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Original Research Article

Metabolizable Energy Content of Breastmilk Supports Normal Growth in Exclusively Breastfed Icelandic Infants to Age 6 Months

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A B S T R A C T

Background: Neither the global population nor individual countries have reached the World Health Organization (WHO) target of ≥50% of infants exclusively breastfed (EBF) until 6 mo. This may partly be because of the perceptions of insufficient milk and energy supply to meet rapid growth and development needs.

Objectives: In a longitudinal observational study, we aimed to determine whether breastmilk energy content is sufficient to support growth during EBF until 6 mo.

Methods: A sample of 27 EBF infants was dosed with doubly labeled water (DLW) at 5.6 mo to measure body composition, breastmilk intake, energy intake, and the metabolizable energy (ME) content of their mother's breastmilk over the following week. *Z*-scores were calculated for anthropometry using WHO reference data and for fat-free mass (FFM) and fat mass (FM) using United Kingdom reference data.

Results: Anthropometric *z*-scores from birth indicated normal weight and length growth patterns. At ~6 mo, the mean ± standard deviation (SD) FFM *z*-score was 0.22 ± 1.07, and the FM *z*-score was 0.78 ± 0.70, significantly >0. In the 22 infants with acceptable data, the mean ± SD measured intake of breastmilk was 983 ± 170 g/d and of energy, 318 ± 60 kJ/kg/d, equivalent to 75.9 ± 14.3 kcal/kg/d. The mean ME content of breastmilk was 2.61 kJ/g [standard error (SE) 0.1], equivalent to 0.62 kcal/g (SE 0.02). Mothers were positive toward breastfeeding, on paid maternity leave (planned mean 10 mo), and many (56%) had received specialized breastfeeding support.

Conclusions: The evidence from this study confirms that when mothers are motivated and supported without economic restraints, breastmilk intake and the energy supplied by breastmilk to EBF infants at 6 mo of age is sufficient to support normal growth patterns. There was no evidence of constraint on FFM, and other studies show that high FM in EBF infants is likely to be transient. These data further support the recommendation for EBF ≤6 mo of age for body composition.

This trial was registered at clinicaltrials.gov as NCT02586571.

Keywords: body composition, breastfeeding, exclusive, growth, milk, human, infant, nutrition

Introduction

The WHO recommends exclusive breastfeeding (EBF) for 6 mo, that is, feeding an infant solely breastmilk and, if needed, necessary vitamins, minerals, medicines, and/or oral rehydration [1]. This recommendation is based on strong evidence relating to the optimal growth, development, and health of infants [2] and is widely endorsed and adopted by many organizations, governments, and agencies in Europe and North America [3–9]. However, globally only ~40% of infants are EBF until 6 mo of age [10], with a lower prevalence in the United States (26%) [11] and Europe (ranging from 1%–49% in individual countries with a median of 13%) [12]; far from the WHO global target of 50% at 6 mo [13]. A commonly stated reason for earlier cessation of EBF is a maternal concern for an insufficient milk supply

Abbreviations: DSR, ^{2}H:^{18}O dilution space ratio; EBF, exclusively breastfeeding; E_{growth}, energy costs of growth; rCO_2, carbon dioxide production rate; RCT, randomized controlled trial.

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[14,15], whereas scientific researchers have expressed uncertainty over whether EBF for 6 mo can adequately meet infant energy requirements to sustain optimal growth [16].

A systematic review of studies published between the late 1970s and early 2000s aimed to evaluate evidence on ME consumption in EBF infants aged 3–6 mo [17]. Most studies calculated the gross energy content of breastmilk using bomb calorimetry of breastmilk samples or by summing the energy contributions of macronutrients. A limitation of such work is that it cannot evaluate the ME content of breastmilk that is actually consumed by the infant, taking into account that some breastmilk energy is either not absorbed by the gut or used in immune function rather than energy metabolism [18,19]. The systematic review revealed a gap in the published evidence base and called for more empirical studies of the metabolizability of breastmilk and milk transfer data from 5–6 mo old EBF infants [17].

To our knowledge, only 1 longitudinal study, performed in Glasgow, UK, has investigated breastmilk energy in 5.6 mo old infants using the DLW technique [20]. This technique directly measures the ME content of breastmilk, taking into account the efficiency of energy absorption, whereas also circumventing the variability inherent in other measurement techniques related to changing breastmilk energy content and macronutrient composition within and between feedings. The Glasgow study found no evidence of insufficient milk supply among EBF infants almost 6 mo old [20]. Three randomized controlled trials (RCTs) on EBF until 4 compared with 6 mo of age have further supported the energy adequacy of EBF until 6 mo of age. Two RCTs of EBF Honduran infants reported no anthropometric differences between the groups and greater breastmilk intake in the 6-mo group [21,22]; 1 RCT in Icelandic infants reported similar results and, in addition, showed no differences in growth outcomes between the groups followed into early childhood [23,24]. The RCTs measured breastmilk intake but were not able to evaluate the ME intake of breastmilk.

However, the DLW method has only been applied to UK infants in 3 studies examining the ME content of breastmilk; at 11 and 12 wk of age [25,26] and at 5.6 mo of age [20]. Equivalent evidence is lacking from other populations, especially for infants aged 6 mo, that is, the age when there is particular interest in establishing whether EBF can support healthy growth. The present study aimed to use the DLW isotopic technique to quantify body composition, breastmilk intake, and ME intake among EBF infants of well-nourished mothers at 5.5–6 mo of age and determine the ME content of the breastmilk. The goal was to evaluate if the breastmilk supplied sufficient energy at 6 mo of age in a population characterized by high birth weight.

Methods

The sampling of biomarkers and collection of other relevant data for the present study were part of the Iceage2 study (Growth and Body Composition in Breastfed Infants: Study on Age of Introduction of Complementary Foods in Iceland). Iceage2 is a longitudinal observational study aiming to investigate breastmilk and breastfeeding characteristics that contribute to the growth and development of body composition among infants who are exclusively or partially breastfed at age 5.5–6 mo. Most infants were dosed with deuterium oxide at 5.5–6 mo to estimate body composition, whereas a subgroup of EBF infants was dosed with DLW to further quantify breastmilk intake, ME intake, and ME content of breastmilk. Figure 1 shows the flowchart of the Iceage2 study from recruitment at 5 mo until completion of the first part of the study at 6 mo. Ethical approval was obtained from the National Bioethics Committee in Iceland (VSN 13-146) and the participating healthcare centers. Parents provided written informed consent to participate. The study was registered at clinicaltrials.gov (https://clinicaltrials.gov/ct2/show/NCT02586571).

Sample size

The primary outcome measure for the present study was the ME content of breast milk. Using a weighted mean ME content of breastmilk of 2.6 kJ/g and an SD of 0.4 kJ/g (mean from all studies in a systematic review of cross-sectional data on ME consumption in 3–6

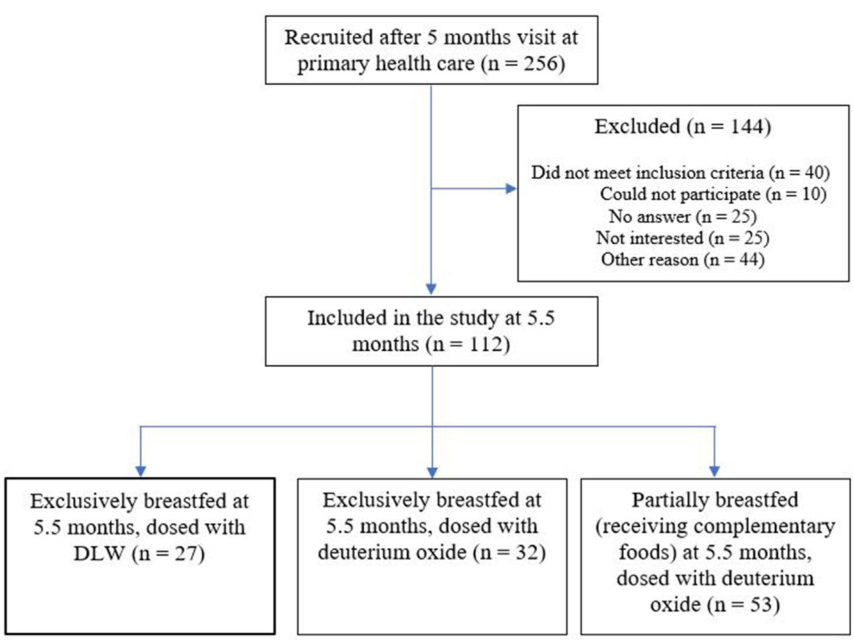

FIGURE 1. Flowchart of the IceAge2 prospective longitudinal study from recruitment at 5 mo until completion of the first part of the study at 6 mo. DLW, doubly labeled water.

mo old EBF infants from the developed world) [17], we considered a sample of 25 infants sufficient for a SE of 0.080 kJ/d and a 3.1% error on the mean value. To address potential unsuccessful measurements, 27 infants were dosed with DLW. These were selected from the total sample of 59 EBF infants by convenience sampling; that is, every second EBF infant recruited was assigned to be dosed with DLW until the target number of 27 was reached.

Recruitment

Mother-infant pairs were recruited in 5 primary healthcare centers in the Reykjavik Capital Area and neighboring municipalities Iceland from October 2014 to February 2019. During routine Well-baby clinic visits at infant age 5 mo, nurses weighed infants and screened for potential eligibility: singleton birth, gestational age 37–42 wk, birth weight >2500 g, healthy (ie, absence of congenital abnormalities or chronic health issues likely to affect growth or development), and mothers planning to continue EBF until infant age of 6 mo. The long recruitment period for the study rests on several constraints, both related to the participating mother-infant pairs (eg, eligibility criteria and burden of participation) and issues related to the health system (eg, staff). The operational definition of EBF was breastfeeding with no additional liquid or solid foods other than vitamins and medications, although up to a maximum of 5 feedings of formula or water since birth was allowed because of the practicalities of EBF; for example, neonates are at times given formula or sugar water at birth or during sickness.

If eligibility criteria were met, the mother or both parents were informed orally about the study and given study handouts. If interested in the study, the parents were recruited by giving written informed consent and permission to be contacted by the research staff at the Unit for Nutrition Research (Reykjavik, Iceland). Close to age 5.5 mo, the research staff contacted the mother/parents, confirmed that the eligibility criteria still applied (ie, the infant was still EBF, and the mother planned to continue EBF until the infant was 6 mo), and scheduled a home visit. During the initial home visit, parents received more detailed information about the study, confirmed their commitment to participation, and received equipment and instructions for taking a predose urine sample from the infant on a certain day. Only thereafter formal data collection commenced.

Isotope technique

The DLW method was used to measure milk intake, EI, and the ME content of breast milk [25]. The method has been validated against indirect calorimetry in preterm and term infants in hospital settings [27, 28]. The DLW was purchased as mixed sterilized >99.9 atom% ^2H_2O and 10.40 atom% $H_2^{18}O$ (Rotem Industries Ltd.) and dosed at 2.5 g/kg body weight. The measurement involved predose urine sampling (day 1), a dosing visit (day 0), postdose urine sampling (days 1–7), and a postdose visit (day 7) (Figure 2).

Dose administration procedure

On the dosing day (day 0), the required amount of DLW (calculated as 2.5 g/kg body weight at the 5-mo postnatal visit +1 g to account for additional growth since then) was filtered into a sterile dose bottle. After gentle mixing for 1 min, a 1 mL dose sample was collected and stored in a freezer at –80°C until analysis. At the dosing visit, the DLW was administered to the infant orally through a 10 mL syringe ($n = 22$) or, in the case of uncooperativeness or mother's request, by a combination of 10 mL syringe and infant's feeding bottle ($n = 5$) [29]. Spills were collected in tissues that were part of the dosing equipment. The amount of dose consumed was determined using preweighing and postweighing of dosing equipment on a precision scale (Mettler Toledo, model EL202; accuracy ±0.01 g). If the infant's own feeding bottle was used, it was reweighed at the laboratory. Infants were weighed naked without a diaper on an infant scale (Tanita, model 1583; accuracy ±10 g) on the dosing day (day 0) and 7 d later (day 7).

Urine sampling

Urine samples were collected by placing cotton pads in the diaper and checking every 30 min to determine the sample collection time [30]. The time of urination was taken as the midpoint between the last time it was dry and the time it was wet. The cotton pads were placed inside a syringe to express the urine into 2 mL cryogenic tubes. Two predose urine samples were collected (on days 1 and 0) and 8 postdose urine samples (5 h after dosing and thereafter daily on days 1–7) (Figure 2). The mean uncertainty in sample collection time ranged from 24 min (day 7) to 33 min (day 2), which gave a maximum error on the timing of the sample of 16.5 min and was considered satisfactory for the modeling of results. The urine samples were stored in the home refrigerator until picked up by research staff on day 0 (predose urine samples) and day 7 (postdose urine samples) and thereafter stored in a freezer at –80°C until analyzed.

Isotope analysis

Urine samples were analyzed for background abundance and isotope elimination by isotope ratio MS (Delta Plus XP; Thermofisher Scientific). Briefly, 500 μL urine samples were flush-filled for 7 min at 75 mL/min with 2% H_2 in He mixture and equilibrated for a minimum of 5 h using platinum catalyst rods (Thermo) for $^2H/^1H$ analysis and with 0.3% carbon dioxide in He for $^{18}O/^{16}O$ analysis, with a minimum of 24 h equilibration. Samples were analyzed in duplicate, with all enrichments normalized to values for international standard water samples (deltas) and the mean value used in subsequent calculations. The median difference in $^{18}O/^{16}O$ enrichment between duplicates was

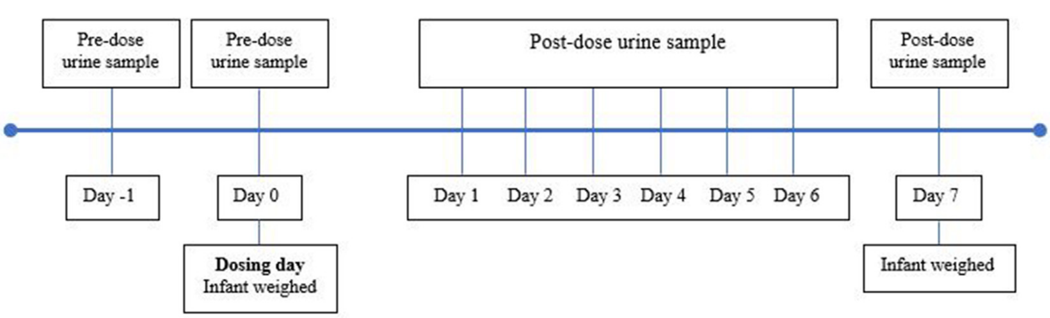

FIGURE 2. Flowchart of measurements.

0.16‰; for $^{2}H/^{1}H$, it was 1.5‰. Where the difference between duplicates was >0.5 ‰ ($^{18}O/^{16}O$) or 5.0 ‰ ($^{2}H/^{1}H$), sample analysis was repeated if sufficient urine remained.

Dilution spaces and flux rates of the ^{2}H and ^{18}O tracers were calculated to allow subsequent calculation of TEE using 2 alternative equations. The carbon dioxide production rate (rCO_2) was calculated using the equations of Speakman et al. [31], now recommended for universal use to harmonize studies. To aid comparability with the literature, we also calculated rCO_2 using the older equations of Livingstone et al., used in several previous studies of infant energetics [20, 25,32]. In each case, oxygen consumption was predicted from rCO_2 using a respiratory quotient of 0.85. The rCO_2 was converted to TEE using Weir's equation [33]. To ensure that only high-quality isotopic data were included, values for the isotope dilution space ratio (DSR) had to be within the range of 1.00–1.08.

Modeling

Breastmilk intake

Because all oral water intake was assumed to comprise breastmilk, we calculated breastmilk intake from the ^{2}H rate constant and dilution space as follows [34]:

$$\text{Breastmilk intake} = [0.937 * ((N_d * k_d)/0.99) + W_{stored}] / 0.96 \tag{1}$$

where N_d is the hydrogen dilution space, k_d is the ^{2}H rate constant, water stored (W_{stored}) was calculated from mean daily weight gain and the percentage of body water in weight on day 1, the constant of 0.96 takes into account the water content of breastmilk, and the constant of 0.937 corrects for environmental water influx.

TEE

TEE was calculated using the equation of Speakman et al. [31]. We first calculated rCO_2 from isotope rate constants and dilution spaces using the following equation:

$$rCO_2 = [0.45859 * N * (k_o - (DSR * k_d))] * 22.26 \tag{2}$$

where N is the isotope dilution space, k_0 is the ^{18}O rate constant, DSR is the ^{2}H:^{18}O DSR, and k_d is the ^{2}H rate constant. Values for DSR were predicted from infant weight using the equation of Speakman et al.

$$DSR = 1.036 - 0{:}05 * [\exp(-0.5249 * \text{weight})] \tag{3}$$

Taking an assumed value of 0.85 for the respiratory quotient, the energy equivalent of carbon dioxide ($E_{eq}CO_2$) was determined using Weir's equation (1949)

$$E_{eq}CO_{2.}\ (kJ/L) = (15.457/\ \text{respiratory quotient}) + 5.573 \tag{4}$$

Then TEE (kJ/d) is calculated from the rCO_2 as

$$TEE = rCO_2 * E_{eq}CO_2 * 22.4 \tag{5}$$

where 22.4 is the conversion factor for moles of carbon dioxide.

Body composition

Data on infant body composition (FFM and FM) at baseline (day 0, D0) were obtained based on the measurement of total body water, adjusted for the hydration fraction of fat-free tissue [35]. FM was calculated as the difference between FFM and weight.

ME intake

ME intake was calculated as the sum of TEE and the energy costs of growth (E_{growth}), calculated using a method described previously [32]. E_{growth} relates to the deposition of fat and protein, each of which has a specific energy content per gram [36].

We assumed that body composition on day 7 (D7) had the same ratio of FFM and FM as on D0, allowing us to estimate the gains in FM and FFM over the 7 d. Protein mass was calculated from FFM at both time points, taking into account age- and sex-specific values for the protein fraction of FFM [35]. Daily protein and fat gain values were calculated as day 7 values minus baseline values, divided by the 7-d measurement period. E_{growth} was calculated as the product of protein mass or FM gain and their respective energy content.

ME content

The ME content of breastmilk, expressed in kJ/100 mL of milk, was calculated as follows:

$$\text{ME content} = (\text{ME intake} / \text{breastmilk intake}) * 100 \tag{6}$$

Background characteristics

Data on maternal age, weight, education, parity, whether the parents were cohabiting/married, planned duration of maternity leave, gestational length of the infant, delivery (vaginal or cesarean), initiation of breastfeeding, specialized breastfeeding support provided by a lactation consultant or midwife (additional to the general support following birth and during routine Well-baby clinic visits) and breastfeeding attitude were gathered via questionnaire answered by the mother at infant age 5.6 mo. Data on infant weight and length at birth, 3 and 5 mo, were gathered from health records.

Statistical analyses

For descriptive analysis, data were presented as mean and SD, or *n* and percentage. Statistical analysis was done in Microsoft Excel and SPSS version 28 (IBM Corporation). Weight and length were converted to *z*-scores using the WHO Infant Growth Standards [37]. To evaluate body composition outcomes, data were converted to *z*-scores using published UK reference data obtained using the same stable isotope method [38]. Paired t-tests were used to evaluate whether mean *z*-scores differed from 0.

To compare the TEE calculated by the equations of Speakman (now recommended) compared with those of Livingstone and Coward used in previous isotopic studies of breastmilk energetics [20,25,32], we calculated the mean difference between the equations and their 95% CIs, as well as the correlation between mean and difference, using the method of Bland and Altman [39].

Results

Characteristics of the 27 infants (18 girls and 9 boys) dosed with DLW and their mothers are shown in Table 1. Most mothers initiated breastfeeding within the first hour of birth (93%), had received specialized breastfeeding support in the months following birth (56%), and had a positive attitude toward breastfeeding. The planned duration of maternity leave was until the mean infant age of 10 mo (SD 2). The infants showed normal growth relative to the WHO Child Growth Standards; that is, all measured length and weight values were within 2 SD of the mean. Mean weight and length at birth were 3.73 kg (SD 0.49) and 51.1 cm (SD 2.2), whereas mean *z*-scores for weight, length, and BMI (kg/m^2) at 5 mo were 0.73 (SD 0.88), 1.04 (SD 0.82) and 0.06 (SD 0.89), respectively.

TABLE 1
Characteristics and growth of participants included in the current analysis (n = 27)

	Mean ± SD
At birth	
Gestational length (wk)	40.4 ± 1.1
Birth weight z-score[2]	0.92 ± 1.00
Birth length z-score[2]	0.90 ± 1.16
At 3 mo	
Weight-for-age z-score[1]	0.46 ± 1.12
Length-for-age z-score[1]	1.15 ± 0.72
BMI-for-age z-score[1]	−0.01 ± 0.89
Weight-for-length z-score[1]	−0.17 ± 0.89
At 5 mo	
Weight-for-age z-score[1]	0.73 ± 0.88
Length-for-age z-score[1]	1.04 ± 0.82
BMI-for-age z-score[1]	0.06 ± 0.89
Weight-for-length z-score[1]	0.10 ± 0.89
Mothers	
Age (y)	30.4 ± 4.1
Weight (kg)	69.3 ± 13.1
BMI (kg/m^2)	24.3 ± 4.3
Parity	
Primiparous	9 (33)[2]
Multiparous	18 (67)[2]
Completed university degree (BSc./BA/BEd., or higher)	21 (78)[2]
Cohabiting with other parents	27 (100)[2]
Vaginal delivery	23 (85)[2]

BMI, body mass index; SD, standard deviation; WHO, World Health Organization.
[1] Relative to the WHO Child Growth Standards.
[2] Data presented as n (%).

At the time of DLW dosing, the mean infant age was 24.5 wk (SD 0.6) or 5.6 mo. Samples from all 27 infants gathered over the following week were analyzed. We rejected isotope data from 3 infants for being outside the defined acceptable DSR range (Supplementary Figure 1). After calculating the ME content of breastmilk for the remaining 24 infants, an inspection of the values suggested 2 biologically implausible values with high estimates (Figure 3). Excluding those 2 values resulted in a ME content with SD more in line with published literature (Supplementary Figure 2). Therefore, 22 infants remained with successful and plausible analyses.

Energy balance variables and values for absolute body composition outcomes in kg and z-scores at ~6 mo in the 24 infants within the acceptable DSR range and the 22 infants with successful and plausible analyses are given in Table 2. In the 22 infants with acceptable data, the mean FFM z-score was 0.22 (SD 1.07), not significantly different from 0, whereas the mean FM z-score was 0.78 (SD 0.70), significantly >0. Very similar mean z-scores were obtained if the 2 discarded infants were included. Values for breastmilk intake, ME intake, and ME content of breastmilk for the 22 infants at ~6 mo are shown in Table 3. The mean ME intake of 317.5 kJ/kg/d is equivalent to 75.9 kcal/kg/d, and the mean ME content of 2.61 kJ/g (SE 0.1) is equivalent to 0.62 kcal/g (SE 0.02).

Supplementary Figure 3 presents the Bland Altman plot to compare the Speakman and Livingstone calculation methods. The mean bias was –16 kJ (P = 0.14), and the limits of agreement were –475 and 443 kJ. There was no significant correlation between the difference and the mean TEE (r = 0.07; P = 0.7).

Discussion

To our knowledge, this study is the second [20] to use the state-of-the-art DLW technique to investigate breastmilk intake, ME intake, and ME content in infants aged ~6 mo who were EBF from birth to this age. This population is of particular interest, given scientific discussion and the concerns of individual mothers over how long EBF can support healthy infant growth.

The infants in this study showed normal growth relative to the WHO Child Growth Standards, with weight-for-age and length-for-age z-scores close to 1 in the first 6 mo of life and BMI-for-age z-scores close to 0. This is similar to what we previously reported among EBF

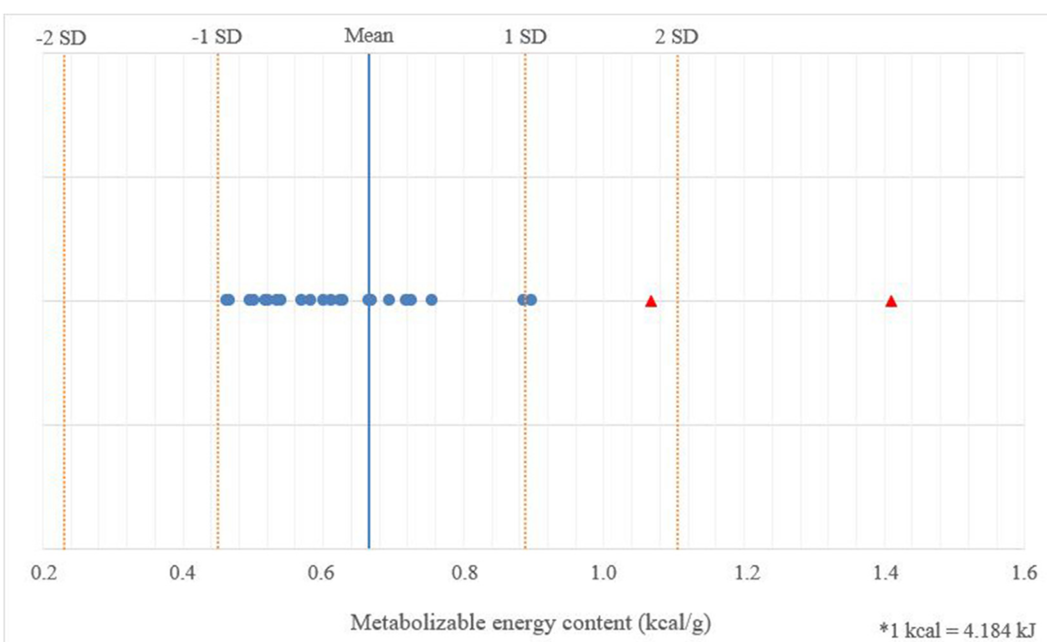

FIGURE 3. ME content of breastmilk (n = 24). Values identified as implausible outliers based on a review of the literature are depicted as triangles, others as circles. ME, metabolizable energy; SD, standard deviation.

TABLE 2
Energy balance variables and breastmilk intake at ~6 mo

	Participants with high-quality isotopic data (n = 24) Mean ± SD	Participants with high-quality and biologically plausible isotopic data (n = 22) Mean ± SD
At the time of DLW dosing		
Age (wk)	24.5 ± 0.6	24.5 ± 0.6
Weight-for-age z-score[1]	0.70 ± 0.96	0.68 ± 1.00
Weight (kg)	8.05 ± 0.92	8.03 ± 0.94
Total body water baseline (kg)	4.30 ± 0.53	4.31 ± 0.56
FFM baseline (kg)	5.41 ± 0.67	5.42 ± 0.70
FFM baseline, z-score[1]	0.20 ± 1.03	0.22 ± 1.07
FM baseline (kg)	2.65 ± 0.59	2.61 ± 0.59
FM baseline, z-score[1]	0.83 ± 0.72[2]	0.78 ± 0.70[2]
Gain for 7 d following DLW dosing		
Weight gain (g/d)	19.8 ± 13.8	19.4 ± 14.0
FFM gain (g/d)	12.5 ± 9.3	12.3 ± 9.6
Protein gain (g/d)	2.3 ± 1.5	2.3 ± 1.6
FM gain (g/d)	7.2 ± 4.7	7.0 ± 4.7
Protein stored (kJ/d)	54.9 ± 35.8	54.3 ± 36.9
Protein stored (kcal/d)	13.1 ± 8.5	13.0 ± 8.8
Fat stored (kJ/d)	280.4 ± 183.4	271.2 ± 182.3
Fat stored (kcal/d)	67.0 ± 43.8	64.8 ± 43.6
Energy stored (kJ/d)	335.2 ± 216.4	325.5 ± 216.5
Energy stored (kcal/d)	80.1 ± 51.7	77.8 ± 51.7
TEE (kJ/d)	2330.1 ± 558.3	2214.2 ± 414.8
TEE (kcal/d)	556.9 ± 133.4	529.2 ± 99.1
Dilution space ratio	1.030 ± 0.02	1.030 ± 0.02

DLW, doubly labeled water; FFM, fat-free mass; FM, fat mass; SD, standard deviation; TEE, total energy expenditure.
[1] Relative to published UK reference data obtained using the same stable isotope method.
[2] Different from 0 $P > 0.05$ according to paired t-tests.

infants from the same population [24]. Compared with UK reference data, the sample showed normal FFM but elevated FM, indicating no constraint of fat-free tissue accretion in the first 6 mo but higher concentrations of fat deposition. However, systematic reviews have shown that high body fat concentrations in EBF infants appear to be transient [40] and have not been associated with long-term outcomes such as obesity or noncommunicable disease [41].

The ME content of breastmilk was 2.61 kJ/g (SE 0.1) or 0.62 kcal/g at ~6 mo, which is comparable to a UK study investigating breastmilk energy content for EBF infants at 5.6 mo using the same isotopic method (2.60 kJ/g) [20]. Our findings are also consistent with a systematic review that included studies published up to 2002 on EBF infants aged 3–6 mo from the developed world (2.6 kJ/g) [17], as well as a more recent study from Poland on EBF aged 6 mo (2.56 kJ/g) [42]. Although milk energy content was determined using isotopic methods in the current and UK studies, all other studies cited in the systematic review and the Polish study used bomb calorimetry, or direct measurements of macronutrient content, to evaluate breastmilk energy content and thus did not analyze the breastmilk actually consumed by the infant. This issue is important, as the degree of gross energy absorption by breasted infants is difficult to assess, whereas the energy content of breastmilk is known to vary substantially within and between feeds in any given infant [43–46].

Breastmilk intake in our population at ~6 mo (983 g/d) was comparable to an EBF UK population of the same age using the same isotopic method (999 g/d), and the ME intake from breastmilk (2540 kJ/d or 607 kcal/d) showed similar comparability (UK study: 2577 kJ/d) [20]. The UK study showed higher breastmilk and EIs in boys than girls [20], which may contribute to the small observed difference between the studies, as we had more girls than boys. Both studies report higher breastmilk intake values than a previous Icelandic study using the deuterium-dose-to-the-mother method (901 g/d for 6 mo old EBF infants) [23] and literature values (894 g/d at 6 mo) [17]. As previously mentioned, the literature values are largely based on the test-weighing method, which may be prone to imprecision in situations of frequent feedings and involves a risk of underreporting, which isotopic measures avoid [17]. A mean difference of 66 g/d (95% CI: 11, 123 g/d) in milk intake between the test-weighing method and isotopic methods has been estimated [17]. Our value for ME intake per kg body weight (317.5 kJ/kg/d or 75.9 kcal/kg/d) was not far from the WHO reference value of an energy requirement of 328 kJ/kg/d for 6 mo old breastfed infants[47].

In isotopic measurements, the DSR has a major impact on calculating rCO_2 and TEE. In children below 2 y of age, an inappropriate DSR value may lead to an underestimation of rCO_2 and TEE. A recent review reported a new estimate of the mean DSR (1.031) at younger ages [31]. To further evaluate the accuracy, Speakman et al. [31] plotted the DSR for individuals ranging in size range from 2.4–10 kg against body weight and showed a positive association between weight and DSR. This led to a new equation that combines the body-weight dependency of the DSR with the standard DLW equation. When the 3 outliers were excluded from our data analysis, the mean DSR was 1.030, which is consistent with Speakmans' theory. The rCO_2 values calculated using the Speakman and Livingstone equations showed a trivial mean difference of 16 kJ, not significantly different from 0, although the limits of agreement in individuals were relatively wide (−475 to 443 kJ). This indicates that the methods are comparable at the group level, which is most important for this study, but do not give comparable individual results.

The study's main strength is using a state-of-the-art isotopic technique to investigate breastmilk intake, ME intake, and ME content in a population of infants nearing 6 mo, the age when concern over the energy adequacy of EBF is greatest. As for representativeness of the sample population, mothers participating in the present study were relatively affluent, with a high level of university education, cohabiting with the other parent, a long paid maternal leave, and over half had received specialized breastfeeding support. They had chosen to EBF their infants until 6 mo and had a positive attitude toward breastfeeding. Whether the results of this study extend to different settings, eg, low-income countries, is not known, but the study could build a knowledge base preparing for work in such settings. A study in Brazil reported higher levels of infant TEE in infants of lower compared with

TABLE 3
Milk intake, EI, and ME content of breastmilk at ~6 mo (n = 22)

	Mean ± SD	95% CI
Breastmilk intake (g/d)	982.5 ± 169.7	907.3, 1057.8
Breastmilk intake (g/kg/d)	122.6 ± 17.6	114.8, 130.4
ME intake (kJ/d)	2539.7 ± 537.6	2301.4, 2778.1
ME intake (kcal/d)	607.0 ± 128.5	550.0, 664.0
ME intake (kJ/kg/d)	317.5 ± 59.6	291.1, 344.0
ME intake (kcal/kg/d)	75.9 ± 14.3	69.6, 82.2
ME content (kJ/g)	2.61 ± 0.48	2.40, 2.82
ME content (kcal/g)	0.62 ± 0.11	0.57, 0.68

CI, confidence interval; EI, energy intake; ME, metabolizable energy; SD, standard deviation.

higher maternal socioeconomic status [48], but whether this scenario extends to the ME content of breastmilk is unknown.

Among the study's limitations is the small sample size, although the SE of breastmilk energy content was relatively low. The reported EBF status of infants cannot be validated in the DLW technique, but participants were well aware of the importance of continued EBF until the end of urine sampling. There were 3 failed dosings, although these are expected in this age group and population, and 2 implausible values for ME content of breastmilk, which are likely to indicate an undetected problem either with the dosing or the mass-spectrometric analysis. These values were, however, sufficiently extreme outliers relative to all previous research on this topic to justify their exclusion. The study is underpowered to detect differences between girls and boys and did not measure the adequacy of all aspects of EBF to 6 mo, such as the effect on iron. An earlier RCT in Iceland found higher SF concentrations at 6 mo among infants who received small amounts of complementary foods in addition to breastmilk from 4 mo of age as compared with infants EBF until 6 mo, but no difference between groups in iron deficiency, IDA or iron depletion [49]. Finally, the isotopic method requires modeling for outcome variables using equations and constants that may be subject to some sources of error.

In conclusion, even in affluent countries with good support systems to encourage breastfeeding, EBF until 6 mo is uncommon. Many factors contribute to the earlier cessation of breastfeeding, but the perception of insufficient milk and energy supply to the infant remains an important factor. When mothers are motivated and supported to follow the WHO recommendation, milk (close to 1000 g/d) and EIs (317.5 kJ/kg/d or 75.9 kcal/kg/d in our population) are sufficient and appear to support growth requirements in infants showing normal growth. The ME content of breastmilk was 2.61 kJ/g or 0.62 kcal/g at ~6 mo, thereby strengthening the validity of the values shown in other studies using the same or other techniques. The evidence provided by this study should be helpful in the promotion of a greater duration of EBF and contribute toward achieving the global nutrition targets in 2025.

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Author contributions

The authors' responsibilities were as follows – BT, GG, IT, MSF, JCKW, and REK: designed the research; BT and TO: conducted the research; REK, SE, and JCKW: provided the essential materials; SE and AV-V: analyzed the data; BT, TO, and JCKW: performed the statistical analysis; BT, TO, JCKW: wrote the paper; BT: had the primary responsibility for the final content and all authors: read and approved the final manuscript.

Conflict of interest

MSF has received an unrestricted donation for infant nutrition research from Philips, unrelated to this project. All other authors report no conflicts of interest.

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Data availability

Data described in the manuscript, code book, and analytic code will not be made available because it is part of an ongoing study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2023.06.005.

References

[1] World Health Organization, Infant and young child nutrition. 55th World Health Assembly, World Health Organization, Geneva, Switzerland, 2002.
[2] N.F. Butte, M.G. Lopez-Alarcon, C. Garza, Expert consultation on the optimal duration of exclusive breastfeeding. Nutrient adequacy of exclusive breastfeeding for the term infant during the first six months of life / Nancy F. Butte: Mardia G. Lopez-Alarcón: Cutberto Garza, World Health Organization, Geneva, Switzerland, 2002.
[3] Nordic Council of Ministers, Nordic nutrition recommendations 2012. Integrating nutrition and physical activity, 2012. Copenhagen, Denmark.
[4] EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), J. Castenmiller, S. de Henauw, K.I. Hirsch-Ernst, J. Kearney, H.K. Knutsen, et al., Appropriate age range for introduction of complementary feeding into an infant's diet, EFSA J 17 (9) (2019), e05780.
[5] M. Fewtrell, J. Bronsky, C. Campoy, M. Domellöf, N. Embleton, N. Fidler Mis, et al., Complementary feeding: a position paper by the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) committee on nutrition, J. Pediatr. Gastroenterol. Nutr. 64 (1) (2017) 119–132.
[6] J.Y. Meek, L. Noble, Section on Breastfeeding [policy statement]. Policy statement: breastfeeding and the use of human milk, Pediatrics 150 (1) (2022), e2022057988.
[7] ACOG Committee Opinion No, 756: optimizing support for breastfeeding as part of obstetric practice, Obstet, Gynecol 132 (4) (2018) e187–e196.
[8] American Academy of Family Physicians, Breastfeeding, family physicians supporting (position paper), 2023. Available from: https://www.aafp.org/about/policies/all/breastfeeding-position-paper.html.
[9] J.N. Critch, Canadian Paediatric Society, Nutrition and Gastroenterology Committee. Nutrition for healthy term infants, six to 24 months: an overview, Paediatr. Child Health. 19 (10) (2014) 547–552.
[10] World Health Organization, United Nations Children's Fund (UNICEF), Global breastfeeding scorecard 2021: protecting breastfeeding through bold national actions during the COVID-19 pandemic and beyond, 2021. Available from, https://www.who.int/publications/i/item/WHO-HEP-NFS-21.45.
[11] R.L. Bailey, J.S. Stang, T.A. Davis, T.S. Naimi, B.O. Schneeman, K.G. Dewey, et al., Dietary and complementary feeding practices of US infants, 6 to 12 months: A narrative review of the federal nutrition monitoring data, J. Acad. Nutr. Diet. 122 (12) (2022) 2337–2345.e1.
[12] A.T. Bagci Bosi, K.G. Eriksen, T. Sobko, T.M. Wijnhoven, J. Breda, Breastfeeding practices and policies in WHO European Region Member States, Public Health Nutr 19 (4) (2016) 753–764.
[13] World Health Organization, Global targets 2025: to improve maternal, infant and young child nutrition, 2012. Available from, https://www.who.int/teams/nutrition-and-food-safety/global-targets-2025.
[14] Q. Wu, N. Tang, C. Wacharasin, Factors influencing exclusive breastfeeding for 6 months postpartum: a systematic review, Int. J. Nurs. Knowl. 33 (4) (2022) 290–303.
[15] L. Gatti, Maternal perceptions of insufficient milk supply in breastfeeding, J. Nurs. Scholarsh. 40 (4) (2008) 355–363.

[16] J.J. Reilly, J.C. Wells, Duration of exclusive breast-feeding: introduction of complementary feeding may be necessary before 6 months of age, Br. J. Nutr. 94 (6) (2005) 869–872.

[17] J.J. Reilly, S. Ashworth, J.C. Wells, Metabolisable energy consumption in the exclusively breast-fed infant aged 3-6 months from the developed world: a systematic review, Br. J. Nutr. 94 (1) (2005) 56–63.

[18] T.R. Fenton, S. Elmrayed, The importance of reporting energy values of human milk as metabolizable energy, Front Nutr 8 (2021) 655026.

[19] B. Lönnerdal, Bioactive proteins in breast milk, J. Paediatr. Child Health. 49 (Suppl 1) (2013) 1–7.

[20] S.B. Nielsen, J.J. Reilly, M.S. Fewtrell, S. Eaton, J. Grinham, J.C. Wells, Adequacy of milk intake during exclusive breastfeeding: a longitudinal study, Pediatrics 128 (4) (2011) e907–e914.

[21] R.J. Cohen, K.H. Brown, J. Canahuati, L.L. Rivera, K.G. Dewey, Effects of age of introduction of complementary foods on infant breast milk intake, total energy intake, and growth: a randomised intervention study in Honduras, Lancet 344 (8918) (1994) 288–293.

[22] K.G. Dewey, R.J. Cohen, K.H. Brown, L.L. Rivera, Age of introduction of complementary foods and growth of term, low-birth-weight, breast-fed infants: a randomized intervention study in Honduras, Am. J. Clin. Nutr. 69 (4) (1999) 679–686.

[23] J.C. Wells, O.H. Jonsdottir, P.L. Hibberd, M.S. Fewtrell, I. Thorsdottir, S. Eaton, et al., Randomized controlled trial of 4 compared with 6 mo of exclusive breastfeeding in Iceland: differences in breast-milk intake by stable-isotope probe, Am. J. Clin. Nutr. 96 (1) (2012) 73–79.

[24] O.H. Jonsdottir, R.E. Kleinman, J.C. Wells, M.S. Fewtrell, P.L. Hibberd, G. Gunnlaugsson, et al., Exclusive breastfeeding for 4 versus 6 months and growth in early childhood, Acta. Paediatr. 103 (1) (2014) 105–111.

[25] A. Lucas, G. Ewing, S.B. Roberts, W.A. Coward, How much energy does the breast fed infant consume and expend? Br. Med. J. (Clin. Res. Ed.). 295 (6590) (1987) 75–77.

[26] J.C. Wells, Energy metabolism in breastfed and formula-fed infants [PhD thesis], University of Cambridge, 1994.

[27] S.B. Roberts, W.A. Coward, K.H. Schlingenseipen, V. Nohria, A. Lucas, Comparison of the doubly labeled water (2H2(18)O) method with indirect calorimetry and a nutrient-balance study for simultaneous determination of energy expenditure, water intake, and metabolizable energy intake in preterm infants, Am. J. Clin. Nutr. 44 (3) (1986) 315–322.

[28] P.J. Jones, A.L. Winthrop, D.A. Schoeller, P.R. Swyer, J. Smith, R.M. Filler, et al., Validation of doubly labeled water for assessing energy expenditure in infants, Pediatr. Res. 21 (3) (1987) 242–246.

[29] S.B. Nielsen, J.C. Wells, C. Slater, M.S. Fewtrell, J.J. Reilly, Administering labelled water to exclusively breast-fed infants in studies involving stable isotope dilution techniques, Isotopes Environ, Health Stud 47 (1) (2011) 18–25.

[30] S.B. Roberts, A. Lucas, Measurement of urinary constituents and output using disposable napkins, Arch. Dis. Child. 60 (11) (1985) 1021–1024.

[31] J.R. Speakman, Y. Yamada, H. Sagayama, E.S.F. Berman, P.N. Ainslie, L.F. Andersen, et al., A standard calculation methodology for human doubly labeled water studies, Cell Rep. Med. 2 (2) (2021) 100203.

[32] J.C. Wells, P.S. Davies, Estimation of the energy cost of physical activity in infancy, Arch. Dis. Child. 78 (2) (1998) 131–136.

[33] J.B. Weir, New methods for calculating metabolic rate with special reference to protein metabolism, J. Physiol. 109 (1-2) (1949) 1–9.

[34] J.C. Wells, P.S. Davies, Correction for environmental water influx in measurement of milk volume intake by deuterium turnover in infants, Early Hum. Dev. 41 (3) (1995) 177–182.

[35] S.J. Fomon, F. Haschke, E.E. Ziegler, S.E. Nelson, Body composition of reference children from birth to age 10 years, Am. J. Clin. Nutr. 35 (5) (1982) 1169–1175.

[36] S.B. Roberts, V.R. Young, Energy costs of fat and protein deposition in the human infant, Am. J. Clin. Nutr. 48 (4) (1988) 951–955.

[37] WHO Multicentre Growth Reference Study Group, WHO Child Growth Standards based on length/height, weight and age, Acta Paediatr Suppl 450 (2006) 76–85.

[38] J.C.K. Wells, P.S.W. Davies, M.S. Fewtrell, T.J. Cole, Body composition reference charts for UK infants and children aged 6 weeks to 5 years based on measurement of total body water by isotope dilution, Eur. J. Clin. Nutr. 74 (1) (2020) 141–148.

[39] J.M. Martin Bland, D.G. Altman, Statistical methods for assessing agreement between two methods of clinical measurement, Lancet 327 (8476) (1986) 307–310.

[40] C. Gale, K.M. Logan, S. Santhakumaran, J.R. Parkinson, M.J. Hyde, N. Modi, Effect of breastfeeding compared with formula feeding on infant body composition: a systematic review and meta-analysis, Am. J. Clin. Nutr. 95 (3) (2012) 656–669.

[41] B.L. Horta, C. Loret de Mola, C.G. Victora, Long-term consequences of breastfeeding on cholesterol, obesity, systolic blood pressure and type 2 diabetes: a systematic review and meta-analysis, Acta. Paediatr. 104 (467) (2015) 30–37.

[42] A. Bzikowska-Jura, A. Czerwonogrodzka-Senczyna, G. Olędzka, D. Szostak-Węgierek, H. Weker, A. Wesołowska, Maternal nutrition and body composition during breastfeeding: association with human milk composition, Nutrients 10 (10) (2018).

[43] O. Ballard, A.L. Morrow, Human milk composition: nutrients and bioactive factors, Pediatr. Clin. North. Am. 60 (1) (2013) 49–74.

[44] S. Khan, A.R. Hepworth, D.K. Prime, C.T. Lai, N.J. Trengove, P.E. Hartmann, Variation in fat, lactose, and protein composition in breast milk over 24 hours: associations with infant feeding patterns, J. Hum. Lact. 29 (1) (2013) 81–89.

[45] B. Kociszewska-Najman, B. Borek-Dzieciol, M. Szpotanska-Sikorska, E. Wilkos, B. Pietrzak, M. Wielgos, The creamatocrit, fat and energy concentration in human milk produced by mothers of preterm and term infants, J. Matern. Fetal. Neonatal. Med. 25 (9) (2012) 1599–1602.

[46] E. Ward, N. Yang, B.S. Muhlhausler, G.E. Leghi, M.J. Netting, M.J. Elmes, et al., Acute changes to breast milk composition following consumption of high-fat and high-sugar meals, Matern. Child Nutr. 17 (3) (2021), e13168.

[47] N.F. Butte, Energy requirements of infants, Public Health Nutr 8 (7A) (2005) 953–967.

[48] H. Haisma, W.A. Coward, G.H. Visser, R. Vonk, J.C. Wells, A. Wright, et al., Socio-economic and environmental factors influence energy utilization in Brazilian breast-fed infants, J. Nutr. 136 (11) (2006) 2945–2951.

[49] O.H. Jonsdottir, I. Thorsdottir, P.L. Hibberd, M.S. Fewtrell, J.C. Wells, G.I. Palsson, et al., Timing of the introduction of complementary foods in infancy: a randomized controlled trial, Pediatrics 130 (6) (2012) 1038–1045.