Estimation of percent body fat based on anthropometric measurements in children and adolescents with congenital adrenal hyperplasia due to 21-hydroxylase deficiency

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Summary

Background & aim: Congenital adrenal hyperplasia due to 21-hydroxylase deficiency is associated with a high risk for obesity. Anthropometric measures are simple and inexpensive methods to assess body fat. However, the accuracy of alternative methods in these patients is unknown. This study aims to develop and evaluate the accuracy of predictive anthropometric equations in the estimation of percent body fat in individuals with congenital adrenal hyperplasia due to 21-hydroxylase deficiency.

Methods: A total of 31 female and 22 male patients, aged 7–20 years, were evaluated. Dual-energy X-ray absorptiometry was used as the reference method for body fat, and anthropometric measurements were performed.

Results: Three new predictive equations showed similar results: Equation (1) ($R^2 = 0.85; \text{SEE} = 2.89\%$), Equation (2) ($R^2 = 0.86; \text{SEE} = 2.82\%$), and Equation (3) ($R^2 = 0.86; \text{SEE} = 2.81\%$). Internal cross-validation procedures showed a high $R^2$ (range, 0.84–0.85) and low SEE (<3%). The limits of agreement ranged from −5.6% to 5.6% and no trend was observed.

Conclusion: In children and adolescents with congenital adrenal hyperplasia due to 21-hydroxylase deficiency, three new predictive equations were validated for the estimation of percent body fat, with dual-energy X-ray absorptiometry as the reference method.

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1. Introduction

Congenital Adrenal Hyperplasia (CAH) represents a group of diseases characterized by the deficiency of one of the enzymes responsible for cortisol biosynthesis.\(^1\,^2\) The most common impairment of CAH is the deficiency of the 21-hydroxylase enzyme (CAH-21OHD), accounting for more than 90% of cases. The disease results in low production of glucocorticoids and excess androgens, with or without mineralocorticoid insufficiency.\(^3\) Congenital adrenal hyperplasia due to 21-hydroxylase deficiency (CAH-21OHD) is a common genetic endocrine disorder.\(^1\,^2\) The classic form of CAH-21OHD has an incidence of 1:15,000 live births and diverges according to ethnicity and country.\(^1\,^2\) High rate was reported in Brazil, about 1:10,325 live births.\(^2\) Inadequate therapy may cause early puberty, short stature and infertility due to androgen excess (hyperandrogenism) while a high dose treatment may cause insulin resistance, osteoporosis and increased body fat due to the effects of hypercortisolism.\(^6\,^9\)

Body fat assessment is an important tool in the evaluation of nutritional status and monitoring the treatment of individuals with CAH, mainly because these patients present higher risk of...
obesity. Several methods have been used to assess body composition in children and adolescents. Dual-energy X-ray absorptiometry (DXA) has emerged as one of the most widely accepted methods in the assessment of body composition, and is considered a valid and reliable method for assessing body composition in children and adolescents; however, this assessment involves extended laboratory facilities. Consequently, it is important to identify alternative methods that are convenient, reliable and accurate in measuring body composition in this special population. Anthropometry may provide an alternative as it is a simple, noninvasive, and inexpensive method, especially when compared with other laboratory-based methods. However, it is difficult to find the appropriate balance in glucocorticoid replacement therapy, which can compromise the accuracy and applicability of this method in patients with CAH, once these patients may present changes in body fat due to the effects of glucocorticoid therapy, or greater lean mass reflecting the adverse effects of prolonged androgen exposure.

Models based on anthropometric measurements are widely used to predict total body fat in children and adolescents, but these are generally based on samples of healthy individuals. To our knowledge, a predictive equation based on anthropometric measurements for CAH patients does not exist, and its accuracy has not yet been determined in this population.

Therefore, the aim of the present investigation was to develop and evaluate the accuracy of percent body fat (%BF) predictive equations based on anthropometric measurements in individuals with classic CAH-21OHD using dual-energy X-ray absorptiometry (DXA) as the reference method.

2. Materials and methods

2.1. Subjects

A total of 53 patients (31 females and 22 males), aged 7–20 years were evaluated. All of the patients were diagnosed with classic CAH due to 21-hydroxylase deficiency (21OHD) confirmed by clinical, hormonal, and molecular analyses. Of the total, sixteen subjects were diagnosed with the simple-virilizing (SV) form, and 38 were diagnosed with the salt-wasting (SW) form. All of the patients were diagnosed and followed in the Outpatient Pediatric Endocrinology Clinic of the Clinical Hospital, State University of Campinas (UNICAMP), Brazil. Pubertal development was classified according to Marshall and Tanner criteria, by a single pediatric endocrinologist. All measurements were carried out in the same day while the patients visited the outpatient clinic. All subjects and parents or tutors were informed about the possible risks of the investigation before giving their written informed consent to participate. All procedures were approved by the Research Ethics Committee of UNICAMP and conducted in accordance to the declaration of Helsinki for human studies of the World Medical Associations. In brief, the procedures are described below.

2.2. Glucocorticoid replacement

The patients received glucocorticoid replacement with hydrocortisone \( (n = 36) \), prednisone \( (n = 6) \), dexamethasone \( (n = 7) \) or a combination of hydrocortisone plus dexamethasone \( (n = 4) \). For the data analysis, the doses of the various glucocorticoids were converted using a growth-retarding cortisol equivalent (GRCE) formula: 80 mg hydrocortisone = 16 mg prednisone = 1 mg dexamethasone. Forty-five patients (38 SW and 7 SV) additionally received fludrocortisones (FC) twice a day.

2.3. Anthropometry

Anthropometric measurements were performed using standardized procedures and conditions. The measurements were performed by a highly trained technician in standardized conditions. Skinfolds (SKF) were measured three times to the nearest 0.1 mm and averaged for analysis. All SKF measurements (triceps, medial calf, subscapular, biceps, and suprailliac) were made on the right side of the body, according to the standardized anatomic locations and methods, using a Lange caliper (Cambridge Scientific Instruments, Cambridge, MA). These data were used to calculate body mass index (BMI; kg/m²), the sitting height/waist circumference ratio (SH/WC), sum of triceps and biceps SKF, sum of triceps and subscapular SKF, sum of triceps and medial calf SKF and sum of triceps, biceps, subscapular and suprailliac SKF. The BMI was used to classify the patients into normal, overweight, and obese according to age- and sex-specific cutoff points proposed by the International Obesity Task Force (IOTF). On the basis of the test–retest reliability using eight subjects, the technical error of measurement (TEM) for the anthropometric measurements ranged from 1.3% to 3.8%.

2.4. Dual-energy X-ray absorptiometry (DXA)

To assess %BF, DXA measurements were made on a fan beam Hologic model Discovery Wi densitometer, software version 12.7 (Hologic, Bedford, MA). According to the procedures recommended by the manufacturer, the densitometer was calibrated daily using a step phantom with six fields of acrylic and aluminum of varying thickness and known absorptive properties was scanned alongside each subject to serve as an external standard for the analysis of different tissue components. The same laboratory technician positioned the subjects, performed the scans and executed the analysis according to the operator’s manual using the standard analysis protocol. Based on test–retest, the TEM was 0.498% for %BF, and the coefficient of variation was 1.32%, in our laboratory.

2.5. Statistical analysis

For the statistical analyses, SPSS (Statistical Package for the Social Sciences, Chicago, IL, USA) version 18.0 was used. The normal distribution of the data was tested using the Shapiro–Wilk test. Independent–sample t-tests were used to compare variables between genders, and Mann–Whitney U tests were used as the alternative if the data did not present a normal distribution. Scatter plots and Pearson’s correlation coefficient were used to assess the relationship between the values of each independent variable (predictor) and dependent variable (%BF). Stepwise regression analysis was performed to identify which combination of variables would best predict %BF measured by DXA. The adjusted coefficient of determination \( R^2 \) and SEE were estimated. A variance inflation factor for each independent variable was also calculated to evaluate multicollinearity. Simple regression analysis was performed to determine the relationships between %BF predicted by the new equations and %BF assessed by DXA. Slopes and intercepts were examined. The adequacy of the final prediction models was assessed by testing the normality of the residuals and the correlation of the absolute residuals with the variables in the models. Differences between the reference method (DXA) and each new predictive equation were calculated using paired-sample t-tests. Agreement between the predictive equations and reference method was assessed using the Bland–Altman method. The three models were then internally cross-validated in all of the models using the predicted residual sum of squares (PRESS) statistics method. The PRESS statistic is obtained by fitting a regression equation with one observation excluded, (2)
obtaining the predicted value of the excluded observation, (3) calculating the residual for that predicted value (observed – predicted), (4) repeating steps 1–3 for all observations, and (5) taking the sum of squares of all of the residuals. Finally, the PRESS statistic is a function of these residuals, and alternative measures of model adequacy ($R_{\text{PRESS}}^2$ and $\text{SEE}_{\text{PRESS}}$) were calculated. The PRESS statistic was performed using SigmaPlot for Windows version 11.0 (Systat Software, Erkrath, Germany). For all of the tests, statistical significance was set at $p < 0.05$.

### 3. Results

The general characteristics and body composition results of the CAH-21OHD patients are presented in Table 1. Eighteen patients were classified as prepubertal and 35 as pubertal or postpubertal. The median (range) of daily doses (mg) of glucocorticoids and fluorocortisone were 22.0 (10.0–55.0) and 0.05 (0.01–0.08) respectively.

Females had significantly higher values for biceps SKF, triceps SKF, calf SKF, sum of triceps and calf SKF, sum of triceps and biceps SKF ($p < 0.05$), and %BF measured by DXA ($p = 0.001$). In our sample, the prevalence of overweight was 28.3% (27.3% for males and 29% for females), and the prevalence of obesity was 9.4% (9.1% for males and 9.7% for females).

Table 2 presents the regression parameters, and cross-validation results between %BF measured by DXA and the predictive anthropometric equations.

The %BF values estimated by the three predictive equations demonstrated a significantly high correlation ($R > 0.90, p < 0.001$) with the reference method. The regression analysis demonstrated that the %BF estimated by the new three predictive equations explained 85%, 86% and 86% of the variance in DXA %BF values for Equations (1)–(3), respectively. The standard error of estimation (SEE) for the predictive anthropometric equations was less than 2.9% for all of the equations. The variance inflation factor (VIF) ranged from 1.11 to 2.75. The accuracy of the cross-validation results from the PRESS method ($R_{\text{PRESS}}^2 \geq 0.84; \text{SEE}_{\text{PRESS}} \geq 2.94\%$) was similar to the performance of the developed models (Table 2).

### 3.1 General characteristics

Table 1

General characteristics of the patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency and correlation with the percentage of body fat mass.

<table>
<thead>
<tr>
<th></th>
<th>Males (n = 22)</th>
<th>Females (n = 31)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>13.2 ± 4.2</td>
<td>13.9 ± 4.1</td>
<td>0.15</td>
</tr>
<tr>
<td>Treatment time (years)</td>
<td>11.1 ± 4.4</td>
<td>13.4 ± 4.3</td>
<td>0.17</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>151.6 ± 17.4</td>
<td>147.3 ± 13.3</td>
<td>0.27</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>50.8 ± 15.5</td>
<td>48.7 ± 16.3</td>
<td>0.60</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.5 ± 3.1</td>
<td>21.8 ± 4.5</td>
<td>0.72</td>
</tr>
<tr>
<td>Normal/Overweight/Obese</td>
<td>14/6/2</td>
<td>–</td>
<td>0.001</td>
</tr>
<tr>
<td>Sitting height</td>
<td>81.7 ± 8.9</td>
<td>78.8 ± 7.3</td>
<td>0.23</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>68.8 ± 7.7</td>
<td>66.6 ± 9.7</td>
<td>0.70</td>
</tr>
<tr>
<td>SH/WC (mm)</td>
<td>1.10 ± 0.10</td>
<td>1.05 ± 0.11</td>
<td>0.86</td>
</tr>
<tr>
<td>Triceps SKF (mm)</td>
<td>12.8 ± 4.5</td>
<td>16.0 ± 5.2</td>
<td>0.84</td>
</tr>
<tr>
<td>Biceps SKF (mm)</td>
<td>6.1 ± 3.0</td>
<td>8.6 ± 3.7</td>
<td>0.80</td>
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<tr>
<td>Suprailiac SKF (mm)</td>
<td>20.2 ± 9.1</td>
<td>25.5 ± 12.3</td>
<td>0.84</td>
</tr>
<tr>
<td>Subscapular SKF (mm)</td>
<td>11.4 ± 4.8</td>
<td>13.3 ± 7.2</td>
<td>0.58</td>
</tr>
<tr>
<td>Calf SKF (mm)</td>
<td>14.0 ± 5.2</td>
<td>18.0 ± 5.6</td>
<td>0.76</td>
</tr>
<tr>
<td>Triceps + Subscapular (mm)</td>
<td>24.2 ± 8.3</td>
<td>29.3 ± 11.6</td>
<td>0.85</td>
</tr>
</tbody>
</table>

BMI: Body mass index, SH/WC: sitting height/waist circumference ratio, Triceps + Subscapular: sum of triceps and subscapular SKF thickness (mm), Triceps + Calf: sum of triceps and medial calf SKF thickness, Triceps + Biceps: sum of triceps and biceps SKF thickness (mm), Sum 4SKF: sum of triceps, subscapular, medial calf and biceps SKF thickness (mm), DXA: Dual-energy X-ray absorptiometry.

### 3.2 Regression parameters

Table 2

Regression parameters and cross-validation results between %BF measured by dual-energy X-ray absorptiometry (DXA) and the predictive anthropometric equations.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
<th>$\beta$</th>
<th>SE</th>
<th>VIF</th>
<th>$p$</th>
<th>SE</th>
<th>VIF</th>
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</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>55.24</td>
<td>53.27</td>
<td>12.65</td>
<td>9.54</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Triceps</td>
<td>0.77</td>
<td>0.52</td>
<td>0.21</td>
<td>2.18</td>
<td>0.15</td>
<td>2.54</td>
<td></td>
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</tr>
<tr>
<td>$R^2$</td>
<td>0.50</td>
<td>0.79</td>
<td>0.52</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEE (%)</td>
<td>2.91</td>
<td>2.70</td>
<td>0.91</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>$R_{\text{PRESS}}^2$</td>
<td>0.82</td>
<td>0.79</td>
<td>0.52</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{SEE}_{\text{PRESS}}$ (%)</td>
<td>3.00</td>
<td>2.77</td>
<td>0.91</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equation (2)</td>
<td>Intercept</td>
<td>56.34</td>
<td>49.15</td>
<td>10.83</td>
<td>9.76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triceps + Biceps</td>
<td>0.51</td>
<td>0.36</td>
<td>0.12</td>
<td>1.92</td>
<td>0.10</td>
<td>2.86</td>
<td></td>
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<tr>
<td>SH/WC</td>
<td>-37.75</td>
<td>-25.65</td>
<td>8.30</td>
<td>7.53</td>
<td>2.86</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>$R^2$</td>
<td>0.86</td>
<td>0.82</td>
<td>0.91</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SEE (%)</td>
<td>2.70</td>
<td>2.62</td>
<td>0.91</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>$R_{\text{PRESS}}^2$</td>
<td>0.85</td>
<td>0.81</td>
<td>0.91</td>
<td>0.001</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>$\text{SEE}_{\text{PRESS}}$ (%)</td>
<td>2.78</td>
<td>2.68</td>
<td>0.91</td>
<td>0.001</td>
<td></td>
<td></td>
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<tr>
<td>Equation (3)</td>
<td>Intercept</td>
<td>53.74</td>
<td>53.08</td>
<td>10.69</td>
<td>10.05</td>
<td></td>
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<tr>
<td>Triceps + Calf</td>
<td>0.40</td>
<td>0.26</td>
<td>0.09</td>
<td>1.97</td>
<td>0.08</td>
<td>2.63</td>
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<tr>
<td>SH/WC</td>
<td>-36.32</td>
<td>-29.19</td>
<td>8.10</td>
<td>7.38</td>
<td>2.63</td>
<td></td>
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<tr>
<td>$R^2$</td>
<td>0.94</td>
<td>0.90</td>
<td>0.91</td>
<td>0.001</td>
<td></td>
<td></td>
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<tr>
<td>SEE (%)</td>
<td>2.61</td>
<td>2.75</td>
<td>0.91</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R_{\text{PRESS}}^2$</td>
<td>0.86</td>
<td>0.79</td>
<td>0.91</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>$\text{SEE}_{\text{PRESS}}$ (%)</td>
<td>2.71</td>
<td>2.83</td>
<td>0.91</td>
<td>0.001</td>
<td></td>
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</tbody>
</table>

$\beta$: beta (regression coefficient), SE: standard error, VIF: variance inflation factor, $R^2$: coefficient of determination, $R_{\text{PRESS}}^2$: coefficient of determination from prediction of sum of squares, $\text{SEE}_{\text{PRESS}}$: standard error of the estimate from prediction of sum of squares, Triceps: triceps SKF thickness (mm), SH/WC: sitting height/waist circumference ratio, Triceps + Biceps: sum of triceps and biceps SKF thickness (mm), Triceps + Calf: sum of triceps and medial calf SKF thickness.

Significantly different between genders $p$ by $t$-test.

Significantly different between genders $p$ by Mann–Whitney $U$ test.

Significant correlation with Body Fat Mass DXA ($p < 0.001$).

Comparison between genders performed by Mann–Whitney $U$ test; correlation performed on log-transformed value.
where, Triceps: triceps SKF thickness (mm), SH/WC: sitting height–waist circumference ratio, Triceps + Biceps: sum of triceps and biceps SKF thickness (mm), Triceps + Calf: sum of triceps and medial calf skinfolds thickness. Gender: Males = 0 and females = 1.

4. Discussion

Our data demonstrated that the %BF calculated by the three new anthropometric predictive equations that used SKF measurements, the SH/WC ratio, and gender as predictors were highly associated (R) with the reference method, reflected by the elevated power of explanation (R^2) and low errors (SEE). Furthermore, at the individual level, the equations presented nonsignificant bias and acceptable limits of agreement, with no trend observed between the differences and the mean of the newly developed models and the reference method. This latter finding indicates that the differences between the models and the criterion method were not dependent on the level of adiposity, which is considerably high in this population.

The assessment of body fat is very important for children and adolescents because of the high association between excess body fat and the increased risk of developing several diseases. In this context, these newly developed models should be part of clinical care in patients with CAH, considering that these individuals may have higher body fat content attributable to treatment with glucocorticoids.

Several studies reported a strong relationship between SKF thickness and body fatness in children and adolescents as predictors of body fat. Several studies reported a strong relationship between SKF thickness and body fatness in children and adolescents as predictors of body fat. In the present study, the SKF were highly correlated with %BF measured by DXA, while BMI and waist circumference showed moderate and low associations, respectively. BMI is a very practical and important tool in studies that use large numbers of individuals, but BMI cannot distinguish between body fat and lean mass, and this may affect its use in individuals with CAH, considering that these subjects may have increased body fat attributable to excess corticosteroids or increased fat-free mass attributable to excess androgens. Waist circumference is an important indicator of childhood obesity related to visceral fat. This may be especially important in patients with CAH who can develop increased trunk fat caused by glucocorticoid treatment.

We included the SH/WC ratio in our analysis because of the probability that these patients will accumulate fat in the trunk, which has been reported in previous studies, indicating that these patients may have compromised growth in the lower limbs. Our results showed that the SH/WC ratio has a high association with %BF measured by DXA and was a significant predictor (p < 0.001) in the three new models. Moreover, because sitting height is used as an indicator of maturity, we decided to include this variable as a predictor in the developed model and consider the possible effect of maturation in the derived models.

The new equations demonstrated very similar accuracy at both the group level and individual level. Notably, the three new models...
are relatively easy to apply in clinical settings because they involve only four anthropometric measures that should cause minimal discomfort to the patients, especially women because undressing is unnecessary.

We assessed the multicollinearity for each predictor variable, detected by calculating the VIF. Multicollinearity affects the precision of the regression coefficients and thus the accuracy of the prediction when the equation is applied to other samples. No definitive criteria indicate what the VIF should be for each predictor variable. In the present study, the VIF values ranged from 1.1 to 2.7. Some authors argue that if the VIF value exceeds 10, then multicollinearity may affect the regression estimates. Additionally, cross-validation was performed using PRESS. This internal cross-validation procedure is an alternative to data splitting and convenient when insufficient independent data are available. PRESS is a useful case diagnostic tool. The PRESS of each subject in the total dataset is excluded one at a time as the regression analysis is performed. In the present study, the $R^2_{\text{PRESS}}$ value remained high, and $\text{SEE}_{\text{PRESS}}$ remained less than 3%, demonstrating good accuracy. Validation using the PRESS procedure is similar to applying the equation to an independent sample because the PRESS residual is obtained for the observations that are not included in the data when the equation is derived.

Because of the relative simplicity and low cost, these three new predictive equations may be used as alternative methods in the evaluation of body fatness in clinical settings where the use of other reference methods is limited. Importantly, however, the present study has some limitations. Because of the reduced number of subjects included in this research, the large variability in the subjects’ ages may not have the required representation. Additionally, the accuracy of these models for tracking changes in adiposity need further research that uses a longitudinal design. Further work is needed to validate these models in similar samples, especially with regard to different glucocorticoid replacement therapies that may affect fat patterning.

5. Conclusion

The high cost and the time consuming of assessment adiposity by DXA, as well as the discomfort of the patients precludes its use in most clinical settings. Considering that the newly developed anthropometric-based models are quick and easy to perform, this study highlights the usefulness of this technique as a valid alternative method to assess body fat in 7- to 20-year-old individuals with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency.

Statement of authorship

All of the authors have made substantial contributions to the study EMG: (1) the conception and design of the study, acquisition of data, analysis and interpretation of data, (2) drafting the article,
(3) final approval of the version to be submitted. **AMS:** (1) the conception and design of the study, and interpretation of data, (2) revising the manuscript critically for important intellectual content, (3) final approval of the version to be submitted. **CNM:** (1) interpretation of data, (2) revising the manuscript critically for important intellectual content, (3) final approval of the version to be submitted. **SHVL-M:** (1) the conception and design of the study, acquisition of data, analysis and interpretation of data, (2) revising the manuscript critically for important intellectual content, conception and design of the study, and interpretation of data, (2) revising the manuscript critically for important intellectual content, (3) final approval of the version to be submitted. **AOS:** (1) interpretation of data, (2) revising the manuscript critically for important intellectual content, (3) final approval of the version to be submitted. **GG-J:** (1) the conception and design of the study, acquisition of data, analysis and interpretation of data, (2) revising the manuscript critically for important intellectual content, (3) final approval of the version to be submitted.

**Conflict of interest**

None of the authors has declared a conflict of interest.

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