

Evaluation of equations for fat-free mass based on anthropometry in infants and young children in South Asia

Bandana Sen¹, Dilip Mahalanabis^{1*}, Saijuddin Shaikh¹, Anura V. Kurpad² and Kaushik Bose³

¹Society for Applied Studies, CF-198, Salt Lake City, Sector 1, Kolkata 700 064, India

²St John's Medical College, Institute of Population Health and Clinical Research, St John's National Academy of Health Sciences, Bangalore 560 034, India

³Department of Anthropology, Vidyasagar University, Paschim Midnapore 721 102, India

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Rapid postnatal growth in low-birth weight infants increases the risk of hypertension, CHD and type 2 diabetes in adult life. To provide validated tools to study the growth in South Asian infants, we evaluated two published equations to measure total body water (TBW) and fat-free mass (FFM) based on anthropometry in 6- to 24-month-old infants, using ²H₂O dilution. In a method-comparison study in seventy-eight infants aged 6–24 months (forty-two girls and thirty-six boys) from the urban poor attending an immunisation clinic of a hospital in Kolkata, we measured their length to the nearest 0.1 cm, weight to the nearest 10 g and TBW using ²H₂O dilution. The calculated TBW in kg (TBWkg) and FFM in kg (FFMkg) using two equations based on the length and weight were each compared with TBWkg and FFMkg calculated from ²H₂O dilution. The mean FFMkg were 7.31 (SD 1.11), 7.13 (SD 1.08) and 7.26 (SD 1.13) by the ²H₂O dilution method, and the anthropometry equations of Mellits and Cheek (AN-1) and Morgenstern *et al.* (AN-2), respectively. The mean of the paired difference in FFMkg was 0.18 (SEM 0.06) and 0.04 (SEM 0.07) between ²H₂O, and AN-1 and AN-2, respectively. There is a good agreement for FFM derived by AN-2 with ²H₂O dilution. The former is 1% lower than that obtained from the reference method (*P*=0.28). The AN-2 equation is useful for evaluating FFM in infants in India.

Total body water: Fat-free mass: ²H₂O dilution technique: Infants

Measuring fat-free mass (FFM) in infants and young children is of scientific and public health interest. Recent findings on the relationship between intra-uterine growth retardation and diseases in adults such as hypertension, type 2 diabetes and CHD generated renewed interest in studying the developmental indicators of infants and young children^(1–4). Furthermore, it has been shown that more rapid postnatal growth (catch-up growth) in infants born with intra-uterine growth retardation is also a risk factor for these adult diseases^(5–8). In South Asian countries, the rate of low birth weight is very high largely due to intra-uterine growth retardation⁽⁹⁾. Furthermore, the incidence of hypertension, type 2 diabetes and CHD is higher among South Asians compared with the Caucasians⁽¹⁰⁾. We therefore need population-based studies on growth and its components like FFM in infancy and early childhood. The absence of validated equations for measuring FFM in infants in South Asia based on simple measurements such as height/length and weight is a known constraint. Are the existing equations suitable for use in infants and young children in South Asia?

In the light of that mentioned earlier, measurement of body composition such as FFM assumes importance. The practical methods for use in clinics and in the field to measure FFM in infants and children are largely based on anthropometry

(such as height/length and weight) and equations for children have been developed mainly in developed countries. We found two such validated equations in literature based on anthropometry^(11,12) for use in children. Before we can use these equations with confidence on a population different from that on which these equations were developed, it is appropriate to validate them before they are applied on a large scale in infants and children in countries in South Asia. To address this question, we evaluated these two published equations for FFM based on anthropometry. We compared the total body water (TBW) and FFM derived by these equations with a reference method based on isotope dilution using ²H₂O.

Subjects and methods

Subjects

The present study was conducted in apparently healthy children among the urban poor attending an immunisation clinic of a large charitable government hospital in the city of Kolkata. The hospital service is provided free. The immunisation clinic is held once a week. Each week we interviewed the first four mothers who registered their children

Abbreviations: FFM, fat-free mass; FFMkg, FFM in kg; TBW, total body water; TBWkg, TBW in kg.

* **Corresponding author:** Dr Dilip Mahalanabis, fax +91 33 2337 0709, email sas_kolkata@vsnl.net

in the weekly clinic, and we aimed to recruit up to two children each week. The recruitment was spread over a period of 1 year.

The eligibility criteria for inclusion in the present study were age 6–24 months of either sex, absence of illness during the preceding 1 month, absence of gross congenital anomalies and chronic diseases, and parents' willingness to participate. The socio-economic and demographic features of the families are given in Table 1. These children are from low socio-economic status and records on birth weight and gestation were not available for consideration. Eighty-six children, aged 6–24 months, participated in the present study.

Written informed consent was obtained from the parents and the present study was approved by the ethical review committee of the Society for Applied Studies, Kolkata, India.

Sample size

Our plan was to evaluate simple prediction equations of measuring FFM for large-scale use, based on anthropometry method. The reference method is based on $^2\text{H}_2\text{O}$ (a stable isotope) dilution. For an ideal prediction equation, the difference in the measured quantity between the reference method and the method under study should be zero. The sample size will depend on the magnitude of deviation from zero and its CI that will be acceptable for the purpose the method is to be used and therefore can only be indicative. The comparisons will be paired, i.e. the results from the two methods on each subject will be compared. The degree of deviation of the mean of the differences from zero is of interest for the present study. We have calculated the sample size to detect 1.5% or more deviation from zero difference with a standard deviation of 2. To detect this degree of deviation with 95% confidence and 90% power, we need a sample of seventy-one subjects in each group⁽¹³⁾. Since the same subject will provide data for FFM by both the reference and the comparison methods, the sample size remains the same, i.e. seventy-one subjects. We added 12% to this number to give a sample size of eighty subjects.

Anthropometric measurements

The anthropometric measurements were made using recommended protocols^(14,15) and are briefly described. The measurements were taken on the same day the infant underwent $^2\text{H}_2\text{O}$ dilution test.

Table 1. Socio-economic status of the families

Variables	Number (%)
House with cement floor, wall and roof	8 (10%)
Lives and cooks in one room	51 (65%)
Family income, £/month (median, quartiles)	22 (18, 31)
Mother's education	
Illiterate	10 (13%)
1–5 Years of school	27 (35%)
6–10 Years of school	33 (42%)
> 10 Years of school	8 (10%)

Length

Recumbent length was measured with a wooden measuring board as described earlier⁽¹⁶⁾. The board was made sufficiently broad to cover the shoulder blades. The reading was taken to the nearest 0.1 cm.

Weight

Weight was measured nude, using an electronic platform balance with a precision of 10 g. The balance was checked regularly for accuracy using standard weights.

^2H -labelled water dilution method

$^2\text{H}_2\text{O}$, water with a stable isotope of hydrogen, was used. This measurement took place between 09.00 and 14.00 hours. Because of their age, the infants were not fasted. A vast majority of them were breast-fed (88%). No food other than breast-feeding was allowed for 3 h before administering the $^2\text{H}_2\text{O}$. Breast-feeding was stopped 1 h before administering the $^2\text{H}_2\text{O}$. As expected, the infants were normally hydrated at the time of the test. Children's baseline saliva was collected by a disposable syringe. We took a measured volume of $^2\text{H}_2\text{O}$ (99.9%; Sigma, St Louis, MO) approximately equivalent to 50 mg/kg body weight in a disposable container. $^2\text{H}_2\text{O}$ has a specific gravity of 1.107. We then weighed the $^2\text{H}_2\text{O}$ dose using a precision balance and the weight with an accuracy of 0.1 mg was recorded and used in calculating TBW. It was mixed with 20 ml of distilled water and the child drank it from a disposable syringe. The empty container was rinsed with two consecutive lots of 15 ml of distilled water and the child drank both lots from the same disposable syringe. Time was allowed to equilibrate the $^2\text{H}_2\text{O}$ into the body fluid. No food or fluid including breast milk and water was permitted during the equilibration period of 3 h. At the end of the equilibration period, the second sample of saliva was collected and analysed for ^2H using a dual-inlet mass spectrometer (Europe Scientific, Crewe, UK), using the zinc reduction technique. The results of $^2\text{H}_2\text{O}$ concentration in the saliva samples were used for calculating the TBW.

The following equation was used:

$$\text{TBW in kg} = (\text{TBW in moles} \times \text{molecular weight of } \text{H}_2\text{O (i.e. 18.0153)}) / 1000.$$

TBW in moles was calculated as follows:

$$\text{TBW in moles} = (F_1 \times N_1) \div (F_2 \times 1.041),$$

where F_1 = (concentration of $^2\text{H}_2\text{O}$ in atom per cent (i.e. 99.9) $\times 10^6 / 100 - 150$) ppm of $^2\text{H}_2\text{O}$, F_2 = ppm after dose – ppm before dose of $^2\text{H}_2\text{O}$ (using concentrations in saliva samples) and N_1 = dose of $^2\text{H}_2\text{O}$ in gram/molecular weight of $^2\text{H}_2\text{O}$ (i.e. 20.0274).

In using ^2H for estimating TBW, it should be noted that this isotope undergoes some exchange with non-aqueous hydrogen and a correction factor of 1.041 was used. The FFM was calculated as TBW divided by an age- and sex-specific hydration factor for FFM⁽¹⁷⁾.

Anthropometry-based published equations

The prediction equations for TBW using weight and length that we evaluated in the present study are given later.

Anthropometry-based equation AN-1⁽¹¹⁾:

$$\text{For boys: TBW (kg)} = -1.927 + 0.465W + 0.045H.$$

$$\text{For girls: TBW (kg)} = 0.076 + 0.507W + 0.013H,$$

where H = length in cm, W = weight in kg and when $H \leq 132.7$ cm for boys and $H \leq 110.8$ cm for girls. These equations were derived from studies on the US subjects aged 1–34 years.

Anthropometry-based equation AN-2⁽¹²⁾:

$$\text{For boys: TBW (kg)} = 0.0846 \times (H \times W)^{0.65}.$$

$$\text{For girls: TBW (kg)} = 0.0846 \times 0.95 \times (H \times W)^{0.65}.$$

These equations were derived from studies on the US subjects aged 3 months to 13 years.

The same hydration factors for FFM⁽¹⁷⁾ were used for calculating FFM from TBW derived by the anthropometry-based equations and ²H₂O dilution technique.

Statistical analysis

Epi Info version 6.0⁽¹⁸⁾ and Stata version 7.0⁽¹⁹⁾ were used for data entry and statistical analysis. For anthropometric data, a software package based on National Center for Health Statistics database as provided with the Epi Info software was used. As is conventionally done, correlation and regression analysis were used to compare the TBW based on anthropometry with the reference method, i.e. ²H₂O dilution technique. We plotted the results along the 'line of equality' (i.e. the line at a 45° angle) to visually examine the concordance⁽²⁰⁾.

Furthermore, the difference in TBW for each subject between each of the two anthropometry equations and ²H₂O dilution (paired data) was evaluated for their difference from zero by t -test. Mean (SD 2) was calculated to derive the range within which 95 % of the values were expected to lie⁽²⁰⁾.

Results

Eighty-six children participated in the present study. In eight of them, the ²H₂O procedures were incomplete because of vomiting or being fed during the equilibration period. They were dropped from the study. The study infants were predominantly breast-fed (88 %). Characteristics of the study children are shown in Table 2. Weight-for-age, length-for-age and weight-for-length standard deviation scores suggest some degree of underweight, wasting and stunting. Using the two equations, i.e. AN-1⁽¹¹⁾, AN-2⁽¹²⁾, and by ²H₂O dilution, we derived TBW in kg (TBWkg). Mean TBW based on AN-1 was 1.86 % lower than the reference method ($P=0.002$) and TBW derived by AN-2 was 0.75 % lower than the reference method ($P=0.2$). Calculated TBW and FFM using ²H₂O dilution technique and the two anthropometry equations are shown in Table 3. Mean FFM derived by AN-1 was 2.30 % lower than the reference method ($P=0.002$) and FFM derived by AN-2 was 0.79 % lower than ²H₂O dilution ($P=0.29$). As expected, the mean FFM in boys tended to be higher than that in the girls for all the three methods. The differences however did not reach statistical significance. The mean, SD values of the difference in FFM in kg (FFMkg) and percentage of body weight between those derived by ²H₂O dilution and each of the two equations under study and 95 % CI of the difference are shown in Table 4. The results show that the FFMkg derived by the equation AN-2⁽¹²⁾ gives a better agreement with the reference method; 95 % limits of agreement with the reference

Table 2. Characteristics of subjects

Characters	Boys (n 36)		Girls (n 42)	
	Mean or number	SD	Mean or number	SD
Age, months (range)	15.3 (6.2–23.8)	5.6	16.2 (7–23.6)	5.3
< 12 months	13		10	
≥ 12 months	23		32	
Body weight, kg (range)	8.9 (6.4–12.1)	1.3	8.8 (6.2–11.9)	1.4
Length, cm (range)	76.6 (65.5–86.5)	6.0	76.0 (63.8–86.0)	6.1
WHZ*	−1.37	1.05	−1.01	0.70
WHZ < −2	10		3	
WHZ ≥ −2 and < −1	14		17	
WHZ ≥ −1	12		22	
WAZ*	−1.57	1.10	−1.33	0.95
WAZ < −2	12		10	
WAZ ≥ −2 and < −1	16		18	
WAZ ≥ −1	8		14	
HAZ*	−0.87	1.05	−0.87	1.22
HAZ < −2	4		7	
HAZ ≥ −2 and < −1	10		13	
HAZ ≥ −1	22		22	
Mid-arm circumference (range)	14.1 (12.0–16.5)	1.1	13.9 (12.5–16.0)	0.9
Biceps (range)	3.3 (2.0–6.0)	0.9	3.4 (2.0–5.8)	0.7
Triceps (range)	5.4 (3.2–9.0)	1.3	5.6 (4.0–8.0)	0.9
Subscapular (range)	6.2 (4.0–10.0)	1.3	6.0 (4.0–10.0)	1.2
Suprailiac (range)	4.8 (2.5–8.0)	1.1	4.6 (3.0–7.0)	1.0

*WHZ, WAZ and HAZ are weight-for-height, weight-for-age and height-for-age standard deviation scores, respectively, compared with National Center for Health Statistics reference.

Table 3. Mean and standard deviation (sd) of TBWkg, FFMkg and as percentage of body weight derived by the two equations under study and by the ²H-labelled water dilution technique (reference method)*

Methods	Mean, TBWkg, sd (TBW%, sd)			Mean, FFMkg, sd (FFM%, sd)			P FFMkg (FFM%)
	Total (n 78)	Boys (n 36)	Girls (n 42)	Total (n 78)	Boys (n 36)	Girls (n 42)	
² H ₂ O	5.75, 0.84 (65.04, 5.64)	5.89, 0.92 (65.91, 4.40)	5.63, 0.76 (64.29, 6.48)	7.31, 1.11 (82.6, 7.20)	7.49, 1.21 (83.79, 6.04)	7.15, 1.00 (81.65, 8.04)	0.18 (0.19)
AN-1	5.60, 0.81 (63.17, 1.58)	5.67, 0.84 (63.46, 1.93)	5.54, 0.79 (62.92, 1.16)	7.13, 1.08 (80.3, 2.40)	7.21, 1.12 (80.65, 3.24)	7.06, 1.04 (80.07, 1.38)	0.52 (0.30)
AN-2	5.70, 0.85 (64.29, 2.44)	5.90, 0.82 (66.08, 2.21)	5.53, 0.85 (62.75, 1.35)	7.26, 1.13 (81.8, 3.50)	7.51, 1.11 (84.08, 3.56)	7.05, 1.11 (79.92, 2.03)	0.07 (<0.001)

* FFM, fat-free mass; TBW, total body water; FFMkg, FFM in kg; TBWkg, TBW in kg; AN-1, anthropometry equations of Mellitts and Cheek; AN-2, anthropometry equations of Morgenstern et al.

Table 4. Mean, SD of the difference in FFM (of paired values) in kg and percentage of body weight between those derived by ²H-labelled water dilution and by each of the two equations under study and 95% CI of the difference

	Mean difference in kg (as % body weight)	SD	95% CI	P*
² H ₂ O-AN-1 ^a	0.18 (2.30)	0.57 (6.39)	0.05, 0.31 (0.86, 3.74)	0.007 (0.002)
² H ₂ O-AN-2 ^b	0.04 (0.79)	0.57 (6.51)	-0.09, 0.17 (-0.67, 2.26)	0.505 (0.285)

FFM; fat-free mass.

^a Difference between ²H₂O and anthropometry methods (AN-1).

^b Difference between ²H₂O and anthropometry methods (AN-2).

* The t test evaluates the difference in the paired values from zero.

method (i.e. mean (SD 2)) were -0.96 to 1.32 and -1.10 to 1.18 for AN-1 and AN-2, respectively. Distribution of points for TBWkg along the line of identity between ²H₂O and AN-1 and ²H₂O and AN-2 methods is shown in Fig. 1 (a,b). Similarly, FFMkg are plotted in Fig. 2 (a,b). Bland-Altman plots of the differences in TBWkg in each participant between ²H₂O and AN-1 and ²H₂O and AN-2 methods against average TBWkg are shown in Fig. 1 (c,d); mean differences were 0.15 and 0.05, respectively. Similarly, for FFMkg, the differences are plotted in Fig. 2 (c,d); mean differences were 0.18 and 0.04, respectively. In Fig. 3, individual data points for percentage of FFM derived by the two anthropometry equations and by the reference method are plotted against age in months. Age- and sex-specific reference percentage of FFM in healthy American infants derived by multicomponent models⁽¹⁷⁾ are also plotted for comparison. The percentage of FFM in the study infants derived by all three methods are consistently higher than the reference values in the US infants, indicating that the infants under study are considerably leaner and have lower fat mass percentage.

Discussion

The weight-for-height indices such as Z-scores have not been evaluated for their ability to predict FFM. Weight-for-height indices and recently introduced BMI Z-score for children do not distinguish fat and lean masses. In fact there is a 2-fold range of variation in fatness for a given BMI value in individual children⁽²¹⁾. Based on skin-fold measurement, Yajnik and colleagues^(10,22) have shown that Indian babies are not only small at birth but they also have less muscle mass and relatively more fat mass, the so-called 'thin-fat baby' syndrome. They further showed that thin-fat babies grow up to become thin-fat adults with thinner limbs and high waist-hip ratio; they appear to be fetally programmed and predisposed to diabetes. They also showed that the smallness and thinness of the Indian babies is present at birth and an unusual thin-fat body composition is associated with the insulin-resistance syndrome^(10,23). To further understand and study such phenomena, validated prediction equations for FFM based on anthropometry should be of value. The conventional weight-for-height indices and BMI percentile are likely to be inadequate to understand the growth and development of FFM.

The purpose of the present study was to evaluate predictive equations for the percentage of FFM derived from TBW by

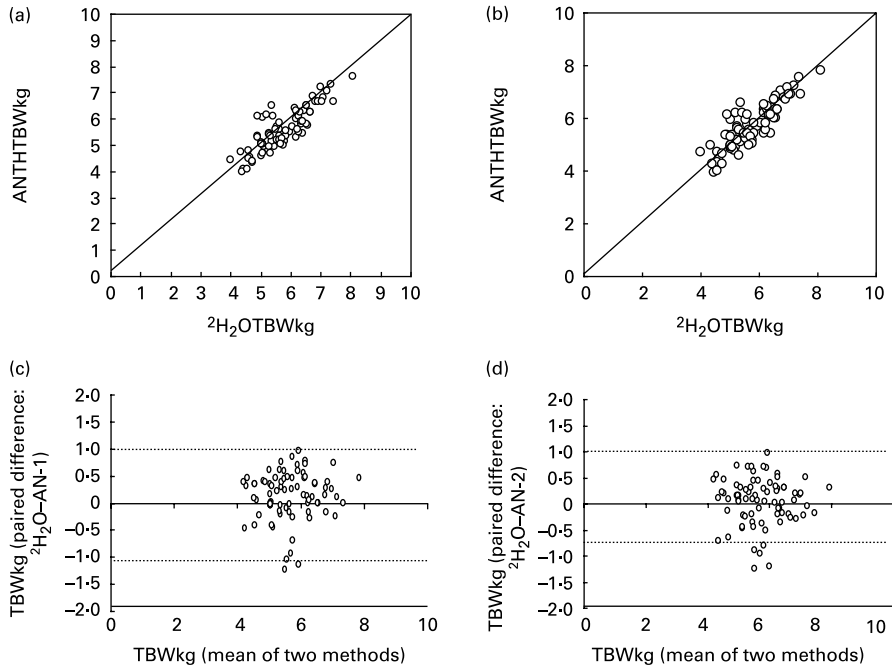


Fig. 1. (a,b) TBWkg: Individual data points comparing values derived by the reference method (^2H -labelled water dilution) with each of the anthropometric equations under study are plotted along the 'line of identity' (at 45° angle). ANHTTBWkg: TBWkg derived by the two equations based on anthropometry (AN-1 and AN-2), plotted against $^2\text{H}_2\text{O}$ method ($R^2 = 0.73$ and 0.74 , respectively). $^2\text{H}_2\text{O}$ TBWkg: TBWkg derived by ^2H -labelled water dilution method^(11,12). (c,d) Bland–Altman plots of the difference (mean (SD 2)) in each participant between TBWkg derived by ^2H -labelled water reference method and each of the methods under study plotted against average of the TBWkg by ^2H -labelled water method and each of the study methods^(11,12). ^2H -labelled water–AN-1: mean difference = 0.15 , $\text{SD} = 0.44$, $P = 0.005$, ^2H -labelled water–AN-2: mean difference = 0.05 , $\text{SD} = 0.45$, $P = 0.372$.

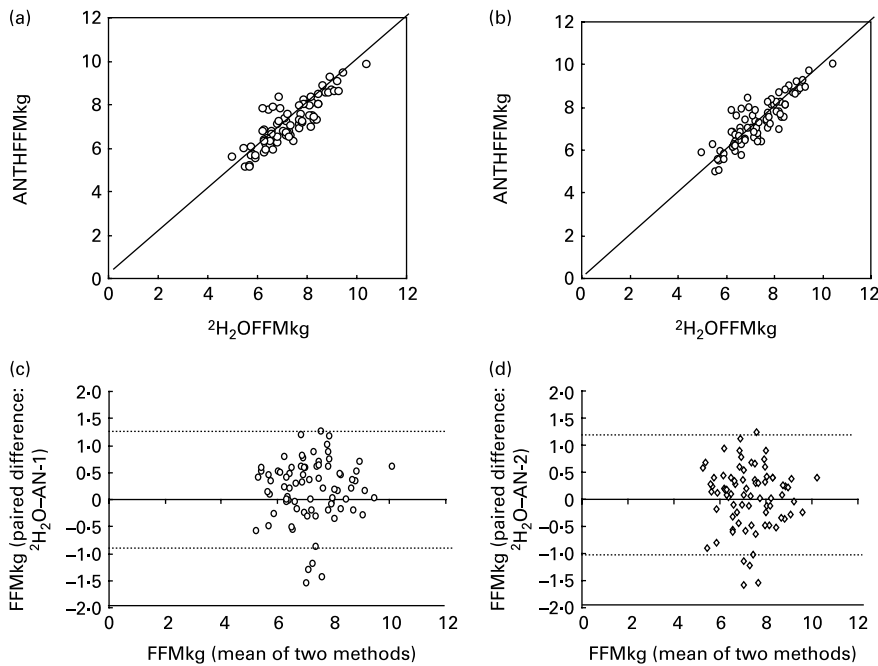


Fig. 2. (a,b) FFMkg: Individual data points comparing values derived by the reference method (^2H -labelled water dilution) with those by the two equations under study are plotted along the 'line of identity' (at 45° angle). ANTHFFMkg: FFMkg derived by the two equations based on anthropometry (AN-1 and AN-2), plotted against ^2H -labelled water method ($R^2 = 0.75$ and 0.76 , respectively). $^2\text{H}_2\text{O}$ FFMkg: FFMkg derived by ^2H -labelled water dilution method^(11,12). (c,d) Bland–Altman plots of the difference (mean (SD 2)) in each participant between FFMkg derived by ^2H -labelled water as the reference method and each of the methods under study plotted against average of the FFMkg by ^2H -labelled water method and each of the study methods^(11,12). ^2H -labelled water–AN-1: mean difference = 0.18 , $\text{SD} = 0.57$, $P = 0.007$, ^2H -labelled water–AN-2: mean difference = 0.04 , $\text{SD} = 0.57$, $P = 0.505$.

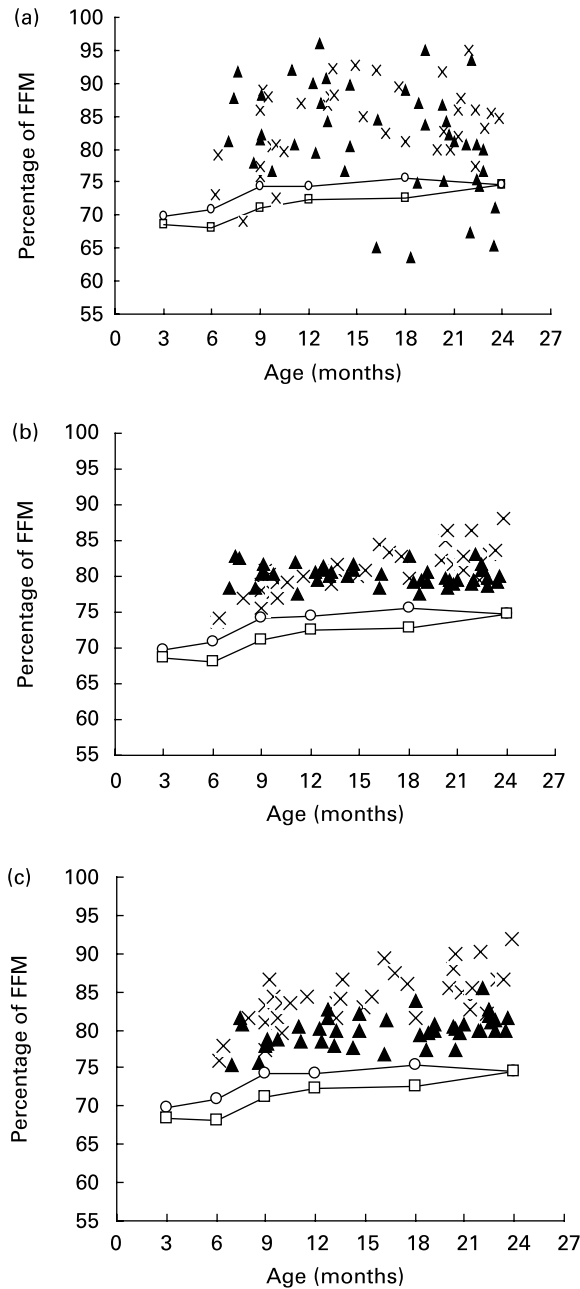


Fig. 3. Individual data points for percentage of FFM derived by (a) ^2H -labelled water dilution method and (b,c) the two anthropometry equations^(11,12) are plotted against age in months. Age- and sex-specific reference percentage of FFM in healthy American infants derived by multicomponent models⁽¹⁷⁾ are plotted for comparison (data points are connected). Boys (X), girls (▲), boys (—○—, Butte⁽¹⁷⁾), girls (—□—, Butte⁽¹⁷⁾).

the anthropometry method for use on South Asian infants and young children. We found two equations in the literature for measuring TBW based on anthropometry of infants and children aged 6–24 months. Comparing these two equations with the reference method, we note that the TBW by AN-2⁽¹²⁾ gave better agreement than the one by AN-1⁽¹¹⁾. The study to develop AN-2⁽¹²⁾ used a larger sample and includes data from several studies including those of Mellits and Cheek⁽¹¹⁾. However, due to relatively wide limits of agreement (i.e. large SD) with the reference method, the

equation is less useful for individual infants. However, given that the mean difference of the paired values of FFM is nearly zero and the 95% CI is within a narrow range, the method of Morgenstern *et al.* should be eminently suitable for comparing groups. Butte *et al.*⁽¹⁷⁾ have recently provided reference data on the body composition of children aged 0.5–24 months from the USA. Fig. 3 shows that the percentage of FFM is consistently higher in the study infants compared with those in the USA. This is so, by both the anthropometry and reference methods based on $^2\text{H}_2\text{O}$ dilution. These findings are consistent with the degree of undernutrition present in these infants (Table 2).

Some comment on the prediction equations we have evaluated is pertinent. Widely used anthropometry equations developed by Mellits & Cheek were derived on children with a wide age range of 3 months to 9 years (estimated). Their data include twenty-three boys and nine girls under 1 year of age. Morgenstern *et al.* used data on 167 children of a wide age range of 3 months to 13 years derived from several studies and included the data of Mellits & Cheek and it appears from the data plots that they have had a reasonable number under 2 years of age (though not stated).

Techniques for validation such as underwater weighing are not feasible in infants and young children. Use of the methods that involve any degree of radiation (e.g. dual-energy X-ray absorptiometry, radioactive tracers) is also not desirable for infants. We therefore used a stable isotope dilution technique as the reference standard. Variation exists among laboratories in the way TBW is calculated from $^2\text{H}_2\text{O}$ dilution measurements. One of us (S. S.) has had the opportunity to review the isotope dilution methods with Dr Tom Preston of the Stable Isotope Biochemistry Laboratory of the Scottish Universities Environmental Research Centre, and based on this consultation, we used a unified approach for calculating TBW using $^2\text{H}_2\text{O}$ dilution as described by Schoeller & Jones⁽²⁴⁾. Isotope dilution methods use a two-component model to measure FFM and fat mass and are generally safe, reliable, accurate and feasible in infants and children. To derive FFM, one has to use age- and sex-specific hydration factors derived by multicomponent models^(17,25). While the hydration of FFM changes with age and maturation, use of age- and sex-specific hydration factor largely minimises errors associated with maturation. This however may not be true in some clinical situations such as dialysis patients. For this age group, the hydration factor for FFM ranged from 80.7 to 77.0 for boys and 80.7 to 78.0 for girls⁽¹⁷⁾. The hydration factors for FFM proposed earlier for 1- to 5-year-old children give closely similar results^(25,26). Measurement of TBW using $^2\text{H}_2\text{O}$ dilution methods has been used as a reference method in many of the classic studies of body composition in children^(27–30).

Recently, Tennefors & Forsum have evaluated the usefulness of measuring body fatness derived from skin-fold thickness and BMI using TBW by $^2\text{H}_2\text{O}$ dilution as the reference method. Both these approaches were found to be inadequate in placing 9- and 14-month-old children in the correct body fatness group. It may be noted that TBW is better used to measure FFM, and evaluation of fatness by this method is indirect³¹.

To conclude, among the presently available prediction equations based on anthropometry for measuring FFM in infants and young children, the one by Morgenstern and colleagues

is suitable for studying infants in India for prospective cohort studies and intervention studies such as the impact of breast-feeding programmes on chronic illness in later life⁽³²⁾.

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