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Human milk intake and growth in exclusively breast-fed infants

Milk intake and growth in 45 exclusively breast-fed infants were documented during the first 4 months of life. Energy and protein intakes were substantially less than current nutrient allowances. Energy intake declined significantly from 110 ± 24 kcal/kg/day at 1 month to 71 ± 17 kcal/kg/day at 4 months. Protein intake decreased from 1.6 ± 0.3 gm/kg/day at 1 month to 0.9 ± 0.2 gm/kg/day at 4 months. Infant growth progressed satisfactorily, compared with National Center for Health Statistics standards. A reevaluation of energy and protein intakes and allowances during infancy is merited. (J PEDIATR 104:187, 1984)

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NUTRIENT ALLOWANCES DURING INFANCY are based on empirical observations of the intakes of thriving infants.[1,2] There is a discrepancy, however, between the amounts of human milk required to meet these allowances and the usual rates of milk production. The Food Agricultural Organization/World Health Organization and National Research Council recommendations for energy and protein require that an infant weighing 4.55 kg and younger than 3 months of age consume 817 gm milk per day to meet energy needs estimated at 120 kcal/kg/day. The infant weighing 6.65 kg and aged 3 to 5 months would require 1141 gm milk per day to meet the recommended amount of 115 kcal/kg/day. The latter quantity is not representative of observed rates of human milk production, which range from 600 to 900 gm/day,[3-8] although extraordinarily high milk outputs akin to levels produced by a wet nurse[9] have been cited.[10,11] The amount of human milk necessary to fulfill protein recommendations requires similarly high yields. In spite of the discrepancy between reported rates of usual milk production and amounts estimated to meet nutrient allowances, exclusive breast-feeding can support adequate infant growth throughout the first 4 to 6 months of life.[12] There is insufficient information on the volume and composition of milk produced under optimal nutritional and environmental conditions to reconcile this discrepancy. The importance of verification of maternal breast-feeding skills and the tendency to include a disproportionate number of higher milk producers in later lactation have not been appreciated in most previous studies.

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NPN	Nonprotein nitrogen
PN	Protein nitrogen
TN	Total nitrogen

Investigations of human milk production generally have been cross-sectional and thus not suitable for an examination of the relationship between milk intake and growth performance. In longitudinal studies, the relationship between intake and growth rate is most likely discernible during rapid growth, but may be obscured by modifying factors such as infection or the marked variation in normal growth patterns. Nevertheless, documentation of voluntary intakes and growth performance of healthy breast-fed infants is important for a definition of nutrient requirements and to establish the duration of adequacy of breast-feeding.

Our study was designed to document longitudinally voluntary intakes and growth of exclusively breast-fed infants in order to obtain normative data on human milk production and to elucidate the discrepancy between observed levels of human milk intake and current nutrient recommendations.

MATERIALS AND METHODS

Study design. Expectant mothers who planned to breast-feed exclusively for at least 4 months were recruited prenatally through the Baylor Milk Bank Program. Participants were required to meet the following selection criteria: healthy, nonsmoking, aged 18 to 36 years, no long-term medications, and parity 1 or 2. Infants were required to be healthy, term, and appropriate size for gestational age. Informed consent to the study protocol, approved by the Institutional Human Experimentation Committees, was obtained after delivery.

Mothers were visited postpartum in the hospital by a breast-feeding consultant for review of basic breast-feeding skills. Throughout the study period, mothers had access to the consultants for advice. Anthropometric measurements were obtained on the mothers and infants at this time. Pertinent information regarding maternal health, labor and delivery, and infant status was extracted from hospital records.

At approximately 2 weeks postpartum, mothers were oriented to the study procedures in their homes. Explicit instructions were given on the collection and freezing of milk. Written records to be kept by the mothers throughout the study were explained.

Although the study protocol specified exclusive breast-feeding, the feeding of occasional food supplements was anticipated. Written records of the type and amount of supplements used were kept by the mothers. The use of vitamin, iron, and fluoride supplements was also ascertained. Written records of maternal and infant illness were kept. Study procedures were postponed if either mother or infant was ill.

The determination of milk intake, sampling of milk for compositional studies, and monitoring of infant growth were performed at monthly intervals. The actual ages of the infants at the times of observation were 35 ± 5, 64 ± 3, 91 ± 4, and 119 ± 6 days. Study procedures were conducted in the homes, with the exception of the anthropometric measurements, which were performed at the Medical Center by one investigator.

Subjects. Forty-five women participated. Data are missing for two subjects at month 3 because of maternal illness and a scheduling oversight. Two subjects moved and failed to complete the last month of the study. Two subjects discontinued their participation after 2 months for personal reasons. Data for these six subjects were included in the analysis.

The women were in the middle to upper socioeconomic stratum. Mean (±SD) maternal age was 28.0 ± 3.1 years; mean level of education was 15.4 ± 1.8 years. Ethnic background was as follows: 41 white, two Hispanic, one Asian, and one West Indian. Most (82%) of the women were employed in professional or technical positions prior to delivery.

Thirty-eight (84%) infants were delivered vaginally, and seven (16%) by cesarian section. The mean birth weight of the 45 infants was 3.58 ± 0.45 kg (range 2.56 to 4.57 kg). The mean gestational age according to Dubowitz was 39.2 ± 1.8 weeks (range 37 to 42 weeks). There were 27 boys and 18 girls. Apgar scores averaged 8.4 ± 0.7 and 9.2 ± 0.5 at 1 and 5 minutes, respectively. Forty-two percent of the infants were first born; the remainder were second born.

Human milk intake. The amount of milk ingested over a 24-hour period was determined by weighing the infant before and after each feeding. The mothers were instructed in the use of an automatic, electronic balance (Sartorius 3804 MP), which integrates repetitive weighings every 0.5 second and displays a figure when consistent, successive readings are registered. The precision stated by the manufacturer is ±0.05 gm. The mothers were asked to change the infants' diapers before each feeding, not to change the infants' clothing during a feeding, not to alter the frequency or duration of their usual lactation pattern, and to record any losses of milk, urine, and feces not retained on the infants. Losses were not measured, but noted to qualify the test-weighing procedure. The accuracy of the test-weighing procedure was evaluated in a formula-fed infant. The mean difference in milk intakes calculated from weighing the infant and weighing the bottle before and after nine successive feedings was 3.2 ± 3.1 gm. The difference observed in infant weight before and after a feed always was less than that recorded for the bottle, as would be expected because of insensible water losses during a feed.

Routinely, the test-weighing procedure was conducted over a 24-hour period. However, 10 women agreed to a 48- to 96-hour consecutive test weighing session in order to study individual daily variation in intake.

Milk sampling. Milk was collected for compositional studies within 3 days after the test-weighing procedure. Mothers were instructed not to alter their usual feeding routine. At each feeding over a 24-hour period, the infant was offered one breast, and the contralateral breast was emptied of its entire contents with the use of an Egnell (Cary, IL) electrical breast pump. Breasts were alternated for feeding and pumping with successive feeds. If necessary, infants were given supplements of human milk that had been collected and frozen in advance.

Milk samples were refrigerated in sterile, acid-washed polypropylene bottles for a maximum of 24 hours and transported on ice to the laboratory, where volumes were measured and 24-hour pooled samples composed.

Table I. Human milk and nutrient intakes of exclusively breast-fed infants during first 4 months of life

	Age (mo)			
	1 (n = 37)	*2 (n = 40)*	*3 (n = 37)*	*4 (n = 41)*
Feedings (n/day)	8.3 ± 1.9	7.2 ± 1.9	6.8 ± 1.9	6.7 ± 1.8
Human milk				
Gm/day	751.0 ± 130.0	725.0 ± 131.0	723.0 ± 114.0	740.0 ± 128.0
Gm/kg/day	159.0 ± 24.0	129.0 ± 19.0	117.0 ± 20.0	111.0 ± 17.0
Protein				
Gm/day	7.6 ± 1.7	6.5 ± 1.7	6.1 ± 1.3	6.1 ± 1.4
Gm/kg/day	1.6 ± 0.3	1.1 ± 0.2	1.0 ± 0.2	0.9 ± 0.2
Lactose				
Gm/day	48.5 ± 8.9	47.8 ± 9.0	48.0 ± 8.0	49.3 ± 9.2
Gm/kg/day	10.3 ± 1.6	8.5 ± 1.3	7.8 ± 1.4	7.4 ± 1.2
Fat				
Gm/day	28.0 ± 8.5	25.2 ± 7.1	23.6 ± 7.2	25.6 ± 8.6
Gm/kg/day	5.9 ± 1.7	4.4 ± 1.2	3.8 ± 1.2	3.8 ± 1.3
Energy				
Kcal/day	520.0 ± 131.0	468.0 ± 115.0	458.0 ± 124.0	477.0 ± 111.0
Kcal/kg/day	110.0 ± 24.0	83.0 ± 19.0	74.0 ± 20.0	71.0 ± 17.0

At onset of study, milk intake was estimated by deuterium dilution, a technique later determined to be inaccurate.[10] For this reason, data are missing at 17 time points during first 3 months.
Data expressed as mean ± SD.

Table II. Human milk composition

Age (mo)	*n*	*Total nitrogen (mg/gm)*	*Protein nitrogen (mg/gm)*	*NPN (mg/gm)*	*Lactose (mg/gm)*	*Fat (mg/gm)*	*Energy (kcal/gm)*
1	37	2.17 ± 0.30	1.61 ± 0.24	0.56 ± 0.28	64.7 ± 2.4	36.2 ± 7.5	0.68 ± 0.08
2	40	1.94 ± 0.24	1.42 ± 0.17	0.52 ± 0.20	65.8 ± 2.5	34.4 ± 6.8	0.64 ± 0.08
3	37	1.84 ± 0.19	1.34 ± 0.15	0.50 ± 0.13	66.5 ± 2.3	32.2 ± 7.8	0.62 ± 0.09
4	41	1.80 ± 0.21	1.31 ± 0.17	0.48 ± 0.14	66.6 ± 2.4	34.8 ± 10.8	0.64 ± 0.10

Data expressed as mean ± SD.

Biochemical analysis. The heats of combustion of the milk specimens were determined in an adiabatic bomb calorimeter (Parr Instrument, Moline, IL).[13] A weighed amount of milk (approximately 0.2 gm) was combusted with a known amount of mineral oil. Nitrogen was analyzed by the Kjeldahl method before and after trichloroacetic acid (10% 1:1 volume) precipitation of protein; protein nitrogen was determined on the solubilized precipitant, and nonprotein nitrogen was estimated from the difference between total and protein nitrogen[4]; PN was converted to protein by the factor 6.25. Fat was determined gravimetrically after methylene chloride extraction, a modification of the Roese-Gottlieb method[14]; lactose was determined using an automated analyzer (YSI Model 27, Yellow Springs, OH) that detects the hydrogen peroxide produced during the oxidation of galactose.

Infant nutrient intakes were calculated from the 24-hour milk intakes and from milk compositional data.

Anthropometry. Infant weight was recorded on the electronic balance before feeding. Infant length was measured on a recumbent infant board. Two research assistants were required to extend the infant satisfactorily.

Statistical analysis. Data were entered into a Scientific Information Retrieval Data Base Management System.[15] Data records were interfaced with Minitab and analyzed by regression and by Pearson correlation coefficient.[16] Trends over time were described by fitting polynomial regressions to individuals and testing the slopes for significance. Milk intake, nutrient concentrations, and measurements of growth were subjected to trend analysis. Growth was evaluated according to National Center for Health Statistics reference standards[17] and by relative shifts in birth percentiles. The data on intake and growth were analyzed according to sex; however, the high variability in the data obscured detection of statistical significant differences between the sexes.

RESULTS

Human milk intake. Intake of milk (gm/day) plateaued over the 4 months of observation, with an overall mean of 733 ± 89 gm/day (Table I). Mean coefficient of variation of milk intake (gm/day) between infants of the same age

Table III. Infant growth and NCHS percentile rankings during first 4 months of life

Age (mo)	*n*	*Weight (kg)*	*Length (cm)*	*Weight gain*		*Length accretion (cm/mo)*
				(gm/day)	*(gm/kg/day)*	
0	45	3.58 ± 0.45	50.9 ± 2.5			
1	45	4.76 ± 0.52	55.7 ± 2.3	37.3 ± 12.4	10.6 ± 3.8	4.6 ± 2.1
2	44	5.62 ± 0.67	59.0 ± 2.6	32.3 ± 13.8	6.9 ± 3.0	3.7 ± 1.6
3	42	6.30 ± 0.30	61.8 ± 2.4	22.4 ± 7.6	4.0 ± 1.4	2.8 ± 1.3
4	41	6.78 ± 0.80	63.7 ± 2.4	18.3 ± 8.1	2.9 ± 1.3	2.5 ± 1.7

Data expressed as mean ± SD.

was 17.0 ± 1.0%. Consumption decreased significantly relative to body weight, from 159 ± 24 gm/kg/day at 1 month to 111 ± 17 gm/kg/day at 4 months ($P < 0.001$). The number of feedings per day declined slightly throughout the 4 months of observation.

The linear regression describing milk intake (Y) (gm/kg/day) against age (X) (days) was $Y = 171.69 - 0.553X$. The second-degree polynominal term for age was significant ($P < 0.03$). The quadratic equation defined was $Y = 199.08 - 1.48X + 0.0066X^2$.

Ten test-weighing sessions conducted for 48 to 96 consecutive hours provided some indication of the individual daily variation in intake. The overall mean day-to-day variation in intake was 7.9 ± 3.6% (i.e., mean coefficient of variation of the 10 consecutive test-weighing sessions).

Milk composition. The regimen followed for the collection of representative 24-hour milk samples theoretically should have yielded 50% of the daily milk production. The actual milk collections were equivalent to 49 ± 17% of the 24-hour milk production determined by test-weighing. The number of samples included in the 24-hour milk aliquots was equal to 90 ± 22% of the number of feedings recorded during the test-weighing sessions.

The concentration of PN (Table II) decreased significantly at a monthly rate of 0.01 mg/gm milk ($P < 0.001$); NPN, comprising 27% of TN, declined at a monthly rate of 0.03 mg/gm milk ($P < 0.08$). Lactose concentration increased slightly ($P < 0.001$), from 64.7 ± 2.4 mg/gm at 1 month to 66.6 ± 2.4 mg/gm at 4 months. Fat, which averaged 34.3 ± 6.9 mg fat/gm, did not decrease significantly over the 4 months ($P < 0.07$). The energy content of the milk decreased significantly, from 0.68 kcal/gm at 1 month to 0.64 kcal/gm at 4 months ($P < 0.001$).

There were positive correlations (r ranged from 0.33 to 0.43) between the caloric content of the milk and the volume of the aliquot of milk expressed for analysis at months 1 to 3 ($P < 0.05$). If the 24-hour aliquot was less than 40% of usual milk production determined by test-weighing, the mean caloric content was 0.63 ± 0.09 kcal/gm; if the aliquot represented 40% to 60% of usual milk production, the caloric value was 0.66 ± 0.10 kcal/gm; and if the milk aliquot exceeded 60%, the caloric content was 0.68 ± 0.09 kcal/gm. The theoretical energy content of the milk calculated from the conversion factors[18] (5.65 kcal/gm protein, 3.95 kcal/gm lactose, 9.25 kcal/gm fat) was equivalent to 100.2 ± 6.8% of the value determined directly by bomb calorimetry.

Few statistically significant correlations between TN, PN, NPN, lactose, fat, and energy content of the milk were noted. Lactose was related inversely to fat ($r = -0.37$, $P < 0.01$). Energy was positively associated with fat ($r = 0.84$, $P < 0.01$). A positive association was demonstrated between milk intake (gm/day) and the energy density of the milk (kcal/gm) at months 1 ($P < 0.05$) and 3 ($P < 0.01$).

The concentrations of TN, PN, lactose, fat, and calories were significantly correlated (r ranged from 0.42 to 0.74, $P < 0.01$) to their subsequent monthly values, indicating a degree of consistency in milk consumption within mothers.

Nutrient intakes. Energy intake (kcal/day) did not vary significantly throughout the first 4 months of life (Table I). The overall mean intake was 476 ± 90 kcal/day. Energy intake declined significantly in terms of body weight, from 110 ± 24 kcal/kg/day at 1 month to 71 ± 17 kcal/kg/day at 4 months ($P < 0.001$). The linear regression of energy intake (kcal/kg/day) on age (days) was $Y = 118.95 - 0.44X$. The quadratic equation ($Y = 146.95 - 1.40X + 0.01X^2$) was statistically significant ($P < 0.01$).

The absolute amount of protein ingested decreased from 7.56 ± 1.69 gm/day at 1 month to 6.06 ± 1.38 gm/day at 4 months ($P < 0.001$). Protein intake declined relative to body weight, from 1.63 ± 0.31 gm/kg/day at 1 month to 0.88 ± 0.19 gm/kg/day at 4 months ($P < 0.001$). The linear regression describing protein intake (Y) (gm/kg/day) against age (X) (days) was $Y = 1.71 - 0.0073X$. The quadratic equation defined was $Y = 2.14 - 0.0218X + 0.0010 X^2$ ($P < 0.001$). Protein provided 8% of the total energy intake. The mean NPN intake varied from 438 ± 234 mg/day at 1 month to 360 ± 134 mg/day at 4 months. If the infant utilized NPN, the available nitrogen

Weight-for-age			*Length-for-age*			*Weight-for-length*		
Median	*25th Percentile*	*75th Percentile*	*Median*	*25th Percentile*	*75th Percentile*	*Median*	*25th Percentile*	*75th Percentile*
72	40	86	51	18	86	45	27	64
76	60	89	67	31	83	67	41	79
76	57	89	63	34	88	65	37	82
71	53	86	63	46	89	58	40	71
69	42	86	55	37	82	49	34	72

would have been augmented by approximately 27%. The overall mean intake of lactose was 48.4 ± 6.5 gm/day, which supplied 40% of the energy intake. The daily fat intake was 25.2 ± 6.3 gm/day; 49% of the calories were provided by fat. The ingestion of lactose and fat relative to body weight decreased with advancing age ($P < 0.001$).

Supplementation. There were 13 reported instances of food supplementation among eight infants. Six of the infants received inconsequential amounts of formula, oatmeal, glucose water, or rice water for 1 or 2 days only. The supplement contributed no more than 60 kcal/day on these occasions. Apple juice (40 kcal/day) was given on 13 occasions to one infant attending a day care center. Another infant required formula and rice supplementation because of apparently inadequate milk production; this infant received an additional 90 kcal/day for 6 days during the fourth month. Vitamin supplements were given to 40% of the infants on a regular basis. Iron supplements were given to 7%, and fluoride supplements to 26% of the infants throughout the study period.

Growth. The mean birth weight was 3.58 ± 0.45 kg, and birth length 50.9 ± 2.5 cm, (Table III). Although mean weights of these infants were consistently greater than the mean weights of the NCHS study population[17] ($P < 0.01$), weight-for-age percentile decreased after the first month at a rate of 2.6 percentile per month ($P < 0.01$). Infant lengths, although not significantly different at birth, were significantly greater than the NCHS sample at the 4 successive months ($P < 0.02$). Excluding birth measurements, length-for-age percentile did not vary significantly throughout the study, and weight-for-length percentile declined at a rate of 3.9 percentile per month ($P < 0.01$).

Growth performance also was evaluated by examining shifts in relative frequency distributions of the growth percentiles. At birth 7% of the infants, and at 1 month 2%, fell below the 10th percentile weight-for-age; thereafter, none of the infants was below the 10th percentile ranking. On the upper end, 19% of the infants were above the 90th percentile at birth, and 14% were above at 4 months. The remainder of the infants tended to aggregate within the 50th to 90th percentiles. There were no dramatic shifts in length-for-age percentiles during the 4 successive months; however, with the exclusion of birth measurements, there was a preponderance of infants above the 50th percentile. Length-for-age percentiles were correlated positively with weight-for-age and negatively for weight-for-height ($P < 0.01$).

The linear equation describing weight gain (gm/kg/day) against age (X) (days) was $Y = 13.55 - 0.095X$. As expected, the rates of weight gain and linear growth decreased with progressive age (Table III). The rate of weight gain (gm/day) was correlated positively at each month to the infant's weight-for-age percentile ($P < 0.05$) (r ranged from 0.29 to 0.55).

Intake/growth. Infant weight was correlated with milk intake (gm/day) at months 1 ($P < 0.10$), 2 ($P < 0.01$), and 4 ($P < 0.02$); infant weight, however, explained only 16% of the variability in intake.

Milk intake (gm/day) was correlated with the rates of weight gain (gm/day) at months 1 ($P < 0.10$), 2 ($P < 0.001$), and 4 ($P < 0.001$). Milk intake accounted for 10%, 30%, and 28% of the variability, respectively, in the rate of weight gain at these 3 months. The mean slope of these three regression equations was 0.041 ± 0.016, which indicates that the ingestion of 100 gm milk was associated with a mean weight gain of 4.1 gm. The variables energy and protein concentration did not account for any more of the variability seen in growth rate by multiple-regression analysis. Thus the amount of milk, and not the concentration of its constituents, exerted the greatest impact on growth rates.

Infants were subdivided into quadrants according to their percentile distribution of milk intake (gm/kg/day), and the corresponding rates of weight gain (gm/kg/day) of the infants in each quadrant were computed (Table IV). Conversely, milk intakes were computed for infants categorized according to their percentile distribution of rate of weight gain (gm/kg/day) (Table V). A tendency for growth rate to increase with increasing intake was observed.

The ratios of weight gain to energy intake were 7.5 ±

Table IV. Corresponding weight gain of infants classified according to percentile distribution of milk intake

	Percentile distribution of milk intake (gm/kg/day)			
	<25th	26th to 50th	51st to 75th	76th to 100th
Age (mo)	Weight gain (gm/kg/day)			
1	11.6 ± 4.3	11.4 ± 5.2	9.5 ± 4.3	10.8 ± 1.8
2	6.0 ± 2.7	6.4 ± 3.1	6.6 ± 3.3	8.5 ± 3.4
3	4.4 ± 1.0	3.3 ± 1.2	4.3 ± 1.8	3.9 ± 1.2
4	2.6 ± 1.0	2.0 ± 1.3	3.2 ± 1.1	3.8 ± 1.1

Data expressed as mean ± SD.

Table V. Corresponding milk intakes of infants classified according to percentile distribution of weight gain

	Percentile distribution of weight gain (gm/kg/day)			
	<25th	26th to 50th	51st to 75th	76th to 100th
Age (mo)	Milk intake (gm/kg/day)			
1	149 ± 14	169 ± 27	173 ± 31	151 ± 20
2	122 ± 19	124 ± 21	128 ± 13	142 ± 16
3	117 ± 24	119 ± 17	111 ± 19	122 ± 23
4	101 ± 16	110 ± 13	111 ± 18	121 ± 17

Data expressed as mean ± SD.

3.0, 7.0 ± 2.5, 5.2 ± 2.1, and 3.8 ± 1.5 gm weight gained for 100 kcal ingested for the 4 consecutive months.

DISCUSSION

Human milk intake. The milk intakes of these exclusively breast-fed infants were consistent with previous reports of breast-fed infants reared under privileged conditions. Typical milk yields have ranged from 600 to 900 gm/day through the first 4 months of life.[3-8] Contrary to other reports[3,5,6,8] of a gradual, steady increase in intake, a plateau in intake was observed throughout our study. Longitudinal studies with high attrition rates and cross-sectional studies may tend to select for the more abundant producers in later months, and may result in progressively higher estimates of output.

Few studies from well-nourished communities have documented the energy[8] and protein[4,8] intake of exclusively breast-fed infants. The levels of protein intake we observed were in agreement with those of investigations in Sweden.[4] The energy and protein intakes reported by Dewey,[8] however, were considerably higher than those we recorded.

The variability in milk intake (mean coefficient of variation) observed in this study was 17% between infants of the same age and 8% within individuals, as estimated from consecutive test-weighings. The variance associated with milk intake was greater between infants than between months or within infants. A wide range of interindividual variability in intakes of infants of the same age (11% to 29%) is evident from the literature.[3-8] One investigator[3] calculated the daily individual variation in intake from 48-hour test-weighing sessions to be less than 5% in two thirds of the study sample. The capacity to regulate intake is remarkable among breast-fed infants.

The validity of the determinations of human milk intake depends on the precision and accuracy of the test-weighing procedure. The integrating function of our scales eliminates the interference of the infant's movements during weighing. Procedural errors, such as omission of feedings, mistranscribing figures, and unaccounted losses of urine, feces, and vomitus may occur. Except for transcribing errors, these procedural problems result in an underestimation of milk intake. Occurrence of such errors was not eliminated, but minimized in our study by having the mothers report any problems during the test-weighing session. There were no reported losses of urine or feces. Mothers estimated the amount of vomitus, which generally was inconsequential, quantitatively. The possibility exists that feedings were omitted, but the conscientiousness of these women and the patterns of feedings do not support this conjecture. The only systematic error of which the investigators were aware was insensible evaporative loss during a feeding.

Milk composition. The close agreement between caloric values as determined by bomb calorimetry and as calculated from energy conversion factors confirms the high reliability of the determinations. Because milk composition is known to vary throughout the day and throughout a feeding, the real problem in the documentation of nutrient intakes of breast-fed infants is obtaining a representative sample of the milk ingested.[19] The sampling scheme used in our study to obtain a 24-hour representative sample permitted typical feeding patterns and the stimulus of infant suckling. This regimen functioned well, although the fat content was unavoidably subject to the emptying proficiency of the mother.

The concentration of TN and its partition between PN and NPN were similar to those in other reports.[4,20] The constant proportionality of NPN to TN suggests a regulatory mechanism beyond simple diffusion from the maternal serum. The inverse relationship between fat and lactose concentration has been noted by others.[19,21] Fat and lactose provide approximately 90% of the calories in human milk. The synthesis of these constituents may be subject to counterregulatory controls that result in a constant energy concentration. The lower energy density observed in months 2 to 4 (0.64, 0.62, and 0.64 kcal/gm, respectively) is attributable to a lower fat concentration. The fat concentrations reported here are in accordance with some investigations,[19,22] but at variance with others.[23,24] Sampling times and technique markedly influence fat determinations, which may explain conflicting lipid values. From the positive correlation observed between caloric content and the aliquot volume expressed for analysis, it may be inferred that the samples expressed were not always complete. Because fat concentration is highest in the hind milk,[19] lower caloric values would be obtained with incomplete emptying.

The energy values reported in our study are presented in terms of gross energy, contrary to usually reported values of metabolizable energy derived from physiologic fuel constants. Metabolizable energy derived from human milk or from cow milk formula has been estimated from balance studies to be 92% of gross energy.[25]

Nutrient recommendations. The energy intakes reported are notably less than current recommended dietary allowances,[1,2] which are based on the voluntary intakes of formula-fed infants.[26-31] Mean metabolizable energy intakes recorded for formula-fed infants decreased from approximately 120 kcal/kg/day at 1 month to 95 kcal/kg/day at 4 months.[30] Thus by 4 months of age, the breast-fed infants in our study consumed 25% fewer calories than the reference formula-fed infants. More recent studies using dietary recall[32] or record[33] methods indicate that the quantities of newer formulations consumed by infants have decreased. An understanding of the functional consequences in terms of growth and development is required to assess the significance of these changes in infant feeding practices.

Despite the lower nutrient intakes, the growth performance of this group of exclusively breast-fed infants compared favorably with NCHS reference standards. There was a slight downward trend in weight-for-age percentiles, which was particularly evident in the fourth month. This trend may represent merely an adjustment to the infant's genetic growth potential after the prenatal effects on birth weight have subsided. None of the infants, however, displayed clinically significant deviations from normal growth patterns. The NCHS growth standards were derived primarily from growth curves of formula-fed infants; the applicability of these standards for breast-fed infants has not been evaluated. Comparable growth among breast-fed and formula-fed infants has been documented,[34-38] notwithstanding conflicting reports.[39-42]

Statistically significant relationships between milk intake and growth rate were demonstrated at months 2 and 4. Definitive relationships between energy intake and growth are difficult to determine, because energy is partitioned between requirements for growth, maintenance, and activity. The estimation of the fraction allocated for growth during the first 4 months of life is 33%.[43] Variability in the remaining needs could disguise any clear relationship between intake and growth. Regresion analysis on the data at hand explained only 23% of the variability in growth rate by milk volume.

The efficiency of energy utilization for growth has been examined carefully in formula-fed infants[30]; however, incongruous age groupings preclude direct comparison with our study. Despite lower energy intakes after the first month of life, the breast-fed infants gained weight at rates similar to those of formula-fed infants. The ratios of weight gain (gm) per 100 calories were approximately 10% to 30% higher among the breast-fed infants than in the formula-fed infants,[30] which suggests a more efficient utilizatin of energy. Infants may respond to varying levels of energy intake by alterations in activity, basal metabolism, body composition, metabolic cost of growth, or diet-induced thermogenesis. Further study is required to determine the relative contributions of these various components to differences in energy utilization.

The protein intakes observed in our study were considerably less than recommended allowances for infants.[1,2] The protein consumption of reference formula-fed infants ranged from 2.0 gm/kg/day at 1 month to 1.6 gm/kg/day at 4 months.[30] Balance studies have demonstrated that formula-fed infants retain more nitrogen per kilogram body weight than breast-fed infants.[25,44] This finding suggests different rates of chemical maturation between the two groups of infants. The influence of diet on the chemical maturation of body composition has been substantiated in growing animals.[45,46]

Recommended levels[1,2] of protein are set at 2 SD above observed mean intakes of formula-fed and breast-fed infants[47] to allow for individual variability and to cover the needs of the majority of the population. Recommendations were based on mean intakes of 282 ± 46 mg N/kg/day for infants younger than 3 months, and 236 ± 30 mg N/kg/day for infants aged 3 to 6 months. The mean total nitrogen intake of the infants in this study ranged from 348 ± 68 mg TN/kg/day at 1 month to 198 ± 42 mg TN/kg/day at 4 months. The total nitrogen intakes in this study differed slightly from the mean intakes on which official recommendations are based. However, the utilization of NPN from human milk has not been evaluated. Free amino acids and peptides, which comprise approximately 10% to 20% of NPN, probably are metabolized. The extent to which urea, the major component of NPN, is utilized is unknown. Children have retained nonspecific nitrogen efficiently when ingesting low-protein diets.[48,49] Confronted with a limited supply of dietary protein, infants may conserve nitrogen by decreasing protein turnover, reutilizing endogenous amino acids, altering the composition of newly accrued tissues, or utilizing nonprotein sources of nitrogen.

Our findings indicate that breast-fed infants attain adequate growth with nutrient intakes substantially less than current dietary recommendations. Further information on the mechanisms by which infants adjust to a given plane of nutrition and adapt to less optimal environmental conditions is required before revision of dietary standards should be undertaken.

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