CHAPTER 6

Body fat measurement in adolescent athletes: multicompartment molecular model comparison[3]

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ABSTRACT

The purpose of this study was to assess the accuracy of air displacement (ADP) and DXA percent fat mass (%FM) estimations in comparison with a reference five-compartimental (5C) model used as the reference method. A total of 32 girl (age: 15.1±0.3 years; BMI: 20.2±2.6 kg/m^2) and 46 boy (age: 15.3±1.2 years; BMI: 22.0±2.5 kg/m^2) athletes were measured. Body volume was assessed by ADP, bone mineral content by DXA, and total body water by deuterium dilution. Statistical analyses included examination of the coefficient of correlation (r), standard error of estimation (SEE), slope, intercept, and pure error (PE) and the agreement between models. For boys and girls, differences between the 5C model and ADP %FM were 0.2 and 1.7% (r=0.86 and 0.98, SEE=2.50 and 1.55%, PE=2.77 and 2.23%), respectively. Differences between the 5C model and DXA %FM were –1.0 and –3.7% (r=0.85 and 0.91, SEE=2.60 and 2.91%, PE=2.90 and 4.66%), respectively for boys and girls. For girls, regression between ADP and DXA against the reference method did not differ from the line of identity ($p>0.05$) while for boys differences were found ($p<0.05$). DXA overestimated %FM, particularly in girls. For both genders, large limits of agreement were found between reference method and both techniques, with the exception of ADP in female athletes. We conclude that the two techniques were not precise for individual %FM prediction, though ADP revealed a better agreement for girls. However, considering all performance criteria for mean group, our analysis highlights ADP as an accurate and nonbiased tool in the evaluation of body composition in adolescent athletes.

"To improve is to change; to be perfect is to change often."
Winston Churchill

[3] European Journal of Clinical Nutrition, (2005). *In Press.*

INTRODUCTION

Estimates of body composition are widely used in young athletes to prescribe desirable body weights, to optimise performance, and to assess the effects of training. Despite the recognized needs of body composition measurement in the paediatric population, it is difficult to assess body components with accuracy and precision, particularly in young athletes. There are several body fat measurement methods that can be applied in the clinical setting (1). One traditional method is to evaluate body fat based on a two-compartment (2C) model (2), by hydrostatic weighing and more recently, by air displacement plethysmography (ADP), (3, 4). The 2C model is formulated on assumed constant densities of fat and fat-free body mass (2). ADP, which depends on the assumptions of a 2C model, can quickly and safely evaluate body composition in a wide range of subject types, including those who are often difficult to measure, such as the elderly, children, and obese individuals (5). Therefore, ADP has been widely validated in healthy adults (6-15). However, few studies have validated ADP against multicompartment models during growth and maturation, to address issues related to body composition changes, specifically fat-free mass (FFM) density and respective fractions of water, protein, and mineral (13, 16). Body volume measurements that are usually estimated by hydrostatic weighing or ADP serve as the basis for the 2C model of body composition (17). The addition of total body water allows the development of a 3C molecular model. The 3C molecular model can then be extended to a 4C molecular model by adding an estimate of bone mineral by Dual energy X-ray Absorptiometry (DXA) (18-21). Three and four compartmental molecular models are now widely applied in body composition research. However, both 3C and 4C molecular models do not include a discrete estimation of soft mineral, a small but important component at the molecular level.

Therefore, more recently, a new approach to obtain this component was proposed (22), enabling the use of a 5C molecular model, which divides body mass into fat, water, bone mineral, soft tissue mineral, and protein.

DXA has emerged as one of the most widely accepted methods in the assessment of body composition in children and adolescents (23-25). This method has advantages over other laboratory techniques as it provides whole-body and regional estimates of body composition into fat, lean tissue mass (LTM), and bone mineral content (BMC). Because DXA does not rely on the assumptions of a 2C model to provide estimates of body composition and because it does not depend on subject performance, DXA is sometimes regarded as a standard against which other methods can be validated (26-29). However, like most other methods for measuring body composition, DXA is also subject to error (30-33). Few studies have been conducted to validate DXA against multicompartment models during growth and maturation (16, 34-38).

In addition, the physiological changes due to the interaction of growing and the training process in athletes may alter the FFM composition, namely the protein and water constituents, and in turn the assumption that FFM hydration and density are constant. Studies on a few small groups of athletes have found systematic differences between estimates from body density (39-41), indicating that FFM density is different from 1.1 g/cm^3, whereas studies of other groups have not reached the same conclusion (40, 42-44).

Therefore, uncertainties about FFM composition in growing athletes and its implication on body fat estimates remain and efforts to overcome methodological limitations using robust multicompartment models are warranted. It is relevant that the 5C molecular model approach has not yet been used in a paediatric athletic

population. As a result, the purpose of the present study was to compare percent body fat estimations using ADP and DXA with the gold standard 5C molecular model in adolescent athletes.

METHODS

Subjects

Body composition data were collected in 32 girls (age: 15.1 ± 0.3 y; weight: 56.2 ± 14.2 kg; stature: 1.65 ± 0.13 m; BMI: 20.2 ± 2.6 kg/m^2) and 46 boys (age: 15.3 ± 1.2 y; weight: 71.5 ± 12.3 kg; stature: 1.80 ± 0.12 m; BMI: 22.0 ± 2.5 kg/m^2) 13 pubescent (8 boys and 5 girls) and 64 post-pubescent (38 boys and 27 girls), who volunteered to participate in this study. Subjects were recruited from five sports clubs in Lisbon (Portugal) and were involved in a variety of professional sports (swimming, basketball, rugby, gymnastic, and judo). All subjects were informed about the research design and signed a consent form according to the regulations of the Ethical Committee of the Faculty of Human Movement, Technical University of Lisbon. After a 12-hour fast, subjects came to the laboratory where all measurements and testing were carried out, in the same morning.

Maturation

Subjects were grouped by puberty stage, determined by self-assessment according to Tanner stage (45) and adapted by Ross and Marfell-Jones (46). A self-evaluation method, with figures, was used to identify the degree of development of the genital organs, breast, and pubic hair.

Reliability

Reproducibility was performed on 2 days approximately one week apart in 10 subjects, 2 males and 8 females, ranging in age from 25 to 44 yrs (mean ± SD: 35.0 ±

6.8 yrs) and BMI from 20.8 to 27.9 kg/m^2 (mean ± SD: 24.6 ± 2.4 kg/m^2) for the following measurements: body volume, bone mineral content, total-body water, and the propagation of measurement error calculation.

Body composition Measurements

Body volume (BV) was assessed by air displacement plethysmograph (BOD POD®, Life Measurement Inc, Concord, CA, USA). Each subject wore a swimming suit and their body mass was measured to the nearest 100 g by an electronic scale connected to the plethysmograph computer. BV was computed based on the initial body volume corrected for thoracic gas volume and a surface area artefact computed automatically. Measured thoracic gas volume was obtained in all subjects. Body density (Bd) was then calculated as body mass divided by BV. Percent body fat ($\%FM_{ADP}$) was estimated from body density based on a two-compartment model using the Siri's equation (2):

$$\%FM_{ADP} = [(4.95/B) - 4.50]x100 \quad (1)$$

Also, Lohman's age-adjusted constants (47) were used to convert body density into %FM ($\%FM_{Lohman}$). All measurements were conducted with the BOD POD® software version 1.68. The technical error of measurement (TEM) and the coefficient of variation (CV) for BV were 0.17 L and 0.5 %, respectively.

Total body bone mineral was estimated using DXA (QDR-1500, Hologic, Waltham, USA, pencil beam mode, software version 5.67 enhanced whole body analysis). The attenuation of x-rays pulsed between 70 and 140 kV synchronously with the line frequency for each pixel of the scanned image. The same lab technician positioned the subjects, performed the scans and executed the analysis according to the operator's manual using the standard analysis protocol. Considering that bone mineral content (BMC) represents ashed bone, BMC was converted to total body

bone mineral (TBBM) by multiplying it by 1.0436 (48). The TEM and CV for BMC in our laboratory were 0.02 kg and 1.6 %, respectively.

Total body water (TBW) was assessed by deuterium dilution technique using a stable Hydra gas isotope ratio mass spectrometer (PDZ, Europa Scientific, UK). After a completed 12h fast, an initial urine sample was collected and immediately administrated a deuterium oxide solution dose (^2H_2O) of 0.1g/kg of body weight diluted in 30 mL of water. After a 4 h equilibration period, new urine sample was collected. Abundances of ^2H_2O in dilutions of the isotope doses were analyzed. Urine and diluted dose samples were prepared for $^1H/^2H$ analysis using the equilibration technique of Prosser and Scrimgeour (49). After the tubes were filled they were equilibrated at 20 ± 1ºC overnight for 3 days. The tubes were then introduced sequentially into a helium flow that was dried by magnesium perchlorate, and then analyzed by a Hydra gas isotope ratio mass spectrometer set to detect $^1H/^2H$. The enrichments of equilibrated local water standards were calibrated against SMOW (Standard Mean Ocean Water). Based on delta SMOW, TBW was estimated including a 4% correction due to the recognized amount corresponding to deuterium dilution in other compartments(50). The TEM and CV for TBW with the stable isotope ratio mass spectrometry in this laboratory were 0.26 L and 1.3 %, respectively.

Total-body Soft Mineral was assessed with the equation, developed by Wang and colleagues (22):

$$Ms\ (kg) = 0.0129 \times TBW \qquad (2)$$

where Ms is total-body soft mineral in kg and TBW is total body water in kg.

Five-component model

A multi-compartment model, 5C molecular model, was used to estimate %FM. Rather than a single total mineral component, this 5-component model accounts for both the mineral and soft mineral components (22). However, it is important to note that this model will provide near equivalent results to the more commonly used 4C molecular models (48).

Accordingly, FM was assessed with the following equation:

$$FM\ (kg)=2.748\times BV - 0.715\times TBW + 1.129\times Mo + 1.222xMs - 2.051\times BM \quad (3)$$

where BV is body volume (L), TBW is total body water (kg), Mo is total-body bone mineral (kg), Ms is total-body soft tissue mineral (kg) and BM is body mass (kg).

Calculation of FFM density

The density (D) of the FFM (D_{FFM}) was estimated from TBW, Mo, Ms, and protein (protein is equal BM minus FM from the 5C model, TBW, Mo, and Ms) contents of the FFM (estimated as BM minus FM from the 5C model) and their respective densities (0.9937 g/cc, 2.982 g/cc, 3.317 g/cc, and 1.34 g/cc, respectively for TBW, Mo, Ms, and Protein) using the following equation:

$$D_{FFM}= 1 / [(TBW/D_{TBW}) + (Mo/D_{Mo}) + (Ms/D_{Ms}) + (Protein/D_{protein})] \quad (4)$$

Propagation of measurement error

In the present study we selected ADP to assess BV, DXA to estimate Mo, and deuterium dilution to estimate TBW. The propagation of measurement errors associated with the determination of BV, TBW, and Mo can be calculated by assuming that the squared errors (TEM^2) are independent and additive (48). Accordingly,

$$TEM = [TEM^2 \text{ for effect of ADP on \%FM} + TEM^2 \text{ for TBW on \%FM} + TEM^2 \text{ for Mo on \%FM}]^{0.5} \quad (5)$$

using equation 3,

$$TEM = [0.73^2 + 0.26^2 + 0.04^2]^{0.5} = 0.79 \text{ \%FM from TEM values}$$

The test-retest reliability data collected in the present study thus yields a value of ~1 %FM units.

Statistical analysis

Paired t-tests were used to compare %FM from ADP and DXA with the 5C model, for boys and girls. An independent t-test was used to compare FFM composition and density between boys and girls. Whenever a normal distribution in body composition variables was found among the sports (by gender), one-way ANOVA was used to perform comparison of means. Otherwise, a non-parametric test, Kruskal-Wallis, was employed. For each gender, if no more than 2 sports presented a sufficient number of athletes, the comparison of means was performed using a paired-t test (when a normal distribution was found) or the Wilcoxon test (when the distribution was different from normality). Simple linear regression analysis was performed when comparing %FM estimates by ADP and DXA, as the independent variables, with the reference method, as the dependent variable. Multiple regression analysis was performed to test the influence of the maturation level alone and in interaction with %FM from each method ADP and DXA, separately. For each method, if maturation level and the interaction between this factor with DXA and ADP %FM were non-significant, simple linear regression analysis would be performed to explain %FM from the reference method. The Standard Error of Estimation (SEE), the coefficient of correlation (r) and the coefficient of variation (CV) were analysed. The SEE is used as a measure of validation to assess the lack of association between two methods (reference method vs. DXA; reference method vs. ADP). The CV is the standard deviation of duplicate measurements standardized for

the mean value of the duplicate measurements. Also, the pure error (PE) was assessed, as another measure of validation, using the following equation $(\Sigma(\ddot{Y}-Y)^2/n)^{1/2}$, where $\ddot{Y}$ is the predicted %FM, Y is the observed %FM and n is the number of subjects (51). Agreement between methods was assessed (52) including the 95% limits of agreement. Technical error was assessed with the following equation $(\Sigma d^2/2n)^{1/2}$, where d stands for the difference between repeated measurements and n is the number of paired repeated measurements. Statistical significance was set at $p<0.05$.

RESULTS

Sample descriptive characteristics, %FM from ADP, DXA, the reference method ($\%FM_{5C}$), FFM composition and density, and gender differences for all variables are reported in **Table 6.1**. Although similar ages were found among boys and girls, height and weight were significantly higher in boys ($p<0.001$), and girls were significantly fatter than boys were ($p<0.001$). For boys, %FM obtained from the several methods did not differ among the four representative male sports (Judo, Basketball, swimming, and Rugby) ($p>0.05$) while gymnastics females were significantly fatter than basketball female players ($p<0.001$) (data not shown). For girls, FFM density and composition was not significantly different ($p>0.05$) between basketball players and gymnastic players (data not shown). However, male swimmers presented a significant lower FFM density than male basketball players ($p=0.016$) but no differences were found with judo and rugby male players ($p>0.05$). For the male sample, a significant lower mineral fraction of the FFM was found in swimmers compared to basketball players ($p=0.002$) (data not shown).

For boys and girls, %FM from DXA was significantly higher than $\%FM_{5C}$ (boys: $p=0.014$; girls: $p<0.001$). For boys, %FM from ADP did not differ from

$\%FM_{5C}$. However, for girls, %FM from ADP was significantly lower ($p<0.001$) than $\%FM_{5C}$.

Lohman's age-adjusted constants were used to convert body density to %FM ($\%FM_{Lohman}$). After applying these equations, body fatness was underestimated in both genders, especially in girls (boys: -1.70 %, $p<0.001$; Girls: -4.04 %, $p<0.001$). FFM density from the reference method was higher than Lohman's age-adjusted models and differed ($p<0.001$) by 0.004 and 0.012 g/cc, in boys and girls, respectively (data not shown).

Results from the FFM composition revealed that girls showed a significant smaller water fraction ($p=0.003$) and a significant higher protein fraction ($p=0.007$) compared with boys, resulting in a superior FFM density in the female sample ($p=0.001$).

Table 6.1. Physical characteristics, %FM using ADP, DXA, $\%FM_{5C}$, FFM composition and density, and gender differences (Mean ± SD)

	Girls (n=32)	Boys (n=46)
	Mean± SD	
Age (years)	15.1 ± 0.3	15.3 ± 1.2
Weight (kg)	56.2[2] ± 14.2	71.5 ± 12.3
Stature (m)	1.65[2] ± 0.13	1.80 ± 0.12
BMI (kg/m^2)	20.2[2] ± 2.6	22.0 ± 2.5
$\%FM_{5C}$	19.8[2] ± 7.0	13.1 ± 4.9
$\%FM_{ADP}$	18.2[1,2] ± 6.7	12.9 ± 5.5
Bias ($\%FM_{5C}$ - $\%FM_{ADP}$)	1.65[3] ± 1.53	0.21 ± 2.79
$\%FM_{DXA}$	23.5[1,2] ± 6.8	14.1[1] ± 5.1
Bias ($\%FM_{5C}$ - $\%FM_{DXA}$)	-3.69[3] ± 2.89	-1.04[3] ± 2.75
$\%FM_{Lohman}$	15.8[1,2] ± 7.3	11.6[1] ± 5.5
Bias ($\%FM_{5C}$ - $\%FM_{Lohman}$)	-4.04[3] ± 1.63	-1.70[3] ± 2.73
$FFM_{density}$ (g/cc)	1.105[2] ± 0.004	1.100 ± 0.007
Water/FFM (%)	70.9[2] ± 1.2	72.4 ± 1.7
Total Body Mineral/FFM (%)	6.3 ± 0.7	6.1 ± 0.5
Bone Mineral/FFM (%)	5.4 ± 0.7	5.2 ± 0.5
Soft tissue Mineral/FFM (%)	0.92[2] ± 0.02	0.93 ± 0.02
Protein/FFM (%)	22.8[2] ± 1.6	21.5 ± 1.5

[1] Significantly different from $\%FM_{5C}$, $p<0.05$
[2] Significantly different between boys and girls, $p<0.05$
[3] Significantly different from 0, $p<0.05$

For each method, maturation was tested and did not present a significant contribution to the explained %FM variability from the reference method using ADP %FM (boys: p=0.657; girls: p=0.517) and DXA %FM (boys: p=0.139; girls: p=0.807), as the independent variables. In addition, no significant interactions between maturation level with ADP %FM (boys: p=0.700; girls: p=0.821) and DXA %FM (boys: p=0.168; girls: p=0.892) were found across the models.

ADP and DXA *vs.* the 5C model

Group means results

The performance of ADP and DXA as predictor variables of $\%FM_{5C}$ is illustrated in **Figure 6.1**. For boys and girls, ADP explained 74 and 96% respectively, of the variance of the standard multicompartment model with relatively small SEEs, 2.50 and 1.55 %, respectively.

Using %FM from DXA to predict $\%FM_{5C}$, we found that DXA explained 72 and 83% of the variance, respectively for boys and girls, presenting SEEs higher than those obtained by ADP (Boys: 2.60 %; Girls: 2.91 %).

The pure error for %FM estimates using ADP was smaller than using DXA, ranging from 2.23 to 2.77 % for ADP, and from 2.91 to 4.66 % for DXA. As described above, no differences were found between ADP and the reference method for boys (bias=0.2 %FM, p=0.946). However, %FM was significantly underestimated in girls using ADP (bias=1.7 %FM, $p<0.001$). DXA overestimated %FM in relation to the reference method (Boys: bias=-1.0 %FM, p=0.015; Girls: bias=-3.7 %FM, $p<0.001$), particularly in girls.

Slopes and intercepts from the regression between ADP and DXA with $\%FM_{5C}$, did not differ from the line of identity ($p>0.05$) for the female sample, while for adolescent male athletes, slopes and intercepts differed from the line of identity ($P>0.05$), as indicated in **Table 6.2**.

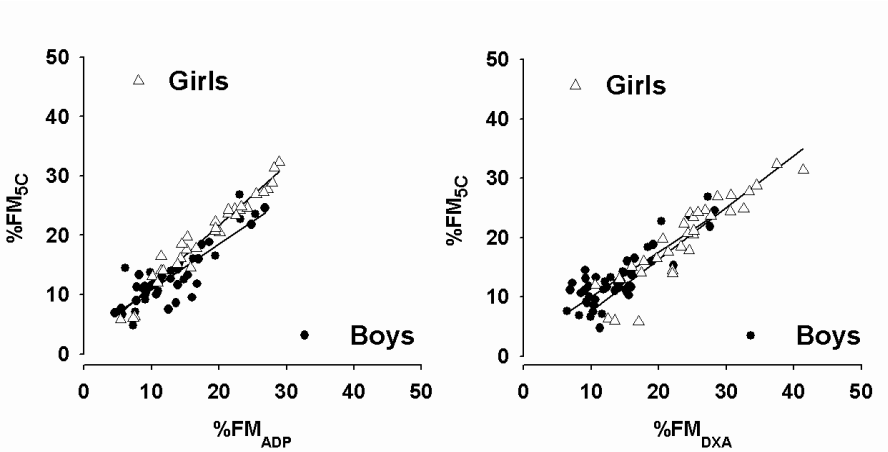

Figure 6.1 – Regression of %FM estimation using the reference method ($\%FM_{5C}$) by ADP in the left panel and by DXA in the right panel for boys and girls.

Table 6.2. Performance criteria: slope, intercept, coefficient of correlation (r), standard error of estimation (SEE), pure error (PE), coefficient of variation (CV) and the agreement (bias, limits and trend) between %FM from the reference method and the two techniques: ADP and DXA

							Agreement		
	Slope	Intercept	r	SEE	PE	CV	Bias	Limits	Trend
$\%FM_{ADP}$									
Girls (n=32)	1.02	1.31	0.98	1.55	2.23	8.5	1.7[1]	4.6, -1.3	0.19
Boys (n=46)	0.76[2]	3.25[1]	0.86	2.50	2.77	19.4	0.2	5.7, -5.3	-0.23
$\%FM_{DXA}$									
Girls (n=32)	0.94	-2.27	0.91	2.91	4.66	12.3	-3.7[1]	2.0, -9.4	-0.11
Boys (n=46)	0.81[2]	1.66[1]	0.85	2.60	2.91	18.4	-1.0[1]	4.3, -6.4	-0.22

[1] Significantly different from 0, $p<0.05$
[2] Significantly different from 1, $p<0.05$

Individual results

The agreement between the 5C model with ADP and DXA is illustrated in **Figure 6.2**. For boys, no trend line was presented for ADP (r= -0.231; p=0.122) and the limits of agreement ranged from -5.3 to 5.7 %. Also for girls, no association was

found between the differences of the reference method and ADP with the mean of both methods (r=0.194; p=0.288) and smaller limits were obtained (-1.3 to 4.6 %) compared to the male group. For DXA, considerable larger limits of agreement compared to ADP were found, ranging from -6.4 to 4.3% for boys and from -9.4 to 2.0 % for girls. No association was shown between the differences of $\%FM_{5C}$ and DXA with the mean of both methods, for boys (r=0.215; p=0.151) and girls (r=0.112; p=0.541).

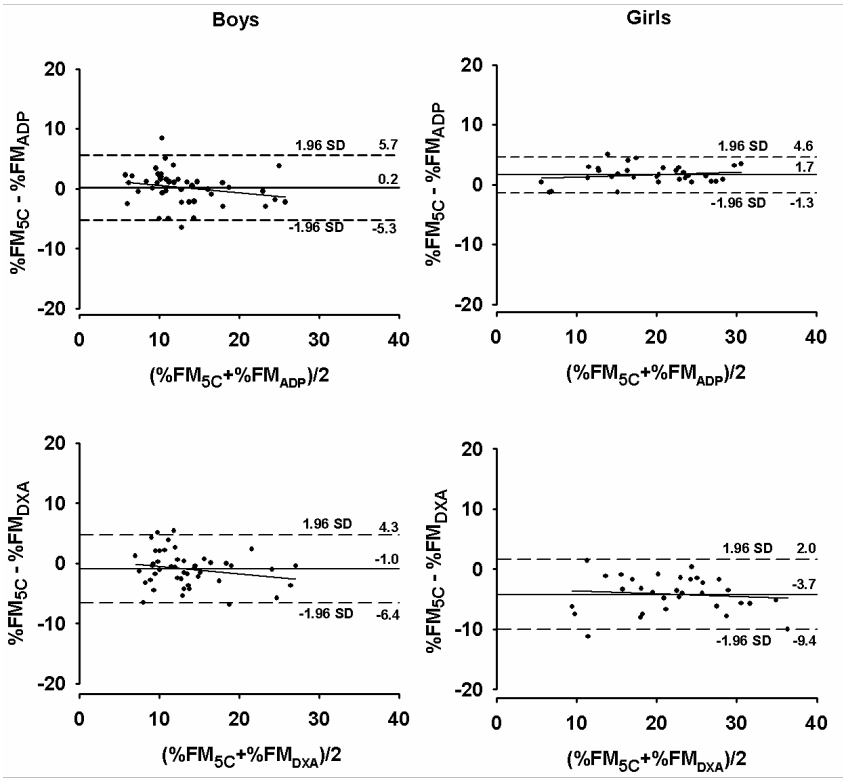

Figure 6.2 - Agreement between the reference method ($\%FM_{5C}$) and the 2 techniques: ADP (Siri equation) and DXA, for boys and girls. The solid line represents the mean differences between the reference method with ADP (upper panels) and DXA (lower panels). The dashed lines represent 95% limits of agreement (±1.96 SD). Trend line represents the association between the differences of the methods and the mean of both methods.

DISCUSSION

To our knowledge, this is the first study to address the validity of percent body fat estimations using DXA and ADP with a multicompartment molecular model, in male and female adolescent athletes.

Validity of DXA

DXA has been evaluated against an independent criterion method for measuring body fatness in children and adolescents, such as the multicompartment models, but not in adolescent athletes (16, 34-38). In the current study, results from the regression analysis of DXA with the 5C molecular model point out the precision of this technique. The r, SEE, and PE ranged from 0.85 to 0.91, 2.60 to 2.91 %FM, and 2.91 to 4.66 %FM, respectively. These results were similar to the findings reported by other authors in prepubescent and pubescent individuals (16), female adolescents (38), obese children and adolescents (34), and in a large paediatric population (36). All these studies were conducted with a 4C molecular model as reference (53). We found that %FM estimation using DXA was systematically higher in boys and girls, in agreement with previous studies (16, 34, 35, 38). Likewise, in our study, these findings were particularly marked in females. The relatively higher body fatness in females may explain the consistency of these results by possibly emphasizing errors of the software assumptions for DXA. Conversely, a study conducted with a sample of 30 children aged 8-12 y (37), indicated that DXA showed negligible mean errors in estimating FM regarding the 4C molecular model. However, the prototype Hologic DXA software for children may have overcome artifacts of body size (37). Moreover, using the same DXA manufacturer (Hologic) other authors referred that body fat overestimation in this equipment resulted from the assumed hydration status of 73.2% (35, 38). According to Pietrobelli and colleagues, DXA

methodological limitations are related with the stability of the lean soft tissue R value which depends, in part, on the constancy of constituent water, protein, and soft tissue mineral proportions (54).

The agreement between DXA and the 5C molecular model showed large limits and a considerable mean bias without a significant trend across different levels of fatness, which also matches with findings reported by other authors (35, 38). The large limits of agreement in our study (Boys: -1.0 ± 5.4 % and Girls: -3.7 ± 5.7 %) could cause an individual %FM value to be underestimated by 4.3 % for boys and 2.0 % for girls, or overestimated by 6.4 % for boys and 9.4 % for girls, though no relation between the differences of the methods and adiposity was present. Using a 4C model, similar findings (bias ± 2SD) were obtained in females (-3.9 ± 6.7 %) (38), prepubescent and pubescent ranging from -1.13 ± 8.41 % to -3.43 ± 9.09 % (35) in a large pediatric sample -1.0 ± 8.9 % (36), showing that DXA could cause an individual %FM value to be overestimated by ~13 %. To a lesser extent, Gately and colleagues, reported bias (± 2SD) for obese children ranging from - 1.7 ± 3.1 to -2.2 ± 4.4%, respectively for boys and girls (34).

To further understand the relatively higher variability on DXA %FM estimates in relation to the reference method, consideration was given to the association between the differences of the methods and the hydration of FFM (TBW/FFM). For girls, no relationship was found between the two variables (r=-0.084; p=0.649) while for boys a significative inverse association was found (r=-0.394; p=0.007) (data not shown). Therefore, it appears that the lower the TBW/FFM hydration, the higher the overestimation of body fatness in the male group. The reason for this finding is unclear and requires further investigation.

Sources of potential error have been corrected by the introduction of improved software (26-29). A recent investigation (55) revealed an overestimation of FFM after updating the software. Therefore, concerns are still warranted, as DXA estimates of fat/lean tissue are assumed to be in the same proportion in pixels containing bone as they are in the adjacent non-bone-containing. To overcome the systematic bias in DXA %fat measurement we recommend the development of descriptive calibration models for DXA group mean %FM estimates in adolescent athletes, even though individual %FM estimates cannot be performed. The applicability of this recommendation to DXA instruments made by other manufacturers or to DXA instruments that use different scans modes and software is not known, though a few studies have shown a lack of interchangeability in DXA systems to assess soft tissue (56-59).

Validity of ADP

Compared with the 5C molecular model, ADP results for mean %FM estimates using Siri's equation (2) to convert body density to % FM showed a slightly underestimation in the female group but no difference in boys. However, Lohman's age-adjusted constants used to convert body density derived from ADP into %FM remarkably underestimated %FM in both genders. Therefore, results obtained from ADP %FM estimates using Siri equation will be discussed throughout this section rather than the use of Lohman age-adjusted models.

For group means, ADP was a precise and valid technique in the determination of body composition in adolescent athletes as indicated by the regression parameters, particularly in the female sample (**Table 6.2**). The r, SEE, and PE ranged from 0.86 to 0.98, 1.55 to 2.50 %FM, and 2.23 to 2.77 %FM, respectively, which closely agree with those observed by others investigators in prepubescents and pubescents (16) and

obese children and adolescents (34), which used a 4C molecular model as reference (53). However, these authors calculated %FM from ADP by converting body density with Lohman's age-adjusted constants (47). Considering the regression parameters, ADP was even more precise in our male sample compared with the findings of Fields and Goran (16). Nevertheless, it is important to note that these authors (16) examined precision and accuracy of %FM estimation using ADP in children of both sexes as a whole, and not separately as in the present work.

No bias was found between this technique and the reference method for boys, but larger limits were found, compared to girls, indicating that this method displays higher variability in individual %FM estimation in adolescent males. However, a small but significant underestimation was found for girls. Body fatness was not related with the differences between ADP and the reference method, which emphasizes the use of ADP in a sample of adolescents athletes that present a recognizable wide range of body fatness (~5 to ~30 %FM). No association with body fatness was also reported by other investigations (16) but a significant mean underestimation was found in prepubescent and pubescent (16) while for overweight and obese children no mean bias was found (34).

Effect of FFM composition and density

One critical issue is the constancy of the FFM density, which is relevant for %FM estimation using a 2C model. Our findings show that adolescent male athletes as a group did not have a FFM density significantly different from the assumed value of 1.1 g/cm^3, although the composition of the FFM differed somewhat from what is commonly assumed. The mean %FM underestimation found between ADP and the reference method for girls resulted from a significantly higher FFM density than the assumed value of 1.100 g/cm^3 (2). Studies have pointed out that the mineral and the

water fractions of the FFM increase and decrease, respectively, during growth and maturation. This effect promotes an increase on FFM density during this period, as total body mineral density is higher than water density (3.038 vs. 0.9937 g/cm^3). Based on a few studies (60-62), Lohman proposed the use of age- and gender-specific equations (47), which account for maturation-related changes in the density of the FFM for children and youth. Our subjects showed a smaller water fraction and a higher protein fraction than previous studies (60-62). As a result, FFM density was clearly underestimated ($p<0.001$) by Lohman's models (47), due to the lower water and the higher residual/protein fractions, which has an assumed greater density than water (1.34 vs. 0.994 g/cc). For females, FFM density was even higher than the adult value while for males matched it. Therefore, Lohman's age-adjusted constants for %FM estimation in this specific sample were ineffective. However, it is important to note that 17% and 73% of our subjects were pubescent and postpubescent, respectively, resulting in a higher maturation level and consequently a higher FFM density. Considering that gymnastic females are more than half of our sample, the higher FFM density found in our study extends the results presented by Prior and colleagues (40). It is important to note that our female basketball players also presented a similar FFM density. These higher FFM density found in our female sample, caused by lower TBW/FFM and higher protein/FFM than assumed, was particularly interesting, because it differed from the pattern observed in other sports (39-41). The lower TBW/FFM could have reflected dehydration. However, there was no indication that they did not adhere to the instructions designed to ensure normal hydration. Regarding the lower mineral fraction of the FFM in our male swimmers, though significant only compared to basketball players, they corroborate results from a previous study (40). The FFM density in our study ranged from 1.096 ± 0.008 g/cc

for male swimmers to 1.105 ± 0.004 g/cc for male basketball players and from 1.104 ± 0.005 g/cc for gymnastic females to 1.105 ±.0.003 g/cc for basketball players, which certainly indicates deviations in the composition of the FFM. Thus, our results may indicate that different types of athletic training may have different effects on the density and composition of the FFM.

The 4C and 5C molecular models have obvious advantages over 2C models because the reliance on a constant for the proportions and densities of the fat free mass is eliminated. The 5C molecular model used in this study ameliorates the effects of maturation, hydration, mineralization and protein status of the fat-free mass, in the estimation of body fatness, as the hydration status decreases and bone mineralization increases with age (53). In the present study we have assumed that 5C analysis provides the most accurate estimates of %FM. Heymsfield and colleagues (48) provide further details on the assumptions for multicompartment models development. Moreover, the use of ADP to estimate body density may be a limiting factor for the use of the 5C model in the present study. It is important to note that there is no true gold standard of body composition analysis. Therefore, body composition studies conducted are a comparison of methods, which are obviously based on some assumptions.

CONCLUSION

The cost of the 5C molecular model does not allow wide implementation of this model in most laboratories. Therefore, less expensive and laborious techniques, such as DXA and ADP, should instead be used in clinical settings. These methods are easily and quickly performed, safe, and progressively more available. Our results showed a strong relationship between body fatness assessed by the reference method

and both techniques. However, the techniques were less precise at an individual analysis though ADP revealed a better precision for individual %FM estimation in adolescent females. In addition, we conclude that body density converted to %FM by using Lohman's age adjusted constants were not useful in this specific population by markedly underestimating body fatness. Moreover, athletes in selected sports may have systematic deviations in D_{FFM} from the value of 1.1 kg/cc assumed in the Siri equation, resulting in-group mean errors for %FM estimation. The cause of these variations is complex and further research is required, especially during growth and maturation.

Although mean %FM estimates differed somewhat between the criterion-5C model and the DXA system, our findings on adolescent athletes suggest a strong and predictable relationship, though a specific DXA calibration is required for Hologic QDR-1500. Considering the performance criteria for group means, the current analysis supports ADP as a valid, precise, and nonbiased tool in the evaluation of body composition in adolescent athletes.

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