NAHRES-79

Vienna, 2004

CO-ORDINATED RESEARCH PROJECT

ON

ISOTOPIC EVALUATIONS IN INFANT GROWTH MONITORING – A COLLABORATION WITH WHO (PARTLY RCA)

Report on the Second and Final Research Co-ordination Meeting

Vienna, Austria, 12-14 November 2003

INTERNATIONAL ATOMIC ENERGY AGENCY

Isotopic evaluations in infant growth monitoring – a collaboration with WHO (partly RCA)

Report on the Second and Final Research Co-ordination Meeting

Vienna, Austria, 12-14 November 2003

NAHRES-79, IAEA, Vienna (2004)

Prepared by the

Section of Nutritional and Health-Related Environmental Studies
Division of Human Health
Department of Nuclear Sciences and Applications
International Atomic Energy Agency
P.O. Box 100, A-1400 Vienna, Austria
Scientific Secretary: Beatriz Miranda-da-Cruz

Single copies of this report are available cost-free on request from the above address

TABLE OF CONTENTS

PART I:
SUMMARY REPORT

SUMMARY REPORT

Participants: H. HAISMA (BRA), J. ALVEAR (CHI), Z.A. BHUTTA (PAK), AND J. WELLS (UK)

B. MIRANDA-DA-CRUZ (Scientific Secretary), N. MOKHTAR, G.V. IYENGAR
Section of Nutritional and Health-Related Environmental Studies, Division of Human Health, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, P.O. Box 100, A-1400, Austria.

INTRODUCTION

In the last few years, the World Health Organisation (WHO) has organised the development of new reference data for infant growth, reflecting the fact that previous growth reference data were based in part on formula-fed infants. With the benefits of breast-feeding for infant health increasingly recognised, and the differences in growth rate between breast-fed and formula-fed infants replicated in many populations, growth reference data from breast-fed infants became a priority. The importance of early growth for adult health is also increasingly appreciated, with numerous studies linking early nutrition and growth patterns to the risk of cardiovascular disease, obesity, type 2 diabetes and stroke in later life.

To support these new reference data, it is also important to assess the breast-milk and nutrient intakes of normally-growing breast-fed infants, in order to revise recommendations for their energy requirements. This coordinated research project (CRP) was therefore initiated by the International Atomic Energy Agency to investigate milk intake and growth in several populations from developing countries. A secondary aim was to continue the technological development of methodologies that allow the required measurements to be undertaken in free-living mother-infant pairs in field conditions.

The measurement of breast-milk intake was until recently undertaken using test-weighing, whereby the infant is weighed before and after each feed during one or more 24 hour periods. This method is intrusive, cumbersome, and unsuitable for many populations under field conditions. The development of the dose-to-the-mother deuterium oxide turnover method, by which maternal breast-milk transfer can be assessed by isotopic kinetics, has transformed the ability to measure milk intake in wholly or partially breast-fed infants in developing countries. The current CRP aimed to apply this technology to a variety of populations, ensuring that the data were comparable between countries through establishment of a standardised protocol.

The participating countries in this CRP were Bangladesh, Brazil, Chile and Pakistan for the measurement of growth and breast-milk intake. These countries, currently undergoing nutritional transition, all have active programmes for the promotion of breast-feeding. Results of the studies from Brazil, Chile and Pakistan were discussed during this final Research Coordination Meeting (RCM). Technological aspects of the measurements, with a view to future applications, were also discussed.

This is the report of the final RCM for the CRP on “Isotopic evaluations in infant growth monitoring – a collaboration with WHO”, held in Vienna, Austria between 12 – 14 November, 2003.

Brazilian study:

Energy requirements are a function of age, sex, and feeding mode. Work from others has demonstrated increased total energy expenditure (TEE) and sleeping metabolic rate (SMR) in formula-fed infants as compared to breast-fed infants. However, in practice many breast-fed infants also receive additional formula or cows’ milk. At 4 months, we found that energy intake (kcal/d) in breast-fed infants also receiving cows’ milk (BCFM) was 18% higher than that of infants who receive breast milk as the only source of milk (BM) (BCFM infants, 97.9 kcal/kg/d, BM infants, 83.1 kcal/kg/d, p=0.008). At 8 months increased minimal observable energy expenditure (MOEE) and SMR were found in BCFM infants (MOEE: BCFM infants, 51.6 kcal/kg/d, BM infants, 48.0 kcal/kg/d, p=0.041). TEE was also slightly higher, but not significantly so. Fat mass and fat mass index were higher in BCFM infants (p=0.016 and p=0.013).

Reference data for growth or energy requirements have been based on infants from high SES to assure that they would be growing optimally with no constraints to health. In developed countries, obesity used to be a problem in the higher SES families. In countries in transition, there is a shift of the prevalence of obesity towards lower SES infants, but this depends on the stage of transition. On the other hand, poor living conditions may result in growth faltering, and an increase in TEE and ER. We observed higher TEE in low SES as compared to high SES infants (high SES, 62.9 kcal/kg/d, low SES, 75.9 kcal/kg/d, p=0.005), MOEE was not increased, and the higher TEE in low SES infants should be contributed to high activity energy expenditure. Analysis of covariance showed that this should be contributed increased crowding in the low SES families.

In conclusion, the development of universally applicable values for energy requirements based on data from infants selected from particular social groups, without reference to feeding pattern, may not be acceptable.

At both 4 and 8 months, energy requirements of breast-fed infants in southern Brazil are well below existing international recommendations (20-25%), but similar to the new and recently published American estimated energy requirements (FNB), and values likely to emerge from recent WHO/FAO/UNU consultations.

The higher total energy intake of infants receiving cows' milk, their higher fat mass index at 8 months, and the low activity energy expenditure of high SES babies with a tendency towards a higher prevalence of obesity may have implications in relation to the current epidemic of obesity later life in Latin America and elsewhere. High SES babies receiving cows' milk may be a population group that particularly need attention in this respect.

Chilean study:

In the past few years there has been a great interest in the growth and energy requirements of breast fed infants, because their growth is different from that of artificially fed infants. In Chile, during the last two decades the number of mothers who breast feed their infants has increased: the last figures obtained in a country sample of 10,000 mother-infant pairs, showed that 45% of the mothers attending the Public Health System are exclusively breast-feeding their infant at 6 months of age. This figure could increase if our infants, could be evaluated by the adequate charts. The anthropometric study proposed by WHO was thought to benefit considerably from adding the determination of breast milk intake using isotopic dilution of deuterium to measure and compare intake of breast milk and water from non breast milk sources in exclusively breast fed infants. The method also provides the determination of body composition of the mother. We also included measurements of milk composition (fat, protein and lactose) and energy density of milk to correlate to energy supply, milk volume and growth.

The study was conducted in 328 mothers of a middle class community in Santiago de Chile selected at the prenatal clinic of Consultorio Recreo. Mean maternal age was 26.5 ±3 years, and pregnancy weight gain was 14.7±2 kg. The infants were 40 girls of 3248 ± 422 gr and 49 ± 2 cm at birth and 34 boys of 3451,1 ± 393 gr and 50,03 ± 2 cm at birth by normal delivery. All of them completed the protocol and received the dosis of 10gr of deuterium oxide (99%) Urine samples from the infant were collected on days 1,2,3,5,13,14 and from the mother on day 1,2,14. An additional dose of 0.2 gr/kg body weight was given to the infant at the end of the dose to mother protocol for the determination to total body water (TBW) in the infant. We followed the infants for 24 months. The antropometric data showed excellent growth of the group. The composition of the milk was comparable to data published in the literature. The body composition data showed that there is a slight difference in body composition between mothers that are exclusively breast feeding their infants compared to the ones that give some supplement. There is a positive correlation between amount of milk produced and growth both in weight and in size.

Pakistan study:

There is considerable evidence to support the view that the current growth standards for infants, which are in-use globally, may be inappropriate. This is based on the observation that these were derived from largely formula-fed western populations and recent studies documenting that exclusively breastfed young infants exhibit a lower growth trajectory. However, there are few studies objectively evaluating energy metabolism, body composition and growth in exclusively breastfed infants, and none in developing countries. We evaluated the growth pattern and breast milk and fluid intake patterns longitudinally in a representative sample of exclusively breast fed newborn infants in Pakistan. These newborn infants were well characterized at birth and sequential measurements of growth, body composition and energy expenditure were made using bio-impedance analysis and indirect calorimetry. In addition, breast milk intake was quantified using deuterium administration to the mother in a standard dose (10g) and estimation of urinary enrichment in exclusively breastfed infants and correlated with weight gain and growth.

A consecutive 112 mother baby pairs were studied longitudinally and breast milk intake was quantified in a subset of 12. The mean (± SD) maternal age was 27.5 ± 4.8 years and the weight 64.8 ± 7.9 kg, indicating the appropriate nutritional status of the population. The mean birth weight of the cohort was 3.13 ± 0.36 kg and the gestational age 3.13 ± 0.36 weeks. The mean body weight of the cohort at 2, 4, 6 and 12 months of age was 4.87 ±0.63, 6.40 ± 0.6, 7.56 ± 0.69 and 11.18 ± 1.05 kg respectively, which was better or comparable to many developed country cohorts of breastfed infants. The mean breast milk intake at 2 weeks, 3 months and 6 months of age was 591.7 ± 144.2, 794 ± 133 and 677.6 ± 298.6 ml/day respectively representing a range of 73-133 ml/kg/day. The maternal fluid intake for these corresponding time periods was 4.19 ± 1.12, 4.15 ± 1.25 and 3.96 ± 1.15 L/day respectively. The estimated non-breast milk fluid intake for the cohort for this period ranged from 6-18 ml/kg/day.

These data indicate that the growth performance and breast milk intake of exclusively breastfed infants in Pakistan was comparable to that observed among healthy breastfed newborn infants in developed countries and further support the continuation of these initiatives to improve early child health and development.

United Kingdom study:

Isotope probes (^{2}H, ^{18}O) can be used to investigate body composition, energetics and milk transfer in either mother or infant. The most comprehensive picture would be obtained where these measurements were made in both parties. This can be achieved either by successive measurements over a period of time, or by dosing the mother and simultaneously measuring isotope kinetics in both mother and infant. Simultaneous measurement of maternal milk transfer and infant energy expenditure could be made by dosing the mother with ^{2}H and the infant with ^{18}O, assuming that the infant ^{2}H kinetics can be quantified with sufficient precision to determine rate constants for both breast-milk influx and total water turnover. Nine mother-infant pairs were measured in Brazil, with error on energy expenditure found to be acceptable providing over 50% of total water intake was from breast-milk. Simultaneous dosing of mother and infant may therefore prove useful in some circumstances, particularly where rapid environmental changes might cause mismatches between measurements of energy intake and expenditure made in successive time periods.

Acknowledgement

The investigators would like to record their warm thanks for the many contributions of Dr Andy Coward in this CRP. Dr. Coward developed the dose-to-the-mother deuterium oxide turnover probe central to the CRP. He was instrumental in setting up the technique in each participating country; in providing technical and practical support throughout the duration of the work; in the mass-spectrometric analysis of the biological samples; in analysing and interpreting the data; and in aiding the participants to become familiar with the broader principles of stable isotope probes in human nutrition.

MAIN OUTCOMES

The main contribution of this CRP can be described as the successful application of the dose-to-the-mother deuterium oxide turnover method to assess breast-milk intake in field settings in several different developing countries. This has allowed the collection of objective data on breast-milk output and infant growth in free-living mother-infant pairs.

This CRP resulted in the following important contributions

1. Breast-milk volume intakes were observed to be similar both between the participating countries, and to data from industrialised countries.

2. Growth patterns were also observed to be similar between the participating countries, and to new reference data for breast-fed infants.

3. Variability between countries in these outcomes was observed to increase in later infancy, when the introduction of non-breast-milk foods/fluids increasingly influenced infant energetics and body composition.

4. Social-economic status significantly influenced infant energy utilisation in Brazil, as measured by doubly-labelled water; this finding has major implications for the universal applicability of recommendations based solely on infants of high socio-economic status.

5. Lactation counselling proved successful in promoting exclusive breast-feeding in Pakistan and Brazil, but there was no effect on milk volume output itself in Brazil.

6. The method proved readily applicable in diverse settings and populations, most of which had not previously utilised isotopic techniques; coordination between countries was also achieved.

7. Stable isotope probes can continue to be adapted and refined for utilisation in field settings, to increase the potential range of their applications.

8. This CRP has helped create a network of investigators who have worked successfully together during the project, and who will continue to participate in collaborative studies of this nature in the future.

9. With research increasingly emphasising (1) the benefits of breast-feeding for infant health, and (2) the associations early nutrition and growth and the risk of many childhood and adult diseases (obesity, type 2 diabetes, cardiovascular disease; stroke), the investigators believe that this research field is one of major importance for improving global health.

RECOMMENDATIONS

In view of the successful achievements of this CRP, the investigators feel further work in this area is of great importance. Specific recommendations are as follows:

1. Following initial investment in these unique cohorts of infants, follow-up studies are advocated in order to examine the relationship between early breast-milk intake and later growth and body composition.

2. The present studies addressed cohorts where maternal nutritional status was within the normal range; the application of this technique to (1) malnourished mothers and (2) low birth weight infants is now a priority.

3. Stable isotope measurements of body composition represent a high-quality approach for epidemiological studies of the relationship between early nutrition and later growth and body fatness.

SUMMARY OF RESULTS FROM THE PARTICIPANTS OF THIS CRP

Countries	**Total number of infants**	**Age group**	**Growth (kg, cm)**	**Milk volume intake (ml/d)**	**Other metabolic measurements**	**Comments**
BRAZIL	70	*4 months* BM BCFM	 6,700 – 63.3 6,600 – 62.6	 810 556	%fat mothers, 34% EI: BM, 83.1 kcal/kg/d; BCFM, 97.9 kcal/kg/d	%fat mother was not correlated to milk output in EBF babies
	62	*8 months* BM BCFM	 8,300 – 69.8 8,500 – 70.1	 783 512	MOEE: BM, 48.0 kcal/kg/d; BCFM, 51.6 kcal/kg/d FMI: BM, 4.5 kg/m^2; BCFM, 5.5 kg/m^2	BCFM infants have a higher MOEE, fat mass, and fat mass index
	67	High SES Low SES	8,600 – 70.5 8,200 – 69.4	673 650	TEE: high SES, 62.9 kcal/kg/d; low SES, 75.9 kcal/kg/d	High SES infants have lower TEE, due to reduced AEE; prevalence of obesity higher in high SES infants
CHILE	74 74 74	1 month 3 months 6 months	4,250 – 54.4 6,180 – 61.0 7,800 – 66.5	810 1,000 890	%fat mothers, 32% energy content milk 67 kcal/100 ml	Growth was positively correlated to milk intake in EBF babies; %fat mother was *positively* correlated to milk output

PAKISTAN	Total sample n=112, isotope work in n=12 12 12 12	 0.5 month 3 months 6 months	 4.47 - 55.2 5.74 - 60.9 6.75 - 68.6	 592 794 678	RMR* using indirect calorimetry (n=112) 48.8 kcal/kg/d 57.1 kcal/kg/d 70.2 kcal/kg/d	Growth rates were comparable to those reported from breastfed infants in developed countries and also those reported from affluent children in India All mothers were able to exclusively breastfeed for six months and estimated amounts of extra fluid intake were small Mean weight of the cohort at 12 months of age was 11.18 ± 1.05 kg which was higher than reported values from Brazil and Chile
UK/ BRAZIL	9	6-18 months	*	*	*	Methodological analysis demonstrated that measurements of milk intake can be combined with measurements of infant energy expenditure in order to study the relationship between energetics and milk transfer

*measurements were done in a supine position, but activity was increasing with age.

Abbreviations: AEE, activity energy expenditure; TEE, total energy expenditure; RMR, resting metabolic rate; MOEE, minimal observable energy expenditure; BCFM, breast-fed infants also receiving cows' milk or formula; BM, breast-fed infants not receiving any other milk; EBF, exclusively breast-fed infants; SES, socio-economic status

Part II:
Country Reports

ENERGY INTAKE AND REQUIREMENTS OF INFANTS IN SOUTHERN BRAZIL – THE INFLUENCE OF BREAST-FEEDING PATTERN AND SOCIO-ECONOMIC STATUS

H. HAISMA[1,5], W.A. COWARD[2], G.H. VISSER[3,4], R. VONK[3,4], J.C.K. WELLS[6,] C.G. VICTORIA[1]

[1]Universidade Federal de Pelotas, Departamento de Medicina Social, Pelotas, RS, Brazil
[2]MRC Human Nutrition Research, Elsie Widdowson Laboratory, Cambridge, UK;
[3]Groningen University, Zoological Laboratory, Haren, the Netherlands
[4]Centre for Isotope Research, Groningen, the Netherlands
[5]Groningen University, Dept of Nutrition and Metabolism, Groningen, the Netherlands;
[6]Institute of Child Health, MRC Childhood Nutrition Research Group, London, UK

Abstract

Energy requirements are a function of age, sex, and feeding mode. Work from others has demonstrated increased total energy expenditure (TEE) and sleeping metabolic rate (SMR) in formula-fed infants as compared to breast-fed infants. However, in practice many breast-fed infants also receive additional formula or cows' milk. At 4 months, we found that energy intake (kcal/d) in breast-fed infants also receiving cows' milk (BCFM) was 18% higher than that of infants who receive breast milk as the only source of milk (BM) (BCFM infants, 97.9 kcal/kg/d, BM infants, 83.1 kcal/kg/d, p=0.008). At 8 months increased minimal observable energy expenditure (MOEE) and SMR were found in BCFM infants (MOEE: BCFM infants, 51.6 kcal/kg/d, BM infants, 48.0 kcal/kg/d, p=0.041). TEE was also slightly higher, but not significantly so. Fat mass and fat mass index were higher in BCFM infants (p=0.016 and p=0.013).

Reference data for growth or energy requirements have been based on infants from high SES to assure that they would be growing optimally with no constraints to health. In developed countries, obesity used to be a problem in the higher SES families. In countries in transition, there is a shift of the prevalence of obesity towards lower SES infants, but this depends on the stage of transition. On the other hand, poor living conditions may result in growth faltering, and an increase in TEE and ER. We observed higher TEE in low SES as compared to high SES infants (high SES, 62.9 kcal/kg/d, low SES, 75.9 kcal/kg/d, p=0.005), MOEE was not increased, and the higher TEE in low SES infants should be contributed to high activity energy expenditure. Analysis of covariance showed that this should be contributed increased crowding in the low SES families.

In conclusion, the development of universally applicable values for energy requirements based on data from infants selected from particular social groups, without reference to feeding pattern, may not be acceptable.
At both 4 and 8 months, energy requirements of breast-fed infants in southern Brazil are well below existing international recommendations (20-25%), but similar to the new and recently published American estimated energy requirements (FNB), and values likely to emerge from recent WHO/FAO/UNU consultations. The higher total energy intake of infants receiving cows' milk, their higher fat mass index at 8 months, and the low activity energy expenditure of high SES babies with a tendency towards a higher prevalence of obesity may have implications in relation to the current epidemic of obesity later life in Latin America and elsewhere. High SES babies receiving cows' milk may be a population group that particularly need attention in this respect.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

Growth charts are widely used throughout the world for assessing the nutritional status of young children. They serve the purpose of a diagnostic tool in public health services. Both excess weight and underweight of an individual relative to the reference can be diagnosed, and action taken. Comparisons between populations can also be made. Currently used growth reference data (National Center for Health Statistics, NCHS, (1)) are based on US infants who were predominantly bottle-fed. Breast-fed infants appear to grow faster during the first 3 months of life, and gain less weight during the second half of infancy (6 to 12 months) (2-7). The apparent growth-faltering of breast-fed infants after 3 months of age relative to the NCHS reference has been thought to be a reason for early introduction of complementary foods. In developing countries, with poor sanitary conditions, this practice is known to increase infant morbidity and mortality, and this has been one of the reasons for the World Health Organisation (WHO) to undertake the construction of new growth reference curves for infants and young children until 71 months of age (8). The new growth reference curves will be based on breast-fed infants raised under optimal circumstances with no constraints to growth. In Brazil these are children from the high and middle socio-economic groups, and the database constructed will be normative (as opposed to descriptive). Pelotas was the first site where the Multicenter Growth Reference Study (MGRS) was implemented in 1998. Food intake data were also obtained to allow estimations of energy and macronutrient intake that correspond to the new growth curves.

In the past estimations of energy intake have been used by WHO/FAO/UNU as the basis for estimations of energy requirements (9). However, accurate assessment of food intake is difficult, and, in a review that was published in 1996 as a result of a meeting of the International Dietary and Energy Consultancy Group (IDECG), it was concluded that the 1985 WHO/FAO/UNU recommendations were overestimating metabolic needs by 9-39% (10). In the light of the increasing prevalence of obesity and associated degenerative diseases, such as non-insulin dependent diabetes, world-wide, this is a matter of growing concern. WHO was advised to modify the recommendations, and to base the estimations of energy requirements on measurements of energy expenditure and an added component for the energy cost of growth.

Energy requirements (ER) of infants and young children are defined as the energy intake (EI) that will balance energy expenditure at a level of physical activity consistent with normal development and allow for deposition of tissues at a rate consistent with health. ER are known to be a function of age, size, gender and feeding mode. TEE appears to be lower in breast-fed as compared to formula-fed infants (11-13), and this appears to be at least partly due to a difference in metabolic differences (higher sleeping metabolic rate in the latter (11)). Total energy expenditure (TEE) can be measured using doubly labelled water ($^2H_2{}^{18}O$, DLW), and be used as a basis for calculations of ER (14). Exclusively breast-fed (EBF) infants are the only group in whom energy utilisation can be accurately assessed from measurements of breast milk intake using the dose-to-the-mother deuterium-oxide (^2H_2O) turnover method (15-17). In combination with breast milk composition data (assuming that this is accurately known), energy intake can then be calculated. The 1996 review included an update of the estimations of energy requirements for infants based on measurements of TEE using DLW, but data during the second half of infancy were scarce, and an expansion of the database would be needed for WHO to justify a modification of the 1985 WHO/FAO/UNU estimations (10). Meanwhile additional studies of TEE in infants have been done, and in 2002, the Food and Nutrition Board (FNB) of the National Academy of Sciences (USA) published an update of the 1985 estimated ER (18), and the WHO/FAO/UNU modification is currently underway.

TEE is made up of basal metabolic rate (BMR), thermal effect of feeding (TEF), thermoregulation, energy cost of growth, and activity energy expenditure (AEE). Measurements of BMR require a high level of standardisation including 12-h fast, measured supine but awake. For obvious reasons, this standardised protocol is not feasible or ethical in infants. Sleeping metabolic rate (SMR) and minimal observable energy expenditure (MOEE) have been used in infants as approximations of BMR. MOEE is a more standardised entity than is SMR, and in the work described here MOEE has been used as the closest approximation of BMR in infants. AEE is calculated as the difference between TEE and MOEE.

It could be argued that in line with the new growth references (8, 19, 20), energy requirements should also be derived from breast-fed infants from high and middle socio-economic status (SES). However, 11.6% of the Brazilian population live in extreme poverty (income < 1 US dollar/day), with a prevalence of malnutrition and common infections such that they can be regarded as part of ordinary life, and this is known to increase ER (9). A study of the effect of SES on ER would provide insight into the impact of this reality. It has been generally accepted that feeding pattern (breast- versus formula-fed) influences energy utilisation, and modified recommendations of energy intake would take feeding pattern into account, however, no studies have addressed a possible effect of SES on TEE and ER. Such a study could be done in many countries, including European, where social inequity exists. Brazil is one of the countries with the highest level of social inequity in the world. By Latin American standards, the south of Brazil is relatively wealthy, but extreme poverty also exists. Thus Pelotas in Rio Grande do Sul, which is a city of 300.000 habitants was an appropriate site to study a possible effect of SES on energy requirements in infants. It was decided to use maternal education rather than income as a criterion for classification of infants by SES. The reason for this was that earlier work in Pelotas showed an association between maternal education and child health outcomes independent of family income. Low SES was defined as a maximum of 3 years of maternal education, and high SES included infants whose mothers had completed at least 8 years of education. To allow investigations of the effect of feeding pattern on TEE as a continuous variable, postulated as being a function of breast milk intake and intake of formula, cows' milk, or solid foods, all infants in the study were at least partially breast-fed.

The overall objective of the work was to assess energy intake (EI), total energy expenditure (TEE) and energy requirements (ER) of breast-fed infants in southern Brazil.

Specific objectives were:

1. To measure the effect of feeding pattern on EI, TEE, MOEE, AEE, anthropometric measures and body composition;
2. To measure the effect of SES on EI, TEE, MOEE, AEE, anthropometric measures and body composition.

Two studies were conducted to address these objectives:

1. Breast milk and energy intake in breast-fed infants aged 4 months of age with or without additional intake of formula or cows’ milk;
2. Components of energy expenditure in breast-fed infants aged 8 months of age from high and low SES.

2. METHODS

2.1. The effect of breast-feeding pattern on components of energy expenditure in 4 and 8 month old infants

2.1.1. Inclusion criteria

For the study of infants aged 4 months of age, the study used the same inclusion criteria as applied in the WHO Multicenter Growth Reference Study (8) as previously carried out at the Pelotas research centre. Every day of the week, mother-infant pairs were recruited from three main hospitals. Eligibility criteria were (19): 1) the mothers were living in the urban area of Pelotas, were non-smokers and were willing to breast-feed; 2) the babies were single births, gestational age was between 37 and 42 weeks and the post-natal stay at the intensive care unit was <24 hours; 3) family income was more than 800 R$ (reais). (At the time of the MGRS R$ 800 was equivalent to about USD 800; at the time of the our study this was USD 500 due to currency devaluation). Mothers who introduced formula or cow milk during the first 14 days after birth and those who started smoking during this period were excluded from participation.

For the study in infants aged 8 months of age, infants born single and term, with a birth-weight ≥ 2,500 kg, living in urban Pelotas, with no malformations were selected from a birth registry database (SINASC). As the study was initially designed to study differences in socio-economic classes (see section 2.2) rather than differences between feeding pattern, and subjects were selected on the basis of SES, all analyses studying a feeding group effect were initially adjusted for SES. However, the adjustment made no difference to the analyses, and results are therefore presented unadjusted.

2.1.2. Classification by feeding pattern

The infants were classified by feeding pattern as follows: 1) infants receiving breast milk as their only source of milk (BM infants); 2) breast-fed infants also receiving cows' or formula milk (BCFM infants).

2.1.3. Measurement of breast milk intake and energy expenditure

The dose-to-the-mother ^2H_2O turnover method (15-17, 21) was used to measure breast milk intake in infants aged 4 and 8 months of age, but at 8 months 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 (21), but in short the method involves the administration of 0.5 M (10 grams) of 99,8% deuterium to the mother, and collection of saliva samples from the mother (day 0 (pre-dose),1,3,13,14) and urine samples from the baby (day 0,1,2,3,13,14). 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 day 14 shortly after the collection of the day 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 pre-weighed tissues. Exact dose administered was calculated from the difference in weight of the dosing vial, syringe, nasogastric tube, and tissues pre- and post-dosing, and was on average 84% of the dose prepared. Subsequently urine samples were collected from the baby on days 15,16,17,20,21. During the field work, samples were stored on ice, and thereafter at –20°C. Samples were shipped unfrozen to the laboratory in Cambridge, UK for analysis using isotope ratio mass spectrometry.

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

For the mother:

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

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 Kmm is water turnover in the mother (1/d).

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

$$E_{b(t)} = \begin{cases} E_{m(0)}\left(\dfrac{F_{bm}}{V_{b(t)}}\right)\left(\dfrac{e^{-K_{mm}t} - e^{-\frac{F_{bb}}{V_{b(t)}}t}}{\dfrac{F_{bb}}{V_{b(t)}} - K_{mm}}\right) & t < t_{D2} \\ E_{m(0)}\left(\dfrac{F_{bm}}{V_{b(t)}}\right)\left(\dfrac{e^{-K_{mm}t} - e^{-\frac{F_{bb}}{V_b}t}}{\dfrac{F_{bb}}{V_{b(t)}} - K_{mm}}\right) + E_{b(D2)} e^{-\frac{F_{bb}}{V_{b(t)}}(t - t_{D2})} & t \geq t_{D2} \end{cases} \qquad 2$$

where $E_{b(D2)}$ is the initial ^2H isotopic enrichment (ppm) appearing as a consequence 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, mole*)* 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 (mole/d) and F_{bb} is total water loss in the baby (mole/d).

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

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

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, mole*)* 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 (mole/d).

Experimental data was simultaneously fitted to equations 1, 2 and separately to 3 using the "Solver" function in Excel® to minimise 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.* (21). For TEE, CO_2 production (r_{CO_2}, mole/d) was first calculated assuming that a constant proportion of the infants water turnover was fractionated (22):

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

where rate constants for isotope disappearance are: $K = \frac{F}{V}$ for ^{2}H and $K' = \frac{F'}{V'}$ for ^{18}O; normalised isotope distribution spaces (*N*, based on ^{2}H dilution, mole) are: $N = \left(\frac{V}{1.04} + \frac{V'}{1.01}\right)(0.5 \times 1.01)$ and (*N'*, based on ^{18}O dilution, mole) $N' = \left(\frac{V}{1.04} + \frac{V'}{1.01}\right)(0.5 \times 1.01)$;

fractionation factors are: $f_1 = 0.941$, $f_2 = 0.991$, $f_2 = 1.037$;
proportion of water losses fractionated (*x*) is assumed to be 0.2 for growing infants (23).

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

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

RQ was estimated from the average food quotient calculated from the composition of the total diet of the infants per study group (24). This was 0.87 in this study.

2.1.4. Complementary food intake

Intake of complementary foods was assessed using a frequency questionnaire (4 months) or by 1-day food weighing (8 months). Standardised Brazilian food composition tables were used for calculation of energy and macronutrient intake (25)

2.1.5. Total body water and body composition

Infant total body water (TBW) was calculated as the average of the isotope distribution spaces corrected for non-aqueous isotope exchange:

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

Fat free mass (FFM) was calculated using a hydration coefficient of 79.7% (26), and fat mass (FM) as the difference between body weight and fat free mass:
Fat free mass (kg) = TBW/0.797
Fat mass (kg) = Body weight at day 14 – FFM
A fat free mass index (FFMI), and fat mass index (FMI) were calculated from fat free mass (kg)/ height (m)^2, and fat mass (kg)/ height (m)^2 respectively (27).

2.1.6. Sleeping metabolic rate

Sleeping metabolic rate was measured by respirometry using a Deltatrac™ MBM-100. The head and part of the body of the infant were covered with a transparent plastic canopy, and the adult mixing chamber with an air-flow of 40 l/min was used to avoid accumulation of carbon-dioxide in the canopy during the time of the measurement. The use of this adult set-up of the Deltatrac in infants has been validated by Wells (13). Oxygen consumption, carbon-dioxide production and respiratory quotient were calculated by the Deltatrac software from the constant air flow and the down-stream gas concentrations, and the data were printed every minute. These data were subsequently entered into a computer and energy expenditure (cal/min) was calculated using Weir's formula (28): $1.106 * VCO_2 + 3.941 * VO_2$, where VCO_2 is carbon-dioxide produced (ml/min), and VO_2 is oxygen consumed (ml/min). SMR (kcal/d) was defined as the average of energy expenditure during the whole measurement period (40 minutes – 1 hour), and MOEE (kcal/d) was assessed as the average of the five consecutive lowest one-minute values for energy expenditure. Measurements were done at a time the baby would usually sleep. This could be any time of the day or night. Measurements done from 22.00 pm to 8.00 am were classified as night measurements. It was common that the baby was fed before the measurements, and then soothed to sleep. The length of a sleep cycle was assessed for the first 10 infants, and was found to be about 40 minutes. Subsequent measurements were therefore conducted for at least 40 minutes, but if possible measurements were continued for 1 hour. MOEE was considered to be the best standardised approximation of BMR, and analysis was primarily based on MOEE.

2.1.7. Classification of infant obesity

The prevalence of infant obesity was assessed using body mass index (BMI, kg/m^2). At the age of 12 months infants were classified as overweight or obese using cut-off points as suggested by Cole *et al.* (29). For girls cut-off points of 19.0 and 21.0 were used for overweight and obesity; for boys 19.5 and 21.5 were used.

2.2. The effect of feeding pattern and SES on components of TEE in infants aged 8 months of age

2.2.1. Classification by SES

An effect of maternal education independent of family income and education of the husband on late child health outcomes was observed from the 1982 Pelotas birth cohort (30). Maternal education was therefore used as a proxy for SES. A linear association was found between years of education and percentage of stunting at 6 months. Stunting was defined as a height-for-age < -2 Z-scores of the NCHS reference. The prevalence of stunting (n=1416) was 9.3% in infants whose mothers completed less than 4 years of education; 4.1% in infants whose mothers completed between 4 and 8 years of education; and 3.1% in infants whose mothers completed 8 or more years of education. The first category was defined as low SES, the last as high SES. The middle category was not included.

The studies were approved by the ethical committee of the Universidade Federal de Pelotas, and informed consent was given by the parents. At the end of the studies sent to the mothers for their information.

3. RESULTS

3.1. Effect of feeding pattern

3.1.1. Energy intakes of breast-fed infants 4 months old

3.1.1.1. Nutritional status and body composition

Table I shows the nutritional status of the infants. Weight gain since birth was not different between BM and BCFM infants (p=0.866). There were no differences in nutritional status or BMI between BM and BCFM infants.

TABLE I. ANTHROPOMETRIC INDICES OF 4-MONTH OLD INFANTS BY FEEDING PATTERN.

	BM (n=54)	BCFM (n=16)	p-value
Birth weight (kg)	3.2 (0.3)*	3.1 (0.3)	0.407
Length at birth (cm)	48.5 (1.9)	48.4 (1.2)	0.761
Weight at 4 mo. (kg)	6.7 (0.8)	6.6 (0.7)	0.612
Length at 4 mo. (cm)	63.3 (2.2)	62.6 (1.7)	0.211
Weight-for-age Z-score	0.27 (0.9)	0.28 (0.8)	0.961
Height-for-age Z-score	0.04 (0.8)	-0.10 (0.6)	0.514
Weight-for-height Z-score	0.19 (1.0)	0.37 (0.9)	0.516
Body mass index (kg/m^2)	16.7 (1.6)	16.9 (1.6)	0.781
Weight gained since birth (kg)	3.5 (0.7)	3.5 (0.7)	0.866

* means and SD

3.1.1.2. Food intake

Food intake data of those infants not receiving solids are presented in Table II. Breast milk intake was highest in BM infants (p=0.000), but total energy intake in BCFM infants was 18% higher as compared to BM infants (p=0.008). BCFM infants received 57.4% of their energy intake through breast milk, and 37.2% through formula (only 1 out of 11 infants received cows' milk); the remaining proportion was from tea or juices. As formula contains more protein and carbohydrates than breast milk, the percentage of energy provided by these macronutrients was higher in BCFM infants. On the other hand, the percentage of energy provided by fat was higher in BM infants, reflecting the higher fat content of breast milk. The slightly lower body weight of BCFM infants reversed this difference if fat intake per kg body weight was compared between groups. However, the difference was not significant (p=0.240). Protein intake expressed in g per kg body weight in BCFM infants was almost twice as high as in BM infants (p=0.000), and carbohydrate intake was also higher in BCFM infants (p=0.000).

TABLE II. MACRONUTRIENT INTAKE OF 4-MONTH OLD INFANTS BY FEEDING PATTERN.

	BM* (n=50)	BCFM* (n=11)	p-value
Breast milk intake (ml/d)‡	808 (756-859)†	556 (445-668)	*0.000*
Energy (kcal/kg/d)‡	83.1 (78.6-87.7)	97.9 (88.3-107.6)	*0.008*
Energy% from breast milk	97.1 (96.5-97.7)	57.4 (39.2-75.6)	*0.000*
Energy% from formula	0.1 (0.0-0.2)	37.2 (19.1-55.4)	*0.000*
Energy% from protein	6.5 (6.3-6.7)	9.1 (7.7-10.5)	*0.000*
Energy% from fat	52.0 (51.8-52.3)	46.1 (43.5-48.7)	*0.000*
Energy% from carbohydrate	41.2 (40.5-41.8)	44.2 (42.1-46.2)	*0.001*
Protein (g/kg/d)	1.3 (1.3-1.4)	2.3 (1.7-2.8)	*0.000*
Fat (g/kg/d)	4.8 (4.6-5.0)	5.2 (3.9-6.4)	0.240
Carbohydrate (g/kg/d)	9.0 (8.7-9.4)	11.7 (9.3-14.0)	*0.000*

* Infants receiving solids not included
† means and 95% CI
‡ adjusted for “mother working out of the house”

3.1.2. Components of energy expenditure in infants aged 8 months of age

3.1.2.1. Nutritional status and body composition

Table III presents results for nutritional status and body composition by feeding pattern. There were no differences in weight or length at birth and 8 months of age between BM and BCFM infants. BCFM infants tended to have gained more weight since birth as compared to BM infants (p=0.182). Body composition was different between the two feeding groups in that the BCFM infants had more fat. Both fat mass (kg) and fat mass index were higher in BCFM as compared to BM infants (p=0.016 and p=0.013). Overweight (as assessed by BMI using cut-off points as mentioned in section 2.1) was prevalent in 4.8% of the BM and 11.4% of the BCFM infants (p=0.402), and the odds ratio was 2.6 (p=0.291). Obesity was not present in any of the infants.

TABLE III. ANTHROPOMETRIC INDICES OF INFANTS AGED 8 MONTHS OF AGE AND THEIR MOTHERS BY FEEDING PATTERN.

	BM (n=42)	BCFM (n=35)	p-value
Birth weight (kg)	3.3 (0.4)*	3.3 (0.4)	0.559
Length at birth (cm)	48.7 (1.9)	48.8 (2.1)	0.770
Weight at 8 months (kg)	8.3 (1.0)	8.5 (1.1)	0.299
Length at 8 months (cm)	69.8 (2.3)	70.1 (3.0)	0.648
Weight gained since birth (kg)	4.9 (0.9)	5.2 (1.1)	0.182
Weight-for-age Z-score	-0.23 (1.0)	-0.01 (1.1)	0.355
Height-for-age Z-score	-0.07 (0.9)	0.05 (1.1)	0.730
Weight-for-height Z-score	-0.18 (0.8)	0.04 (1.0)	0.280
Fat mass (kg)	2.2 (0.8)	2.7 (0.9)	*0.016*
Fat free mass (kg)	6.2 (0.9)	6.0 (0.8)	0.471
Fat mass index (kg/m^2)	4.5 (1.5)	5.5 (1.7)	*0.013*
Fat free mass index (kg/m^2)	12.6 (1.6)	12.2 (1.0)	0.194

* means and SD

3.1.2.2. Food intake

In Table IV results of food and macronutrient intake are summarised. Breast milk intake was 783 ml/d in BM infants and 512 ml/d in BCFM infants (p=0.000). There was no difference in energy intake between BM and BCFM infants. The percentage of energy provided by breast milk was 69.3% in BM as compared to 43.6% in BCFM infants (p=0.000); 1.4% of the energy intake in the BM group was from cows' milk as compared to 19.7% in the BCFM group (p=0.000). The fact that even BM infants have some cows' milk intake will have been partly the result of the difference in time between the generation of the classification criteria (day 0 of the study), and the day of food-weighing (between day 7 and day 14 of the study). BCFM infants tended to get more energy from solids (p=0.115). The contribution of protein to energy intake was higher in BCFM as compared to BM infants (p=0.000), and the opposite was observed for the percent of energy percent provided from fat (p=0.005). Similarly, protein intake as expressed per kg body weight was higher in BCFM infants (p=0.008), and fat intake was higher in BM infants (p=0.007).

TABLE IV. ENERGY INTAKE IN 8-MONTH OLD INFANTS BY FEEDING PATTERN.

	BM (N=37)	BCFM (n=33)	p-value
Breast milk intake (ml/d)	783 (218)*	512 (372)	*0.000*
Energy intake (kcal/kg/d)	93.2 (25.4)	88.0 (25.6)	0.396
Energy% from breast milk	69.3 (21.8)	43.6 (31.8)	*0.000*
Energy% from cow’s milk	1.4 (3.7)	19.7 (19.7)	*0.000*
Energy% from solids	29.3 (20.3)	37.5 (22.4)	0.115
Energy% from protein	9.0 (1.7)	12.3 (3.5)	*0.000*
Energy% from fat	42.5 (8.8)	36.4 (8.7)	*0.005*
Energy% from carbohydrates	51.5 (7.0)	52.5 (6.0)	0.526
Protein intake (g//kg/d)	2.1 (0.7)	2.7 (1.1)	*0.008*
Fat intake (g/kg/d)	4.3 (1.2)	3.5 (1.2)	*0.007*
Carbohydrate intake (g/kg/d)	12.9 (4.2)	12.4 (4.3)	0.618

* means and SD

3.1.2.3. Components of energy expenditure

The results for energy expenditure are presented in Table V. TEE and AEE were not different between BM and BCFM infants, but MOEE was higher in BCFM as compared to BM infants. The difference was observed whether MOEE was expressed in kcal/d or kcal/kg/d or kcal/kg FFM/d (p=0.000, p=0.041, p=0.015).

TABLE V. COMPONENTS OF TEE IN 8-MONTH OLD INFANTS BY FEEDING PATTERN.

	BM	BCFM	p-value
TEE (kcal/d)°	568 (522-615)*	607 (556-657)	0.270
(kcal/kg/d)	68.4 (62.6-74.1)	69.8 (63.6-75.9)	0.744
(kcal/kg FFM/d)	94.1 (86.1-102.0)	101.4 (92.8-110.0)	0.218
MOEE (kcal/d)	399 (385-414)	444 (424-464)	*0.000*
(kcal/kg/d)	48.0 (46.0-50.1)	51.6 (48.6-54.6)	*0.041*
(kcal/kg FFM/d)	67.3 (64.0-70.5)	73.8 (69.5-78.1)	*0.015*
AEE (kcal/d)°	151.5 (95.8-207.1)	162.3 (100.1-224.5)	0.795
(kcal/kg/d)	19.2 (12.6-25.8)	18.6 (11.2-26.0)	0.904
(kcal/kg FFM/d)	26.0 (16.8-35.2)	26.8 (16.5-37.1)	0.905

°values are adjusted for ethnicity
*means and 95% CI

Analysis of covariance showed the effect of potential mediators (i.e. those with a correlation to MOEE p<0.10) on the difference in MOEE between feeding groups. Protein intake was the strongest mediator, reducing the difference between BM and BCFM infants from –44.0 to –27.7 (kcal/d) (p=0.028). The addition of weight into the model further reduced the difference to –23.8 (kcal/d) (p=0.059). MOEE was best explained by the following equation:

MOEE (kcal/d) = 251 + 2.3 * protein intake (g/d) + 15.8 * weight (kg) – 23.8 * feeding group,

where feeding group = 0 for BCFM infants, and 1 for BM infants.

3.2. Effect of socio-economic status

3.2.1. Components of energy expenditure in infants aged 8 months of age

3.2.1.1. Indicators of socio-economic status

Maternal education was used as a proxy of SES (see section 3.1). By selection, all mothers from high SES had at least 8 years of education, and all mothers from low SES no more than 3 years of education. On average, high SES mothers had 10.6 years and low SES mothers 2.0 years of education (p=0.000). Fathers in the high SES group had 9.5 years of education as compared to 3.8 years in the low SES group (p=0.000). Of the low SES families the majority (50.0%) had an monthly income of less than 1 MS, and 44.7% had an income between 1.1 and 3 MS; 5.3% had an income between 3.1 and 6 MS. In the high SES category, there were no families with less than 1 MS per month. The majority of high SES families (46.2%) had an income between 1.1 and 3 MS, and another 25.6% had an income of 3.1 to 6 MS; the remainder (38.2%) had an income higher than 6 MS, a category that was absent in the low SES families.

Working out of the house was more common amongst high SES mothers as compared to low SES mothers (p=0.047). Almost 50% of the low SES mothers smoked, as opposed to 17.9% of the high SES mothers (p=0.014). Tap water and a flushing toilet were available in all high SES houses, but of the low SES households tap water was available in 76.3% and flushing toilet in 68.4%.

3.2.1.2. Nutritional status and body composition

Table VI shows nutritional status and body composition by SES. There were no statistically significant differences in nutritional status and body composition between infants from high and low SES. However, high SES infants tended to have a higher birth weight (p=0.119), a higher weight at 8 months (p=0.123), they tended to be taller at 8 months (p=0.089), and to have gained more length since birth (p=0.163). BMI was not different between high and low SES infants (high SES, 17.2; low SES, 16.9; p=0.398). Overweight was prevalent in 12.8% of high SES and 2.6% in low SES infants, and the odds for overweight tended to be higher for high SES as compared to low SES infants (OR=5.6, p=0.131).

TABLE VI. ANTHROPOMETRIC INDICES OF INFANTS AGED 8 MONTHS BY SES.

	High SES(n=39)	Low SES (n=38)	p-value
Birth weight (kg)	3.4 (0.5)*	3.2 (0.3)	0.119
Length at birth (cm)	48.9 (2.1)	48.6 (1.9)	0.466
Weight at 8 months (kg)	8.6 (1.0)	8.2 (1.0)	0.123
Length at 8 months (cm)	70.5 (2.4)	69.4 (2.8)	0.089
Weight gained since birth (kg)	5.2 (1.0)	5.0 (1.0)	0.338
Length gained since birth (cm)	21.6 (2.1)	20.8 (2.6)	0.163
Weight-for-age Z-score	0.05 (1.0)	-0.31 (1.0)	0.123
Height-for-age Z-score	0.14 (0.9)	-0.22 (1.0)	0.097
Weight-for-height Z-score	-0.02 (1.0)	-0.14 (0.7)	0.553
Fat mass (kg)	2.6 (0.9)	2.3 (0.8)	0.218
Fat free mass (kg)	6.2 (0.7)	6.0 (1.0)	0.191
Fat mass index (kg/m^2)	5.1 (1.7)	4.8 (1.6)	0.396
Fat free mass index (kg/m^2)	12.5 (1.1)	12.4 (1.6)	0.773

* means and SD

3.2.1.3. Food intake

Food intake by SES is presented in Table VII. At the age of 8 months, breast milk intake was 662 (SD 324) ml/d, and still provided 58% (SD 29) of the energy intake. No statistically significant differences were observed between high and low SES infants, but energy intake (p=0.174) and fat intake (p=0.151) tended to be higher in low SES infants.

TABLE VII. ENERGY INTAKE IN 8-MONTH OLD INFANTS BY SES.

	HIGH SES (N=37)	Low SES (n=33)	p-value
Breast milk intake (ml/d)	673 (330)*	650 (323)	0.765
Energy intake (kcal/kg/d)	86.9 (23.9)	95.2 (26.8)	0.174
Energy% from breast milk	60.5 (31.1)	54.6 (27.8)	0.414
Energy% from cow's milk	7.7 (15.5)	12.2 (17.0)	0.252
Energy% from solids	31.9 (24.9)	34.2 (17.1)	0.656
Energy% from protein	10.7 (3.2)	10.2 (3.2)	0.581
Energy% from fat	39.2 (9.4)	40.4 (9.2)	0.596
Energy% from carbohydrates	52.6 (6.9)	51.2 (6.1)	0.370
Protein intake (g/kg/d)	2.3 (0.9)	2.4 (1.0)	0.574
Fat intake (g/kg/d)	3.7 (1.3)	4.2 (1.3)	0.151
Carbohydrate intake (g/kg/d)	12.3 (3.9)	13.1 (4.6)	0.404

* means and SD

3.2.1.4. Components of energy expenditure

Table VIII presents the components of TEE. TEE was higher in low as compared to high SES infants. The difference was significant if TEE was expressed non-normalised (p=0.054), or normalised for weight (p=0.005), or fat free mass (p=0.015). MOEE (kcal/d) was also different between groups (p=0.031), but this was attributed to the slightly higher weight and fat free mass in high SES infants, and the difference disappeared after normalisation for weight and fat free mass. AEE was higher in low SES infants, irrespective of whether data were expressed normalised for weight (p=0.022), fat free mass (p=0.030) or non-normalised (p=0.053).

TABLE VIII. COMPONENTS OF TEE IN 8-MONTH OLD INFANTS BY SES.

	High SES (n=33)	Low SES (n=34)	p-value
TEE (kcal/d)*	549 (498-599)†	622 (572-672)	0.054
TEE (kcal/kg/d)*	62.9 (56.8-68.9)	75.9 (69.9-81.8)	0.005
TEE (kcal/kg FFM/d)*	89.5 (80.9-98.0)	105.2 (96.8-113.6)	0.015
MOEE (kcal/d)	435 (416-453)	405 (384-425)	0.031
MOEE (kcal/kg/d)	49.5 (46.9-52.1)	49.8 (46.9-52.7)	0.865
MOEE (kcal/kg FFM/d)	70.7 (67.4-74.1)	69.5 (65.0-74.1)	0.661
AEE (kcal/d)*	112 (52 – 172)	204 (141-267)	0.053
AEE (kcal/kg/d)*	13.1 (6.0-20.2)	26.2 (18.7-33.6)	0.022
AEE (kcal/kg FFM/d)*	18.2 (8.4-28.0)	35.1 (24.9-45.4)	0.030

†means and 95% confidence intervals
*adjusted for ethnicity

Analysis of covariance showed that the effect of SES on TEE was mediated by energy, fat, and carbohydrate intake (they increased the difference between high and low SES infants); weight gained during the 21 days of the study was a positive mediator, i.e. it decreased the difference in TEE between high and low SES infants (p=0.079).

AEE was found to be mediated by crowdedness as shown in Table 13. Inclusion of crowdedness into a covariance model reduced the effect of SES on AEE to an extent that it was no longer significant (p=0.494). The association between AEE and crowdedness was described by the following regression equation:

AEE (kcal/d) = 48.7 + 48.7 * crowdedness (R^2=0.349, p=0.010)

4. DISCUSSION

4.1. Effect of feeding pattern

Growth and energy expenditure are known to be a function of sex, age, body size and feeding mode. Studies of the effect of feeding mode have focussed on the classification of infants into breast- or formula-fed infants, and found increased weight (2-6) and higher energy expenditure (12, 13, 31) of formula-fed infants. However, in practice in many countries breast-fed infants may also receive formula or cows' milk in addition to breast milk. The study of the difference in growth or energy expenditure between two sub-groups of breast-fed infants (i.e. BM and BCFM) was one of the aims of this work. BCFM infants at 4 months included mainly formula fed infants, whereas at 8 months all but one infant were fed cows' milk. The composition of different milks is presented in Table IX, and shows that the protein content of cows' milk and formula is higher than in breast milk. The contrary is true for fat, and carbohydrate content is higher in formula than in breast milk, but lower in cows' milk as compared to breast milk. In Brazil, at the time the studies were done, formula milk was higher in protein (2.2 g/100 ml) than the formula used at present in Brazil (1.5 g/100 ml) and in other parts of the world, and closer to cows' milk (3.6 g/100 ml) then most humanised milks at present. For the calculation of energy intake at 4 months, the value published by WHO of 67 kcal/100 ml was used (32). As evidence is increasing, that this may be an overestimation, in the later study in infants aged 8 months of age, 65 kcal/100 ml was used (18). Lower values have still been suggested (33).

TABLE IX. COMPOSITION OF INFANT MILKS.

Per 100 ml	**Breast milk - WHO**	**Breast milk - FNB**	**Cow's milk - IBGE**	**Formula – NAN Nestlé**
Energy (kcal)	67	65	61	67
Protein (g)	1.05		3.6	2.2
Fat (g)	3.9		3.0	2.9
Carbohydrates (g)	7.2		4.9	7.9

At 4 months of age we observed an 18% higher energy intake in BCFM infants as compared to BM infants. This difference was no longer found at 8 months, but there was a tendency for higher TEE in BCFM infants, suggesting that their energy utilisation may still be higher. The percentage of energy from protein was higher in BCFM infants as compared to BM infants at both 4 and 8 months, reflecting the higher protein content of formula and cows' milk. Percent energy from fat was lower in BCFM infants, due to the lower fat content of formula and cows' milk as compared to breast milk. Energy percentage from carbohydrate at 4 months was higher in BCFM, reflecting the higher carbohydrate content of formula as compared to breast milk. But at 8 months this difference was no longer observed.

Although energy intake was 18% higher in BCFM infants as compared to BM infants at 4 months, there was no difference in nutritional status or body composition at that age. At 8 months BCFM infants had more fat, a higher fat mass index, and a tendency towards more overweight. It would be tempting to conclude that the BCFM infants were overfed, and the above suggests that this is likely to be at least partly true, but alternatively energy intake needed to meet requirements could have been higher in the latter group for two possible reasons:

1) bioavailibility of nutrients from formula or cow milk is less than from breast milk;

2) BCFM infants may have higher energy requirements compared to BM infants because of metabolic differences. Although there is substantial evidence that absorption of nutrients from breast milk is higher compared to formula or cow milk (Food and Nutrition Board (FNB), 2002), this alone could not have explained the large differences in macronutrient and energy intake found. The second mechanism seems to be a more plausible explanation of the differences in intake between BM and BCFM found. At 8 months MOEE was higher in BCFM as compared to BM infants, and this is conform findings from Butte *et al.* (34) who found a higher SMR and MOEE in formula-fed as compared to breast-fed infants. TEE also tended to be higher in BCFM infants, although not significantly so. AEE was not different between groups.

In the latest review on energy requirements for infants, an 11% difference between breast- and formula fed infants at 4 months was described (Butte, 1996). At that age, we calculated total energy intake of BM infants to be 83 kcal/kg/d, which is about the same as the suggested modified energy requirements of breast-fed infants by Butte (10) and FNB (18). Total energy intake of BCFM infants was 100 kcal/kg/d, which is 20% above ER for breast-fed infants (10), and also 20% above the latest American estimated ER (18).

A covariance model showed that at 8 months the effect of feeding group on MOEE was mediated by: (1) protein intake, (2) body weight. The mediating effect from protein on MOEE could be partly explained by its contribution to TEF, of which protein intake is known to be the most important determinant (35). However, Butte *et al.* (34) actually measured TEF in breast- and formula-fed infants, and found no difference. In our 8 month old infants, we estimated that 20% of the difference in MOEE between feeding groups could be attributed to TEF. An additional explanation for the feeding group effect is from recent work from Hoppe *et al.* (36). They showed an association of intake of animal protein and circulating IGF-I. It was suggested by Rolland-Cachera *et al.* (37) that the increase of IGF-I would influence maturation and trigger adipocyte multiplication. They observed an association between high protein intake at the age of 2 years and a higher incidence of obesity at the age of 8 years. Similar results were obtained by Scaglioni *et al.* (38). Hoppe *et al.* (39) found that high intake of cows' milk, but not meat, increased s-IGF-I in 8-year old boys (39). This suggests that some bioactive factor in cows' milk other than protein could have been responsible for the metabolic differences found between BM and BCFM infants. An alternative explanation for the effect of feeding group found may be through breast milk or the process of breast-feeding. As all infants in the study were breast-fed, any influence of the feeding process itself would have to be related to time spent on the breast, or the volume of breast milk intake. Breast milk intake was negatively correlated with MOEE, but did not mediate the feeding group effect. Time spent on the breast was not measured in this study, but one would expect it to be related to the volume of breast milk intake. Nevertheless, some mothers would let their infants suckle for a long time after the real feeding process had stopped, and we cannot exclude the possibility of MOEE being reduced by time suckling. Apart from breast milk volume, the composition of breast milk as compared to cows' milk could also have contributed to the lower MOEE in BM infants. The fat content of breast milk is higher than in cows' milk, and this was reflected by the tendency for higher fat intake of BM infants. However, fat intake was not associated with MOEE in this study, and was not a mediator of the effect of feeding group. Breast milk is also known to contain benzodiazepine-like sedative compounds (40), and this has been suggested to decrease energy expenditure in BM infants (41). TEE (kcal/kg FFM/d) also tended to be higher in BCFM infants as compared to BM infants although not significantly so.

The definition of ER implies a condition of long term health. The discrepancy that exists in growth, fatness, and TEE between BM, BCFM, and CFM infants raises questions about the ER of each group. At least part of the difference in TEE between BM and CFM (31) or BM and BCFM can be explained by a higher MOEE of the CFM and BCFM infants, but their higher weight gain during the first year may be a matter of some concern. Rapid growth early in life has been linked to the development of obesity later on (42, 43), and the larger weight gain in formula-fed infants as compared to breast-fed infants during the first year of life may be a risk factor for the development of obesity, and suggests that their intake may have exceeded the requirements, resulting in fat storage. ER can therefore not simply be deduced from TEE and energy cost of growth. The question should always be: was growth healthy on the long term? Studies on this subject have however been inconclusive. Some (37, 38) suggest a role for early macronutrient intake and childhood obesity, whereas others (44, 45) find that factors other than infant feeding practices influence childhood obesity.

4.2. Effect of SES

The second aim was to study the effect of SES on growth and energy expenditure. For the purpose of development of references, measurements are usually done in infants from middle and high SES, assuring that growth would not be compromised by sub-optimal living conditions. Several criteria, such as income or education, but also more complex classification systems including income, profession, housing conditions have been used for the classification by SES. As suggested by Monteiro *et al.* (46), these classifications do not always give the same conclusions. They found a protective effect of maternal education on the prevalence of obesity in Brazilian adults, but income was a risk factor. In contrast, Post *et al.* (47) showed a protective effect of income on obesity (as assessed by WHZ>2) in 12 months old infants in Pelotas. Reanalysis of the data using BMI to categorise infants gave the same conclusions. However, analysis of the same cohort of infants using maternal education to categorise infants into three SES classes, showed increased obesity in the highest SES group (with maternal education more than 8 years, corresponding to the high SES group in our study). This indicates that whenever conclusions are made about the influence of SES on health outcomes, and comparisons between studies made, the criteria used for classification by SES should be taken into account.

Growth of middle and high SES infants (based on income) have been considered optimal, and for this reason these infants have been included in the WHO Multicenter Growth Reference Study (8). However, the above illustrates that the definition of optimal or sub-optimal living conditions in countries in transition is not straight forward, and decisions on which socio-economic classes to include for the development of reference data are difficult. The work described here aimed to provide insight into the differences between socio-economic classes as assessed by maternal education. Maternal education was chosen as the criterion for classification by SES, as an effect of maternal education independent of family income on child health outcomes had been observed in earlier Pelotas (30).

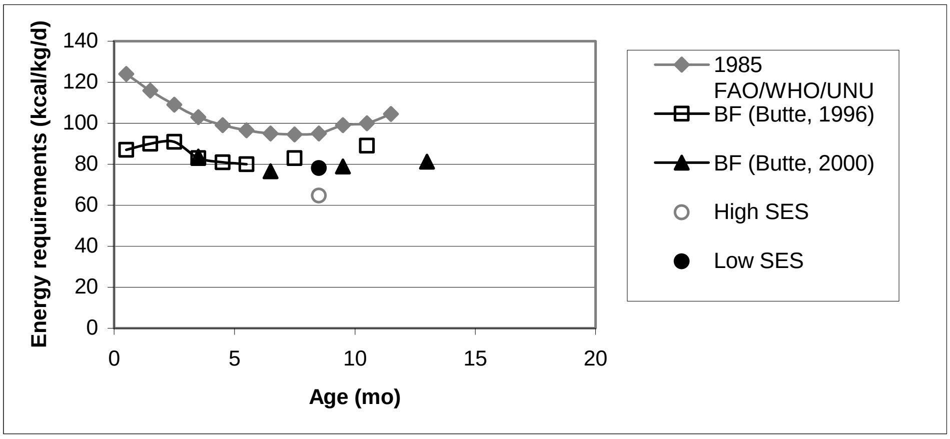

FIG.1. Energy requirements of breast-fed infants from high and low SES as compared with reference values.

The effect of SES on growth and energy expenditure was only studied at 8 months because in the study of 4 month old infants, all infants were from high SES. Energy intake tended to be higher in low SES infants, but the difference as compared to high SES infants was not significant. Similarly, high SES infants tended to be taller and heavier at 8 months, although not significantly so. Post-hoc analysis of statistical power using means and standard deviations for weight and length, and sample sizes achieved, resulted in a power of 42%. Lack of power seems therefore to have been the reason for the inability to detect a significant difference in weight and length in the study population aged 8 months of age. Within a larger sample of the Pelotas 1993 birth cohort, a significant difference in both weight and length was found at all ages throughout infancy, but BMI and prevalence of overweight or obesity was not different between categories of SES (results not shown). In contrast, in 8 months old infants, overweight (though not obesity) was prevalent in 12.8% of the high SES infants as compared to only 2.6% in the low SES infants, with an odds ratio of 5.6 (p=0.131).

With respect to the components of energy expenditure in high and low SES infants, TEE was found to be significantly higher in low SES infants. *A priori*, we had hypothesised that TEE and ER could possibly be higher in low SES infants as results of the strain of the environment, thereby increasing basal metabolic needs for example due to infections or for catch-up growth. This was not confirmed by our findings. Rather, it was AEE that explained a difference in TEE between high and low SES infants. Analysis of covariance showed that the difference in AEE was mediated by crowdedness. Its inclusion into a multivariate model reduced the difference between groups to an extent that it was no longer significant. Crowdedness is expected 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. The tendency towards a higher prevalence of overweight in high as compared to low SES infants, suggests that the low TEE in high SES infants may not be a desirable situation. Child development, however, was better in high SES infants, and this shows that a high AEE does not necessarily mean that more energy is directed towards development.

Comparison of TEE observed in this study with findings from others at this age (see Figure 1) showed that TEE in high SES infants was about 20-25% lower than usually observed, whereas TEE in low SES infants was similar to findings from others. The low values found for SES infants in this study are not significantly different (within 95% CI) from values published by Butte (48), and although a difference in TEE between categories of SES has not been described before, it is possible that a distinction between high and low SES infants in other studies would reveal similar results (10). The average of TEE of both groups of infants was 69.0 (SD 17.6).

It could be argued that the difference in AEE and TEE between high and low SES infants is typical only at this specific age. The age of 8 months is an age the infants start to sit, to play, to crawl, i.e. it is an age at which the infant's activity still depends to a large extent on the initiative from carers. Hypothetically, the difference could disappear at an age the infants start crawling and walking and become more independent. However, the association between AEE and crowdedness, and also the lack of an association between child development and TEE (49), suggest that it is life style that influences TEE. The Brazilian Institute for Geography and Statistics uses crowdedness as an indicator of quality of life (50) and it seems reasonable to assume that low SES infants will continue to live under the same circumstances that apparently result in higher AEE and TEE, and consequently low TEE in high SES infants may also persist in the future.

Whether or not low TEE is a risk factor for the development of obesity later in life is a matter of some controversy. Roberts *et al.* (51) did find a higher prevalence of overweight in infants 1 year of age with low TEE, whereas Davies *et al.* (52) and Wells *et al*. (53) did not find an association between TEE at 3 months and fatness at 2-3.5 years of age. Others found that energy intake at 3 months, not expenditure, is a determinant of body size at 1 year of age (54).

In conclusion, energy intake is higher in BCFM as compared to BM infants aged 4 months old. Although the difference in EI is no longer significant at 8 months, BCFM infants are fatter, and MOEE is higher in BCFM infants. The higher protein content in cows' milk explains some of the difference in MOEE between BCFM and BM infants, but some other bioactive factor in cows' milk is expected to contribute to the difference in energy metabolism.

SES also influences energy requirements, in that they are higher in low SES infants. The difference in TEE between high and low SES infants is attributed to AEE, and reflects the difference in life style between the categories of SES. The combination of the low TEE, low AEE and the tendency towards a higher prevalence of overweight in high SES infants is a matter of concern, that should be addressed by public health services.

REFERENCES

[1] Hamill PV, Drizd TA, Johnson CL, Reed RB, Roche AF. NCHS growth curves for children birth-18 years. United States. Vital Health Stat 11 1977:i-iv, 1-74.

[2] Dewey KG, Peerson JM, Brown KH, et al. Growth of breast-fed infants deviates from current reference data: a pooled analysis of US, Canadian, and European data sets. World Health Organization Working Group on Infant Growth. Pediatrics 1995;96:495-503.

[3] Haschke F, van't Hof MA. Euro-Growth references for breast-fed boys and girls: influence of breast-feeding and solids on growth until 36 months of age. Euro-Growth Study Group. J Pediatr Gastroenterol Nutr 2000;31 Suppl 1:S60-71.

[4] Victora CG, Morris S, Barros FC, Horta BL, Weiderpass E, Tomasi E. Breast-feeding and growth in Brazilian infants. Am. J. Clin. Nutr. 1998;67:452-8.

[5] Whitehead RG, Paul AA. Growth charts and the assessment of infant feeding practices in the Western world and in developing countries. Early Hum. Dev. 1984;9:187-207.

[6] Whitehead RG, Paul AA, Cole TJ. Diet and the growth of healthy infants. J Hum Nutr Diet 1989;2:73-84.

[7] WHO. Working Group on Infant Growth. An evaluation of infant growth. WHO/NUT/94.8 1994.

[8] WHO. Working Group on the Growth Reference Protocol. A Growth Curve for the 21st Century: The WHO Multicentre Growth Reference Study. Protocol. 1998.

[9] WHO. Energy and protein requirements. Report of a Joint FAO/WHO/UNU Expert Consultation. Technical Report Series 1985;724.

[10] Butte NF. Energy requirements of infants. Eur. J. Clin. Nutr. 1996;50:S24-S36.

[11] Butte NF, Wong WW, Ferlic L, Smith EO, Klein PD, Garza C. Energy expenditure and deposition of breast-fed and formula-fed infants during early infancy. Pediatr Res 1990;28:631-40.

[12] Davies PSW, Ewing G, Coward WA, Lucas A. Energy metabolism in breast-fed and formula-fed infants. In: Atkinson SA, Hanson LA, Chandra RK, eds. Breast-feeding, nutrition, infection and growth in developed and emerging countries. St John's Newfoundland: Arts Biomedical, 1990:521.

[13] Wells JCK. Energy metabolism in breast-fed and formula-fed infants. Cambridge: Cambridge University, 1994.

[14] Coward WAAJCNsS-S, 1998. Contributions of doubly labeled water method to studies of energy balance in the Third World. Am J Clin Nutr 1998;68:962S-969S.

[15] Butte NF, Wong WW, Patterson BW, Garza C, Klein PD. Human-milk intake measured by administration of deuterium oxide to the mother: a comparison with the test-weighing technique. Am. J. Clin. Nutr. 1988;47:815-21.

[16] Coward WA, Cole TJ, Sawyer MB, Prentice AM. Breast-milk intake measurement in mixed-fed infants by administration of deuterium oxide to their mothers. Hum. Nutr. Clin. Nutr. 1982;36:141-8.

[17] Orr-Ewing AK, Heywood PF, Coward WA. Longitudinal measurements of breast milk output by a 2H2O tracer technique in rural Papua New Guinean women. Hum. Nutr. Clin. Nutr. 1986;40:451-67.

[18] Food and Nutrition Board (FNB) IoMI. Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Protein and Amino Acids (Macronutrients). Washington, D.C.: National Academies Press, 2002.

[19] De Onis M, Victora CG, Garza C, Frongillo E, Cole T. A new international growth reference for young children. In: Dasgupta P, Hauspie R, eds. Perspectives in human growth, development and maturation. Dordrecht, the Netherlands: Kluwer Academic Publishers, 2001.

[20] WHO. Working Group on the Growth Reference Protocol and WHO Task Force on Methods for the Natural Regulation of Fertility. Growth patterns of breastfed infants in seven countries. Acta Paediatr. 2000;89:215-22.
[21] Haisma H, Coward WA, Albernaz E, et al. Breast milk and energy intake in exclusively, predominantly and partially breast-fed infants. Eur J Clin Nutr in press.
[22] Prentice AM, ed. The doubly labelled water method for measuring energy expenditure. Technical recommendations for use in humans. A consensus report by the IDECG Working Group. Vienna: International Atomic Energy Agency, 1990.
[23] Vasquez-Velasquez L. Energy metabolism in children. Darwin College. Cambridge, UK: University of Cambridge, 1988.
[24] Black AE, Prentice AM, Coward WA. 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.
[25] IBGE. Estudo Nacional da Despesa Familiar - ENDEF - Tabelas de composição de alimentos. Rio de Janeiro, 1981.
[26] Butte NF, Wong WW, Hopkinson JM, Smith EO, Ellis KJ. Infant feeding mode affects early growth and body composition. Pediatrics 2000;106:1355-66.
[27] Van Itallie TB, Yang MU, Heymsfield SB, Funk RC, Boileau RA. Height-normalized indices of the body's fat-free mass and fat mass: Potentially useful indicators of nutritional status. Am J Clin Nutr 1990;52:953-959.
[28] Weir JBdV. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol 1949;109:1-9.
[29] Cole T, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ 2000;320:1-6.
[30] 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.
[31] 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.
[32] WHO. Complementary feeding of young children in developing countries: a review of current scientific knowledge. WHO/NUT/98.1 1998.
[33] Lucas A, Ewing G, Roberts SB, Coward WA. How much energy does the breast fed infant consume and expend? Br Med J (Clin Res Ed) 1987;295:75-7.
[34] Butte NF, Smith EO, Garza C. Energy utilization of breast-fed and formula-fed infants. Am J Clin Nutr 1990;51:350-8.
[35] Kleiber M. Calorigenic effect of food. The fire of life. New York: John Wiley & Sons, Inc., 1961:266-278.
[36] Hoppe C, Udam TR, Lauritzen L, Molgaard C, Juul A, Michaelsen KF. Intake of animal protein, s-IGF-I and growth in healthy 2,5 year old Danish children. FASEB. San Diego, California, 2003.
[37] Rolland-Cachera MF, Deheeger M, Akrout M, Bellisle F. Influence of macronutrients on adiposity development at follow up study of nutrition and growth from 10 months to 8 years of age. Int J Obes 1995;19:573-8.
[38] Scaglioni S, Agostoni C, De Notaris R, et al. Early macronutrient intake and overweight at five years of age. Int J Obesity 2000;24:777-781.
[39] Hoppe C, Molgaard C, Juul A, Schack-Nielsen L, Michaelsen KF. High intakes of milk protein increase s-IGF-I in eight-year-old Danish boys. Int J Obesity 2001;25 (Suppl 2):S65.

[40] Dencker SJ, Johansson G, Milsom I. Quantification of naturally occurring benzodiazepine-like substances in human breast milk. Physchopharmacology 1992;107:69-72.
[41] Wells JCK. Parent-offspring conflict theory, costly begging behavior, signaling of nutritional need, and differential weight gain in early life. Quarterly review of biology 2003;78:169-202.
[42] Stettler N, Zemel BS, Kumanyika S, Stallings VA. Infant weight gain and childhood overweight status in a multicenter, cohort study. Pediatrics 2002;109:194-9.
[43] Stettler N, Kumanyika SK, Katz SH, Zemel BS, Stallings VA. Rapid weight gain during infancy and obesity in young adulthood in a cohort of African Americans. Am J Clin Nutr 2003;77:1374-8.
[44] Zive MM, McKay H, Frank-Spohrer GC, Broyles SL, Nelson JA, Nader PR. Infant-feeding practices and adiposity in 4-y old Anglo- and Mexican-Americans. Am J Clin Nutr 1992;55:1104-8.
[45] Agras WS, Kraemer HC, Berkowitz RI, Hammer LD. Influence of early feeding style on adiposity at 6 years of age. J Pediatr 1990;116:805-9.
[46] Monteiro CA, Conde WL, Popkin BM. Independent effects of income and education on the risk of obesity in the Brazilian adult population. J Nutr 2001;131:881S-886S.
[47] Post CL, Victora CG, Barros FC, Horta BL, Guimarães PRV. Desnutrição e obesidade infantis em duas coortes de base populacional no Sul do Brasil: tendências e diferenciais (Infant malnutrition and obesity in two population-based birth cohort studies in southern Brazil: trends and differences). Cad. Saúde Públ. 1996;12:49-57.
[48] 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.
[49] Haisma H, Coward WA, Visser GH, Vonk R, Wells JC, Victora CG. Influence of socio-economic and environmental factors on total and activity energy expenditure in breast-fed infants in southern Brazil. submitted.
[50] IBGE. Censo Demográfico 2000. 2000.
[51] 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.
[52] Davies P, Day J, Lucas A. Energy expenditure in early infancy and later body fatness. Int J Obesity 1991;15:727-31.
[53] 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.
[54] 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.

BREASTFEEDING AND GROWTH IN A GROUP OF SELECTED 0 TO 24 MONTHS INFANTS

ALVEAR J., SALAZAR G., BERLANGA R., ANCIANI A., PIZZARRO F

Instituto de Nutricion y Tecnologia de los Alimentos University of Chile, Santiago de Chile

Abstract

In the past few years there has been a great interest in the growth and energy requirements of breast fed infants, because their growth is different from that of the artificially fed infants. In Chile during the last two decades the number of mothers who decide to breast feed their infants has increased: the last figures obtained in a country sample of 10,000 mother-infant pairs, shows that 45% of the mothers attending the Public Health System are exclusively breast-feeding their infants at 6 months of age. This figure could increase if our infants, could be evaluated by the adequate charts. The anthropometrics study proposed by WHO was thought to benefit considerably from adding the determination of breast milk intake using isotopic dilution of deuterium to measure and compare intake of breast milk and water from non breast milk sources in exclusively breast fed infants. The method also provides the determination of body composition of the mother. We also include milk composition (fat, protein and lactose) and energy density of milk to correlate energy supply, milk volume and growth. The study was conducted in 328 mothers of a middle class community in Santiago de Chile selected at the prenatal clinic of "Consultorio Recreo". Mean maternal age was 26.5+-3 years, and pregnancy weight gain was 14.7+- 2 Kg. All of them completed the protocol and received the doss of 10gr of deuterium oxide (99(%) Urine samples were collected on days 1,2,3,5,13,14,on the infant and 1,2,14 on the mother. An additional dose of 0.2 gr/kg body weight was given to the infant at the end of the dose to mother protocol for the determination to total body water (TBW) in the infant. We followed the infants for 24 months. The anthropometrics data showed excellent growth of the group. The composition of the milk was comparable to the published in the literature. The body composition data showed that there is a slight difference in body composition between mothers that are exclusively breast feeding their infants compared to the ones that give some supplement. There is a positive correlation between amount of milk produced and growth both in weight and in size.

1. INTRODUCTION

In the past few years, there has been great interest in the growth and energy requirements of breast fed infants. Breast fed infants tend to gain weight more in the first trimester of life but are lighter than artificially fed infants after 6 months of age [1,2], for that reason WHO is undertaking a combined growth study in several countries, in order to establish adequate growth curves for breast-fed infants. Present growth charts in use, were obtained in a sample of breast and artificial feeding infants, which have overestimated the growth needs. In our country during the last two decades the number of mothers who decide to breast-feed their infants has permanently grown, due to the implementation of health care programs that promote, mother-infant interaction right after birth, in all Public Hospitals, and education programs for the mothers, during both antenatal and post natal control of mother and child. The last figures obtained in a country sample of 10,000 mother-infant pair, shows that 45% of the mothers are exclusively breast-feeding their infants at 6 months of age. This figure could increase if our infants were evaluated by the adequate charts, because it is of common knowledge that many health professionals suggest to stop breast feeding because the infant is not growing according the charts in use, [3,4,5,6].

The anthropometric study proposed by WHO was thought to be benefited considerably by adding the determination of breast-milk intake using isotopic dilution of deuterium, a standard procedure proposed by Coward et al [6-11] and extensively used in our country [11-

15], to measure and compared intake of breast milk and water from non breast milk sources in exclusively breast fed infants. The proposed study will also include milk composition (fat, protein and lactose) and energy density of milk to correlate energy supply, milk volume and growth. The data collected at each site must be comparable with respect to selection of the study population, reliability of measurements and compliance with the recommendations on breast feeding and absence of constraints on growth.

2. METHODS

The study was conducted in Santiago de Chile as part of a multicentric WHO study that combines a longitudinal design from birth to 24 months with a cross sectional study of children aged 18-71 months from the same site.. The project activities started in March of 2000, with the definition and selection of the Health area in which the project was going to be done, and the selection and capacitating of the personnel. The number of subjects to be studied and the protocol was redefined after the Co-ordinated Research Project at Vienna meeting in May of 2000.

3. SUBJECTS

The study was done in a population with no socio-economic constraints on growth, low mobility, 50 % or more of the mothers following feeding recommendations and access to breast feeding support. Mothers (n=328) were selected at the prenatal clinic of Consultorio Recreo in the San Joaquin County located in Santiago Metropolitan Region at 780m of altitude (Servicio de Salud Metropolitano Sur) that has 37500 people under control of a 50000 population 50% of them being females. During 1999 a total of 150 women assisted monthly to prenatal control to the prenatal clinic and 230 infants were born at the Maternity of the Barros Luco Troudeau that serves that Health area. This Hospital joined to the UNICEF and Chilean Ministry of Health program " Iniciativa Hospitales Amigos del niño y de la Madre IHANM", in 1992 (3) From the 150 women recruited, it is expected to have at least 75 mother-infant ending the study. The whole sample will be in exclusive lactation at 1 month (n=150) in 60% of sample by sixth month (n=90), and in partial breastfeeding, 50% of sample (n=75) by month 12th.

3.1. Inclusion criteria

Individual mothers selected for the study were of middle socio-economic status willing to follow feeding recommendations, non-smoking and non drug abuser. In the case of alcohol consumption, this was recorded, but alcoholics were not included. All infants were delivered at term and had no chronic health problems at birth expected to influence growth.

Feeding recommendations were: predominant breast-feeding that is breast-feeding alone or with non milk fluids for at least six months, introduction of appropriate complementary foods by six months and partial breast feeding for 12 months or more. As an additional request, the selection will consider:

- Those with a previous good breastfeeding experience;
- House wives (or women working at home);

- Term and vaginal single birth deliveries whithout significant perinatal morbidity (a stay of 12 or more hours in the intensive care unit will inhibit the baby to be incorporated to the sample).

3.2. Exclusion criteria

Lack of compliance with feeding recommendations;
Serious illness;
Voluntary exclusion;
Infants recruited into the study, whose mothers fail to comply, were followed throughout the two years in order to compare their growth with compliers.

Women who were followed during the prenatal clinic, received feeding recommendation and were willing to participate in the protocol were asked to participate in the study.

3.3. Follow-up

The mother infant dyad was visited and controlled in the first 36 hours after delivery at the maternity wards of the Hospital Barros Luco Troudeau that is the local Hospital that gives attention to this population.
They were visited at home and controlled at the out patient clinic on weeks 1,2,4,6 and 8 and monthly from 3 to 12 months; in the second year visits were controlled every two months.

1) Anthropometric determinations at every visit from birth onwards: (According WHO protocol)[16]

- Weight and length, hip and waist circumferences at birth;
- Arm, calf, circumferences at every visit starting 7 days of age;
- Skinfold thickness: (triceps, biceps, hip, subscapular, and calf) measured at every visit starting at 6 months;
- Head circumference was measured 1 week after birth, due to head moulding;
- Height of both parents were measured once at the first control at the out patient clinic and the mother weighed at each visit.

2) Socio-economic demographic and environmental characteristics, were measured using the Alvarez test. This test has been validated for our population since 1975 and measures: family structure, education, goods and working conditions.

3) Measurement of breast-milk volume by deuterium dilution

This evaluation was done at 1, 3, 6, 12 months. To this effect, the dose-to-mother method was used, which is able to measure maternal milk intake, as non-milk liquid ingested by the infant. [6,7]

A basal sample will be collected in both infant and mother on day 0, before the administration to the mother, of a standard dose of 10 g of deuterium oxide (99.9%). Urine samples were collected on days 1,2,3,5,13,14 on the infant and 1,2,14 on the mother.
An additional dose of 0.2g/kg weight was administered to the infant at the end of the dose-to-mother protocol, for the determination of total body water (TBW) in the infant.

4) Body composition of infant-mother dyad was determined by deuterium dilution at month 1,3,6,12.
The procedures described above, will permit the determination of body composition in the mother (dose-to mother protocol) and the infant (dose-to-infant), by establishing total body water (TBW) by the back-extrapolation procedure.
Using hydration coefficients, fat-free mass and fat mass (by difference to weight) will be established in the mother and the infant
Analysis of samples will be conducted in a HYDRA mass spectrometer, Europe Scientific, (Crewe UK) in the Mass Spectrometry Section of the Energy Metabolism and Stable Isotopes Laboratory, INTA, University of Chile.

5) Milk composition and energy density: fat, protein and lactose
Individual milk samples were pooled by collection of samples before and after the baby was fed during the day. Milk was stored at -20ºC.

Energy content of milk was obtained by bomb-calorimeter. Content of fat and protein was determined by standard procedures at The Food Analyses Centre, INTA, University of Chile. Lactose was determined by difference with energy in content of milk.

3.4. Ethical considerations

The family participating was asked to sign a written consent form (ethical approval by local and international standards).

The whole sample was in exclusive lactation at 1 month (n=150) in 60% of sample by sixth month (n=90), and in partial breastfeeding, 50% of sample (n=75) by month 12th.

3.5. Macronutrient intake

The volume of intake of water, juice formula or supplemented formula was obtained using a Food frequency and amount questionnaire (FQ). For this study, estimation of macronutrient intake was restricted to EBF, PBF and BF+supplemented FM, and solid food. Intake of energy (kJ/day) protein (g/day, fat (g/day) and carbohydrates (g/day) from food other than BM was estimated using Chilean food composition table (Tabla de Composición Química de Alimentos Chilenos. Facultad de Ciencias Químicas y Farmacológicas, U. de Chile, 7a Edición, 1985.

3.6. Measurement of breast milk intake

Breast milk intake was measured using the dose to the mother deuterium- oxide turnover technique (Coward et al) 1982 Orr-Ewing et al, 1986 Butte et al 1988. This technique also allows estimation of non-breast milk water intake, and the mothers body composition. A baseline sample of 2ml of urine from the mother and a urine sample from the child were collected on day 0, after mother received an oral dose of 10g (0,5mol) deuterium, the quantity being determined to the nearest 0.01g using an analytical scale. A further three urine samples from the mother (days 1,4,14) and another five urine samples from the baby (days 1,3,4,13,14) were then collected over a 14 day period. The time of collection was recorded. Small pieces of cotton wool balls were used to collect urine. The cotton balls were placed in clean diapers, which were checked every 10 m. After urination the sample was released by compressing them in a syringe. The samples were stored in special glass containers and then in boxes provides by us and kept in the mothers freezers before taking them to our laboratory were they were stored a –20ºC, until the end of the study. All the samples were then analysed in our Laboratory.

Calculation
Intake of breast milk and water from non milk sources was calculated by fitting the isotopic (tracer) data to a model for water (tracee) turnover the mothers and infants and the transfer of milk from the mother to the baby [6,7]).

3.7. Anthropometry

Anthropometry at birth, and follow up of each baby was done at the Hospital in the first 24 hours after birth and in each control at the out patient clinic by the Paediatrician responsible of the investigation and by a trained field worker. The deuterium study and mother infant anthropometry was done by the same group. The infants were weighted at the outpatient clinic without clothes using a portable electronic scale accurate to a 0.1gr. Length was measured using a standarise anthropometer CMS Weighing equipment LTD London UK. Mothers were weighed at each prenatal control day 7 post partum and in every infant control, without shoes but with a minimun clothing using a Neta scale.accurate to 0.1kg. The maternal weight was calculated as the difference between the weigh with clothes and the weigh of clothes. Maternal height was measured to the nearest millimetre using a stadiometer. Maternal body mass index was calculated from: weight(kg) /length $(m)^2$.

The Skinfold thickness: (triceps, biceps, hip, subscapular, and calf) measured at every visit starting at 6 months with a Holtain calliper.

4. RESULTS

We interviewed 700 pregnant women at the prenatal clinic and we could select 328 of them to be included as part of the study. Mothers' age was 26.5 +- 3 years, in most of the cases this was their second child and they had a previous good breastfeeding experience. During pregnancy they gained 14.7 +-2 Kg of weight. The infants were 40 girls of 3248 +- 422 gr and 49 +- 2 cm at birth and 34 boys of 3451,1 +- 393 gr and 50,03 +- 2 cm at birth by normal delivery. After infant birth, we could follow up 74 mother-infant pairs for two years. All infants were exclusively breastfed on demand during the first month of life 46 of them were EBF for 6 months and received breast milk as the only milk provided, up to one year of age. Growth in breast fed infants was excellent, if we compare them with a group of selected European breastfed infants (figures 1-4). EBF infants grows in height in good condition up to 6^{th} months and then desacelerate to regain growth at 12 months (figure 5). There is a relationship between milk volume and height increment (figure 6 and 7). It seems that mothers BMI is related to Milk volume (figure 8). Mothers milk composition at months 1,3,6 is comparable to the published data in literature(Table I). Most of the infants followed had very good health and none of them required Hospital admission during the time of the study, only a 5% of them had minor health problems.

5. DISCUSSION

All the patients followed showed a very good compliance with the program. We think that is was related with the fact that all mothers were asked to participate during pregnancy and some of them invited their friends to participate in it. Growth of the group of the EBF infants both in size and weight is adequate according published standards. We found a relationship between amount of breast milk and size, (height) and we think that is most interesting, and it would be most important to study in detail. Stables isotopes have proved being very useful for field studies on breast milk intake and body composition. They also allow measuring energy expenditure in infants. Establishing energy requirements is of great importance in breast fed infants and the data collected by us in Chile is in agreement with the results from different groups of researchers, suggesting that adequate growth may be sustained on a smaller supply of energy than what was recommended. This fact is most relevant stressing the necessity to continue to promote exclusive breast feeding during the first months of age, specially after the growing evidence that breast feeding protects from obesity that is a mayor problem in developing and developed countries. In our experience the amount of milk produced and the growth of exclusively breast fed infants are comparable to the data published in the literature [19,20]. With our data we can conclude that the intake of non- breast milk liquids of infants reported as being EBF is negligible ad the difference in breast milk intake between EBF and PBF is small and they grow almost the same way. Breast fed infants appear to have lower requirements that formula fed infants (Butte 1996 Salazar 2000) the surplus of energy is stored, resulting in increased weight gain.

6. ACKNOWLEDGEMENTS

We thank the mothers’ infants pairs and their families that made possible this study, all the professionals of the out patient Clinic where the study was done. We thank professor Coward for helping us with the calculation of data.

7. FINAL REMARKS

It must be noticed that it as actually report is based on half of the mothers who finished the protocol. This due to several factors:

a) The protocol is quite ambitious for only two person working at field level
b) The amount of samples collected, to be measured and analysed, exceeded the time frame and laboratory capacities
c) Given the excellent information already collected, we think necessary to finish the whole group incorporated within year 2004.
d) For that, we would like NARHES to consider an extension of the CRP Project, and additional support.

REFERENCES

[1] Dewey KG, et al.(1995) Growth of breast-fed infants deviates from current reference data: a pooled analysis of US, Canadian and European data sets World Health Organisation working group on infant growth Paediatrics 96 495-503

[2] DEWEY KG et al.(1992). Growth of breastfeeding and formula fed infants from 0 to 18 months: the DARLING study. Paediatrics;89:1035-41.

[3] CHILE MINISTERIO DE SALUD Situación de la salud en Chile: tarjeta de presentación Santiago: Ministerio de Salud, 1999

[4] CASTILLO C et al.(1996). Breast-feeding and the nutritional status of nursing children in Chile. Bulletin of PAHO;30:125-133.

[5] DIAZ S. et al (1995) Breast feeding duration and growth of fully breastfed infants in poor urban Chilean population. Am J Clin Nutr 62 1-6

[6] CASTILLO C et al. (1996) Feeding practices and nutritional status in children under age 18 months. Rev Chil de Pediatria;;67:22-28.

[7] COWARD WA et al. (1982) breast milk measurement in mixed-fed infants by administration of deuterium oxide to their mothers. Hum Nutr Clin Nutr;36C:141-81

[8] COWARD WA (1984) Measuring milk intake in breast fed babies J Pediatr Gastroenterol. Nutr. ;3, 275-279

[9] SCHOELLER DA et al, (1980) Total body water measurement, in humans, with 18O and 2H labelled water. Am J Clin Nutr;2686-93.

[10] FJIELD CR, BROWN KH & SCHOELLER DA (1988) Validation of the deuterium oxide method for measuring average daily milk intake in infants Am J. Clin. Nutr.48,671-679

[11] BUTTE NF, WONG WW, PATTERSON BW, GARZA C, KLEIN PD (1988). Human milk intake measured by administration of deuterium oxide to the mother: a comparison with the test weighting technique Am J Clin Nutr;47: 815-21

[12] BUTTE NF, WONG WW, FERLIC l,,O BRIEN,SMITH E, KLEIN PD & GARZA C. (1990) Energy expenditure and deposition of breast-fed and formula fed infants during early infancy. Pediatr Res 28 631-640.

(13) SALAZAR G et al, (1994) Deuterium equilibration time in infant's body water. Eur J Clin Nutr;48:475-481

[13] INFANTE C et al. (1991)The-dose-to-mother method to measure milk intake in infants by deuterium dilution: A validation study. Eur J Clin Nutr;45:121-9

[15] INFANTE C, LARA W & VIO F (1985) Isotope dilution measurements of breast milk production in Chilean urban mothers Hum Nutr Clin Nutr 39C 379-386

[16] VIO F SALAZAR G INFANTE C (1991)Smoking during pregnancy and lactation and its effects on breast –milk volume Am J Clin Nutr Dec 54:6 1011-6

[17] Salazar G., Vio F., Garcia C., Aguirre E &Coward W.A (2000), Energy requirements in Chilean infants Arch . Dis. Child. Fetal Neonatal De 83 F120-F123

[18] WHO MULTICENTRE GROWTH REFERENCE STUDY PROTOCOL Appendix B-pg 1 1999

[19] HARZER G DIETERICH I HAUG M (1984) Effects of the diet on the composition of human milk Ann Nutr Metab 28: 4 231-9

[20] DEWEY KG, FINLEY DA, LONNERDAL B. (1984) Breast milk volume and composition during late lactation (7-20 months) J Pediatr Gastroenterol Nutr Nov 3:5 713-20

Weight Growth chart in EBF girls
0-6m z score

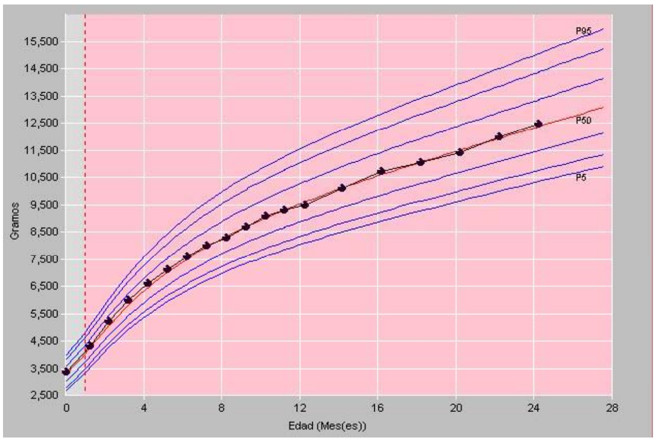

FIG. 1.: Weight Growth chart in EBF girls; 0-6m z score.

Weight Growth chart in EBF boys
0-6m z score

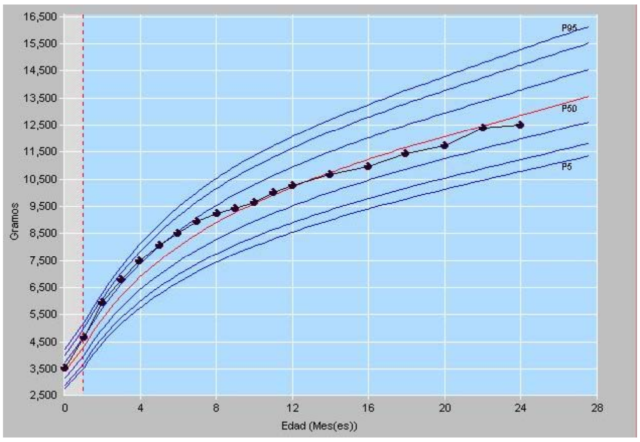

FIG 2. Weight Growth chart in EBF boys; 0-6m z score.

Height Growth chart in EBF girls
0-6m z score

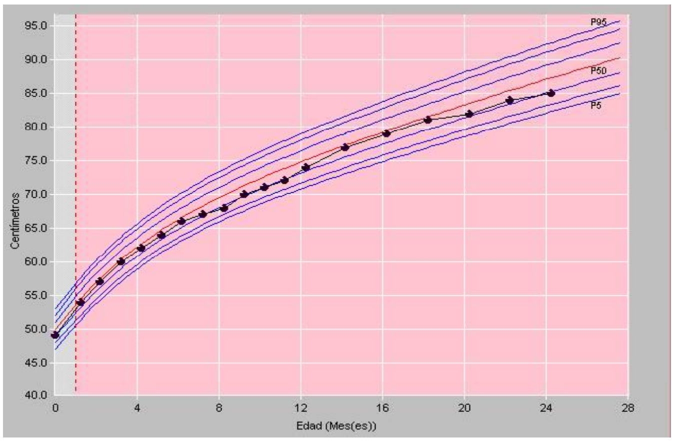

FIG 3. Height Growth chart in EBF girls; 0-6m z score.

Height Growth chart in EBF boys
0-6m z score

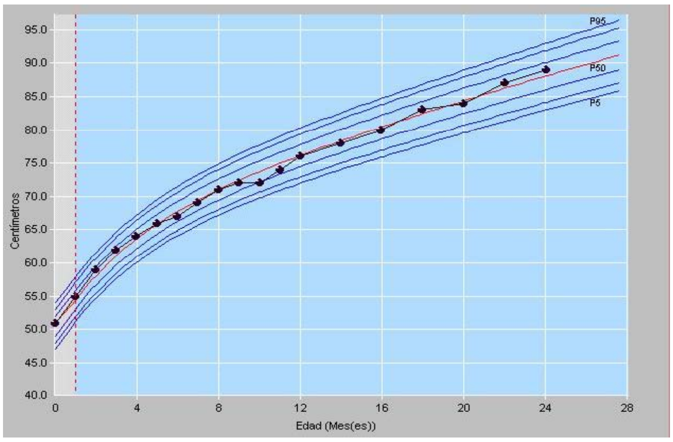

FIG. 4. Height Growth chart in EBF boys; 0-6m z score.

TABLE I: MATERNAL MILK COMPOSITION 1º, 3º , 6º MONTHS

Maternal Milk composition
1º, 3º , 6ºmonths

Months	hum	prot	fat	ashes	tot	ENN	Cal
1st month	86.83+-1.58	1.41+-0.24	3.89+-1.42	0.22+-0.04	92.3+-0.46	7.65+-0.46	71.23+-13.38
3rd month	87.69+-1.82	1.20+-0.1	3.25+-1.97	0.2+-0.04	87.1+-21.9	7.53+-0.41	65.31+-17.04
6 th month	87.52+-1.86	1.17+-0.36	3.58+-1.75	0.18+-0.04	92.4+-0.4	7.55+-0.4	67.22+-16.14

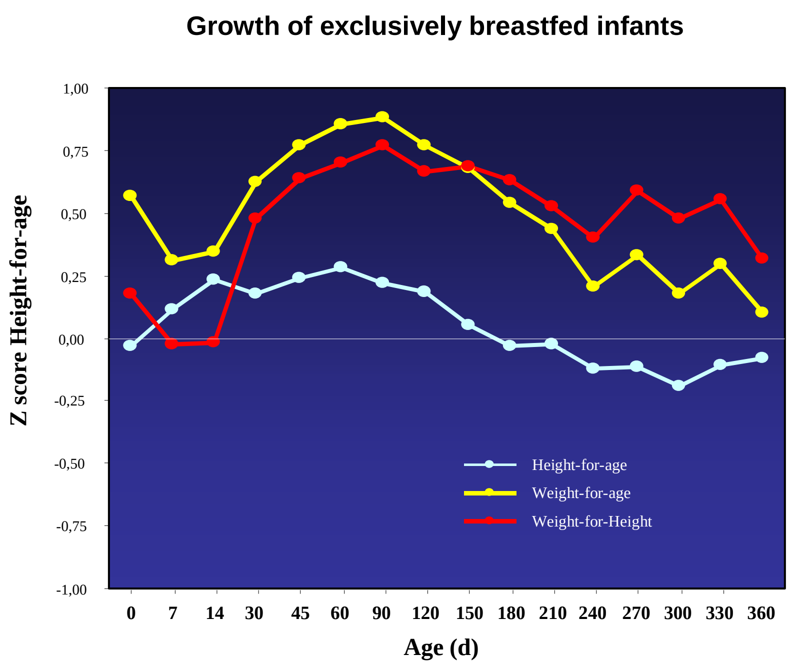

FIG 5. Growth of exclusively breastfed infants.

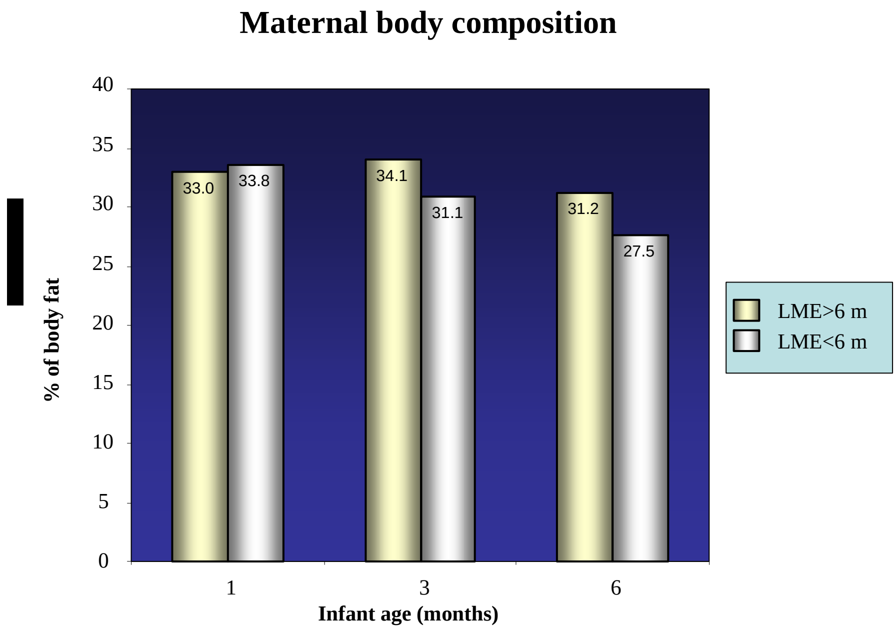

FIG. 6. Maternal body composition.

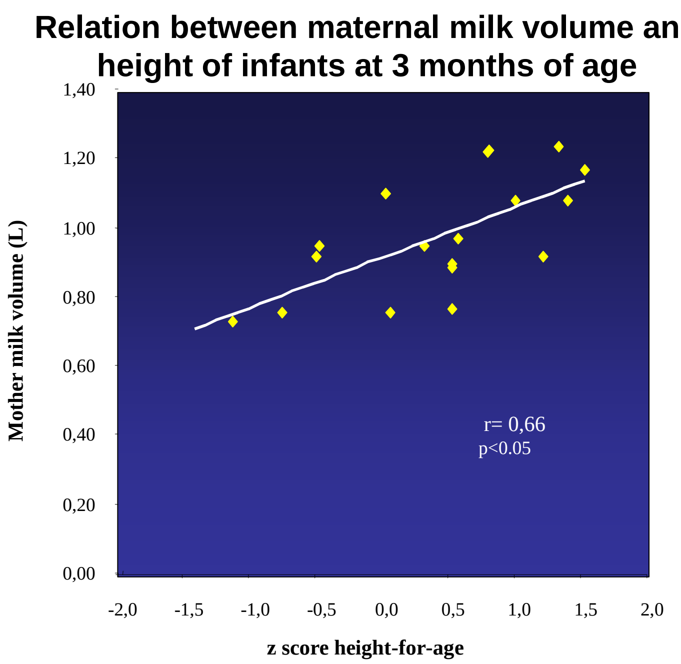

FIG 7. Relation between maternal milk volume and height of infants at 3 months of age.

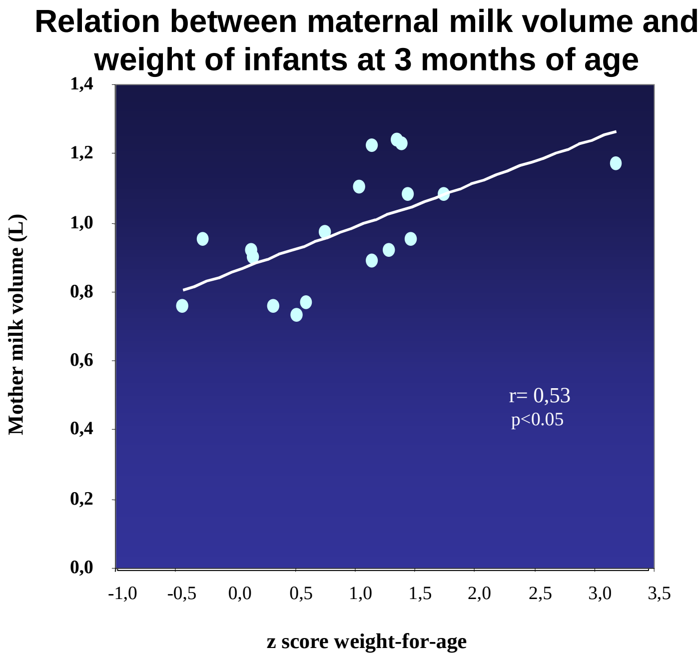

FIG 8. Relation between maternal milk volume and weight of infants at 3 months of age.

ISOTOPIC EVALUATION OF BREAST MILK INTAKE, ENERGY METABOLISM, GROWTH AND BODY COMPOSITION OF EXCLUSIVELY BREASTFED INFANTS IN PAKISTAN

ZULFIQAR AHMED BHUTTA[1], SAMREEN ABBASS[1], ANTONY WRIGHT[2], ANDY COWARD[2]

[1]The Aga Khan University & Medical Center, Department of Paediatrics Karachi, Pakistan
[2]MRC Dunn Nutrition Laboratories, Cambridge (UK)

Abstract

There is considerable evidence to support the view that the current growth standards for infants, which are in-use globally, may be inappropriate. This is based on the observation that these were derived from largely formula-fed western populations and recent studies documenting that exclusively breastfed young infants exhibit a lower growth trajectory. However, there are few studies objectively evaluating energy metabolism, body composition and growth in exclusively breastfed infants, and none in developing countries. We evaluated the growth pattern and breast milk and fluid intake patterns longitudinally in a representative sample of exclusively breast fed newborn infants in Pakistan. These newborn infants were well characterized at birth and sequential measurements of growth, body composition and energy expenditure were made using bio-impedance analysis and indirect calorimetry. In addition, breast milk intake was quantified using deuterium administration to the mother in a standard dose (10g) and estimation of urinary enrichment in exclusively breastfed infants and correlated with weight gain and growth.

A consecutive 112 mother baby pairs were studied longitudinally and breast milk intake was quantified in a subset of 12. The mean (± SD) maternal age was 27.5 ± 4.8 years and the weight 64.8 ± 7.9 kg, indicating the appropriate nutritional status of the population. The mean birth weight of the cohort was 3.13 ± 0.36 kg and the gestational age 3.13 ± 0.36 weeks. The mean body weight of the cohort at 2, 4, 6 and 12 months of age was 4.87 ±0.63, 6.40 ± 0.6, 7.56 ± 0.69 and 11.18 ± 1.05 kg respectively, which was better or comparable to many developed country cohorts of breastfed infants. The mean breast milk intake at 2 weeks, 3 months and 6 months of age was 591.7 ± 144.2, 794 ± 133 and 677.6 ± 298.6 ml/day respectively representing a range of 73-133 ml/kg/day. The maternal fluid intake for these corresponding time periods was 4.19 ± 1.12, 4.15 ± 1.25 and 3.96 ± 1.15 L/day respectively. The estimated non-breast milk fluid intake for the cohort for this period ranged from 6-18 ml/kg/day.

These data indicate that the growth performance and breast milk intake of exclusively breastfed infants in Pakistan was comparable to that observed among healthy breastfed newborn infants in developed countries and further support the continuation of these initiatives to improve early child health and development.

1. SCIENTIFIC BACKGROUND OF THE PROJECT

The NCHS -WHO growth reference standards (1) are widely used as reference growth centiles in the developing world. Despite numerous efforts to define separate regional or national growth standards which tend to accept lower birth weight and growth trajectories as normal (2,3), recent evidence indicates that it may be inappropriate to do so (4). Several major concerns have been expressed about the current/NCHS WHO reference standards including the following

1. The standards were drawn from a single, ethnically homogenous caucasian population of artificially fed infants.

2. Measurements were taken at relatively wide intervals with outdated curve-fitting procedures.

3. Several studies of exclusively breastfed infants have revealed lower growth trends in comparison with NCHS/WHO reference standards.

A major multi-center international growth reference study sponsored by the WHO, is presently underway to study the growth of exclusively or predominantly breastfed children longitudinally for the first 24 months of life. This standard will be drawn from a comparable multiethnic population of children in different parts of the world and may provide a valid international reference standard. This infant growth data from 6-8 sites in different countries will be combined with cross-sectional data from children aged 18-71 months, in order to construct a new international reference growth standard (de Onis M, personal communication 2002).

While several studies have established the immunological advantages of exclusive breastfeeding, reduced infectious disease morbidity (5,6) and improved neurological outcome (7), few have addressed the issue of body composition. In drawing up these new standards, it will be important to document that these breastfed infants, even though following a lower growth trajectory (8), are normal in terms of their body composition. Similarly it will be important to correlate growth and variations thereof, to the absolute breast-milk intake of the reference population. Classically breast milk intake has been estimated by test weighing (9) but this is a cumbersome technique and impractical for such evaluation in community settings. The recently developed methods of evaluating breast milk intake by using stable isotopes like deuterium ($^{2}H_2O$) (10-12) and measurement of energy metabolism by using the doubly labeled water ($^{2}H_2{}^{18}O$) (13-16), offer the exciting possibility of non-invasive evaluation of the relationship of human lactation, growth and energy metabolism. In addition, relatively sophisticated techniques like indirect calorimetry are now available in some developing country settings and can be potentially used in ambulatory settings for the assessment of energy expenditure and body metabolism.

There has also been considerable recent interest in immunostimulation and growth faltering in developing countries, but few longitudinal studies have attempted to scientifically evaluate this possibility. Data from the recently concluded CRP on the use of stable isotopes to assess amino acid metabolism following immunostimulation strongly suggest this possibility. It will be extremely useful to correlate dynamic studies of <u>energy metabolism</u>, coexisting morbidities and growth during this longitudinal study.

There are few studies correlating growth during early infancy with energy metabolism in developing countries. While studies employing indirect calorimetry are clearly impractical for field studies, the doubly labeled water method is uniquely suitable for field based studies. Of an estimated 250 studies evaluating this technique globally, only about 12 have been performed in developing country situations and of these only 4 have addressed issues of infant nutrition (17).

2. METHODOLOGY

The trial procedures and protocol were approved by the Ethics Review Committee at the Aga Khan University. The Aga Khan University Medical center (AKUMC) in Karachi represents the leading teaching hospital and referral center of Karachi and caters to a wide range of the population. There are approximately 4000 intra-mural births annually and a referral birth cohort from affiliated maternity homes of approximately 20,000.

We planned to prospectively include a consecutive 150 mothers-infant pairs in the study on the basis of fulfillment of inclusion criteria, motivation to exclusively breastfeed for 6 months and ease of monitoring. This was based on the assumption that of these at least 100 would fulfill criteria of exclusively breastfeeding for 6 months and appropriate introduction of complementary foods thereafter. These mothers belonged to the middle to high socio-economic class, and agreed to participate in the study and exclusively breastfeed for at least 6 months. The study protocol and procedures were explained to the mothers and written consent obtained for inclusion in the study.

Following an initial examination the infants were examined at home by a team consisting of a paediatrician and a research technician, and using standardized procedures for assessment of weight (Seca scales, sensitivity 10 g), infant stadiometer (AHRTAG baby length measures, London, UK), and skin fold thickness (Holtain, Crymych,UK). Mothers were weighed at baseline and at each subsequent month without shoes but with clothes using a Seca scale with sensitivity of 100g. Maternal height was measured to the nearest millimetre using a standard portable stadiometer. Maternal body mass index was calculated from: weight (kg)/length $(m)^2$.

The type and mode of feeding was assessed at monthly visits by 24 hours dietary recall and the mother and newborn infant assessed for any recent or concurrent morbidity. The anthropometric measurements were carried out at fortnightly and monthly intervals as per the International Growth Reference Study Protocol. We randomly selected 12 mothers from the above 100 mothers-infant dyads for detailed evaluation of lactation performance and body composition/energy metabolism by using deuterium (^2H_2O) as per the following protocol.

Actual breast milk intake was quantified in a randomly selected subset of exclusively or predominantly breastfed infants by administration of measured doses of deuterium (10 g) to the mother at time points A, B, C (Figure 1).

	A	B	C
Days	15-30	90-105	165-180
Administration of Deuterium to mother,	↑	↑	↑
Breastfeeding, collection of urinary and salivary samples	•••• • •	•••• • •	••••• •

FIG 1. Measurement of breast milk intake through administration of Deuterium to the mother.

In addition these we obtained bioimpedance data (Xytron Hydra) as well as estimates of energy metabolism by indirect calorimetry (Deltatrac II) in a standardized fashion.

2.1. Measurements of breast milk intake

Breast milk intake was measured using the dose-to-the mother deuterium-oxide turnover technique (18-20). This technique also allows estimation of non-breast milk water intake, and the mother's body composition. A baseline sample of 2ml of saliva from the mother and a urine sample from the child were collected on day 0, after which the mother received an oral dose of 10 g (0.5 mol) ^2H_2O, the quantity being determined to the nearest 0.01 g using an analytical Sartorius scale. A further three saliva samples from the mother (days 1,4,14) and another five urine samples from the baby (days 1,3,4,13,14) were then collected over a 14-day' period. Saliva collection was carried out after having been assured that the mother did not eat or drink in the previous 0.5 h. The time of collection was recorded. Small pieces of cotton wool pads were used to collect saliva samples (2 ml), after which saliva was released by placing them in salivettes and centrifuging them. For urine collection, urine samples (2 ml) were obtained using small urine collection bags and additional cotton wool balls to collect spillage (21). Urine and saliva samples were collected and stored on ice during transportation by the ambulatory research team and thereafter stored at -70°C until analysis. All samples were thereafter transported to UK unfrozen for further analysis at the collaborating laboratory in Cambridge. ^2H enrichment in the saliva and urine samples was measured by isotope ratio mass spectrometry after equilibration with H_2 gas (22). Each sample was measured in duplicate. The precision of the measurements was 0.26 ppm.

2.2. Calculations

Intake of breast milk and water from non-milk sources was calculated by fitting the isotopic (tracer) data to a model for water (tracee) turnover in the mothers and infants and the transfer of milk from mother to the baby (18, 19). For the mother, data were fitted to

$$E_{m(t)} = E_{m(0)} e^{-K_{mm} t}$$

where $E_{m(t)}$ is isotopic enrichment above background at time t (ppm), $E_{m(0)}$ is the zero time isotope enrichment (ppm), t is time postdose (day) and Kmm is water turnover in the mother (1/day).

For the infant, data were fitted to

$$Eb(t) = E_{m(0)} \left(F_{bm}/Vb \right) \{e^{-K_{mm} t} - e^{(fbb/Vb)t} / (F_{bb} = V_b) - K_{mm}\}$$

where *Eb(t)* is isotopic enrichment above background at time t (ppm), *Fbm* is the transfer of water from the mother to them baby via breast milk (kg or L/day), V_b is the infant's total ^2H_2O distribution space (kg) and F_{bb} is total water loss in the baby (kg/day).

Curve fitting was performed by using the 'Solver' function in Excels to minimise the sum of the squares of the differences between observed and fitted values for mother and baby data combined. Parameters fitted were Em(0), Fbm, Kmm and Fbb. Vb was assumed to change linearly with weight (W, kg) during the experimental period and was related to infant was

$$Vb = 0{:}84W^{0:82}$$

Subsequent calculations and their basis for calculating maternal water, infant breast milk and non-breast milk intake are defined elsewhere (23-25).

3. RESULTS

Of potential 340 eligible mothers approached for inclusion in the study between April 2002 and June 2002, a total 128 agreed to participate in the study. Of these a further 16 dropped out for either withdrawal of consent (6 women) or a move away from Karachi (10 families). Thus a total 112 women participated in the study.

Table I indicates the baseline characteristics of the mothers participating in the study indicating that this group represented a well nourished and motivated group of women.

Table II and figures 2-6 represent the anthropometric growth data on this cohort and the corresponding serial observations on weight for age, length for age, occipito-frontal circumference for age, mid-arm circumference and left mid-arm skin fold thickness for age for the cohort.

Table III indicates the maternal fluid intake, breast milk and non-breast milk intake for the subgroup that underwent the stable isotope study. The mean breast milk intake at baseline (2 weeks) was 591.7 ± 144.2 ml/day, 794 ± 133 ml/day and 677.6 ± 298.6 ml/day at 3 and 6 months of age respectively. The amount of non-breast milk fluid intake at these time points was small and did not represent a significant energy intake for the sub group.

Table IV indicates the metabolic data on the entire cohort at different time points and Figure 7 indicates the respective energy expenditure data for the cohort for the study duration.

4. DISCUSSION

Our data support the findings from the multicountry growth reference study (Bhandari et al 2002) that infants born to well nourished and upper socio-economic class women in developing countries grow at the same rate as healthy newborn infants in developed countries. These findings further support exclusive breastfeeding for six months as the growth of these infants was adequate and comparable to that reported from other studies in both developed (Dewey K Darling) and developing countries (23, Kramer).

The impact of additional small amounts of fluids taken by the infants at 3 and 6 months of age was small and was only detected in the subgroup of infants studied by deuterium enrichment. These findings are consistent with the recent findings by Victora et al (29) and supported by the WHO Working Group on the Growth Reference Protocol (30) showing no difference in growth between exclusively and predominantly breastfed infants.

These data have enormous potential implications supporting exclusive breastfeeding as the norm in Pakistan and other developing countries. The importance of exclusive and early breastfeeding as the means for reducing the burden of infant and early child mortality (through reduction of the burden of infection and diarrhea in developing countries) has been recently underscored (31). Available data from Pakistan underscores the enormous burden of diarrhea and lack of breastfeeding as determinants of poor growth in deprived settings (32). These data support the additional importance of exclusive breastfeeding as the means of supporting early child growth in such settings as the growth of these infants was fully comparable to that reported from developed country settings. Recent data from India on promotion of exclusive breastfeeding also support the feasibility and benefits of promoting this intervention in community settings (33).

Some limitations of these data must be recognized however. It is important that these studies be replicated in a diverse group of mothers and infants that reflect the norm in developing countries. Recent data from south Asia highlight the importance of intrauterine growth retardation with almost 25-40% of all births falling in this category (34, 35). In addition to the data emerging on differences in body composition among small newborn infants in south Asia that may impact on growth and metabolism in later life (36), there is the need to define better and more sensitive measures of growth and body composition in these infants.

REFERENCES

[1] US Department of Health Education and Welfare. National Center for Health Statistics (NCHS) growth curves for children, birth-18 years. DHEW Publication NO (PHS) 1997:78-1650.

[2] Hauspie RC, Das SR, Preece MA, Tanner JM. A longitudinal study of the growth in height of boys and girls of West Bengal, aged six months to 20 years. Ann Hum Biol 1980;7:429-41.

[3] Roma RJ, Chinn S. National Study on Health and Growth: Social and biological factors associated with height of children of ethnic groups living in England. Ann Hum Biol 1986;13:453-71.

[4] Kelly AM. Growth of Pakistani children in relation to the 1990 growth standards. Arch Dis Child 1997;77:401-5.

[5] Dewey KG, Heinig MJ, Nommsen-Rivers LA. Differences in morbidity between breastfed and formula-fed infants. J. Pediatr 1995;126:696-702.

[6] Howie PW, Forsyth JS, Ogston SA et al. Protective effect of breastfeeding against infection. Br Med J. 1990;300-11-16.

[7] Lucas A, Morby R, Cole TJ et al. A randomized multicenter study of human milk versus formula and later development in preterm infants. Arch Dis Child 1994;70:F141-F146.

[8] Dewey KG, Shaw NJ, Thomas AMC, Pyasent PB, Baker DJ. Growth of breastfed formula fed infants from 0-18 months: the DARLING study. Pediatrics 1992;89:1035-41.

[9] Vio F, Salazar G, Infante C. Smoking during pregnancy and lactation and its effect on breast milk volume. Am J. Clin Nutr 1991;54:1011-6.

[10] Orr-Ewing AK, Heywood PF, Coward WA. Longitudinal measurements breastmilk output by an 2HO technique in rural Papua New Guinean Women. Hum Nutr: Clin Nutr 1986;40C:451-67.

[11] Coward WA, Cole TJ, Sawyer MB, Prentice AM. Breastmilk intake measurement in mixed fed infants by administration of deuterium oxide to their mothers. Hum Nutr: Clin Nutr 1982;36C:141-8.

[12] Villalpando SF, Brutte NF, Wong WW, Flores-Huerta S, de Hermandez-Beltran M, Smith EO, Garza C. Lactation performance of rural Mesoameinudians. Eur J. Clin Nutr 1992;46:337-48.

[13] Roberts SB, Coward WA, Schlingenseipen KH, Nohria V, Lucas A. Comparison of the doubly labeled water (2H218O) method with indirect calorimetry and a nutrient-balance study for suiltaneous determination of energy expenditure, water intake and metabilizable energy intake in preterm infants. Am. J. Clin Nutr 1986;44:315-22.

[14] Jensen CL, Butte NF, Wong WW, Moon JK. Determining energy expenditure in preterm infants: comparison of 2H218O method and indirect calorimetry. Am J. Physiol 1992;263:R685-92.

[15] Coward WA, Cole TJ. The doubly labeled water method for the measurements of energy expenditure in humans: risk and benefits. In Whitehead RJ, Preutice AJ (eds) in New Techniques in Nutritional Research, New York, Academic Press 1991;pp.139-76.

[16] Coward WA. The doubly labeled water 2H218O method: principles and practice. Proc Nutr Soc 1988;47:209-18.

[17] Coward WA. Contributions of the doubly labeled water method to studies of energy balance in the Third World. Am. J. Clin Nutr 1998;68(Suppl):962S-969S.

[18] Coward WA, Cole TJ, Sawyer MB & Prentice AM. Breast-milk intake measurement in mixed-fed infants by administration of deuterium oxide to their mothers. Hum. Nutr. Clin. Nutr. 1982;36, 141–148.

[19] Orr-Ewing AK, Heywood PF & Coward WA. Longitudinal measurements of breast milk output by a 2H2O tracer technique in rural Papua New Guinean women. Hum. Nutr. Clin. Nutr. 1986;40:451–467.
[20] Butte NF, Wong WW, Patterson BW, Garza C & Klein PD. Human-milk intake measured by administration of deuterium oxide to the mother: a comparison with the test-weighing technique. Am. J. Clin. Nutr. 1988;47: 815–821
[21] Roberts SB & Lucas A. Measurement of urinary constituents and output using disposable napkins. Arch. Dis. Child. 1985;60: 1021–1024.
[22] Hoffman DJ, Sawaya AL, Coward WA, Wright A, Martins PA, de Nascimento C, Tucker KL & Roberts SB. Energy expenditure of stunted and nonstunted boys and girls living in the shantytowns of Sao Paulo, Brazil. Am. J. Clin. Nutr. 2000;72:1025–1031.
[23] H Haisma, WA Coward, E Albernaz, GH Visser, JCK Wells, AWright, CG Victora. Breast milk and energy intake in exclusively, predominantly, and partially breast-fed infants. Eur J Clin Nutr 2003 (in press)
[24] Holland B, Welch AA, Unwin ID, Buss DH, Paul AA & Southgate DAT. The Composition of Foods, 1991. McChance and Widdowson. 5th Edition. Cambridge, UK.
[25] Wells JC & Davies PS (1995): Correction for environmental water influx in measurement of milk volume intake by deuterium turnover in infants. Early Hum. Dev. 1995;41:177–182.
[26] Bhandari N, Bahl R, Taneja S, de Onis M, Bhan MK. Growth performance of affluent Indian children is similar to that in developed countries. Bull WHO 2002;80:189-195.
[27] Dewey KG et al. Growth of breastfeeding and formula fed infants from 0 to 18 months: the DARLING study. Pediatrics 1992;89:1035-41.
[28] Kramer MS, Tong G, Platt RW, Sevkovskya Z, Dzikovich I, Collet J-P, Shapiro S, Chalmers B, Hodnett E, Vanilovich I, Mezen I, Ducruet T, Shishko G, Bogdanovich N. Infant growth and health outcomes associated with 3 compared with 6 mo of exclusive breastfeeding. Am J Clin Nutr 2003;78:291-5.
[29] Victora CG, Morris S, Barros FC, Horta BL, Weiderpass E & Tomasi E. Breast-feeding and growth in Brazilian infants. Am. J. Clin. Nutr.1998;67:452–458.
[30] Working group on the growth reference protocol, WHO task force on methods for the natural regulation of fertility.Growth of healthy infants and the timing, type, and frequency of complementary foods. Am. J. Clin. Nutr. 2002;76: 620–627.
[31] Jones G, Steketee RW, Black RE, Bhutta ZA, Morris SS and the Bellagio Child Survival Study Group. How many child deaths can we prevent this year? Lancet 2003; 362:65-71.
[32] Jalil F, Karlberg J, Hanson LA, Lindblad BS. Growth disturbance in an urban area of Lahore, Pakistan related to feeding patterns, infections and age, sex, socio-economic factors and seasons. Acta Paediatr Scand Suppl 1989;350:44-54
[33] Bhandari N, Bahl R, Mazumdar S, Martines J, Black RE, Bhan MK, and the infant feeding study group. Effect of community-based promotion of exclusive breastfeeding on diarrhoeal illness and growth: a cluster randomised controlled trial. Lancet 2003;361:1418-23.
[34] Bhutta ZA. The enigma of maternal and childhood malnutrition in South Asia: can we break the vicious cycle? Journal of College of Physicians & Surgeons Pakistan. 2002;12: 327-330.
[35] Gillespie S, ed. Malnutrition in South Asia: A regional profile. Kathmandu: UNICEF Regional Office for South Asia ,1997.
[36] Yajnik CS, Fall CH, Coyaji KJ, Hirve SS, Barker DJ, Jogleka, Kellingray S. Neonatal anthropometry: the thin-fat Indian baby. The Pune maternal Nutrition Study. Int J Obes Relat Metab Disord. 2003; 27:173-80.

TABLE I. BASELINE CHARACTERISTICS OF STUDY COHORT
N = 100

Characteristics	Mean (SD)	Range
Maternal age (years)	27.5 ± 4.8	19 - 42
Maternal weight (kg)	64.8 ± 7.9	48.5 - 84.2
Maternal height (cm)	157.5 ± 5.4	148.0-168.0
Maternal mid-arm circumference (cm)	26.5 ± 3.3	19.0 - 35.0
Parity	2.2 ± 1.4	1.0 – 7.0
Antenatal visits	6.7 ± 2.9	0 - 15
Gestational age at birth (weeks)	38.8 ± 1.2	36.0 – 41.0
Infant weight at birth (kg)	3.13 ± 0.36	2.50 – 4.28
Infant length (cm)	48.9 ± 1.8	44.0 – 53.0
Infant OFC (cm)	34.2 ± 1.0	32.0 – 36.5
Infant SFT (mid-triceps) mm	5.3 ± 0.9	3.4 – 7.4

TABLE II. MATERNAL AND INFANT SEQUENTIAL ANTHROPOMETRY DATA

Characteristics	Baseline	1 month	2 month	3 month	4 month	5 month	6 month	12 month
Mat Weight (kg)	64.8 ± 7.9	62.3 ± 7.9	61.8 ± 7.9	61.9 ± 8.1	61.6 ± 8.0	61.6 ± 7.9	61.0 ± 10.0	61.1 ± 8.3
Infant Weight (kg)	3.13 ±0.36	4.04± 0.56	4.87 ±0.63	5.64±0.64	6.40 ± 0.6	6.91 ± 0.57	7.56 ± 0.69	11.18 ± 1.05
Infant Length (cm)	48.9 ± 1.8	53.1± 5.6	56.9±2.9	63.5 ± 3.0	63.5 ± 3.0	66.0 ± 2.7	67.9 ± 8.4	74.6 ± 3.8
Infant OFC (cm)	34.2 ± 1.0	37.3 ± 3.5	39.1± 1.1	39.6 ± 1.4	40.4 ± 5.2	42.2 ± 1.0	43.2 ± 1.0	45.6 ± 1.0
Infant MAC (cm)	9.7 ± 1.2	10.5 ± 1.5	11.4 ± 1.1	11.8 ± 1.2	12.2 ± 1.4	12.9 ± 1.0	13.4 ± 1.6	15.4 ± 1.0
Infant MTC (cm)	14.0 ± 1.8	15.2 ± 1.8	16.1 ± 1.9	17.1 ± 2.0	18.0 ± 2.3	19.0 ± 2.0	19.7 ± 2.9	23.8 ± 1.9
Infant SFT MT (L)	5.3 ± 1.0	5.9 ± 1.2	6.2 ± 1.2	6.7 ± 1.3	7.5 ± 3.8	7.5 ± 1.3	7.7 ± 1.3	9.7 ± 1.3
Infant SFT MT (R)	5.3 ± 0.9	4.8 ± 1.0	6.7 ± 1.3	6.7 ± 1.3	7.8 ± 4.0	7.5 ± 1.3	7.8 ± 1.2	9.8 ± 1.3
Infant SFT (SS)	4.2 ± 0.7	4.7 ± 1.1	5.1 ± 1.1	5.6 ± 1.1	6.0 ± 1.1	6.3 ± 1.1	6.7 ± 1.0	8.4 ± 1.2
Infant SFT (Abd)	3.7 ± 0.7	4.4 ± 1.0	4.8 ± 1.1	5.3 ± 1.1	5.8 ± 1.2	6.3 ± 2.2	6.8 ± 1.9	8.2 ± 1.2

TABLE III. FLUID INTAKE, BREAST MILK INTAKE AND NON BREAST MILK FLUID DATA
N=12

	Baseline (2 weeks)	**3 months**	**6 months**
Maternal weight (kg)	62.1 ± 8.7	61.8 ± 9.3	61.6 ± 8.5
Maternal height (cm)	157.7 ± 4.5	157.7 ± 4.5	157.7 ± 4.5
Maternal BMI	25.0 ± 3.5	25.0 ± 3.5	25.0 ± 3.5
Maternal Fluid intake (L/day)	4.19 ± 1.12	4.15 ± 1.25	3.96 ± 1.15
Infant weight (kg)	4.47 ± 0.51	5.74 ± 0.71	6.75 ± 0.36
Infant length (cm)	55.2 ± 2.5	60.9 ± 3.0	68.6 ± 3.5
Breast milk intake (ml/day)	591.7 ± 144.2	794 ± 133	677.6 ± 298.6
Breast milk intake (ml/kg/day)	132.6 ± 27.7	73.3 ± 10.2	117.3 ± 49.4
Non-breast milk fluid intake (ml/day)	6.7 ± 1.6	18.0 ± 1.8	18.2 ± 25.9

TABLE IV. METABOLIC DATA ON THE COHORT

	Baseline (2 weeks)	2 months	4 months	6 months	12 months
Infant weight (kg)	3.13 ±0.36	4.87 ±0.63	6.40 ± 0.6	7.56 ± 0.69	11.18 ± 1.05
Infant height (cm)	48.9 ± 1.8	56.9±2.9	63.5 ± 3.0	67.9 ± 8.4	74.6 ± 3.8
VO2	19.2 ± 5.9	26.7 ± 7.0	38.5 ± 9.2	48.2 ± 11.3	55.9 ± 13.1
VCO2	17.3 ± 6.7	24.3 ± 7.9	36.5 ± 9.8	46.1 ± 11.7	53.5 ± 12.8
Energy expenditure	194.0 ± 46.7	263.4 ± 65.2	405.4 ± 121.0	525.5 ± 180.5	579.9 ± 229.2

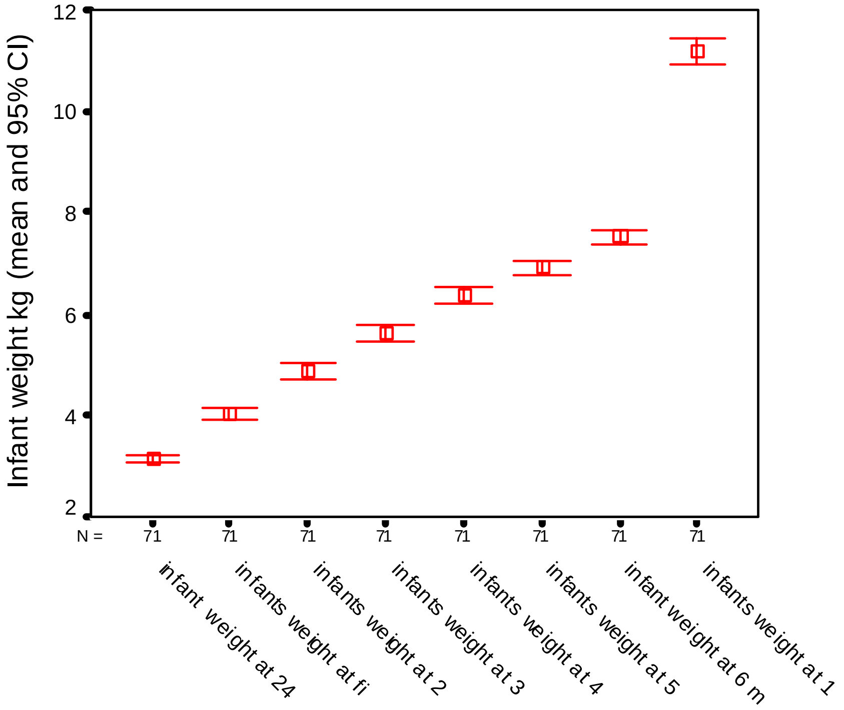

FIG. 2. Body weight in the birth cohort.

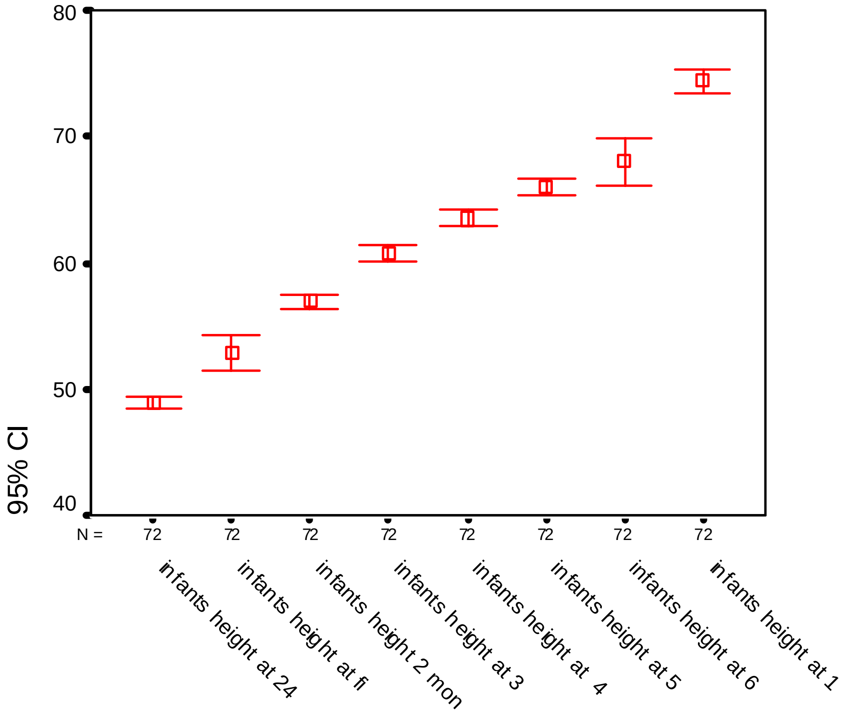

FIG. 3. Length for age in the birth cohort.

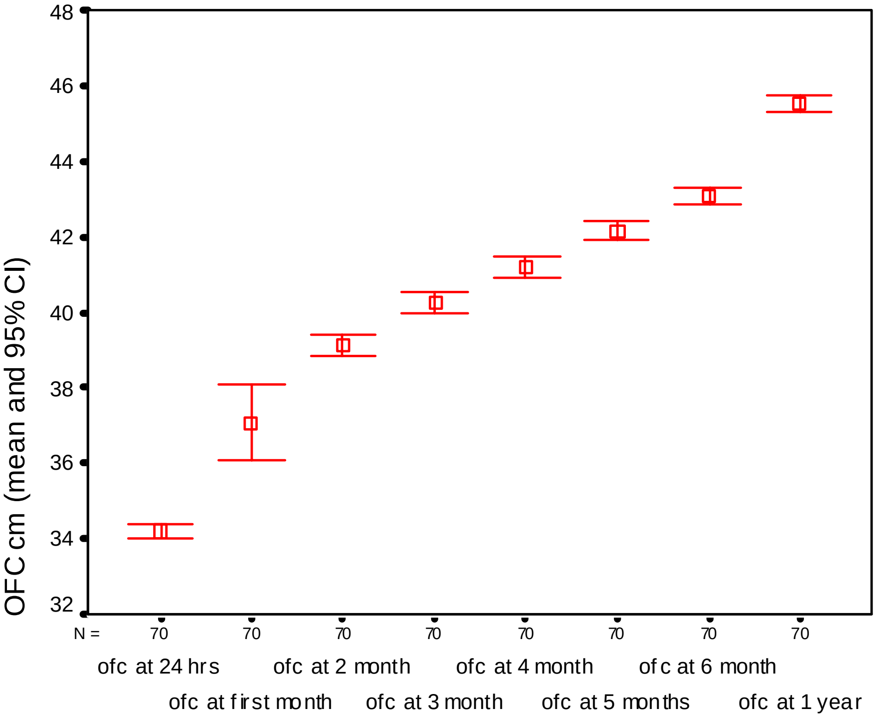

FIG. 4. OFC increment in the cohort.

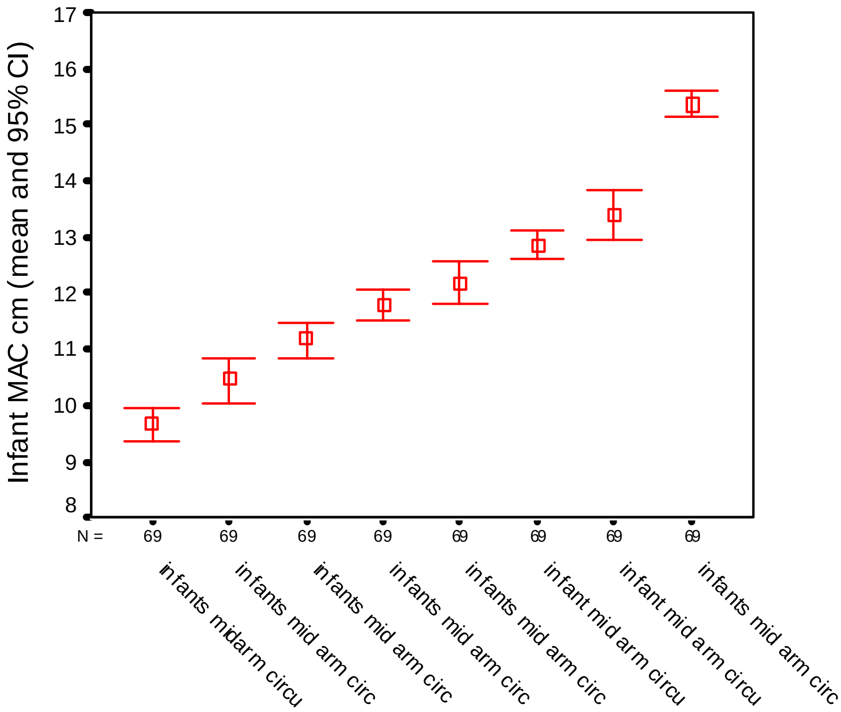

FIG. 5. Mid arm circumference in the cohort.

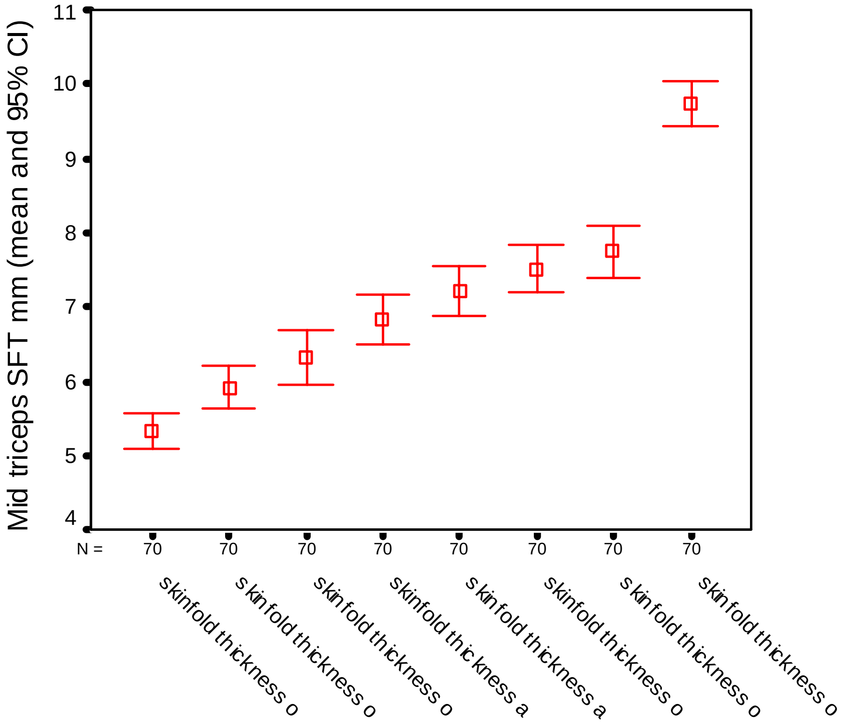

FIG. 6. Mid-triceps skin fold thickness in infancy.

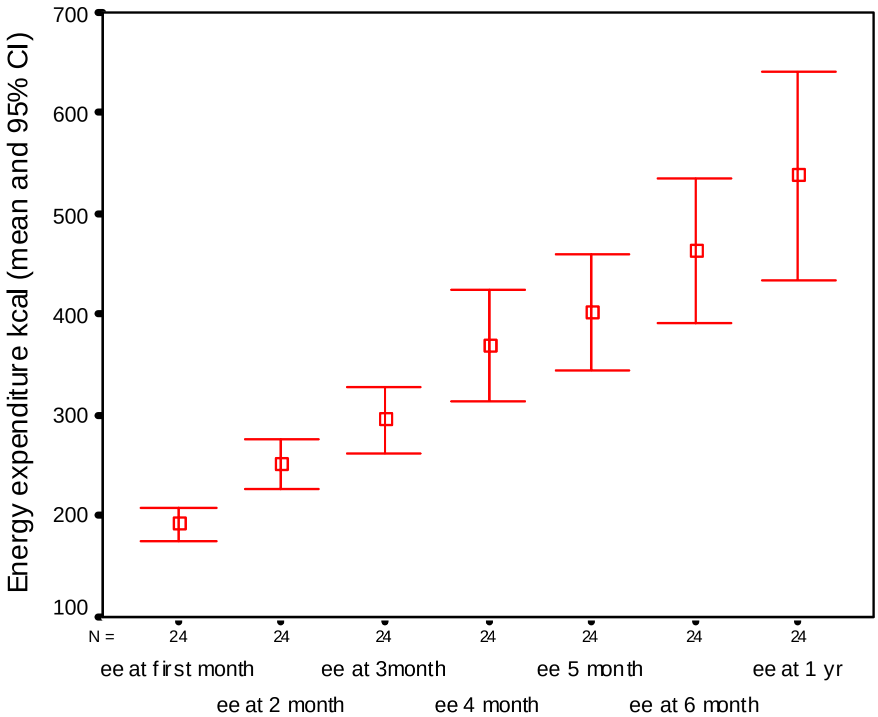

FIG. 7. Energy expenditure in infancy.

SIMULTANEOUS MEASUREMENT OF MILK INTAKE AND TOTAL ENERGY EXPENDITURE IN MIXED-FED INFANTS: METHODOLOGICAL APPROACH AND PREDICTION OF TOTAL BODY WATER

[1]J.C.K. WELLS, [2]H. HAISMA, [3]A. WRIGHT, [3]W.A. COWARD

[1]Childhood Nutrition Research Centre, Institute of Child Health, 30 Guilford Street London WC1N 1EH, United Kingdom
[2]Groningen University, Zoological Laboratory, Haren, The Netherlands
[3]MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road Cambridge CB1 9NL, United Kingdom

Abstract

Isotope probes (^2H, ^{18}O) can be used to investigate body composition, energetics and milk transfer in either mother or infant. The most comprehensive picture would be obtained where these measurements were made in both parties. This can be achieved either by successive measurements over a period of time, or by dosing the mother and simultaneously measuring isotope kinetics in both mother and infant. Simultaneous measurement of maternal milk transfer and infant energy expenditure could be made by dosing the mother with ^2H and the infant with ^{18}O, assuming that the infant ^2H kinetics can be quantified with sufficient precision to determine rate constants for both breast-milk influx and total water turnover. Nine mother-infant pairs were measured in Brazil, with error on energy expenditure found to be acceptable providing over 50% of total water intake was from breast-milk. Simultaneous dosing of mother and infant may therefore prove useful in some circumstances, particularly where rapid environmental changes might cause mismatches between measurements of energy intake and expenditure made in successive time periods.

1. INTRODUCTION

The isotopes ^2H and ^{18}O can be used to investigate a number of variables in nutritional research, including water turnover, body composition, total energy expenditure and breast-milk transfer. Body composition, milk transfer and total energy expenditure (TEE) may be measured in the mother, to improve understanding of the energetics costs of lactation, or in the infant, to investigate the energetics of early growth and health. Given that maternal and offspring energetics are closely related during early life, the most comprehensive picture would be obtained where these variables were measured in both parties. Such an approach would for example improve understanding of the effect of maternal energy balance on milk output and the energy content of breast-milk. In practice, multiple measurements are more problematic, for two reasons: first they may complicate data collection in the infant, as both isotopes continue to be transferred via breast-milk from the mother throughout the measurement period; second, larger isotope doses are required if the mother is dosed, which for ^{18}O greatly increases the expense.

Where multiple measurements are required, several options are available. First, the measurements could be undertaken in separate time periods. For example, breast-milk transfer could first be assessed using the dose-to-the-mother method, followed by measurement of infant TEE by dosing the infant with doubly-labelled water (DLW). This approach, utilised in Brazil by Hasima et al. (this report), requires correction of the infant DLW ^2H rate constant for the continued transfer of D_2O in breast-milk. Alternatively, the order of the measurements could be reversed, such that maternal breast-milk transfer kinetics would require correction for the infant DLW ^2H rate constant. These corrections are not especially problematic, and the main limitation is that the entire period required for both measurements is 3 weeks, during which energy metabolism and milk intake are not necessarily constant due to the rapid rate of development in early life. Both normal growth patterns, and environmental effects such as infection or weaning, might cause changes in energy utilisation, such that there could be a significant mismatch between milk intake and TEE data collected in successive time periods.

To resolve this dilemma, milk intake and TEE could be measured simultaneously. Again, there are two options. First, both isotopes could be given to the mother. Two rate constants would then need to be extracted from the infant's kinetics for each isotope: the rate of influx from the mother, and the rate of loss in the infant. From this approach, TEE could be measured in both mother and infant, along with milk transfer to the infant. However, this would require a large ^{18}O dose for the mother, and the infant measurements of TEE might be unreliable as no measurement of infant total body water would be possible. Second, it is possible to dose the mother with ^{2}H and the infant with ^{18}O. Again, two ^{2}H rate constants would require extraction from the isotopic data of the infant. This approach would measure maternal and infant body composition, breast-milk transfer, and infant TEE. It would not be unduly expensive, and since the ^{18}O is given directly to the infant, both milk transfer and TEE data could have acceptable accuracy.

The aim of our study was therefore to investigate this latter approach, by determining the precision with which the two ^{2}H rate constants (milk transfer and water turnover) can be extracted from the infant's isotopic data if the deuterium dose is given to the mother.

2. METHODS

Deuterium kinetics for mother and infant were calculated using a standard application of the dose-to-the-mother method for measurement of breast-milk transfer, the only adaptation comprising an additional number of post-dose urine samples being collected from the infant in order to increase precision of the rate constants. The isotopic data were fitted to different models [1,2] in order to calculate the required rate constants and pool sizes.

2.1. Subjects and measurements

One mother was initially recruited from Cambridge, UK, as a pilot study. Nine mothers were then recruited for the full study from Pelotas, Brazil, from within an ongoing study of breast-milk intake. Ethical permission was obtained from Cambridge Local Research Ethics Committee, and the ethical committee of the Federal University of Pelotas.

Weight was recorded at the start of the study in both mother and infant. Baseline samples of saliva (mother) or urine (infant) were collected, after which a dose of approximately 10g of 99.9% $^{2}H_2O$, weighed accurate to 0.01g using electronic scales (Sartorius), was given orally to the mother. Additional saliva samples were collected from the mother on days 1, 2, 3, 13 and 14 post-dose. Additional urine samples were collected from the infant 12 hours post-dose, twice daily on days 1, 2 and 3 post-dose, and then daily on days 4, 5, 6, 7, 9, 12 and 14 post-dose. Maternal saliva samples were collected using cotton mouth swabs, ensuring the mother had not eaten or drunk in the half hour before the sample was taken. Infant urine samples were collected using cotton balls left in the infant nappy, and checked for urination every hour. One infant also received an oral dose of ^{18}O (0.15 g per kg infant body weight) at the same time as the maternal dosing.

The samples were stored frozen prior to shipping to the UK analysis laboratory. ^{2}H and, where appropriate ^{18}O, analysis was undertaken by isotope-ratio mass-spectrometry as described previously [3].

2.2. Stable isotope kinetics in infant and mother

For the mother, data were fitted to

$$E_{m(t)} = E_{m(0)} e^{-K_{mm} t} \qquad \text{(equation 1)}$$

where $E_{m(t)}$ is isotopic enrichment above background level at time *t*; $E_{m(0)}$ is the time-zero enrichment; *t* is the time post-dose in days; and K_{mm} is maternal water turnover in litres per day. All isotopic data are expressed in parts per million relative to standards of known enrichment. Maternal total body water, calculated from the time-zero enrichment, was divided by the hydration fraction 0.73 to calculate lean mass. This was then subtracted from weight to calculate maternal fat mass.

For the infant, data were fitted to

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

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

(equations 2 and 3)

where $E_{b(t)}$ is enrichment at time *t*; F_{bm} is water transfer from mother to infant via breast-feeding (kg/day); V_b is the infant isotopic distribution space; and F_{bb} is infant water turnover (kg/day) [4]. Equation 2 has been rewritten in equation 3, with the term F_{bb}/V_b replaced by the term K_{bb}, the rate constant under investigation in the current study.

2.3. Calculations

Curve fitting was undertaken using the 'Solver' function in Excel, in order to minimise the sum of squares of the differences between observed and fitted values for mother and infant combined. The fitted parameters comprised $E_{m(0)}$; F_{bm}; K_{mm}; and F_{bb}. V_b was predicted from infant weight using an equation generated from previous measurements of total body water in infants [5], and assumed to change in linear fashion during the measurement period. A further calculation was then performed, using SAAM software, to calculate the precision of the best-fit rate constant values.

Corrections were then made for the water content of breast-milk; the water released during the oxidation of breast-milk; the increase in total body water due to growth over the measurement period; fractionation of infant water losses; water influx through non-oral routes; and non-milk oral water intake [4]. These corrections allowed calculation of breast-milk transfer.

Total energy expenditure (TEE) is calculated using the following equation

$$TEE = N(k_O - k_D)/2 \quad \text{(equation 4)}$$

In the dual-dosing method, k_D is calculated as k_{bb} in equation 3 above. In order to estimate the effect of this approach on TEE, the effect of precision of k_{bb} on TEE was evaluated as follows. Error on k_O (the ^{18}O rate constant) was assumed to be proportional to the k_{bb} error. These error values were then applied to typical k_D and k_O values for infants (0.188 and 0.236 respectively). Finally, this error was then related to the proportion of total infant water intake derived from breast-milk using correlation and graphic analysis.

3. RESULTS

Data from the first pilot study have not been included in this report, which therefore refers to the 9 subjects from Brazil. A description of the sample is given in Table 1. All subjects satisfactorily completed the protocol. Isotopic parameters are given in Table 2. The average proportion of water intake derived from breast-milk was 54%, ranging from 9% to 84%. The average error on TEE was 11.0 (SD 9.5) %. The correlation between the proportion of water intake derived from breast-milk and error on TEE was -0.93, ($p<0.0001$). Figure 1 shows a plot of the data, where the error on TEE remains <8% so long as at least half of daily water intake is derived from breast-milk.

4. DISCUSSION

4.1. Isotopic analyses

Although breast-milk intake and infant energetics have been extensively studied during the first three months post-partum, less attention has been directed to later infancy, when breast-milk is increasingly supplemented by other foods and fluids. Only recently have isotopic method been applied to this issue. Recently, Haisma et al [4] reported that partially breast-fed infants at 4 months received significantly less breast-milk than predominantly or exclusively breast-fed infants in Brazil. That study indicates that the effect of introducing supplements has significant effects on breast-milk intake even in the first few months, and similar information is required for later infancy. This is particularly important as the risk of infection increases after weaning. A major limiting factor for work in this area has been the capacity to measure both milk transfer and energetics in the same infants.

Stable isotope methodologies represent a major advance in our ability to make both types of measurement. Non-invasive and applicable relatively easily in free-living populations, they are transforming our understanding of nutrition in early life. Until recently, data on milk intake were obtained using test-weighing. This procedure is laborious and difficult to apply over full 24 hour periods. Compared to early studies using test-weighing, recent stable isotope studies show substantially greater breast-milk intakes in infants from most developing countries. However, many issues remain, in particular those relating to the energetics of breast-feeding.

The amount of energy transferred to the infant is a function of several variables: the volume of milk consumed; the energy content of the milk; and the capacity of the infant to capture the available energy. Some previous studies have suggested that the energy content of breast-milk declines in proportion to maternal nutritional status [6-8], although other studies have not reported such an association [9]. Thus, the volume of milk consumed is not necessarily an adequate measure of nutritional intake in all circumstances. Furthermore, recent work has suggested that in some populations, gut function is reduced following infection, such that the ability to absorb all available energy is compromised. Hence, via either of these two pathways, infants may fail to obtain sufficient energy for growth and health despite consuming relatively high milk intakes.

Both these issues may be addressed by measuring infant energy expenditure as well as milk volume intake. Ideally, the measurements should be made over the same time period. Environmental factors such as infection can have marked short-term effects on appetite, such that comparison of two periods, one characterised by health and one by infection, could involve significant mismatches between TEE and milk intake. However, in order to make simultaneous measurements of milk intake and TEE in partially breast-fed infants, the precision with which rate constants can be quantified must be investigated.

In the present study, we measured deuterium kinetics in infants with a wide range of variability in the proportion of dietary intake supplied by breast-milk (9 to 84%). Using a method that minimises the lack of fit in both maternal and infant isotope kinetics simultaneously, we determined the precision of the DLW infant ^{2}H rate constant and propagated error on TEE, assuming an equal error on the ^{18}O kinetics. Error may be calculated in more than one way, and the approach we used is likely to have under-estimated the true error on k_{bb}. This potential bias is offset by our assumption that error on K_D is the same as that on k_O, since infant ^{18}O kinetics, negligibly influenced by maternal kinetics, ought to be quantified with greater precision than infant ^{2}H kinetics. However, our main finding, that the error on TEE increases markedly when breast-milk intake accounts for below 50% of total water intake, is likely to be robust to the choice of method for calculating error. Typically, TEE error averages around 5% in infants, while in the present study for those infants receiving >50% of water from breast-milk the average was 6.3%.

Our study therefore suggests that the dual dosing procedure, for simultaneous measurement of milk transfer and TEE in partially breast-fed infants, may have acceptable accuracy where breast-milk remains a major component of total dietary intake, but that precision of TEE is likely to become unacceptable as the contribution of breast-milk declines.

4.2. The relationship between TBW and anthropometry, age and sex in infancy

The deuterium dose to the mother method does not directly measure infant total body water, a parameter required in the calculation of milk transfer. Infant TBW could be measured by ^{18}O dilution, but is usually predicted from body weight. In our previous report, we derived new prediction equations for UK infants aged 1.5 to 12 months, providing separate equations for breast-fed and formula-fed infants to reflect well-established differences in growth rate. Other published equations for predicting total body water in infants [10,11] have likewise come from industrialised population. These equations are now used in other populations when undertaking milk transfer calculations.

Recently, data from India have appeared which strongly suggest that a single equation is unlikely to be adequate for all populations. In Indian neonates, birth weight is markedly lower than in western populations (2.7 vs 3.5 kg) [12]. However, the deficit is primarily in lean mass, especially viscera, rather than fat mass (the "thin-fat" phenotype). Equations generated in European infants and applied in Indian infants are therefore likely to over-estimate total body water, and hence to over-estimate milk intake. It is not clear to what extent the Indian scenario reflects low birth weight more generally, or is specific to Asian populations. Any bias in predicted total body water will have the same effect on calculated milk transfer, and errors of >10% are possible in populations characterised by the thin-fat phenotype. Further work on this issue is therefore required, to establish the magnitude of the bias.

5. CONCLUSIONS

Dual dosing of mother and infant can yield acceptable data on multiple isotopic measurements provided that breast-milk contributes the majority of water intake in the infant. The value of this approach is likely to be highest when there is a risk that measurements in successive time periods will be confounded by changes in infant state between the periods. Where such changes are not anticipated, the application of successive measurements may provide improved accuracy due to the reduced influence of feeding patterns on infant isotope kinetics.

REFERENCES

[1] COWARD, W.A., COLE, T.J., SAWYER, M.B., PRENTICE, A.M., Breast-milk intake measurement in mixed-fed infants by administration of deuterium oxide to their mothers, Hum. Nutr. Clin. Nutr. 36 (1982) 141–148.

[2] ORR-EWING, A.K., HEYWOOD, P.F., COWARD, W.A., Longitudinal measurements of breast milk output by a 2H2O tracer technique in rural Papua New Guinean women, Hum. Nutr. Clin. Nutr. 40 (1986) 451-67.

[3] HOFFMAN, D.J., SAWAYA, A.L., COWARD, W.A., WRIGHT, A., MARTINS, P.A., DE NASCIMENTO, C., TUCKER, K.L., ROBERTS, S.B., Energy expenditure of stunted and non-stunted boys and girls living in the shantytowns of Sao Paulo, Brazil, Am. J. Clin. Nutr. 72 (2000) 1025-1031.

[4] HAISMA, H., COWARD, W.A., ALBERNAZ, E., VISSER, G.H., WELLS, J.C.K., WRIGHT, A., VICTORA, C.G., Breast milk and energy intake in exclsuively, predominantly, and partially breast-fed infants, Eur. J. Clin. Nutr. 2003 - in press.

[5] WELLS, J.C.K., DAVIES, P.S.W., COWARD, W.A., Simultaneous measurement of milk intake and total energy expenditure in mixed fed infants: methodological approach and prediction of total body water, International Atomic Energy Agency, NAHRES-55 report: Isotopic evaluations in infant growth monitoring – a collaboration with WHO (2000) 11-20.

[6] PRENTICE, AM., PRENTICE A., WHITEHEAD,R.G., Breast-milk fat concentrations of rural African women. 2. Long-term variations within a community, Br. J. Nutr. 45 (1981) 495-503.

[7] VILLALPANDO, S., BUTTE, N.F., WONG, W.W., FLORES-HUERTA, S., HERNANDEZ-BELTRAN, M. DE J., O'BRIAN SMITH, E., GARZA, C., Lactation performance of rural Mesoamerindians, Eur. J. Clin. Nutr. 46 (1991) 337-348.

[8] BROWN, K.H., AKHTAR, N.A., AHMED, M.G., Lactational capacity of marginally nourished mothers: relationships between maternal nutritional status and quantity and proximate composition of milk, Pediatr. 78 (1986) 909-919.

[9] KHIN-MUANG-NAING, TIN-TIN-OO, KYWE-THEIN, NEW-NEW-HLAING, Study on lactation performance of Burmese mothers, Am. J. Clin. Nutr. 33 (1980) 2665-2668.

[10] FRIIS-HANSEN, B., Changes in body water compartments during growth, Acta Paediatr. 46 Suppl. 110 (1957) 1–68.

[11] BUTTE, N.F., WONG, W.W., GARZA, C., Prediction equations for total body water during early infancy, Acta Paediatr. 81 (1992) 264–265.

[12] YAJNIK, C.S., FALL C.H., COYAJI K.J., HIRVE S.S., RAO S., BARKER D.J., JOGLEKAR C., KELLINGRAYS., Neonatal anthropometry: the thin-fat Indian baby. The Pune Maternal Nutrition Study, Int. J. Obes. 27 (2003) 173-180.

TABLE I. DESCRIPTION OF THE SAMPLE

		Mean	SD
Mother	Weight (kg)	54.4	7.2
	Total body water (l)	25.5	2.3
	% fat	34.9	9.6
Infant	Weight (start) (kg)	9.2	1.2
	Weight (end) (kg)	9.3	1.2
	Predicted total body water (l)	5.1	0.5
	Predicted % fat	29.2	1.9

TABLE II. INDIVIDUAL VALUES FOR BREAST-MILK WATER AND TOTAL WATER TURNOVER, AND ESTIMATED ERROR ON TEE

Infant	Fbb	Fbm	Ratio	TEE error (%)
1	1.08	0.73	0.67	6.3
2	1.12	0.57	0.51	6.1
3	0.93	0.66	0.71	4.5
4	0.88	0.49	0.55	7.7
5	0.72	0.61	0.84	6.0
6	1.28	0.18	0.14	23.0
7	0.71	0.49	0.69	7.2
8	0.82	0.55	0.67	6.5
9	2.04	0.18	0.09	31.5
Average	1.07	0.50	0.54	11.0
SD	0.41	0.19	0.26	9.5

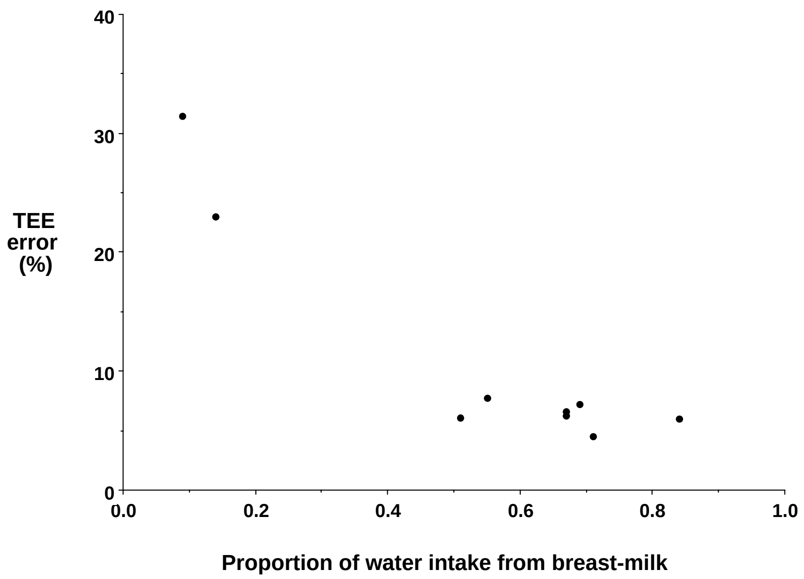

FIG. 1. Relationship between proportion of total water intake derived from breast-milk, and predicted error on total energy expenditure calculated by doubly-labelled water.

PART III: APPENDICES

APPENDIX 1: AGENDA OF THE MEETING

SECOND AND FINAL RESEARCH CO-ORDINATION MEETING (RCM,) FOR THE CO-ORDINATED RESEARCH PROJECT (CRP) ON ISOTOPIC EVALUATIONS IN INFANT GROWTH MONITORING – A COLLABORATION WITH WHO (PARTLY RCA),

_RC-812.2. / E4.30.10._

AGENDA

WEDNESDAY, 12 NOVEMBER 2003

9:00 – 9:30 Registration

9:30 – 9:45 Opening of the meeting

B. Miranda-da-Cruz, Nutritional and Health-Related Environmental Studies Section (NAHRES)
- Election of the rapporteur
- Adoption of the agenda
- Administrative arrangements for the meeting

9:45 – 10:00 Coffee break

10:00 – 12:30 SESSION 1: PROJECT REPORTS AND DISCUSSION

Brazil (*H. Haisma*):
Breastmilk intake according to exclusivity of breastfeeding and to presence of lactation counselling

Chile (*J. Alvear*):
Breast feeding and growth in a group of selected 0 to 24 months infants

12:30 –14:00 Luncheon

14:00 – 16:00 SESSION 2: PROJECT REPORTS (continuation)

Pakistan (*Z. Bhutta*):
Isotopic evaluation of breast milk intake, energy metabolism growth and body composition exclusively of breastfed infants in Pakistan

United Kingdom (*J. Wells*):
Energy metabolism and body composition in breast-fed infants

16:00 – 16:15 Coffee break

16:15 – 16:45 General discussions

16:45 Reception

THURSDAY, 13 NOVEMBER 2003

9:30 – 12:00 SESSION 3: DRAFTING REPORTS

Preparation for drafting:
(1) CRP summary for the NAHRES report, and
(2) evaluation/assessment of the CRP.

12:00 –14:00 Luncheon

14:00 – 17:00 SESSION 4: DRAFTING REPORTS (continuation)

Coffee break as appropriate

FRIDAY, 14 NOVEMBER 2003

9:30 – 14:00 SESSION 5: FINALIZING REPORTS AND FINAL DISCUSSIONS

(1) Presentation and adoption of the reports,
(2) Presentation of conclusions and recommendations.

CLOSING OF THE MEETING

APPENDIX 2: LIST OF PARTICIPANTS

Dr. Hinke HAISMA
Groningen University
Zoological Laboratory
Kerklaan 30
9751 NN Haren
THE NETHERLANDS
Tel: +31-50-363 2029
Fax: +31-50-363 5202
E-mail: hhaisma@zonnet.nl

Dr. Jorge ALVEAR
Instituto de Nutricion y Tecnologia de los Alimentos
Universidad de Chile
Nutricion Clinica
Laboratorio de Isotopes Estables
Macul 5540
Santiago de Chile
CHILE
Tel: +56-2-678 1400
Fax: +56-2-221 4030
E-mail: jalvear@uec.inta.uchile.cl

Dr. Zulfiqar A. BHUTTA
Professor of Paediatrics and Child Health
The Aga Khan University
P.O. Box 3500, Stadium Road
Karachi 74800
PAKISTAN
Tel: +92-21-493 0051, ext. 4721
Fax: +92-21-493 4294
E-mail: zulfiqar.bhutta@aku.edu

Dr. Jonathan WELLS
Institute of Child Health
MRC Childhood Nutrition Research Centre
30 Guilford Street
London WC1N 1 EH
UNITED KINGDOM
Tel: +44-171-905 2389
Fax: +44-171-831 9903
E-mail: J.Wells@ich.ucl.ac.uk

Scientific Secretary:
Dr. Beatriz Miranda-da-Cruz
Section of Nutritional and Health-Related
Environmental Studies
Division of Human Health
International Atomic Energy Agency (IAEA)
P.O. Box 100
A-1400 Vienna
AUSTRIA
Tel: 0043-1-2600-21635
Fax: 0043-1-2600-7-21635 or 0043-1-26007
E-mail: b.miranda-da-cruz@iaea.org