

## Estimation of fat-free mass in Asian neonates using bioelectrical impedance analysis

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### Abstract

The aims of this study were to develop and validate a prediction equation of fat-free mass (FFM) based on bioelectrical impedance analysis (BIA) and anthropometry using air-displacement plethysmography (ADP) as a reference in Asian neonates and to test the applicability of the prediction equations in an independent Western cohort. A total of 173 neonates at birth and 140 at two weeks of age were included. Multiple linear regression analysis was performed to develop the prediction equations in a two-third randomly selected subset and validated on the remaining one-third subset at each time point and in an independent Queensland cohort. FFM measured by ADP was the dependent variable, and anthropometric measures, sex and impedance quotient ( $L^2/R_{50}$ ) were independent variables in the model. Accuracy of prediction equations was assessed using intra-class correlation and Bland–Altman analyses.  $L^2/R_{50}$  was the significant predictor of FFM at week two but not at birth. Compared with the model using weight, sex and length, including  $L^2/R_{50}$  slightly improved the prediction with a bias of 0.01 kg with 2 sd limits of agreement (LOA) (0.18, –0.20). Prediction explained 88.9% of variation but not beyond that of anthropometry. Applying these equations to the Queensland cohort provided similar performance at the appropriate age. However, when the Queensland equations were applied to our cohort, the bias increased

**Abbreviations:** ADP, air-displacement plethysmography; BIA, bioelectrical impedance analysis; FFA, fat-free mass; FM, fat mass; GUSTO, Growing Up in Singapore Towards Healthy Outcomes; S, sex; L, length; LOA, limits of agreement; TBW, total body water; W, weight.

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slightly but with similar LOA. BIA appears to have limited use in predicting FFM in the first few weeks of life compared with simple anthropometry in Asian populations. There is a need for population- and age-appropriate FFM prediction equations.

**Key words: Bioelectrical impedance analysis: Air-displacement plethysmography: Validation: Asian neonates**

Research on developmental origins of health and disease has pointed to the importance of body size and composition at birth, which reflect the adaptation of the fetus to its intra-uterine environment, and is linked to the risk of subsequent metabolic diseases<sup>(1)</sup>. Neonatal body composition has been a focus for many research studies as it may be a modifiable factor for health and diseases later in life<sup>(2)</sup>. Body composition can be measured by a variety of methods, which differ in accuracy, feasibility, cost and complexity. However, some methods are not suitable for neonates, including under-water weighing, stable isotope dilution and dual-energy X-ray absorptiometry (DXA), whereas others methods (such as MRI) are limited by their cost, especially for large epidemiological studies.

The measurement of body composition in neonates or infants is challenging, as the distribution of fluid in the body changes rapidly during the first few days of life, with 5–10% reduction in body weight attributable to the change in total body water (TBW) and body composition<sup>(3,4)</sup>. In addition, highly accurate techniques (e.g. MRI, stable isotope dilution) in neonates and infants generally require high compliance from the subject. This can be difficult to achieve in neonates and infants. Air-displacement plethysmography (ADP) for healthy infants has been validated in several studies using the <sup>2</sup>H dilution method for body water and a four-compartment body composition model as the reference methods<sup>(5–7)</sup>. ADP does not require the infant to be restrained during the 2-min measurement. However, the infant ADP device is expensive, large and not readily portable and generally not available outside of major hospitals and research facilities. Therefore, an inexpensive, portable and reliable method to measure body composition is clearly required at this age.

Bioelectrical impedance analysis (BIA) is a simple, non-invasive and useful method for estimating body composition in epidemiological and clinical research<sup>(8)</sup>. BIA measures impedance of the body to an imperceptible, harmless electric current transmitted through electrodes placed on the hands and the feet. Impedance (Z) of a conductor is proportional to  $L^2/Z$ . Thus, impedance of the whole body can be used to estimate the volume of TBW in the aqueous tissue compartments. Fat-free mass (FFM) can be calculated from TBW by using a hydration fraction for FFM, usually assumed to be 0.732<sup>(9)</sup>. Fat mass (FM) can be estimated by subtracting FFM from body weight.

As BIA was first introduced as a method for estimating body composition, many studies have demonstrated its validity to measure body water in healthy populations of older children and adults with normal fluid distribution<sup>(8,10–17)</sup>. However, research on its usefulness for predicting body composition in neonates is limited, especially in Asians. Although a few small studies have reported the use of BIA in infants, most of them have not compared the performance of equations developed from BIA neither with that of simple anthropometry nor did

they use an independent validation group<sup>(18–21)</sup>. In addition, the studies were on infants requiring intensive care or on low birth weight infants in whom the body composition profile may be different from that of healthy infants. Lingwood *et al.*<sup>(22)</sup> developed prediction equations for FFM based on BIA in a cohort of seventy-seven healthy infants in the first few months of life, and reported that the contribution of the BIA parameter in their model was not statistically significant and did not improve the prediction over that using weight alone until their study infants reached 3 months of age. In a recent study on predicting FFM using BIA reference to that of ADP also suggested the potential use of BIA in infants, and the subjects used to develop the prediction equation were of mixed age range – that is, from 0 to 6 months<sup>(23)</sup>. These studies on predicting FFM in infants using impedance from BIA were conducted in the Western population, and thus may not necessarily be applicable to the Asian population. A prediction equation for FFM using BIA in Asian infants would be a valuable tool, especially in large cohorts or longitudinal cohort studies.

Our aims were to develop a prediction equation for FFM during the early neonatal period based on BIA and to validate that prediction using the ADP device, PEA POD infant body composition system, in Asian neonates. The applicability of this equation will also be tested in the independent validation group in this cohort as well as in an independent Western cohort of children from a study conducted at the University of Queensland.

## Methods

### Study design and setting

The neonates were participants of Growing Up in Singapore Towards Healthy Outcomes (GUSTO), a prospective birth cohort study in Singapore<sup>(24)</sup>. Pregnant women attending maternity units of two major public hospitals in Singapore, the KK Women's and Children's Hospital (KKH) and National University Hospital (NUH), were recruited in their first trimester between June 2009 and September 2010. All the participants were Singapore citizens or permanent residents, intended to deliver either in KKH or NUH and to reside in Singapore for the next 5 years. Both participant and her partner were of Chinese, Malay or Indian ethnicity with homogeneous parental ethnic background. Participants receiving chemotherapy or psychotropic drugs and those having type I diabetes mellitus were excluded. Recruited women were followed-up throughout pregnancy and delivery, and their children were followed-up postnatally.

### Subjects

Participating neonates were born between November 2009 and May 2011 to GUSTO mothers. A total of 173 healthy singleton



neonates who completed the BIA and ADP measurements at birth (0–3 d), and 140 who completed them at week 2 (5–17 d), were included in this analysis. In total, fifty-two neonates were tested at both ages. Most neonates were born at term, although four neonates in each group were born at 35–37 completed weeks of gestation.

### Ethics

Study ethics approvals were obtained from the Centralized Institutional Review Board, SingHealth and Institutional Review Board of the National Health Care Group of Singapore.

### Anthropometry

All anthropometric measurements at birth were carried out within 24 h after delivery (median: 1 d) and at week 2 (median: 10 d), respectively. The weight (W) of the neonate was measured using an integrated scale in the PEA POD infant body composition system. Recumbent length (L) was measured to the nearest 0.1 cm using a SECA infant mat (SECA 210 Mobile Measuring Mat; SECA Corp.). Recumbent length was measured in duplicate for reliability, and a third measurement was performed if the difference between the first two measurements was >1 cm. The average of the 2 or 2 closest of the 3 measurements was used for the analysis.

### Body composition measurement by PEA POD Infant body composition system

The PEA POD Infant Body Composition System version 3.1.0 (Cosmed) was used to measure body composition – that is, FM and FFM. The system was calibrated each day before measuring the neonates. A cylinder with known volume was used to calibrate the chamber, and a 2-kg weight was used to calibrate the scale. Clothing was removed and a tight-fitting cap was placed on the head to minimise the amount of air trapped in the hair. Items on the neonate that could not be removed – for example, the umbilical clamp or the hospital identification bracelet – were used to tare the scale for body weight and volume measurements. The neonate was then placed on the scale to measure body mass, and then placed inside the chamber for body volume measurement, which required approximately 2 min. Weight and volume measurements by the PEA POD system are very precise with CV for repeated measurements of 0 and 0.02–0.09%, respectively<sup>(5)</sup>. Infant percent body fat was computed by software integral to the PEA POD system. Body mass and body volume of the neonate were used to calculate body density ( $D_B$ ). The raw  $D_B$  was adjusted for thoracic volume and surface area artefact. The values for the densities of FM and FFM were used to calculate % fat and % FFM<sup>(25,26)</sup>. The density of fat, 0.9007 kg/l, remains constant throughout life. This value is used by the PEA POD for fat density estimation<sup>(26)</sup>. Contrary to fat, the density of FFM changes from birth onwards. Thus, age- and sex-specific densities of FFM based on multi-compartment studies are used by the PEA POD<sup>(25,26)</sup>.

ADP is suggested to be more accurate for body fat measurement than DXA<sup>(27)</sup>. There is good agreement for within-subject measurements using the PEA POD with a mean differences of <1%<sup>(7,28)</sup>. Likewise, there is good agreement between measurements of % fat in term and preterm neonates using ADP and measurements using labelled water and a four-compartment model. A study on forty-nine healthy infants demonstrated no significant difference in the mean % fat measured by ADP and that by the four-compartment model ( $r^2$  0.73, standard error of the estimate (SEE) = 3.7% fat)<sup>(7)</sup>. A similar study compared % fat by ADP and H<sub>2</sub><sup>18</sup>O dilution in seventy preterm infants and nine term infants and also reported good agreement ( $r^2$  0.63, SEE = 1.65% fat)<sup>(28)</sup>. The regression between the two different % fat measurements did not deviate significantly from the line of identity for both studies.

### Bioelectrical impedance analysis

Bioimpedance was measured using the ImpediMed SFB7 (ImpediMed), a single-channel, tetra-polar bioimpedance spectroscopy device and its electrode 292-STE single-tab electrodes. The device measures resistance and reactance at 256 frequencies between 3 and 1000 kHz. We chose to use resistance at 50 kHz as this frequency is used in single-frequency analysers. Therefore, the prediction equation developed will be applicable to predict FFM in infants using the more common single-frequency analysers.

Neonates were placed in the supine position. Insulating materials (e.g. thin cotton blanket) were used to prevent skin-to-skin contact among the body parts. Sense electrodes were placed on the dorsum of the wrist and dorsum of the ankle at the level of the styloid process and the medial malleolus. Source electrodes were placed on the palm and sole over the metacarpals and metatarsals. Good connection between electrodes and the lead as well as skin contact of the electrodes were ensured. Ten consecutive BIA measurements were completed within 2 min.

### Statistical analysis

A random number generator was used to divide the subjects at each study visit (birth and week 2) into two groups: a model development group and a model validation group. The model development group included approximately two-thirds of the subjects and was used to develop prediction equations for FFM ( $n$  116 and 96 for birth and week 2, respectively). The model validation group comprised approximately one-third of the subjects ( $n$  57 and 46 at birth and week 2, respectively). FFM prediction equations based on BIA or simple anthropometry developed from 'model development groups' were applied to model validation groups. Subsequently, these predicted FFM were compared with FFM measured by ADP (FFM<sub>ADP</sub>) at respective time points.

For prediction of FFM, either simple anthropometric measurements alone or in combination with the impedance quotient,  $L^2/R_{50}$  (cm<sup>2</sup>/Ω), were used as predictors;  $R_{50}$  is the resistance at 50 kHz and was used for all analyses. In the model development group, the prediction equation for FFM was



developed by multiple linear regression analysis using FFM<sub>ADP</sub> as the dependent variable; predictors were W (kg), sex (S); (male = 1, female = 2) together with L (cm) alone or L<sup>2</sup>/R<sub>50</sub>. Weight, length and sex were used in the prediction as these are significant predictors at least at one of the 2 time points (for length and sex). In addition, weight and length reflect the body size of the neonate. Sex was included as it is well accepted that there is sex difference in body composition; R<sub>50</sub> is the resistance at 50 kHz and was used for all analyses, and 50 kHz is the frequency most commonly used in single-frequency impedance analysers.

Standardised regression coefficients were used to assess the independent contribution of each variable to the dependent variable (FFM). The developed prediction equation was then used to derive FFM in the validation group within the GUSTO cohort as an independent assessment of the performance of the prediction equation.

Agreement between FFM<sub>ADP</sub> and the predicted FFM by GUSTO equation (FFM<sub>GUSTO</sub>) either using the impedance quotient (W+S+L<sup>2</sup>/R<sub>50</sub>) or simple anthropometry (W+S+L) was assessed using the intra-class correlation coefficient (ICC). Bland–Altman analysis was then used to compare FFM<sub>ADP</sub> and FFM derived from prediction equations. The bias or mean difference of FFM between the two methods was used to determine underestimation (negative bias) or overestimation (positive bias) of the prediction equation. The possible extent of the variation was determined by limits of agreement (LOA) (mean of differences (SD 1.96)) in which the agreement between two methods would lie in 95% of the repeated samples. The slope of the regression line defined whether there is a systematic variation of the difference with the mean of FFM values

by two methods. Analysis was carried out using IBM SPSS 22 and MedCalc version 15 (MedCalc Software). Scattered plots and Bland–Altman plots were produced using MedCalc version 15.

The GUSTO prediction equations developed at birth and week 2 (FFM<sub>GUSTO</sub>) were also applied to an independent cohort from the University of Queensland to cross-validate the performance of the developed prediction equation in an independent Western population. This Queensland cohort included seventy-seven neonates at birth, fifty-four at 6 weeks, fifty-five at 3 months and fifty-three at 4.5 months of age<sup>(22)</sup>. Intra-class correlations between FFM<sub>ADP</sub> and FFM<sub>GUSTO</sub> in the Queensland cohort, as well as bias and LOA, were compared with correlations between FFM<sub>ADP</sub> and predicted FFM using the Queensland cohort's own equation developed at birth.

## Results

Table 1 presents the characteristics of the neonates at birth and at week 2 after delivery. The physical characteristics and body composition of the neonates were not different in the model development and validation groups at either time point.

Resistance was negatively associated with FFM<sub>ADP</sub> both at birth and at week 2. The strength of the association between FFM<sub>ADP</sub> and R<sub>50</sub> was low at birth ( $r = -0.204$ ,  $P = 0.007$ ) but stronger at week 2 ( $r = -0.438$ ,  $P < 0.001$ ). The correlations between FFM<sub>ADP</sub> and weight were  $r = 0.947$  and  $0.946$  (both  $P < 0.001$ ) and between FFM<sub>ADP</sub> and length were  $r = 0.750$  and  $0.753$  (both  $P < 0.001$ ) at birth and week 2, respectively.

**Table 1.** Characteristics of the study subjects (Numbers and percentages; mean values and standard deviations)

	Birth (n 173)					2 weeks after delivery (n 140)				
	Model development group (n 116)		Validation group (n 57)		P	Model development group (n 94)		Validation group (n 46)		P
	n	%	n	%		n	%	n	%	
Sex					0.162					0.053
Male	52	44.8	32	56.1		45	47.9	30	65.2	
Female	64	55.2	25	43.9		49	52.1	16	34.8	
Ethnicity					0.179					0.580
Chinese	59	50.9	21	36.8		32	34.0	20	43.5	
Malay	36	31.0	21	36.8		43	45.7	18	39.1	
Indians	21	18.1	15	26.3		19	20.2	8	17.4	
Age groups*					0.647					1.000
Age group I	85	73.3	38	66.7		7	7.4	3	6.5	
Age group II	16	13.8	9	15.8		81	86.2	42	91.3	
Age group III	15	12.9	10	17.5		6	6.4	1	2.2	
	Mean	sd	Mean	sd	P	Mean	sd	Mean	sd	P
Gestational age (weeks)	38.9	1.1	38.8	1.1	0.480					
Age on the day of BIA and ADP (d)	1.0	0.0	1	0.0	0.392	10.0	2.0	10.0	2.0	0.762
Weight (kg)	3.11	0.37	3.15	0.41	0.500	3.20	0.40	3.20	0.40	0.847
Recumbent length (cm)	48.2	2.0	48.5	1.8	0.267	49.7	1.8	49.7	1.7	0.881
Percentage fat-free mass (%)	90.3	3.5	89.3	3.8	0.103	87.8	3.7	88.4	3.5	0.323
Percentage fat mass (%)	9.7	3.5	10.7	3.8	0.103	12.2	3.7	11.6	3.1	0.332
Fat-free mass (kg)	2.77	0.30	2.77	0.29	0.958	2.80	0.31	2.80	0.35	0.912
Fat mass (kg)	0.30	0.13	0.34	0.15	0.094	0.40	0.15	0.38	0.15	0.439

\* Age group at birth: I: day 0, II: day 1, III: day 2–3. Age group at week 2: I: <1 week, II: 1–2 weeks, III: >2 weeks.

Significant difference ( $P < 0.05$ ). P values were based on between-group comparisons of two sample *t* tests for continuous variables and  $\chi^2$  test for categorical variables.

**Table 2.** Multiple regression analysis of weight (W; kg), sex (S) and length (L) or impedance quotients for predicting fat-free mass (FFM) in the model development group

Model	Predictive variables	Overall <i>r</i>	<i>R</i> <sup>2</sup>	Adjusted <i>R</i> <sup>2</sup>	Standardised coefficients			Prediction equations for FFM (kg)
					W (kg)	S	L (cm) or L <sup>2</sup> /R <sub>50</sub> (cm <sup>2</sup> /Ω)	
Birth								
1	L <sup>2</sup> /R <sub>50</sub>	0.541	0.293		0.541**			1.527 + 0.319 L <sup>2</sup> /R <sub>50</sub>
2	W	0.949	0.901		0.949**			0.386 + 0.775W
3	W + S	0.952	0.906	0.904	0.941**	-0.077**		0.478 + 0.769W - 0.047S
4	W + S + L	0.952	0.906	0.903	0.943**	-0.078*	-0.004	0.502 + 0.771W - 0.047S - 0.001L
5	W + S + L <sup>2</sup>	0.952	0.906	0.904	0.914**	-0.071*	0.038	0.396 + 0.747W - 0.043S + 0.0006L <sup>2</sup>
6	W + S + L <sup>2</sup> /R <sub>50</sub>	0.952	0.906	0.903	0.932**	-0.074*	0.017	0.459 + 0.762W - 0.045S + 0.010L <sup>2</sup> /R <sub>50</sub>
Week 2								
1	L <sup>2</sup> /R <sub>50</sub>	0.769	0.592		0.769**			1.333 + 0.402L <sup>2</sup> /R <sub>50</sub>
2	W	0.935	0.874		0.935**			0.430 + 0.741W
3	W + S	0.936	0.876	0.873	0.934**	-0.040		0.469 + 0.741W - 0.024S
4	W + S + L	0.943	0.888	0.885	0.803**	-0.035	0.173**	-0.660 + 0.637W - 0.022S + 0.029L
5	W + S + L <sup>2</sup>	0.943	0.889	0.885	0.800**	-0.036	0.177**	0.058 + 0.634W - 0.022S + 0.0003L <sup>2</sup>
6	W + S + L <sup>2</sup> /R <sub>50</sub>	0.948	0.899	0.895	0.783**	-0.027	0.214**	0.375 + 0.621W - 0.016S + 0.112L <sup>2</sup> /R <sub>50</sub>

L<sup>2</sup>/R<sub>50</sub>, impedance quotients.

\* *P* < 0.05, \*\* *P* < 0.01 statistically significant standardised regression coefficient from multiple linear regression.

### Birth

**Model development group.** The linear regression analysis revealed that weight and sex are both significant predictors of FFM at birth (Table 2). Weight was the strongest contributor to the prediction of FFM. The standardised coefficient for L<sup>2</sup>/R<sub>50</sub> was greater than that for L in the models, but contribution of L<sup>2</sup>/R<sub>50</sub> was not significant in the model including weight and sex. Weight explained 90.6% of the variance in FFM at birth. Adding sex to the model resulted in minimal improvement in predicting FFM, whereas adding L or L<sup>2</sup>/R<sub>50</sub> led to no improvement.

**Validation group.** The scatterplot for the associations between FFM<sub>ADP</sub> and predicted or FFM<sub>(W+S+L)</sub> are shown in Fig. 1(a) and (b), respectively. The ICC between FFM<sub>ADP</sub> and was 0.970 (*P* < 0.001) and for FFM<sub>(W+S+L)</sub> it was 0.971 (*P* < 0.001). Fig. 2 shows the Bland–Altman plots for the validation group. The plots show the mean of FFM<sub>ADP</sub> and predicted FFM either by W + S + L<sup>2</sup>/R<sub>50</sub> (Fig. 2(a)) or W + S + L (Fig. 2(b)) on the x-axis and the difference between the two methods on the y-axis. The mean bias of the predicted was 0.03 kg – that is, 0.7% of FFM at birth (2.77 kg). The LOA were 0.22 to -0.16 kg. The root mean square error (RMSE) of the prediction equation using W + S + L<sup>2</sup>/R<sub>50</sub> at birth was 0.10 kg. The mean bias of FFM prediction by W + S + L was 0.11 kg, larger than that of the model using W + S + L<sup>2</sup>/R<sub>50</sub>. The LOA were +0.30 to -0.08 kg. RMSE for this prediction by W + S + L was 0.15 kg. No significant relationship was observed between the mean and the difference of measured *v.* predicted FFM.

### Week 2

**Model development group.** Unlike the prediction at birth, the prediction models at week 2 revealed that weight and either length or L<sup>2</sup>/R<sub>50</sub> were significant predictors of neonatal FFM at week 2 but sex was not (Table 2). Adding L<sup>2</sup>/R<sub>50</sub> to the model

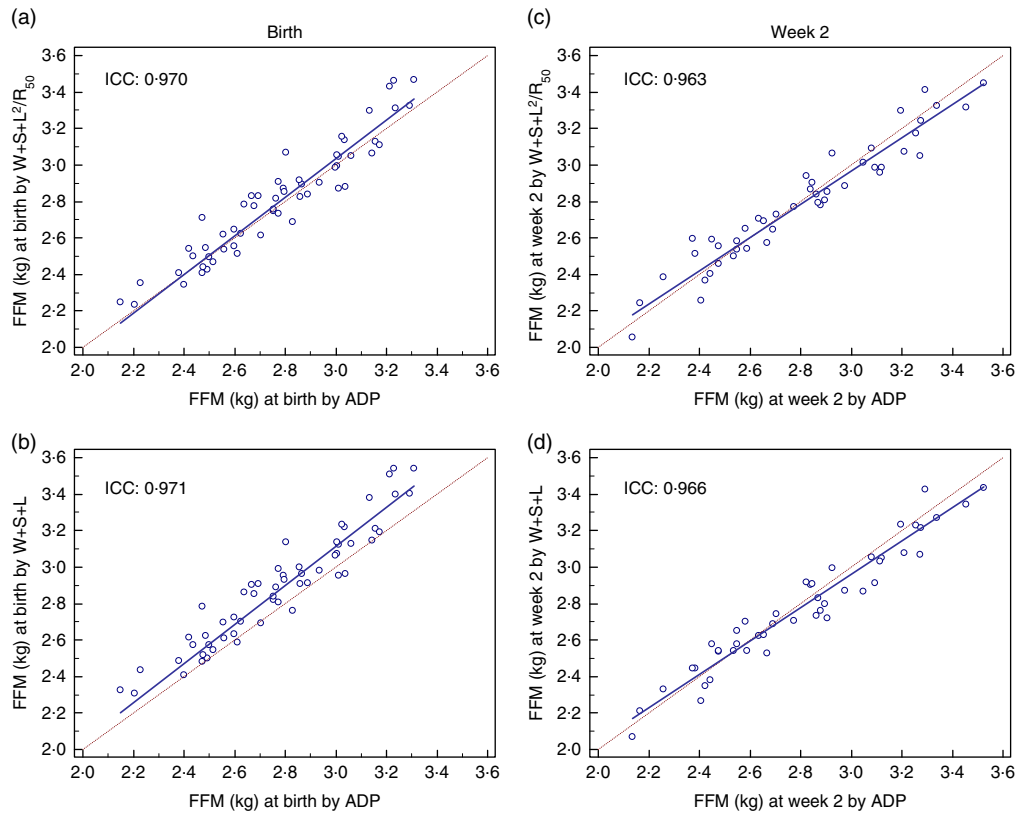
explained 89.9% of the variance in FFM at week 2 in neonates compared with 87.4% with weight alone and 88.8% with W + S + L.

**Validation group.** The scatterplots between FFM<sub>ADP</sub> and that predicted FFM by W + S + L<sup>2</sup>/R<sub>50</sub> or W + S + L in the validation group are shown in Fig. 1(c) and (d), respectively. The ICC between the FFM<sub>ADP</sub> and and FFM<sub>(W+S+L)</sub> were 0.963 and 0.966 (*P* < 0.001), respectively.

The Bland–Altman analysis shows that the mean bias of FFM (kg) at week 2 by ADP and W + S + L<sup>2</sup>/R<sub>50</sub> was -0.01 kg – that is, 0.3% of FFM at week 2 (2.80 kg). The LOA for the GUSTO equation at week 2 were +0.18 to -0.20 kg (Fig. 3(a)). Bias for prediction of FFM<sub>(W+S+L)</sub> was slightly larger (-0.03 kg, 0.9% of FFM). The LOA of this prediction were 0.15 to -0.21 kg (Fig. 3(b)). RMSE for prediction was similar to prediction using L<sup>2</sup>/R<sub>50</sub> at 0.096 kg. No significant relationship was observed between the mean and the difference of measured *v.* predicted FFM.

**Cross-validation with the independent cohort.** The GUSTO prediction equations using simple anthropometry or L<sup>2</sup>/R<sub>50</sub> were applied to an independent cohort from the University of Queensland. When GUSTO prediction equations at birth were applied to the Queensland cohort at birth (Table 3), the ICC, bias and LOA of the predictions were very close to those values obtained in own GUSTO cohort, and again inclusion of the impedance quotient did not improve the prediction. However, when the Queensland equations were applied to the GUSTO cohort, the bias increased slightly, although the LOA were similar.

When GUSTO predictions equation developed at 2 weeks were applied at 6 weeks in the Queensland cohort, the bias and LOA were slightly smaller compared with results using the Queensland 6-week equation. However, when the GUSTO 2-week equation was applied to the Queensland cohort at



**Fig. 1.** Scatterplot of fat-free mass (FFM) (kg) of neonates measured by air-displacement plethysmography (ADP) and FFM derived from Growing Up in Singapore Towards Healthy Outcomes prediction equations based on weight (W), sex (S) and impedance quotient ( $L^2/R_{50}$ ) and W, S and recumbent length (L) in the validation group at birth (a and b) and week 2 (c and d). —, Lines of identity. ICC, intra-class correlation coefficient.

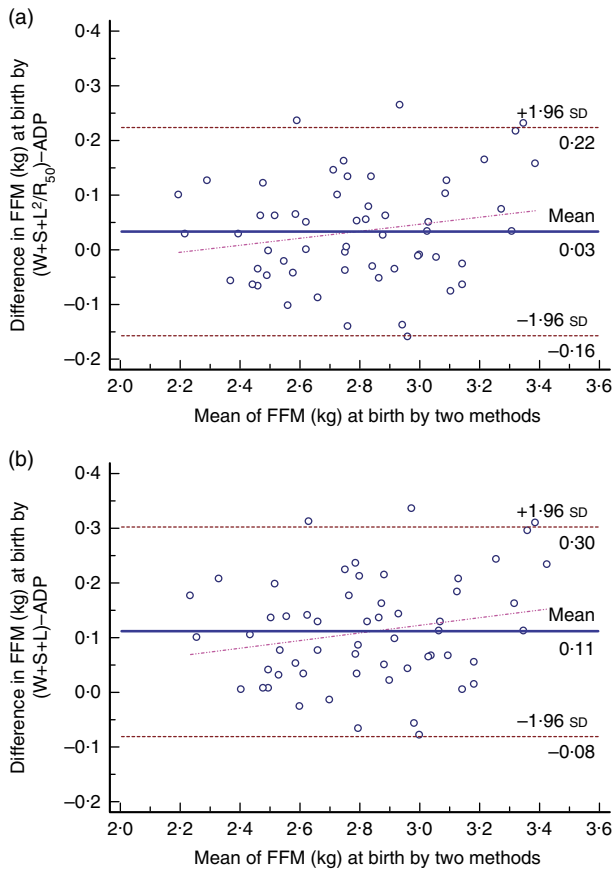
3 months and 4.5 months of age, the bias was larger and LOA were wider compared with results using the Queensland's age-appropriate equations (Table 4). Agreement worsened with increasing age.

### Discussion

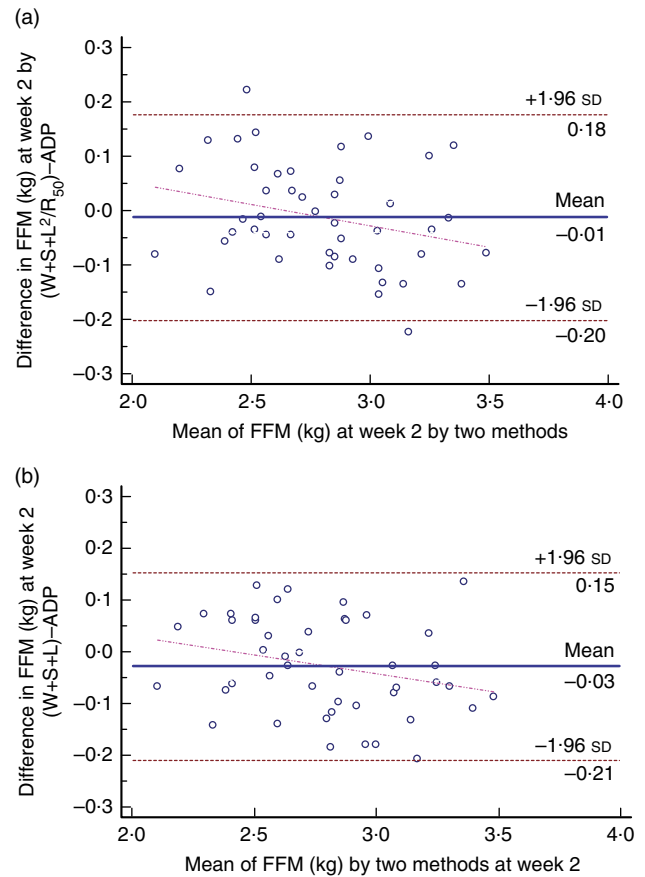
A simple and reliable method is required to measure body composition in newborn infants participating in large epidemiological longitudinal studies<sup>(29)</sup>. Our findings add to the limited available evidence on the validity of BIA estimates of body composition in neonates. We developed prediction equations for FFM using BIA ( $W+S+L^2/R_{50}$ ), as well as simple anthropometric measures ( $W+S+L$ ), and validated these against the criterion method ADP (PEA POD). At birth, we found that  $L^2/R_{50}$  did not contribute significantly to the prediction of FFM. Unlike the findings at birth, the prediction then improved substantially over the subsequent week or two. At 1–2 weeks of age, both L and  $L^2/R_{50}$  became significant predictors of FFM. Their contributions to FFM prediction were similar and much greater than the non-significant contribution of sex at this age. The rapid changes in body water status and body composition in the first few days of life may impact on hydration status of FFM, thus confounding the conversion of TBW to FFM<sup>(30)</sup>. In the Queensland cohort,  $L^2/R_{50}$  did not become a significant predictor of FFM until 4.5 months, and its

contribution was similar to the significant contribution of sex<sup>(22)</sup>. In addition, this finding of significant contribution of  $L^2/R_{50}$  in GUSTO may imply that the hydration status in Asian infants is different from the Western populations studied previously.

Although the  $L^2/R_{50}$  contributed significantly to the prediction at 2 weeks of age, it did not appear to contribute much more than that provided by simple anthropometry. There is a debate in the literature as to whether  $L^2/R_{50}$  performs better than simple anthropometry in predicting FFM. BIA is widely used in adults, adolescents and older children but much less frequently in infants. A few small studies have suggested that BIA is useful for estimating TBW or FFM in infants, but they were performed in low birth weight infants or infants requiring intensive care<sup>(30)</sup>, whose fluid distributions were likely to be different from healthy term infants. Moreover, those studies did not validate their equations in an independent group, nor did they compare prediction using BIA with the use of simple anthropometric measures alone<sup>(19,21)</sup>. Raghavan *et al.*<sup>(20)</sup> reported high correlation between predicted TBW using BIA and that based on a dilution technique, but the prediction equation did not perform better than did weight alone. Tang *et al.*<sup>(21)</sup> reported that weight and impedance index together improved an association ( $r^2$ ) slightly *v.* weight alone but they did not test that finding in a validation group. Lingwood *et al.*<sup>(22)</sup> concluded that it did not improve the prediction compared with simple anthropometry in infants younger than 3 months of age, whereas a slight improvement was present with the prediction using  $L^2/R_{50}$  at



**Fig. 2.** Bland–Altman plots comparing fat-free mass (FFM) (kg) of neonates measured by air-displacement plethysmography (ADP) and from prediction equations based on weight (W), sex (S) and impedance quotient ( $L^2/R_{50}$ ) (a) and W, S and recumbent length (L) (b) in the validation group at birth ( $n$  57). —, The bias (mean difference) between the two methods; - - - - - , the limits of agreement (mean bias (sd 1.96)); — — — — , the slope of the regression line between difference and mean of the measured and predicted FFM.



**Fig. 3.** Bland–Altman plots comparing fat-free mass (FFM) (kg) of neonates measured by air-displacement plethysmography (ADP) and from prediction equations based on weight (W), sex (S) and impedance quotient ( $L^2/R_{50}$ ) (a) and W, S and recumbent length (L) (b) in the validation group at week 2 ( $n$  46). —, The bias (mean difference) between the two methods; - - - - - , the limit of agreement (mean bias (sd 1.96)); — — — — , the slope of the regression line between difference and mean of measured and predicted FFM.

3 and 4.5 month of age. The study of Ethiopian infants by Wibaek *et al.*<sup>(23)</sup>, although with a wide age range of infants from 0–6 months, also found that the  $L^2/R_{50}$  improved prediction for infants older than 3 months of age. Therefore, our findings consistent with the previous findings suggest that BIA has limited value in very early life in predicting FFM, above and beyond that of anthropometry (weight and length) alone.

The failure of impedance measurements to improve the prediction of FFM at early infancy means that without DXA or ADP, prediction is limited to anthropometric measures: weight, length and skinfold thickness (SFT). However, weight or length has inherent limitations for predicting body composition, because these measures do not differentiate between fat and lean mass. The use of weight alone assumes that FM and FFM have similar proportions over time and in different populations, which is not true. Even individuals with similar weight and length can have very different body composition<sup>(31)</sup>. Measurements of SFT have been shown to correlate with body fat measured in older children and infants<sup>(32–35)</sup>. A previous study in GUSTO showed that prediction of FM using subscapular SFT together with W, S and gestational age (GA), referenced to  $FM_{ADP}$ , only slightly improved prediction compared with

prediction using W, S and GA only ( $r^2$  0.811 *v.* 0.774)<sup>(36)</sup>. However, the validity of SFT in infants is being questioned due to rapid changes in hydration status and the variability in skinfold compressibility among neonates<sup>(13,37)</sup>. In addition, training of study team members, standardisation and quality control are a challenge, especially for large longitudinal studies.

Yajnik *et al.*<sup>(29,38)</sup> have described the thin-fat phenotype of South Asian neonates: lower birth weight but greater adiposity, reflecting different intra-uterine trajectories in fat *v.* lean tissues. Studies on total or regional adiposity in Indian infants compared with European infants of UK suggested that Indian-born babies have greater adiposity compared with their Western counterparts<sup>(29,39,40)</sup>. Other studies have shown that this phenotype at birth persists into childhood and later in life<sup>(41–43)</sup>. In addition, the first few weeks of life may be a critical period for the development of obesity later in life<sup>(44–48)</sup>. These findings may also reflect a different trajectory of FM and FFM growth in Asian populations. It would be of great value if a simple method could be developed to predict FFM beyond that of simple anthropometry during infancy.

When GUSTO prediction equations developed at birth and week 2 were applied to the respective age groups in the

**Table 3.** Cross-validation of performance of fat-free mass prediction equations developed from the GUSTO and Queensland cohorts at birth

Origin	Age of prediction	Parameters	Equation	Cohort applied	Age group applied	Bias (kg)*	LOA (kg)*	ICC
GUSTO	Birth	W + S	0.478 + 0.769W - 0.047S	GUSTO	Birth	0.03	0.22, -0.16	0.971
UQ	Birth	W + S	0.864 + 0.682W - 0.093S	UQ	Birth	-0.03	0.20, -0.25	0.966
GUSTO	Birth	W + S + L	0.502 + 0.771W - 0.047S - 0.001L	GUSTO	Birth	0.08	0.27, -0.10	0.952
				GUSTO	Birth	0.11	0.30, -0.08	0.940
UQ	Birth	W + S + L	0.507 + 0.646W - 0.089S + 0.009L	UQ	Birth	-0.05	0.18, -0.27	0.963
GUSTO	Birth	W + S + L <sup>2</sup> /R <sub>50</sub>	0.459 + 0.762W - 0.045S + 0.010L <sup>2</sup> /R <sub>50</sub>	GUSTO	Birth	0.06	0.24, -0.13	0.963
				GUSTO	Birth	0.03	0.22, -0.16	0.970
UQ	Birth	W + S + L <sup>2</sup> /R <sub>50</sub>	0.822 + 0.669W - 0.081S + 0.016L <sup>2</sup> /R <sub>50</sub>	UQ	Birth	-0.02	0.20, -0.24	0.968
				GUSTO	Birth	0.08	0.26, -0.10	0.954

LOA, limit of agreement; ICC, intra-class correlation coefficient; GUSTO, Growing Up in Singapore Towards Healthy Outcomes; UQ, Queensland cohort.  
\* Bias and LOA were based on Bland-Altman analysis.

**Table 4.** Performance of fat-free mass prediction equations at week 2 and the Queensland's own age-appropriate equations at specific ages in the Queensland cohort

Origin	Age of prediction	Parameters	Equation	Cohort applied	Age group applied	Bias (kg)*	LOA (kg)*
GUSTO	Week 2	W + S + L <sup>2</sup> /R <sub>50</sub>	0.375 + 0.621W - 0.016S + 0.112L <sup>2</sup> /R <sub>50</sub>	UQ	6 weeks	-0.06	0.32, -0.45
					3 months	-0.20	0.25, -0.66
					4.5 months	-0.29	0.42, -0.99
UQ	6 weeks	W + S + L <sup>2</sup> /R <sub>50</sub>	1.248 + 0.584W - 0.142S + 0.002L <sup>2</sup> /R <sub>50</sub>	UQ	6 weeks	-0.10	0.32, -0.52
	3 months		1.458 + 0.498W - 0.197S + 0.067L <sup>2</sup> /R <sub>50</sub>		3 months	-0.03	0.41, -0.48
	4.5 months		2.203 + 0.334W - 0.361S + 0.185L <sup>2</sup> /R <sub>50</sub>		4.5 months	0.07	0.59, -0.45

LOA, limit of agreement; GUSTO, Growing Up in Singapore Towards Healthy Outcomes; UQ, Queensland cohort.  
\* Bias and LOA were based on Bland-Altman analysis.

Queensland cohort, the performance of the prediction equations seemed to be similar to its own GUSTO cohort. When the Queensland prediction equations developed at birth were applied to the same age group of the GUSTO cohort, the results were similar with a slightly larger bias and wider LOA. Therefore, although the performance of the GUSTO equation in the Queensland Cohort may suggest that prediction equations are transferrable, results from application of the Queensland equations to the GUSTO cohort suggest that FFM prediction equations are best used in the population in which they were originally developed.

When GUSTO FFM prediction equations developed at 2 weeks were applied to 6-week-old infants of the Queensland cohort, the bias and LOA were slightly smaller compared with results using the Queensland 6-week equation. However, when the GUSTO 2-week equations were applied to 3- and 4.5-month-old infants of the Queensland cohort, the bias was larger and LOA were wider than when using Queensland's equations at 3 months and 4.5 months of age, and agreement decreased with age. This finding implies that prediction equations are best applied in the age group for which they were developed.

A limitation of this study is that FFM density data produced by Fomon *et al.*<sup>(25)</sup> and Butte *et al.*<sup>(26)</sup> were based on Western populations. It is possible that FFM density of Asian infants will be different, which may impact on body composition data derived from the PEA POD.

In conclusion, BIA appears to have limited use in predicting FFM in the first few weeks of life compared with simple anthropometry. There is still a need for population- and

age-appropriate FFM prediction equations. Future studies should assess the age at which electrical impedance provides a significant improvement over simple anthropometry in predicting body composition, especially in Asian populations.

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