ARTICLE

Assessment of body composition in lactating mothers in a rural African community using deuterium oxide

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Background and objective. The deuterium oxide (DO) dilution method for measuring body composition was validated against the widely used skinfold (SF) measurement-based equation of Durnin and Womersley. The study involved 10 lactating women living in a rural community in Nandi, Kenya and participating in a cross-sectional study aimed at determining their iron and vitamin A status.

Methods. The selection criteria were exclusive breast-feeding, infants between 2 and 4 months of age, maternal parity < 4, birth weight more than 2 500 g and no congenital abnormalities. Maternal and infant anthropometric measurements were taken.

DO (approximately 0.1 g ^2H_2O/kg body water) was given orally to each mother accordingly to the Maastricht protocol (DO) for total body water (TBW) determination. The ^2H enrichment of the urine was measured using gas-isotope-ratio mass spectrometry. Blood samples were collected. Serum retinol and ferritin were determined using high- performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA) respectively. Body mass index (BMI) was determined as weight/height (m^2). The Bland-Altman pair-wise comparison was used to compare maternal fat-free mass (FFM), body fat (BF) and percentage body fat (% BF) that were determined based on the DO and SF techniques.

Results. Maternal mean (± standard deviation (SD)) for parity, age, BMI, haemoglobin (Hb), serum ferritin and serum retinol were 3 (2), 26 (4) years, 23.4 (4), 12.1 (1.8) g/dl, 10.3 (4.0) µg/l and 0.696 (0.300) µmol/l respectively. The FFM, BF and % BF accordingly to the DO and SF methods respectively were 44.0 (4.7) kg v. 42.9 (5.9) kg, 16.7 (8.8) kg v. 17.8 (7.5) kg and 26.2 (8.1)% v. 28.4 (6.4)%. Limits of agreement for underestimation of FFM and % BF were 4.4 kg (SEE 3.4) and 11.6% (SEE 5.8) respectively. Bias in the measurement of FFM and % BF was 1.1 kg (SEE 1.9), and –2.2% (SEE 3.3) respectively.

Conclusion. The variability (5.8 - 17.5%) observed in the SF technique may result in lower prediction of % BF. This may be an important factor for community-based nutritional interventions that aim at improving the body composition of vulnerable groups such as pregnant and lactating women or subjects with severe undernutrition.

The first report on poverty in Kenya indicates that the majority of the population (73%) live in rural areas and 23% are likely to have inadequate food intake and hence a compromised body composition.[1] Responses of the body to reduced food intake include low body weight and body mass index (BMI). There is marked reduction in body fat (BF), and fat-free mass (FFM).[2,3] Very few studies have been done on changes likely to occur in the body composition of individuals in developing countries living on long-term marginal food intake.[4] Assessment of nutritional status based on determination of variations in BF, FFM and percentage body fat (% BF) will help to bring the magnitude and implications of low food intake into focus. This factor is critical in determining the impact of community-based nutritional interventions that aim at improving the body composition of vulnerable groups such as lactating mothers or those with severe wasting due to acute and frequent infections.

Techniques need to be accurate and practical in field conditions. This factor, plus the cheapness and ready availability of deuterium oxide (DO) (^2H_2O), has led to its extensive use in body composition research.[5-8] Use of the Maastricht protocol[7] under field conditions provides the same accuracy as obtained in carefully controlled laboratory settings. A limiting factor in DO research has been the complexity of the laboratory meaurements, which require use of a mass spectrometer and trained personnel. Because of this most DO validation studies tend to be small in sample size[9,10] In our study, transportation and analysis costs determined our sample size of 10 lactating women from a rural community in Nandi, Kenya. In future this may change. A welcome recent development has been a faster and easy measurement of deuterium oxide (DO) sample enrichments using Fourier transformed infrared spectrophotometer (FTIR).[11,12]

Anthropometric measurements are widely used in the estimation of body composition indices. Compared with laboratory-based methods they are relatively fast, non-invasive, require the minimum of equipment and most important of all the measurements can be done anywhere.[13] The skinfold (SF) thickness equations developed for predicting body composition indices use measurements usually obtained from well-nourished subjects living in developed countries.[14,15] To date the validity of using the SF thickness equations in predicting body composition of lactating mothers in a rural community in Africa has not been established. Therefore the objectives of this study were: (*i*) to use the DO-based method under field conditions in a rural Kenyan community to determine the body composition of lactating women; and (*ii*) to determine the degree of agreement between a widely used SF thickness method and the DO-based Maastricht protocol for the assessment of body composition.

Subjects and methods

Study area

The study was conducted in seven villages based in a rural community in Nandi, about 30 km south-west of Eldoret in Kenya. The subjects were lactating women and their infants. These women were participants in a cross-sectional study aimed at determining iron and vitamin A status during lactation.[16] In the sampling strategy we used a cluster of seven Nandi villages. The selection criteria for inclusion in the study were exclusive breast-feeding, infants between 2 and 4 months of age, maternal parity < 4, birth weight more than 2 500 g and no congenital abnormalities. Maternal body composition was assessed and recorded for 10 women randomly selected from each village by quota. Maternal body composition indices were determined based on the isotope dilution technique and anthropometric measurements. Clearance to conduct the study was obtained from Moi University, Eldoret, and the Kenyan government, and informed consent was obtained from all 10 subjects.

Anthropometry and blood collection

Maternal body weight was measured to the nearest 0.1 kg using an electronic scale (SECA) and height was measured to the nearest 0.1 cm using a height meter. BMI was computed as weight/height (m^2). The mid upper arm circumference (MUAC) was measured using standard techniques. A Holtain SF thickness calliper (UK) was used to measure quadruple biceps, triceps supra-iliac and subscapular SF thickness.[17] One investigator did all the measurements. The infant's body weight was measured to the nearest 0.1 kg using a baby weighing scale and height was measured to the nearest 0.1 cm using a height stadiometer. Five millilitre venous blood samples were taken and stored on ice for transportation to the laboratory. Serum was separated from blood by centrifugation at 3 000 revolutions for 10 minutes at room temperature on arrival and samples were stored at –70°C until analysed for serum retinol and ferritin.

Urine sample collection and processing

A background urine sample was collected in the evening 5 - 10 minutes before the DO intake. The next morning 1 - 3 hours before the final urine sampling the mothers were asked to empty their bladders. This part of the urine was not collected. A sample was collected from a second urine voiding 1 hour later. As far as possible the time between drinking of DO and the last urine sampling was 10 hours (± 10 minutes). The urine samples were frozen and stored at –20°C until transported to the analytical laboratory in Maastricht.

Determination of body composition

DO: The stock mixture of DO (^2H_2O) was prepared from separate (un) labelled water. The subjects were given a standard dose of 70 ml water with 5 APE DO[8] after preparation using the Pt equilibration technique;[18] the samples were analysed using an isotope ratio mass spectrometry (Aqua Sira VG, UK).

DO was measured in hydrogen gas. Hydrogen gas was produced from the sample on-line using the hot uranium technique.[19] The FFM was calculated as total body water (TBW)/0.724 as suggested by van Raaij.[20] Maternal BF and % BF were calculated from BW by difference. Thus BF = BW – FFM and % BF = (BF × 100) /BW.

SF measurement: Bicep, tricep, subscapular and supra-iliac SF measurements were used to estimate body density.[14] The Siri equation[21] was used to determine BF and % BF. Based on maternal body weight (BW) the estimates for FFM were determined as

BW – BF.[13,15] A body weight < 52 kg and FFM < 42.8 kg were considered indicative of mild to chronic undernutrition.[4]

Biochemical analysis

A Coulter counter, model 560 was used to determine haemoglobin (Hb). Retinol levels in serum were assayed using high-performance liquid chromatography. Meaurement of serum ferritin was performed using the enzyme-linked immunosorbent assay (ELISA) (Boehringer Mannheim Immundiagnostike, Mannheim, Germany). Nutritional anaemia was considered present if Hb was less than 12 g/dl.[22] Depletion of iron stores was based on the levels of serum ferritin concentration as follows: severe < 12 µg/l, marginal 12 - 29 µg/l and normal ≥ 30 µg/l.[23] Vitamin A status was based on serum retinol concentration as follows: deficient < 0.35 µmol/l (10 µg/dl), and marginal < 0.70 µmol/l (20 µg/dl).[24]

Statistical analyses

Means, standard deviations (SDs) median and ranges were computed. The Bland-Altman pair-wise comparison[25] was used to compare BF, FFM and % BF estimated by using the SF and DO techniques respectively. The degree of bias and the limits of agreement between the two methods were determined. Data were analysed using the SPSS/PC statistical package version 11.1.

Results

Mean values for nutritional and biochemical characteristics of the 10 lactating mothers are given in Table I. Infant growth is comparable to NCHS/World Health Organisation reference standards. For this group of 10 lactating Nandi women, Hb status was within the normal range, vitamin A status was marginal and iron stores were severely deficient. Based on BMI (< 20) 20% of the mothers would be considered underweight. The mean values for body composition are shown in Table II. Using a FFM cut-off point of 42.8 kg, 60% of the mothers would be considered undernourished based on SF calculations compared with 40% based on the DO method. Although not significantly different, the % BF estimates derived from SF measurements were higher while those for FFM were lower compared with the DO method.

Table I. **Nutritional and biochemical characteristics of 10 lactating Nandi women**

	Mean*
Age	25.8 ± 4.7
No. of children	3.4 ± 2.4
Infant weight/age *z*-score†	0.067 ± 0.860
Lactation duration (months)	4.0 ± 3.3
Weight (kg)	60.7 ± 12.4
Height (m)	1.6 ± 0.1
BMI (m^2)	23.4 ± 4.0
MUAC (cm)	25.3 ± 3.1
Hb (g/dl)	12.1 ± 1.8
Serum ferritin (µg/l)‡	10.3 ± 4.0
Serum retinol (µmol/l)	0.696 ± 0.300

*Mean ± SD.
†Based on NCHS World Health Organisation reference standards.
‡Logarithmic values.
BMI = body mass index (weight/height); MUAC = mid upper arm circumference.

Table II. **Body composition indices of 10 lactating Nandi women**

	Mean*	**Minimum**	**Maximum**
Skinfold thickness			
Biceps (mm)	9.9 ± 6.7	5.0	24.6
Triceps (mm)	16.7 ± 6.7	8.0	29.5
Supra-iliac (mm)	13.4 ± 7.7	5.2	29.6
Subscapular (mm)	20.6 ± 10.5	8.3	38.5
Total body water (TBW)	31.8 ± 3.4	25.9	35.8
Body composition (SF)†			
BF (kg)‡	17.8 ± 7.5	8.7	34.0
FFM (kg)‡	42.9 ± 5.9	33.4	51.2
% BF	28.4 ± 6.4	18.2	39.9
Body composition (DO)§			
BF (kg)‡	16.7 ± 8.8	8.1	36.7
FFM (kg)‡	44.0 ± 4.7	35.7	49.5
% BF	26.2 ± 8.1	16.8	43.1

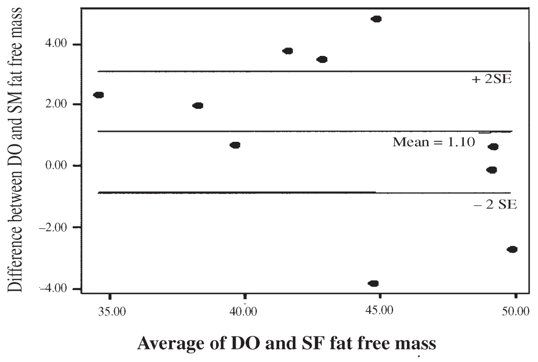
*Mean ± SD.
†Estimates based on skinfold thickness measurements (SF).
‡ Body fat (BF), fat-free mass (FFM), percentage body fat (% BF).
§ Estimates based on deuterium oxide (DO).

The body composition indices derived from the DO and SF measurements were all significantly ($p < 0.01$) and positively correlated for BF ($r = 0.95$), FFM ($r = 0.88$) and % BF ($r = 0.82$). For the DO-derived body composition indices (mean ± SD) maternal TBW, FFM, and BF were 53.4 (5.9)%, 73.8 (8.08)% and 26.15 (8.1)% expressed as a percentage of body weight respectively. The estimates for BF, FFM and % BF derived from the DO method account respectively for 91%, 78%, and 66% of the total variability observed in the values derived from the SF measurements.

The relation between differences in body composition indices obtained from the DO and SF methods and the average of the values from these two methods are shown in Fig. 1. The estimates for % BF had the biggest bias, with a mean (± SD) difference of –2.2% (4.7). Across the range of body composition indices represented by this population of lactating mothers, the Bland-Altman relation of inter-method differences and means indicate considerable lack of agreement between the two methods and some bias in the body composition indices derived from the SF method.

The limits of agreement and differences in accuracy levels for the two methods are shown in Table III. The range of agreement between the two methods is large for all the body composition indices. The limits of agreement estimates (± 95% confidence interval (CI))

(a) Maternal fat free mass (FFM)



(b) Maternal % body fat (%BF)

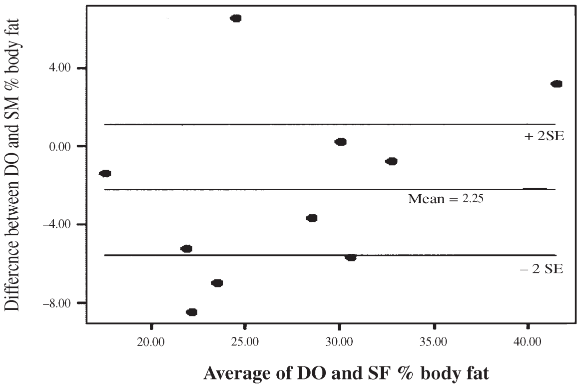


Fig. 1. Mean difference and 95% CI for body composition indices derived from deuterium oxide (DO) and from skinfold-thickness measurements (SF) plotted against the mean value for both the methods.

for % BF were –2.2 ± 9.4 resulting in a SF % BF estimation that is 11.6% (SEE 5.8) below the DO value for % BF. For FFM the limits of agreement (± 95% CI) between the two methods was 1.1 ± 5.6 indicating that the SF and FFM estimates were likely to be 6.7 kg (SEE 3.5) above the estimates made from the DO method. For % BF and FFM limits of precision (± 95% CI) were –2.2 ± 3.4 and 1.1 ± 1.9 respectively. The accuracy of the SF estimates for % BF may be up to 5.6% below the values derived from DO. The accuracy for FFM above the DO-derived values could be as high as 3.1 kg.

The variability (5.8 - 17.5%) observed in the SF technique may result in lower prediction of % BF. The Maastricht protocol for assessment of body composition was applied with relative ease under the field conditions in a rural community in Kenya. The combination of this method with other nutritional status indicators can provide valuable information for monitoring community-based nutritional interventions.

Discussion

Studies of body composition in developing countries require accurate and practical techniques that interfere minimally with the busy physical life of rural communities. All the subjects in this study were lactating mothers living in a rural community in Kenya. Their TBW was etimated based on the Maastricht protocol for determination of body composition.[7,8] Since it is administered in the evening, the method does not interfere with the lactating mother's busy daytime routine. The very minimal field laboratory requirements and ease of sample collection will make researchers in developing countries appreciate the tremendous strides that the Maastricht protocol entails. Its application in a field situation in a rural community provides an opportunity to make a fairly accurate assessment of nutritional status. In this study the body composition indices derived on the basis of SF thickness from the DO dilution technique were only compared with body composition indices derived from the age- and sex-specific logarithmic equation of Durnin and Wormersley.[14] The purpose of the study was to provide further validation for the use of the isotope dilution technique in the estimation of body composition indices.

FFM is assumed to contain all the TBW that comprises 730 g/kg FFM. Isotope dilution provides an indirect measure for TBW. Hence by measuring TBW the FFM can be estimated as TBW/0.730.[20] An 8 - 10-hour equilibration time for determination of DO enrichment gives more accurate estimates of TBW. The Maastricht protocol recommends 10 hours as the optimal time for isotopic equilibrium for DO.[6,8] Its application under field

Table III. **Mean differences and variation in the limits of precision and agreement between maternal body composition indices estimated from the DO dilution technique and anthropometric measurements of skinfold thickness for the 10 rural lactating mothers**

Body composition	Mean*	95% CI for Precision†	95% CI for Agreement‡	95% CI for limits of agreement‡ < DO value	95% CI for limits of agreement‡ > DO value
BF (kg)§	–1.11 ± 2.80	–1.11 ± 1.99	–1.11 ± 5.6	6.71 ± 3.46	4.49 ± 3.46
FFM (kg)§	1.11 ± 2.80	1.11 ± 1.99	1.11 ± 5.6	4.49 ± 3.46	6.71 ± 3.46
% BF§	–2.25 ± 4.70	–2.25 ± 3.34	–2.25 ± 9.4	11.65 ± 5.81	7.15 ± 5.81

*Mean difference ± SD.
†Mean difference ± (t x SE).[25]
‡Mean difference ± 2SD (t x 3SE).[25]
§Body fat (BF), fat-free mass (FFM), percentage body fat (% BF).

conditions seems quite feasible. The various levels of accuracy and limits of agreement examined in this study show considerable variability in etimates for FFM and % BF. In this study using DO as the more accurate method, evaluation of the relative accuracy of the SF method shows that the limits of agreement for FFM were 1.1 ± 5.6 kg. This gives a SF overestimation of 6.7 kg (SEE 3.5) compared with the limits of agreement for % BF which were –2.2 ± 9.4 % leading to an 11.7% (SEE 5.8) SF underestimation of % BF. The advantages of the DO dilution technique are the relative ease with which it can be applied in developing country field conditions and the possibility of combining this type of evaluation with other nutritional indices providing valuable information for nutritional interventions. The main disadvantage may be the complexity of the laboratory measurements, which requires use of a mass spectrometer and trained personnel. This limitation may now be reduced by using the Fourier transformed infrared spectrophotometer (FTIS) to measure DO sample enrichment.[11,12] This new development will facilitate wider use of the DO method.

As shown in this study, the DO method can be combined with other nutritional indicators, thus providing a better assessment of the subject's nutritional status. The method offers the opportunity to determine maternal body composition and breast-milk output simultaneously,[26] a factor critical in studying breast-feeding practices. A good example of this is the study done on 30 Otomi Indians from Mexico[27] where it was observed that lactation performance correlated significantly with maternal size and body composition. In a number of investigations of energy requirements and expenditure FFM has been used as a primary predictor of basal metabolic rate (BMR). A general prediction equation of BMR = 370 + 21.6 (FFM) has been suggested by Cunningham.[28] This equation explains 65 - 90% of the variation in BMR. It predicts 1 235 kcal (5.17 MJ day) for a standard individual of 40 kg FFM living in a developing country. In our study the DO estimation for maternal FFM was 44.0 kg. When the Cunningham prediction equation is applied it yields a maternal BMR of 1 320 kcal/day (5.52 MJ/day). The validity of generalised regression equations is debatable and the use of predictive equations applicable to the subjects under study is generally recommended.[29,30] The DO FFM combined with local estimations of BMR can be used to generate local prediction equations for BMR. Average energy expenditure may then be estimated from BMR multiplied by an appropriate activity factor that varies from 1.2 to 1.4.[30] This may be useful in studies of energy requirements and expenditure in developing countries where pregnant and lactating women tend to be undernourished, are overworked due to too many demands on their time, and often have limited access to resources that could help them cope with their circumstances.

Accurate assessment of body composition indices continues to be necessary because determining changes in body size and composition provides important information on the consequences of lowered energy intake, especially if sustained at low levels as is often the case in rural communities in the developing world. The most pronounced outcome of this study is that the observed limits of agreement for FFM and % BF can be attributed to the variation in SF-derived body composition indices. Compared with the DO-derived body composition indices, when the Durnin and Womersly SF thickness equation is used individual % BF can be underestimated by as much as 11.6% (SEE 5.81) or overestimated by up to 7.1% (SEE 5.8). The variability (5.8 - 17.5%) in the SF measurements lower prediction of % BF must be taken into account in the evaluation of undernutrition in a rural African community.

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1. Central Bureau of Statistics. *The First Report of Poverty in Kenya: Incidence and Depth of Poverty*. Ministry of Planning and National Development (CBS), vol. 1. Nairobi: CBS, 1998.
2. Shetty PS. Adaptive changes in basal metabolic rate and lean body mass in chronic under-nutition. *Hum Nutr Clin Nutr* 1984; **38:** 573-581.
3. Barac-Nieto M, Spurr GB, Lotero H, Maksud MG. Body composition in chronic undernutrition. *Am J Clin Nutr* 1978; **31:** 23-40.
4. Shetty PS. Adaptation to low energy intakes: the responses and limits to low intakes in infants, children, and adults. *Eur J Clin Nutr* 1999; **53:** Suppl 1, S14-33.
5. Lukaski HC, Johnson PE. A simple, inexpensive method of determining total body water using a tracer dose of D_2O and infrared absorption of biological fluids. *Am J Clin Nutr* 1985; **41:** 363-370.
6. van Marken Lichtenbelt WD, Westerterp KR, Wouters L. Deuterium dilution as a method for determining total body water: effect of test protocol and sampling time. *Br J Nutr* 1994; **72:** 491-497.
7. Westerterp KR, Wouters L, van Marken Lichtenbelt WD. The Maastricht protocol for the measurement of body composition and energy expenditure with labeled water. *Obes Res* 1995; **3:** suppl, 49-57.
8. Westerterp KR. Body composition, water turnover and energy turnover assessment with labelled water. *Proc Nutr Soc* 1999; **58:** 945-951.
9. Infante C, Hurtado J, Salazar G, Pollastri A, Aguirre E, Vio F. The dose-to-mother method to measure milk intake in infants by deuterium dilution: validation study. *Eur J Clin Nutr* 1991; **45:** 121-129.
10. Fjeld CR, Brown KH, Schoeller DA. Validation of the deuterium oxide method for measuring average daily milk intake in infants. *Am J Clin Nutr* 1988; **48:** 671-679.
11. Caire G, Calderon de la Barca AM, Bolanos AV, *et al*. Measurement of deuterium oxide by infrared spectroscopy and isotope ratio mass spectrometry for quantifying daily milk intake in breast fed infants and maternal body fat. *Food Nutr Bull* 2002; **23:** Suppl 3, 38-41.
12. Cisse AS, Bluck L, Diaham B, Dossou N, Guiro AT, Wade S. Use of Fourier transformed infrared spectrophotometer (FTIR) for determination of breast milk output by the deuterium dilution method among Senegalese women. *Food Nutr Bull* 2002; **23:** Suppl 3, 138-141.
13. Roche AF, Siervogel RM, Chumlea WC, Webb P. Grading body fatness from limited anthropometric data. *Am J Clin Nutr* 1981; **34:** 2831-2838.
14. Durnin JVG, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements in 481 men and women aged from 16 to 72 years. *Br J Nutr* 1974; **32:** 77-97.
15. Brodie DA. Techniques of measuring body composition. Part I. *Sports Med* 1988; **5:** 11-40.
16. Ettyang GA, van Marken Lichtenbelt WD, Oloo A, Saris WHM. Serum retinol, iron status and body composition of lactating women in Nandi, Kenya. *Ann Nutr Metab* 2003; **47:** 276-283.
17. Gibson RS. Anthropometric measurement of body composition. In: Gibson RS, ed. *Principles of Nutritional Assessment.* New York: Oxford University Press, 1990: 187-208.
18. Scrimgeour CM, Rollo MM, Mudambo SMKT, Handley LL, Prosser SJ. A simplified method for deuterium/hydrogen isotope ratio measurements on water samples of biological origin. *Biological Mass Spectrometry* 1993; **22:** 383-387.
19. Wong WW, Lee LS, Klein PD. Deuterium and ^{18}oxygen measurements on microlite

samples of urine, plasma, saliva and human milk. *Am J Clin Nutr* 1987; **45:** 905-991.

20. Raaij JMA, van Peek MEM, Vermaart-Miedema SH, Schonk CM, Hautvast JGAJ. New equations for estimating body fat mass in pregnancy from body density or total body water. *Am J Clin Nutr* 1988; **48:** 24-29.
21. Siri WE. Body composition from fluid spaces and density: analysis of methods. In: *Techniques For Measuring Body Composition*. Washington, DC: National Academy of Sciences, National Research Council, 1961: 223-224.
22. ACC/SCN. *Fourth Report on the World Nutrition Situation. Nutrition throughout the life cycle*. Geneva: ACC/SCN in collaboration with IFPRI, 2000.
23. Yip R. Iron deficiency: Contemporary scientific issue and international programmatic approaches. *J Nutr* 1994; **124:** 1479S-1490S.
24. World Health Organisation. *Indicators for Assessing Vitamin A Deficiency and Their Application in Monitoring and Evaluating Intervention Programmes.* WHO/NUT/96.10 Geneva: WHO, 1996.
25. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; **1:** 307-310.
26. 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.
27. Villalpando SF, Butte NF, Wong WW, *et al*. Lactation performance of rural Mesoamerindians. *Eur J Clin Nutr* 1992; **46:** 337-348.
28. Cunningham J. Body composition as a determinant of energy expenditure: a synthetic review and a proposed general prediction equation. *Am J Clin Nutr* 1991; **54:** 963-969.
29. Norgan NG, Ferro-Luzzi A. The estimation of body density in man: are general equations general? *Ann Hum Biol* 1985; **12:** 1-15.
30. Durnin JVGA. Energy requirements: general principles. *Eur J Clin Nutr* 1996; **50:** Suppl, 52-9; discussion 9-10.