 days lactation to 4.3 g/dL at 15 to 18 days lactation. In agreement, Guerrini et al (1981) found that fat concentrations averaged 2.5 g/dL in samples collected over the first 6 days of lactation. Reasons for these higher values are unclear but may reflect a lesser amount of colostrom in the samples. The method used by Anderson et al (1981) could give higher fat concentrations because the extraction recovers phospholipids more effectively than other methods, however the fat concentration found

in term milk in their study was significantly lower than in the PT milk and did reflect the effect of colostrom.

Weekly average fat contents of D milks ranged from 3.1 g/dL to 4.2 g/dL and for the six week period the mean fat content was 3.9 g/dL. The fat content of D milk was similar to PT milk over the six week study but because D milk collections were from mothers of infants over six weeks of age the low fat content typical of colostrom was not observed in week one. When week one fat values were omitted the mean fat content of the PT milk was 3.8 g/dL, a value very similar to the 3.9 g/dL fat content of D milk indicating that fat concentrations in PT and D milk would be very similar over time.

Although the donor milk samples were not representative 24 hour samples of mature milk, the average fat content was almost the same as that determined for pooled 24 hour samples from 200 mothers, a value of 3.8% as reported by Macy et al (1953). A higher average fat content, 4.5%, was reported by Hambraeus (1977) and a lower content, 2.10 to 2.95%, by Fomon (1974). The fat content in D milk is often highly variable making standardization of D milk difficult. Much of the variability relates to the fact that milk is collected after varying periods of lactation, at different times during the day and at varying periods of time after the donor's feeding of her own infant. The fat concentrations in D milk in this study were not as variable as the 1.7 to 7.9 g/dL reported by D. Anderson et al (1983) for spot D milk specimens but were not from one feeding from one donor.

Reliability of the method of fat analysis used in this study (Nakai and Le 1970), has been questioned by Dorea et al (1981) who observed a correlation of only 0.471 between the turbidometric and

Roese-Gottlieb methods. However Hundrieser et al (1984) reported a high correlation coefficient, 0.979, between the two methods. Dorea et al (1981) analyzed samples that had been frozen and held at -20°C. Freezing often causes flocculation making accurate measurement of small samples difficult and the freezing and thawing activates lipases in milk (Jensen et al, 1978) possibly influencing values obtained by this method after freezing.

4.1.4. Lactose Content

The mean lactose content of the PT milk collected during the first week of feeding was 6.3 g/dL and for weeks two to six ranged from 6.7 to 7.0 g/dL (Table 15). The lactose content of the PT milk was significantly lower in week one than in weeks four to six. For the six week period the mean lactose content of PT milk was 6.8 g/dL (Table 16).

Lactose values ranging from 5.04 to 6.20 g/dL have been reported for PT milk during the first week of lactation, as was shown in Table 6. In all three studies which reported lactose values for both days 1 to 3 and 4 to 6 (Gross et al, 1980, G. Anderson et al, 1981 and D. Anderson et al, 1983) the lactose concentrations were lowest during the first week and increased with progressing lactation similar to the results of the present study. G. Anderson et al (1981) suggested that the lower lactose concentration was a consequence of the higher nitrogen throughout their study. This inverse relationship could be relevant in the first week of this study when lactose concentrations were significantly lower and TN significantly higher than concentrations determined with progressing lactation. Since both lactose and TN are part of the water soluble fraction, the higher nitrogen may effectively decrease the lactose concentration in the milk.

Weekly average lactose contents of D milk ranged from 6.6 to 7.4 g/dL, and for the six week period the mean lactose content was 6.9 g/dL. A similar lactose concentration, 6.74 g/dL, was determined in milk collected after one month postpartum by Lauber and Reinhardt (1979) using a modification of the phenol-sulfuric method used in this study.

The mean lactose content of the PT/D milk collected during the first week of feeding was 5.9 g/dL and for weeks two to six increased from 6.5 to 6.9 g/dL (Table 15). The lactose content of the PT/D milk was significantly lower in week one than in weeks two to six. For the six week period the mean lactose content of PT/D milk, 6.6 g/dL, was significantly lower than the mean lactose content of D milk. 6.9 g/dL. The TN concentration of the PT/D milks was high and the lactose content very low during week one lending further support to the inverse relationship between TN and lactose concentration.

4.1.5. Energy Content

The mean energy content of the PT milk during the first week of lactation was 55.2 kcal/dL, for weeks two to five ranged from 67.0 to 71.8 kcal/dL and for week six was 75.1 kcal/dL (Table 15). The energy content of the PT milk was significantly less in week one than in weeks two to six and in week three significantly less, than in week 6. For the six week period the mean energy content of the PT milk was 68.8 kcal/dL (Table 16).

Other recent reports have documented a similar range of energy values in PT milk (Table 7). Energy content is largely dependent on fat concentrations in the milk and factors used to calculate energy can influence values reported. The majority of studies on PT milk have also observed low total energy concentrations during the first week of

lactation. Gross et al (1980), G. Anderson et al (1981) and D. Anderson et al (1983) reported low energy concentrations of 52.4, 58 and 49 kcal/dL, respectively, at days 3 to 5 increasing to 67.4, 71 and 67 kcal/dL, respectively, at day 7 to 10 and then remaining constant. This pattern is similar to the energy concentrations in this study and appears to be a reflection of fat concentration. Because of sampling techniques described earlier, Lemons et al (1982) did not observe the low energy concentrations in their first sample.

The energy in this study was calculated from the measured concentrations of protein (6.25 TN), fat and lactose using the formula: kcal/dl=protein (g/dL)x5.65 + fat (g/dL) x 9.25 + lactose (g/dL) x 3.95. These factors are based on the heats of combustion for the individual nutrients and thus are comparable to bomb calorimetry values. G. Anderson et al (1981) found that calculated energy values using heats of combustion averaged 97% of those determined by bomb calorimetry and D. Anderson et al (1983) also found no significant difference between calculated energy content and energy content determined with bomb calorimetry. However,Lemons and coworkers found the mean energy content of the milk samples as determined by bomb calorimetry to be 18.5% greater than the energy content based upon calculation from nutrient content using the factors of 4.27, 8.79 and 3.87 for protein, fat and lactose respectively. The factors used by Lemons et al (1982) estimate available energy based on metabolic studies which could account, in part, for the discrepancy between calculated values and bomb calorimetry. The mean total energy content of PT milk as determined by Lemons et al (1982) using the bomb calorimeter, 73.2 kcal/dL, was higher than the total energy content reported in this study and by other researchers. A precise conversion factor for available energy from a

milk diet for infants is difficult to determine as it is likely to vary under different physiological conditions such as gestational age, postnatal age and illness.

In the present study weekly average energy contents of D milk ranged from 66.5 to 72.6 kcal/dL and for the entire six week period the mean energy content was 69.3 kcal/dL. The energy content of D milk was similar to PT milk over the six week study but was higher in week one because of the higher fat concentration. Energy values ranging from 60-75 kcal/dL and a mean of 70 kcal/dL have been reported for mature milk (Jenness et al, 1977; Brostrom, 1981). Energy content of the mature D milk sampled in this study was more variable than PT milk and confirmed the high variability of previously reported values for mature milk (Anderson et al, 1983).

The energy content of the combined PT and D milks was similar to the PT milk with a low value, 48.8 kcal/dL during week one and values ranging from 62.5 to 72.5 during weeks two to six.

4.1.6. Sodium Content

The mean sodium content of the PT milk was 11.5 meq/L during week one and decreased to 5.4 meq/L during week 6 (Table 17). For the entire six week period the mean sodium content of PT milk was 8.4 meq/L. (Table 16). The mean sodium content of the combined PT and D milks was 8.0 meq/L similar to the sodium content of PT milk.

Other recent reports have demonstrated a similar decrease in sodium concentrations over time in PT milk but the values in this study were generally lower than those reported in the literature. Gross et al (1980) reported that sodium content decreased from 26.6 meq/L at day 3 to 12.6 meq/L at day 28 and Atkinson et al (1980) reported that the

Table 17

MACROMINERAL CONTENT[1] OF PRETERM (PT), DONOR (D) AND PRETERM/DONOR (PT/D)[2] MILK FED TWENTY PREMATURE INFANTS

Nutrient	Milk Source	Week 1	2	3	4	5	6
Sodium (meq/L)	PT	11.5±1.7 (6)[3]	10.4±1.8 (6)	6.5±1.1 (5)	7.3±3.0 (5)	8.8±3.2 (5)	5.4±0.8 (6)
	D	4.2±0.0 (2)	4.0±0.4 (7)	3.5±1.2 (2)	- -	7.8±3.7 (2)	4.1 (1)
	PT/D	- -	9.9±0.4 (3)	10.7±1.4 (6)	5.6±0.4 (5)	6.5±1.2 (4)	5.6±0.1 (2)
Calcium (mg/dL)	PT	40.6±4.3 (6)	38.6±2.1 (6)	34.5±1.9 (5)	34.0±2.3 (5)	35.2±1.8 (5)	38.7±3.2 (6)
	D	45.5±2.5 (2)	42.2±2.0 (7)	38.4±2.2 (2)	- -	45.1±5.9 (2)	51.0 (1)
	PT/D	- -	33.5±2.7 (3)	39.9±3.2 (6)	37.1±2.6 (5)	42.1±2.4 (4)	33.4±6.8 (2)
Phosphorus (mg/dL)	PT	8.6±0.6 (6)	8.8±0.6 (6)	9.0±0.6 (5)	8.8±0.7 (5)	8.8±0.7 (5)	9.6±0.6 (6)
	D	9.8±0.7 (2)	8.5±0.4 (7)	7.5±0.8 (2)	- -	9.5±0.6 (2)	10.2 (1)
	PT/D	- -	7.6±0.2 (3)	8.5±0.7 (6)	8.7±0.6 (5)	9.5±0.5 (4)	7.9±0.6 (2)

[1] Mean±SEM

[2] The percent of PT in PT/D milk ranged from 90% (wk 1) to 52% (wk 6)

[3] Number in brackets is the number of samples analyzed

initial sodium content at days 3 to 5, 23.0 meq/L decreased significantly to 13.5 meq/L at days 13 to 17 and then remained constant. Schanler and Oh (1980) found that sodium content decreased, from 22.2 meq/L at days 1 to 3 to 7.6 meq/L at days 26 to 29 and Lemons et al (1982) also reported that sodium concentrations in PT milk decreased as duration of lactation increased.

Weekly average sodium concentrations in D milk ranged from 3.5 to 7.8 meq/L and for the entire six week period the mean sodium content was 4.5 meq/L, a value lower than the 6.4 meq/L reported by the Department of Health and Social Security (1977), or other values of 8.1 meq/L and 11.2 meq/L reported by Atkinson et al (1980) and by Schanler and Oh (1980) respectively. PT milks in the present study were consistently much higher in sodium content than D milks. The difference between the means for the entire six week period, 8.4 meq/L for PT milk and 4.5 meq/L for D milk, was highly significant. A preterm infant fed his own mother's milk would receive approximately 85% more sodium than if fed an equal volume of mature D milk during the first six weeks. Lemons et al (1982) found that based upon an average sodium content in mature breast milk, a preterm infant being fed his own mother's milk would receive approximately 75% more sodium than when fed pooled mature milk.

Considerable variability in milk sodium concentrations occurs between lactating women and the variability appears to be greater in milk from women delivering prematurely (Atkinson et al, 1980 and Gross et al, 1980). Sodium concentrations in individual samples ranged from 2.4 to 19.0 meq/L during this study. However, Curran (1979) reported values ranging from <2 to >20 meq/L with a median of 7 meq/L and Gross

et al (1981) reported values from <10 meq/L to >50 meq/L during their four week study of PT milk.

4.1.7. Calcium Content

The mean calcium content of the PT milk ranged from 34.0 to 40.6 mg/dL for the first six weeks of feeding (Table 17). For the entire six week period the mean calcium content of PT milk was 37.9 mg/dL (Table 16). The calcium concentrations in PT milk did not change significantly during the period of lactation studied (Figure 2). PT/D milks contained almost exactly the same mean calcium content, 38.0 g/dL, and also showed no significant change over the six weeks.

Most recent reports (Gross et al, 1980, Lemons et al, 1982 and Chan, 1982) found that calcium concentrations remained constant throughout the first month of lactation and reported values ranging from 20.8 to 35.9 mg/dL (Table 9). However, Atkinson et al (1980) reported that calcium concentrations decreased significantly from 32.2 mg/dL at the 3 to 5 day stage to 27.5 mg/dL at the 13 to 17 day stage.

Weekly average calcium contents of D milk ranged from 38.4 to 51.0 mg/dL and for the entire six week period the mean calcium content was 43.2 mg/dL. PT milks were consistently much lower in calcium content than D milks and the difference between the means for the entire period, 37.1 mg/dL for PT milk and 43.2 mg/dL for D milk, was significant. Conversely, Schanler and Oh (1980) found that PT milk was generally higher in calcium content, than donated, pooled milk expressed between one week and eight months postnatally throughout their study.

Different sampling procedures, sample preparation and methodology could account for the discrepancies between the findings of the present study and other reports. One interesting possibility is the use of

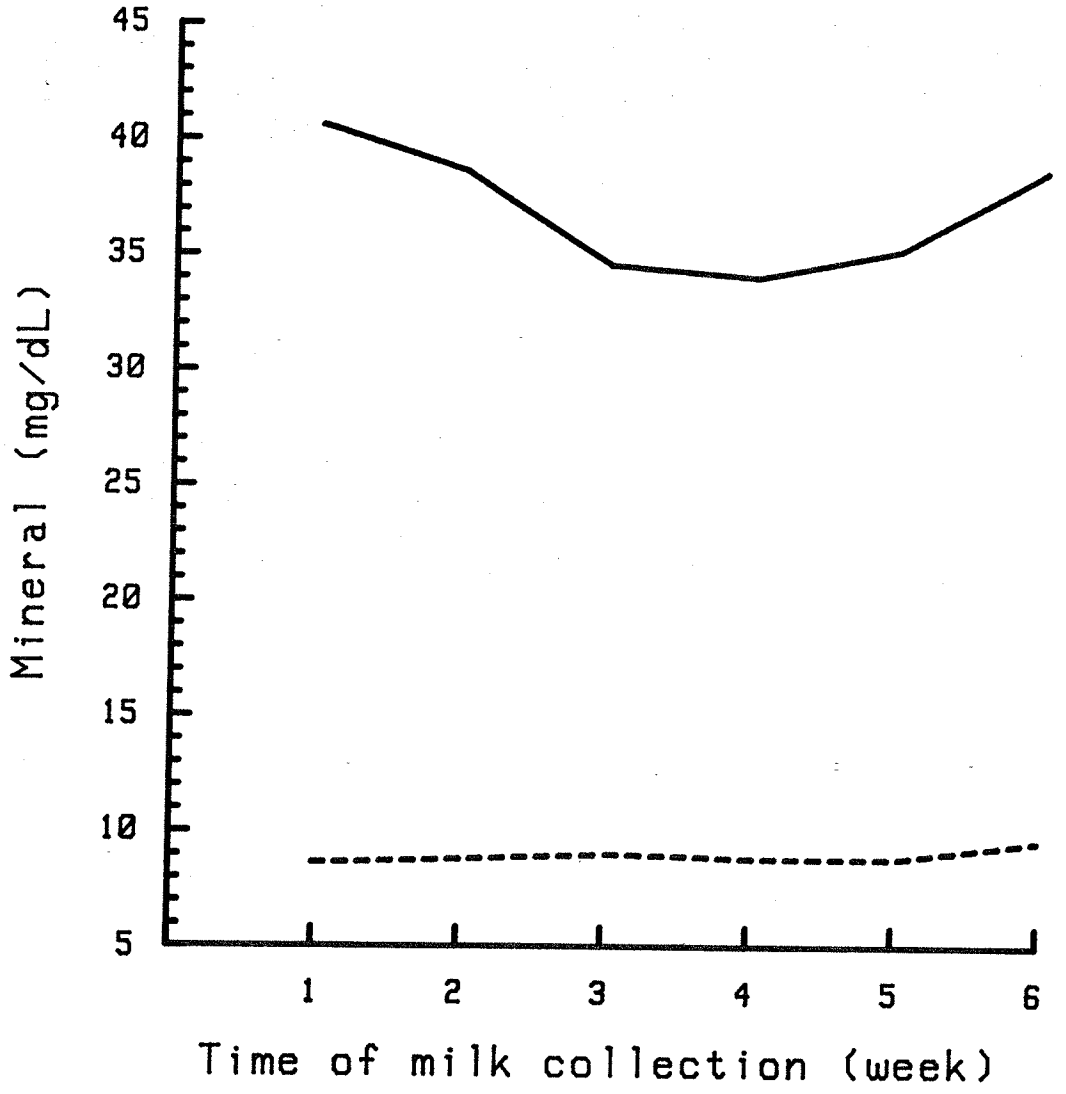

Figure 2. Comparison of calcium and phosphorus in preterm milk.
Calcium ———— , Phosphorus -------- .

whole milk versus defatted milk in calcium determinations. Fransson and Lonnerdal (1984) found that 10% of total calcium is bound to membrane proteins in human milk fat. Use of whole milk in the present study could account for the somewhat higher calcium values.

Variability occurs in mineral composition during the course of a single feed, because of diurnal variations and between mothers and could also account for the discrepancies. Many investigators have examined the calcium content in human milk and found variation from mother to mother. Jelliffe and Jelliffe (1978a) found a difference in calcium concentration in milk from well nourished mothers, 28.6 to 34.0 mg/dL, and poorly nourished mothers, 20.8 to 34.2 mg/dL. Atkinson et al (1980) found that considerable variability in calcium concentrations occurred between lactating women with the variability in milk from mothers giving birth prematurely being greater than in the milk from mother giving birth at term. They reported calcium concentrations ranging from 18 to 48 mg/dL at the 3 to 5 days interval whereas calcium concentrations ranged from 24 to 52 mg/dL during the six week period in this study.

4.1.8. Phosphorus Content

The mean phosphorus content of the PT milk ranged from 8.6 to 9.6 mg/dL for the first six weeks of feeding (Table 17). For the entire six week period the mean phosphorus content of PT milk was 8.9 mg/dL and of PT/D milk was 8.6 mg/dL (Table 16). The phosphorus concentration in PT milk and in PT/D milk did not change significantly during the period of lactation studied.

Other recent reports have documented higher phosphorus concentrations in PT milk (Table 10). Gross et al (1980) reported that

phosphorus remained constant throughout the first month of lactation and ranged from 9.5 mg/dL to 14.9 mg/dL. Atkinson et al (1980) found no significant changes in phosphorus concentrations in PT milk and reported values of 15.5 mg/dL to 16.7 mg/dL. Lemons et al (1982) reported that the phosphorus concentration, 13.2 mg/dL, did not change over the period of lactation studied but Chan (1982) found that phosphorus content in PT milk was significantly lower at the 26 to 31 day stage of lactation, 17.0 mg/dL, than at the 2 to 4 day, 22.0 mg/dL.

Weekly average phosphorus contents of D milk ranged from 7.5 to 10.2 mg/dL and for the entire six week period the mean phosphorus content was 8.8 mg/dL. The phosphorus content of D milk was similar to that of PT milk over the six week study.

Different sampling procedures, sample preparation and methodology could account for the discrepancies between the findings of the present study and other reports. In this study phosphorus was determined on digested samples prepared for atomic absorption spectroscopy and the method of digestion using perchloric acid and hydrogen peroxide may have resulted in some loss of phosphorus. This method of digestion was used for the samples for all mineral determinations because the amount of sample remaining for these analyses was very small.

4.2. Effect of Nutrient Intakes on Growth of Premature Infants.

Test of the hypotheses related to the second objective of the study indicated there were positive linear correlations between both protein and energy intakes and the growth of premature infants with birthweights less than 1500 g during the first six postnatal weeks.

4.2.1. Growth Measurements

The weight, length and head circumference (HC) of the 20 study

infants were measured weekly by the nurse coordinator at the Health Sciences Centre. These measurements are reported in Appendices G,H, and I. Means of weight gain, length gain and HC gain for the first six weeks postnatal, and the time to regain birthweight are listed in Table 18. There was a strong positive linear correlation between weight and HC ($r=0.94$) and weight and length ($r=0.86$). Difficulty obtaining accurate length measurements with a tape could account for the lower coefficient of correlation (r) for weight and length. Weight gain was used as the dependent variable in the analysis of the growth data because the strong positive correlation of weight with HC and length made use of the additional measures unnecessary.

4.2.2. Protein and Energy Intakes and Growth

Mean weight gains for the 20 premature infants are reported in Appendix J. These correspond to weight gains reported in the literature (Chessex et al, 1983; Shenai et al 1980). Energy and protein intakes for the individual infants during six weeks postnatal are reported in Appendices K to M. During the first week the mean total energy intake was 55.7 kcal/kg/d. Parenteral nutrition, consisting of a 10% glucose solution routinely provided at birth, provided 49% of these calories. By week 2, parenteral nutrition consisting of glucose, amino acids, and vitamin and mineral solutions provided only 14% of the 114 kcal/kg/d total energy intake for the 20 infants. Means of the energy and protein intakes from milk and the total energy intake for each infant are listed in Table 19.

Weight gain (g/kg/d), energy intake (kcal/kg/d) and protein intake g/kg/d) for the 20 infants during their first six weeks postnatal are

Table 18

DAYS TO REGAIN BIRTHWEIGHT AND MEAN WEIGHT, LENGTH AND HEAD CIRCUMFERENCE (HC) GAINS OVER SIX WEEKS FOR TWENTY PREMATURE INFANTS

Infant	Weight gain g/kg/d	Length gain cm/wk	HC gain cm/wk	Time to regain birthweight d
1	13.3	.83	.67	16
2	13.1	.67	.83	21
3	13.6	.92	.75	16
4	5.1	.67	.25	36
5	11.4	.92	.58	15
6	11.2	.67	.83	16
7	12.9	1.00	1.00	16
8	17.4	.50	.67	17
9	12.6	1.00	.75	11
10	16.7	.80	1.00	6
11	12.0	1.10	.67	14
12	15.0	.40	1.00	13
13	12.0	.50	.67	14
14	17.5	1.00	.92	6
15	10.2	1.00	.90	16
16	6.2	.58	.63	24
17	9.1	.50	.58	23
18	2.6	.58	.50	23
19	5.4	1.00	.50	25
20	11.7	.75	.83	16
Mean	11.5	.77	.73	17

Table 19

MEAN DAILY ENERGY AND PROTEIN INTAKES OF TWENTY PREMATURE INFANTS DURING FIRST SIX WEEKS POSTNATAL

Infant	Energy Intake kcal/kg/d		Protein g/kg/d
	milk	total	milk
1	87.6	104.4	2.83
2	112.7	119.3	2.72
3	116.3	119.9	2.85
4	86.1	110.6	1.73
5	124.6	137.5	3.03
6	106.7	108.8	2.45
7	97.2	110.2	2.00
8	136.1	136.9	2.91
9	125.3	130.1	2.54
10	134.3	145.7	3.05
11	127.3	129.9	2.85
12	103.6	115.0	2.31
13	113.4	124.9	2.36
14	153.4	160.2	3.53
15	124.2	130.4	2.51
16	35.5	93.2	0.75
17	82.8	116.9	1.96
18	86.8	103.0	1.45
19	87.5	102.8	1.64
20	103.8	107.0	2.65
Mean	107.3	120.3	2.41

plotted in Figures 3 and 4. In the first two weeks of life there was a sharp increase in all parameters with weight gain and protein intake peaking in the third week and energy intake peaking in the fourth week. This was followed by a flattening of the curves when a weight gain of approximately 18.5 g/kg/d, energy intake of 141 kcal/kg/d and protein intake of approximately 3.0 g/kg/d was achieved. The graphs show that the weight gain closely followed the energy and protein intakes during the first six postnatal weeks. These curves are similar to those reported by Chessex et al (1981) who found that the relationships between metabolic rate, energy intake, weight gain and age all followed a similar pattern. Weight gains and energy intakes for the 13 very LBW, formula-fed infants in their study also increased in the first two weeks of life and subsequently stabilized at 18 g/kg/d and 155 kcal/kg/d respectively.

The similarity of the age-weight gain, age-energy intake and age-protein intake (Figures 3 and 4) indicate that protein and energy intake correlated with weight gain. A significant linear correlation ($r=0.82$) of increasing weight gain with increasing protein intake is demonstrated in Figure 5. A significant linear correlation is similarly shown between weight gain and total energy intake ($r=0.68$) in Figure 6 and weight gain and energy intake from milk ($r=0.72$) in Figure 7.

These results support previously published work that has shown the importance of protein intake on the growth of premature infants. The LBW premature infant is predicted to have higher protein requirements in relation to energy intakes. The requirement of protein necessary to cover maintenance, losses and growth has been estimated from fetal accretion rates to be approximately 3 g/kg/d (Fomon et al, 1977). The

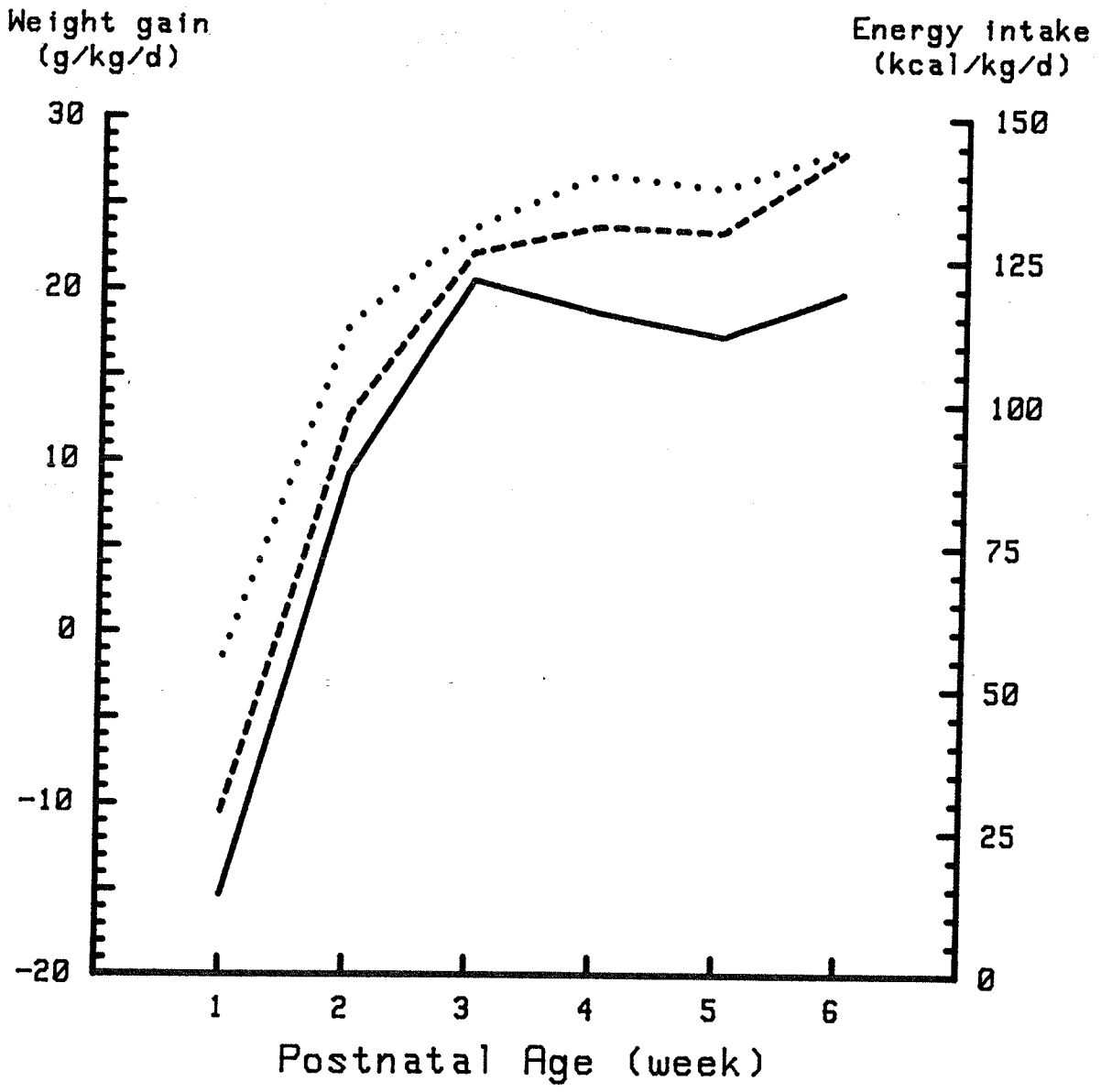

Figure 3. Mean weight gain and mean energy intakes (total and from milk) of twenty premature infants during first six weeks postnatal.
Weight gain ————, milk energy intake --------, and total energy intake ·····.

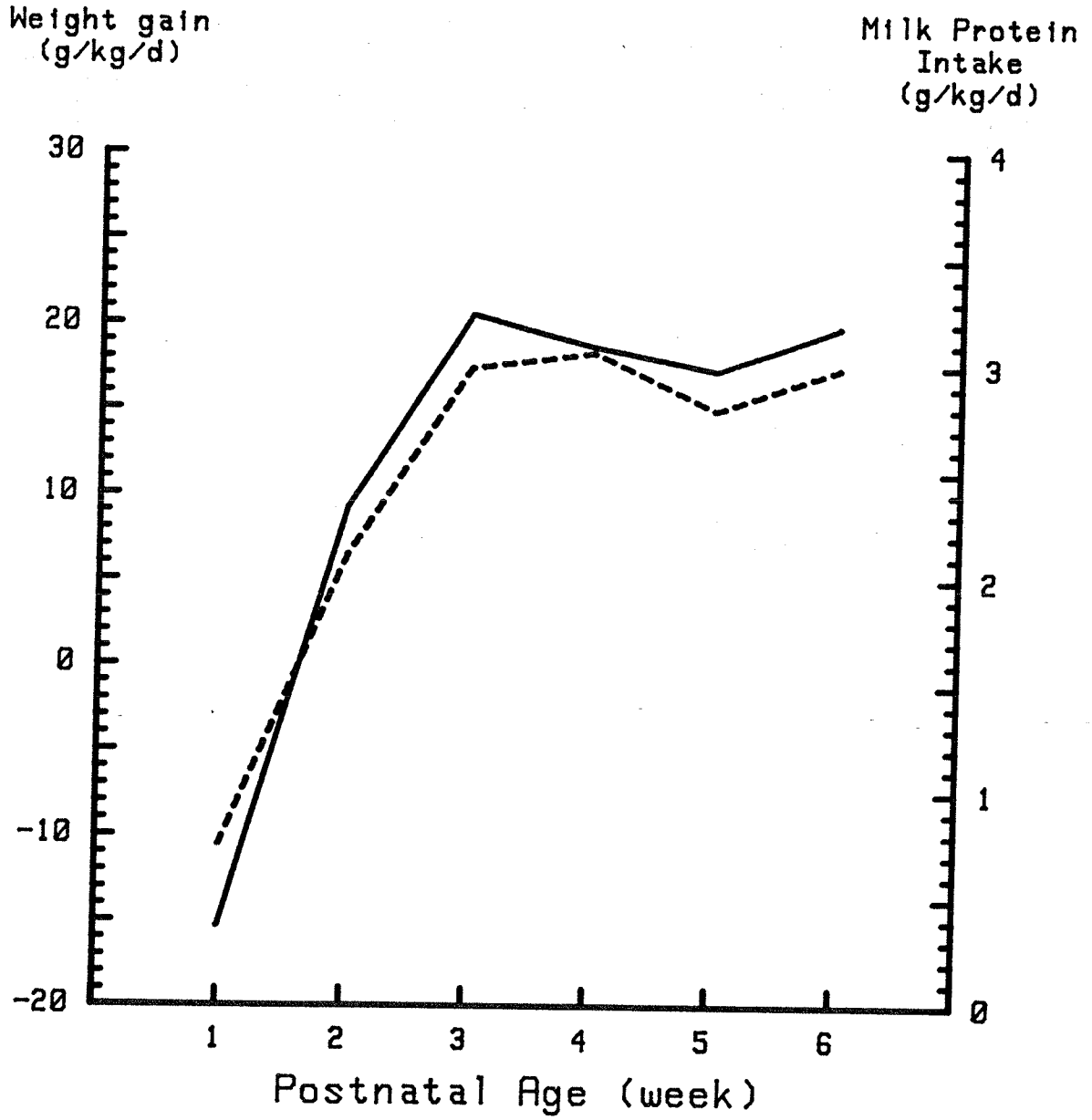

Figure 4. Mean weight gain and milk protein intake of twenty premature infants during first six weeks postnatal.
Weight gain ———— , protein intake - - - - - - - - .

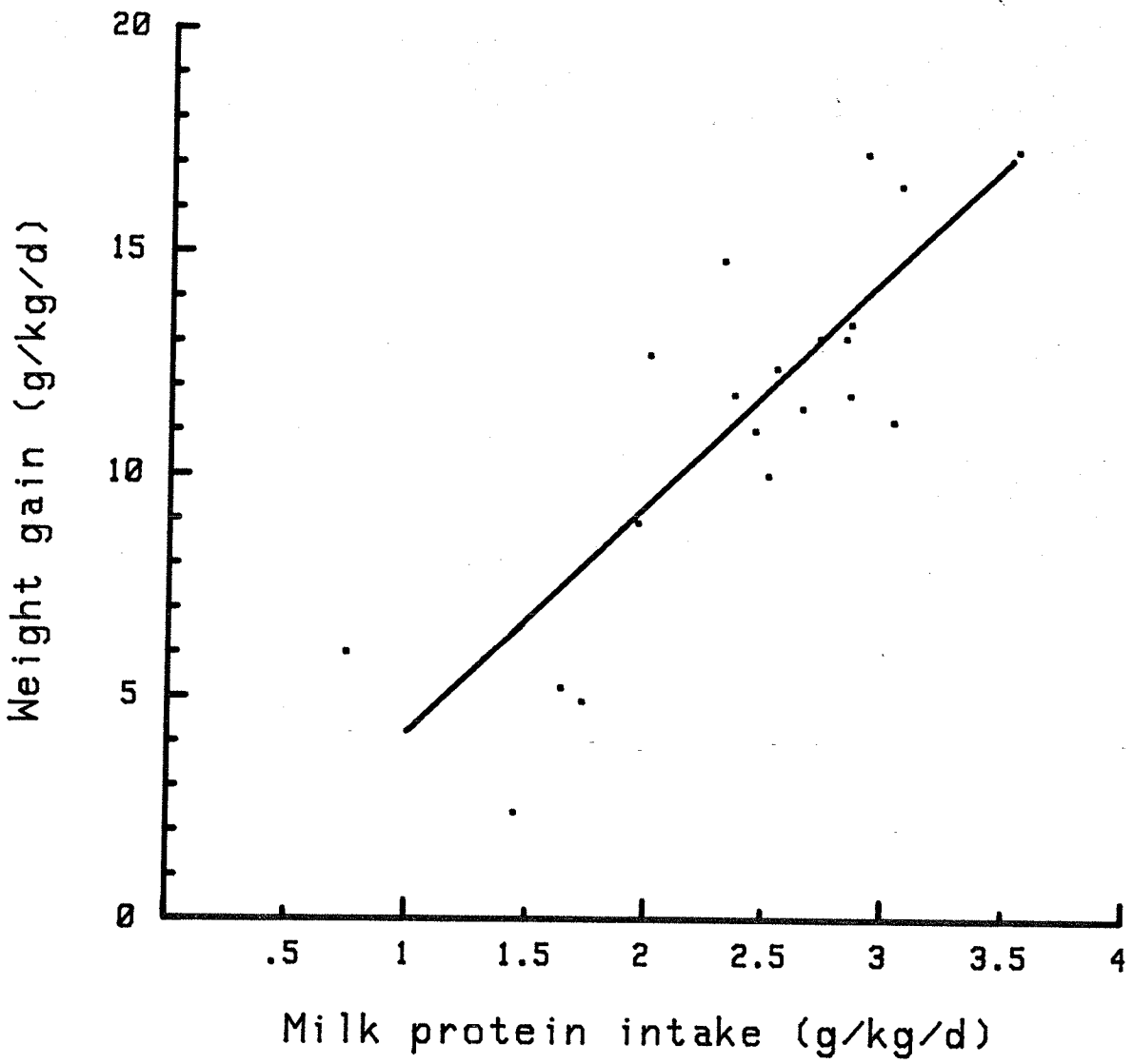

Figure 5. Relationship between milk protein intake and weight gain during initial six week postnatal period. R = .82

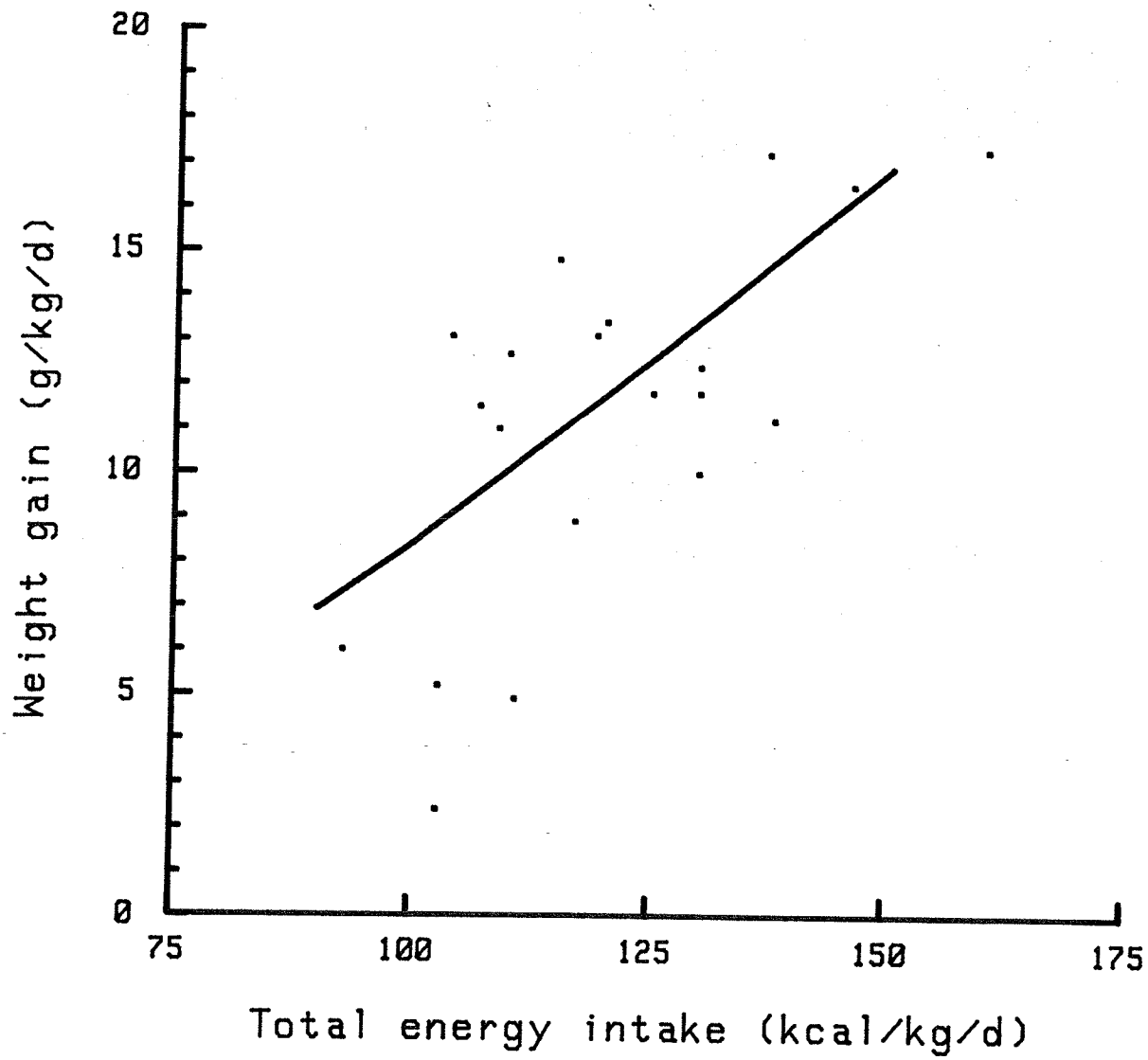

Figure 6. Relationship between total energy intake and weight gain during initial six week postnatal period. R = .68

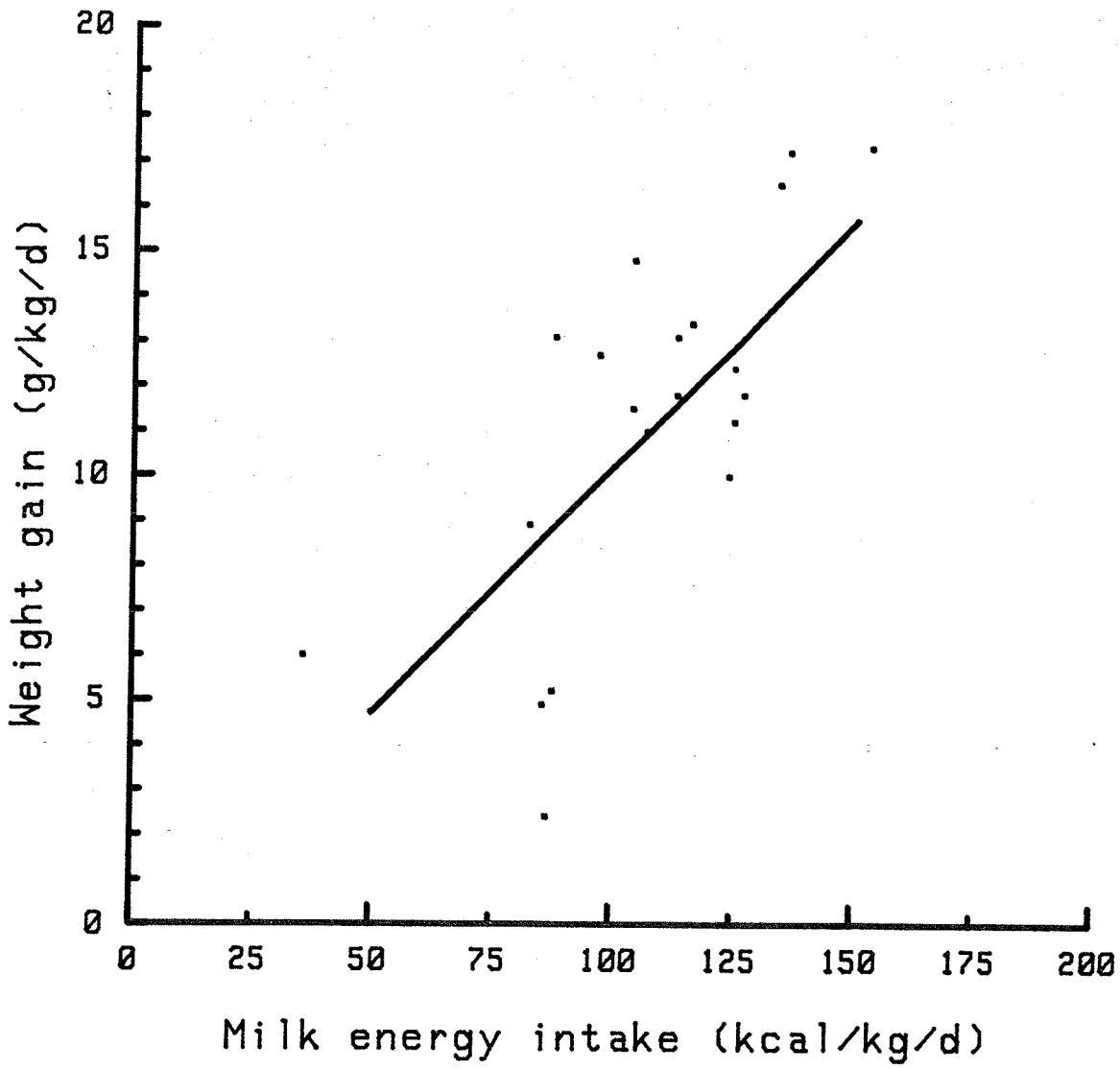

Figure 7. Relationship between milk energy intake and weight gain during initial six week postnatal period. R = .72

mean protein intake of the premature infants in this study increased during the first two weeks subsequently stabilizing at approximately 3 g/kg/d for the remainder of the study, however, weight gain was generally less than the in utero gain used in the estimations of requirements.

4.2.3. Infant Growth Compared to Fetal Growth

Several authorities (Ziegler, et al 1981; Committee on Nutrition, 1977) have suggested that the "optimal" diet for the LBW infant is one that supports a rate of growth similar to that of the fetus in utero. The rate of weight gain is greater in the fetus between 26 and 36 weeks of gestation than at any other time in the normal human lifespan. The true scale of this burst of growth becomes apparent when the weight gain is expressed in relation to body size. The growth involves cell division as well as cell enlargement and it is possible that in some organs, especially the brain, there are critical periods for the accomplishment of cell division. The proper feeding and nutrition of prematurely born infants if they are to survive unimpaired is a cause for real concern. The desirable intakes of major nutrients such as protein and the principal energy sources are uncertain because, although minimum intakes have been defined and a certain amount is known about the protein and energy cost of growth (Fomon et al, 1977) there is no agreement about the desirable rate and quality of growth. It has been assumed that after a short cessation of growth, it is desirable that the premature infant should have a rate of weight gain similar to that of the fetus.

In order to compare weight gains of the infants in this study, with weight gains of what would be expected based on growth of the reference

fetus (Ziegler et al, 1976; Appendix N) percent of the expected weight gain was calculated for the 18 infants with gestational ages of 28 to 32 weeks. (Appendix O). Infants numbers 10 and 14 were excluded from this study because gestational age was 36 weeks.

Weight gains of the infants did not approach those of the reference fetus until the sixth week of life (Table 20). The overall mean percent of expected weight gain for the 18 infants was 75% ranging from 18 to 138% for individual infants over the six week period. At week 2 postnatal, when the infants were 30 to 34 weeks postconceptual age they had gained only 45% of the weight gain expected of the fetus. However, at week 6 when the infants were 34 to 38 weeks postconceptual, weight gain had increased to 96% of the weight gain expected of the fetus.

It appears that utilizing intrauterine growth rates as a goal is somewhat unrealistic. Atkinson et al (1983) also found that the infants studied had generally lower weight gains than the in utero rate of 23 g/d used as a standard by Ziegler et al (1981) for estimation of the requirements of premature infants. Chessex et al (1983) also reported that the mean growth rate of preterm infants who were fed their own mothers' milk was slightly lower than that of the placentally fed fetus. Ideal rates of growth have not been determined for the LBW premature infant and expecting the premature infant to continue to grow at the same rate as that which would have occurred had the infant remained in utero, fails to take into account the many physiological and metabolic changes which occur at birth. While the intrauterine rate of growth may represent a reasonable goal for the nutritional management of premature infants, such growth is rarely achieved. Moreover, there is no evidence

Table 20

PERCENT OF EXPECTED WEIGHT GAIN[1] FOR PREMATURE INFANTS DURING FIRST SIX WEEKS POSTNATAL

Infant	GA (wk)	Postnatal Age (wk) 1	2	3	4	5	6	Mean
1	29	0[2]	41	89	158	33	96	86
2	31	0	90	14	86	100	124	83
3	31	0	21	76	91	96	112	79
4	29	0	0	67	16	33	118	47
5	28	0	68	71	67	37	133	75
6	31	0	95	76	91	71	88	84
7	28	0	111	94	89	95	57	89
8	30	0	11	200	86	125	267	138
9	31	0	63	86	96	42	60	69
11	30	0	56	129	102	114	-	100
12	31	0	69	110	136	146	91	110
13	28	0	37	59	0	132	148	75
15	32	0	48	73	92	112	64	78
16	32	0	0	6	33	96	27	32
17	32	0	14	9	50	88	136	59
18	29	0	0	0	47	0	41	18
19	29	0	24	56	48	14	32	37
20	30	0	61	127	90	127	29	87
Mean	30	0	45	75	77	81	96	75

[1]Expected for reference fetus (Ziegler et al. 1976, Appendix N)

[2]May represent negative values.

that failure to achieve such growth is particularly harmful to the infant (Heird, 1977).

4.2.4. Effect of Preterm Milk and of Mature Donor Milk and Formula Combinations on Growth

Various human milk and formula combinations were fed to the 20 study infants. Four infants (PT) were fed their own mothers' milk exclusively during their first six weeks postnatal. Sixteen infants (C) were fed combinations of own mothers' milk, mature donor milk, Special Care and Similac formulas (Ross Laboratories, Montreal, Quebec). Clinical data for the infants are reported in Table 21. The mean gestational age for the PT and C infants were similar, 30.5 and 30.7 weeks, respectively and there was no difference between the birthweight of the PT infants, 1285 g and the C infants, 1204 g. Mean length and mean head circumference (HC) were similar for the two groups. The PT infants regained birthweight in 14.3 days and the C infants in 17.9 days, however these differences were not significant ($p < 0.11$).

Energy intake, protein intake and weight change for the two groups of infants are reported in Table 22. In week 1, the mean daily protein intake for the PT infants, 1.1 g/kg/d was significantly greater than for C infants, 0.5 g/kg/d and in week 2, the mean intakes of milk energy, total energy and protein for the PT infants, 125 kcal/kg/d, 130 kcal/kg/d and 3.2 g/kg/d, respectively, were significantly greater than the intakes for the C infants, 91 kcal/kg/d. 110 kcal/kg/d and 1.8 g/kg/d. During week 1 the PT and C infants lost 12.9 and 16.0 g/kg/d, respectively and by the end of the second week of life PT infants had regained their initial weights but the C infants were only 93% of their initial weights (Figure 8). This suggests that PT milk may be a

Table 21

CLINICAL DATA[1] FOR PREMATURE INFANTS FED PRETERM MILK (PT) OR COMBINATION OF HUMAN MILK AND FORMULA (C)

	PT (4)[2]	C (16)[2]
Gestational age (wk)	30.5±0.9	30.7±0.6
Birthweight (g)	1285±95.3	1204±48.5
Length (cm)	40.2±1.2	38.3±0.8
Head circumference (cm)	26.9±0.1	26.5±0.4
Regained birthweight (d)	14.3±1.2	17.9±1.8

[1]Mean±SEM

[2]Number of infants in each group

Table 22

INTAKE AND WEIGHT GAIN OF FOUR INFANTS FED PRETERM MILK (PT) AND SIXTEEN INFANTS FED COMBINATION HUMAN MILK AND FORMULA (C)[1]

	Infant	Week 1	2	3	4	5	6	Overall Mean
Energy intake (milk) (kcal/kg/d)	PT	33±7.3	125±5.6[2]	136±4.0	136±14.0	142±10.3	133±16.6[3]	117±8.8
	C	20±4.0	91±9.6	116±12.8	130±12.6	126±9.4	147±10.6	104±5.9
Energy intake (total) (kcal/kg/d)	PT	57±4.9	130±2.8[2]	136±4.0	141±10.3	145±11.2	133±16.6[3]	124±7.2
	C	55±3.0	110±5.0	129±8.9	140±8.1	136±6.2	148±11.1	118±4.5
Protein intake (g/kg/d)	PT	1.1±0.3[2]	3.2±0.2[2]	2.9±0.1	2.6±0.2	2.6±0.1	2.1±0.3[3]	2.4±0.2
	C	0.5±0.1	1.8±0.3	2.8±0.3	2.8±0.2	3.2±0.3	3.2±0.3	2.3±0.2
Weight gain (g/kg/d)	PT	-12.9±3.5	14.2±3.0	16.8±2.2	15.3±5.2	18.4±4.2	17.0±4.2	11.5±2.7
	C	-16.0±2.9	8.0±2.8	21.4±3.1	19.5±2.0	16.9±2.3	20.5±3.1	11.6±1.7

[1]Mean±SEM

[2]PT group was significantly different from C group ($p<0.02$)

[3]Complete intake not recorded for 2 infants receiving PT milk who were nursed during week 6.

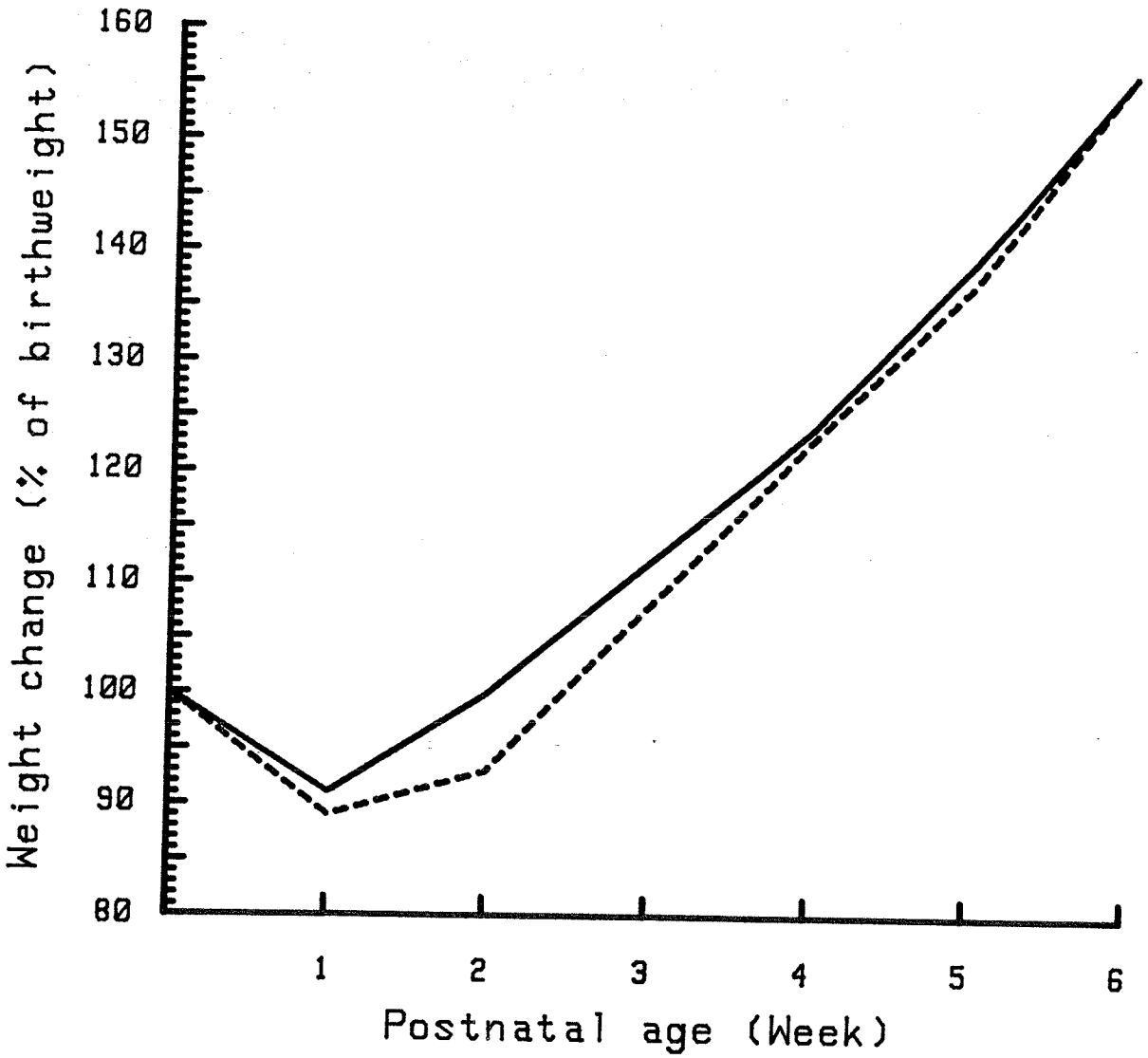

Figure 8. Weight change for infants receiving their own mothers' milk (PT) and infants receiving various combinations of human milk and formula (C). PT———— , C -------- .

superior source of nutrition compared to the mature donor milk fed the infants in this study during the first two weeks. These results are similar to Atkinson et al (1981) where by the end of the second week infants fed PT milk regained their initial weights, but infants fed pooled donor milk were only 94% of their initial weights.

Energy intake, protein intake and weight gain of the two groups of infants were similar during weeks 3 to 6. Many C infants were fed a premature infant formula high in protein (2.2 g/dL) whereas the protein content of PT milk decreased from 2.4 g/dL in week 1 to 1.4 g/dL in week 4 and remained stable. After six weeks the overall mean weight gain was 11.5 and 11.6 g/kg/dL for PT and C infants, respectively representing 158 and 152 percent of their initial weights.

4.3 Effect of Sodium Intake on Hyponatremia

Tests of the hypothesis related to the third objective of the study indicated that sodium intakes did influence the incidence of hyponatremia in premature infants.

The occurrence of hyponatremia (serum sodium <130 meq/L) was examined in 19 low birthweight (LBW) premature infants. Infant number 16 was omitted from this study because of renal failure.

Contributory factors to hyponatremia investigated in this study included birthweight, gestational age, time to regain birthweight and sodium intakes (Table 23). There were no significant differences in birthweight or time to regain birthweight between the normal and hyponatremic infants. However, the difference between the means of the gestational age, 31.4 weeks for normal infants, and 29.4 weeks for hyponatremic infants was significant. Premature infants are prone to hyponatremia in the first few weeks of life because of high urinary

Table 23

CLINICAL DATA AND SODIUM VALUES IN NORMAL AND HYPONATREMIC INFANTS[1]

Variable	Normal (n=11)	Hyponatremic (n=8)	p value[2]
Birthweight	1281±58.2	1130±61.4	0.096
Gestational Age (wk)	31.4±0.7	29.4±0.5	0.048
RGBW (d)	14.9±1.6	20.0±2.9	0.136
Sodium Intake (meq/kg/d)			
Milk	1.30±0.1	0.9±0.1	0.036
Bicarbonate	0.13±0.1	0.22±0.1	0.182
TPN	0.08±0.0	0.44±0.2	0.183
Other	0.26±0.1	0.36±0.1	0.209
Total	1.77±0.1	1.92±0.1	0.152

[1]Mean±SEM

[2]Exact p value for differences between means

sodium loss probably caused by renal immaturity (Roy et al, 1976). It has been demonstrated that the percentage of fractional sodium excretion was inversely related to postnatal age in the LBW infant and reflects sodium losses through the kidneys (Curran, 1979).

Hyponatremia occurred frequently in 8 infants (5 appropriate for gestational age (AGA), 3 small for gestational age (SGA) between the ages of birth and four weeks (Table 24). Two infants had only one episode of hyponatremia but in 6 infants, hyponatremia recurred after treatment for the sodium deficit. Of the 11 infants who did not experience episodes of hyponatremia 6 were AGA and 5 SGA. The AGA Infants in this study had more severe instances of hyponatremia (serum sodium 120 to 124 meq/L) than did the SGA infants. This is in agreement with Day et al (1976) who reported hyponatremia in 23 of 30 infants (14 AGA and 9 SGA) between the ages of 2 and 6 weeks.

Sodium intake was measured from the milks fed the infants and calculated from other sources. Total sodium including that from intravenous fluids and medication did not differ between the two groups over the four weeks. Sodium intakes from milk alone, however, were different ($p<0.05$), being lower in the milk fed infants who had episodes of hyponatremia. Sodium requirements vary greatly between infants, however most studies reported that hyponatremia occurred frequently in LBW infants receiving a sodium intake $\leq$ 2 meq/kg/d (Roy et al, 1976; Day et al, 1976). Roy et al (1976) found hyponatremia in approximately one-third of LBW infants fed formulas providing < 2 meq/kg/d sodium between the second and sixth week of life. The hyponatremia was corrected by supplementing the formula to provide a total daily sodium intake of 3 meq/kg/d. Chance et al (1977) modified a standard formula

Table 24

INCIDENCE AND SEVERITY OF HYPONATREMIA IN PREMATURE INFANTS[1]

	Number of Infants	Number infants with hyponatremia	Total episodes of hyponatremia	Episodes of hyponatremia with serum sodium as indicated	
				125-129 meq/L	120-124 meq/L
AGA	11	5	8	5	3
SGA	8	3	6	5	1
Total	19	8	14	10	4

[1] AGA: appropriate for gestational age.

SGA: small for gestational age.

to supply sodium at 3 meq/kg/d to infants < 1.3 kg birthweight. Of the dietary manipulations used in their study only sodium intake was shown to influence the rate of weight gain due to growth in lean body mass. Kumar and Sacks (1978) found that the usual maintenance intake of 2 to 3 meq/kg/d of sodium chloride was rarely sufficient in the extremely premature infant as LBW infants cannot adequately conserve urinary sodium.

The hyponatremia in LBW infants in the first 6 weeks of life is due to the combined influence of renal immaturity which permits relatively high urinary sodium loss and insufficient sodium intake due to the low sodium content of human milk and humanized formulas. Supplementation of human milk and formulas to provide adequate intakes of sodium for premature infants is indicated.

4.4 Effect of Calcium and Phosphorus Intakes on Bone Mineralization

Tests of the hypotheses related to the fourth objective of the study indicated that calcium and phosphorus intakes did influence the incidence of delayed bone mineralization in premature infants. However there was no relationship between calcium and phosphorus intakes and serum calcium, serum phosphorus or serum alkaline phosphatase levels. Also there was no relationship between serum calcium, phosphorus or alkaline phosphatase levels and the incidence of delayed bone mineralization.

Radiographs of the radius-ulna obtained from 19 premature infants at three months of age or at discharge from hospital were assessed as to degree of mineralization by a pediatric radiologist. Infant number 4 was omitted from this study because no radiograph of the radius-ulna was obtained. Five infants, 3 appropriate for gestational age (AGA) and 2

small for gestational age (SGA) were assigned to the normal bone mineralization group. Thirteen infants (7 AGA and 6 SGA) were assigned to the delayed bone mineralization group. One infant (SGA) was assigned to the overt rickets group. The bone texture was better defined and the cortices were somewhat wider in the normal bone mineralization group than in the delayed bone mineralization group. Coarse trabeculation indicating lack of bone mass was evident in the radiograph of the infant with rickets.

Intrauterine accretion of calcium and phosphorus increases dramatically during the last trimester of pregnancy. During that time the fetus lays down approximately 100 mg calcium/kg bodyweight/day in the form of new bone (Ziegler et al, 1976; Shaw, 1976). The more immature a premature infant is, the more predisposed he or she may be to calcium and phosphorus deficiency especially when a rapid rate of growth is achieved.

The mean birthweight of the normal bone mineralization group, 1374 g, was significantly greater than the mean birthweight of the delayed bone mineralization group, 1188 g (Table 25). The birthweight of the infant with overt rickets was 890 g. The difference between the means of gestational age, 32.9 and 30.0 weeks for the normal and delayed bone mineralization group, respectively, was highly significant. The gestational age of the infant with rickets was 29 weeks. The difference between the means of time to regain birthweight for the normal bone mineralization group and the delayed bone mineralization group, 11.0 days and 17.5 days respectively, was also highly significant. The time to regain birthweight for the infant with rickets was 25 days. The

Table 25

CLINICAL DATA AND SERUM VALUES FOR INFANTS WITH NORMAL AND DELAYED BONE MINERALIZATION

Delayed	Mineralization[1]		
	Normal (5)[2]	Delayed (13)	p values[3]
Birthweight (g)	1374±58.1	1188±49.7	0.052
Gestational Age (wk)	32.9±1.2	30.0±0.4	0.009
RGBW (d)	11.0±2.1	17.5±1.1	0.009
Weight Change [3] (g/kg/d)	14.8±1.7	11.3±1.0	0.085
Serum Concentrations:			
Calcium (mg/dL)	9.6±0.2	9.6±0.1	0.887
Phosphorus (mg/dL)	6.4±0.3	5.6±0.3	0.136
Alkaline Phosphatase (IU/L)	448±91.7	501±64.0	0.664

[1]Mean±SEM

[2]Number in bracket represents number of infants in group

[3]Exact p value for differences between means

difference in the rate of weight change for the two groups was not significant.

There was no difference in the serum calcium, phosphorus or alkaline phosphatase values between the two groups. Normal serum values for preterm infants are calcium > 7 mg/dL, phosphorus 4 to 7 mg/dL and alkaline phosphatase 100 to 300 IU (Children's and General Normal Values for Laboratory Procedures, Health Sciences Centre, Winnipeg, Manitoba). The infants in both groups had relatively normal serum calcium and phosphorus concentrations and elevated serum alkaline phosphatase. Normal serum calcium and phosphorus concentrations were also found in premature infants with rickets (Kulkarni et al, 1980; Steichen et al, 1981). The elevated alkaline phosphatase in the normal bone mineralization groups is disturbing and its use as a determinant of delayed bone mineralization needs investigation. Both Kulkarai et al (1980) and Steichen et al (1981) found serum alkaline phosphatase remained elevated during treatment of rickets and only gradually decreased.

The mean daily intakes of energy, calcium and phosphorus for the normal bone mineralization group were significantly greater than the intakes for the delayed bone mineralization group (Table 26). The mean daily intake of energy, calcium and phosphorus for the infant with rickets was 88 kcal/kg/d, 67 mg/kg/d and 15 mg/kg/d respectively. There was no difference in the calcium phosphorus ratio, vitamin D intake or parenteral intakes between the two groups.

Fetal calcium accretion is approximately 130 mg/kg/d at 28 weeks' gestation increasing to 140 mg/kg/d at 32 weeks and 151 mg/kg/d at 36 weeks (Ziegler et al, 1976). If extrauterine growth must proceed at

Table 26

MEAN DAILY NUTRIENT INTAKES OF INFANTS WITH NORMAL AND DELAYED BONE MINERALIZATION

Variable	Mineralization[1]		
	Normal (5)	Delayed (13)	p values[3]
Enteral Intakes:			
Energy (kcal/kg/d)	128±8.0	102±7.1	0.054
Calcium (mg/kg/d)	121±22.2	79±8.6	0.046
Phosphorus (mg/kg/d)	59±13.5	30±5.5	0.030
Ca:P Ratio	2.7±0.3	3.5±0.2	0.100
Vitamin D (IU/d)	274±20.3	266±12.6	0.738
Parenteral Intakes:			
Calcium (mg/kg/d)	3.2±1.4	8.7±2.4	0.067
Phosphorus (mg/kg/d)	1.3±0.6	2.9±1.5	0.343

[1]Mean±SEM

[2]Number in bracket represents number of infants in group

[3]Exact p value for differences between means

the same rate as intrauterine growth the premature infant must receive an adequate supply of nutrients. One of the most important determinants of the amount of calcium absorbed by infants appears to be the length of gestation (Shaw, 1976). It is assumed that infants in the normal bone mineralization group with greater gestational age and higher birthweight would absorb more calcium than infants in the delayed bone mineralization group. Assuming a 40% absorption of dietary calcium an intake of 200 to 400 mg calcium/kg/d would be required to provide the premature infant with sufficient calcium to approach intrauterine accretion. It is evident that the premature infants in this study did not receive calcium intakes comparable to those received in utero. Shenai et al (1980) found that higher calcium intakes may result in higher total calcium absorption and reported a calcium absorption rate of 73% in 10 LBW infants fed a formula providing 160 mg calcium/dL. Even if the absorption rate for infants in the present study were 73%, calcium intakes would still not be great enough to approximate the in utero calcium accretion of 150 mg/kg/d. Calcium intakes of 205 mg/kg/d would be necessary. Thus the normal bone mineralization of 5 infants in this study may be the result of greater gestational age and birthweight.

The main factor underlying delayed bone mineralization is probably the interruption of the transplacental mineral supply at a time when the needs of the fetus are high. The importance of providing premature infants with an adequate calcium and phosphorus intake must be stressed. Human milk and most formulas do not supply adequate calcium and phosphorus for normal bone mineralization and supplementation of the diets of LBW infants with calcium and phosphorus is worth examining. However the exact mineral requirements of premature infants have not

been established and the optimal form and quantity of calcium and phosphorus which are required to support adequate mineralization of rapidly growing bone in LBW infants need to be precisely defined.

Chapter 5

SUMMARY AND CONCLUSIONS

The development of intensive care neonatal units has resulted in the survival of an increasing number of low birthweight infants. The ideal milk for these premature infants remains a controversial issue and considerable attention has been directed to the type of milk best suited to their nutritional needs. This study was designed to determine how nutrient levels in milks fed to premature infants differed, depending on the milk source, whether milk from mothers delivering prematurely or mature milk from donors. The relationships of these differences to growth parameters, incidence of hyponatremia and of delayed bone mineralization were also examined. This study was unique because the samples analysed were aliquots of the actual intake of milk fed to the premature infants, and therefore exact nutrient intakes could be determined. The large number of analyses performed contributed to the validity of the nutrient data.

Levels of major nutrients in the milk of mothers delivering prematurely (PT) differed from the nutrient levels in donor breast milk (D) in a number of instances. These values confirmed earlier reports and interestingly often fell in the middle of the range of representative milk sample values reported. Most notable was the difference in total nitrogen (TN) and therefore in protein content of PT and D milk. TN in PT milk was extremely high during the first two weeks and although the concentration decreased with progressing lactation the value at week six was still higher than that for TN in D milk. This would suggest that premature infants fed their own mothers' milk rather

than D milk would receive twice as much protein during the first two weeks of life and 64% more protein during the six weeks studied. The PT/D mixture also contained high levels of TN even when the quantity of PT milk in the mixture had decreased. The higher TN content of the PT and PT/D milks compared to the D milk would provide the premature infant with higher amounts of protein, nonprotein nitrogen, urea, and free amino acids, all of which are potentially utilizable for synthesis of tissue protein. Mothers giving birth prematurely should be strongly encouraged to provide their own milk for their infants even when volumes are relatively small.

Nonprotein nitrogen (NPN) concentrations in PT and PT/D milks were constant over the six weeks and were always greater than NPN in D milk. The role of NPN and its contribution to growth is not known and it remains to be investigated whether the higher NPN in the PT milk will influence growth. PT milk NPN values were generally lower than reported values sometimes by as much as 50%, but this difference could have resulted from the preanalysis treatment of the samples. Samples in this study were analysed fresh whereas samples in most other studies were frozen at -20^{o}C and thawed before analysis. Freezing and thawing could disrupt the protein micelles, stimulate proteolysis and result in higher NPN values. In one study samples to be analysed were stored at -80^{o}C and in this case NPN values were identical to those in this study. An investigation of the effect of freezing of human milk at various temperatures is necessary.

There were no significant differences between the mean levels of fat, lactose and energy in PT and D milk over the six week period. However the effect of colostrom in week one in PT and PT/D milks was

quite evident with lower concentrations of fat and lactose and subsequently lower energy contents being recorded. These lower concentrations may be physiologically advantageous to the premature infant born with an immature digestive system and the progressive increases in fat and lactose over the first six weeks of lactation may parallel the postnatal development of lipase and lactase enzyme activities. The initially lower lactose may also be a consequence of the higher nitrogen content of the PT milk. Since both the protein and lactose are a part of the water soluble fraction, the higher nitrogen may effectively decrease the lactose concentration of the milk. The lower energy content during week one was primarily a consequence of the lower fat concentration.

Levels of mineral concentrations in PT milk did differ from levels in D milk in some instances. Sodium concentrations were higher, calcium concentrations were lower and there were no differences in phosphorus concentrations. The sodium levels in PT milk decreased over time but were always higher than in D milk. If fed their own mothers' milk during the first six weeks of life, premature infants would receive approximately 75% more sodium than if fed D milk. The relatively high sodium concentrations especially in the first two weeks may protect the infant against hyponatremia. The calcium and phosphorus concentrations in PT and PT/D milks were constant over the six weeks and calcium concentrations were lower than in D milk. The amounts of calcium and phosphorus available would be inadequate for growing premature infants to attain in utero accretion rates and a need for calcium and phosphorus supplementation is predicted. There are some significant discrepancies between the absolute mineral values found in the present study and other

reports. The reason for these discrepancies is unclear but may reflect differences in methodology and possibly some systematic error in the measurements. Further investigation is necessary to substantiate these observations.

Some of the differences in nutrient levels between PT and D milks may be of potential benefit to the premature infant during the early weeks of life. It is apparent from this study that energy and protein intake are important determinants of weight gain. Premature infants are predicted to have high protein requirements in relation to energy intake and a significant linear correlation of increasing weight gain with increased protein intake was found in this study. A significant linear correlation was similarly found between weight gain and energy intakes. The desirable intakes of protein and energy for premature infants are uncertain because although minimum intakes have been defined and a certain amount is known about the protein and energy cost of growth, there is no agreement about the desirable rate and quality of growth. There are no growth curves for premature infants and the relevance of intrauterine growth curves to extrauterine patterns of growth has yet to be established. Premature infants in this study did not approach weight gains similar to those in utero until age six weeks postnatal. The study confirms other reports that PT milk is a more appropriate feeding than D milk during the early weeks of life. Infants receiving PT milk had significantly greater intakes of protein and energy than infants receiving D milk and by the end of week two had regained their initial weight while the infants fed D milk were only 94% of their initial weight. However, when D milk was supplemented with premature formula in

week three the intakes and weight gains were similar. The results suggest that mothers of premature infants are the best source of human milk for their infants and if D milk is used it should be supplemented.

Neither human milk nor most infant formulas provide adequate amounts of sodium, calcium or phosphorus if infants are growing at rates that approximate intrauterine growth curves. However, the sodium intake from milk alone in this study was greater in infants who were normal than in infants who had episodes of hyponatremia. The hyponatremia was probably caused by high urinary sodium loss due to renal immaturity as well as insufficient sodium intake. Calcium and phosphorus intakes were greater in infants with normal bone mineralization than in infants with delayed bone mineralization or rickets although the normal bone mineralization may also be a consequence of greater gestational age, birthweight and less time to regain birthweight. Further investigation is required to determine the ideal quantities of calcium and phosphorus that must be supplemented to infants fed human milk or humanized formulas.

Results of the present investigation indicate that a premature infant's own mother's milk is a more appropriate source of nutrients than mature donor milk. However, calcium and phosphorus are apparently present in insufficient amounts to support normal growth. Appropriate supplementation of the milk may be necessary for these and possibly other nutrients. More research is necessary to determine the best possible source of milk for premature infants.

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Appendix A

CLINICAL DETAILS OF TWENTY LOW-BIRTHWEIGHT INFANTS

	Sex	Gestational Age (wks)	Size[1]	Birth Measurements Weight (g)	Length (cm)	HC[2] (cm)
1	M	29	AGA	1080	37.0	27.0
2*	F	31	SGA	1180	38.5	26.0
3*	F	31	SGA	1200	38.0	27.0
4	M	29	AGA	1200	36.0	26.0
5*	F	28	AGA	980	36.0	24.5
6	F	31	AGA	1400	43.0	27.0
7	F	28	AGA	1000	36.0	25.0
8	F	30	AGA	1480	43.0	29.0
9	F	31	SGA	1090	37.0	27.0
10	F	36	SGA	1230	41.0	27.0
11	F	30	AGA	1420	41.5	28.5
12	F	31	AGA	1490	42.0	27.0
13	F	28	AGA	1160	41.0	26.5
14	F	36	SGA	1240	40.0	28.5
15	F	32	AGA	1490	40.0	27.0
16	M	32	SGA	1260	41.0	27.0
17	M	32	SGA	1380	40.5	27.5
18*	F	29	SGA	890	34.0	24.0
19*	F	29	SGA	890	33.0	25.0
20	M	30	AGA	1340	35.0	25.0
Mean		30.7		1220	38.7	26.6

*multiple birth

[1]Size: AGA, appropriate for gestational age
SGA, small for gestational age

[2]HC: Head Circumference

APPENDIX B

MATERNAL MILK - GROWTH STUDY

Name -

DAY		FEED NUMBER								
		1 (8 am)	2	3	4	5	6	7	8	
	Maternal Milk									
	Donor Milk									
	Formula									
	Maternal Milk									
	Donor Milk									
	Formula									
	Maternal Milk									
	Donor Milk									
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	Formula									
	Maternal Milk									
	Donor Milk									
	Formula									

Head Circumference: ______________________ Date: ______________________

Length: ______________________ Date: ______________________

Appendix C

MICRO-KJELDAHL METHOD FOR TOTAL NITROGEN (TN) DETERMINATION

Duplicate 0.2 mL samples of human milk were transferred to 30 mL digestion flasks containing 1 g titanium dioxide catalyst. Boiling beads and 2 mL concentrated sulfuric acid were added. When the samples became clear they were digested for 30 minutes.

The flasks were cooled and a minimum volume of water was added to dissolve the solids. The digest and boiling beads were then transferred to the distilling apparatus, the flask was rinsed three times with 1-2 mL of water. A 50 mL beaker containing 5 mL saturated boric acid solution and two drops methyl red-methylene blue indicator was placed under the condenser with tip extending below the surface of the solution. Nine mL 50% NaOH was added to the still. Approximately 15 mL distillate was collected and titrated to end-point with 0.01N HC1.

Calculation:

TN (mg/dL) = (mL HC1 x normality x 14 x 100) / 0.2 mL sample.

Appendix D

PROTEIN PRECIPITATION FOR NONPROTEIN NITROGEN (NPN) DETERMINATION

The pooled human milk samples were defatted by centrifugation and the defatted layer was removed with a Pasteur pipette. The proteins were precipitated by mixing 1 mL of the defatted milk samples and 1 mL 24% trichloroacetic acid (TCA). After 1 h the mixture was centrifuged for twenty minutes in an IEC International Centrifuge model CS (International Equipment Company, Boston, Mass.), the supernatant was filtered through Whatman number 4 filter paper and 1 mL 12% TCA was mixed with the filtrate and centrifuged as before. This step was repeated and the filtrate was diluted to 10 mL. Concentrations of NPN of the human milk samples were determined by micro-Kjeldahl method using 2 mL of the filtrate. Duplicate analyses were performed and the mean of the two titration readings was used to calculate the amount of NPN in the sample. Calculation formula for amount of NPN in the sample:

$$\text{NPN (mg/dL)} = (\text{mL HCl} \times \text{normality} \times 14.0 \times 100) / 0.2 \text{ mL}$$

Appendix E

TURBIDOMETRIC METHOD FOR FAT DETERMINATION

A clear solution was obtained by adding 5 mL of 97% acetic acid to a 0.05 mL sample of fresh human milk in a test tube. Turbidity was developed by adding 2.5 mL of a solution containing 20% urea and 0.2% imidazole. The solution was mixed on a Vortex Mixer and allowed to stand at room temperature for 30 minutes. Absorbance was measured on a Bausch and Lomb Spectronic 20 Spectrophotometer (Fisher Scientific, Winnipeg, Manitoba) against a reagent blank. Duplicate analyses were performed on each combined fresh milk sample. The mean of the two absorbance readings was used to estimate fat content of the sample from a standard curve.

Appendix F

PHENOL-SULFURIC METHOD FOR LACTOSE DETERMINATION

A premeasured 1 mL sample of frozen milk was transferred to a 100 mL volumetric flask and dilution completed to 100 mL with distilled water. A 0.1 mL sample of this diluted milk was transferred to a test tube and 4.9 mL distilled water was added making a 1/5000 dilution. A 2 mL sample of diluted milk was placed in a 20 mL screw cap tube and 1 mL of 5% phenol was added. Then 5 mL sulfuric acid was added rapidly; for complete mixing the acid was directed against the liquid surface rather than the side of the tube. The tubes were capped and allowed to stand at room temperature ten minutes and then mixed on a Vortex Mixer. After thirty minutes more standing time, the absorbance was measured on a Bauch and Lomb Spectronic 20 spectrophotometer (Fisher Scientific, Winnipeg, Manitoba) at 490 nm against a water blank. Duplicate analyses were performed on each sample. The mean of the two absorbance readings was used to estimate the lactose content of the sample from a standard curve.

Appendix G

WEIGHT DATA (G) FOR STUDY INFANTS DURING FIRST SIX WEEKS POSTNATAL

Infant	Postnatal Age (wk) 0	1	2	3	4	5	6
1	1080	930	1000	1160	1460	1530	1770
2	1180	980	1150	1180	1370	1610	1920
3	1200	1120	1160	1320	1520	1750	2030
4	1200	950	920	1040	1070	1140	1400
5	980	750	860	980	1100	1170	1450
6	1400	1210	1390	1550	1750	1920	2140
7	1000	780	960	1120	1280	1460	1580
8	1480	1300	1320	1700	1880	2155	2430
9	1090	1020	1140	1320	1530	1630	1780
10	1230	1300	1300	1700	1980	2090	2295[1]
11	1420	1270	1370	1615	1830	2080	2220
12	1490	1440	1570	1800	2100	2450	2580
13	1160	1120	1180	1280	1280	1530	1840
14	1240	1250	1220	1540	1890	2290	2400[1]
15	1490	1300	1400	1560	1780	2060	2130
16	1260	1120	1070	1200	1280	1520	1580
17	1380	1270	1300	1320	1440	1660	1960
18	890	820	800	800	890	890	980
19	890	720	760	860	970	1000	1070
20	1340	1210	1320	1560	1750	2030	2100
Mean	1107	1093	1160	1330	1508	1698	1883

[1] Extrapolated value.

Appendix H

LENGTH DATA (CM) FOR STUDY INFANTS DURING FIRST SIX WEEKS POSTNATAL

Infant	Postnatal Age (wk) 0	1	2	3	4	5	6
1	37.0	37.0	39.0	38.0	39.0	40.0	42.0
2	38.5	37.0	38.0	39.0	40.5	40.5	42.5
3	38.0	40.0	39.0	39.0	41.0	41.0	43.5
4	36.0	36.5	39.0	38.0	40.0	40.0	40.0
5	36.0	36.0	38.0	39.0	40.0	40.0	41.5
6	43.0	41.0	44.0	44.0	46.5	46.0	47.0
7	36.0	36.0	39.0	40.0	41.0	42.0	42.0
8	43.0	42.5	41.5	42.5	43.0	43.0	45.0
9	37.0	38.0	40.0	40.0	43.0	42.5	43.0
10	41.0	41.0	42.0	43.0	45.5	45.0	NM[1]
11	41.5	41.5	41.5	42.0	45.5	47.0	NM
12	42.0	42.0	43.0	42.0	43.0	44.0	NM
13	41.0	40.0	40.0	42.0	43.0	42.0	43.0
14	40.0	41.0	39.5	43.0	44.0	47.0	46.0
15	40.0	39.0	41.3	41.0	44.0	46.0	NM
16	41.0	38.5	38.5	40.0	39.0	40.5	42.0
17	40.5	40.5	41.0	41.5	41.0	43.5	41.5
18	34.0	34.5	36.5	36.0	36.0	36.0	37.5
19	33.0	33.0	36.0	36.0	36.0	37.5	39.0
20	35.0	43.0	43.0	44.0	46.3	46.3	47.5
Mean	38.7	38.9	40.0	40.5	41.9	42.5	42.7

[1] NM = not measured

Appendix I

HEAD CIRCUMFERENCE DATA (CM) FOR STUDY INFANTS

DURING FIRST SIX WEEKS POSTNATAL

Infant	Postnatal Age (wk) 0	1	2	3	4	5	6
1	27.0	25.0	26.0	27.0	NM[1]	29.0	31.0
2	26.0	NM	26.5	27.0	28.5	29.5	31.0
3	27.0	26.5	27.0	28.0	28.8	30.5	31.5
4	26.0	26.0	25.5	26.5	27.0	27.5	27.5
5	24.5	25.0	NM	25.3	26.0	26.5	28.0
6	27.0	27.0	28.0	28.0	30.0	31.0	32.0
7	25.0	NM	27.0	29.5	30.0	30.0	31.0
8	29.0	28.0	28.5	30.0	31.0	31.8	33.0
9	27.0	26.8	27.5	28.0	30.0	31.0	31.5
10	27.0	27.0	28.0	31.0	31.5	33.0	NM
11	28.5	27.0	27.8	28.5	29.5	31.0	NM
12	27.0	28.5	28.5	30.8	32.0	33.0	NM
13	26.5	24.5	27.0	27.5	28.5	29.0	29.5
14	28.5	29.0	29.0	31.0	33.0	33.5	34.0
15	27.0	28.0	28.0	29.5	31.0	31.5	NM
16	27.0	26.0	26.0	27.0	28.0	29.0	29.8
17	27.5	28.0	28.5	29.0	29.0	30.0	31.0
18	24.0	24.0	NM	25.0	25.5	25.8	27.0
19	25.0	25.5	25.5	25.5	26.0	27.3	28.0
20	25.0	27.5	28.0	30.0	31.0	32.0	32.5
Mean	26.6	26.6	27.4	28.2	29.3	30.1	30.5

[1]NM = not measured

Appendix J

MEAN DAILY WEIGHT GAINS (g/kg/d) OF TWENTY STUDY INFANTS

Infant	Week					
	1	2	3	4	5	6
1	-19.8	10.8	22.9	36.9	6.8	22.4
2	-24.2	24.8	3.7	23.0	25.0	27.5
3	-9.5	5.0	19.7	21.6	21.6	22.9
4	-29.8	-4.5	18.6	4.1	9.3	32.6
5	-33.5	21.0	19.9	17.5	9.1	34.2
6	-19.4	21.3	16.4	18.4	13.9	16.4
7	-31.4	33.0	23.8	20.4	20.1	11.7
8	-17.4	2.2	41.1	15.1	20.9	42.5
9	-9.2	16.8	22.6	22.7	9.3	13.1
10	8.1	0.0	44.0	23.5	7.9	32.9
11	-15.1	11.2	25.5	19.0	19.5	19.8
12	-4.8	12.9	20.9	23.8	23.8	13.3
13	-4.9	7.7	12.1	0.0	27.9	28.9
14	1.2	-3.4	37.5	32.5	30.2	6.9
15	-18.2	11.0	16.3	20.1	22.5	9.7
16	-15.9	-6.4	17.4	9.5	26.8	5.6
17	-11.4	3.4	2.2	13.0	21.8	25.8
18	-11.2	-3.5	0.0	16.1	0.0	14.4
19	-27.3	7.9	18.8	18.3	4.4	10.0
20	-13.9	13.0	26.0	17.4	22.9	4.9
Mean	-15.4	9.2	20.5	18.6	17.2	19.8

Appendix K

MEAN ENERGY INTAKE FROM ALL SOURCES (kcal/kg/d)

OF TWENTY STUDY INFANTS

Infant	Week					
	1	2	3	4	5	6
1	61.7[1]	110.0[1]	124.6	85.0[1]	108.3[1]	136.8
2	45.8[1]	116.2[1]	103.8[1]	153.4	146.4	150.1
3	49.1[1]	108.1[1]	117.8[1]	142.0	149.1[1]	153.5
4	52.9[1]	86.4[1]	119.8	131.2	83.3[1]	190.2
5	59.0[1]	144.4[1]	139.3	136.0	159.4[2]	187.0[2]
6	52.9[1]	137.5[1]	127.8	132.5	111.7[2]	90.1[2]
7	45.3[1]	128.5[1]	117.8	135.3	117.0	117.5[1]
8	52.0[1]	123.7[1]	134.2	144.7	142.4	224.2
9	48.9[1]	124.4[1]	145.1[1]	141.6	157.9	162.8
10	85.4[1]	119.7	209.0[1]	178.5	136.1	143.2[3]
11	49.7[1]	120.4[1]	167.1	172.1	140.0	146.4[3]
12	79.4[1]	116.8[1]	139.3	143.4[1]	136.1[1]	74.7
13	55.1[1]	130.4[1]	130.2	119.8[1]	158.7[1]	155.2
14	61.7[1]	107.2[1]	198.9	217.3	190.3	186.1
15	71.1[1]	127.1[1]	139.4[1]	168.5[1]	151.8[1]	124.5[1]
16	45.0[1]	68.2[1]	89.1[1]	102.0[1]	132.0[1]	122.6[1]
17	47.7[1]	76.0[1]	125.3[1]	155.7[1]	151.4[1]	145.1[1]
18	49.4[1]	101.4[1]	89.0[1]	113.1[1]	116.8	148.0
19	49.2[1]	130.4[1]	99.6	110.2	111.5	116.0
20	52.0[1]	104.2	95.3	116.9	15.10	122.9
Mean	55.7	114.0	130.6	140.0	137.6	144.8

[1]Includes parenteral feeding.

[2]Total intake not recorded; infant breast fed.

[3]Extrapolated value.

Appendix L

MEAN ENERGY INTAKES FROM MILK (kcal/kg/d) OF TWENTY STUDY INFANTS

Infant	Week					
	1	2	3	4	5	6
1	28.1	110.0	124.6	50.1	75.7	136.8
2	13.7	108.9	103.8	153.4	146.4	150.1
3	31.9	108.1	113.0	142.0	149.1	153.5
4	00.0	25.2	119.8	131.2	50.2	190.2
5	6.8	119.1	139.3	136.0	159.4[1]	187.0[1]
6	40.8	137.5	127.8	132.5	117.4[1]	90.1[1]
7	00.0	107.8	117.8	135.3	117.0	105.2
8	47.6	123.7	134.2	114.7	142.4	224.2
9	23.0	121.6	145.1	141.6	157.9	162.8[2]
10	43.0	119.7	194.2	178.5	136.1	143.2[2]
11	37.0	120.4	167.1	172.1	140.0	146.4
12	13.9	114.2	139.3	143.4	136.1	74.7
13	18.4	130.3	130.2	110.4	146.1	155.2
14	28.3	99.3	198.9	217.3	190.3	186.1
15	49.4	111.5	139.4	168.5	151.8	124.5
16	15.1	19.7	00.0	1.2	57.6	119.6
17	00.0	8.2	50.0	151.2	144.5	143.0
18	25.9	80.3	58.5	91.1	116.8	148.0
19	00.0	87.7	99.6	110.2	111.5	116.0
20	32.4	104.2	95.3	116.9	151.0	122.9
Mean	28.5	97.9	126.2	130.9	129.9	143.9
% of total intake	51	86	97	94	94	99

[1]Total intake not recorded; infant breast fed.

[2]Extrapolated value.

Appendix M

MEAN PROTEIN INTAKES FROM MILK (g/kg/d) OF TWENTY STUDY INFANTS

Infant	Week					
	1	2	3	4	5	6
1	1.25	3.02	3.24	1.17	1.95	3.46
2	0.29	2.15	3.04	3.70	3.50	3.67
3	0.67	2.16	3.26	3.52	3.70	3.80
4	0.00	0.48	2.26	1.96	0.97	4.71
5	0.34	3.84	3.67	2.80	3.06[1]	4.46[1]
6	1.55	3.47	2.91	2.76	2.31[1]	1.70[1]
7	0.00	2.38	2.69	2.48	2.54	1.89
8	0.71	1.93	3.03	3.65	3.20	4.92
9	0.72	3.57	3.03	2.77	2.76	2.40
10	0.64	2.19	4.90	4.51	2.99	2.95[2]
11	0.52	2.07	4.20	4.40	3.10	3.03[2]
12	0.28	2.25	3.26	3.43	3.00	1.65
13	0.65	3.26	2.68	2.10	2.86	2.64
14	0.44	1.86	4.89	5.49	4.42	4.08
15	1.64	2.56	3.10	2.79	2.44	1.54
16	0.75	0.58	0.00	0.00	1.11	2.06
17	0.00	0.12	0.56	3.83	3.61	3.61
18	0.32	0.91	1.18	1.69	2.20	2.41
19	0.00	0.86	2.22	2.33	2.24	2.20
20	1.24	2.62	2.69	2.85	3.81	2.71
Mean	0.75	2.11	2.99	3.06	2.79	2.99

[1]Total intake not recorded; infant breast fed.

[2]Extrapolated value

Appendix N

DAILY INCREMENTS OF BODY WEIGHT OF THE REFERENCE FETUS[1]

Age Interval (weeks)	Weight Gain/day (g)
29-30	23.1
30-31	24.3
31-32	25.7
32-33	27.1
33-34	30.0
34-35	31.4
35-36	34.3
36-37	35.7
37-38	31.4

[1]Adapted from Ziegler et al, 1976

Appendix O

CALCULATION OF PERCENT OF EXPECTED WEIGHT GAIN

$$\text{Expected weight gain \%} = \frac{\text{weight gain (g/d) of infant}}{\text{weight gain (g/d) of R fetus}} \times 100$$

$$= 22.9^{1} \div 25.7^{2} \times 100$$

$$= 89\%$$

[1]Weight gain of infant 1, postnatal age 3 wk, postconceptual age 32 wk (Appendix J).

[2]Weight gain of reference fetus, postconceptual age 32 wk (Appendix N)