The Journal of Nutrition
Community and International Nutrition

Socio-Economic and Environmental Factors Influence Energy Utilization in Brazilian Breast-Fed Infants[1,2]

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Abstract

Energy intake recommendations for infants are based on data from industrialized countries. FAO/WHO/UNU expressed the need for studies on total energy expenditure (TEE) and basal metabolic rate from developing countries covering current and changing lifestyles. For this observational study, 65 infants of differing socioeconomic status (SES) ($n = 32$ middle SES, $n = 33$ low SES) were selected in Pelotas, southern Brazil, aiming to: *1*) compare TEE, minimum observable energy expenditure (MOEE), activity energy expenditure (AEE) between breast-fed infants 8.7 mo of age from middle and low SES; and *2*) investigate the effect of potential mediating factors on TEE and AEE. TEE and total body water were measured with doubly labeled water, MOEE with respiration calorimetry, breast milk intake using the dose-to-the-mother deuterium-oxide turnover method, food intake using 1-d food weighing, and prevalence of overweight using BMI Z-scores. TEE adjusted for ethnicity was 257 (95% CI 232–281) kJ/(kg · d) in middle SES infants vs. 318 (95%CI 294–342) kJ/(kg · d) in low SES infants ($P = 0.001$). MOEE did not differ between groups and the difference in TEE was therefore attributed to AEE ($P = 0.008$). The effect of SES on AEE was mediated by the number of persons per bedroom (crowding). Prevalence of overweight tended to be higher in middle SES infants ($P = 0.054$) than in low SES infants. The difference in TEE and AEE between SES groups emphasizes the importance of an accurate description of the SES of any population in which TEE is studied and questions the extent to which TEE data from middle-class infants in transitional countries should be considered normative. J. Nutr. 136: 2945–2951, 2006.

Introduction

The recently modified FAO/WHO/UNU estimations of energy requirements of infants (1) were based on studies in infants from the United Kingdom (2–7), the United States (8–10), the Netherlands (11), Chile (12) and China (13). There is an urgent need to study the effect of changes in lifestyle on both total energy expenditure (TEE)[9] and basal metabolic rate in developing countries and countries in transition. In developing countries, energy requirements (ER) are estimated to be 8–14% higher for catch-up growth in children who are recovering from disease (14,15). Butte et al. (16) showed increased TEE in Mexican infants who were living under poor conditions, and Vasquez-Velasquez (17) observed increased TEE and sleeping metabolic rate (SMR) in malnourished infants in the Gambia. Indirect evidence suggests that the process of transition also affects energy balance. In Brazil, for example, the prevalence of obesity and degenerative diseases is increasing, mainly in the poor (18–20). The study described in this article focuses on 2 strata of the Brazilian population that are in different phases of nutrition transition. Very poor people are probably still in the pretransition phase and may represent an imbalance in energy toward undernutrition. In contrast, the middle-class population might be undergoing the nutrition transition, with a possible imbalance toward overnutrition. Our study provides a unique set of data on energy metabolism, including both TEE and SMR, in 2 strata of socio-economic status in a country in transition. Measurement of TEE and basal metabolic rate at the same time allows calculation of activity energy expenditure and this is important for the interpretation of the TEE data (21).

[1] Supported by the International Atomic Energy Agency (RC10981/R2) and the Directorate General for International Cooperation, Netherlands Ministry of Foreign Affairs.

[2] Supplemental Tables 1 and 2 are available with the online posting of this paper at jn.nutrition.org.

[9] Abbreviations used: AEE, activity energy expenditure (kJ/d); DLW, doubly labeled water ($^2H_2{}^{18}O$); ER, energy requirements (kJ/d); FFM, fat free mass (kg); FFMI, fat free mass index (kg/m^2); FM, fat mass (kg); FMI, fat mass index (kg/m^2); MOEE, minimal observable energy expenditure (kJ/d); SES, socio-economic status; SMR, sleeping metabolic rate (kJ/d); TEE, total energy expenditure (kJ/d).

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Manuscript received 7 April 2006. Initial review completed 14 May 2006. Revision accepted 1 September 2006.

Subjects and Methods

An earlier publication on the association between cow's milk consumption and sleeping metabolic rate was based on the same sample of infants (22). As subjects and methods were mainly the same, this section provides a brief general description of the study design and subject selection followed by a more detailed description of only those methods that are additional to or different from the earlier publication.

Study design. The study was conducted in Pelotas, a city of ~330,000 habitants and 6000 births/y in the extreme south of Brazil (32° S and 52° W). The study was designed as a cross-sectional study to compare TEE, minimal observable energy expenditure (MOEE), and activity energy expenditure (AEE) among infants 8 mo of age from middle and low socio-economic status (SES). Participation of each mother-infant pair was for a period of 3 wk. All measurements were done by qualified fieldworkers at the homes of the participating babies. During the first 14 d of data collection, breast-milk intake was measured using the dose-to-the-mother ^2H_2O turnover method. During 1 d in the second wk of the study, food intake was measured by food-weighing. On d 14, a dose of doubly labeled water ($^2H_2{}^{18}O$, DLW) was given to the baby, and urine samples were collected until d 21. During this last week, sleeping metabolic rate and child development were also assessed. Morbidity was monitored throughout the study period.

Subjects. An electronic database (SINASC), including all birth registrations in Pelotas, was used for the random selection of mother-baby pairs. Mother-baby pairs were selected on the basis of maternal education: ≤3 y, low education, low SES group; or maternal education ≥8 y, middle education, or middle SES group. All babies were healthy at the beginning of the study.

Environmental factors, indicators of socio-economic status. A standardized questionnaire was used to collect data on parental education, family income, employment, availability of water and sanitation, crowding, parity, and smoking behavior. Schooling of the father was categorized in the same way as for the mothers (≤3 y; 3.1–7.9 y; ≥8 y). Income was categorized using minimum wages for Brazil (at the time of the study this was R$180/mo, or ~$80/mo). Crowding was defined as:

$$\text{Crowding} = \frac{\text{number of persons living in the house}}{\text{number of bedrooms} + 1}.$$

Prevalence of obesity. The prevalence of obesity was assessed on the basis of BMI (kg/m^2). At 8 mo of age, infants were classified as being overweight or obese using cut-off points corresponding to a BMI of 25 for overweight and 30 for obesity at age 18 y, as suggested by Cole et al. (23). Z-scores were calculated from the BMI data available from our population of infants:

$$\text{Z} - \text{score} = \frac{\text{BMI value} - \text{BMI by sex}}{\text{SD by sex}}.$$

For girls Z-scores of 1.19 and 2.0 were used as cut-off points for overweight and obesity, respectively, and for boys these values were 1.30 and 2.0.

Intake of complementary foods, minimal observable energy expenditure, anthropometry, and morbidity. Intake of complementary foods was measured by 1-d food weighing using a mechanical scale calibrated against standard weights. SMR and MOEE were measured by respiration calorimetry using a Deltatrac MBM-100. SMR (kJ/min) was defined as the mean of energy expenditure during a period of 40 min to 1 h of sleep, and MOEE (kJ/min) was defined as the mean of the 5 consecutive lowest 1-min values for energy expenditure (kJ/d). Measurements were made at a time the infant would usually sleep. Weight and length were measured. Morbidity questionnaires were applied twice weekly. Details of these measurements can be found elsewhere (22).

Breast-milk intake and total energy expenditure. The dose-to-the-mother ^2H_2O turnover method was used to measure breast-milk intake, but this was combined with the subsequent measurement of TEE using $^2H_2{}^{18}O$. Details of the basic breast-milk measurements have been described elsewhere (22,24), but, in short, the method involves the administration of 0.5 mol/L (10 g) of 99.8% deuterium to the mother, and collection of saliva samples from the mother immediately before dose administration on d 0 (predose), and subsequently on d 1, 3, 13, and 14. Urine samples were collected from the baby on d 0 (predose), and on d 1, 2, 3, 13, and 14. For the measurement of TEE an oral dose of 0.18 g/kg $H_2{}^{18}O$ and 0.10 g/kg ^2H_2O was administered to the infant on d 14 shortly after the collection of the d 14 sample for the breast-milk estimates. The dose was slowly fed into the baby's mouth using a nasogastric tube attached to a syringe. Any spillage was collected using preweighed tissues. The exact dose administered was calculated from the difference in weight of the dosing vial, syringe, nasogastric tube, and tissues pre- and postdosing, and was, on average, 84% of the dose prepared. Subsequently, urine samples were collected from the baby on d 15, 16, 17, 20, and 21. During the field work, samples were stored on ice, and thereafter at –20°C. Samples were shipped unfrozen to Cambridge, for analysis.

Calculations. For the measurement of ^2H_2O kinetic parameters, ^2H enrichment above d 0 baseline, measured at the defined times in the period from d 0 to d 14 for the mother and d 0 to 21 for the baby, were fitted to the basic lactation model described by Haisma et al. (24) but including the additional ^2H isotopic dose at d 14. In this way residual ^2H reaching the baby from the mother could be accounted for during the TEE measurement phase (d 14–21). For the mother,

$$E_{m(t)} = E_{m(0)} e^{-K_{mm}.t}, \tag{Eq. 1}$$

where $E_{m(0)}$ is ^2H isotopic enrichment above background (ppm) immediately after the first isotope dose, $E_{m(t)}$ is subsequent enrichment, t is time after the isotopic dose (d) and K_{mm} is water turnover in the mother (d^{-1}).

For the infant, data for ^2H was fitted to:

when $t < t_{D2}$,

$$E_{b(t)} = E_{m(0)} \left(\frac{F_{bm}}{V_b}\right) \left(\frac{e^{-K_{mm}t} - e^{-\frac{F_{bm}}{V_b}t}}{\frac{F_{bb}}{V_b} - K_{mm}}\right);$$

and when $t \geq t_{D2}$,

$$E_{b(t)} = E_{m(0)} \left(\frac{F_{bm}}{V_b}\right) \left(\frac{e^{-K_{mm}t} - e^{-\frac{F_{bm}}{V_b}t}}{\frac{F_{bb}}{V_b} - K_{mm}}\right) + E_{b(D2)} e^{-\frac{F_{bb}}{V_b}(t - t_{D2})}, \tag{Eq. 2}$$

where $E_{b(D2)}$ is the initial ^2H isotopic enrichment (ppm) appearing as a consequence of the second isotopic dose given at time t_{D2} (d) after the first dose. $E_{b(D2)}$ was used to calculate V_b (the ^2H distribution space, mol) at this time and values at other times ($E_{b(t)}$) were assumed to be in the same proportion of body weight changing linearly over the measurement period. F_{bm} is the transfer of water from the mother to the baby via breast milk (mol/d) and F_{bb} is total water loss in the baby (mol/d).

For the infant ^{18}O data were fitted to:

$$E'_{b(t)} = E'_{b(D2)} e^{-\frac{F'_{bb}}{V'_{b(t)}} \cdot (t - t_{D2})}, \tag{Eq. 3}$$

where $E'_{b(D2)}$ is the initial ^{18}O enrichment following the second isotopic dose. $E'_{b(D2)}$ was used to calculate V'_b (the ^{18}O distribution space, mol) at this time and values at other times ($E'_{b(t)}$) were assumed to be in the same proportion of body weight changing linearly over the measurement period. F'_{bb} is total water plus water equivalents of CO_2 loss in the baby (mol/d).

Experimental data were simultaneously fitted to equations 1, 2, and separately to 3 using the "Solver" function in Excel to minimize the sum of the squares of the differences between observed and fitted values for mother and baby data combined. Parameters fitted were $E_{m(0)}$, $E_{b(D2)}$, $E'_{b(D2)}$, F_{bm}, K_{mm}, F_{bb}, and F'_{bb}.

Calculation of the parameters of breast-milk and other water intake was performed from the fitted data as described by Haisma et al. (24). For TEE, CO_2 production (r_{CO_2}, mol/d) was first calculated assuming that a constant proportion of the infants' water turnover was fractionated (25):

$$r_{CO_2} = \frac{K'N'}{2f_3} - \frac{KN(xf_2 + 1 - x)}{2f_3(xf_1 + 1 - x)},$$

where rate constants for isotope disappearance are: $K = \frac{F}{V}$ for ^{2}H and $K' = \frac{F'}{V'}$ for ^{18}O; normalized isotope distribution spaces (N, based on ^{2}H dilution, mol) are:

$$N = \left(\frac{V}{1.04} + \frac{V'}{1.01}\right)(0.5 \times 1.04),$$

and (N', based on ^{18}O dilution, mol) $N' = \left(\frac{V}{1.04} + \frac{V'}{1.01}\right)(0.5 \times 1.01)$; fractionation factors are: $f_1 = 0.941$, $f_2 = 0.991$, and $f_3 = 1.037$; proportion of water losses fractionated (x) is assumed to be 0.2 (26).

r_{CO_2} was then converted to TEE (kJ/d) from the equation:

$$TEE = r_{CO_2}\left(\frac{82.88}{RQ} + 29.71\right).$$

RQ was estimated from the mean food quotient calculated from the composition of the total diet of the infants per study group (27). This was 0.87 in this study. AEE was calculated from the difference between TEE and MOEE.

Total body water and body composition. Infant total body water was calculated as the mean of the isotope distribution spaces of ^{2}H and ^{18}O corrected for nonaqueous isotope exchange:

$$\text{Total body water (kg)} = (V/1.04 + V'/1.01)/2.$$

Fat free mass (FFM, kg), fat mass (FM, kg), fat free mass index (FFMI, kg/m^2), and fat mass index (FMI, kg/m^2) were calculated from total body water as described elsewhere (22) using a hydration coefficient of 79.7% for the infant (28) and 73% for the mother (25).

Sample size. The main study outcome was TEE. The only data available from 8-mo-old breast-fed infants were from Dutch infants, and these data were used for sample size calculations. The TEE of these infants at 8 mo was 347 ± 40.4 kJ/(kg · d) (11). The same authors found an 8% difference in TEE at 8 mo of age between breast-fed and formula-fed infants. To detect a significant difference of the same magnitude in TEE between middle and low SES infants, assuming a standard deviation of 40.4 kJ/(kg · d), the study required 35 infants in each group. These calculations assume a Type I error (alpha) of 5%, 2-tailed, and a Type II error (beta) of 20%, that is, a statistical power of 80%.

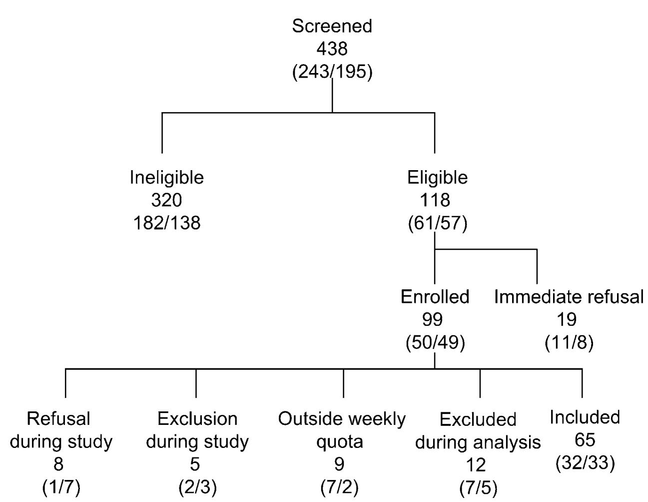

Figure 1 Sampling scheme. Parenthetical numbers represent middle/low SES.

To allow for any unforeseen reduction in sample size, we increased the total sample size by 8 (4 middle SES, 4 low SES infants), i.e., 39 middle SES and 39 low SES mother-infant pairs were enrolled.

Figure 1 shows how the participating mother-infant pairs were obtained. Of the 78 infants enrolled, TEE was measured successfully in 65 infants. This sample was used as the basis of analysis. SMR and MOEE data were available from 52 of 65 infants. Food intake data were available from 60 of 65 infants. Breast-milk intake, child development and behavior, and morbidity were evaluated in all 65 infants included.

Statistics. Differences between infants from middle and low SES were studied using a Student's t test for independent samples. In case the distribution was not normal, a nonparametric test was used (Mann-Whitney U). Sex and ethnicity were studied for a possible confounding effect on TEE and AEE as described by Rothman and Greenland (29). Factors studied for a potential mediating effect on TEE were: *1*) environmental characteristics, such as crowding, numbers of hours the mother was working away from the child, the availability of water and sanitation, mother smoking, mother working away from home, type of birth (vaginal vs. Caesarean); *2*) maternal characteristics, such as maternal age, maternal weight, maternal height, maternal BMI, maternal FM, maternal FFM, percentage body fat, FMI, FFMI, parity; and *3*) child characteristics, i.e., birth weight, length at birth, baby's weight, length, head circumference, BMI, FM, FFM, FMI, FFMI, breast milk, and cow's milk intake, morbidity, motor development and child behavior scores. For continuous variables, Pearson's correlation coefficient was used to study associations between the potential mediating factors and TEE; for categorical variables 1-way ANOVA was used. Those variables that were associated with TEE at $P < 0.10$ were subsequently entered into a model of covariance to determine the extent to which the SES effect was mediated by them. A variable was considered a definite confounder or mediator if its inclusion in the model resulted in a minimum change of 10% in the crude difference in the outcome variable between study groups. The same procedure was repeated for AEE. The SPSS software package, version 11.0, was used. Values in the text are means ± SD.

Ethics. The study was approved by the Ethical Committee of the Universidade Federal de Pelotas, affiliated with the National Commission on Research Ethics or the Brazilian Ministry of Health, and signed informed consent was provided by the mother.

Results

Subjects. **Table 1** shows anthropometric indices of the infants by SES. The prevalence of overweight (based on BMI Z-scores) tended to differ ($P = 0.054$) between study groups (middle SES, 6/32 (18.8%); low SES, 1/33 (3.0%), $P = 0.054$). None of the infants were classified as being obese. Of the 65 infants, 30 were male with no difference in sex distribution between the middle SES (46.9% male) and low SES (45.5% male) groups. Ethnicity differed between the middle and low SES group, in that the middle SES group consisted of a larger percentage of white infants (middle SES, 87.5%; low SES, 42.4%, $P < 0.001$). Overall, 65% of the infants studied were white, 28% black, and 8% mixed. Body composition did not differ due to sex or ethnic group.

Mothers of the 2 groups did not differ in age, weight, height, % fat or FMI, but FFMI was lower in middle SES mothers (15.9 ± 1.8) than in low SES mothers (17.3 ± 2.9, $P = 0.035$). Middle SES mothers had 2.1 ± 1.5 children, whereas the low SES mothers had 3.6 ± 1.8 ($P = 0.001$).

Refusals and drop-outs. Socio-economic, environmental, and anthropometric characteristics did not differ between mother-infant pairs who completed the study and those who refused before or during the study, or were lost due to problems at the stage of isotope analysis.

TABLE 1 Anthropometric indices of 8-mo–old breast-fed infants by SES[1]

	Middle SES, $n = 32$	Low SES, $n = 33$	P-value
Birth weight, *kg*	3.4 ± 0.5	3.2 ± 0.3	0.057
Length at birth, *cm*	49.0 ± 2.2	48.7 ± 2.0	0.469
Weight at 8 mo, *kg*	8.7 ± 1.0	8.2 ± 1.1	0.034
Length at 8 mo, *kg*	70.7 ± 2.5	69.3 ± 2.9	0.050
Head circumference at 8 mo, *cm*	45.0 ± 1.3	44.3 ± 1.6	0.054
Weight-for-height Z-score	0.124 ± 1.01	−0.137 ± 0.76	0.245
Growth velocity,[2] *g/21 d*	10.1 ± 9.1	11.4 ± 10.2	0.616
Weight gained,[2] *kg/21 d*	0.21 ± 0.19	0.24 ± 0.21	0.616
Weight gained from birth, *kg*	5.3 ± 1.0	4.9 ± 1.0	0.164
Body mass index,[2] *kg/m²*	17.4 ± 1.6	16.9 ± 1.3	0.140
Body mass index Z-score	0.29 ± 1.13	−0.08 ± 0.90	0.141
Fat free mass at 8.5 mo, *kg*	6.2 ± 0.6	5.9 ± 0.9	0.151
Fat mass at 8.5 mo, *kg*	2.7 ± 0.7	2.4 ± 0.8	0.129
Percentage fat at 8.5 mo	29.7 ± 6.0	28.4 ± 7.8	0.458
Fat mass index,[2] *kg/m²*	5.3 ± 1.4	4.9 ± 1.5	0.266
Fat free mass index,[2] *kg/m²*	12.4 ± 1.0	12.2 ± 1.3	0.648

[1] Values are means ± SD.
[2] Determined during the 21 d of the study.

Confounding factors. Gender was not a confounder of the association between TEE and SES. Normalized to weight, TEE was 284 ± 65.7 in boys vs. 291 ± 79.1 kJ/(kg · d) in girls (P = 0.672). In contrast, ethnicity differed between SES groups (see Subjects section), and TEE [kJ/d, kJ/(kg · d), kJ/(kg FFM · d)] differed between white and nonwhite infants. Normalized to weight, TEE was 272 ± 76.4 kJ/(kg · d) in white infants as compared with 316 ± 56.8 kJ/(kg · d) in nonwhite infants (P = 0.019). Further analysis (see below) showed that the inclusion of ethnicity in the regression model resulted in a 10% decrease in the crude difference of TEE between study groups (middle vs. low SES). Ethnicity was therefore treated as a confounder in subsequent analyses of TEE, and all analyses concerning TEE were adjusted for ethnicity.

Indicators of socio-economic status and environmental characteristics. All indicators of SES and environmental characteristics included in the questionnaire differed between SES groups (**Table 2**).

TEE by SES group. The difference in fractional turnover rates of ^{2}H and ^{18}O as measured by the DLW method differed between SES groups (**Table 3**). TEE [kJ/d, kJ/(kg · d), kJ/(kg FFM · d)] was significantly different between middle and low SES infants. Crude means were 2239 ± 613 kJ/d for middle SES compared with 2653 ± 505 kJ/d for low SES infants (P = 0.004). Normalized to weight, TEE was 254 ± 69.0 for middle SES infants vs. 321 ± 60.7 kJ/(kg · d) in low SES infants (P < 0.001); normalized to FFM, TEE was 366 ± 104 kJ/(kg FFM · d) in middle SES compared with 454 ± 85.0 kJ/(kg FFM · d) in low SES infants (P < 0.001). Values for TEE were adjusted for ethnicity (**Table 4**). Adjustment for ethnicity reduced the difference between groups from 67.4 to 61.2 kJ/(kg · d).

Minimal observable energy expenditure and sleeping metabolic rate. Table 4 also shows results for MOEE and SMR by SES. Normalized to weight, the difference was not significant. MOEE [kJ/d, kJ/(kg · d), kJ/(kg FFM · d)] did not differ between the sexes, nor between white and nonwhite infants. Comparisons of

TABLE 2 Indicators and environmental characteristics of middle and low SES households in Pelotas, Brazil[1]

	Middle SES, $n = 32$	Low SES, $n = 33$	P-value
Indicators of socio-economic status			
Family income, *reais/* mo	1038 ± 1010	195 ± 175	<0.001
Paternal education, *y*	9.7 ± 3.3	3.7 ± 1.8	<0.001
Maternal education, *y*	10.7 ± 2.3	2.0 ± 1.2	<0.001
Environmental determinants			
Crowding[2]	1.6 ± 0.7	3.1 ± 1.2	<0.001
Mother working away from home, *%*	25.0	6.1	0.044
Mother smoking, *%*	15.6	42.4	0.028
Tap water, *%*	100	75.8	0.005
Flushing toilet, *%*	100	66.7	<0.001

[1] Values are means ± SD or %.
[2] Number of persons in a household/(number of bedrooms + 1).

SMR [kJ/d, kJ/(kg · d), kJ/(kg FFM · d)] among SES groups, sexes, or white and nonwhite infants gave similar results.

Activity energy expenditure. AEE [TEE-MOEE, kJ/d, kJ/(kg · d), kJ/(kg FFM · d)] differed between study groups. Unadjusted means were 394 ± 616 kJ/d in middle SES infants and 927 ± 583 kJ/d in low SES infants (P = 0.002). Normalized to weight, these values were 46.3 ± 70.8 kJ/(kg · d) for middle SES infants and 117 ± 70.4 kJ/(kg · d) for low SES infants (P = 0.001); and normalized by FFM, AEE was 66.2 ± 100.5 kJ/(kg FFM · d) in middle SES infants and 159 ± 97.6 kJ/(kg FFM · d) in low SES infants (P = 0.001). AEE did not differ between sexes. Ethnicity was a confounding factor in the association of AEE and SES, but differences remained significant even after adjustment for this variable (Table 4). Comparisons based on AEE calculated as the difference between TEE and SMR gave similar results.

Energy and macronutrient intake. Breast milk provided 55.7 ± 28.1% of the infants' energy intake; cow's milk, 10.4 ± 17.3%, and solids, 33.9 ± 18.7%. In terms of nutrients, 51.9 ± 6.7% of the energy was from carbohydrates, 39.8 ± 9.3% from fat, and 10.5 ± 3.1% from protein. There were no differences between middle and low SES infants.

Breast milk intake did not differ between the middle SES (689 ± 334 mL/d) and low SES, (638 ± 325 mL/d, P = 0.535), but intake of cow's milk tended to be higher in low SES (162 ± 224 mL/d) compared with middle SES (69.1 ± 174 mL/d, P = 0.079) infants. The percentage of infants receiving breast milk as the only source of milk (i.e., not receiving any cow's milk) did

TABLE 3 Isotope dilution spaces, fractional turnover rates of ^{2}H and ^{18}O in breast-fed infants from middle and low SES in Pelotas, Brazil[1]

Measure	Middle SES, $n = 32$	Low SES, $n = 33$	Overall	P-value
N_D, *kg*	5.18 ± 0.56	4.95 ± 0.80	5.06 ± 0.69	0.170
N_O, *kg*	5.00 ± 0.51	4.80 ± 0.77	4.89 ± 0.65	0.214
N_D/N_O	1.037 ± 0.022	1.032 ± 0.031	1.034 ± 0.027	0.455
k_D, *1/d*	0.224 ± 0.030	0.216 ± 0.024	0.220 ± 0.027	0.241
k_O, *1/d*	0.266 ± 0.027	0.264 ± 0.025	0.265 ± 0.026	0.760
k_O-k_D	0.043 ± 0.010	0.049 ± 0.009	0.046 ± 0.010	0.008

[1] Values are means ± SD.

TABLE 4 Components of energy expenditure in breast-fed infants in Pelotas, Brazil by SES[1]

	Middle SES	Low SES	*P*-value
TEE,[2] *kJ/d*	2267 (2054,2480)	2627 (2418,2836)	0.027
TEE,[2] *kJ/(kg·d)*	257 (232,281)	318 (294,342)	0.001
TEE,[2] *kJ/(kg FFM·d)*	371 (336,407)	448 (413,483)	0.006
MOEE, *kJ/d*	1820 (1739,1902)	1690 (1601,1778)	0.029
MOEE, *kJ/(kg·d)*	206 (195,217)	209 (196,221)	0.763
MOEE, *kJ/(kg FFM·d)*	298 (285,312)	294 (274,313)	0.664
SMR, *kJ/d*	2034 (1959,2109)	1908 (1828,1988)	0.021
SMR, *kJ/(kg·d)*	230 (218,243)	235 (223,248)	0.553
SMR, *kJ/(kg FFM·d)*	334 (320,348)	331 (313,350)	0.830
AEE,[2,3] *kJ/d*	429 (175,683)	890 (624, 1155)	0.024
AEE,[2] *kJ/(kg·d)*	49.4 (19.4,79.3)	114 (82.5,145)	0.008
AEE,[2] *kJ/(kg FFM·d)*	71.0 (29.0,113)	154 (110,198)	0.014

[1] Values are means (with 95% CI), $n = 27$ middle SES, 25 low SES except TEE, $n = 32$ middle SES, 33 low SES.
[2] Values are adjusted for ethnicity.
[3] AEE calculated from TEE-MOEE.

not differ between groups (middle SES, 53.1% vs. low SES, 51.5%; $P = 0.897$). Energy and macronutrient intakes did not differ between middle SES [367 ± 89.0 kJ/(kg · d)] and low SES [404 ± 114 kJ/(kg · d); $P = 0.163$] infants (**Supplemental Table 1**), and were not correlated with weight, FFM, or FM. Ethnicity and sex were not confounding factors in the association of energy intake and SES.

Morbidity. There were no differences in morbidity between middle and high SES infants (**Supplemental Table 2**).

Mediating factors for TEE. Of the potential mediating factors, TEE (kJ/d) was positively correlated with intake of cow's milk (mL/d, $r = 0.272$, $P = 0.037$). TEE tended to be correlated with weight ($r = 0.200$, $P = 0.130$) and FFM (kg, $r = 0.200$, $P = 0.129$), but not with FM ($r = 0.07$, $P = 0.575$). TEE (kJ/d) tended to be negatively correlated with breast-milk intake (mL/d, $r = -0.230$, $P = 0.068$). Health status was not associated with TEE (kJ/d), nor were the number of days an infant presented diarrhea, fever, running nose, or cough correlated with TEE [kJ/d or kJ/(kg · d)].

Inclusion of the intake of breast milk or cow's milk into the model had no effect on the difference in TEE between groups.

Crowding as a mediating factor of AEE. AEE (kJ/d) (adjusted for ethnicity) was not correlated with any of the indicators of nutritional status and morbidity. AEE (kJ/d) tended to be positively correlated ($P < 0.10$) with crowding, and maternal weight. Adjustment for crowding reduced the difference in AEE between groups by >10%, to an extent that it was no longer significant; the unadjusted difference between SES groups was –460 (95% CI −856 to −64.7) kJ/d ($P = 0.024$); adjusted for crowding, the difference was reduced to –352 (95% CI −867 to 184) kJ/d ($P = 0.177$). Inclusion of maternal weight into the model had no effect on the difference in AEE between groups.

Discussion

For the purpose of development of growth references (30), measurements are usually done in infants from middle and high SES, to ascertain that growth is not compromised by suboptimal living conditions, and, for the same reason, the latest FAO/WHO/UNU estimations of energy requirements in infants have been restricted to developed countries (1). The authors of the FAO/WHO/UNU report, however, acknowledge the urgent need for TEE and BMR studies in developing countries, covering prevailing and changing lifestyles. Our study aimed to provide insight into the factors that might affect TEE in infants living under different socio-economic circumstances in a country undergoing the nutrition transition. Maternal education was chosen as the criterion for classification by SES because this information (but not income) could be obtained from the electronic birth registry (SINASC), and because an effect of maternal education independent of family income on child health outcomes had been observed in a 1982 birth cohort in Pelotas (31).

The study has both limitations and strengths. A strength of the study was the large sample size per study group. Post hoc power calculations, using means and SD of TEE observed, showed a statistical power of 96%. The large sample size also allowed an investigation of associations between components of TEE and ER and environmental, maternal, and infant characteristics. The cross-sectional study design could be considered a limitation, but for the purpose of the study (i.e., to compare TEE and ER between different socio-economic groups), adequate power at a fixed age was considered more important than having smaller samples at various ages.

The DLW methodology used in this study was modified from the usual procedure in that a dose of DLW was administered after first applying a dose-to-the-mother ^2H_2O dilution method for measuring breast-milk intake. The reason for administering the DLW dose after completing the breast-milk intake measurement was to avoid the possibility that expensive $H_2{}^{18}O$ would be given to infants who would subsequently drop out of the study. The consequence of this procedure was that there was ^2H_2O influx from breast milk during the DLW experiment. Ample consideration has been given to this issue in the analysis. The model applied to the isotopic data accounted for the ^2H_2O influx but because it is more complex than that normally used, the larger population CV of TEE [kJ/(kg · d)] observed in this study [25% compared with the 19% observed by Butte et al. (10) in 9-mo–old infants] is not unexpected. Isotope dilution spaces and fractional turnover rates indicate no methodological difficulties. The difference in TEE between SES groups was found in all terciles of the breast-milk intake distribution (results available from the author). Thus, the modified method may have been the origin of the larger population CV of TEE [kJ/(kg · d)] observed in this study but is unlikely to have contributed to a general or group-specific bias.

A priori, we hypothesized that TEE and ER could possibly be higher in low SES infants due to the strain of the environment, thereby increasing basal metabolic needs, for example, as the result of infections or for catch-up growth. However, although TEE was higher in low SES infants, we did not find a difference in MOEE or SMR, and therefore our a priori hypothesis was not confirmed. Rather, the low TEE in middle SES infants was the result of low AEE in those infants. Weight and height were higher in middle SES infants, as was the prevalence of overweight. Although energy intake did not differ between groups in this sample of infants, in the larger sample of 78 infants that were initially enrolled, energy intake tended to be lower in the high SES infants ($P = 0.08$). The differences in body composition and weight gain did not reach statistical significance, but were in the expected direction, and may well become significant in a larger sample, with adequate power. This may be a lesson for planning of future studies.

MOEE and AEE were available only from 52 of 65 infants. To maximize statistical power, 65 infants were included in the analysis for comparison of TEE and anthropometric variables. Results of these variables were similar in the subgroup of 52 infants from whom MOEE and AEE were available.

Analysis of covariance showed that the difference in AEE was mediated by crowding. Its inclusion into a multivariate model reduced the difference between groups to an extent that it was no longer significant. Crowding was the only environmental variable that had a significant impact on the difference in AEE between groups. Whether crowding refers just to the number of people per bedroom, or whether it should be considered in a broader sense, including other living or housing conditions, cannot be concluded from this study simply because of the limited number of socio-environmental factors included. The Brazilian Institute for Geography and Statistics uses crowding as an indicator of quality of life (32). We expect crowding to be inversely related to time spent sleeping. Infants from low SES live in small houses, sometimes sleep with 8 people in one room, and it is likely that they sleep or rest less and thus spend more energy on activity. Crowding is also likely to be related to housing facilities such as ventilation and heating but the real significance of the "crowding factor" remains to be unraveled.

Ethnicity was a confounder in the association of SES and TEE and AEE, and analyses were therefore adjusted for this factor. However, this does not imply that there is a genetic origin for the differences in TEE between white and nonwhite infants. Rather, if a regression model is used to explain differences between ethnic groups, SES appears as a highly significant mediator (results not shown). We should therefore conclude that the difference between ethnic groups is mediated through SES, and is phenotypic rather than genetic.

Comparison of mean TEE observed in this study (288 ± 72.8 kJ/(kg · d)) with findings from others at this age (see **Table 5**, 10,11,15,17,26,33–35) showed that it is slightly lower than usual found, though within the 95% CI of values published by Butte (10), and is approximately on the 25th percentile as published by Reichman et al. (35).

Whether low TEE is a risk factor for the development of obesity later in life is a matter of some controversy. Roberts et al. (36) found a higher prevalence of overweight in infants 1 y of age with low TEE, whereas Davies et al. (37) and Wells et al. (38) did not find an association between TEE at 3 mo and fatness at 2–3.5 y of age. Others found that energy intake at 3 mo, not expenditure, is a determinant of body size at 1 y of age (9). A follow-up of the Brazilian infants who participated in this study should contribute to understanding this question.

In conclusion, energy expenditure was found to be 24% higher in breast-fed infants from low SES compared with breast-fed infants from middle SES at 8 mo of age. The difference in TEE between middle and low SES infants is attributed to AEE, and reflects the difference in lifestyle between the categories of SES. Prevalence of overweight was also higher in middle SES infants, and although the longer-term implication of these findings is not known, it may be a matter of concern in relation to the development of obesity later in life. Furthermore, the findings question the extent to which TEE data, based on middle SES infants, should be considered normative for the age group.

TABLE 5 Summary of TEE values for infants at ~8 mo of age[1]

References and study specifics	Total energy expenditure, kJ/(kg · d)
This study, Southern Brazil	
$n = 33$ BF, MF, middle SES, 8.5 mo of age	258 ± 75
$n = 34$ BF, MF, low SES, 8.5 mo of age	318 ± 63
Gambia (26)	
$n = 19$ MF, age 6–9 mo of age	335 ± 65
$n = 8$ MF, age 9–12 mo of age	335 ± 50
Peru (15)	
$n = 22$ FF, age 3–18 mo of age, early catch-up phase	377 ± 50
$n = 19$ FF, age 3–18 mo of age, late catch-up phase	351 ± 42
UK (33)	
$n = 19$ BF, age 9.2 mo of age	326 ± 59
UK (35)	
$n = 16$ MF, 9 mo of age	310 ± 55
The Netherlands (11)	
$n = 22$ BF/FF, 8 mo of age	333 ± 40
USA (10)	
$n = 23$ BF, 9 mo of age	320 ± 50
Sweden (34)	
$n = 30$ MF, 9 mo of age	323 ± 34

[1] Values are crude means ± SD. BF, breast fed; MF, mixed fed; FF, formula fed.

Acknowledgments

We thank Mara Santos for general assistance, Marcia Carvalho for screening the subjects, Verina Silva for DLW administration, Mirian Franz and Clarita Alves for urine and saliva collection, Danton Duro Filho for measuring MOEE, Josiane Katrein and Ana Beatriz Palmeira for food-weighing, Patricia Vianna for application of the Bayley Scales of Infant Development, and Wilian Trinidade for data entry. We thank Dr. Elaine Albernaz for her help in finding the best method for applying DLW to the infants.

Literature Cited

1. FAO. Human Energy Requirements. Report of Joint FAO/WHO/UNU Expert Consultation. Rome, 17–24 October 2001. FAO Food and Nutrition Technical Report Series 1. 2004.
2. Lucas A, Ewing G, Roberts SB, Coward WA. Measurement of milk intake by deuterium dilution. Arch Dis Child. 1987;62:796–800.
3. Roberts SB, Coward WA, Ewing G, Savage J, Cole TJ, Lucas A. Effect of weaning on accuracy of doubly labeled water method in infants. Am J Physiol. 1988;254:R622–7.
4. Wells JC, Davies PS. Diet and behavioural activity in 12-week-old infants. Ann Hum Biol. 1995;22:207–15.
5. Davies PS, Wells JC, Hinds A, Day JM, Laidlaw A. Total energy expenditure in 9 month and 12 month infants. Eur J Clin Nutr. 1997;51:249–52.
6. Davies PS, Ewing G, Lucas A. Energy expenditure in early infancy. Br J Nutr. 1989;62:621–9.
7. Wells JC, Cole TJ, Davies PS. Total energy expenditure and body composition in early infancy. Arch Dis Child. 1996;75:423–6.
8. Butte NF, Wong WW, Ferlic L, O'Brian Smith E, Klein PD, Garza C. Energy expenditure and deposition of breast-fed and formula-fed infants during early infancy. Pediatr Res. 1990;28:631–40.
9. Stunkard AJ, Berkowitz RI, Stallings VA, Schoeller DA. Energy intake, not energy output, is a determinant of body size in infants. Am J Clin Nutr. 1999;69:524–30.
10. Butte NF, Wong WW, Hopkinson JM, Heinz CJ, Mehta NR, Smith EO. Energy requirements derived from total energy expenditure and energy deposition during the first 2 y of life. Am J Clin Nutr. 2000;72:1558–69.
11. De Bruin NC, Degenhart HJ, Gal S, Westerterp KR, Stijnen T, Visser HK. Energy utilization and growth in breast-fed and formula-fed infants measured prospectively during the first year of life. Am J Clin Nutr. 1998;67:885–96.
12. Salazar G, Vio F, Garcia C, Aguirre E, Coward WA. Energy requirements in Chilean infants. Arch Dis Child Fetal Neonatal Ed. 2000;83:F120–3.

13. Jiang Z, Yan Q, Su Y, Heson KJ, Thélin A, Piguet-Welsch C, Ritz P, Ho Z. Energy expenditure of Chinese infants in Guangdong Province, south China, determined with use of the doubly labelled water method. Am J Clin Nutr. 1998;67:1256–64.
14. Fjeld CR, Schoeller DA. Energy expenditure of malnourished children during catch-up growth. Proc Nutr Soc. 1988;47:227–31.
15. Fjeld CR, Schoeller DA, Brown KH. A new model for predicting energy requirements of children during catch-up growth developed using doubly labeled water. Pediatr Res. 1989;25:503–8.
16. Butte NF, Villalpando S, Wong WW, Flores-Huerta S, Hernadez-Beltran M, Smith EO. Higher total energy expenditure contributes to growth faltering in breast-fed infants living in rural Mexico. J Nutr. 1993;123:1028–35.
17. Vasquez-Velasquez L. Energy expenditure and physical activity of malnourished Gambian infants. Proc Nutr Soc. 1988;47:233–9.
18. Monteiro CA. D'A Benicio MH, Conde WL, Popkin BM. Shifting obesity trends in Brazil. Eur J Clin Nutr. 2000;54:342–6.
19. Peña M, Bacallao J. Obesity and poverty. A new public health challenge. Washington, DC: Pan American Health Organization. Pan American Sanitary Bureau, Regional Office of the World Health Organization; 2000.
20. Mondini L, Monteiro CA. The stage of nutrition transition in different Brazilian regions. Arch Latinoam Nutr. 1997;47:17–21.
21. Coward WA. Contributions of doubly labeled water method to studies of energy balance in the Third World. Am J Clin Nutr. 1998;68: 962S–9S.
22. Haisma H, Wells JC, Coward WA, Duro Filho D, Vonk R, Wright A, Visser GH. Complementary feeding with cows' milk alters sleeping metabolic rate in breast-fed infants. J Nutr. 2005;135:1889–95.
23. Cole T, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ. 2000;320:1240–3.
24. Haisma H, Coward WA, Albernaz E, Visser H, Wells JCK, Wright A, Victora CG. Breast milk and energy intake in exclusively, predominantly and partially breast-fed infants. Eur J Clin Nutr. 2003; 57:1633–42.
25. IAEA. The doubly labeled water method for measuring energy expenditure. Technical recommendations for use in humans. A consensus report by the IDECG working group. Vienna, Austria; 1990.
26. Vasquez-Velasquez L. Energy metabolism in children [PhD thesis]. Cambridge, UK: University of Cambridge; 1988.
27. Black AE, Coward WA, Prentice AM. Use of food quotients to predict respiratory quotients for the doubly labelled water method of measuring energy expenditure. Hum Nutr Clin Nutr. 1986;40:381–91.
28. Butte NF, Wong WW, Hopkinson JM, Smith EO, Ellis KJ. Infant feeding mode affects early growth and body composition. Pediatrics. 2000; 106:1355–66.
29. Rothman KJ, Greenland S. Modern epidemiology. 2nd ed. Philadelphia (PA): Lippincott Williams & Wilkins; 1998.
30. WHO. Working group on the growth reference protocol. A Growth Curve for the 21st Century: The WHO Multicentre Growth Reference Study. Protocol. 1998.
31. Victora CG, Huttly SR, Barros FC, Lombardi C, Vaughan JP. Maternal education in relation to early and late child health outcomes: findings from a Brazilian cohort study. Soc Sci Med. 1992;34:899–905.
32. IBGE. Censo demográfico 2000. 2000 [cited 2006 Sept 17]. Available from http://www.ibge.gov.br/censo/.
33. Davies PS, Ewing G, Coward WA, Lucas A. Energy metabolism in breast-fed and formula-fed infants. In: Atkinson SA, Hanson LA, Chandra RK, editors. Breast-feeding, nutrition, infection and growth in developed and emerging countries. St John's Newfoundland: Arts Biomedical; 1990. p. 521.
34. Tennefors C, Coward WA, Hernell O, Wright A, Forsum E. Total energy expenditure and physical activity level in healthy young Swedish children 9 or 14 months of age. Eur J Clin Nutr. 2003;57:647–53.
35. Reichman CA, Davies PS, Wells JC, Atkin L-M, Cleghorn G, Shepherd RW. Centile reference charts for total energy expenditure in infants from 1 to 12 months. Eur J Clin Nutr. 2003;57:1060–7.
36. Roberts SB, Savage J, Coward WA, Chew B, Lucas A. Energy expenditure and intakes of infants born to lean and overweight mothers. N Engl J Med. 1988;318:461–6.
37. Davies P, Day J, Lucas A. Energy expenditure in early infancy and later body fatness. Int J Obes. 1991;15:727–31.
38. Wells JC, Stanley M, Laidlaw AS, Day JM, Davies PS. The relationship between components of infant energy expenditure and childhood body fatness. Int J Obes Relat Metab Disord. 1996;20:848–53.