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A novel approach to assess body composition in children with obesity from density of the fat-free mass --Manuscript Draft--

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Abstract:	<p>Background & Aims: Assessment of Fat Mass (FM) and fat free mass (FFM) using Air-displacement plethysmography (ADP) technique assumes constant density of FFM (DFFM) by age and sex. It has been recently shown that DFFM further varies according to body mass index (BMI), meaning that ADP body composition assessments of children with obesity could be biased if DFFM is assumed to be constant. The aim of this study was to validate the use of the calculations of DFFM (rather than constant density of the FFM) to improve accuracy of body composition assessment in children with obesity. Methods: cross-sectional validation study in 66 children with obesity (aged 8 to 14 years) where ADP assessments of body composition assuming constant density (FFMBODPOD and FMBODPOD) were compared to those where DFFM was adjusted in relation to BMI (FFMadjusted and FMBadjusted), and both compared to the gold standard reference, the 4-component model (FFM4C and FM4C). Results: FFMBODPOD was overestimated by 1.50kg (95%CI -0.68kg, 3.63kg) while FMBadjusted was 0.71 kg (-1.08kg, 2.51kg) (percentage differences compared to FFM4C were 4.9% ($\pm 2.9\%$) and 2.8% ($\pm 2.1\%$), respectively ($p < 0.001$)). Consistently, FM was underestimated by both methods, representing a mean difference between methods of 4.0% ($\pm 2.9\%$) and 6.8% ($\pm 3.8\%$),</p>

respectively, when compared to the reference method. The agreement and reliability of body composition assessments were improved when adjusted using calculations (adjusted models) rather than assuming constant DFFM. Conclusions: The use of constant values for fat-free mass properties may increase bias when assessing body composition (FM and FFM) in children with obesity by two-component techniques such as ADP. Using adjusted corrections as proposed in the present work may reduce the bias by half.

A novel approach to assess body composition in children with obesity from density of the fat-free mass

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31 **ABSTRACT**

32 **Background & Aims:** Assessment of Fat Mass (FM) and fat free mass (FFM) using Air-
33 displacement plethysmography (ADP) technique assumes constant density of FFM (D_{FFM}) by age
34 and sex. It has been recently shown that D_{FFM} further varies according to body mass index (BMI),
35 meaning that ADP body composition assessments of children with obesity could be biased if D_{FFM}
36 is assumed to be constant. The aim of this study was to validate the use of the calculations of
37 D_{FFM} (rather than constant density of the FFM) to improve accuracy of body composition
38 assessment in children with obesity. **Methods:** cross-sectional validation study in 66 children
39 with obesity (aged 8 to 14 years) where ADP assessments of body composition assuming
40 constant density (FFM_{BODPOD} and FM_{BODPOD}) were compared to those where D_{FFM} was adjusted in
41 relation to BMI ($FFM_{adjusted}$ and $FM_{adjusted}$), and both compared to the gold standard reference,
42 the 4-component model (FFM_{4C} and FM_{4C}). **Results:** FFM_{BODPOD} was overestimated by 1.50kg
43 (95%CI -0.68kg, 3.63kg) while $FFM_{adjusted}$ was 0.71 kg (-1.08kg, 2.51kg) (percentage differences
44 compared to FFM_{4C} were 4.9% ($\pm 2.9\%$) and 2.8% ($\pm 2.1\%$), respectively ($p < 0.001$)). Consistently,
45 FM was underestimated by both methods, representing a mean difference between methods of
46 4.0% ($\pm 2.9\%$) and 6.8% ($\pm 3.8\%$), respectively, when compared to the reference method. The
47 agreement and reliability of body composition assessments were improved when adjusted using
48 calculations (adjusted models) rather than assuming constant D_{FFM} . **Conclusions:** The use of
49 constant values for fat-free mass properties may increase bias when assessing body composition
50 (FM and FFM) in children with obesity by two-component techniques such as ADP. Using
51 adjusted corrections as proposed in the present work may reduce the bias by half.

52

53 **INTRODUCTION**

54 Many health and disease conditions are related to body composition status and changes therein,
55 both in adults and children (1). For this reason, important applications of body composition
56 assessment include the diagnosis of disease, monitoring clinical progress and tailoring
57 treatment.

58 Body mass index (BMI) is widely considered as the accepted clinical standard to classify
59 nutritional status; it is commonly used as the screening tool for overweight and obesity and has
60 been recommended for this purpose by the World Health Organization (WHO) due to it being
61 inexpensive, simple and fast to carry out (2). BMI represents the ratio of body weight to height-
62 squared; however, it cannot distinguish between body weight components, namely the fat-free
63 mass (FFM) and fat mass (FM). In addition, BMI does not have a constant association with body
64 composition across the range of age, sex or ethnicity (3), and this can lead to misclassification of
65 nutritional status.

66 The gold standard method to assess body composition *in vivo* is the 4-component (4C) model,
67 which divides body weight into fat, protein, mineral and water. To perform this analysis, several
68 individual 2-component techniques are needed: air-displacement plethysmography (ADP) to
69 obtain body volume (BV); dual-energy X-ray absorptiometry (DXA) to obtain bone mineral
70 content (BMC); and isotopic dilution with deuterium (DD) to obtain total body water (TBW).
71 Commonly, to simplify the protocol, these techniques are often used in isolation to assess body
72 composition. However, this practice requires the use of several assumptions such as the
73 assumption of constant FFM properties (density and hydration) (8). These assumptions can
74 introduce a degree of bias in body composition assessment due to different physiological and
75 pathological factors (9), including age (10), sex, ethnicity, hormone cycle, pregnancy, fasting,
76 nutritional status (11–13), kidney or gastrointestinal diseases, etc. In fact, the current methods
77 available have greater error in those with obesity, and this error tends to increase with obesity
78 level (14).

79 ADP studies in adults have concluded that the individual variation in FFM properties such as
80 hydration and density could influence the accuracy of body composition results (15,16). ADP has
81 been validated to assess body composition in children with relatively high precision (17,18).
82 However, a study comparing ADP with a 3-component model concluded that ADP showed high
83 precision at group level, but indicated that biological individual characteristics such as hydration
84 could increase bias at the individual level (17). The standard approach in densitometric methods
85 such as ADP is to calculate body composition assuming that values for the density of fat mass

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86 (FM) and FFM are constant by age and sex (19). Recently, it has been shown in children that the
87 hydration of FFM increases, and the density of FFM decreases, with the degree of obesity (12).
88 This study reported a predictive equation to estimate the density and hydration of FFM, which
89 could be used when using a 2-component model to assess children and adolescents with
90 different degrees of obesity. Our hypothesis is that calculating the density of the FFM adjusted
91 in this way for BMI as well s age and sex (12), rather than using a constant value by age and sex,,
92 may improve the body composition analyses in subjects with obesity.

93 The aim of this study was to validate the use of FFM density calculations in body composition
94 assessmentsby air-displacement plethysmography (ADP) against the 4-component model to
95 improve the accuracy and precision of the body composition predictions in children and
96 adolescents with obesity.

97 **MATERIALS and METHODS**

98 ***Design***

99 This is a cross-sectional validation study, secondary to a clustered randomized clinical trial on a
100 motivational intervention to treat children with obesity. To perform the present validation
101 study, we used the baseline body composition data of the participants enrolled in the
102 OBEMAT2.0 clinical trial (20).

103

104 ***Participants***

105 Data from 66 children with obesity (35 males; 31 females) aged 8 to 14 years were obtained
106 from the clinical trial OBEMAT2.0 at baseline. Children were recruited from June 2016 to March
107 2018 from primary healthcare centres belonging to the “Camp de Tarragona” healthcare area.
108 Obesity was categorised according to BMI values $\geq 97^{\text{th}}$ percentile of the Hernández references
109 from 1988 (21) defined by the national Guidelines for Clinical Practice on the Prevention and
110 Treatment of Childhood and Adolescent Obesity (22). At recruitment, patients were excluded
111 from the motivational intervention if they had known eating disorders according to the primary
112 care paediatrician (such as bulimia), were participating in another randomized clinical trial, were
113 receiving corticoid or ADHD treatment, or presented with neuropathies and/or
114 endocrinopathies (Cushing Syndrome, Prader Willi Syndrome, hypothyroidism, etc. previously
115 known by the paediatrician or revealed by blood sample analyses at the baseline visit).

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117 **Body composition analyses**

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2 118 Examinations were taken between 8:00 a.m. and 10:00 a.m. after an overnight fast. The physical
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4 119 examination consisted of basic anthropometric measurements: weight (HT), height (HT) and
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6 120 body mass index (BMI); and body composition assessment using: DXA which was performed by
7
8 121 a specialist trained technician using a General Electric (GE) Lunar Prodigy Advance (Madison, Wi,
9
10 122 USA) with GE, Axial Lunar Prodigy Full Advance (encore 2014 version 15.20.002) software to
11
12 123 obtain BMC; ADP with a BOD POD® device (COSMED, Life Measurements, Inc, Concord, CA) to
13
14 124 obtain BV, FM_{BODPOD} and FFM_{BODPOD}; and DD analysis where the participants had an oral dose
15
16 125 equivalent to 1g/kg body weight of deuterium oxide (D₂O, 99.8 %, CK Isotopes Ltd., Ibstock,
17
18 126 Leicestershire, UK). Urine samples collected over the following 5 days were analysed by isotope
19
20 127 ratio mass spectrometry (Sercon ABCA-Hydra 20-22, Sercon Ltd, Crewe, Cheshire, UK) to obtain
21
22 128 TBW with a precision of 0.94 L. Further, FM and FFM were calculated by the 4C model using the
23
24 129 equation of Fuller (1992) (23):

$$130 \quad FM_{4C} = (2.747 \times BV) - (0.710 \times TBW) + (1.460 \times BMC) - (2.050 \times WT)$$

25
26 131 where FM = fat mass in kg; BV= body volume (L) from ADP; TBW= total body water volume (L)
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28 132 from deuterium dilution; BMC = bone mineral content (kg) from DXA and WT = body weight (kg).
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30 133 FFM_{4C} was then calculated as the difference of FM from body weight, in kg.

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34 135 Body composition measures from ADP (FM_{BODPOD} and FFM_{BODPOD}) assuming a constant density of
35
36 136 the FFM (D_{FFM}) was compared to body composition measures based on predicted density of the
37
38 137 FFM (FM_{adjusted} and FFM_{adjusted}) and both compared to the gold standard reference 4C model
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40 138 (FM_{4C} and FFM_{4C}).

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44 140 *Steps for the calculation of adjusted measures of body composition from Air Displacement*
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46 141 *Plethysmography*

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48 142 Derived values for the density of fat-free mass (D_{FFM}) were used with an assumed constant
49
50 143 density of fat mass to generate age specific constants (C1 and C2), which are needed in the
51
52 144 generic Siri equation (24) to calculate the percentage of body fat (%BF) as follows.

53
54 145 *1. Density of the fat-free mass (predicted)*

55
56 146 Density of the FFM (D_{FFM}) was calculated using the following predictive equation (12):

$$57 \quad D_{FFM} = 1.0791 + (0.009 \times \text{age}) + (0.0021 \times \text{gender}) - (0.0014 \times \text{BMISDS})$$

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148 where age is in years; gender 1 = male and 2 = female; BMISDS = body mass index in z-score.

149 2. $C1$ and $C2$ were calculated as (25):

$$150 \quad C1 = \frac{(D_{FFM} \times D_{FM})}{(D_{FFM} - D_{FM})}$$

$$151 \quad C2 = \frac{(D_{FM})}{(D_{FFM} - D_{FM})}$$

152 where $D_{FM} = 0.9007$ kg/L (assumed constant) and D_{FFM} was predicted in the previous step.

153

154 3. *Percentage of body fat (%BF) calculated using the generic Siri equation (24):*

$$155 \quad \%BF = \left(\frac{C1}{BD} - C2 \right) \times 100$$

156 where BD = body density, and was calculated as:

$$157 \quad BD = \frac{WT}{BV}$$

158 where BV = body volume in L, obtained from the BOD POD output.

159

160 4. *Calculation of $FM_{adjusted}$ and $FFM_{adjusted}$:*

161 FM (kg) derived from the density of FFM and ADP body volume measurements ($FM_{adjusted}$) was

162 further calculated as:

$$163 \quad FM_{adjusted} = \frac{\%BF \times WT}{100}$$

164 where WT = body weight in kg.

165 Then, $FFM_{adjusted}$ was calculated as the difference of $FM_{adjusted}$ from body weight, in kg.

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167 **Statistical analysis**

168 All statistical analyses were performed using IBM SPSS Statistics for Windows (version 25.0; IBM

169 Corp., Armonk, NY, USA). Descriptive characteristics for the overall sample (weight, height, BMI,

170 BMI SDS, body volume, total body water, FFM density and FM and FFM from the different

171 methods used) are shown as Mean \pm Standard Deviation (SD). The Kolmogorov-Smirnov test for

172 normality was applied to assess the distribution of the variables. Differences between boys and

173 girls in anthropometric and body composition parameters were explored using Student's T-test

174 or Mann Whitney U-Test, depending on the distribution. The degree of difference between
175 techniques was given as mean percentage with limits of agreement calculated as ± 2 standard
176 deviations of the bias. We assessed the linear association between FFM and FM measurements
177 from BodPod output (FM_{BODPOD} and FFM_{BODPOD}) and calculations derived from the predicted
178 density of FFM ($FM_{adjusted}$ and $FFM_{adjusted}$), with the reference method (4C) by Pearson correlation
179 coefficients. Reliability was obtained from Cronbach's α analysis. Concordance was given as the
180 intraclass correlation coefficient (ICC) with a confidence interval (CI) of 95%. Bland and Altman
181 plots were performed to assess agreement between methods and bias trends, and the limits of
182 agreement for FM and FFM against the reference method (4C) were calculated.

184 **Ethics**

185 The study followed the rules of the Declaration of Helsinki (26). Ethical committees of all
186 involved study centres (CEIC Hospital Universitari de Tarragona Joan XXIII, CEIC Hospital
187 Universitari Sant Joan de Reus (29th January 2016, code 16-01-28/1ass2), CEIC IDIAP Jordi Gol
188 (26th November 2015, code PI14/116)) approved the protocol. All parents or legal guardians
189 signed informed consent prior to study enrolment. Children aged 12 years or above also gave
190 written informed assent.

192 **RESULTS**

193 Physical characteristics of the participants are shown in **Table 1**. Children were of white
194 European ancestry, with an average age of 10.7 ± 1.5 y (8 to 13.3 years) and BMI SDS ranging
195 from 1.86 to 3.08 SDS. We only found statistically significant differences between males and
196 females in BMI SDS ($p = 0.038$) and D_{FFM} ($p < 0.001$).

197 Differences between $FFM_{adjusted}$ and FFM_{BODPOD} were analysed compared to the reference
198 method (FFM_{4C}) (**Figure 1**). $FFM_{adjusted}$ was slightly overestimated by 0.71 kg (limits of agreement
199 -1.08 kg, 2.51 kg) showing a mean difference of $2.8\% \pm 2.1\%$ ($p < 0.001$). FFM_{BODPOD} was
200 overestimated by 1.50 kg (limits of agreement -0.68 kg, 3.63 kg), showing a two-fold percentage
201 of difference when compared to 4C ($4.9\% \pm 2.9\%$; $p < 0.001$) than $FFM_{adjusted}$.

202 Consistently, FM was underestimated by both methods, $FM_{adjusted}$ by -0.71 kg (limits of
203 agreement 1.1 kg, -2.5 kg) and FM_{BODPOD} -1.4 kg (limits of agreement 0.9 kg, -3.6 kg) representing a
204 mean difference of $4.0\% \pm 2.9\%$ and $6.8\% \pm 3.8\%$, respectively, when compared to the reference
205 method (FM_{4C}) (**Figure 1**) (**Table 2**). This meant that the degree of bias from the method

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206 following adjustment for density of FFM ($FM_{adjusted}$) was two-fold lower than the FM_{BODPOD}
207 (consistently with FFM measures).

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209 **Table 3** displays the correlations and reliability coefficients of both techniques, showing that
210 when body composition measurements were adjusted for FFM density, the concordance and
211 reliability of the assessment were improved as compared to the gold standard method.

212

213 **DISCUSSION**

214 The aim of the present study was to assess the accuracy of body composition assessment in
215 patients with obesity using ADP, following a correction for FFM density compared to not
216 following the correction. To assess the accuracy of both methods, we used the gold standard
217 method to assess body composition *in vivo*, the 4-component model. To our knowledge, this is
218 the first study to include individual calculations for the density of fat-free mass when assessing
219 body composition using ADP, and to then compare the results with the 4-component model in
220 children with obesity.

221 Children with obesity may have a significantly lower density of FFM than normal weight children
222 (12), however, the BOD POD internal algorithms assume constant values of FFM density by age
223 and sex. Our study has demonstrated that this assumption may increase the degree of bias when
224 assessing body composition in children with obesity.

225 Previously published data has suggested that assumptions of the properties of FFM could be the
226 cause of bias in the evaluation of FM by ADP (15,17). However, there are few studies which have
227 used a multi-compartment model to compare ADP measurements, and furthermore, most of
228 the previous studies were conducted in adults.

229 In a study of 42 healthy British females, Fields *et al.* (15) reported that, compared to the 4C
230 model, body fat percentage measured using BOD POD calculations was underestimated,
231 although both techniques were well correlated. They also investigated the relative hydration of
232 FFM as a possible explanation for such differences and found that indeed the hydration of FFM
233 was associated with the magnitude of the difference between the techniques.

234 In agreement with Fields *et al.*, Millard-Stafford *et al.* (16) conducted a similar study in 50 young,
235 healthy adults of varying ethnicity (males n = 40; Caucasians n = 35; African-Americans n = 15)
236 and found that %FM obtained from BOD POD was underestimated when compared to the 4C
237 model and other methods such as under water weighing (UW) or DXA. They concluded that FFM

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238 density and its fractional components (i.e. minerals, water and protein), were important
239 considerations when determining FM and FFM. These findings are consistent with the findings
240 of our present study in children.

241 To our knowledge, the only existing study comparing BodPod ADP and other techniques with
242 the 4C model was conducted by Fields and Goran (27) with 25 healthy British children. They did
243 not find significant bias between ADP and 4C, but no BMI data was included in their analysis.
244 Furthermore, the sample was homogenous in age ($11.4 \pm 1.4y$) and anthropometric
245 characteristics.

246 Wells *et al.* (17) evaluated 28 British healthy children aged 5 to 7 years using ADP and compared
247 it to the 3C model. They found high accuracy of ADP when compared to the 3C model for body
248 composition measurements in groups but highlighted the need to improve bias in individuals.
249 They concluded that the bias between methods could be due to methodological precision or
250 biological variability in hydration. In addition, according to an earlier finding, Wells *et al.* (8)
251 reported that the calculated density of FFM was slightly increased for both sexes, but
252 significantly so only for girls, when compared to Lohman's 1989 reference data. Thus, they
253 showed significant bias in %FM compared to the 3C model when using predicted values for the
254 density of FFM. This implies that BOD POD calculations should be adjusted by specific FFM
255 properties to increase its accuracy. A recent publication from a large dataset compiled from
256 several UK studies confirmed that FFM hydration and density varied according to age and BMI
257 (12). In the present study we have shown how to apply this recent knowledge to increase the
258 accuracy of more simple techniques to assess body composition in children with obesity.

259 The results of our work are consistent with those previously presented by Wells *et al* (8). FM and
260 FFM assessment had a narrower agreement with 4C model measurements when calculations
261 were adjusted by specific FFM density than BOD POD outputs, which did not consider the
262 nutritional status of the subjects and assumed a constant density of FFM. Thus, this study
263 demonstrates and validates the use of corrections of FFM for density when using BOD POD. As
264 the 4-component model is not usually feasible in clinical settings nor in big epidemiology studies,
265 ADP correcting for FFM properties might be one of the best 2-component models in children,
266 especially in children with obesity. The translation of the present results to a clinical setting could
267 be easily done by simple calculations as shown in steps 1 to 4 in the methods section. First,
268 calculating the predicted density of D_{FFM} according to BMI z scores; second, using the predicted
269 D_{FFM} values in the C1 and C2 equations (rather than using constant values) and third, using those
270 C1 and C2 values in the customized version of Siri's equation, together with body density
271 (derived from body volume provided by the ADP device), to estimate body fat percentage. To

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272 facilitate calculations, we provide an excel file with all the steps as supplementary online
273 material.

274 In addition, using these adjustments would also improve the longitudinal assessment of
275 patients with obesity; if the degree of error for the Bod Pod outputs ranged 4.1 to 7.5% for FM
276 and FFM, these biases could be greater than real changes between visits in a follow up. If this
277 bias could be minimized to 2.3 to 4.7% by applying the proposed corrections, this would improve
278 the sensibility of the method to changes in energy balance and improve clinical assessment.

279 The small sample size is a possible limitation of the present study; however, it remains one of
280 the biggest sample sizes published for this type of analysis in children.

281 The main strength of the present study is the high quality of the methodology used: we used a
282 highly precise technique to assess body composition, and compared it to the gold standard 4-
283 component model in order to reduce the bias of ADP in children with obesity. In addition, the
284 conditions of the measurements were highly controlled as all the measurements being
285 performed at the same time between 8 and 10:00 a.m. after an overnight fast.

286 In conclusion, the use of constant values for fat-free mass properties may increase bias when
287 assessing body composition in children with obesity using two-component based techniques like
288 air-displacement plethysmography. Using corrections for the density of fat-free mass (as
289 proposed with the 4 steps in the methodology section) reduces the bias in fat mass and fat-free
290 mass measurements derived from ADP in children with obesity. This approach should be
291 considered not only in children with obesity. Further studies should demonstrate whether this
292 approach would improve assessments in the general population and in longitudinal studies
293 where small changes between repeated measures should be quantified with reduced bias.

294

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328 supervised the overall project; MV and PS performed isotopic analyses; DG, JM, MZ, MG, CR,
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414 **TABLES**

415 **Table 1.** Descriptive characteristics of the participants.

	Male (n = 35)		Female (n = 31)	
	Mean ±SD	Range	Mean ±SD	Range
Age (y)	10.6 ± 1.6	8.0 - 13.3	10.7 ± 1.4	8.3 - 13.3
Weight (kg)	55.1 ± 11.2	37.8 - 83.1	57.5 ± 11.4	37 - 81.8
Height (cm)	146.4 ± 10.0	131.6 - 167.0	147.2 ± 10.7	125.5 - 170.3
BMI (kg/m ²)	25.6 ± 2.60	21.7 - 32.5	26.2 ± 2.4	21.9 - 30.6
BMI SDS (WHO 2007) [†]	2.72 ± 0.45	1.86 - 4.20	2.52 ± 0.31	1.89 - 3.08
Body Volume (BodPod - L)	54.8 ± 11.3	37.5 - 83.7	57.4 ± 11.2	36.1 - 81.1
Density FFM predicted (kg/L) [‡]	1.087 ± 0.002	1.083 - 1.091	1.089 ± 0.001	1.087 - 1.092
FM _{D&BV} (kg)	21.6 ± 5.9	13.0 - 38.2	23.8 ± 5.2	11.- -32.1
FFM _{D&BV} (kg)	33.5 ± 6.1	23.7 - 46.2	33.7 ± 7.8	20.2 - 53.7
FM _{BODPOD} (kg)	21.3 ± 6.0	12.7 - 38.4	22.7 ± 5.0	11.4 - 30.4
FFM _{BODPOD} (kg)	34.0 ± 5.9	24.1 - 46.2	34.8 ± 7.5	21.2 - 51.9
FM _{4C} (kg)	22.1 ± 6.0	12.5 - 38.7	24.7 ± 5.3	11.6 - 33.8
FFM _{4C} (kg)	33.0 ± 6.1	23.5 - 45.6	32.8 ± 7.4	20.3 - 50.6
Total Body Water (DD-kg)	25.0 ± 4.6	17.9 - 34.9	24.3 ± 5.3	15.7 - 36.5

416 Significance: [†]p = 0.038; [‡]p < 0.001.

417 Abbreviations: BMI = body mass index; SDS = standard deviation score; FM = fat mass; FFM = fat
 418 free mass; D&BV = measurements derived from the density of FFM calculated with the new
 419 equation and body volume; 4C = four-component model; DD = deuterium dilution.

420

421 **Table 2.** Analyses of differences (%) between body composition outcomes extracted from the
 422 BodPod (adjusted using calculated values for the density of fat free mass) versus the reference
 423 4-component model (n=66).

	MEAN DIFFERENCE (max, min 95% CI; p-value)	SD
FFM _{adjusted}	2.80% (2.29-3.30; p<0.001)	±2.06%
FM _{adjusted}	3.97% (3.25-4.69; p<0.001)	±2.92%
FFM _{BODPOD}	4.87% (4.15-5.59; p<0.001)	±2.92%
FM _{BODPOD}	6.77% (5.60-7.45; p<0.001)	±3.77%

424

425 **Table 3.** Correlations and reliability of fat-free mass measurements against the 4-component
 426 model (n=66).

	Correlation coefficient (p- value)	Cronbach's alfa	ICC (CI 95%; p-value)
FFM _{adjusted}	0.992 (p<0.001)	0.996	0.993 (IC 95% 0.967-0.997; p<0.001)
FFM _{BODPOD}	0.987 (p<0.001)	0.993	0.981 (IC 95% 0.640-0.995; p<0.001)

427

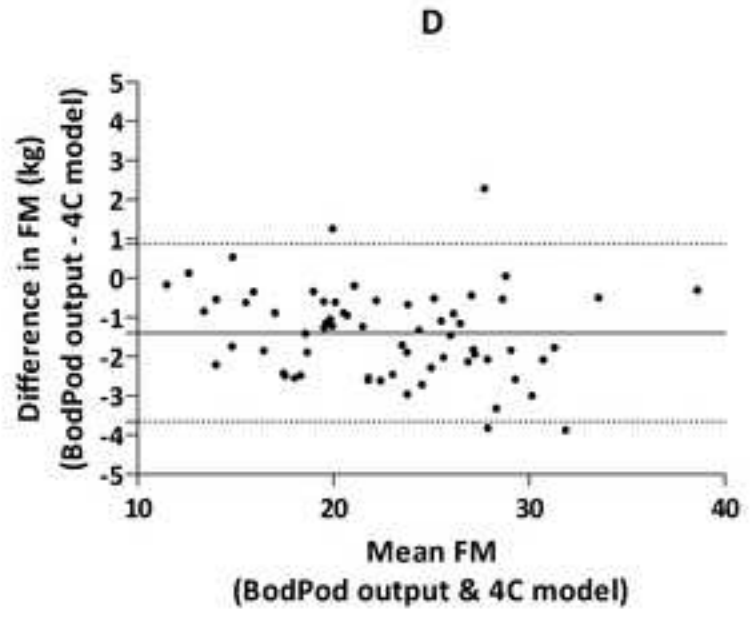
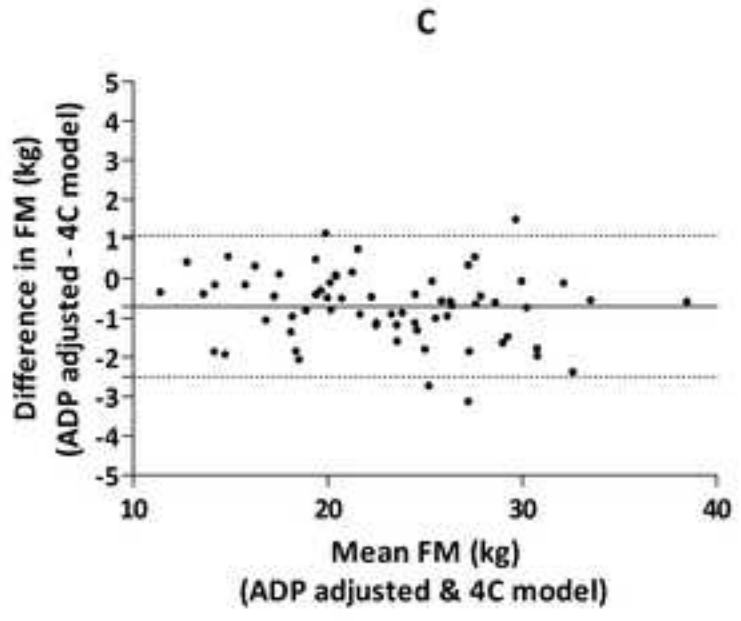
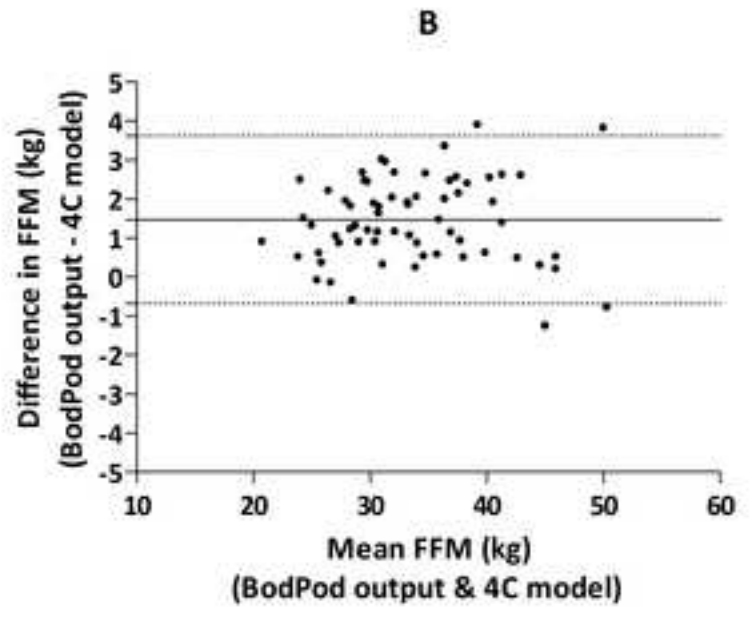
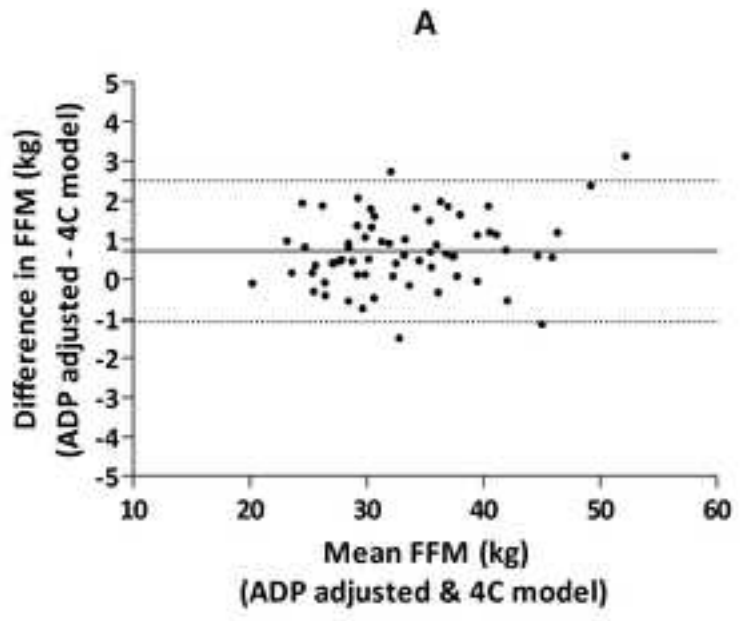
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428 **Figures**

429 **Figure 1. Agreement between methods.** Bland and Altman plots of the difference between fat-free
430 mass (FFM) and fat mass (FM) (kg) as obtained from the BodPod assuming constant values of the FFM
431 (FFM_{BODPOD} and FM_{BODPOD}) (B and D) or obtained from body volume from air displacement
432 plethysmography and further corrections using adjusted density of the fat free mass) (A and C), all
433 compared to the reference method, 4C model (four component model).

434 Bland and Altman plots show that body composition measures derived from air displacement
435 plethysmography adjusted using the calculated density of the fat free mass have narrower limits of
436 agreement than when assuming a constant density of the fat free mass.

437



Body Composition Analysis
Adjusted fat free mass properties
in Air Displacement Plethysmography

Step 0 Complete the subjects characteristics

Age (years), decimal points can be used			years
Body weight			kg
Height			m
Add the BMI z score of the subject			sds
Gender (1 if a boy, 2 if a girl)			
Body volume (L) from BodPod			L

Automatic calculations from that point onwards, please do not modify cells.

Step 1 Density of the fat-free mass (predicted) 1.0791 kg/L

Step 2 C1 and C2 calculation

C1	5.4481
C2	5.0488

Step 3 Body Fat (%)

Body density	#DIV/0!
Body Fat (%)	#DIV/0!

Step 4 Adjusted Fat Mass and Fat Free Mass (kg)

Fat Mass (kg)	#DIV/0!	kg
Fat Free Mass (kg)	#DIV/0!	kg
Fat Mass Index (kg/m ²)	#DIV/0!	kg/m ²
Fat Free Mass Index (kg/m ²)	#DIV/0!	kg/m ²

Reference: Gutiérrez-Marín D, Escribano J, Closa-Monasterolo R, Ferré N, Venables M, Singh P, Well JCK, Muñoz-Hernando J, Zaragoza-Jordana M, Gispert-Llauradó M, Rubio-Torrents C, Alcázar M, Núñez-Roig M, Monné-Gelonch R, Feliu A, Basora JM, Alejos AM, Luque V. A novel approach to assess body composition in children with obesity from density of the fat-free mass. Clin Nutr 2020.