Assessing the reliability of FTIR spectroscopy measurements and validity of bioelectrical impedance analysis as a surrogate measure of body composition among children and adolescents aged 8–19 years attending schools in Kampala, Uganda

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Abstract

Background: Accurate measurement of body composition in children and adolescents is important as the quantities of fat and fat-free mass have implications for health risk. The objectives of the present study were: to determine the reliability of Fourier Transform Infrared spectroscopy (FTIR) measurements and; compare the Fat Mass (FM), Fat Free Mass (FFM) and body fat percentage (%BF) values determined by bioelectrical impedance analysis (BIA) to those determined by deuterium dilution method (DDM) to identify correlations and agreement between the two methods.

Methods: A cross-sectional study was conducted among 203 children and adolescents aged 8–19 years attending schools in Kampala city, Uganda. Pearson product-moment correlation at 5% significance level was considered for assessing correlations. Bland Altman analysis was used to examine the agreement between FTIR measurements and between estimates by DDM and BIA. Reliability of measurements was determined by Cronbach’s alpha.

Results: There was good agreement between the in vivo D2O saliva enrichment measurements at 3 and 4 h among the studied age groups based on Bland-Altman plots. Cronbach’s alpha revealed that measurements of D2O saliva enrichment had very good reliability. For children and young adolescents, DDM and BIA gave similar estimates of FFM, FM, and %BF. Among older adolescents, BIA significantly over-estimated FFM and significantly under-estimated FM and %BF compared to estimates by DDM. The correlation between FFM, FM and %BF estimates by DDM and BIA was high and significant among young and older adolescents and for FFM among children.

Conclusions: Reliability of the FTIR spectroscopy measurements was very good among the studied population. BIA is suitable for assessing body composition among children (8–9 years) and young adolescents (10–14 years) but not among older adolescents (15–19 years) in Uganda. The body composition measurements of older adolescents determined by DDM can be predicted using those provided by BIA using population-specific regression equations.

Keywords: Body composition, Bioelectric impedance analysis, Deuterium dilution method, Children, Adolescents, agreement, reliability

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Background
Nutrition-related non-communicable diseases (NCDs) such as hypertension, high blood glucose and cholesterol levels, diabetes and cardiovascular diseases, are increasing and are predicted to be the major cause of morbidity and mortality in most developing nations by 2020 [1]. In children and adolescents, the most common risk factors for nutrition-related NCDs include overweight, obesity, physical inactivity and unhealthy diets [2]. Pediatric adolescent overweight and obesity are the driving force behind metabolic syndrome risk that has become a growing public health concern in low and middle-income countries (LMICs) [3]. This calls for interventions to prevent and manage childhood and adolescent overweight and obesity. These interventions’ design, monitoring and evaluation rely on correct identification of overweight and obese individuals. Therefore, there is need for accurate body composition measures to correctly identify overweight and obese individuals.

Body mass index (BMI) is the commonly used technique to determine nutrition status because it is inexpensive, fast and non-invasive [4]. However, it is a poor index of fatness and has poor sensitivity and inaccuracy for categorizing of obesity and overweight [5]. These limitations make BMI a poor outcome for research on the efficacy of nutrition programs. A number of reference methods are used to estimate body composition, including underwater weighing (UWW) technique, air displacement plethysmography (ADP), dual-energy X-ray absorptiometry (DEXA) and Deuterium Dilution Method (DDM) [6]. While DDM has widely been used due to its simplicity and relatively low cost, no published study was found on FTIR spectroscopy measurements’ reliability among any population in Uganda. Reliability is defined as the degree of consistency and the lack of error in a measurement [7]. Despite the scarcity of studies on the reliability of FTIR measurements, it is a prerequisite for investigators aiming to validate a device or technique to evaluate the reliability of the reference method, as lack of reliability often masks the actual effects and leads to misinterpretation [8]. Internal reliability which measures repeatability of a tool is determined by Cronbach’s alpha [7] and by Bland-Altman analysis [9].

BIA is a rapid, cheap, safe and simple technique for measuring body composition both in the field and in clinical settings [10], based on population-specific predictive equations [11]. Since the validity of BIA measurements varies with age and ethnicity [12], a number of studies have assessed the validity of BIA devices in various populations of children and adolescents commonly using DEXA and DDM as reference techniques [13–16]. However, BIA’s validity for assessment of body composition and agreement with reference techniques like DDM has not been assessed among any population in Uganda, including children and adolescents. Therefore, the objectives of the present study were to: i) assess the reliability of FTIR spectroscopy measurements of saliva D2O enrichment for determination of body composition and; ii) compare the body composition variables determined by BIA and DDM and, identify possible correlations and agreement between the two methods.

Methods
Subjects
In a cross-sectional study, 203 apparently healthy (based on self-proclamation) participants attending primary and secondary schools in Kampala city, Uganda were selected through a two-stage cluster sample design. The Ministry of Education and Sports provided an up to date list of all the primary and secondary schools in Kampala from which schools to participate in the study were randomly selected. Due to homogeneity between schools and between students in divisions of Kampala, schools were treated as clusters. Sampling of students from schools followed probability proportion to size procedure and a sample of 203 participants aged 8–19 years was randomly selected using random numbers.

Since sample size determination for validation studies is rarely ever justified a priori [17], for this study validation sample size was based on recommendations of researchers in the field of validity studies and from sample sizes used in previous validity studies as stated below. For a study of agreement between two methods of measurement, a sample size of 100 subjects is sufficient, giving a 95% CI of about +/− 0.34 s, where s is the standard deviation of the differences between measurements by the two methods. A sample of 200 subjects is better since it gives a 95% CI of about +/− 0.24 s [18]. A sample size of 100 to 200 subjects is a reasonable size for validation studies as it’s adequate for a range of likely degrees of validity and allows for appropriate deletion of some subjects [19]. Furthermore, a minimum of 80 subjects for validity studies provides highly representative estimates of the main study samples [20]. For most studies, sample sizes used have often been small, ranging from 15 to 189 subjects [21–25]. Against this background, for this study, 203 participants were selected. At least four subjects were targeted for each age. The subject to item ratio (n = 4) is the frequently recommended approach when performing an exploratory factor analysis [17]. In a similar study to assess body composition in Mexican school children of different geographical regions and ethnicity, two children per age and ethnic group were regarded as sufficient [26].

The selected subjects’ nutritional status was evaluated by anthropometric measurements: BMI, waist circumference, waist to hip ratio and weight to height ratio and their body composition was assessed by BIA and DDM. Immediately after the anthropometric and BIA
measurements were taken, saliva samples were collected from the subjects and D₂O doses were given to them. This permitted the assessments to be performed at the same time and under the same conditions, with a consequent constant state of hydration during all methods of body composition assessment used in the study.

**Assessing height and weight**
Height and weight were taken by trained researchers using standard equipment. Body weight was measured to the nearest 0.1 kg using a weighing scale, (Seca 899; Seca Weighing and Measuring Systems, Model No. 8691321004, SECA GmbH & Co. Germany made in China) with minimal clothing and no shoes. Height was measured to the nearest 0.1 cm using a height board (Shorr-board, height board, Weight and Measure LLC, Irwin J. Shorr, MPH, MPS. Olney, Maryland USA) without shoes. BMI (kg/m²) was calculated as weight in kilogram divided by the square of height in meters.

**Assessing waist and hip circumferences**
Waist circumference (WC) was measured to the nearest 0.1 cm in standing position at the midpoint between the lowest rib and the iliac crest and at the end of normal expiration, using a measuring tape. Hip circumference (HC) was measured to the nearest 0.1 cm in standing position at the widest point of the hips using a measuring tape (Luftkin Executive Diameter Steel Tape; 2 m Thinline Model W606 PM, Apex Tool Group, LLC NC 27502, USA).

**Body composition assessment by bioelectrical impedance analysis**
Body composition by BIA was measured using a BIA (Tanita SC-331S Body Composition Analyzer; Tanita Inc., Arlington Heights, IL) instrument, which provides a measure of fat mass and fat-free mass using in-built manufacturers’ equations. Impedance was measured with the subject standing barefoot on the metal foot-plates of the machine for approximately 1 min. The subject’s age, gender, and height were entered into the machine, and a standard 0.5 kg was entered as an adjustment for clothing weight for all participants.

**Body composition assessment using deuterium dilution technique**
A baseline saliva sample was collected from participants 2 hours after their last meal. Each participant then received an oral dose of D₂O (0.5 g/kg body weight). Two endpoint saliva samples were collected at 3 and 4 h after D₂O dose ingestion. Samples were stored in plastic saliva vials at −20 °C until they were analyzed for D₂O using FTIR spectroscopy instrument (FTIR-8400S, Shimadzu Corporation, Japan) according to manufacturer’s instructions. The instrument was housed in the Department of Biochemistry, Makerere University Kampala, Uganda. The instrument settings were: measurement mode: absorbance; apodization: square triangle; number of scans: 32; resolution: 2.0 and; range (cm⁻¹): minimum 2300 - maximum 2900.

A ‘background’ scan was performed using the unenriched drinking water that was used to make the calibration standard (zero standard) and the instrument was calibrated using a prepared D₂O standard (1000 mg/kg). Total body water (TBW) was calculated from the saliva sample by plateau method, based on the assumption that this plateau was reached at 3 or 4 h. FM and %BF were estimated from TBW while FFM was calculated from FM.

**Statistical analysis**
Descriptive statistics (means and confidence intervals) were used for presentation of measurements data for D₂O enrichment, participants’ characteristics and body composition (FFM, FM, and %BF) by DDM and BIA. Normality of variables was inspected visually using normal histogram plots. Box plots were used to inspect for data outliers 8 of which were removed. To show the relationship between saliva D₂O enrichment at 3 and 4 h after ingestion of the D₂O dose when equilibration is achieved, Pearson product-moment correlation was used. Reliability of the two FTIR measurements was verified using the Bland-Altman analysis by plotting the differences between the two measurements of each subject against the mean value of the two measurements. Mean differences and limits of agreement were determined according to Bland Altman procedures. Limits of agreement were considered as the mean of differences between the measurements at 3 and 4 h ± 1.96 × their standard deviation. Cronbach’s alpha was used to assess the level of reliability of the FTIR spectroscopy measurements at 3 and 4 h after D₂O dose ingestion. Cronbach’s α values between 0.7–0.9 were considered representative of good reliability, while values above 0.9 were considered representative of very good reliability [27].

Paired t-tests were used to compare mean measures of FFM, FM, and %BF by BIA and DDM. To show the relationship between DDM and BIA, Pearson product-moment correlation was considered. The Bland Altman plots examined the agreement between DDM and BIA for measuring FFM, FM, and %BF. Mean differences and limits of agreement were calculated according to Bland Altman procedures. Limits of agreement were considered as the mean of differences between measurements by DDM and BIA ± 1.96 × their standard deviation. The analyses were done using with STATA version 13 software and the level of significance was set at P < 0.05.
Results

There were wide ranges for body weight, height, BMI, waist circumference and hip circumference across the different age groups (Table 1). In the current study, 16 children aged 8–9 years, 112 young adolescents aged 10–14 years and 67 older adolescents aged 15–19 years; 84 males and 111 females with mean (95% confidence interval) age 13.44 (12.98 to 13.90) years, weight 44.61 (42.92 to 46.31) kg, height 1.51 (CI: 1.50, 1.53) m, waist circumference 65.87 (CI: 65.02, 66.71) cm and hip circumference 82.48 (CI: 80.97, 83.99) cm participated.

Cronbach’s alpha values for the two measurements of saliva D_2O enrichment were high (0.999, 0.997 and 0.996 for children, young and older adolescents, respectively) (Table 2).

The correlation coefficients for deuterium enrichment at 3 and 4 h were high and positive among children, young and older adolescents at \( r = 0.998, 0.995, \) and 0.993 respectively (Fig. 1a). The Bland-Altman plots showed random nature of spread with no detectable proportional bias for saliva D_2O enrichment at 3 and 4 h among the different age groups. For children and young adolescents, FFM, FM and %BF estimates by DDM were not statistically significantly different from those measured by BIA (Table 3). Among older adolescents, DDM significantly underestimated FFM (\( P < 0.0001 \)) and significantly overestimated FM and %BF at \( P < 0.0001 \) and \( P < 0.0001 \) respectively compared to BIA. Among young and older adolescents, the correlations between FFM, FM and %BF estimates by DDM and BIA were high and significant at \( r > 0.7 \) and \( P < 0.0001 \) (Figs. 3a and 4a). The Bland-Altman plots for FFM, FM, and %BF showed a random nature of spread with no detectable significant negative bias for FFM, FM and %BF values estimated by DDM and BIA among the different age groups (Figs. 2b, 3b, and 4b).

DDM and BIA exhibited generally narrower limits of agreement for FFM, FM or %BF among children and young adolescents than among older adolescents (Fig. 2b, 3b, and 4b). Older adolescents (15–19-years) exhibited the largest mean differences for FFM (−2.84 kg), FM (2.84 kg), and %BF (5.01) while young adolescents (10–12 years) showed the smallest mean differences for FFM (−1.45 kg), FM (0.29 kg), and %BF (2.26).

Table 1 Participants’ characteristics

| Characteristic | Children (8–9 years) Mean (95% Confidence Interval) | Young adolescents (10–14 years) | Older adolescents (15–19 years) | Overall |
|---------------|-------------------------------------------------|---------------------------------|---------------------------------|---------|
| N             | 16                                              | 112                             | 67                              | 195     |
| Male          | 7                                               | 51                              | 26                              | 84      |
| Female        | 9                                               | 61                              | 41                              | 111     |
| Age           | 8.34 (8.11 to 8.64)                             | 11.80 (11.57 to 12.04)          | 17.39 (17.09 to 17.69)          | 13.44 (12.98 to 13.90) |
| Weight (kg)   | 28.31 (25.88 to 30.75)                          | 40.40 (38.74 to 42.05)          | 55.55 (53.48 to 57.62)          | 46.61 (42.92 to 46.31) |
| Height (m)    | 1.31 (1.28 to 1.35)                             | 1.48 (1.47 to 1.50)             | 1.62 (1.60 to 1.64)             | 1.51 (1.50 to 1.53) |
| BMI (kg/m²)   | 16.30 (15.65 to 16.95)                          | 18.16 (17.69 to 18.63)          | 21.23 (20.53 to 21.93)          | 19.07 (18.64 to 19.50) |
| Waist circumference (cm) | 58.56 (56.83 to 60.28) | 65.07 (64.06 to 66.08) | 68.94 (67.62 to 70.26) | 65.87 (65.02 to 66.71) |
| Hip circumference (cm) | 68.43 (66.25 to 70.60) | 79.20 (77.70 to 80.71) | 91.32 (89.17 to 93.47) | 82.48 (80.97 to 84.26) |
| Waist height ratio | 0.44 (0.44 to 0.46) | 0.44 (0.43 to 0.44) | 0.43 (0.42 to 0.44) | 0.44 (0.43 to 0.44) |
| Waist hip ratio | 0.86 (0.84 to 0.88) | 0.82 (0.82 to 0.83) | 0.76 (0.74 to 0.78) | 0.80 (0.80 to 0.81) |
| 3 h deuterium enrichment (ppm) | 722.86 (626.83 to 818.88) | 796.77 (772.52 to 821.03) | 790.85 (744.72 to 836.99) | 788.67 (766.49 to 810.86) |
| 4 h deuterium enrichment (ppm) | 720.06 (622.18 to 817.94) | 799.78 (775.74 to 823.83) | 798.69 (753.26 to 844.11) | 792.87 (770.84 to 814.89) |
| DDM Total body water (litres) | 17.18 (15.90 to 18.47) | 24.97 (24.07 to 25.87) | 32.05 (30.75 to 33.35) | 26.76 (25.84 to 27.68) |
| DDM Total body water (%) | 60.92 (59.38 to 62.47) | 62.29 (61.44 to 63.14) | 58.25 (56.21 to 60.28) | 60.79 (59.09 to 61.68) |
| DDM Fat free mass (kg) | 23.48 (21.72 to 25.23) | 34.11 (32.88 to 35.34) | 43.78 (42.00 to 45.56) | 36.56 (35.30 to 37.82) |
| BIA Fat free mass (kg) | 24.13 (22.10 to 26.16) | 33.89 (32.72 to 35.06) | 46.62 (45.05 to 48.19) | 37.46 (36.13 to 38.79) |
| DDM Fat mass (kg) | 4.84 (3.90 to 5.77) | 6.29 (5.61 to 6.96) | 11.77 (9.97 to 13.58) | 8.05 (7.23 to 8.87) |
| BIA Fat mass (kg) | 4.18 (3.48 to 4.88) | 6.51 (5.80 to 7.22) | 8.93 (7.54 to 10.32) | 7.15 (6.50 to 7.81) |
| DDM Fat (%) | 16.77 (15.65 to 16.95) | 14.90 (13.74 to 16.06) | 20.43 (17.64 to 23.71) | 16.95 (15.74 to 18.17) |
| BIA Fat (%) | 14.61 (12.83 to 16.39) | 15.34 (14.21 to 16.45) | 15.42 (13.32 to 17.52) | 15.30 (14.34 to 16.27) |
| Impedance | 660.00 (626.16 to 693.84) | 596.63 (581.35 to 611.90) | 515.04 (500.91 to 529.16) | 573.79 (561.67 to 585.92) |
14-years) exhibited lowest mean differences for FFM (0.22 kg), FM (−0.22 kg) and %BF (−0.44) (Table 3). Furthermore, the mean differences between DDM and BIA for measures of FFM, FM and %BF for older adolescents exhibited largest 95% confidence intervals compared to those for children and young adolescents. The Bland Altman plots for FFM, FM, and %BF for older adolescents exhibited largest limits of agreement compared those of children and young adolescents (Fig. 2b, 3b and 4b).

**Discussion**

Prior to this work, no published studies were found on the reliability of FTIR saliva D\textsubscript{2}O enrichment measurements among populations in Uganda. In this study, the reliability of FTIR spectroscopy measurements among children and adolescents in Uganda was assessed. The high and positive correlation between 3 and 4-h FTIR spectroscopy measurements is indicative of similarity and reproducibility of the two sets of measurements. The Bland-Altman plots that showed no apparent trend in error differences between the measurements taken after 3 and those taken after 4 h imply that saliva D\textsubscript{2}O enrichment measurements were reproducible among the study population. The high Cronbach's alpha value (> 0.9) among all studied age groups indicates very good repeatability of the FTIR spectroscopy saliva D\textsubscript{2}O enrichment measurements among children, young and older adolescents in Uganda. The FTIR spectroscopy instrument can, therefore, provide reliable measures for D\textsubscript{2}O saliva enrichment and thus suitable for validation of other body composition assessment techniques for more accurate assessment of body composition among children and adolescents in Uganda. Furthermore, the FTIR spectroscopy technique has several advantages in assessing body composition including simplicity to carry out, minimal subject cooperation requirements, acceptability in all age groups [28], non-invasiveness, relatively low cost, easy administration of tracers, and easy collection of samples [29].

In this study, the ability of the inbuilt equations from the Tanita SC-331S BIA instrument to assess body composition of children and adolescents in Uganda by using DDM as a reference method also was investigated. Prior to this work, no published studies were found comparing the body composition estimates obtained by BIA to those obtained by DDM among children and adolescents in Uganda. Since estimates for body composition had varying agreement across the studied age groups, DDM and BIA are generally not interchangeable across children and adolescents in Uganda. The none-statistically significantly different (P > 0.05) FFM, FM and %BF measures by DDM and BIA among children and young adolescents imply possibility for agreement between the two

| Age category | Cronbach's Alpha |
|--------------|------------------|
| 8–9 years    | 0.999            |
| 10–14 years  | 0.997            |
| 15–19 years  | 0.996            |

Table 2 Cronbach’s alpha values of the two readings for the different age groups
methods in these age categories. For children and young adolescents, the generally narrow limits of agreement, the small mean discrepancies (biases) for the FFM, FM and %BF estimates and their narrow 95% confidence intervals of means imply that DDM and BIA estimates for FFM, FM, and %BF agree and can be used interchangeably for FM, FFM, or %BF for these age categories in Uganda. These findings are similar to those by Mehta and others who found agreement between BIA and DDM for FFM, FM and %BF among children 14 years of age or younger with Intestinal Failure [23]. In a study to validate 2 portable BIA devices; the Inbody 230 and the Tanita BC-418 for body composition assessment in healthy Taiwanese school-age children, Bland-Altman analysis showed clinically acceptable agreement between the Inbody 230 device and DEXA for FFM measurements [15].

On the other hand, the statistically significantly different mean values ($P < 0.05$) for FFM, FM and %BF among older adolescents imply no possibility for agreement between the two methods. The wide limits of agreement for FFM, FM, or %BF exhibited by Bland Altman plots

| Table 3 | Body composition mean values (CI), mean difference (CI) and $P$-values between DDM and BIA among different age groups |
|---------|----------------------------------------------------------------------------------------------------------------------------------|
| Body composition means | DDM | BIA | Mean difference | $P$-value |
| **Children (8–9 years)** | | | | |
| FFM (kg) | 23.48 (21.72–25.23) | 24.13 (22.10–26.16) | $-0.657 (-1.474–0.160)$ | 0.1071 |
| FM (kg) | 4.84 (3.90–5.77) | 4.18 (3.48–4.88) | $0.657 (-0.160–1.474)$ | 0.1071 |
| %BF | 16.77 (14.66–18.88) | 14.61 (12.83–16.39) | $2.157 (-0.397–4.711)$ | 0.0920 |
| **Young adolescents (10–14 years)** | | | | |
| FFM (kg) | 34.11 (32.88–35.34) | 33.89 (32.72–35.06) | $0.224 (-0.111–0.559)$ | 0.1876 |
| FM (kg) | 6.29 (5.61–6.96) | 6.51 (5.80–7.22) | $-0.224 (-0.559–0.111)$ | 0.1876 |
| %BF | 14.90 (13.74–16.06) | 15.34 (14.21–16.45) | $-0.436 (-1.239–0.367)$ | 0.2846 |
| **Older adolescents (15–19 years)** | | | | |
| FFM (kg) | 43.78 (42.00–45.56) | 46.62 (45.05–48.19) | $-2.841 (-3.983–-1.699)$ | < 0.0001 |
| FM (kg) | 11.77 (9.97–13.58) | 8.93 (7.54–10.32) | $2.841 (1.699–3.983)$ | < 0.0001 |
| %BF | 20.43 (17.64–23.71) | 15.42 (13.32–17.52) | $5.006 (3.068–6.944)$ | < 0.0001 |

Fig. 2 Regression and Bland-Altman plots for FFM (left), FM (middle) and % body fat (right) determined by DDM and BIA among children.
for older adolescents, the big mean discrepancies (biases) for the FFM, FM and %BF estimates and their wide 95% confidence intervals in this age group imply limited agreement between the two methods. This reveals that DDM and BIA are not directly interchangeable for either FM, FFM, or %BF among older adolescents (15–19 years) in Uganda. Similar to this study’s findings where BIA overestimated FFM among older adolescent and underestimated their FM and %BF are those by Resende and others who reported
that BIA overestimated the measures of FFM and underestimated the measures of FM compared to those provided by DDM among obese adolescents in Brazil [5]. Resende and others reported a high, positive and significant correlation between FFM and FM values determined by DDM and BIA but there was no agreement between the two methods among obese adolescents [5] as was the case for older adolescents in this study. In a study to validate predictive equations of BIA to FFM estimation in army cadets aged 17–24 years, Langer and others observed significant differences between FFM values from 8 predictive BIA equations and no good agreement with DXA [11]. Also, among healthy Indian children and adolescents aged 5–18 years, there was no agreement between BIA and DXA in assessment of body composition [30]. A possible explanation for the discrepancy of body composition among older adolescents by the BIA system’s inbuilt prediction equations is that they are normally based on Western European or North American populations, which may differ in body composition and proportion when compared to the population under study [30]. Growth involves the deposition of both fat mass (FM) and fat-free mass (FFM) components and human body composition is ethnicity dependent [31]. No literature was found regarding the age- and sex-related pattern of changes in body composition for populations in Uganda.

While the study was the first of its kind among populations in Uganda, it was not without limitations. DDM was used as the reference method which, although widely validated as a reliable estimate, is not a gold standard for body composition. Ideally, a four-component model would have been used as the reference method, but this was not possible in our study setting. While DDM has the advantage that it is relatively easy to perform, it is not without limitations: one assumption is the hydration of FFM, which may vary among persons by age, sex, maturation and ethnicity and to estimate FFM from TBW, age, and sex-specific hydration fractions were used [6]. But the hydration of FFM values used for computation of TBW to estimate FFM, FM, and %BF were not Uganda specific. Higher hydration factors have been observed among African American adults compared to whites using a four-component model [32]. However, there is no information on the hydration factors of FFM for Ugandan populations.

Conclusions
The reliability of the FTIR spectroscopy saliva D₂O enrichment measurements was very good among the studied population. This technique can be used as a reference technique in the validation of field techniques like BIA for more accurate estimation of body composition in resource-poor countries that cannot afford four-compartment (gold standard) techniques.

The other results of the study showed that DDM and BIA can be used interchangeably for FFM, FM, and %BF for children and young adolescents aged 8–14 years in Uganda but not interchangeable for the assessment of body composition in older adolescents aged 15–19 years in Uganda. For that reason, among older adolescents in Uganda, BIA is not a valid measure for body composition, so deriving population-specific BIA equations may be a suitable approach for assessing body composition.

The study, therefore, revealed BIA’s limitations in assessing body composition among children and adolescents in Uganda.

Abbreviations
%BF: Body fat percentage; ²H: Deuterium; ADP: Air displacement plethysmography; BIA: Bioelectrical impedance analysis; BMI : Body mass index; D₂O: Deuterium oxide; DDM: Deuterium dilution method; DEXA: Dual-energy x-ray absorptiometry; FFM: Fat-free mass; FM: Fat mass; FTIR: Fourier transform infrared; IAEA: International atomic energy agency; LMICs: Low and middle-income countries; NCDs : Non-communicable diseases; TBW: Total body water; UWW : Underwater weighing; VD: Dilution space; WHO: World Health Organization

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions
CTN, DI, JHM, JEA, and DN conceived, designed, and revised the manuscript. CTN did the literature search. SMAS, RB, and BO did the statistical analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The purpose and objectives of the study were carefully explained to each participant and their parents. Informed consent to the study was obtained from participants’ parent/guardian to affirm their willingness or not. The parents or guardians of participants provided consent to allow their children to take part in the study while participants signed assent accepting to participate in the study. Ethical clearance to engage human subjects was obtained from Makerere University School of Biomedical Sciences Higher Degrees, Research and Ethics Committee and Uganda National Council for Science and Technology under reference numbers: SBS 291 and HS 1950 respectively.

Competing interests
The authors declare that they have no competing interests.

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References

1. Celemajer DS, Chow CK, Marijon E, Anstey NM, Woo KS. Cardiovascular disease in the developing world. J Am Coll Cardiol. 2012;60:1207–16. https://doi.org/10.1016/j.jacc.2012.03.074.

2. Raj M. Obesity and cardiovascular risk in children and adolescents. Indian J Endocrinol Metab. 2012;16:13. https://doi.org/10.4103/2230-8216.91176.

3. WHO. Obesity and overweight @ wwwwho.int. 2017. http://www.who.int/es/news-room/fact-sheets/detail/obesity-and-overweight. Accessed 1 June 2018.

4. Akindele MO, Phillips JS, Igumbor EU. The relationship between body fat percentage and body mass index in overweight and obese individuals in an urban african setting. J Public Health Africa. 2016. https://doi.org/10.4086/jpha.2016.515.

5. Resende CMM, Camelo Júnior JS, Vieira MNCM, Fierroli E, Pfimer K, Perdonà GSC, et al. Body composition measures of obese adolescents by the deuterium oxide dilution method and by bioelectrical impedance. Brazillian J Med Biol Res. 2011;44:1164–70. https://doi.org/10.1590/S0100-797X20110007000022.

6. Srichan W, Yamborisut U, Kijboonchoo K, Thasanasuwon WA. Comparison of bioelectrical impedance analysis with deuterium dilution technique for body fat assessment in school-age children. J Public Health (Bangkok). 2014;44:223–36.

7. Molina KM, Molina KM, Goltz HH, Kowalkowski MA, Hart SL, Latini D, et al. Reliability and validity, Encycl. Behav. Med. New York, NY: Springer New York; 2013. p. 1643–4. https://doi.org/10.1007/978-1-4419-1005-5_1549.

8. Meisal K, Smidt E, Tintner J. Reproducibility of fourier transform infrared spectra of compost, municipal solid waste, and landfill material. Appl Spectrosc. 2008;62:190–6. https://doi.org/10.1366/000370208X8575537.

9. Bland MJ, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet London J Onwhyn, 1823–1970. 1986;327:307–10. doi:https://doi.org/10.1016/S0140-6736(86)90837-8.

10. Böhm A, Heitmann BL. The use of bioelectrical impedance analysis for body composition in epidemiological studies, Eur J Clin Nutr. 2013;67:597–85. https://doi.org/10.1038/ejcn.2012.168.

11. Langer RD, Borges JH, Pascoa MA, Cirolini VX, Guerra-Júnior G, Gonçalves S. Validity of bioelectrical impedance analysis to estimate fat-free mass in the army cadets. Nutrients. 2016;8 https://doi.org/10.3390/nu8030121.

12. Dehghan M, Merchant AT. Is bioelectrical impedance accurate for use in large epidemiological studies? Nutrients. 2013;5:7-26. https://doi.org/10.3390/nu14020726.

13. Chiponkar S, Kajale N, Ekbote V, Mandalik P, Parthasarathy L, Khadilkar V, et al. Validation of bioelectric impedance analysis against dual-energy X-ray absorptiometry for assessment of body composition in Indian children aged 5 to 18 years. Indian Pediatr. 2017;54:219–24.

14. Haroun D, Croker H, Viner RM, Williams JE, Darch TS, Fewtrell MS, et al. Comparison of body composition assessment methods in pediatric intestinal failure. J Pediatr Gastroenterol Nutr. 2014;59:999–105. doi:https://doi.org/10.1097/MPG.0000000000000364.

15. Widen EM, Merchant AT. Is bioelectrical impedance accurate for use in large epidemiological studies? Nutrients. 2013;5:7-26. https://doi.org/10.3390/nu8030121.

16. Mehta NM, Raphael B, Guteirezm I, Quinn N, Mitchell PD, Litman HJ, et al. Comparison of body composition assessment methods in pediatric intestinal failure. J Pediatr Gastroenterol Nutr. 2014;59:999–105. doi:https://doi.org/10.1097/MPG.0000000000000364.

17. Shafer KJ, Siders WA, Johnson K, Lukaski HC. Reliability of segmental multiple-frequency bioelectrical impedance analysis to estimate body composition of adults across a range of body mass indexes. Nutrition. 2009;25:25–32. https://doi.org/10.1016/j.nut.2008.07.004.

18. Widen EM, Strain G, King WC, Yu W, Lin S, Goodpaster B, et al. Validity of bioelectrical impedance analysis for measuring changes in body water and percent fat after bariatric surgery. Obes Surg. 2014;24:847–54. https://doi.org/10.1007/s11695-014-1182-5.

19. Chitra-Emandi A, Dobrescu A, Papa M, Puiu M, Emandi AC. Reliability of measuring Subcutaneous fat Tissue thickness using Ultrasound in non-athletic young adults MEASURING SUBCUTANEOUS FAT TISSUE USING ULTRASOUND. Maedica -a J Clin med Maedica a J Clin med Maedica a J Clin Med. 2015;10:204–9.

20. IAEA. Nutritional and Health-Related Environmental Studies (NAHRES). Using nuclear techniques to assess the role of nutrition-sensitive agri-food systems in improving diet, health and nutritional status of vulnerable populations. 2015. http://www-naweb.iaea.org/nahu/NAHRES/crp/e43029.html. Accessed 14 Feb 2018.

21. Lee SY, Gallagher D. Assessment methods in human body composition. Curr Opin Clin Nutr Metab Care. 2008;11:566–72. https://doi.org/10.1097/MOC.0b013e3282f3f533.

22. Wickkamaschinghe V, Lamabadusurya S, Cleghorn G, Davies P. Assessment of body composition in Sri Lankachildren: validation of a bioelectrical impedance prediction equation. Eur J Clin Nutr. 2008;62:1170–7. https://doi.org/10.1038/ejcn.2012.89.

23. Xiong K-Y, He H, Zhang Y-M, Ni G-X. Analyses of body composition charts among younger and older Chinese children and adolescents aged 5 to 18 years. BMC Public Health. 2012;12:835. https://doi.org/10.1186/1471-2458-12-835.

24. Prins M, Hawkesworth S, Wright A, Fulford a JC, LM a J, Prentice a M, et al. Use of bioelectrical impedance analysis to assess body composition in rural Gambian children. Eur J Clin Nutr. 2008;62:1065–74. https://doi.org/10.1038/ sj.ejcn.1602830.