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Energy utilization and growth in breast-fed and formula-fed infants measured prospectively during the first year of life[1–3]

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ABSTRACT This study is the first to report approximations of energy requirements for male and female breast-fed and formula-fed infants based on individual estimates of total daily energy expenditure (TDEE) and energy deposition derived from total body fat (TBF) and fat-free mass (FFM) gain as determined by total-body electrical conductivity. In 46 healthy, full-term infants the effect of ≥4 mo of exclusive breast-feeding compared with formula feeding on macronutrient and energy intake, TDEE, energy deposition, and growth were investigated prospectively. Metabolizable energy intake (MEI) was assessed from macronutrient intake by test weighing (MEI-TW) and from the sum of TDEE and energy deposition (MEI-Pred). At 1–2, 2–4, 4–8, and 8–12 mo of age MEI-Pred averaged 431 ± 38, 393 ± 33, 372 ± 33, and 355 ± 21 kJ $\cdot$ kg^{-1} $\cdot$ d^{-1} for boys, and 401 ± 59, 376 ± 25, 334 ± 33, and 326 ± 17 kJ $\cdot$ kg^{-1} $\cdot$ d^{-1} for girls. No significant difference between breast-fed and formula-fed infants was found with respect to weight, length, head circumference, TBF, FFM, and TDEE at all ages, or for gain in length, weight, TBF, and FFM. MEI-TW was significantly different between feeding groups at 1–4 mo of age (formula-fed being greater than breast-fed, $P < 0.005$). This feeding effect, however, was not significant for MEI-Pred (MJ/d). MEI-TW differed from MEI-Pred only in breast-fed infants at 1–4 mo ($P < 0.05$ at 2–4 mo). The data from this study indicate that energy requirements in infants are lower than the recommendations in guidelines currently in use. *Am J Clin Nutr* 1998;67:885–96.

KEY WORDS Infant nutrition, human milk, formula, protein, fat, carbohydrate, energy intake, energy expenditure, energy deposition, growth, body composition, fat-free mass, TOBEC, total-body electrical conductivity, deuterium-to-infant method, doubly labeled water method

INTRODUCTION

The energy intakes of breast-fed (BF) and formula-fed (FF) infants have been reported to differ (1). Whether these differences result in altered energy utilization or growth has been debated for many years. A recent meta-analysis (1) showed that energy expenditure in the first year of life is affected by age and by feeding mode (BF < FF). Growth in BF infants also deviates from current reference standards (2). The former two studies suggest that energy utilization may differ between BF and FF infants. However, energy intake, deposition, and expenditure have never been measured in the same cohort of infants. Difficulties in the estimation of energy deposition as a result of the inaccuracy of existing body-composition methods prohibited such attempts. The appearance of total-body electrical conductivity (TOBEC) (3) as a safe and accurate body-composition method for use with infants now opens the possibility for simultaneous measurement of energy intake, expenditure, and deposition.

Energy requirements in infancy (4) are based on the observed intake data of healthy, well-nourished, thriving infants. In BF infants, the energy content of human milk is usually estimated from expressed breast milk (5). Because this approach is prone to various errors, energy requirements of infants were calculated alternatively from the sum of energy expenditure and energy deposition (6, 7). Energy expenditure was estimated from various combined doubly labeled water data from the literature. Energy deposition was calculated from reference values for body composition (8).

We report the results of the Sophia Study, which, to our knowledge, is the first to simultaneously follow nutrient intake, energy expenditure, growth, and body composition prospectively during the first year of life in 46 healthy, full-term infants exclusively BF or FF for ≥4 mo. Solid foods were introduced after 4 mo. Energy requirements were assessed from the sum of energy expenditure measured by the doubly labeled water method and energy deposition calculated from gain in TOBEC-derived total body fat (TBF) and fat-free mass (FFM).

SUBJECTS AND METHODS

Study design

Pregnant women who intended to exclusively breast-feed or bottle-feed their babies for ≥ 4 mo were recruited with the coop-

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TABLE 1
Characteristics of the infant study population[1]

	Boys		Girls	
	BF (*n* = 9)	FF (*n* = 15)	BF (*n* = 14)	FF (*n* = 8)
Height of father (cm)	187 ± 8[2]	183 ± 8	183 ± 8	184 ± 9
Height of mother (cm)	170 ± 6	169 ± 5	170 ± 7	168 ± 5
Weight of father (kg)	84.1 ± 10.2	80.7 ± 14.9	83.5 ± 13.8	83.0 ± 7.3
Weight of mother (kg)	64.2 ± 6.7	67.5 ± 14.8	64.6 ± 7.8	64.9 ± 9.9
Age of father (y)[3]	34 ± 5	29 ± 4	31 ± 4	29 ± 3
Age of mother (y)	30 ± 4	28 ± 3	28 ± 3	28 ± 4
Father employed (*n*)	8	14	13	8
Mother employed (*n*)	5	8	8	2
Monthly net income (NLG)[4]	3773 ± 1260	3217 ± 1225	3332 ± 1019	3570 ± 1983
Father's education, high/intermediate/low (*n*)	4/4/1	4/8/3	10/3/1	1/3/4
Mother's education, high/ intermediate/low (*n*)	4/4/1	3/5/7	5/6/3	1/4/3
Parity of mother (*n*)				
1	5	8	11	4
2	4	7	3	4
Gestational age (wk)	40.8 ± 1.3	40.5 ± 1.2	40.5 ± 1.5	40.3 ± 1.1

[1] BF, breast-fed; FF, formula-fed.
[2] $\bar{x}$ ± SD.
[3] Significantly different by mode of feeding, $P < 0.05$.
[4] Netherlands guilder = US$0.49.

eration of local midwives. Within the first 2 wk after delivery the mothers were contacted again and informed consent was obtained. Healthy, white infants were selected whose birth weights > 2500 g, and who were born by nonpathologic, vaginal delivery from healthy, nonsmoking mothers who were once or twice parous. Mothers with a history of gestational diabetes, gestational hypertension, preeclampsia, eclampsia, or use of tobacco, alcohol, or drugs during or after gestation were excluded, as were infants with a history of intrauterine growth retardation, asphyxia during or after birth, major infections, or any kind of failure to thrive during the first month of life. Mothers who stopped breast-feeding before 4 mo were excluded from the study.

Of the 92 responding mothers, 42 refused afterward or were excluded after delivery because of incompatibility with the inclusion criteria. Fifty infants were enrolled in the study. Four infants were excluded because the mothers failed to follow the protocol in some respect. The characteristics of the study population are shown in **Table 1**. Measurements of nutrient intake, energy expenditure, growth, and body composition were planned prospectively at 1, 2, 4, 8, and 12 mo of age. The study was approved by the Medical Ethical Committee of the University Hospital Rotterdam.

Energy and macronutrient intake

Recording of food intake

Food intake of the infant was measured at home by the mothers at 1, 2, 4, and 8 mo of age by 5-d test weighing and at 12 mo of age by the "double-portion" method for 3 d. Mothers were asked not to change the infant's feeding mode from ≥ 1 wk before until the end of the measurement period. Human milk intake was measured by weighing the infant before and after a feeding with an electronic integrating balance [precision: 1 g (0–3 kg), 2 g (3–6 kg) , 5 g (6–10 kg); Instru Vaaka Oy, Vaany, Finland]. Mothers were instructed not to change the diaper before the second weighing (after the feeding) was recorded, and not to include the weight of the bib on either of the two weight recordings. The time at which the infant was weighed was noted by the mother. Feeding duration was defined as the period between the two weights before and after the feeding, which did not necessarily equal the actual time the infant spent at the breast. Corrections for insensible water loss (IWL) during the feeding were made assuming a value for IWL of 1.8 $g \cdot kg^{-1} \cdot h^{-1}$ (9).

Twenty-four–hour breast-milk samples were collected within 4 d after the test-weighing period. Mothers mechanically expressed one or two breasts depending on their feeding habit: some gave one breast per feeding whereas others gave both breasts at a feeding. All expressed milk was gently shaken and a ≈20-mL subsample was stored in a glass jar at −20 °C immediately after collection. The remainder was given to the infant by bottle. Mothers were encouraged to mechanically express their milk in the same manner as they fed their babies, ie, one full breast or part of two breasts per feeding. The amount of hind milk (and subsequently the energy content of the total amount of breast milk) varies by the length of the feeding time and the feeding habit of the mothers. Twelve mothers who were not able to follow this procedure were asked to mechanically express 5 mL per breast before the feeding and 5 mL afterward. This resulted in 10 mL of breast milk when mothers gave one breast per feeding and in 20 mL of breast milk when two breasts per feeding were given. Human milk samples were transported at −20 °C to the laboratory. At completion of the study all samples were thawed to prepare pooled 24-h samples and immediately refrozen at −45 °C. Aliquots of milk proportional to the milk intake at each feeding (determined by 5-d test weighing) were then pooled and macronutrient analysis was performed.

Intake of formula was measured by weighing the bottle before and after a feeding. All formula powder came from one batch (Nutrilon Premium; Nutricia Inc, Zoetermeer, Netherlands). Mothers prepared a daily stock, of which immediately after the solution

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was stirred, 20 mL was set apart for analysis of formula density.

Milk intake was corrected for the amount of regurgitation of human milk (after the feeding) and of formula (during and after the feeding). Regurgitation of milk was assessed and recorded on a five-point scale by the mother (one teaspoon = 5 mL, one dinner spoon = 10 mL, one-half cup = 20 mL, one cup = 50 mL; if more than one cup the mothers were asked to assess how many cups).

Intake of nonmilk foods and fluids was determined by test weighing using a balance with 1-g precision. Details on the type of feeding, as well as further information as mentioned above was recorded by the mother on a simple structured preprepared form. Nutrient composition of recorded foods was calculated by using the information given by the manufacturer and in the case of fresh food by using a national food table (10).

At 12 mo of age intake was assessed by the double-portion method for 3 d. Equal portions of all drinks and foods that the infants consumed (assessed with a balance or by visual inspection with a gram- or milliliter-scaled can) were stored in plastic containers, refrigerated at home, and transported at −20 °C to the laboratory where they were stored at −45 °C until analyzed. It was emphasized that only the amount of food or drink that was actually consumed by the child should be deposited into the plastic containers.

Milk intake by the deuterium-to-infant method

The human milk intake was also assessed by using total water output data resulting from the doubly labeled water technique (11). To correct for the environmental water influx on total water milk intake a correction factor of 0.937 was used (12). When 50 g/d (11) was used instead of the correction factor of Wells and Davies (12), a difference of only 1% in total milk intake was found, despite the differences in climate between the two study areas (Houston and Cambridge, United Kingdom).

Nutrient analysis

All macronutrient analyses were performed after completion of the study. Human milk and double-portion samples were dried at 102 °C under vacuum. Fat was determined by the Rose-Gottlieb procedure (human milk and formula samples) or by the Weibull method (double portion samples), total nitrogen by the Kjeldahl method, and lactose by an enzymatic procedure (test kit no. 176303; Boehringer-Mannheim, Mannheim, Germany). Nonprotein nitrogen was assumed to be 20% of total nitrogen for human milk (5, 13) and 13% for formula (14; personal communication, Nutricia Inc, 1997). Protein nitrogen was taken as total nitrogen minus nonprotein nitrogen. Milk protein (human milk and formula) was calculated as protein nitrogen × 6.38 and protein in nonmilk foods as protein nitrogen × 6.25. Carbohydrates were calculated by difference. Gross energy content by test weighing (GEI-TW) was calculated from fat, protein, and total carbohydrate by using the factors 9.25, 5.65, and 3.95 kcal/g (38.7, 23.6, and 16.5 kJ/g), respectively, for human milk and formula and the factors 9.4, 5.65, and 4.15 (39.3, 23.6, and 17.3) for nonmilk foods (15). Metabolizable energy intake by test weighing (MEI-TW) was assumed to be 94% of GEI-TW (1). Energy content of the double portions at 12-mo was also assessed by means of standard bomb calorimetry, which did not give results that were significantly different from macronutrient analysis (3200 ± 580 compared with 3250 ± 470 kJ/L, respectively, in BF infants and 3450 ± 460 compared with 3490 ± 450 kJ/L in FF infants). The correlation between energy content by bomb calorimetry and macronutrient analysis was 0.86 ($P < 0.001$).

Growth and body composition

TOBEC

FFM was measured by TOBEC. TBF was calculated as weight minus FFM. Details about the TOBEC method, its accuracy, reproducibility, calibration, and the calculation of TBF and FFM were discussed earlier (15–18).

Anthropometry

At the time of the TOBEC measurement, the infants were weighed naked on an electronic baby scale (Instru Vaaka Oy) to the nearest 1 g (0–3 kg), 2 g (3–6 kg), or 5 g (6–10 kg). Recumbent crown-heel length was measured to the nearest millimeter on a length board. Frontooccipital head circumference was measured to the nearest millimeter with a 1-cm wide standard plastic measurement tape. Skinfold thickness (triceps, subscapular, and quadriceps) was measured with a Harpenden caliper (HE Morse Co, British Indicators, Ltd, St Albans, United Kingdom) to the nearest 0.1 mm and read at the point of stabilization of the measurement (≈15–60 s after application of the caliper). SD scores of length, weight, and head circumference were based on the Dutch growth reference centiles (19). Most of the measurements in this study (> 90%) were performed by the main observer. The other measurements were performed by a second observer, who was well trained by the first observer. We measured interobserver variation with this second observer (3) and found no significant difference for weight, length, or head circumference, and a small difference for skinfold thickness measurements (< 3%).

Energy expenditure by $^2H_2{}^{18}O$

Energy expenditure was measured by the doubly labeled water method. Details about $^2H_2{}^{18}O$ dosing, urine collection, transport, and storage were described elsewhere (18). For calculation of energy expenditure the time zero (t = 0) intercept, two-point approach was used. A urine sample taken before administration of labeled water was used as a baseline sample. Urinary tracer concentrations were corrected for additional isotope dilution caused by changes in the body water compartment during the 8 d of the experiment, as well as for the timing error of each urine sample caused by mixing of urine with decreasing concentrations of label in the bladder between two subsequent voids (18).

Pool sizes of ^{2}H (N_H) and ^{18}O (N_O) were calculated by extrapolation of concentrations to $t = 0$. Both isotopes have different fractionation factors and were administered concomitantly. The ratio of N_H to N_O (N_H:N_O) is narrowly defined, therefore, and used as a measure for the reliability of the urine sample. Data were excluded when N_H:N_O was > 3 SD from the mean N_H:N_O. This ratio was normally distributed (results not shown), leading to a < 1% loss of correct data that was rejected. Nineteen data points were excluded on this ground. An additional seven measurements were excluded because not all spoiled tracer could be collected (six cases) and because of unclear notation of urine collection times (one case).

The rate of carbon dioxide production (rCO_2) was calculated as described by Schoeller et al (20):

$$rCO_2 = (N/2.078) \times (1.01k_O - 1.04k_H) - 0.0246 \times r_{Gf} \quad (1)$$

where k_O and k_H are elimination rates of ^{18}O and ^{2}H, respectively, and N is the total body water (TBW) volume calculated from the isotope dilution spaces at time zero [(N_O/1.01 + N_H/1.04)/2] and

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TABLE 2
Macronutrient and gross energy intake of infants per day by age[1]

	Boys		Girls	
	BF (*n* = 9)	FF (*n* = 15)	BF (*n* = 14)	FF (*n* = 8)
Milk volume by deuterium-to-infant method (g)[2]				
1 mo[3]	800 ± 209 [6]	779 ± 154 [12]	607 ± 96 [9]	652 ± 57 [7]
2 mo[3]	830 ± 148 [4]	848 ± 89 [13]	687 ± 104 [9]	724 ± 72 [6]
4 mo	922 ± 179 [5]	923 ± 73 [9]	828 ± 134 [10]	851 ± 129 [6]
Milk volume by test weighing (g)				
1 mo[3]	768 ± 147	742 ± 104	635 ± 101	686 ± 95
2 mo[3,4]	799 ± 144	842 ± 86	690 ± 83	811 ± 111
4 mo[3,4]	806 ± 106	920 ± 92	736 ± 120	855 ± 89
Protein (g)				
1 mo[3,5]	8.34 ± 1.44	9.43 ± 1.8	6.82 ± 0.98	8.80 ± 1.59
2 mo[5]	7.54 ± 1.27	10.4 ± 1.7	6.74 ± 0.88	10.4 ± 1.3
4 mo[5]	7.35 ± 1.04	10.9 ± 1.7	6.76 ± 1.21	10.3 ± 0.8
8 mo[3]	23.2 ± 6.9	23.6 ± 5.7	19.5 ± 3.9	19.5 ± 4.1
12 mo	28.8 ± 4.4	30.0 ± 5.9	27.9 ± 6.3	23.6 ± 5.3
Protein (g/kg)				
1 mo	1.83 ± 0.28	2.06 ± 0.39	1.69 ± 0.36	2.10 ± 0.46
2 mo[5]	1.36 ± 0.08	1.91 ± 0.35	1.39 ± 0.19	2.04 ± 0.15
4 mo[5]	1.09 ± 0.11	1.62 ± 0.29	1.16 ± 0.26	1.55 ± 0.14
8 mo	2.67 ± 0.85	2.77 ± 0.75	2.48 ± 0.51	2.28 ± 0.45
12 mo	2.84 ± 0.41	2.96 ± 0.70	3.03 ± 0.71	2.46 ± 0.67
Fat (g)				
1 mo[5]	20.5 ± 8.0	27.3 ± 5.5	19.0 ± 5.2	26.5 ± 5.4
2 mo[5]	21.6 ± 7.0	30.3 ± 4.6	19.9 ± 5.3	31.6 ± 3.7
4 mo[5]	22.5 ± 5.1	31.5 ± 5.3	20.4 ± 7.9	31.6 ± 2.4
8 mo	25.0 ± 6.5	27.2 ± 3.9	27.7 ± 3.2	27.1 ± 5.1
12 mo	21.6 ± 7.9	22.1 ± 5.1	21.6 ± 7.0	19.6 ± 4.2
Fat (g/kg)				
1 mo[5]	4.50 ± 1.68	5.95 ± 1.02	4.62 ± 1.17	6.34 ± 1.52
2 mo[5]	3.85 ± 1.03	5.53 ± 0.57	4.12 ± 1.15	6.21 ± 0.47
4 mo[5]	3.35 ± 0.72	4.66 ± 0.72	3.47 ± 1.31	4.78 ± 0.45
8 mo[6,7]	2.84 ± 0.74	3.16 ± 0.49	3.52 ± 0.44	3.16 ± 0.54
12 mo	2.13 ± 0.81	2.16 ± 0.50	2.35 ± 0.79	2.02 ± 0.49
Carbohydrate (g)				
1 mo[3]	60.2 ± 12.5	51.8 ± 9.6	48.8 ± 7.5	49.1 ± 9.0
2 mo	61.2 ± 12.0	58.2 ± 8.7	53.5 ± 7.5	58.0 ± 7.3
4 mo[3]	64.3 ± 7.7	63.2 ± 8.2	58.3 ± 8.9	59.0 ± 5.1
8 mo	99.6 ± 15.0	102.0 ± 16.6	98.1 ± 12.7	94.6 ± 19.4
12 mo[3]	115.4 ± 18.8	114.4 ± 22.2	103.0 ± 21.0	95.2 ± 18.6
Carbohydrate (g/kg)				
1 mo	13.2 ± 2.4	11.3 ± 1.8	11.9 ± 1.3	11.7 ± 2.6
2 mo	11.0 ± 1.2	10.6 ± 1.4	11.0 ± 1.2	11.4 ± 0.8
4 mo	9.5 ± 0.9	9.4 ± 1.5	10.0 ± 1.8	8.9 ± 0.7
8 mo	11.4 ± 1.5	11.9 ± 2.3	12.5 ± 1.7	11.0 ± 1.8
12 mo	11.3 ± 1.2	11.3 ± 2.7	11.2 ± 2.4	9.8 ± 2.1
Gross energy (MJ)				
1 mo[4]	1.96 ± 0.45	2.13 ± 0.40	1.70 ± 0.26	2.04 ± 0.39
2 mo[5]	2.02 ± 0.48	2.38 ± 0.34	1.81 ± 0.24	2.42 ± 0.29
4 mo[5]	2.11 ± 0.25	2.52 ± 0.30	1.91 ± 0.37	2.44 ± 0.17
8 mo	3.22 ± 0.45	3.36 ± 0.39	3.21 ± 0.39	3.12 ± 0.49
12 mo[3]	3.53 ± 0.61	3.56 ± 0.48	3.29 ± 0.64	2.98 ± 0.49
Gross energy (kJ/kg)				
1 mo[4]	434 ± 88	464 ± 75	414 ± 50	489 ± 113
2 mo[5]	364 ± 59	435 ± 50	372 ± 54	477 ± 33
4 mo[5]	314 ± 29	372 ± 46	326 ± 67	368 ± 29
8 mo[6,7]	368 ± 46	393 ± 54	410 ± 54	364 ± 42
12 mo	347 ± 54	351 ± 63	359 ± 75	309 ± 59

continued

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TABLE 2 (*continued*)

	Boys		Girls	
	BF (n = 9)	FF (n = 15)	BF (n = 14)	FF (n = 8)
Percentage of energy from breast milk (%)				
1 mo	100 ± 0	0	100 ± 0	0
2 mo	100 ± 0	0	100 ± 0	0
4 mo	95 ± 12	0	97 ± 4	0
8 mo	27 ± 2 [3]	0	31 ± 2 [2]	0
Percentage of energy from formula (%)				
1 mo	0	100 ± 0	0	100 ± 0
2 mo	0	100 ± 0	0	100 ± 0
4 mo	0	96 ± 10	0	99 ± 3
8 mo[3]	35 ± 20 [7]	43 ± 15	46 ± 13 [13]	51 ± 11
Food quotient				
1 mo[5]	0.89 ± 0.02	0.86 ± 0.01	0.88 ± 0.03	0.85 ± 0.01
2 mo[5]	0.91 ± 0.06	0.85 ± 0.01	0.90 ± 0.03	0.86 ± 0.01
4 mo[5]	0.89 ± 0.02	0.85 ± 0.01	0.89 ± 0.04	0.85 ± 0.01
8 mo	0.88 ± 0.02	0.88 ± 0.02	0.88 ± 0.01	0.88 ± 0.01
12 mo	0.89 ± 0.01	0.89 ± 0.02	0.90 ± 0.02	0.90 ± 0.01

[1] $\bar{x}$ ± SD. Data at 1, 2, and 4 mo of age for breast-fed (BF) infants corrected for insensible water loss. Values for n are given in brackets if different from those given at the top of the table. FF, formula-fed infants.

[2] Data were derived from a subgroup of exclusively BF or FF infants (for corresponding n values *see* Table 5). Percentage difference in milk intake between test weighing and the deuterium-to-infant method at 1, 2, and 4 mo of age averaged, respectively, 3%, 2%, and −4% in BF and 3%, 2%, and −2% in FF infants (all not significantly different from zero by multiple linear regression analysis). Test weighing data were used for all other measurements.

[3] Significant effect of sex, $P < 0.05$.

[4, 5] Significant effect of mode of feeding: [4] $P < 0.05$, [5] $P < 0.005$.

[6] Significant interaction of sex by mode of feeding, $P < 0.05$.

[7] Significant interaction effect did not result in a significant feeding effect when tested in separate sex groups.

corrected for an exponential change over the observation period (21) by the following:

$$N = [N_{t=0} - N_{end}]/[\ln(N_{t=0}/N_{end})] \qquad (2)$$

where N_{end} is TBW at the end of the observation period. N_{end} was calculated from the difference between body weight at the start and body weight at the end of the observation period (measured within 5 d after the postdose urine sample and interpolated to day 9) by using values for the percentage of body water in weight gain as published by Fomon et al (8). The rate of water loss via fractionated gaseous routes (r_{Gf}) was estimated for the present study to be $1.19(k_O - k_H)$, assuming that breath is saturated with water and contains 3.5% carbon dioxide (fractionated breath water = $1.77 \times rCO_2$) and that transcutaneous fractionated (nonsweat) water loss amounts to ≈65% of breath water. Total daily energy expenditure (TDEE) was calculated from rCO_2 by TDEE = $22.4 \times Eeq_{CO_2} \times rCO_2$, where Eeq_{CO_2} is the energy equivalent of carbon dioxide at a given respiratory quotient (22). The respiratory quotient was estimated as a food quotient from food composition (23). An energy conversion factor of 0.85 for fat was used to correct for lower digestibility in infants than in adults (23). Food quotients were corrected for fat and protein deposition (23) by using data on composition of weight gain from Fomon et al (8), which were applied to the weight gain during the observation period.

Energy deposition

Energy deposition was calculated first by subtraction of TDEE from MEI-TW. Furthermore, energy deposition was calculated from gain in TBF (fat storage) and FFM (protein storage), as measured by TOBEC, in two ways. Method A involved calculation of increments of TBF and FFM between 1–2, 2–4, 4–8, and 8–12 mo. Method B involved calculation of gain in TBF and FFM for each child by using the first derivative at 1, 2, 4, 8, and 12 mo of third-degree polynomial curves through the individual values of TBF and of FFM plotted against age. The first derivative at 1, 2, 4, 8, and 12 mo then represents gain in TBF and FFM for each child at a chosen age.

Protein gain was estimated from FFM accretion by using reference data from Fomon et al (8). Average weights between 1–2, 2–4, 4–8, and 8–12 mo were used for expression of energy deposition on a kilogram per body weight basis. Energy conversion factors of 9.25 for fat and 5.6 for protein were used (24). Carbohydrate storage was assumed to be negligible. For the period of 0–1 mo of age energy deposition was calculated from weight gain. Protein and fat gain for this period were assessed from weight gain by using reference values on composition of weight gain (8).

Data analysis

SPSS for Windows (SPSS Inc, Chicago) was used for most statistical analyses. Data were expressed as means ± SDs. An effect was considered statistically significant if $P < 0.05$. For the different ages separately, differences between sexes and feeding groups were analyzed by multiple linear regression, with sex and feeding group and their interaction term as independent variables in the model. If the interaction was not significant, it was left out of the model. If it was significant, feeding groups were compared within boys and girls separately. By general linear mixed-model regression analysis, using the procedure Proc Mixed of the SAS statistical package (SAS/STAT software, changes and enhancements through release 6.11; SAS Institute Inc, Cary, NC), the dependence of TDEE and predicted metabo-

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TABLE 3
Percentage of total energy intake from protein, fat, and carbohydrate[1]

	Boys		Girls	
	BF (n = 9)	FF (n = 15)	BF (n = 14)	FF (n = 8)
Protein (% of energy)				
1 mo	10 ± 0.9	10 ± 1.0	10 ± 1.7	10 ± 0.3
2 mo[2]	9 ± 1.3	10 ± 1.0	9 ± 0.6	10 ± 0.2
4 mo[2]	8 ± 0.9	10 ± 1.0	8 ± 1.4	10 ± 0.2
8 mo[3]	17 ± 4.2	17 ± 4.3	14 ± 1.8	15 ± 2.1
12 mo	20 ± 2.3	20 ± 3.2	20 ± 3.1	19 ± 3.1
Fat (% of energy)				
1 mo[2]	39 ± 9.2	50 ± 1.6	43 ± 6.8	50 ± 1.2
2 mo[2]	41 ± 5.1	50 ± 1.7	42 ± 6.9	50 ± 0.9
4 mo[2]	41 ± 5.9	50 ± 1.8	40 ± 9.5	50 ± 1.8
8 mo[3]	30 ± 5.8	32 ± 4.5	34 ± 2.0	34 ± 4.6
12 mo	23 ± 6.0	24 ± 5.4	25 ± 5.5	25 ± 4.2
Carbohydrate (% of energy)				
1 mo[2]	51 ± 8.8	41 ± 1.2	48 ± 6.0	40 ± 0.9
2 mo[2]	51 ± 4.0	41 ± 1.3	50 ± 6.6	40 ± 0.7
4 mo[2]	51 ± 5.1	41 ± 1.4	52 ± 8.7	40 ± 1.9
8 mo	52 ± 6.1	50 ± 5.1	51 ± 2.2	51 ± 4.7
12 mo	55 ± 5.6	54 ± 5.9	53 ± 5.3	54 ± 4.9

[1] $\bar{x}$ ± SD. BF, breast-fed; FF, formula-fed.
[2] Significant effect of mode of feeding, $P < 0.0001$.
[3] Significant effect of sex, $P < 0.01$.

lizable energy intake (MEI-Pred) on sex, age, length, weight, and FFM was studied. In these repeated-measures analyses covariance structure was left completely free. This statistical method does not allow the calculation of r^2 values. The periods of exclusive breast-feeding or formula feeding (0–4 mo) and after weaning (>4 mo) were treated as separate periods because of principally different basic growth conditions in relation to feeding mode (exclusive breast-feeding or formula feeding versus a mixed infant diet).

As a practical and financial consequence of the design of the study, which aimed at simultaneous measurement of growth, energy intake, and energy expenditure by the expensive doubly labeled water method, only a limited number of infants could participate. Also, energy expenditure measurements could be performed only on a subset of infants. At 8 and 12 mo of age this inevitably subverted the power of the study as far as the energy expenditure data and their derivatives were concerned. We therefore present these data only as mean values for boys and girls. Here, data have been broken down into feeding mode and sex only when significant differences or interactions were observed.

RESULTS

Macronutrient and energy intake

All infants were exclusively BF and FF from birth to ≥4 mo of age, except for seven BF infants at the start of the measurement period at 4 mo of age: four who started with supplemental formula, two with supplemental fruit, and one with supplemental apple or pear juice. At 4 mo of age, two FF infants started with supplemental fruit, three with supplemental apple or pear juice, and one with supplemental vegetables. In only one infant did supplemental intake exceed 10% of total gross energy intake (24%). These solids

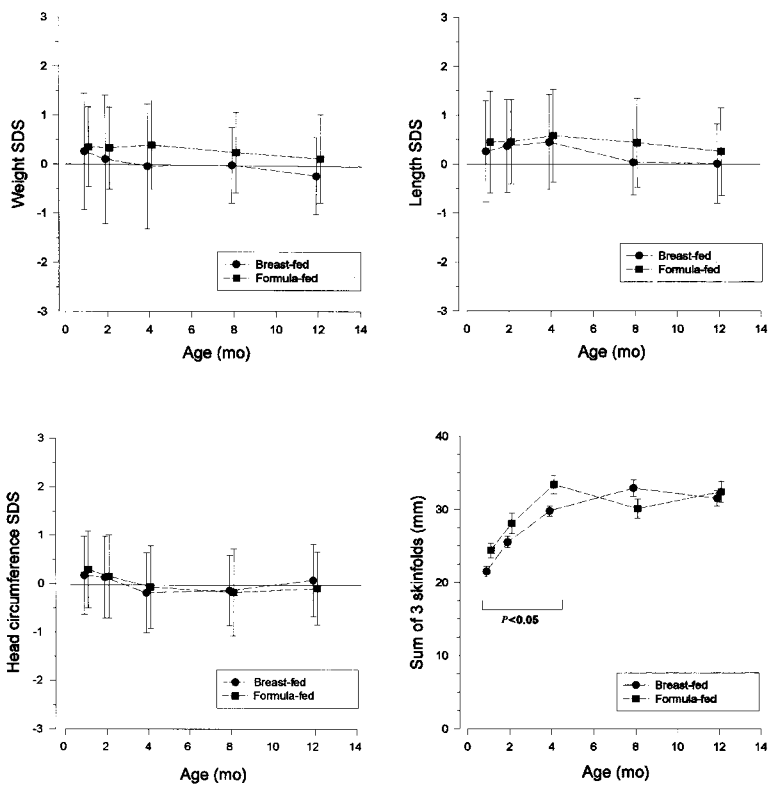

FIGURE 1. SD scores (SDS) of weight, length, head circumference, and average values for the sum of three skinfold thicknesses. $\bar{x}$ ± SD; n = 23 breast-fed and 23 formula-fed infants.

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TABLE 4
Body composition[1]

	Boys		Girls	
	BF (n = 9)	FF (n = 15)	BF (n = 14)	FF (n = 8)
Total body fat (%)				
1 mo	15.2 ± 3.1	14.6 ± 4.2	14.4 ± 3.8	14.2 ± 2.2
2 mo	20.6 ± 5.0	18.9 ± 3.3	19.1 ± 3.2	20.4 ± 2.9
4 mo	24.7 ± 5.6	24.1 ± 3.7	25.0 ± 2.5	27.2 ± 1.9
8 mo	26.7 ± 3.3	25.4 ± 3.7	25.5 ± 3.2	27.3 ± 3.4
12 mo[2]	26.6 ± 1.9	23.7 ± 5.4	24.9 ± 3.0	26.7 ± 3.6
Total body fat (kg)				
1 mo	0.70 ± 0.20	0.68 ± 0.24	0.61 ± 0.20	0.60 ± 0.11
2 mo	1.17 ± 0.40	1.05 ± 0.28	0.95 ± 0.24	1.03 ± 0.15
4 mo	1.71 ± 0.51	1.65 ± 0.42	1.49 ± 0.28	1.80 ± 0.14
8 mo	2.35 ± 0.42	2.21 ± 0.48	2.02 ± 0.32	2.33 ± 0.31
12 mo	2.71 ± 0.31	2.45 ± 0.73	2.30 ± 0.34	2.62 ± 0.46
Fat-free mass (kg)				
1 mo[3]	3.87 ± 0.40	3.91 ± 0.36	3.51 ± 0.42	3.63 ± 0.34
2 mo[4]	4.38 ± 0.41	4.43 ± 0.39	3.95 ± 0.47	4.04 ± 0.36
4 mo[4]	5.08 ± 0.46	5.13 ± 0.39	4.43 ± 0.48	4.83 ± 0.42
8 mo[3]	6.43 ± 0.52	6.43 ± 0.47	5.86 ± 0.37	6.23 ± 0.53
12 mo[4]	7.46 ± 0.60	7.79 ± 0.64	6.92 ± 0.44	7.14 ± 0.55

[1] $\bar{x}$ ± SD. BF, breast-fed; FF, formula-fed.
[2] Significant interaction of sex by mode of feeding, $P < 0.05$.
[3,4] Significant effect of sex: [3] $P < 0.05$, [4] $P < 0.005$.

TABLE 5
Energy expenditure[1]

	Boys	Girls	All
Energy expenditure (MJ/d)			
1 mo[2,3,4]	1.36 ± 0.21 [18]	1.19 ± 0.20 [16][5]	1.28 ± 0.22 [34]
2 mo[6]	1.72 ± 0.22 [17]	1.38 ± 0.22 [15]	1.56 ± 0.28 [32]
4 mo[6]	2.12 ± 0.29 [14]	1.78 ± 0.23 [17]	1.94 ± 0.31 [31]
8 mo[2]	2.91 ± 0.37 [12]	2.56 ± 0.23 [10]	2.75 ± 0.35 [22]
12 mo[2]	3.57 ± 0.23 [8]	3.08 ± 0.36 [8]	3.32 ± 0.38 [16]
($kJ \cdot kg^{-1} \cdot d^{-1}$)			
1 mo	298 ± 46 [18]	288 ± 42 [16]	293 ± 44 [34]
2 mo[2]	315 ± 36 [17]	286 ± 31 [15]	301 ± 37 [32]
4 mo[7,8]	319 ± 42 [14]	292 ± 40 [17]	304 ± 43 [31]
8 mo[9,10]	343 ± 42 [12]	320 ± 35 [10]	333 ± 40 [22]
12 mo[7,11]	341 ± 35 [8]	323 ± 27 [8]	332 ± 31 [16]
($kJ \cdot kg\ FFM^{-1} \cdot d^{-1}$)			
1 mo	351 ± 53 [18]	336 ± 50 [16]	344 ± 51 [34]
2 mo	393 ± 44 [17]	357 ± 48 [15]	376 ± 49 [32]
4 mo	422 ± 51 [14]	395 ± 56 [17]	408 ± 55 [31]
8 mo[9,12]	486 ± 54 [12]	438 ± 40 [10]	455 ± 49 [22]
12 mo	450 ± 40 [8]	441 ± 32 [8]	445 ± 35 [16]

[1] $\bar{x}$ ± SD; n in brackets. BF, breast-fed; FF, formula-fed; FFM, fat-free mass.
[2,6] Significant effect of sex: [2] $P < 0.05$, [6] $P < 0.005$.
[3,9] Significant effect of mode of feeding: [3] $P < 0.005$, [9] $P < 0.05$.
[4] 1.30 ± 0.13 (n = 6), 1.40 ± 0.23 (n = 12), 1.07 ± 0.16 (n = 9), 1.34 ± 0.13 (n=7) MJ/d, respectively, for BF boys, FF boys, BF girls, and FF girls.
[5] Significant effect of mode of feeding (FF > BF) in girls only, $P < 0.005$.
[7] Significant interaction effect (sex by mode of feeding), $P < 0.05$.
[8] 305 ± 48 (n = 5), 326 ± 40 (n = 9), 308 ± 33 (n = 11), 262 ± 37 (n = 6) $kJ \cdot kg^{-1} \cdot d^{-1}$, respectively, for BF boys, FF boys, BF girls, and FF girls.
[10] 357 ± 47 (n = 5), 334 ± 40 (n = 7), 338 ± 35 (n = 6), 293 ± 11 (n = 4) $kJ \cdot kg^{-1} \cdot d^{-1}$, respectively, for BF boys, FF boys, BF girls, and FF girls.
[11] 323 ± 21 (n = 3), 351 ± 40 (n = 5), 340 ± 25 (n = 4), 306 ± 15 (n = 4) $kJ \cdot kg^{-1} \cdot d^{-1}$, respectively, for BF boys, FF boys, BF girls, and FF girls.
[12] 489 ± 58 (n = 5), 453 ± 49 (n = 7), 459 ± 41 (n = 6), 408 ± 4 (n = 4) $kJ \cdot kg\ FFM^{-1} \cdot d^{-1}$, respectively, for BF boys, FF boys, BF girls, and FF girls.

have been incorporated in the macronutrient and energy intake estimations in **Table 2**. None of the infants had been switched from BF to FF or vice versa. At 8 mo of age, five infants were still partially BF (which averaged 28 ± 3% of total energy intake and 16 ± 5% of total protein intake; Table 2). At 12 mo of age none of the infants received breast milk. Feeding time in BF infants at 1, 2, and 4 mo averaged 164 ± 26 min/d. Feeding time decreased with age (significantly at 2 and 4 mo for both sexes). IWL at 1, 2, and 4 mo averaged 25 ± 8 mL. If no corrections for IWL would have been made, total intake would have been underestimated by 3.6 ± 1.1%.

At 1, 2, and 4 mo, respectively, the fat concentration of breast milk was 30.0 ± 9.0, 29.0 ± 8.0, and 27.0 ± 11 g/L; the nitrogen concentration was 2.06 ± 0.24, 1.83 ± 0.18, and 1.65 ± 0.18 g/L; the protein concentration was 11.2 ± 1.6, 9.9 ± 0.9, and 8.6 ± 1.0 g/L; the lactose concentration was 64.5 ± 3.8, 64.3 ± 2.6, and 64.6 ± 2.9 g/L; and the carbohydrate concentration was 79 ± 6, 79 ± 5, and 75 ± 6 g/L. Energy density calculated from fat, protein, and carbohydrate concentrations at 1, 2, and 4 mo, respectively, was 2710 ± 330, 2650 ± 330, and 2490 ± 460 kJ/L (or 650 ± 80, 630 ± 80, and 600 ± 110 kcal/L).

Nutrient intake and food quotients are summarized in Table 2. Except for carbohydrate intake, FF infants showed higher macronutrient and gross energy intakes during the exclusive FF period. The difference was most striking at 2 and 4 mo of age. At 4 mo of age no difference between sexes or feeding groups was found in milk intake volume by the deuterium-to-infant method. The percentage of GEI-TW from protein and fat was lower for BF than for FF infants (**Table 3**).

Growth and body composition

Birth weights were not significantly different between subgroups. The sum of three skinfold thicknesses was higher in FF infants of both sexes at 1 and 4 mo and in girls at 2 mo. Significant differences by feeding mode for weight were found in girls at 4 and 8 mo (BF < FF: 5.9 ± 0.7 compared with 6.6 ± 0.5 kg at 4 mo, and 7.9 ± 0.5 compared with 8.6 ± 0.6 kg at 8 mo of age) but not in boys (6.8 ± 0.8 kg at 4 mo and 8.7 ± 0.8 kg at 8 mo). No significant differences by feeding mode in length and head circumference were observed (**Figure 1**). A significant difference by mode of feeding in TBF and FFM was found only in girls at 4 and 8 mo of age. On average, FFM was higher in boys at all ages (**Table 4**). Weight gain was higher in boys at 0–1 mo (27 compared with 20 g/d in girls, $P < 0.05$). Differences in weight gain by feeding mode were observed only in girls at 2–4 mo (FF > BF: 24 compared with 18 g/d, $P < 0.01$). Length gain was not significantly influenced by sex or feeding mode. Fat gain was significantly higher in FF girls at 1–4 mo (FF > BF: 15 compared with 11 g/d at 1–2 mo and 12 compared with 9 g/d at 2–4 mo, $P < 0.05$). FFM gain was higher in FF infants only from 2 to 4 mo in girls (12 compared with 8 g/d, $P < 0.01$).

Energy expenditure

Energy expenditure (MJ/d) was not significantly different between BF and FF infants, except at 1 mo of age (**Table 5**).

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TABLE 6
Fractional turnover rates and ratio of dilution spaces of hydrogen and oxygen[1]

	1 mo (n = 34)	2 mo (n = 32)	4 mo (n = 31)	8 mo (n = 22)	12 mo (n = 16)
Dose (g)	8.2 ± 0.9	9.9 ± 1.3	11.1 ± 2.1	15.1 ± 3.0	19.1 ± 2.8
k_H	0.240 ± 0.040	0.237 ± 0.018	0.229 ± 0.023	0.202 ± 0.035	0.775 ± 0.032
k_O	0.279 ± 0.041	0.280 ± 0.019	0.274 ± 0.026	0.252 ± 0.036	0.224 ± 0.034
k_O:k_h	1.168 ± 0.028	1.182 ± 0.020	1.200 ± 0.022	1.250 ± 0.038	1.288 ± 0.052
N_H:N_O	1.025 ± 0.005	1.026 ± 0.006	1.027 ± 0.006	1.026 ± 0.006	1.028 ± 0.008
TBW (kg)					
Boys[2]	3.04 ± 0.30	3.43 ± 0.31	3.99 ± 0.28	4.93 ± 0.26	6.15 ± 0.19
Girls	2.78 ± 0.30	2.93 ± 0.26	3.55 ± 0.42	4.50 ± 0.32	5.34 ± 0.56

[1] $\bar{x}$ ± SD. There were no significant differences between feeding modes.
[2] Significant effect of sex, $P < 0.005$.

TDEE per kilogram FFM increased significantly with age from 1 through 8 mo of age ($P < 0.05$, repeated-measures ANOVA) and stabilized thereafter. TBW determined by [^{18}O]water was reported before (18), and was not significantly different between feeding groups. The dose of $^2H_2{}^{18}O$ administered, k_H, k_O, k_O:k_H, and N_H:N_O did not differ between study groups (**Table 6**).

TDEE was regressed against age (mo), weight (kg), and FFM (kg) as follows (sex: boys = 0, girls = 1):

$$\text{TDEE (kJ/d)} = 28.5 + 4.5\text{FFM} + 1.54\ \text{FFM}^2 + 9.98\text{age} - 0.53\text{age}^2 - 8.3\text{sex} \quad (3)$$
$$[\text{TDEE (kcal/d)} = 119 + 19.0\text{FFM} + 6.44\text{FFM}^2 + 41.7\text{age} - 2.21\text{age}^2 - 34.7\text{sex}]$$

$$\text{TDEE (kJ/d)} = 23.3 + 11.7\text{weight} + 4.62\text{age} - 9.93\text{sex} \quad (4)$$
$$[\text{TDEE (kcal/d)} = 97.3 + 48.8\text{weight} + 19.3\text{age} - 41.5\text{sex}]$$

$$\text{TDEE (kJ} \cdot \text{kg FFM}^{-1} \cdot \text{d}^{-1}) = 18.7 + 1.78\text{age} - 0.0957\text{age}^2 - 1.61\text{sex} \quad (5)$$
$$[\text{TDEE (kcal} \cdot \text{kg FFM}^{-1} \cdot \text{d}^{-1}) = 78.0 + 7.46\text{age} - 0.400\text{age}^2 - 6.74\text{sex}]$$

When quadratic terms of FFM, age, or weight were significant, they were included in the equation. TDEE was not significantly affected by feeding mode.

Energy deposition

Energy deposition calculated as TDEE − MEI-TW showed an extremely large error (data not shown). In several cases TDEE exceeded MEI-TW and negative values for energy deposition were found. With use of body-composition data and method A, higher energy deposition in boys at 0–1 mo and no differences thereafter were found (**Table 7**). At 1–2 mo an interaction effect between sex and feeding mode was observed. The low values of energy deposition found between 0–1 mo (calculated partly with use of Fomon et al's reference data) as compared with 1–2 mo of age were not present at 1 and 2 mo as calculated by method B (the first derivative method). With use of body-composition data and method B, no significant differences between study groups were found.

Predicted metabolizable energy intake

We checked the 95% CIs of the P values of the multiple linear regressions for both methods A and B at 8 and 12 mo for TDEE and its derivative parameters and found wide ranges including zero. The power of all tests involving these parameters will undoubtedly be unsatisfactory because of the limited number of infants in whom doubly labeled water experiments were performed. In most instances MEI-Pred was significantly higher in boys than in girls (except at 1–4 mo, when normalized for weight). A significant interaction effect between sex and feeding mode was found for MEI-Pred expressed as kJ · kg^{-1} · d^{-1} at 1, 2, and 8 mo of age ($P < 0.05$). For BF boys and girls, and FF boys and girls, respectively, MEI-Pred was 490 ± 46, 448 ± 54, 401 ± 67, and 501 ± 38 kJ · kg^{-1} · d^{-1} at 1 mo; 442 ± 50, 414 ± 35, 384 ± 39, and 422 ± 33 kJ · kg^{-1} · d^{-1} at 2 mo, and 336 ± 16, 355 ± 33, 358 ± 31, and 309 ± 11 kJ · kg^{-1} · d^{-1} at 8 mo of age (for corresponding n, *see* tables).

MEI-Pred (MJ/d) was linearly related to age (**Figure 2**). With use of data from method B, the relation could be described as follows:

$$\text{MEI-Pred (kJ/d)} = 111.7 + 7.46\text{age} - 18.4\text{sex} \quad (6)$$
$$[\text{MEI-Pred (kcal/d)} = 467 + 31.2\text{age} - 77.1\text{sex}]$$

$$\text{MEI-Pred (kJ/d)} = 45.7 + 15.0\text{weight} - 7.75\text{sex} \quad (7)$$
$$[\text{MEI-Pred (kcal/d)} = 191 + 62.6\text{weight} - 32.4\text{sex}]$$

$$\text{MEI-Pred (kJ/d)} = -50.4 + 3.16\text{length} - 12.9\text{sex} \quad (8)$$
$$[\text{MEI-Pred (kcal/d)} = -244 + 13.2\text{length} - 53.8\text{sex}]$$

$$\text{MEI-Pred (kJ/d)} = 56.7 - 3.54\text{age} + 0.301\text{age}^2 + 14.3\text{weight} - 8.06\text{sex} \quad (9)$$

$$[\text{MEI-Pred (kcal/d)} = 237 - 14.8\text{age} + 1.257\text{age}^2 + 59.8\text{weight} - 33.7\text{sex}]$$

where sex is 0 for boys and 1 for girls, age is in months, weight is in kilograms, and length is in centimeters. When quadratic terms of age, weight, or length were significant, they were included in the equation.

In **Figure 3,** MEI-TW is compared with MEI-Pred by method B in the same subgroup of infants. This comparison shows that MEI-TW was increasingly underestimated in BF infants at 1–4 mo of age.

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TABLE 7
Total daily energy deposition calculated from gain in fat and protein as derived from total-body electrical conductivity body-composition estimates[1]

	Boys (n = 24)	Girls (n = 22)	All (n = 46)
Model A: energy deposition[2]			
(kJ/d)			
0–1 mo[3,4]	288 ± 108	226 ± 83	259 ± 100
1–2 mo[5,6]	585 ± 209	527 ± 176	556 ± 192
2–4 mo	447 ± 146	443 ± 100	447 ± 125
4–8 mo	231 ± 94	217 ± 107	224 ± 99
8–12 mo	127 ± 136	127 ± 84	127 ± 112
(kJ · kg^{-1} · d^{-1})			
0–1 mo[4]	71 ± 29	60 ± 23	66 ± 27
1–2 mo[5,7]	114 ± 36	116 ± 38	115 ± 36
2–4 mo	72 ± 20	80 ± 17	76 ± 18
4–8 mo	31 ± 13	31 ± 16	31 ± 15
8–12 mo	13 ± 14	14 ± 10	14 ± 12
Model B: energy deposition[8]			
(kJ/d)			
1 mo	660 ± 270	648 ± 192	656 ± 230
2 mo	552 ± 184	539 ± 125	548 ± 155
4 mo	368 ± 79	355 ± 79	359 ± 79
8 mo	150 ± 109	142 ± 96	146 ± 100
12 mo	134 ± 234	138 ± 192	134 ± 213
(kJ · kg^{-1} · d^{-1})			
1 mo	142 ± 54	155 ± 43	148 ± 49
2 mo	100 ± 29	109 ± 23	104 ± 25
4 mo	54 ± 11	58 ± 15	56 ± 13
8 mo	18 ± 13	18 ± 12	18 ± 13
12 mo	12 ± 23	14 ± 20	13 ± 21

[1] $\bar{x}$ ± SD. BF, breast-fed; FF, formula-fed.

[2] Calculated with average weights from 1–2, 2–4, 4–8, and 8–12 mo.

[3] Significant effect of sex, $P < 0.05$.

[4] Calculated from actual weight gain data and reference data on composition of weight gain (8).

[5] Significant interaction of sex by mode of feeding, $P < 0.05$

[6] 644 ± 247, 543 ± 180, 472 ± 171, 619 ± 150 kJ/d, respectively, for BF boys, FF boys, BF girls, and FF girls.

[7] 125 ± 46, 109 ± 33, 104 ± 33, 134 ± 38 kJ/d, respectively, for BF boys, FF boys, BF girls, and FF girls.

[8] Calculated for each child from the first derivative at each age of a third-degree polynomial curve through either the values of total body fat or fat-free mass against age.

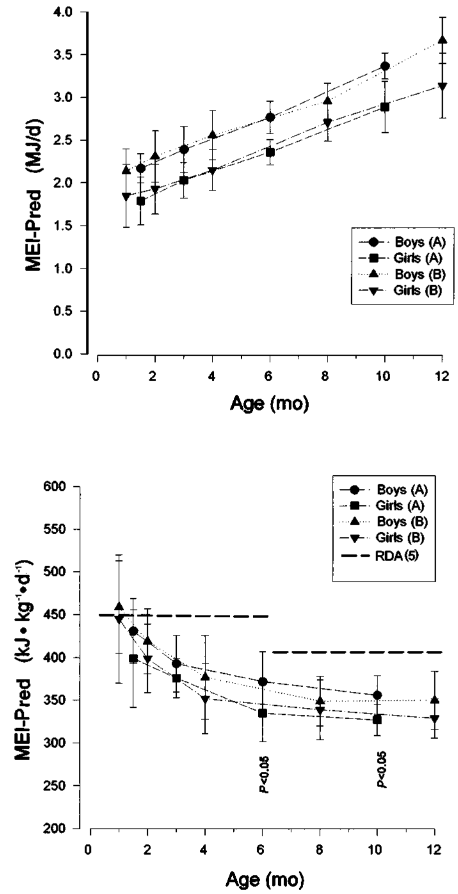

FIGURE 2. Predicted metabolizable energy intake (MEI-Pred), calculated by adding total daily energy expenditure and energy deposition (derived from body-composition data). Dotted lines represent the recommended dietary allowances (RDA; 5). Method A: MEI-Pred calculated by using energy deposition from body-composition differences between months. Method B: MEI-Pred calculated by using energy deposition from the first derivative method. For n, *see* Table 5.

DISCUSSION

Predicted metabolizable energy intake

Our study is the first describing simultaneous measurements of energy intake, TDEE, and body composition in individual infants and direct calculation of energy requirements from TDEE and energy deposition. In our study population MEI-Pred (MJ/d) was not affected by feeding mode and was higher in boys. When MEI-Pred was normalized by weight (kJ · kg^{-1} · d^{-1}), significant interaction effects arose because of small body-composition differences by feeding mode in girls. Former estimates of MEI-Pred (1, 6, 7) were derived from compiled literature data on TDEE and reference data on composition of weight gain (8). MEI-Pred of the present study agrees with data of Prentice et al (6) and of Whitehead (7), who based MEI-Pred estimations on TDEE data of Prentice et al (**Figure 4**). Recently, Butte (1) summarized TDEE data from various studies and summed these with energy deposition extracted from Fomon et al's (8) reference data. Averaged for sex and mode of feeding, Butte's estimates of MEI-Pred at 0–2 mo and 9–12 mo deviate from our data and the other estimates of MEI-Pred (6, 7). A trend toward lower energy deposition in the first month of life as observed by Butte (1) was present in our study as well, and might be due to the applied reference values for composition of weight gain (8) or to the physiologic postnatal weight loss with subsequent lower energy deposition.

Energy intake by test weighing

Our data on gross energy intake by test weighing (GEI-TW) agree with those of Butte et al (25, 26). They found values of 422 ± 67 compared with 493 ± 71 kJ · kg^{-1} · d^{-1} and 301 ± 38 compared with 364 ± 46 kJ · kg^{-1} · d^{-1} for BF compared with FF infants at 1 and 4 mo of age, respectively. However, the magnitude of the sex differences could not be calculated adequately from their paper. Except for some values in FF infants, our data on energy intake agreed with a recent meta-analysis on energy intake of BF and FF infants (1). In the DARLING Study (27), GEI-TW values found at 3 mo of age in BF compared with FF infants were 359 ± 50 compared with 405 ± 59 kJ · kg^{-1} · d^{-1} and 359 ± 38 compared with 418 ± 63 kJ · kg^{-1} · d^{-1} in girls and boys, respectively. These values agree with the average of our 2- and 4-mo values for GEI-TW. For BF infants (but not FF infants)

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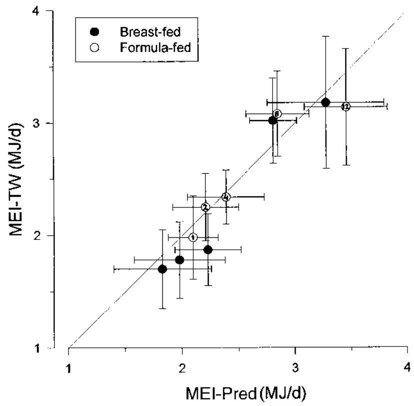

FIGURE 3. Comparison of metabolizable energy intake by test weighing (MEI-TW) and as predicted from energy expenditure and composition of weight gain in the same subset of infants (MEI-Pred). Horizontal and vertical error bars describe the SD of the corresponding variables. Values increased with age (1, 2, 4 , 8, and 12 mo of age) The difference is significant in breast-fed infants at 2 and 4 mo of age ($P = 0.03$ and $P = 0.005$, respectively, Wilcoxon nonparametric test), and in formula-fed infants at 1 mo of age ($P = 0.03$, Wilcoxon nonparametric test).

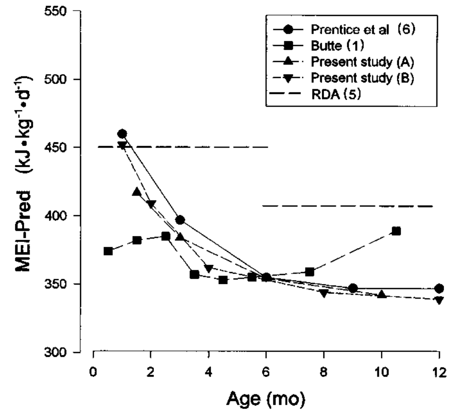

FIGURE 4. Predicted metabolizable energy intake (MEI-Pred): data from the present study compared with the earlier estimations of Prentice et al (6) and Butte (1). Dotted lines represent the recommended dietary allowances (RDA; 5). Method A: MEI-Pred calculated by using energy deposition from body-composition differences between months. Method B: MEI-Pred calculated by using energy deposition from the first derivative method. For *n*, *see* Table 5.

GEI-TW at 8 mo in our study was higher than in the DARLING Study at 9 mo, which may be due to the larger number of infants in the DARLING Study still being breast-fed at that age. Stuff and Nichols (28) reported GEI-TW values of 301 ± 88 and 263 ± 75 $kJ \cdot kg^{-1} \cdot d^{-1}$ at 8 mo for infants who were breast-fed exclusively until 5 and 6 mo of age, respectively. These values are lower than those found in our study at 8 mo of age. As in our study, the above-mentioned studies (27, 28) did not find a sex difference in GEI-TW per kilogram body weight. In our study as well as in these two studies, differences in GEI-TW (at least from birth to 4 mo of age) did not result in changes in length growth or weight gain.

We are aware of the fact that the method of protein determination from nitrogen with assumption of NPN has its limitations. Different NPN values have been reported. However, because of the substantial interindividual variability in NPN and the fact that our protein values agree with other reports we suggest that the assumed value of 20% for NPN (13) is acceptable for our population and methodology.

The Rose-Gottlieb method used for breast-milk fat determination might be a source of error in the discrepancy between the BF-FF differences found in MEI-TW and not in MEI-Pred. The Rose-Gottlieb method measures triacylglycerol and not fatty acids. Because of continuous lipase activity, true fat content might be increasingly underestimated when samples are held in storage. However, the discrepancy would be anticipated to be more at 1–2 mo and less at 2–4 mo, whereas the opposite was true. Another explanation for the increasing discrepancy between MEI-TW and MEI-Pred from 1 to 4 mo might be a difference in the amount of hind milk pumped by the mothers compared with the average amount normally suckled by the child. We asked the mothers to take the same time for expression of milk per breast as they did for letting the baby feed on each breast. Data on the total volume of milk pumped by the mothers was not recorded. We are not able to confirm that the mothers indeed pumped as much hind milk as they usually would have given to their babies. This difference might indeed become more exaggerated at 2 and 4 mo in mothers who might become more hasty and inaccurate in following the exact study protocol. Corrections for IWL during feeding time were made assuming a value for IWL of 1.8 $g \cdot kg^{-1} h^{-1}$ (9). Butte (1) recently summarized published IWL values, which averaged 1.62 ± 0.90 $g \cdot kg^{-1} \cdot h^{-1}$. The difference is too small to account for the discrepancy between MEI-Pred and MEI-TW at 1–4 mo of age.

The percentage of energy intake from fat at 12 mo of age averaged 25%, which was slightly lower than that of two Dutch food intake surveys conducted in 1986 (29) and 1992 (30). In these surveys the percentage of energy intake from fat at 12 mo of age averaged 31.7 ± 6.9% in 1986 ($n = 100$) and 29.9 ± 7.5% in 1992 ($n = 101$), respectively, which was lower than values at ages 2–5 y. At 12 mo of age, none of our study infants showed any sign of chronic nonspecific diarrhea, which has been associated with low fat intake (31). In addition to growth, which was normal according to the Dutch growth percentiles (Figure 1), psychomotor development was normal: Bayley tests on psychomotor development at 18 mo of age were performed on all study infants and no differences between sexes or feeding modes after correction for parental education were found (unpublished data).

Difference between MEI-Pred and MEI-TW

In the exclusively BF infants, MEI-TW was consistently lower than MEI-Pred. Estimation of milliliters of milk intake by test weighing equaled estimates of water intake (converted to milk intake) by the deuterium-to-infant method. This strongly suggests that it is milk energy density instead of milk intake measured by test weighing that results in underestimation of MEI. At 8 mo, test weighing and subsequent self-reporting of nutrient intake by the mothers overestimated MEI-TW in all infants. Parents might be inclined to round off (upward) their baby's food intake. At 12 mo the 3-d double-portion measurement of energy intake matched the MEI-Pred assessment of 8–12 mo well (if extrapolated to 12 mo of age) in all infants. The double-portion method might be preferred over self-reporting by parents of test-weighed food intake in older infants with mixed diets.

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Energy content of breast milk

Lucas et al (24, 32) assessed the metabolizable energy content of breast milk (MEC_{BM}) by an alternative approach with use of MEI-Pred. With use of this approach, MEC_{BM} in our study was 150.7 ± 4.8 kJ/L (630 ± 20 kcal/L) and 160.3 ± 7.2 kJ/L (670 ± 30 kcal/L) between 1–2 and 2–4 mo, respectively. At 2–4 mo MEC_{BM} was higher than breast-milk energy density as measured by test weighing and expression of breast milk (155.5, 150.7, and 143.5 kJ/L, or 650, 630, and 600 kcal/L at 1, 2, and 4 mo, respectively). Lucas et al found lower values for MEC_{BM} than we did: 136.4 ± 12 and 143.5 ± 12 kJ/L (570 ± 50 and 600 ± 50 kcal/L) at 5 and 11 wk of age, respectively, and 141.1 ± 12 kJ/L (590 ± 50 kcal/L) in an earlier study at both 4–6 and 10–12 wk of age (24, 32). Because of the errors involved in each of the various steps of this method, however, these values can be interpreted only as a rough indication of breast-milk energy content. To check the validity of this approach, the metabolizable energy content of formula ($MEC_{formula}$) may serve as an internal check. $MEC_{formula}$ calculated from MEI-Pred equaled $MEC_{formula}$ by test weighing, the latter assumed to be 95% of the laboratory determination of gross energy content of the formula samples (160.3 ± 4.8 compared with 157.9 ± 4.8 kJ/L, or 670 ± 20 compared with 660 ± 20 kcal/L, respectively). $MEC_{formula}$, as stated by the manufacturer, was 148.3 kJ/L (620 kcal/L). The difference between the manufacturer's value and our observed $MEC_{formula}$ might be due to higher powder concentrations used by the mothers. Fat determination by the manufacturer and our study was performed by equivalent laboratory procedures (personal communication from the manufacturer's research laboratory).

Growth and body composition

No consistent differences were found in growth and body composition between BF and FF infants. However, when girls were analyzed separately, BF girls differed in growth status and body composition at 4 and 8 mo of age, as a result of lower weight gain between 2 and 4 mo of age. At 2–4 mo of age, FF girls had higher weight gains, resulting in higher amounts of TBF and FFM at 4 and 8 mo of age. Although this effect was small and the physiologic significance may be questionable, it is interesting to see that in the DARLING Study (33–35) as well as in animal studies in primates (36, 26), similar feeding effects were found that were more apparent in females. Further studies should determine whether this indeed is a physiologic phenomenon.

Although not significant, we observed the same trend in weight and length *z* scores as found in a recent meta-analysis (2). Most studies in this area focused on prolonged breast-feeding. The growth data from our study were not different from those of others when restricted to ≤4 mo. A progressive increase in protein and energy intake and skinfold thickness in FF infants as compared with BF infants was found in the DARLING Study. However, as in our study, length growth was not different, whereas only a small difference in weight gain was present, predominantly in girls (33–35). Comparable differences in MEI-TW in the period when infants were exclusively BF or FF between feeding groups were found in the present study. As in the DARLING Study, in our study a steeper rise in skinfold thicknesses in FF infants compared with BF infants was found. In our study, these differences were significant at 1–4 mo, in the DARLING Study differences in skinfold thicknesses arose after 4 mo of age. These differences, however, were not found by the TOBEC body-composition measurements. This implies that although skinfold-thickness measurements in FF infants are higher than in BF infants, total body fat is not different.

As in our study, Motil et al (38) in a recently published longitudinal study found no differences in growth and body composition between BF and FF infants despite marked differences in nutrient intake. These authors explain the differences by a lower gross efficiency of nutrient utilization in FF infants than in BF infants.

Conclusions

In healthy infants exclusively BF or FF for 4 mo, significant differences in energy intake between feeding groups (determined by test weighing) were not followed by accompanying differences in energy expenditure, growth, or body composition. In a subset of infants in whom doubly labeled water measurements were performed, metabolizable energy intake predicted from the sum of energy expenditure and energy deposition (by direct TOBEC body-composition measurements) did not differ between feeding groups, but the study groups were small, especially at 8 and 12 mo. No differences in volume of milk intake (mL/d) were found between the deuterium-to-infant method and test weighing. The discrepancy between MEI-Pred and MEI-TW may be due to methodologic problems in the accuracy and reproducibility of the estimation of breast-milk energy and fat content by milk expression. The advantage of the assessment of MEI-Pred from energy expenditure and composition of weight gain is that this approach gets around such methodologic obstacles. MEI-Pred estimations can be used alternatively to assess energy requirements in BF and FF infants. The data of the present study are in line with data from other studies (1, 6, 7) and can be used for future guidelines for energy requirements in infants.

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