Body composition by $^2$H dilution in Gambian infants: comparison with UK infants and evaluation of simple prediction methods

Jonathan C. K. Wells$^{1,*}$, Kate Hawton$^1$, Tegan Darch$^1$ and Peter G. Lunn$^2$

$^1$Childhood Nutrition Research Centre, UCL Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK
$^2$Department of Biological Anthropology, University of Cambridge, Pembroke Street, Cambridge CB2 3DZ, UK

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Gambian infants show growth faltering, but the underlying body composition is unknown. The present study aimed to compare body composition in Gambian and UK infants using $^2$H dilution; and to evaluate accuracy of bioelectrical impedance analysis (BIA) and creatinine excretion for estimating lean mass (LM), using $^2$H as the reference. Body composition was measured in thirty Gambian infants, aged 3–18 months, using (1) anthropometry, (2) $^2$H, (3) BIA (equation of Fjeld et al. Pediatr Res (1990), 27, 98–102) and (4) 5 h urinary creatinine excretion. Compared with UK reference data, Gambian infants were light, short and had reduced BMI and skinfolds. The subscapular skinfold standard deviation score (SDS) was greater than the triceps SDS ($P<0.001$), indicating central fat preservation. Both LM and fat mass were reduced in Gambian infants, with or without adjustment for length. However, whereas the Gambia–UK difference in LM increased with age, that in fat mass decreased. Average creatinine excretion was similar to that expected (95.5 (SD 23.2) % recovery), but LM estimates showed unacceptable error in individuals. BIA using Fjeld’s equation overestimated total body water and LM ($P<0.001$), hence a new equation was developed, with standard error of 0.47 kg LM. In conclusion, Gambian infants characterised by growth faltering had LM deficits that increased with age. However, adiposity increased with age, and showed indications of a more central distribution than in the reference infants. A new BIA equation for LM prediction is presented; however, creatinine excretion is not recommended for LM estimation in this population.

Body composition: Isotope dilution: Bioelectrical impedance analysis: Growth faltering

Many developing country populations experience growth faltering in early life$^{(1)}$, associated with increased morbidity and mortality. In the Gambia, for example, growth faltering tends to occur from 3 months$^{(2)}$. Traditionally, faltering has been associated with poor nutrition and infectious disease$^{(3)}$, factors which often compound each other. Public health interventions have aimed to prevent growth faltering, and reduce exposure to infections, though in the Gambia their efficacy has been variable$^{(4,5)}$.

As low-income populations undergo modernisation and pass through the nutrition transition, attention is increasingly directed not only to short-term survival, but also to the longer-term effects of growth faltering. Studies in industrialised populations have linked low birth weight (WT) and poor infant WT gain with increased risk of type 2 diabetes, stroke, hypertension and CVD in adulthood$^{(6)}$, and these associations are now being discerned in modernising countries such as India$^{(7)}$. Both low birth WT and catch-up growth appear to contribute to increased disease risk, with the worst outcome observed in those born small who subsequently become large$^{(6,8,9)}$.

Associations between growth and later health risk may derive in part from the ontogenetic development of body composition. Low birth WT is associated with reduced lean mass (LM) in later life$^{(10)}$, while catch-up growth is associated with a more central fat distribution$^{(11)}$, particularly if fast growth persists beyond infancy into childhood$^{(12)}$. A high fat:lean ratio may therefore be part of the mechanism whereby early growth is associated with later disease risk.

However, very few data are available relating to body composition development during early life. Data from low-WT Indian neonates indicate a ‘fat-thin’ phenotype, where adipose tissue is preserved while abdominal viscera and muscle are sacrificed$^{(13,14)}$. It remains unclear whether such a thin-fat neonate represents a ‘thrifty gene’ effect, possibility characteristic of Asians, or a ‘thrifty phenotype’ effect that is characteristic of fetal undernutrition more generally$^{(15)}$.

Alterations in tissue deposition in relation to nutritional supply are predicted by life history theory, a branch of evolutionary biology which focuses on the allocation of finite resources to competing biological functions$^{(16)}$. Fat mass (FM) represents a short-term benefit for survival, when energy intake is unreliable, but it may exert health costs in the longer term. LM, in contrast, is correlated in the long term with reproductive success and work capacity, but in the short term represents a more costly growth strategy requiring

Abbreviations: BIA, bioelectrical impedance analysis; FM, fat mass; FMI, fat mass index; HT, height; LM, lean mass; LMI, lean mass index; SDS, standard deviation score; TBW, total body water; WT, weight.

* Corresponding author: Dr Jonathan Wells, fax +44 207 831 9903, email J.Wells@ich.ucl.ac.uk
greater energy supply\(^{(17)}\). The optimal allocation of energy to fat vs. lean tissue in early life may therefore vary across different ecological environments, and the prioritisation of fat stores over LM in Indian infants may represent a short-term adaptive response to poor nutritional supply.

The growth trajectory of Gambian infants follows a pattern of normal growth from birth to 3 months, followed by a steep decline in both length and WT relative to healthy European individuals, until a plateau is reached at about 15–18 months\(^{(2)}\). How this stunning and poor WT gain impact on body composition is not known. The primary aim of the present study was to compare the body composition of Gambian infants with that of UK infants, measured using the same isotope-dilution methodology. The secondary aim was to evaluate the accuracy of two simpler methods (bio-electrical impedance analysis (BIA) and creatinine excretion) for estimating LM (here considered synonymous with fat-free mass), using \(^2\)H dilution as the reference method.

**Methods**

Thirty infants, aged between 3 and 18 months, were recruited from three rural villages in the Gambia at the Medical Research Council Nutrition Centre, Keneba during 2006. Estimates of body composition were made using four methods: (1) anthropometry – WT, height (HT), head circumference, mid-upper arm circumference and triceps and subscapular skinfold thicknesses; (2) BIA; (3) \(^2\)H dilution; (4) urinary creatinine excretion assayed over a 5 h period. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects or patients were approved by the Medical Research Council Gambia Ethical Committee. Written or verbal informed consent was obtained from all subjects or patients after the purpose and details of the study had been explained to them in the local language, with verbal consent recorded using a thumbprint.

Crown-to-heel HT was measured supine on a Harpenden measuring table to 0.1 cm and WT measured to 0.01 kg on electrical scales. Triceps and subscapular skinfold thicknesses were measured using Harpenden callipers. Head circumference and mid-upper arm circumference were measured using a tape measure. Each measurement was taken three times each on 2 separate days. Anthropometric data were compared against UK reference data using standard deviation scores (SDS)\(^{(18-20)}\). Differences between triceps and subscapular skinfold SDS were tested using two-sample and paired \(t\) tests.

To determine total body water (TBW), a dose of 0.10 mg of 99.9% atom % \(^2\)H-labelled water was administered per kg body WT. Urine collections were made using a Hollister urine bag. Pre-dose and 5 h and 2d post-dose samples were obtained for TBW calculations, using the back-extrapolation method\(^{(21)}\). We have previously demonstrated the importance of using the back-extrapolation method in infants, by comparing back-extrapolation and plateau calculations based on a 5 h urine sample\(^{(21)}\). Urine samples were frozen at \(\sim 20^\circ\)C before shipment to the UK for analysis. Urine samples were analysed by isotope-ratio MS (Delta Plus XP, ThermoFisher Scientific, Bremen, Germany). Briefly, 500\(\mu\)l urine samples were flush-filled for 7 min at 75 ml/min with 2% \(^2\)H in He mixture and equilibrated for a minimum of 5 h using platinum rods (Thermo, Howell, MI, USA). Samples were analysed in duplicate, with all enrichments normalised to values for international standard water samples, and the average value used in subsequent calculations. The mean SD of \(^2\)H analyses was < 2.5 deltas, inducing imprecision on TBW of < 0.5%.

For calculating TBW, it was assumed that \(^2\)H dilution space overestimated body water by a factor of 1.044\(^{(22)}\).

LM was calculated using the equation:

\[
LM (kg) = \frac{TBW}{H_{LM}}, \quad (\text{equation 1})
\]

where \(H_{LM}\) refers to age- and sex-specific reference values for lean tissue hydration\(^{(23)}\). FM was calculated as the difference of WT and LM. LM and FM were divided by the square of HT in metres in order to calculate the LM index (LMI) and FM index (FMI)\(^{(24)}\). We have previously recommended this size-adjusted approach for evaluating between-subject body composition differences in early life\(^{(25)}\).

Data on LMI and FMI from these thirty infants were compared with those from a large sample of 303 UK infants aged 1.5–24 months, in whom TBW measurements have been described elsewhere\(^{(26)}\). The aim was to establish whether there was any trend with age in the body composition differences between Gambian and UK infants. For the UK sample, sex-specific mean values were calculated using linear interpolation between the values at 1·5, 3, 6, 9, 12 and 24 months. For each Gambian infant, we calculated the difference between the actual LMI or FMI values and those predicted for that age and sex from the UK data, assessing significance using paired-sample \(t\) tests between actual and expected values. These differences were plotted against age, and multiple regression analysis was used to test the significance of age associations, using age and age\(^2\) to evaluate non-linear associations.

Impedance was measured using BIA (Body Stat, Douglas, Isle of Man) in infants who were sleeping or lying still. TBW was calculated using the following equation developed in malnourished infants\(^{(27)}\):

\[
TBW (kg) = 0.76 + 0.18 HT^2/Z + 0.39 WT, \quad (\text{equation 2})
\]

where \(Z\) refers to impedance. Predicted TBW was then converted to LM using the same hydration values (equation 1). To develop our own population-specific BIA equation, TBW and LM were regressed on HT\(^2/Z\).

Creatinine values were calculated following a 5 h urine collection. The creatinine assay was performed using a creatinine reagent kit, containing alkaline picrate (Randox, Crumlin, Co. Antrim, UK). Urine samples were thawed, centrifuged and diluted 1:10 before performing the assay. A standard curve was produced and then the urine samples were compared with the standards with absorbance being read at 1 min intervals for 5 min. Creatinine excretion was calculated in mg per 24 h period, and the following paediatric equation was used to calculate LM:\(^{(28)}\)

\[
LM (kg) = (0.0491 \times \text{creatinine (mg per 24 h)}) + 2.78, \quad (\text{equation 3})
\]

The percentage creatinine recovery was then calculated, using LM by \(^2\)H to calculate the expected level of excretion using equation 3.
Agreement between LM values obtained by different methods was assessed using the Bland–Altman method\(^{29}\). Bias was calculated as the estimated value minus the reference value, and assessed for statistical difference from zero using a paired \(t\) test. The limits of agreement were calculated as twice the standard deviation of the bias. The magnitude of the correlation of the bias between the mean and difference for the two methods was also assessed.

### Results

Of the thirty infants (fifteen of each sex) in the study, anthropometry and creatinine excretion were measured successfully in all thirty infants, impedance in twenty-seven infants, and pometry and creatinine excretion were measured successfully in all thirty infants, they indicate significant deficits in birth TBW in twenty-five infants. The study sample is described in Table 1. Data on birth WT were available for twenty-one infants. Mean birth WT was 2·98 (SD 0·34) kg, equivalent to 1·6 in males and 1·7 in females (\(P<0·01\)). Both skinfold SDS were also significantly reduced, averaging between −1·6 and −2·3 SDS depending on sex and skinfold site. Subcapular SDS values were significantly greater than triceps SDS values (\(\Delta = 0·36\) (SD 0·39), \(P<0·01\) by two-sample \(t\) test; \(P<0·001\) by paired-sample \(t\) test), indicating a more central fat deposition relative to UK infants. There were no significant sex differences in HT, WT, BMI or skinfolds. There was no significant association of age with HT SDS, WT SDS, BMI SDS, triceps SDS, or subcapular SDS.

Two \(^2\)H data points were rejected post hoc, as calculated LM exceeded body WT. Based on the twenty-five accepted data points, Gambian infants had significantly lower LMI than UK infants (\(P=0·002\)). The infants also had significantly lower FMI (\(P=0·009\)). Figures 1 and 2 illustrate the age trend of these Gambia–UK differences in body composition. Figure 1 shows that LMI was similar to UK values at about 3 months of age, but was approximately 1 kg/m\(^2\) less from 6 months onwards. Multiple regression analysis shows that there was a non-linear association between this inter-population difference and age, with the decline steepest in early infancy, and shallower subsequently (Table 2). Figure 2 shows the opposite trend for FMI, which was reduced compared with UK values during the first year, but similar to

#### Table 1. Description of age, anthropometry and body composition in the sample

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>Minimum</td>
</tr>
<tr>
<td>Age (months)</td>
<td>15</td>
<td>3·9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>15</td>
<td>56·7</td>
</tr>
<tr>
<td>Height SDS</td>
<td>15</td>
<td>−5·3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>15</td>
<td>4·4</td>
</tr>
<tr>
<td>Weight SDS</td>
<td>15</td>
<td>−5·0</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>15</td>
<td>13·7</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>15</td>
<td>−3·1</td>
</tr>
<tr>
<td>MUAC (mm)</td>
<td>15</td>
<td>108</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>15</td>
<td>38·9</td>
</tr>
<tr>
<td>TBW (kg)</td>
<td>12</td>
<td>3·17</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>12</td>
<td>3·99</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>12</td>
<td>1·24</td>
</tr>
<tr>
<td>Triceps SDS</td>
<td>15</td>
<td>−2·5</td>
</tr>
<tr>
<td>Subscapular SDS</td>
<td>15</td>
<td>−2·5</td>
</tr>
<tr>
<td>LMI (kg/m(^2))</td>
<td>12</td>
<td>9·9</td>
</tr>
<tr>
<td>FMI (kg/m(^2))</td>
<td>12</td>
<td>3·4</td>
</tr>
</tbody>
</table>

SDS, standard deviation score; MUAC, mid-upper arm circumference; TBW, total body water; LMI, lean mass index; FMI, fat mass index.

**Fig. 1.** Difference between lean mass index (LMI) and the age- and sex-specific value expected from the reference data, plotted against age (\(n=25\)). The data indicate an initial increasing deficit in the Gambian infants, followed by a relative plateau. This interpretation is supported by the statistics presented in Table 2.
Fig. 2. Difference between fat mass index (FMI) and the age- and sex-specific value expected from the reference data, plotted against age (n 25). The data indicate an initial decrease in deficit of the Gambian infants, followed by a relative plateau. This interpretation is supported by the statistics presented in Table 2.

UK values in the second year. Multiple regression analysis again showed a non-linear association, with the difference between populations greater at younger age groups, and reduced subsequently. Similar trends within the first 12 months, but not statistically significant, were observed in the skinfold data. Thus, overall, relative to UK infants, Gambian infants showed decrements in LM but increments in adiposity during the first year, with these differences appearing stable over the subsequent 6 months.

Table 3 provides data for creatinine recovery, which, despite high variability between infants, did not differ significantly from 100 % of the expected value (mean 95·5 (SD 23·2) %). Using the creatinine values, the mean difference between 2H-derived and predicted LM was −0·29 (SD 1·30) kg, not significantly different from zero (P=0·27). A Bland–Altman plot for LM derived by creatinine and 2H dilution is shown in Fig. 3. The limits of agreement in individuals, at ±2·6 kg LM, are very large relative to the range of LM in the population.

The BIA data are shown in Fig. 4. In the twenty infants with acceptable data available for both BIA and 2H, there was a significant overestimation of TBW by BIA (bias 0·24 (SD 0·21) kg; P<0·0001). This was equivalent to a bias of 0·24 (SD 0·27) kg in LM, and a negative bias in FM (P<0·001). There was a weak negative correlation (r −0·28) between bias and mean TBW, which did not achieve significance. Using the data from the present study, new equations for predicting TBW and LM from BIA were derived as follows:

TBW (kg) = 1·569 + 0·521 (HT2/Z);
standard error of the estimate 0·35 kg, r2 0·73. (equation 4)

LM (kg) = 1·930 + 0·669 (HT2/Z);
standard error of the estimate 0·47 kg, r2 0·72. (equation 5)

These standard error of the estimate values are equivalent to approximately 7 % of the average TBW or LM, indicating acceptable accuracy in the prediction of body composition for comparisons of two groups.

Discussion

The results of the present study have shown that after adjusting for their reduced length, Gambian infants were lighter and had less LM and FM compared with UK infants. Importantly, these differences changed with age during the first year of life, but followed different trajectories for fat v. LM. The decrement in LM increased with age, whilst that in FM decreased. Comparison of different skinfold values also indicated a more central distribution of fat than in UK infants. This pattern may indicate a degree of adaptation to the specific environmental conditions.

Growth faltering in postnatal life is well established in Gambian infants and children, and has been attributed in particular to gut enteropathy which predisposes to cycles of malnutrition and infection(30–32). The severe impact of these insults is indicated by the difference in LMI between Gambian and UK infants worsening with age during the first year of life. However, whereas young Gambian infants have less fat than their UK counterparts, probably due to their reduced fetal WT accumulation, by the end of the first year of life they show total FM similar to that of UK infants, indicative of ’catch-up’ in adiposity. Since this recovery in adiposity appeared weaker in the individual subcutaneous skinfold thicknesses, it is possible that intra-abdominal fat increases may contribute to the overall fat accumulation. However, this hypothesis requires confirmation using other measurement techniques.

These patterns of tissue accretion suggest a strategic shift in energy storage from lean to fat tissue, as the stress of malnutrition is imposed during infancy. We have previously shown that Zambian infants, given an energy supplement in the second half of infancy (6–9 months), gained in fat rather than lean tissue(33). This suggests that fat may offer short-term benefits for survival during malnutrition, representing an adaptive response to energy stress. However, this pattern of tissue accumulation may also be influenced by the lack of particular micronutrients which promote LM accumulation, such as Zn. Prioritisation of energy stores in association with malnutrition has also been also observed in Indian neonates(13,14,34). In Pune, India, the mean z-score for WT changed from about −1·6 at birth to −2·3 at 1 year and remained low at −1·7 at 6 years, using National Center for Health Statistics (NCHS)/WHO as the reference data(34).
Skinfold SDS values likewise remained negative, but much less so for subscapular than triceps, indicating the selective preservation of central fat through childhood. Although our sample was small, we observed a similar trend in Gambian infants. Selective preservation of central fat in early life may be a common strategy during malnutrition across different ecological environments.

The main limitation of the present study is the small sample size. However, the sample comprised all the available infants within the specified age range in the three villages, measured within a single month. Whilst there is therefore a seasonal aspect to the dataset, the extent to which the sample is representative is aided by there being no refusals amongst those available to study. Evaluation of their WT-for-age z-scores shows that the sample fits well with the pattern of growth described previously in this population, of growth faltering dramatically in early infancy and then becoming more stable\(^\text{(2,31)}\). A further limitation is that the measurements were cross-sectional, and cannot show how individual infants gained fat or LM during infancy. Further work will be required to study infants longitudinally in the same setting. Nevertheless, our initial findings suggest that LM is affected more than body WT during growth faltering, and justify such further research.

The main strength of the present study is the accurate body composition technique used in both populations. \(^{2}H\) dilution is easy to use in field conditions, and in children and adults shows excellent agreement with the criterion four-component model\(^\text{(35,36)}\). In infants \(^{2}H\) dilution has also shown high agreement with total body electrical conductivity, but less good agreement with dual-energy X-ray absorptiometry (DXA) or K scanning\(^\text{(37)}\). However, from a theoretical perspective addressing variability in lean tissue composition, hydrometry is predicted to be more accurate than K quantification, while DXA is known to suffer from variable bias\(^\text{(38)}\). Thus, \(^{2}H\) represents a high-quality reference method for evaluating other prediction methods.

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In the present study, average creatinine excretion was very similar to that expected for infants based on the isotopic measurements of LM. Percentage recovery of creatinine was 95·5 %, not significantly different from 100 %, although highly variable between infants. However, although this seems to suggest that this method is acceptable for assessment of LM in groups of infants, we suggest that greater caution

Table 3. Creatinine excretion and predicted body composition

(Mean values and standard deviations)

<table>
<thead>
<tr>
<th>Creatinine excretion (mg/d)</th>
<th>Predicted LM (kg)*</th>
<th>Difference between actual and predicted LM (kg)$^†$</th>
<th>Creatinine recovery (%)$^‡$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Males</td>
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<td>21·7</td>
<td>5·50</td>
</tr>
<tr>
<td>Females</td>
<td>50·9</td>
<td>33·8</td>
<td>5·28</td>
</tr>
<tr>
<td>All</td>
<td>53·1</td>
<td>28·0</td>
<td>5·39</td>
</tr>
</tbody>
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LM, lean mass.

* LM predicted using the equation of Cheek\(^\text{(28)}\).
† Difference calculated as (creatinine-derived LM $-^{2}H$-derived LM).
‡ Percentage recovery calculated by estimating expected creatinine excretion for the \(^{2}H\)-derived LM values.

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‡ Percentage recovery calculated by estimating expected creatinine excretion for the \(^{2}H\)-derived LM values.

Fig. 3. Bland–Altman plot of lean mass (LM) by creatinine excretion and \(^{2}H\) dilution in twenty-five infants. Creatinine underestimated LM by $-0·29$ (SD 1·30) kg, not significantly different from zero ($P=0·27$). However, this lack of significance for this bias was due to the wide limits of agreement in individuals ($\pm$2·6 kg).

Fig. 4. Bland–Altman plot of total body water (TBW), measured in twenty infants by bioelectrical impedance analysis (BIA) using the equation of Fjeld et al.\(^\text{(27)}\), and by \(^{2}H\) dilution. BIA overestimated TBW by 0·24 (SD 0·21) kg ($P<0·0001$). There was a weak negative correlation between the magnitude of bias and magnitude of TBW ($r=0·28$; NS).
is warranted. The magnitude of the bias in LM was similar to that observed for BIA, but did not achieve significance for creatinine only because of wide limits of agreement (± 2.6 kg LM). Given such poor accuracy, we do not recommend creatinine excretion as a field method.

Bioelectrical impedance, using the equation of Fjeld et al. (27), produced significant overestimates of LM compared with \(^3\)H. The mean bias was similar to that for creatinine, but the limits of agreement were substantially less, hence the BIA bias achieved statistical significance. We therefore developed new BIA equations for TBW and LM based on this population of Gambian infants. The standard error of these equations was 0.47 kg LM. As already established in numerous other studies, BIA has poorer accuracy than direct body composition measurements by isotope dilution, but the equation presented here may be useful for comparing large groups of Gambian infants. However, further work would benefit from a larger sample size than that used here, and we recommend \(^3\)H dilution wherever possible for this type of research.

In summary, we found that growth faltering in Gambian infants is associated with significant decrements in LM that appear to increase with age. In contrast, whereas adiposity is low in early infancy, the decrement decreases over the first year of life. Within this overall pattern, central adiposity appears to be favoured, as recorded previously in malnourished Indian infants and children. Collectively, these data indicate that the trajectory of LM is affected more severely than the trajectory of WT, indicating that body composition should be evaluated in studies of growth faltering and its response to treatment. Further research is warranted in this population, in particular to evaluate the success of intervention trials intended to improve growth. BIA could be used in such trials to assess body composition trends, but we do not recommend creatinine excretion as a field method for LM estimation due to very poor accuracy in individuals.

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P. G. L. and J. C. K. W. designed the study. K. H. conducted the body composition measurements. T. D. analysed the \(^3\)H data. J. C. K. W. and K. H. wrote the first draft of the manuscript. All authors contributed to analyses of the data and revision of the manuscript.

The authors declare no conflict of interest regarding the study.

References


