Validation of energy requirement equations for estimation of breast milk consumption in infants

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Abstract

Objective: To test equations for calculating infants' energy requirements as a simple and reliable instrument for estimating the amount of breast milk consumed in epidemiological studies where test-weighing is not possible.

Design: Infants' energy requirements were calculated using three different equations based on reference data and compared with actual energy intakes assessed using the 3 d weighed dietary records of breast-fed infants from the DOrtmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study.

Setting: A sub-sample of 323 infants from the German DONALD Study who were predominantly breast-fed for at least the first four months of life, and who had 3 d weighed dietary records and repeated body weight measurements within the first year of life.

Subjects: Healthy, term infants breast-fed for at least 4 months, 0–12 months of age. *Results:* The overall differences between measured energy intake and calculated energy requirements were quite small, never more than 10% of total energy intake, and smaller than the mean variance of energy intake between the three days of recording. The equation of best fit incorporated body weight and recent growth, while the worst fit was found for the equation not considering body weight.

Conclusions: Breast milk consumption in fully and partially breast-fed infants can be reasonably quantified by calculating the infants' individual energy requirements via simple equations. This provides a feasible approach for estimating infant energy intake in epidemiological studies where test-weighing of breast milk is not possible.

Keywords Energy intake Energy requirements Infants Breast milk

Data on the amount of breast milk consumed by exclusively as well as partially breast-fed infants are mandatory for various epidemiological purposes. For instance, such data are required for evaluating the intake of total energy and specific nutrients in infants^(1,2), for investigating the association between breast milk intake and growth^(3,4) and for examining potential factors which influence breast milk consumption^(5,6). Various methods exist to estimate breast milk consumption including measurement of dose-to-the-mother ²H₂O turnover⁽¹⁾, the test-weighing method^(3,4) (where the infant is weighed before and after each breast milk feeding for 24 h), assumption of a specific breast milk volume depending on the feeding duration and age of the baby $^{(5,7)}$, and the assumption of a single volume of breast milk (e.g. 750 ml/d) irrespective of the age of the breast-fed infant^(2,8).

Each of these methods has specific advantages and disadvantages⁽⁹⁾. For example, dose-to-the-mother ${}^{2}H_{2}O$ turnover and test-weighing are reportedly the most accurate methods⁽⁹⁾ but are not feasible in large epidemiological studies due to the high costs, requirement for personnel and logistics involved. On the other hand, less complex and

cheaper methods, such as assuming a single volume of breast milk, do not take into account age, sex, weight, growth or the amount of complementary food consumed in addition to breast milk. Thus, there is a need for a simple and reliable method to estimate breast milk consumption.

It has been shown that, in infants, energy intake (EI) is self-regulated by individual energy requirements $(ER)^{(6,10)}$. This leads to the assumptions that: (i) exclusively breastfed infants consume as much energy via breast milk as determined by their ER; and (ii) partially breast-fed infants consume as much energy via breast milk as determined by the difference between their ER and the EI from foods other than breast milk, e.g. complementary food or formula. Taking self-regulation of EI for granted, data on the measured intake of breast milk, as well as on the intake of all food other than breast milk, are required for healthy, normally growing infants. It would then be possible to calculate their total EI for comparison with their ER. Fortunately, well-documented reference data on the ER (RER) of breast-fed infants are available^(11,12) and provide three basic equations for adapting these data to other populations.

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Data on dietary intake including breast milk are available from 3 d weighed dietary records collected as part of the DOrtmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study^(13,14). Thus, the aim of the present study was to compare the measured EI of breast-fed infants from the DONALD Study with the RER, and to identify which of the three equations best predicts the individual ER. The ultimate objective was to find a simple and reliable instrument for estimating the amount of breast milk consumed in epidemiological studies where test-weighing is not possible.

Methods

Study population

The DONALD Study is an ongoing (open) cohort study that collects detailed data on diet, metabolism, growth and development from healthy subjects, from the age of 3 months until 18 years. Data are collected quarterly for each subject (3, 6, 9 and 12 months) in the first year of life, twice in the second year and annually thereafter.

The study started in 1985 with participants of all age groups. Since 1989 approximately forty new infants enter the study each year. Although the DONALD Study sample is biased in having a relatively high socio-economic status, the BMI distribution of the study sample deviates only slightly from the German references^(14,15). Likewise, the breast-feeding rates and complementary feeding practices are similar to a nationwide breast-feeding survey⁽¹⁶⁾. Additional details about the DONALD Study have been published elsewhere^(13,14).

For the present analysis we selected a sub-sample of term (gestational age >37 weeks), healthy infants who were 'predominantly' breast-fed as defined by the WHO⁽¹⁷⁾ (breast milk only, but water, carbohydrate solutions, fruit juices and medicines are allowed) during the first four months (16 weeks) of life, i.e. the same inclusion criteria as those of the sample used for the RER⁽¹²⁾ calculation.

Dietary assessment

Dietary intake in the DONALD Study was assessed by 3 d weighed dietary records collected on three consecutive days. To measure breast milk intake the mothers were supplied with a baby scale (± 10 g, Soehnle Multina 8300) and instructed by trained dietitians to weigh the child before and after each breast-feed (test-weighing). To correct for the systematic error in test-weighing due to insensible water loss, 5% was added to the test-weighing results⁽¹⁸⁾. Parents were also asked to weigh all solid foods and fluid feeds, such as formula, tea and juices, as well as leftovers, using an electronic food scale (± 1 g, WEDO Digi 2000; Werner Dorsch GmbH, Muenster/Dieburg, Germany, since 2002 also Soehnle Digita 8000; Leifheit SG, Nassau, Germany). Information on recipes

for the preparation of formula (milk powder and water separately) and complementary food (e.g. cereals and fruits separately) was requested. For commercial products labelling details on wrappers, cartons or banderols were also requested. Similarly, details were also collected from labels for medicines and supplements consumed. These were evaluated with the dietary records. Semi-quantitative recording, e.g. numbers of cups and spoons, was allowed if weighing was not possible.

EI from dietary records was calculated using the in-house nutrient database LEBTAB, which is based on the German standard food tables for common foods including mature human milk. LEBTAB also contains detailed data on ingredients, energy and nutrients generated by simulation of recipes of commercial foods and is continuously updated⁽¹⁹⁾.

Anthropometric data

Anthropometric measurements were performed within 14 d before or after the dietary record by trained nurses according to standardized procedures. Body weight was assessed unclothed using an electronic scale (± 100 g; Mettler PS 15; Mettler Toledo, Columbus, OH, USA). Daily weight gain during defined age intervals analogous to the reference data (in months), i.e. 0–3, 3–6, 6–9 and 9–12^(12,20), was calculated as the difference in weight between two consecutive measurements (birth weight in the case of the 3-month examination) divided by the number of days between the measurements.

Statistical analysis

Records from infants whose weight decreased between two consecutive measurements and implausible records, as determined by the ratio of reported EI to estimated $BMR^{(21,22)}$, were excluded. This selection resulted in a sample of 323 children (165 boys, 158 girls), providing 758 3 d dietary records (379 from boys, 379 from girls). In 89.8% of these records more than 90% of recorded food items were weighed, while 10.2% of the records consisted of 50–90% weighed food items. EI (total, breast milk, formula, other food) per day was calculated as the individual mean of the three recording days.

ER was calculated for boys and girls separately, at the time of measurement. Three different equations derived from RER⁽¹²⁾ were used:

- Equation_1: adoption of the RER stated as 'kJ/d' (according to age and sex)⁽¹²⁾.
- Equation_2: product of individual measured body weight and RER stated as 'kJ/kg body weight per d' (according to age and sex)⁽¹²⁾.
- **3.** Equation_3: sum of individual calculated total energy expenditure (TEE) and energy cost of growth (ECG) assuming published values^(12,20), where

TEE $(kJ/d) = 92.8 \times weight (kg) - 152$

and

- ECG (kJ/d) during a 3-month interval
 - = individual weight gain (g/d)
 - \times energy cost of tissuedeposition (kJ/g).

To evaluate growth characteristics, mean body weight was compared with the reference body weight⁽²³⁾ taken from the 50th percentile of the WHO pooled breast-fed data set that was used for calculation of the RER⁽¹²⁾.

Infants in this sample were subdivided into four age categories defined as 3 (± 0.5), 6 (± 0.5), 9 (± 0.5) and 12 (± 0.5) months of age, in accordance with the reference data^(12,20). To investigate the difference between the results from the three equations and EI in each of the four age groups, a paired *t* test (PROC MEANS) was used. To graphically assess the agreement of the three equations with EI, Bland–Altman plots were used. The ratio of ER from each equation to EI was calculated for each subject, as were the mean and standard deviation for the total sample^(24,25). The Statistical Analysis System (SAS) statistical software package version 8.02 (SAS Institute, Cary, NC, USA) was used for data analysis. The level of significance was set at *P* < 0.05.

Ethical considerations

The DONALD Study is purely observational and noninvasive and has been approved by the ethical committee of the Rheinische Friedrich-Wilhelms-University, Bonn, Germany. All examinations and assessments were performed with parental consent and later on with the children's consent.

Results

Growth characteristics of the DONALD sample were very close to the WHO reference population (Fig. 1, Table 1). EI adjusted for body weight (kJ/kg per d) in the DONALD sample decreased during the first half-year of life and tended to plateau afterwards, similar to the RER (Fig. 2)⁽¹²⁾.

The proportion of breast-fed infants decreased during the second six months of life from 86% at the age of 6 months to 17% at 12 months (Table 2). Accordingly, the proportions of breast milk, formula and complementary food contributing to total EI changed with increasing age (Fig. 3, Table 3). Overall EI (kJ/d) increased steadily from 3 to 12 months. EI from assumed fixed volumes of breast milk, as suggested in the literature^(2,8), was higher than the EI from measured breast milk consumption in our sample except for the 3-month-old infants^(2,8) (Fig. 3). The day-to-day variance of EI between the three recorded days ranged from 8% to 11% of the group's mean EI (Table 3).

Equation_1 and Equation_2 for ER underestimated EI for all age groups and both genders (Fig. 4, Table 4). Equation_1 showed significant deviations from the mean EI in boys at all age groups and in girls at 9 and 12 months. Equation_2 showed smaller but significant deviations from EI than Equation_1. In contrast, Equation_3 overestimated EI for 3-month-old and 3- and 6-month-old girls and underestimated EI for older infants similar to Equation_2. Overall, the best agreement between ER and EI was found at the age of 6 months and the largest differences were found at 9 and 12 months.

Bland–Altman plots of the ratio between EI and ER v. the average of EI and ER gave similar results for the three

Fig. 1 Mean values of body weight of infants (\blacksquare , boys; \blacktriangle , girls) from the DONALD (DOrtmund Nutritional and Anthropometric Longitudinally Designed) Study sample at 0, 3, 6, 9 and 12 months of age, together with gender-specific trend lines (—, boys; ---, girls), compared with reference body weight of infants (\square , boys; \triangle , girls) aged 0–12 months from the 50th percentile of the WHO pooled breast-fed data set⁽²³⁾. Trend line in DONALD boys: weight = $-0.0346 \times age^2 + 0.9269 \times age + 3.7073$; trend line in DONALD girls: weight = $-0.0247 \times age^2 + 0.7779 \times age + 3.504$



Table 1 Weight gain (g/d) from the reference data (50th percentile of the WHO pooled breast-fed data set⁽²³⁾) and in the DONALD (DOrtmund Nutritional and Anthropometric Longitudinally Designed) Study sample by gender and age

		DONALD weight gain (g/d)			
Age interval (months)	WHO weight gain (g/d)	Mean	SD		
Boys					
0–3	30	32	8		
3–6	17	20	5		
6–9	11	12	5		
9–12	8	11	4		
Girls					
0–3	28	26	5		
3–6	16	18	5		
6–9	10	12	4		
9–12	6	10	4		



Fig. 2 Energy intake (EI) from measured food consumption adjusted for body weight of infants (\blacksquare , boys; ▲, girls) from the DONALD (DOrtmund Nutritional and Anthropometric Longitudinally Designed) Study sample at 3, 6, 9 and 12 months of age, together with gender-specific trend lines (—, boys; ---, girls), compared with reference energy requirements for boys (\Box) and (\triangle) girls⁽¹²⁾. Trend line in DONALD boys: weight = $1.482 \times age^2 - 26.266 \times age + 454.5$; trend line in DONALD girls: weight = $1.3687 \times age^2 - 25.226 \times age + 448.66$

Table 2 Dietary characteristics of infants from the DONALD (DOrtmund Nutritional and Anthropometric Longitudinally Designed) Study

A = =	Breast milk only		d Breast milk + formula		Breast milk + complementary food		Breast milk + formula + complementary food		Formula + complementary food		Complementary food only	
Age (months)	n	%	n	%	n	%	n	%	n	%	n	%
3	125	100	0		0		0		0		0	
6	37	19	2	1	102	53	26	13	23	12	3	2
9	0		0		62	29	29	14	92	43	30	14
12	0		0		30	13	10	4	91	40	96	42

equations and therefore only the results for Equation_3 are shown (Fig. 5). The mean ratio was 1.03 and the 95% limits of agreement were 0.69, 1.37. Thus, EI was underestimated by up to 31% and overestimated by up to 37% for most measurements. The gender-specific regression lines show a small but significant increase in the ratio as the average increases (boys: EI:ER calculated by Equation_3 = 0.80582 + 0.000075 × mean, $R^2 = 0.04$,

P = 0.0002; girls: EI:ER calculated by Equation_3 = $0.906516 + 0.000043 \times \text{mean}, R^2 = 0.01, P = 0.0223$).

Discussion

The objective of the present study was to facilitate the estimation of breast milk consumption in epidemiological



Fig. 3 Total energy intake (EI) assessed by 3 d weighed dietary records differentiated for breast milk (\blacksquare), formula (\blacksquare) and complementary food (\Box) from the DONALD (DOrtmund Nutritional and Anthropometric Longitudinally Designed) Study sample at 3, 6, 9 and 12 months of age, compared with energy from an assumed amount of breast milk (-, 780 ml (3 and 6 months) or 600 ml (9 and 12 months)^(2,8); - -, 750 ml^(2,8))

Table 3 Total energy inta	ke, energy intake fror	n breast milk, formu	la and complement	tary food, and	mean day-to-day	variance by gender
and age in the DONALD	(DOrtmund Nutritiona	I and Anthropometric	c Longitudinally De	signed) Study	sample	

Ago ostogony	Total energy intake (kJ/d)		Energy intake from breast milk (kJ/d)		Energy intake from formula (kJ/d)		Energy intake from complementary food (kJ/d)			
(months)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	(% of total energy intake)	
Boys										
3	2524	434	2524	434	0	0	0	0	8.5	
6	2707	376	1597	1081	415	739	696	751	9.6	
9	3124	472	396	604	541	599	2187	748	8.4	
12	3462	471	151	468	492	675	2819	835	10.0	
Girls										
3	2216	318	2215	318	0	0	1	5	8.7	
6	2461	330	1680	848	171	443	610	675	8.6	
9	2853	421	332	559	502	551	2019	734	8.1	
12	3163	418	101	322	373	522	2690	640	10.6	



Fig. 4 Difference between total measured energy intake (EI) and energy requirements (ER) estimated using three equations (Equation_1, Equation_2, Equation_3), displayed as a percentage of EI, in the DONALD (DOrtmund Nutritional and Anthropometric Longitudinally Designed) Study sample at 3, 6, 9 and 12 months of age. *P < 0.05, **P < 0.0001

Tab	le 4	Difference be	etween en	ergy intake (EI) and ener	gy requiremer	nts (ER),	calculated i	using the th	ree different	equations,	by gender	' and
age	in tł	ne DONALD	(DOrtmun	d Nutritional	and Anthrop	pometric Long	gitudinally	Designed)	Study				

		EI – ER calculated by Equation_1 (kJ)		EI – ER ca Equatio	alculated by n_2 (kJ)	EI – ER calculated by Equation_3 (kJ)	
reference (months)	п	Mean	Р	Mean	Р	Mean	Р
Boys							
3	60	138.7	0.0162	62.1	0.2262	-144.7	0.0193
6	96	113·2	0.0044	91.9	0.0233	1.4	0.9745
9	117	249.6	<0.0001	206.8	<0.0001	167.9	0.0020
12	115	269.7	<0.0001	171.7	0.0004	113.4	0.0241
Girls							
3	65	31.8	0.4237	45·7	0.2235	-62.0	0.1217
6	100	42.7	0.2007	38.1	0.2946	-24.0	0.5728
9	101	196·2	<0.0001	166.2	0.0002	116·0	0.0141
12	119	242.8	<0.0001	117.0	0.0055	79 ∙0	0.0753

Equation_1: adoption of the published reference ER (RER) stated as 'kJ/d'.

Equation 2: product of the individual measured body weight and RER stated as 'kJ/kg body weight per d'.

Equation_3: sum of individual estimated total energy expenditure (TEE) and energy cost of growth (ECG).



Fig. 5 Bland–Altman plot of the ratio between energy intake (EI) and energy requirements (ER) calculated by Equation_3 *v*. the average of EI and ER calculated by Equation_3 for infants (\circ , boys; \bullet , girls) of the DONALD (DOrtmund Nutritional and Anthropometric Longitudinally Designed) Study sample at 3, 6, 9 and 12 months of age and the gender-specific regression lines (-, boys; - -, girls)

studies among healthy infants where measurement of breast milk intake is not possible. Nutrition in early infancy is increasingly considered to be an important factor influencing later health. However, public health strategies to examine the effects of breast-feeding promotion on breast-feeding habits remain to be conducted. Validated equations to estimate breast milk consumption requiring only body weight measurements would be of great interest.

We based our evaluation on EI measured by 3 d weighed dietary records, which are considered to be the gold standard among nutrition survey methods. Moreover, our sample fulfilled the same starting criteria as those used for the sample from which the RER were derived, and growth was very similar to the WHO references used to construct the RER⁽¹²⁾. We cannot demonstrate whether our assumption of self-regulation of EI by ER was true for our sample, but nevertheless we could draw on

published data from infants aged up to 12 months^(6,10). Self-regulation seems to be less complete in infants or toddlers, when environmental influences such as feeding habits of carers gain more importance^(10,26,27).

The main result of our study was that using an infant's body weight alone (Equation_2) or the infant's body weight together with weight gain (Equation_3) is a reasonable approach for estimating EI throughout the first year of life in infants breast-fed for at least 4 months.

Equation_2 is the more practical of the two equations because only the actual weight of the infant is required. On the other hand, taking weight gain into account (Equation_3) could improve the estimation of ER in the case of infants with fast or slow weight gain.

Equation_1, with the highest differences between ER and EI, is based upon the simple adoption of the RER, stated as 'kJ/d', which takes into account age and sex but not weight. Weight is a crucial predictor of $EI^{(12,18)}$. Since the DONALD

Study sample was marginally heavier than the WHO reference population, a higher EI was to be expected.

The overall differences between EI and ER were rather small, never more than 10% of total energy intake, and smaller than the mean variance of energy intake between the three days of recording. The difference was also much smaller than the standard deviation of the mean TEE in the reference population, which was always higher than 10%. Regarding the individual ratios of EI:ER (Bland– Altman plots), the findings strongly advise caution in applying our approach at an individual level. However, the fact that the mean ratio approached one suggests that our approach is reliable for groups of healthy, normally growing infants during the first year of life.

All three equations for ER underestimated EI for nearly all age groups and both genders. Several explanations are possible. First, correcting breast milk intake by adding 5% for insensible water loss might have led to an overestimation of total EI. The influence of this correction should be larger at younger ages because of a higher percentage of breast milk contributing to the overall intake, but in our sample differences were larger in the older age groups. Second, the discrepancy between the metabolisable energy content and the gross energy content of breast milk was not considered in the RER. If we had used the suggested value for the metabolisable energy content $(259 \text{ kJ}/100 \text{ g})^{(28)}$ instead of the one for gross energy content (289 kJ/100 g), our measured EI would have been lower by approximately 10%, at least for the predominantly breast-fed infants. At present there is no agreement as to whether metabolisable or gross EI of human milk is more appropriate⁽²⁹⁾. Third, the weight gain of the DONALD sample was marginally higher than that of the WHO reference sample for nearly all age groups conditioned by a higher EI, which might have led to a higher body weight at the age of 12 months. Fourth, information on the dietary intake of the reference population is available only for the first four months, so we cannot check for potential differences in dietary characteristics between the reference population⁽¹²⁾ and our sample after this age. Finally, our sample reflects dietary habits in countries where breast-feeding rates at 6 months are much lower than recommended and negligible at the age of 9 and 12 months. It would probably have been more appropriate to use non-breast-fed infants, whose RER are higher than for breast-fed infants, as a reference in the second six months. However, this would imply that the reference needs to be switched during the first year of life although no information on diet is available for the reference infants after the first four months. Future studies could investigate our approach for formula-fed infants or for infants without regard to their type of milk feeding.

Only Equation_3, which took weight gain into account, resulted in an overestimation of EI for the youngest age groups of 3 and 6 months. This is probably a methodological artefact. During this age period the earlier peak

growth rate decreased rapidly, whereas the mean daily individual weight gain during the preceding age interval (for instance, 0–3 months) was used to calculate Equation_3 (at month 3). In reality, the individual growth rate would already be lower at this time point⁽³⁰⁾. Depending on whether a child is in a period of fast growth or not, the energy cost of growth could be unpredictable at the time of measurement.

In general, the significant but clinically not relevant differences between ER and EI in our evaluation suggest that the current, well-documented RER data⁽¹²⁾ can be applied to calculate ER in breast-fed infants whose growth trajectory follows the WHO growth curves. This approach seems to be more valid than the simple assumption of a constant volume of breast milk for a wide age range^(2,8). Our results show that Equation_3 can be used if body weight and weight gain are available and Equation_2 if body weight is measured only once. Equation_1 gives only a rough impression of the RER and should only be used if body measurements are not available.

The dietary guidelines for infant nutrition in Germany are in agreement with the latest statement from the European Society for Paediatric Gastroenterology, Hepatology and Nutrition's (ESPGHAN) Committee on Nutrition, valid for the European region. Thus, the infant feeding style in Germany should be similar to that in other European countries⁽³¹⁾. Therefore, our findings, based on a German study sample, should be applicable to other groups of infants fed according to the European recommendations.

In conclusion, our study, using weighed dietary records in infants from the DONALD Study, has shown that breast milk amounts in predominantly or partially breast-fed infants can be estimated with reasonable precision from calculated ER based on the current RER data⁽¹²⁾. Put into practice, this means: (i) exclusively breast-fed infants consume as much energy via breast milk as given by their calculated ER; and (ii) partially breast-fed infants consume as much energy via breast milk as given by the difference between their ER and the EI of foods other than breast milk. Nevertheless, a possible bias due to the lack of self-regulation should always be considered if our method is applied. Various standard methods such as dietary records or recalls are available to quantify the consumption of other food by infants, e.g. formula, complementary menus and cereals. Together with our proposed method for estimating breast milk consumption, assessing dietary intake in epidemiological studies in infants should be easier in the future and may also support the monitoring of breast milk promotion.

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