DOCTORAL DISSERTATION

LEAN BODY MASS AND ANAEROBIC PERFORMANCE IN CHILDREN AND ADOLESCENTS

Authored by

Lorena Correas Gómez

DOCTORAL STUDIES:
RESEARCH IN PHYSICAL ACTIVITY AND SPORT

Mentors:

Prof. Dr. José Ramón Alvero Cruz

Sports Medicine School
Faculty of Medicine
University of Málaga

Prof. Dr. Elvis Álvarez Carnero

Faculty of Educational Sciences
University of Málaga
Translational Research Institute for Metabolism and Diabetes, Florida Hospital and Sanford, Burnham, Prebys Medical Discovery Institute, Orlando, FL, USA

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AUTOR: Lorena Correas Gómez

http://orcid.org/0000-0002-1838-8993

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TESIS DOCTORAL

INDICADORES DE MASA MUSCULAR ESQUELÉTICA Y RENDIMIENTO ANAERÓBICO EN NIÑOS Y ADOLESCENTES

Lorena Correas Gómez

PROGRAMA DE DOCTORADO: INVESTIGACIÓN EN ACTIVIDAD FÍSICA Y DEPORTE

Directores:

Prof. Dr. José Ramón Alvero Cruz

Escuela de Medicina de la Educación Física y el Deporte
Facultad de Medicina
Universidad de Málaga

Prof. Dr. Elvis Álvarez Carnero

Facultad de Ciencias de la Educación
Universidad de Málaga
Translational Research Institute for Metabolism and Diabetes, Florida Hospital and Sanford, Burnham, Prebys Medical Discovery Institute, Orlando, FL, USA

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25 de junio de 2017

El Dr. José Ramón Alvero Cruz, Profesor Titular del Área de Educación Física y Deportiva de la Facultad de Medicina de la Universidad de Málaga

Certifica que
La Memoria de Tesis Doctoral, titulada: "LEAN BODY MASS AND ANAEROBIC PERFORMANCE IN CHILDREN AND ADOLESCENTS", de Lorena Correas Gómez, ha sido planificada, realizada y supervisada, por lo cual se considera apta para su lectura y defensa

José Ramón Alvero Cruz
Profesor Titular del Área de Educación Física y Deportiva
Universidad de Málaga

UNIVERSIDAD
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Elvis Álvarez Carnero
Profesor Contratado Doctor
Universidad de Málaga

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I would like to dedicate this thesis to all sports professionals who are passionate about working with children and adolescents and committed becoming better professionals every day. Those who coach and help athletes achieving their goals and overcoming obstacles, always believing that dreams can come true.

DEDICATORIA

A todos los profesionales del deporte que trabajan con niños y adolescentes, comprometidos con un trabajo dedicado y riguroso para mejorar la vida de los más jóvenes. Aquéllos que con pasión e ilusión enseñan a través de la práctica deportiva a superarse y conseguir objetivos, creyendo siempre que los sueños se pueden hacer realidad.

ABSTRACT

The present manuscript thesis has been structured in three studies carried out in a sample of children, adolescents, and young adults. The first study aimed to extend previous published data focused on elucidating population differences in skeletal muscle mass (SMM) during growth period using the simple technique of anthropometry, with a focus on the differences among age, sex and sport practice groups after controlling for maturation and body size. The novel contribution was to report normal range values of regional muscularity (arm, thigh, and calf) across age and sexes, and to analyze the relevance of sport participation and maturation on muscle-to-height ratios in a large dataset of Caucasian youth of a wide-range of age.

In the second study, concurrent and construct validity between laboratory and field methods to estimate fat free mass (FFM) and SMM were explored simultaneously in a considerable sample of Spanish young subjects, including analysis among sex and athletic/non-athletic groups. The methods used were whole-body dual-energy X-ray absorptiometry scans (DXA), anthropometry, bioelectrical impedance analysis (BIA), and isotope dilution for total body water (deuterium oxide) converted into FFM by using sex- and age-specific hydration constants. Furthermore, construct validity was explored by the individual associations of SMM and FFM with a variety of strength tests.

Finally, the last study aimed to explore the associations and predictive capacity of regional SMM in isometric strength and water distribution in dynamic strength. Body composition predictive models were analyzed to explain anaerobic performance assessed as lower limbs strength and sprint test accounting for sport participation and maturity status.

STUDY 1. The Relevance of Sport Participation, Body Size and Maturation on Total and Regional Skeletal Muscle Mass Along Childhood and Adolescence.

The acquisition of skeletal muscle mass (SMM) during childhood and adolescence must be a main concern to ensure healthy growth and improved motor development. Over the last few decades, a growing interest in SMM assessment in children has been renewed since independent associations between muscle fitness with metabolic and cardiovascular risk have been described in children and youth. Participation in regular physical activity (PA) programs during the growth period plays a positive role in bone and lean mass accrual in addition to diverse health-derived benefits (Ara et al., 2006; Baxter-Jones, Eisenmann, Mirwald, Faulkner, & Bailey, 2008; Forwood et al., 2006; Fraser et al., 2016; Jackowski et al., 2014; Vicente-Rodriguez et al., 2008). But accounting for biological maturity and body size need to be considered when examining independent effect of PA and explaining differences among adolescents. To our best knowledge, there is a lack in the availability of combined reference data for regional and total SMM derived from field techniques along with the analysis of sport participation and maturation.

Objective. To provide reference data of regional and total skeletal muscle mass (SMM) and fat-free mass (FFM) derived from anthropometry among age, sex and sport participation groups in healthy Caucasian children and adolescents, accounting for body size and maturation.

Study design. Cross-sectional data of 1438 participants aged 8-18y were analyzed. FFM and SMM were estimated from anthropometric variables (skinfolds and circumferences). Regional muscle variables were calculated as corrected limb circumferences at three sites. Sport participation (SP) was obtained by *ad hoc* questionnaires and maturity offset was calculated as predicted years from peak height velocity (PHV). General linear model of mixed factors was used to analyze variance of

FFM or SMM across groups and confounders. Natural logarithmic (ln-ln) regression analysis was applied to describe SMM scaling associations with height.

Results. Positive interactions were found between sex, SP and age for regional muscle variables, which also differed between pre- and post-PHV groups. Only active boys increased significantly their regional muscularity from primary to secondary school (arm, Δ0.78cm, $P<0.01$; thigh, Δ1.44cm; calf, Δ1.16cm) and from secondary to high school (arm, Δ2.81cm; thigh, Δ2.51cm; calf, Δ1.88cm; all $P<0.0001$).

Conclusions. Our findings showed sex differences in the relationship between total SMM or FFM and age, even after adjusting for height. Similar results were observed for upper and lower limbs muscle growth. A novel contribution of this study was that sport practice may affect SMM accrual after puberty, and growth of regional muscle-to-height ratio before PHV. These data must be valuable to use as a reference for similar populations, nevertheless, longitudinal studies are required to confirm these results.

STUDY 2. Concurrent Validity of Methods for Estimating Fat Free Mass and Skeletal Muscle Mass in Children, Adolescents and Young Adults: Anaerobic Performance and Sports Participation Constructs.

Direct SMM measurement is only available from imaging methods which are high cost and time consuming. Alternatively, indirect methodologies have been created to predict SMM and FFM with mathematical models (equations) in field settings at low cost. However, the accuracy and validity of the derived equations depends on which method were validated against and if the methods selected are specific of assessed population to guarantee unbiased conclusions. As there is no single method that is error-free, the relationship between health outcomes or performance and SMM or FFM may be affected.

So, finding the best correlated methodology for SMM or FFM assessment with biological constructs represent an important research area.

Body composition constructs (components) are especially important in weight-sensitive sports but also to monitor athletic performance and training programs. Yet, construct validity has been barely covered in the literature apart from exceptions with health variables. On this sense, anaerobic performance of simple exercise must be the most important FFM and SMM-related construct since this type of exercises are highly dependent of muscle contraction. So, it should be pertinent to analyze if known models to estimate FFM and SMM in children and adolescents have a good association with anaerobic performance (strength) to evaluate construct validity.

Objectives: Firstly, to analyze the validity and agreement between laboratory and field methods to estimate FFM and SMM in a large sample of children, adolescents, and young adults. Secondly, to explore construct validity by the individual associations SMM or FFM and strength tests.

Study design. A series of validation studies were designed using large dataset. Studies were carried in children and adolescents at schools or sport clubs from whom written consent was required. Inclusion criteria were: range of age between 6 to 21y-old, be free of acute or chronic diseases, and not having any total or partial amputated limb. Body composition was assessed by isotope dilution method, dual-energy X-ray absorptiometry (DXA), bioelectrical impedance analysis (BIA) and anthropometry (ANT). Static strength was tested with hand and lower limb portable dynamometers and dynamic strength with sprinting and jumping. Information about sport participation was obtained by *ad hoc* questionnaires and maturation status from predicted age at peak height velocity. Concurrent validity was analyzed by differences between methods, and the effect size. Simple regression analysis between reference and alternative techniques and

the two components of the concordance coefficient correlation (ρ_c) were calculated. Agreement analysis was conducted by Bland and Altman analysis. Simple and partial correlations were carried out between body composition variables and strength values for construct validity.

Results. A total of 531 measures of TBW were analyzed (287 boys; 244 girls). Overall, all FFM measures from alternative techniques underestimated FFM measures from deuterium dilution. FFM from ANT showed the lowest bias (-2.29; 95% CIs, -2.77 to -1.80), and similar values were found for FFM-BIA (bias = -2.42; 95% CIs, -2.82 to -2.02). Concordance between methods was better in the non-athletic group than in the active group. Mean differences between SMM from ANT and DXA were lower in girls than in boys (bias = 0.46, SD ±1.39 vs. 1.98, SD ±1.54, $P<0.05$ and $P<0.0001$, girls and boys respectively). Positive associations were found for all FFM and SMM variables with anaerobic performance tests ($P>0.05$) except FFM-DXA and SJ (r = 0.228, $P=0.051$). The lowest correlation coefficient was found between FFM-REF_W and SJ ($\tau = 0.207$, n = 263) and the highest between SMM-DXA and left handgrip ($\tau = 0.764$, n = 186). In general, strength assessed by dynamometry showed higher correlation coefficients with lean and muscle mass variables than explosive dynamic tests.

Conclusions. The results did not confirm agreement between methods, and associations between FFM and SMM with strength tests showed different magnitudes. Concurrent and construct validity of available methods to estimate FFM and SMM in children and adolescents vary among sex and sport participation groups. There was not a perfect method for all situations. However, we found that BIA was adequate for FFM estimation under controlled circumstances and applying age- and sex-specific equations. Similarly, SMM from anthropometry was found to be an alternative to DXA although with some limitations related to age, sex and sport practice. Regarding construct validity,

all methods correlated better with dynamometry than with explosive dynamic tests possibly due to other factors influencing movement performance. Our results evidence the need for developing specific models to estimate FFM and SMM in physically active prepubertal and pubertal youth and to be validated against reference methods.

STUDY 3. Fluid Distribution and Regional Skeletal Muscle Mass Predict Anaerobic Performance in Adolescents: Results of Sprint and Isometric Strength.

Body composition, including hydration, has been classically associated with aerobic performance. However, the relationships between anaerobic performance assessed by sprints and jumps with hydration have not been extensively analyzed in the literature, particularly in children and adolescents. This is a paradoxical situation because the child is mainly involved in high intensity and short duration exercises in most daily tasks, games or sports events. The analysis of these associations should be useful to describe the importance of hydration and FFM in the performance of healthy adolescents.

On the other hand, the relationship between muscle size and strength has been extensively explored in adults, however, less is known about their actual association in adolescents. The identification of the anthropometric indicators of SMM that best predict strength with age, taking into account the processes of growth and maturation, should be useful in field settings for coaches working with young athletes and talent identification.

Objectives. To explore two paradigms related to anaerobic performance and subcomponents of body composition in adolescents: 1) to analyze the association between 30 meters (m) sprint test and molecular body composition variables in young athletes and non-athletes; 2) to examine the association between lower-limb strength (LLS) and regional SMM variables in young athletes and non-athletes; 3) to identify molecular and cellular body composition indicators successfully predict sprint performance and LLS.

Study design. Two analyses were conducted in subsamples of the larger cross-sectional studies described previously. In the first analysis, called *Intracellular water and Sprint Performance,* a sample of 158 adolescents between 12 and 18y participants (15.0 ± 1.5y) were recruited from local high-schools and local sport clubs. An *ad hoc* questionnaire was used to identify those participants involved in organized and competitive sports (≥3times/week of training plus competition, duration of ≥1hour/session), classified as athletes (AT), and those physically inactive (<3hours/week), classified as non-athletes (NAT) (AT = 58, 27 boys, 31 girls, and NAT = 100, 41 boys, 59 girls).

The second analysis was conducted in 166 healthy adolescents aged 12-18y with at least a valid test of LLS by isometric dynamometry and anthropometric assessment of SMM both total and regional (arm, thigh and calf), and was called *Regional SMM and Lower Limb Strength.* Acute or chronic diseases were the only exclusion criteria. Maturity offset was predicted from the equation of Mirwald et al. (2002). Best performance value out of two measures of 30m-sprint and LLS was selected for each analysis. Sport participants (AT, athletes; NAT, non-athletes) were identified with an *ad hoc* questionnaire. Partial correlations were performed between body composition variables and performance tests. And the best-correlated variables with sprint or LLS were included in a stepwise regression analysis to determine body composition predictors of sprint performance after adjusting for age, sex and sport participation.

Results. *Intracellular water and Sprint Performance.* As expected AT were faster than NAT in 30m (mean difference = 0.43 ± 0.09s; P<0.001), but there were not significant differences in ICW (mean difference = 1.18 ± 0.94%; P>0.05). A significant correlation was found between ICW and 30m-sprint test after controlled by age and kg of BF (P<0.001). SMM (kg), the athlete condition and %ICW were identified as predictors of speed in 30m (P<0.001, R^2 = 0.620, adjusted R^2 = 0.612, SEE = 0.37 m/s). The final model was: Speed 30-m (m/s) = 3.926 + (0.051*SMM) + (0.495*AT) + (0.033*ICW).

Regional SMM and Lower Limb Strength. Only 158 participants met the criteria for variables included in the models (age = 15.03 ± 1.63y; NAT = 92, 38 boys, 51 girls, and AT = 66, 42 boys, 24 girls). AT were stronger than NAT (+23.83kg, 4.81 SE, P<0.001) and they also had greater values of CCG (+1.31cm, 0.49 SE, P<0.01) and SMM (-2.16%, 0.84 SE; both P<0.01). The best correlation between SMM variables and LLS test was for total SMM in NAT after controlling by height (r = 0.631, P<0.000) while non-significant correlations were found in AT. Stepwise regression analysis for the sample pooled together resulted showed similar results for a model including SMM (kg), athlete condition, and height (R^2 = 0.488, P<0.001) and for an alternative model including CCG, athlete condition, sex and height (R^2 = 0.484, P<0.001). Maturity was not included in any model.

Conclusions. One of the main findings of these two analyses was that intracellular water assessed by bioimpedance analysis was a significant predictor of sprint performance in adolescents. On the other hand, a simple measure like corrected calf girth was found to contribute significantly for estimating lower limb strength almost as much as whole lean body mass derived from anthropometry, which may have important implications for assessing in field settings. These results add new information to the body of knowledge in youth sport performance.

RESUMEN

El estudio de la composición corporal tiene por objetivo la cuantificación de los componentes del cuerpo humano, principalmente para el cálculo de dosis en tratamientos médicos, su relación con problemas de salud y, de manera más reciente, con el rendimiento deportivo. Factores influyentes como la nutrición, el crecimiento, la maduración, el envejecimiento y su relación con la salud o enfermedades, ha impulsado el desarrollo de esta área de investigación, la cual debería ser de interés primordial para todos los profesionales que trabajan con poblaciones relacionadas con la salud, como los profesores de educación física, preparadores físicos o entrenadores deportivos.

Actualmente, la composición corporal se organiza en un sistema de cinco niveles, desde el nivel atómico hasta el nivel de cuerpo entero, pasando por el nivel molecular, el celular y el nivel de órganos y tejidos. Cada nivel está compuesto por un número determinado de componentes cuya suma equivale al peso corporal, y para su cuantificación se pueden emplear varios modelos. La utilización de uno u otro modelo, los cuales van desde los dos componentes hasta la cuantificación de 11 elementos en el cuerpo, depende de las técnicas disponibles para medirlos. Así pues, a nivel molecular, el modelo multicomponente (MC) es considerado como el modelo referente para la evaluación de la composición corporal hasta la fecha, ya que la combinación de varios métodos o técnicas de medición de referencia va a proporcionar una medida más precisa de cada componente.

La investigación de la composición corporal durante las etapas de niñez y adolescencia ha estado enfocada principalmente en elaborar valores de corte de referencia para la grasa corporal, especialmente debido al aumento de la prevalencia mundial de obesidad infantil en los últimos 20 años. Sin embargo, la investigación sobre la masa

muscular ha generado menos interés aparte de los estudios tradicionales sobre el estado nutricional de niños y adolescentes, en los que la medida del tamaño del músculo se ha utilizado como índice de reserva proteica. Fue a finales de los años sesenta cuando se realizaron los primeros estudios de referencia en grandes cohortes sobre la evolución de la masa muscular en estos períodos de niñez y adolescencia.

Actualmente, el interés sobre el estudio del músculo esquelético está aumentando debido a su relación con distintos factores de salud; se han encontrado asociaciones positivas entre la fuerza muscular y un menor riesgo de sufrir enfermedades cardiovasculares y posterior desarrollo del síndrome metabólico en la edad adulta. Además, la masa muscular desempeña un importante rol en el desarrollo óseo. Sin embargo, la evaluación del músculo en sujetos jóvenes es bastante limitada en lo que a estudios de campo se refiere, lo que no ha permitido desarrollar gran número de investigaciones que arrojen valores de corte y posteriormente ser relacionados con la salud o el rendimiento. Recientemente, varios estudios han publicado valores de referencia de masa muscular esquelética (MM) y masa libre de grasa (MLG) obtenidos a partir de diferentes métodos de evaluación de laboratorio como el modelo de referencia de 4C, y energía dual de rayos X (DXA), y de campo como el análisis de impedancia bioeléctrica (BIA) en diferentes poblaciones infantiles y adolescentes.

A pesar de la importancia de la MM en el ámbito de la fisiología, la nutrición y el rendimiento, actualmente sólo hay dos técnicas no invasivas para mediciones directas del músculo esquelético, como son la resonancia magnética (RM) y la tomografía axial computarizada (TAC), las cuales son de difícil acceso, alto coste económico y consumo de tiempo en el análisis de las imágenes. Además, el TAC es poco práctico para usar en niños debido a la exposición de éstos a la radiación.

Como alternativa a la MM se utiliza la MLG, la cual incluye todos los componentes del cuerpo excepto la MG, incluyendo músculo, agua, masa mineral, tejido conectivo y órganos internos. En población pediátrica y juvenil, el modelo de cuatro componentes (4C) es considerado como el modelo de referencia a nivel molecular para evaluar la MLG, al ser independiente de la edad y el nivel de desarrollo humano. Los métodos de referencia para evaluar con precisión cada uno de esos componentes son la pesada hidrostática o pletismografía por desplazamiento de aire (volumen corporal) para estimar la densidad corporal (Dc), la hidrometría por dilución de isótopos para el agua corporal total (ACT) y DXA para el contenido mineral óseo. Si la medida de este último no es posible, se usa el modelo de tres componentes (3C).

El uso de varios métodos de referencia para desarrollar un modelo de 3C o 4C no es accesible para todos los profesionales, sobre todo para los que trabajan fuera del ámbito médico o de la investigación, siendo el modelo tradicional de 2C el más usado, en el cual el peso corporal se divide en MG y MLG. Aunque muy extendido, su uso en la evaluación de la MLG es limitado debido a que la densidad e hidratación de sus componentes son asumidas como estables, y esto ocurre en el adulto sano, pero este estado se ve alterado durante el período de crecimiento. Los componentes de la MLG (agua, proteínas, minerales y glucógeno) aumentan en diferentes proporciones durante la niñez y la adolescencia. Por lo tanto, cuando se evalúa la MLG en población pediátrica y juvenil, se deben tener en cuenta estas variaciones, y de este modo, cuantos más componentes se puedan evaluar, mayor validez y precisión tendrá la medida.

En la actualidad, el principal paradigma para la estimación de MLG se basa en la medición o estimación de ACT. El agua es el mayor constituyente del cuerpo y de la MLG, y por ello, la estimación de MLG a partir de agua corporal usando las constantes de hidratación de la MLG por edad y sexo, es considerado un método de referencia tras una medición exacta de agua. El método de dilución de isótopos, basado en el principio de dilución, se utiliza comúnmente como el método de referencia para la evaluación de agua corporal. La medida de agua se obtiene a partir de las diferencias de concentración entre las muestras de fluido fisiológico de orina o saliva (antes y después de administrar un isótopo, generalmente óxido de deuterio (D_2O), utilizando la espectrometría de masas. De esta manera, se puede estimar la MLG dividiendo la cantidad de ACT por las constantes de hidratación de la MLG, y así mejorar la precisión del modelo 2C. Hoy en día, el D_2O es considerado uno de los métodos de referencia para medir ACT, aunque es costoso y requiere mucho tiempo para ser utilizado en la mayoría de contextos.

Debido a estas limitaciones, se han creado metodologías indirectas para predecir MM y MLG con modelos matemáticos (ecuaciones) a partir de técnicas de campo como la antropometría y la BIA. Sin embargo, la fiabilidad y validez de las ecuaciones derivadas depende del método con el que fueron validados y si los métodos seleccionados son específicos de la población evaluada para así garantizar conclusiones imparciales. No hay un método único y libre de errores, lo que puede llegar a afectar la relación entre los resultados obtenidos y las variables de salud o rendimiento.

La intervención en la población de estudio requiere instrumentación de alta resolución (precisión) y reproducibilidad (fiabilidad) para poder evaluar los cambios funcionales. Por lo tanto, la validez y concordancia de las evaluaciones de MLG y MM deben ser preocupaciones principales con el fin de elucidar nuevas asociaciones con el rendimiento deportivo y la salud. Debe ser pertinente encontrar la mejor metodología para la evaluación de la MLG y MM en la población joven. Y, además, analizar si esos modelos tienen una buena asociación con el rendimiento anaeróbico (fuerza) para evaluar la validez de constructo, lo que representa una importante área de investigación.

El desarrollo de la fuerza durante la etapa de crecimiento está relacionado con el tamaño corporal (peso y talla) y, por lo tanto, afectado por muchos factores que interactúan al mismo tiempo, como la edad y la maduración, entre otros. Los jóvenes con piernas más largas pueden tener mayores cantidades de masa muscular para una determina estatura puesto que una gran proporción de la masa muscular de todo el cuerpo se encuentra en las extremidades. La relación entre el tamaño del músculo, entendido como área de sección transversal, y la fuerza ha sido revisada en la literatura principalmente en adultos, sin embargo, la asociación real en niños y adolescentes ha sido menos estudiada.

Durante el periodo de pico máximo de crecimiento en la adolescencia, la madurez puede explicar algunas de las diferencias de fuerza entre jóvenes de la misma edad cronológica, por lo que controlar el tamaño del cuerpo cuando se llevan a cabo comparaciones entre medidas y sujetos es fundamental en población pediátrica, puesto que algunas de esas diferencias se verían reducidas. Existe una amplia gama de métodos disponibles para la evaluación de la maduración en jóvenes, la cual puede ser evaluada como estado o proceso, dos indicadores diferentes que no son intercambiables. Algunos de esos métodos para evaluar el proceso madurativo son caracterizados como no invasivos, es el caso de la edad a la que se alcanza la máxima velocidad de pico de crecimiento. Esta medida requiere de varios registros de estatura durante el período de crecimiento para obtener una medida longitudinal, pero como alternativa para los estudios con diseño transversal se pueden usar ecuaciones simplificadas que sólo requieren una medida para predecir la edad a la que el niño o la niña alcanzará el pico máximo de crecimiento.

El análisis de las asociaciones entre MLG y MM de todo el cuerpo y a nivel regional con la fuerza sería útil para describir la importancia de la masa muscular

esquelética, estudiar y controlar el desarrollo saludable, además del rendimiento deportivo de niños y adolescentes.

La relación entre la composición corporal y el rendimiento se ha asociado clásicamente a masa grasa (MG) y capacidad aeróbica, MM y fuerza, o hidratación y capacidad de resistencia. Otros componentes o indicadores regionales de composición corporal han sido menos estudiados en la literatura científica, particularmente en adolescentes, y su importancia es comúnmente ignorada por profesionales de la salud y el ejercicio físico y deportivo.

Aunque el contenido total de agua corporal (hidratación) se ha asociado tradicionalmente con el rendimiento aeróbico, la importancia de la hidratación en el rendimiento anaeróbico ha recibido más atención en los últimos años. Podría especularse que esta relación es un mecanismo agudo (deshidratación) o está asociado con la MLG (mayor cantidad de MLG, mayor fuerza absoluta), lo que sugiere que el rendimiento anaeróbico está determinado bien por componentes de cuerpo entero, bien por consecuencias relacionadas con la deshidratación (retroalimentación negativa). Aunque, los estudios realizados en el siglo anterior y el presente se inclinan más hacia mecanismos que apoyan la importancia de la distribución crónica de fluidos y la MM regional en el rendimiento anaeróbico. Sin embargo, hay varias preguntas por responder en niños y adolescentes como la relevancia de la distribución de agua y la MM a nivel regional, la importancia de la madurez y las diferencias entre jóvenes deportistas y jóvenes no activos.

Los principales objetivos del presente trabajo de investigación son: describir los valores de MLG y MM de niños y adolescentes caucásicos según su edad, género, práctica deportiva y estado de maduración, y generar valores de referencia en la población española para un amplio rango de edad; por otro lado, analizar la validez concurrente de diferentes métodos de laboratorio y campo para la evaluación de MLG y MM y sus asociaciones individuales con la fuerza para evaluar la validez de constructo en niños, adolescentes y adultos jóvenes; y finalmente, analizar las asociaciones entre las pruebas de fuerza isométrica y dinámica con la composición corporal de atletas jóvenes y no atletas, e identificar predictores de rendimiento anaeróbico.

Para lograr estos objetivos, se realizaron tres estudios separados con una muestra común de niños, adolescentes y adultos jóvenes.

Estudio 1. Relevancia de la participación deportiva, tamaño corporal y maduración con la masa muscular total y regional a lo largo de la infancia y la adolescencia.

La adquisición de MM durante la infancia y la adolescencia debe ser una prioridad para asegurar un crecimiento saludable y un mejor desarrollo motor. Por otra parte, dado que el incremento máximo de MM debe ocurrir durante la juventud, éste determinaría la salud y el rendimiento en la etapa adulta. Aunque algunos indicadores de MM se han estado usando desde los años setenta en la evaluación del estado nutricional, como la circunferencia o área del brazo, no hay datos suficientes obtenidos durante las etapas de infancia y adolescencia para establecer valores de referencia para cada población.

El primer objetivo de este estudio, por lo tanto, fue analizar las tendencias de la MLG y MM regional y total, por grupos de edad y género en niños y adolescentes. El segundo objetivo fue analizar la influencia de la participación deportiva y la maduración en la evolución de la musculatura. Y, por último, proporcionar datos de referencia en jóvenes españoles y portugueses de un amplio rango de edad.

Métodos. El diseño del estudio fue transversal, e incluyó 1438 voluntarios de entre 8 y 18 años de edad de colegios, institutos y clubes deportivos locales. La antropometría se realizó de acuerdo con la metodología ISAK para medir los pliegues tricipital, crural y medial de la pantorrilla, y las circunferencias del brazo, muslo y

pantorrilla. Las áreas musculares regionales se calcularon a partir de las circunferencias corregidas de los miembros. La MLG se estimó mediante las ecuaciones antropométricas de Slaughter et al. (1988). Se aplicaron modelos validados y específicos por edad para obtener los valores de MM basados en variables antropométricas (modelos de Poortmans (<16 años) y Lee (>16 años)). La participación deportiva se obtuvo a partir de cuestionarios *ad hoc* y la maduración se calculó como la edad en el momento del pico máximo de crecimiento a partir de ecuaciones predictivas. Los sujetos fueron agrupados anualmente de 8 a 18 años de edad. Se utilizó un modelo lineal general de factores mixtos para analizar la varianza de la MLG y MM en los distintos grupos y las covariables. Se usó un análisis de regresión logarítmica para describir las relaciones de escala entre MM y estatura.

Resultados. Se encontró interacción positiva entre sexo, participación deportiva y edad para las variables musculares regionales, que también difirieron entre los grupos pre- y post-pico máximo de crecimiento. Sólo los niños activos aumentaron significativamente su musculatura regional de primaria a secundaria (brazo, Δ0.78cm, $P<0.01$, muslo, Δ1.44cm, pantorrilla, Δ1.16cm) y de secundaria a bachillerato (brazo, Δ2.81cm, muslo, Δ2.51 cm, pantorrilla, Δ1.88 cm, todo $P<0.0001$).

Conclusiones. Nuestros hallazgos demuestran que existe una diferencia entre niños y niñas en la relación entre MM total o MLG y edad, incluso después de ajustar para la estatura. Se observaron resultados similares para el crecimiento muscular de las extremidades superiores e inferiores. Una contribución novedosa de este estudio fue que la práctica deportiva puede afectar a la acumulación de MM después de la pubertad, y también el aumento de la relación entre músculo regional y estatura antes del pico de crecimiento. Estos datos deben ser valiosos para usar como referencia para poblaciones

similares, sin embargo, se requieren estudios longitudinales para confirmar estos resultados.

Estudio 2. Validez de los métodos para estimar masa libre de grasa y masa muscular esquelética en niños, adolescentes y adultos jóvenes: Constructos de Rendimiento Anaeróbico y Participación Deportiva.

La evaluación de la MM puede ser una preocupación importante para analizar la relación entre la composición corporal, la fuerza y el rendimiento. En cuanto a los adolescentes, existe una carencia de modelos válidos para estimar con precisión la MM. Teniendo en cuenta este razonamiento, debería ser pertinente analizar si los modelos conocidos para estimar MM tienen una buena asociación con el rendimiento de la fuerza para evaluar su validez de constructo. El propósito del estudio fue comparar métodos de laboratorio con métodos de campo, y sus asociaciones individuales con pruebas de fuerza isométrica y dinámica.

Métodos. Se utilizaron varios estudios de validación para compilar una amplia base de datos con un total de 531 medidas de ACT de 340 participantes (159 chicas y 181 chicos). Los criterios de inclusión fueron no padecer enfermad crónica o aguda y no tener miembros amputados. El consentimiento informado fue firmado por todos los participantes y los padres de los menores. La composición corporal se evaluó mediante método de dilución de isótopos usando deuterio (D_2O), DXA, antropometría (ANT) y BIA. La MM a partir de DXA se calculó con los modelos de Kim (MM_X) para adolescentes (<16 años) y para jóvenes adultos (≥ 16 años). Las estimaciones MM basadas en ANT (MM_A) fueron calculadas con los modelos de Poortmans (<16 años) y Lee (≥ 16 años). La MLG a partir de deuterio se calculó utilizando las constantes de hidratación de Lohman, y la ecuación de Houtkooper para los valores a partir de BIA. La fuerza muscular superior (manos derecha e izquierda) y de miembros inferiores se midió con dinamómetros. Y la fuerza dinámica con un sprint de 30 metros (m) y saltos verticales. El estado de maduración se calculó usando variables predictivas para estimar la edad en el pico máximo de crecimiento. Se evaluó la validez de concurrente y la validez de constructo.

Resultados. En general, todas las medidas de MLG obtenidas por las técnicas alternativas infraestimaron las medidas de referencia a partir de deuterio. La MLG a partir de ANT fue la que menos error mostró (-2.29; 95% ICs, -2.77 to -1.80), y para la BIA se encontraron valores similares (-2.42; 95% ICs, -2.82 to -2.02). La concordancia entre métodos fue mejor en el grupo de deportistas que en el grupo inactivo. Las diferencias medias para MM_X y MM_A fueron inferiores en las chicas que en los chicos (0.46 ± 1.39 DE vs. 1.98 ± 1.54 DE, P<0.05 y P<0.0001 respectivamente). Se encontraron asociaciones positivas para todas las variables de MLG y MM con las pruebas de rendimiento anaeróbico (P>0.05), excepto para la MLG estimada por DXA y el salto vertical squat-jump (r = 0.228, P = 0.051, n = 36). La prueba de sprint mostró coeficientes negativos reflejando que, a mayor cantidad de músculo menor tiempo empleado en el sprint. El coeficiente de correlación más bajo se encontró entre el método de referencia y salto vertical SJ (τ = 0.207, n = 263) y el más alto entre MM_X y prensión de mano izquierda (τ = 0.764, n = 186). En general, la fuerza evaluada por dinamometría mostró mayores coeficientes de correlación con las variables de MM y MLG que las pruebas dinámicas explosivas.

Conclusiones. Nuestros resultados no confirmaron la concordancia entre modelos, y las asociaciones entre MM y la fuerza mostraron diferentes magnitudes. Las diferencias podrían estar parcialmente explicadas por el método de referencia utilizado para validar los modelos, ya que Kim y Poortmans evaluaron MM con MRI y DXA respectivamente. Dado que DXA no se considera un método de referencia para medir MM, la validez de constructo del modelo de Poortmans (MM_A) debe ser menor que el modelo de Kim (MM_X).

Estudio 3. La distribución de fluidos y la masa muscular esquelética regional como predictores del rendimiento anaeróbico en adolescentes: Resultados del sprint y la fuerza isométrica.

La composición corporal, incluida la hidratación, se ha asociado clásicamente con el rendimiento aeróbico. Sin embargo, las relaciones entre el rendimiento anaeróbico evaluado por sprints y saltos, y la hidratación, no se han analizado extensamente en la literatura, particularmente en niños y adolescentes. Ésta es una situación paradójica porque el niño está principalmente involucrado en ejercicios de alta intensidad y corta duración en la mayoría de tareas diarias, juegos o eventos deportivos. El análisis de estas asociaciones debe ser útil para describir la importancia de la hidratación y la MLG en el rendimiento de adolescentes sanos.

Por otro lado, la relación entre el tamaño y la fuerza muscular ha sido estudiado ampliamente en adultos, sin embargo, se sabe menos acerca de su asociación real en los adolescentes. La identificación de los indicadores antropométricos de MM que mejor predicen la fuerza con la edad, teniendo en cuenta los procesos de crecimiento y maduración, debe ser útil en los entornos de campo para el entrenamiento deportivo con jóvenes y la selección de talentos deportivos.

El objetivo principal de este estudio fue explorar dos paradigmas relacionados con el rendimiento anaeróbico y subcomponentes de la composición corporal en adolescentes. En primer lugar, analizar la asociación entre las pruebas de sprint de 30m y la composición del cuerpo molecular en atletas jóvenes y no atletas. En segundo lugar, examinar la asociación entre la fuerza de las extremidades y las variables de MM regionales en atletas jóvenes y no atletas. Finalmente, nuestro propósito fue identificar indicadores moleculares y regionales de MM para predecir con éxito el rendimiento de sprint y la fuerza de piernas después de ajustar para la maduración.

Métodos. Estos estudios se realizaron en submuestras de los estudios más amplios descritos en los estudios anteriores y para los cuales se obtuvieron datos válidos con un consentimiento firmado por los participantes y padres o tutores de los menores. Las enfermedades agudas o crónicas fueron los únicos criterios de exclusión para estos dos análisis. En el primer análisis se seleccionaron todos los participantes con datos válidos para la evaluación de la composición corporal molecular por ANT y BIA, y la prueba de sprint de 30m. Con el fin de facilitar la comprensión del manuscrito nos referiremos a este análisis como *Agua intracelular y rendimiento en el Sprint*. El segundo análisis se realizó en todos los adolescentes con al menos una prueba válida de fuerza de miembros inferiores mediante dinamometría isométrica y evaluación antropométrica de MM tanto total como regional (brazo, muslo y pantorrilla). Los resultados específicos y la discusión de este análisis se indican como *Masa muscular regional y Fuerza de miembros inferiores*.

Agua intracelular y rendimiento en el Sprint. Ciento cincuenta y nueve adolescentes sanos entre 12 y 18 años de edad (15.0 ± 1.5 años) fueron reclutados en institutos de secundaria y clubes deportivos locales. Los atletas (AT) y los no atletas (NAT) fueron identificados mediante un cuestionario *ad hoc* (NAT = 101, 41 niños, 60 niñas y AT = 58, 27 niños y 31 niñas).

Masa muscular regional y Fuerza de miembros inferiores. Ciento sesenta y seis adolescentes sanos entre 12 y 18 años de edad (15.2 ± 1.7 años, índice de masa corporal = 21.9 ± 3.9 kg/m^2) fueron reclutados en institutos de secundaria y clubes deportivos locales. Sólo 158 participantes cumplieron con los criterios de las variables incluidas en los modelos (NAT = 92, 38 varones, 54 niñas y AT = 66, 42 varones y 24 niñas). La MM regional fue evaluada por perímetros corregidos como se informó en el estudio 1. Brevemente, los perímetros de los brazos, muslos y pantorrillas fueron medidos, y los pliegues tricipital, crural y medial de la pantorrilla, de los que se calcularon las circunferencias musculares en cada región. La evaluación de la maduración se calculó usando la ecuación de Mirwald et al. (2002). Se realizó un calentamiento de 5 minutos y dos ensayos submáximos de cada prueba a todos los participantes. Todas las pruebas se realizaron durante la misma mañana y con al menos 30 minutos de reposo entre las pruebas realizadas siguiendo el protocolo: En primer lugar, se utilizó un dinamómetro portátil (J Herrera, Barcelona) para evaluar la resistencia de los miembros inferiores en posición de semi-squat. Se pidió a los participantes que hicieran el ejercicio progresivamente y mantuvieran la fuerza máxima durante 2-3 segundos. En segundo lugar, se utilizaron células fotoeléctricas para medir el tiempo empleado en 30m (SmartSpeedTM, Fusion Sport®, United Kingdom); se pidió a los sujetos que corrieran a máxima velocidad hasta un cono situado a 40m de la línea de inicio (el haz de celdas infrarrojas se cruzó a 30m). Para los análisis se utilizó el mejor valor de resultado de dos intentos. AT y NAT fueron identificados mediante un cuestionario *ad hoc*.

Las diferencias entre los grupos se analizaron mediante una prueba T de muestras independientes. Se realizaron correlaciones parciales para determinar las asociaciones entre las variables de composición corporal y los valores de sprint de 30m y la fuerza de miembros inferiores. Las variables mejor correlacionadas con el rendimiento de sprint o fuerza de miembros inferiores se incluyeron en un análisis de regresión paso a paso para determinar los predictores de composición corporal del rendimiento de sprint después de ajustar por edad, sexo y participación deportiva (AT = 1 o NAT = 0). Se aceptó un error de tipo I del 5% en todas las pruebas estadísticas (*P*<0.05), que se realizaron con el paquete estadístico SPSS® (version 15.0; IBM Corporation, Armonk, NY, USA).

Resultados. *Agua intracelular y rendimiento Sprint*. Como era de esperar, el grupo de AT fue más rápido que el grupo de NAT en el sprint 30m (diferencia media = 0.43 ± 0.09 segundos; *P*<0.001), pero no hubo diferencias significativas en agua intracelular (diferencia media = 1.18 ± 0.94%; *P*>0.05). El grupo de NAT tenía significativamente más grasa y menos agua corporal que el grupo de AT. No se encontraron diferencias significativas entre los grupos para la masa muscular (*P*>0.05). Se encontraron correlaciones significativas entre AIC y 30m sprint test después de controlar para la edad y los kg de masa grasa (*P*<0.001).

Para analizar el impacto de la práctica deportiva, todos los participantes se analizaron conjuntamente; %MM, kg de agua intracelular, y la condición de atleta fueron los predictores significativos del tiempo en 30m en el modelo inicial (R^2 = 0.561, R^2 ajustado = 0.552, EE = 0.38 segundos, *P*<0.001). El segundo análisis de regresión paso a paso se realizó de nuevo con velocidad en 30m como variable dependiente. La MM (kg), la condición de atleta y el % de agua intracelular fueron identificados como predictores (*P*<0.001, R^2 = 0.621, R^2 ajustado = 0.613, EE = 0.37 m/s). El modelo final fue: Velocidad 30m (m/s) = 3.73 + (0.058 * MM) + (0.482 * AT) + (0.037 * agua intracelular). La variable de maduración calculada como años hasta o desde el pico máximo de velocidad de crecimiento no fue incluida en ningún modelo.

Masa muscular regional y Fuerza de miembros inferiores. El grupo AT fue más fuerte que el grupo NAT (80.21 ± 32.57 vs. 56.38 ± 25.52 kg, *P*<0.001) y también obtuvieron mayores valores de circunferencia corregida del gemelo (CCG) (AT 30.99 ± 3.20 vs. NAT 29.68 ± 2.75 cm, *P*<0.01). El grupo NAT tenía significativamente más grasa y menos MM que AT (*P*<0.05). No se encontraron diferencias significativas entre los grupos en cuanto a edad, peso, talla y maduración (*P*>0.05). La mejor correlación se encontró en el grupo NAT entre MM y fuerza controlando para la estatura (*r* = 0.631, *P*<0.000) mientras que en el grupo de AT no se encontraron correlaciones significativas (*P*>0.05).

El análisis de regresión paso a paso se realizó con la fuerza de miembros inferiores como variable dependiente, se encontraron resultados similares para un modelo que incluía MM (kg), condición de atleta y estatura (R^2 = 0.488, *P*<0.001) y para uno alternativo que incluía CCG, condición del atleta, sexo y estatura (R^2 = 0.484, *P*<0.001, EE = 22.49 kg, ecuación 2 incluyendo MM regional). La maduración no fue incluida como variable predictora del rendimiento en ningún modelo.

Fuerza de miembros inferiores (kg) = -110.523 + (1.983 * MM) + (18.266 * AT) + (0.769 * Estatura). Ecuación 1

Fuerza de miembros inferiores (kg) = -182.108 + (1.07 * Estatura) + (16.295 * AT) + (11.208 * Género) + (1.979 * CCG) Ecuación 2

Conclusiones. Los principales hallazgos de estos dos análisis fueron que tanto la distribución de agua como la MM regional fueron predictores significativos del rendimiento anaeróbico, lo que confirma nuestras hipótesis iniciales. Estos resultados pueden ser útiles para explorar los mecanismos del sprint y ejercicios de fuerza en adolescentes. Sin embargo, más investigación con diferentes muestras es necesaria para confirmar nuestros resultados.

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ABBREVIATIONS AND SYMBOLS

ACG	Arm corrected girth
ADP	Air displacement plethysmography
ALST	Appendicular lean soft tissue
ANT	Anthropometry
APHV	Age at peak height velocity
aSMM	Appendicular skeletal muscle mass
AT	Athlete/athletic group
BCM	Body cell mass
BF	Body fat
BIA	Bioelectrical impedance analysis
BMI	Body mass index
C	Component
CCG	Calf corrected girth
CI	Confidence intervals
cm	Centimeters
CT	Computerized tomography
D_2O	Deuterium oxide
Db	Density of the body
DXA	Dual-energy X-ray absorptiometry
ECW	Extracellular water
ES	Effect size
f	Mathematical function
FFM	Fat free mass

FFM-BIA	Fat free mass assessed by bioelectrical impedance analysis using the equation of Houtkooper et al. (1992)
FFM-BIA_d	Fat free mass derived from predicted total body water by bioelectrical impedance analysis using the equation of Kushner et al. (1992)
FFM-DXA	Fat free mass assessed by dual X-ray absorptiometry analysis
FFM-REF_L	Fat free mass derived from reference method (deuterium) using hydration constants proposed by Lohman (1986)
FFM-REF_W	Fat free mass derived from reference method (deuterium) using hydration constants proposed by Wells et al. (2010)
FM	Fat mass
g	Grams
H_{FFM}	Hydration of fat free mass
H-S	High school
Ht	Height
ICW	Intracellular water
IMAT	Inter-muscular adipose tissue
ISAK	International Society for the Advancement of Kinanthropometry
kg	Kilograms
kHz	Kilohertz
LLS	Lower limbs strength
Ln-ln	Natural logarithm
m	Meters
ml	Milliliters
mm	Millimeters
Ma	Muscle area

MC	Multicomponent model
Mc	Muscle circumference
mCSA	Muscle cross-sectional area
MO	Maturity offset
MRI	Magnetic resonance imaging
n	Number of observations
NAT	Non-athlete/non-athletic group
NSP	Non-sport participation
m/s	Meters per second
NSP	Non-sport participant
PA	Physical activity
PHV	Peak height velocity
P-S	Primary school
ρ_c	Concordance correlation coefficient
Q	Quantity
r	Pearson's correlation coefficient
R	Resistance
rho	Spearman's correlation coefficient
R^2	Coefficient of determination
s	Seconds
SD	Standard deviation
SE	Standard error
SED	Standard error of the difference
SEE	Standard error of the estimate
SKF	Skinfold

SMM	Skeletal muscle mass
SMM-ANT_A	Skeletal muscle mass derived from anthropometry using the equation of Lee et al. (2000) in the young adult group (participants ≥16y)
SMM-ANT_P	Skeletal muscle mass derived from anthropometry using the equation of Poortmans et al. (2005) in the pediatric group (participants <16y)
SMM-DXA_A	Skeletal muscle mass derived from DXA using the equation of Kim et al. (2004) in the young adult group (participants ≥16y)
SMM-DXA_P	Skeletal muscle mass derived from DXA using the equation of Kim et al. (2006) in the pediatric group (participants ≥16y)
SP	Sport participation
S-S	Secondary school
τ	Kendall's Tau correlation coefficient
TBW	Total body water
TCG	Thigh corrected girth
TEM	Technical error of measurement
vs	Versus
y	Years
YPHV	Years from peak height velocity
2C	Two-component model
3C	Three-component model
4C	Four-component model
%	Percentage
≈	Approximately equal
π	Pi value
º	Degrees

INDEX

I. INTRODUCTION

INTRODUCTION

Body composition research area aims to quantify total and regional components of the human body mainly for dose calculations in medical treatments, health related issues, and sports performance. The relationship with other interacting factors such as nutrition, growth and maturation, aging, and health or disease status, has driven this area of research, which should be a focus of primary interest not only in the clinical field, but also for field professionals working with health-related populations, namely physical education teachers, physical fitness or sport coaches.

Five different levels can be used to describe body mass composition, from the atomic to the whole-body level, passing through the molecular, cellular and tissue-organ levels (Wang, Pierson, & Heymsfield, 1992). Every level is composed by a determined number of components to sum body weight, and several models have been developed for quantifying each level. At the atomic level, body weight is explained by the concentrations of up to 11 elements. On the other side, the multicomponent molecular-level models range from 2, through 3 and 4, and up to 6 components, and the use of one of those models depends on the techniques available to measure its components. This is considered the reference method to date, as a combination of several reference methods can provide a more accurate measure of each component (Heyward & Wagner, 2004).

At a given level, any model is based on the formula C = *f*(Q), describing the magnitude of the mass of a component (C) as the product of some mathematical function (*f*) and a measurable quantity (Q). To quantify the known component, property-based methods (e.g. body volume) is required. Then, the mathematical function can be applied using regression analysis to derive a predictive equation (empirical). Another option is to use a mathematical function based in well-established model (ratios or proportions of Q assumed as constant; e.g. hydration of fat free mass is 0.73) (Heymsfield, Lohman, Wang, & Going, 2005).

In the pediatric population, the 4C model is the most widely used because it may not be affected by age or human developmental level; it is comprised by fat, water, mineral mass, and protein, being the sum of all components equal to body weight. Therefore, four measurements are required as body weight, volume, water, and bone mineral content. Several reference methods are needed to accurately assess each of the components like underwater weighting or air-displacement plethysmography (ADP) for estimating body density (Db) from body volume, hydrometry by isotope dilution for total body water (TBW), and dual-energy X-ray absorptiometry (DXA) for total body mineral mass. If mineral content assessment is not possible, though, a 3C can be used (Heymsfield et al., 2015).

Although the development of multicomponent models was an important goal in the first phase of history of body composition (Carnero, Alvero-Cruz, García, & Sardinha, 2015), in the second part of the 20th century pediatric body composition research was mainly focused on providing reference data and developing new methods for assessing fat, muscle and bone, while also improving field techniques (Lohman, Hingle, & Going, 2013). Considerably research has been centered on publishing standards for body fat in the young boys and girls, especially with the increase of worldwide prevalence of childhood obesity over the past 20 years (De Onis, Blossner, & Borghi, 2010). Some relatively recent studies were carried out to describe body fat reference data in European young (Nagy et al., 2014, 2016) and Spanish young population (Moreno et al., 2007; Moreno et al., 2005), examining also its relationship with sport activities (Ara et al., 2004, 2006).

Relevance and Measurement of Skeletal Muscle Mass and Fat Free Mass in Youth

The importance of measuring muscularity in children and adolescents has been traditionally related with the assessment of nutritional status because the size of the muscle mass is an indicator of protein reserve (Frisancho, 1974). The seminal growth studies from the last decades of the past century described muscle evolution across childhood and adolescence (Forbes, 1974; Johnston & Malina, 1966; Malina, 1969b; Malina & Johnston, 1967). Currently, there is a growing interest on the role of skeletal muscle in youth and its association with cardiometabolic (Steene-Johannessen et al, 2009; Artero et al, 2011; Andersen et al, 2011; Guinhouya et al, 2011) and cardiovascular risk factors (Grøntved et al., 2013). As well as bone strength (Fricke et al., 2008; Fricke, Beccard, Semler, & Schoenau, 2010; Jackowski, Lanovaz, Van Oort, & Baxter-Jones, 2014), and vascular health (Peplies et al., 2016). However, the measurement of total SMM in youth is still an important limitation in field settings, which has not permitted conduct large cohort studies to find cut-off values related with health or performance outcomes.

The first model to estimate total body skeletal muscle mass (SMM) was initially proposed by Matiegka (Matiegka, 1921), although the most accurate method to measure is analysis of cadavers, by direct chemical analysis, and alternative methods were developed from this latter to allow *in vivo* body composition analysis. At the tissue-organ level, direct measurement of skeletal muscle mass (SMM) is now possible due to advances in technology using imaging methods as magnetic resonance imaging (MRI) and computed-tomography (CT). These imaging methodologies are minimally affect by age or demographic variables, but they have limited availability due to its high cost, time consuming, and CT is not applicable in the pediatric population because of a high radiation exposure. So, its use is reduced mainly for medical diagnosis or research as reference method for validation studies.

Considering the limitations to access to valid and reliable measures of SMM, fat-free mass (FFM) is used alternatively as a surrogate of SMM. However, FFM is heterogeneous component being considered as representative of whole body metabolically active tissue or body cell mass (BCM) (Pierson, Wang, Thornton, & Heymsfield, 1998; Wang et al., 2004). To date, the reference method for FFM assessment is a multicomponent molecular-level model (MC), for which is necessary a combination of several reference methods that can provide a more accurate measure of each component of the FFM. But a combine-methods approach is not always affordable, thus, the traditional and much simple model of two components (2C) is generally used, where body mass is equal to the sum of fat mass (FM) and fat free mass (FFM). The 2C model early developed by Behnke (1942) is based on Db using the Archimedes' principle by underwater weighting, assuming FM and FFM had a constant density. Conversion of Db to %FM was then possible using the equations of the models developed by Siri (1961) and later by Brozek et al. (1963) (Heyward & Wagner, 2004).

Although widely used, the 2C model for assessing FFM during growth period is limited because of the assumptions relative to the density and hydration of its components. The proportional relationship between components of FFM are assumed to remain stable in healthy adults (Snyder, Cook, & Nasset, 1975), but during growth period is altered. The four main components comprising FFM (water, proteins, minerals, and glycogen) increase in different proportions along the childhood and the adolescence. So, when assessing FFM in children and young populations, variations in the components of the FFM must be considered. Consequently, more constituents of the FFM can be estimated better accuracy and validity. Nevertheless, protein content and glycogen are not easy to quantify in vivo because of inaccessible reference method of neutron activation analysis (Wang, Heshka, Wang, & Heymsfield, 2006), and the standard method to measure muscle glycogen requires an invasive muscle biopsy or the complexity and still developing carbon magnetic resonance spectroscopy methodology (Buehler, Bally, Dokumaci, Stettler, & Boesch, 2016).

The first pediatric reference data were provided by the pioneer work of Fomon, who introduced reference values for FFM components in children from birth to 10 years (y) of age (Fomon, Haschke, Ziegler, & Nelson, 1982; Fomon & Nelson, 2002). Later, the group of University of Arizona leaded by Timothy Lohman reported age- and sex-specific constants for conversion of body density, water, and potassium to %FM in children and youth by age groups (7-25y) (Lohman, 1986). More recently, Wells proposed new values and developed a new UK reference child (Wells et al., 2010; Wells et al., 2012).

Currently, the main paradigm for estimating FFM is based in measurement or estimation of TBW. Water is the largest constituent of the body and FFM and the estimation of FFM from TBW relying in age- and sex-specific hydration constants of FFM is considered reference method after an accurate measurement of TBW (Lohman & Going, 2006). The isotope dilution method, based on the dilution principle, is commonly used as the reference method for TBW assessment (after the isotopic tracer is administered, typically deuterium oxide (D_2O)), differences in concentrations between baseline and post-dose physiological fluid samples are measured by mass spectrometry (Schoeller et al., 1980). Thereby, FFM can be estimated dividing TBW by the published age- and sex-specific hydration constants of FFM mentioned above (H_{FFM}) to improve the accuracy of the 2C model. Nowadays, the D_2O is considered one of the reference methods to measure TBW, although it is expensive and time consuming to apply in the majority of the context.

Prediction of Fat Free Mass in Children and Adolescents

Prediction models are the most useful way to evaluate FFM when reference methods are not available, using a mathematical modelling of a normal FFM based on age, gender, fat mass and height (Bosy-Westphal & Mülller, 2015). According to a recent systematic review, from the current available models to predict FFM in children and adolescents only seven equations were cross-validated, of which few reported agreement and none reported the concordance correlation coefficient (Silva, Fields, & Sardinha, 2013). Validation studies for predictive equations against accepted reference methods are still needed in youth populations.

There are assumptions inherent to different methods and techniques that can contribute to differences between methods and lead to biased conclusion. The first aim of the present study was to compare current available methods in a sample of Spanish children, adolescents, and young adults for a more practical application by coaches and physical education teachers. In this regard, we found that after accounting for all advantages and limitations of body composition methods is out of our main objective. Nevertheless, there is extensive literature that describes advantages and limitations of body composition assessment in adult and young populations and different techniques (Ellis, 2000; Ellis, 2001; Heymsfield et al., 2005; Heyward & Wagner, 2004; Kyle, Earthman, Pichard, & Coss-Bu, 2015; Lohman et al., 2013; Wells & Fewtrell, 2006). Additionally, focus on SMM assessment (Al-Gindan, Hankey, Leslie, Govan, & Lean, 2014; Heymsfield, Adamek, Gonzalez, Jia, & Thomas, 2014) and future directions have been recently reviewed (Heymsfield et al., 2015).

The most recently data available in the literature for values of total FFM or SMM in children and youths have been obtained from different methods such as bioelectrical impedance analysis (McCarthy, Samani-Radia, Jebb, & Prentice, 2014), dual-energy X-ray absorptiometry (Webber & Barr, 2012) and a reference 4C model (Wells et al., 2012). The availability of total and regional lean body mass across childhood and adolescence is limited, especially in Spanish population. A summary of the most recent and classical available models (equations) used to estimate FFM are presented in table 1, and those applied for estimating SMM are in table 2.

Table 1. Summary of equations used for estimation of FFM in the present study.

Study	Population	N	Criterion method	Derived method	Estimation variable	Equation	*r* (R^2)	SEE
Slaughter et al., 1988	8-29y, males and females (white and black)	310	Underwater weighing – D_2O Dilution – Photon absorptiometry	ANT	FM (FFM derived from 2C model)	Boys, 0.735 * (Triceps + Calf SKF) + 1.0	0.78	3.8 %
						Girls, 0.610 * (Triceps + Calf SKF) + 5.1		
						Triceps + Subescapular SKF ≥35mm	0.78	3.7 %
						Boys, 0.783 * (Triceps + Subescapular) + 1.6		
						Girls, 0.546 * (Triceps + Subescapular) + 9.7		
Houtkooper et al., 1992	10-14y, males and females (white)	94	Hydrostatic weighing – D_2O Dilution	BIA	FFM	0.61 * ($Height^2$/R) + (0.25 * Weight) + 1.31	(0.95)	2.1 kg
Kushner et al., 1992	0-66y, males and females	87	D_2O Dilution	BIA	TBW (FFM = TBW/H_{FFM})	0.593 * ($Height^2$/R) + (0.065 * Weight) + 0.04	0.99	1.2 kg

D_2O, deuterium oxide; ANT, anthropometry; FM, fat mass; FFM, fat-free mass; BIA, bioelectrical impedance analysis; TBW, total body water; SKF, skinfold thickness; H_{FFM}, hydration of fat-free mass; *r*, correlation coefficient; R, resistance; R^2, coefficient of determination; SEE, standard error of the estimate.

Table 2. Summary of equations used for estimating SMM in the present study.

Study	Population	N	Criterion method	Derived method	Estimation variable	Equation	R^2	SEE
Lee et al., 2000	20-81y, males and females (African American, Asian, white and Hispanic)	244	MRI	ANT	SMM	Height * [(0.0074 * CAG^2) + (0.00088 * CTG^2) + (0.00441 * CCG^2) + (2.41 * Sex) – (0.048 * Age) + Race + 7.84	0.91	2.2 kg
Poortmans et al., 2004	7-24y, males and females (Caucasian)	59	DXA	ANT	SMM	Height * [(0.0064 * CAG^2) + (0.0032 * CTG^2) + (0.0015 * CCG^2)] + (2.56 * Sex) + (0.136 * Age)	0.966	NR*
Kim et al., 2006	5-14y, males and females (African Americans, Asians, whites, Hispanics, other)	65	MRI	DXA	SMM (IMAT-free)	Tanner <5 (model 3) (0.483 * ALST) + (0.042 * Weight) – (0.015 * Height) + [0.003 * (ALST * Height)] + 1.734	0.986	0.50 kg
Kim et al., 2004	18-88y (African Americans, Asians, Caucasians)	270	MRI	DXA	SMM (IMAT-free)	Tanner >5 (adults) (1.18 * ALST) – (0.03*Age) – 0.14	0.965	1.38 kg

*NR: not reported; MRI, magnetic resonance imaging; ANT, anthropometry; DXA, dual-energy X-ray absorptiometry; SMM, skeletal muscle mass; IMAT, inter-muscular adipose tissue; ALST, appendicular lean soft tissue; CAG, corrected arm girth; CTG, corrected thigh girth; CCG, corrected calf girth; R^2, coefficient of determination; SEE, standard error of the estimate.

Constructs Related with Skeletal Muscle Mass and Fat Free Mass

The development of SMM must follow a similar pattern as other body tissues since it is based on chemical components that grow in linear fashion from early childhood to late adolescence (Fomon, Haschke et al. 1982; Lohman and Going, 2006). So, this component must be evaluated like other growth outcomes, i.e. in relation to age- and sex-specific reference ranges until chemical maturity is reached, and suggested to occur at 16y in girls and 18y in boys (Lohman, 1986). This latter information may represent the most relevant and studied construct related with SMM: maturity and chronological age. Nevertheless, the main function of muscle tissue and part of the FFM is related with its capacity to generate tension (strength) and displacement, so functional variables may represent constructs independent of maturity. On this sense, anaerobic performance (strength) of simple exercises must be the most important FFM and SMM-related construct since this type of exercises are highly dependent of muscle contraction.

Body composition constructs (components) are especially important in weight-sensitive sports but also to monitor athletic performance and training programs as well as healthy development in childhood and youth. Accordingly, the associations between methods for total and regional FFM and SMM estimation and anaerobic tests are explored in order to evaluate construct validity.

Strength, Anaerobic Performance and Body Size

The assessment of strength in pediatric population was traditionally characterized by static and dynamic field tests such as sprints, isometric strength by limbs dynamometry or jump capacity (anaerobic performance), which some are still frequently used in school and sports settings as part of the physical fitness condition screening. The relationships between anaerobic physical condition assessed by isometric and dynamic strength tests and SMM have been less analyzed in the literature in contrast to aerobic fitness, particularly among children and adolescents, maybe due to a lack of reference standard criterion measure. This is a paradoxical situation because children are primarily involved in short-term high intensity exercise in almost daily tasks, games or sport events, and the scientific literature is devoted to the study of maximal aerobic power (Van Praagh, 2007).

Strength performance during growth is related to body size (weight and height), and thus, influenced by many interacting factors such as age, sex, growth, and maturation, among others. A large proportion of SMM is present in the extremities (Heymsfield et al., 1990), consequently, subjects with longer legs may have higher amounts of SMM at a given height. The relationship between muscle size as cross-sectional area and strength has been reviewed in the literature, however less is known about actual association for adolescents (Jones, Bishop, Woods, & Green, 2008).

During the adolescent growth spurt, maturity can explain some of the differences between young of the same chronological age, so, controlling for body size to carry out comparisons is a main issue in pediatric exercise science as differences would be reduced (Baxter-Jones, Eisenmann, & Sherar, 2005). Methods to assess biological maturation in young athletes were recently reviewed. It can be assessed as maturity status and/or timing, two different indicators that are not interchangeable. A wide range of methods are available for the assessment of maturity, some of them non-invasive as age at peak height velocity (PHV), which requires longitudinal data of height measures during the period of adolescent growth spurt, and thus obtain a measure of maturity timing. As an alternative for studies with cross-sectional design where collect measures over time is not feasible, simplified equations that only require one measure can be used to predict age at PHV but are not free of limitations (Malina, Rogol, Cumming, Coelho e Silva, & Figueiredo, 2015). The age at PHV can be later converted into a categorical variable classifying subjects as pre-, age at, or post-PHV; also as average, early, or late mature depending on their age of attaining the peak (Malina, Bouchard, & Bar-Or, 2004).

In the first part of the present research (study 1), trends of total-body FFM and SMM derived from the simple and practical approach of anthropometry among sex and age groups are presented. Moreover, regional indicators of muscularity expressed as muscle girths are also presented for 13 specific age groups. Additionally, the interaction of sport participation and maturity offset in the evolution of muscularity is examined in young aged 8 to 18y-old.old

In the second part (study 2), we compare the differences in kilograms of FFM estimated from DXA, BIA and anthropometry with the D_2O dilution method as the criterion measure. Previous studies have validated FFM estimated from D_2O with a 4C model in children aged 8-12y (Wells et al., 1999) and in Mexican children and adolescents aged 6-14y (Ramirez, Valencia, Moya-Camarena, Aleman-Mateo, & Mendez, 2009). On the other hand, to reduce biased results from the alternative techniques, the applied predictive equations in our sample to estimate FFM from anthropometry (Slaughter et al., 1988), BIA (Houtkooper, Going, Lohman, Roche, & Van Loan, 1992), and from TBW estimated by BIA (Kushner, Schoeller, Fjeld, & Danford, 1992) were previously validated against 3C model, 4C model, and D_2O respectively, and are sex-, age- and ethnic-specific as close as possible.

Regarding SMM measures, we explore agreement between two anthropometric equations and two DXA-based models, among the sample separated into two groups, pediatric (<16y) and young adult (≥16y), based on maturational differences in SMM found by Kim et al. (2006). The authors developed a pediatric model to estimate SMM from DXA based on MRI, and found that the adult model performed better in subjects with Tanner 5 (Kim, Shen, Gallagher, & Jones, 2006; Kim, Wang, Heymsfield, Baumgartner, & Gallagher, 2002). Accordingly, we use the Lee et al. equation validated with MRI in adults for the young adult group, and the anthropometric equations developed by Poortmans et al. in the pediatric group (Lee et al., 2000; Poortmans, Boisseau, Moraine, Moreno-Reyes, & Goldman, 2005). Maturity offset variable is used to confirm that all subjects in the young adult group are classified as post-PHV.

Furthermore, the associations between measures of total and regional FFM and SMM with anaerobic tests are analyzed in order to study the construct validity of methods for SMM and FFM assessment.

Finally, the last part (study 3) of this manuscript explores a paradigm barely studied in the literature, which the predictive validity of subcomponents of FFM in prediction of anaerobic performance. In this study, we analyze the impact of TBW, extracellular water (ECW) and intracellular water (ICW) in the anaerobic performance of cyclic explosive strength (sprint) and acyclic explosive strength (jump). Our work explores two paradigms, the first one suggested by Sjøgaard and Saltin (1982) whom found a relationship between an analytical exercise in laboratory and water distribution measured by biochemistry techniques in specimens of muscle biopsy (Sjøgaard & Saltin, 1982). Since this seminal work, there has been other studies where the relationship between anaerobic performance and total body hydration has been analyzed (Kraft et al., 2012), however several questions remain to be answered in children and adolescents such as the relevance of water distribution and regional SMM (second paradigm), the importance of maturity and differences between young athletes and normal adolescents.

In this last study, we explore the association and predictive capacity of regional SMM in lower limbs strength and water distribution in sprint performance.

II. OBJECTIVES AND HYPOTHESES

OBJECTIVES & HYPOTHESES

STUDY 1. The Relevance of Sport Participation, Body Size and Maturation on Total and Regional Skeletal Muscle Mass Along Childhood and Adolescence

1. To describe trends of regional and total SMM derived from anthropometry among age and sex groups in children and adolescents.
2. To examine the influence of sport participation and maturity offset in the evolution of muscularity.
3. To provide reference data for young Caucasians across a wide-range of age.

We hypothesized:

a. FFM and SMM increase with age in a non-linear manner and differ among sex groups.
b. Sport participants have higher amounts of muscularity that their non-active peers.
c. Muscularity varies among maturity groups.

STUDY 2. Concurrent Validity of Methods for Estimating Fat Free Mass and Skeletal Muscle Mass in Children, Adolescents and Young Adults: Anaerobic Performance and Sports Participation Constructs

1. To analyze the validity and agreement between laboratory and field methods to estimate FFM and SMM in a large sample of children, adolescents, and young adults.

We hypothesized:

a. Validity of FFM estimations from DXA, BIA, and anthropometry varies among techniques when compared to the reference method (deuterium oxide dilution (D_2O)).

b. There is no agreement between estimation values of FFM and SMM estimation values assessed by DXA, BIA, and anthropometry, and the criterion method of D_2O.

2. To explore construct validity by the individual associations SMM or FFM and strength tests.

We hypothesized:

a. The relationship between FFM or SMM variables and anaerobic performance changes significantly when we change the technique used to assess FFM or SMM.

STUDY 3. Fluid Distribution and Regional Skeletal Muscle Mass Predict Anaerobic Performance in Adolescents: Results of Sprint and Isometric Strength.

1. To analyze the association between 30m-sprint test and molecular body composition variables in young athletes and non-athletes.

We hypothesized:

a. There is a positive relationship between sprint performance and body composition variables that are common for athletes and non-athletes.

2. To examine the association between lower-limb strength (LLS) and regional SMM variables in young athletes and non-athletes.

We hypothesized:

a. There is a positive relationship between regional indicators of SMM and lower limb performance.

3. To identify molecular and regional SMM indicators successfully predict sprint performance and LLS after adjusted for maturation status.

   We hypothesized:

   a. Subcomponents of FFM such as ICW and regional SMM are independent predictors of sprint and lower limbs isometric strength respectively.

III. METHODOLOGY

METHODS

STUDY 1. The Relevance of Sport Participation, Body Size and Maturation on Total and Regional Skeletal Muscle Mass Along Childhood and Adolescence

Design. Cross-sectional data of anthropometry assessments from several studies conducted in our laboratory were collected from 2006 to 2015. Children and adolescents were from Northwestern, Center and South of Spain. Data from a separate study performed in Lisbon (Portugal) were also pooled together. The volunteers were recruited from local schools and sports clubs through written and oral advertisements. Each participant and one parent were informed about the research procedures; afterwards, written consent was signed by parents of children who accepted to participate in the study. Inclusion criteria in this analysis were: range of age between 8 to 18y, be free of acute or chronic diseases, and not having any total or partial amputated limb.

Sample. The initial cohort was 1780 subjects from whom 1659 were in the range of age 8-18y. The sample was limited to Caucasians (n = 1559) because of the low representability of other ethnicities (other ethnicities n = 57, missing data n = 43). After selecting those with partial or total anthropometry assessments, a final sample of 1438 healthy participants were included in the analysis (66.8% boys and 33.2% girls, mean age = 13.63 ±2.60y; mean body mass index, BMI = 20.22 ±3.50 kg/m^2). A total of 632 participants were recruited from schools and 806 from sport clubs.

Procedures.

Body composition. Body weight was measured using a digital scale (Tanita®, model UM-060) to the nearest 0.1kg wearing light clothes and height was measured by a portable stadiometer (Tanita® Leicester) to the nearest 0.1cm. An anthropometric classical procedure was performed to measure skinfolds thickness and circumferences variables, which were assessed by a caliper (Holtain) and tape respectively. Triceps, thigh and calf skinfolds, and arm, thigh and calf circumferences were obtained in accordance with international standards guidelines by four anthropometrists accredited by the International Society for the Advancement of Kinanthropometry (ISAK). They all met the accepted limits for technical error measurements (TEM) in multicenter studies (<3%) (Stewart, Marfell-Jones, Olds, & de Ridders, 2011).

Total body FFM was calculated using a 2C model based on the equations recommended by Slaughter et al. for children 8-18y of age to estimate FM (Slaughter et al., 1988). The equation including triceps + calf skinfold thickness was preferred (R^2 = 0.78, SEE = 3.8 %; equation 1), but in cases when the sum of triceps + subscapular was higher than 35 millimeters (mm), the alternative equation was used instead (R^2 = 0.78, SEE = 3.7 %; equation 2). After FM equations were applied, the equation 3 was used to calculate FFM. The equations applied were as follows:

$$Boys, FM\ (\%) = 0.735 * (Triceps + Calf) + 1.0$$

$$Girls, FM\ (\%) = 0.610 * (Triceps + Calf) + 5.1 \qquad \textit{Equation 1}$$

$$Boys, FM\ (\%) = 0.783 * (Triceps + Subscapular) + 1.6$$

$$Girls, FM\ (\%) = 0.546 * (Triceps + Subscapular) + 9.7 \qquad \textit{Equation 2}$$

$$FFM\ (kg) = Weight - (FM/100 * Weight) \qquad \textit{Equation 3}$$

where triceps, calf and subscapular refers to skinfold thickness in mm, FM in percentage, and weight in kg.

Total body SMM was calculated applying validated age-specific models to estimate SMM from anthropometric variables (Lee et al., 2000; Poortmans et al., 2005). Lee's model was developed and cross-validated against MRI and the same equation was later used with adapted coefficients for a child and adolescent population by Poortmans et al. (2005). In the present study, Poortmans' model was used for participants aged <16y and Lee's model for those aged 16y and older following the recommendation suggested by Kim et al. for maturation-related variation in SMM (Kim et al., 2006). The authors found that the model developed in adults was applicable in children and adolescents late in pubertal development (Tanner stage 5) due to the variation of skeletal muscle distribution with maturation level. Since developmental stages assessed by Tanner stages were only recorded partially in our sample, we used median age at entry into maturity stage instead to split the sample into two groups in order to analyze the higher number of measures possible (Shumei S Sun et al., 2002; Walvoord, 2010). Thus, it was assumed that all children aged 16.0y and beyond had achieved stage 5 and, consequently, the model of Poortmans' et al. was used for subjects below the age of 16.0y (R^2 = 0.966, SEE not reported; equation 4) and the model of Lee's et al. was applied in subjects aged ≥16.0y (R^2 = 0.91, SEE = 2.2kg; equation 5). The equations were as follows:

$$SMM\ (kg) = Height * [(0.00744 * CAG^2) + (0.00088 * CTG^2) + (0.00441 * CCG)^2 + (2.41 * Sex) - (0.048 * Age) + Race + 7.84 \qquad \textit{Equation 4}$$

$$SMM(kg) = Height * [(0.0064 * CAG^2) + (0.0032 * CTG^2) + (0.0015 * CCG^2)] + (2.56 * Sex) + (0.136 * Age) \qquad \textit{Equation 5}$$

where height was in m, CAG, CTG and CCG in cm, age in years, sex was 0 for female and 1 for male, and race was 0 for white.

Regional FFM. Middle upper arm, thigh and calf cross-sectional muscle areas and muscle circumferences (Mc) were calculated from each limb circumference and skinfold (bone included) as follows:

$$Mc = C - (\pi * SKF) \qquad \textit{Equation 6}$$

where C = limb circumference, and SKF = skinfold thickness, all measures in cm. Then, the muscle area (Ma) was calculated by:

$$Ma = (Mc)^2/(4 * \pi) \quad \textit{Equation 7}$$

Sport Participation (SP). Sport participation information of each subject was obtained by *ad hoc* questionnaires. Participants were classified as sport participants (SP) if they were involved in sport practice a minimum of 3 times per week, and as non-sport participants (NSP), either they practiced sports less than 3 days per week or did not participate. The questionnaires were filled by the participants and revised on site by evaluators. Among those children who could not answer properly or had doubts about their sport practices, we asked for help from their parents, teachers or coaches.

Maturity Offset (MO). Maturity offset was assessed by predicted years from peak height velocity (YPHV) using the alternative models created by Moore et al. that included age x height interaction for predictions without sitting height (Moore et al., 2015). Subsequently, predicted age at PHV (APHV) was calculated by subtracting MO values to age. Maturity was also converted into a categorical variable by classifying subjects who did not yet achieve their PHV (negative MO value) as pre-PHV, and those ones with a value of zero or higher (positive MO value) as post-PHV. Additionally, MO values were used to construct predicted biological age categories using 1y intervals (i.e., from -0.49 pre- to +0.49 post-PHV formed group 0 at PHV). The equations used for boys (R^2 = 0.896; SEE = 0.542) and girls (R^2 = 0.898; SEE = 0.528) were as follows:

$$Boys, MO = -7.999994 + [0.0036124 * (age * height)]$$

$$Girls, MO = -7.709133 + [0.0042232 * (age * height)] \quad \textit{Equation 8}$$

where age was in years and height in m.

Statistical Analyses. Subjects were grouped annually from eight to eighteen years of age and thinness, overweight and obese categories as calculated following the International Obesity Task Force (IOTF) BMI cut-offs (Cole & Lobstein, 2012). For further analysis, participants were classified according to age range for school periods in Spain: <12y-old in Primary School (P-S), 12-15y in Secondary School (S-S) and >15y in High School (H-S). Descriptive data are expressed as means and standard deviation (SD). Simple regression analysis was used to explore the relationship between main variables with age and height, and slopes were compared for sex and SP groups. General linear model of mixed factors was used to analyze variance of FFM or SMM variables among age and school groups; interactions: age x sex, age x SP, age x sex x SP, School periods x sex x SP. Tukey's multiple comparison tests were carried out to observe which groups differed from each other. Additionally, a simple allometric model was applied in natural logarithmic form to describe regional and total muscle mass scaling relations to height. Statistical significance was defined as $P<0.05$ (two tailed) for all tests. Statistical procedures were performed using the Statistical Package for Social Sciences (SPSS®, version 15.0; IBM Corporation, Armonk, NY, USA) and GraphPad Prism (version 7; GraphPad Software, La Jolla, CA, USA).

STUDY 2. Concurrent Validity of Methods for Estimating Fat Free Mass and Skeletal Muscle Mass in Children, Adolescents and Young Adults: Anaerobic Performance and Sports Participation Constructs

Design. A series of validation studies were designed using large dataset of the Biodynamic and Body Composition Laboratory of University of Málaga. Studies were carried in children and adolescents at schools or sport clubs, where a study advertisement during physical education classes and training sessions was done for participants' recruitment. After informing about the research procedures, written consent was signed by all participants and by parents of subjects under age of 18. Data was collected from 2006 to 2014. Subjects were from Southeast and Northwest of Spain. Inclusion criteria were: range of age between 6 to 21y-old, be free of acute or chronic diseases, and not having any total or partial amputated limb.

Validation studies

Concurrent Validity. External validation of FFM assessments by dual-energy X-ray absorptiometry (DXA), biological impedance analysis (BIA) and anthropometry (ANT) methodologies were carried out using deuterium oxide dilution method as reference (REF). Additionally, validity and agreement between SMM estimates by age- and sex-specific predictive equations based on anthropometry and DXA were explored.

Construct Validity. Anaerobic performance assessment paradigm relied in isometric and dynamic explosive strength measurements.

Sample. A total of 531 measures of TBW from 340 volunteers (159 females and 181 males) were included in the initial analysis as reference method of FFM for the study of concurrent validity. Assessments for concurrent validity analysis for alternative FFM methods included a sample distributed as follows: FFM from DXA, n = 92; anthropometry, n = 515; and BIA, n = 487. Ethnicity was present in the analyses as follows: DXA and ANT groups, 480 Caucasians, 15 Hispanics, 10 North-Africans, 4 Gipsies, and 6 Black-Americans; BIA, 457 Caucasians, 12 Hispanics, 9 North-Africans, 4 Gipsies, and 6 Black-Americans. A total of 230 (67 girls,163 boys) had sport practice and 290 (175 girls, 115 boys) did not have it (11 missing).

Regarding SMM comparisons where predicted equations from DXA and anthropometric techniques were applied, the sample size was 225 participants (89 females and 136 males), from whom 39.6% did not practice any sport (32 girls, 28 boys) and the rest were involved in sports (54 girls, 99 boys). Sport participation variable was missed in 12 subjects. The sample was composed by Caucasians (n = 203), Hispanics (n = 7), North-Africans (n = 7), Gipsies (n = 2), Black-Americans (n = 5), and Indians (n = 1).

Procedures.

Body composition assessment. Body weight was measured using a digital scale (Tanita®, model UM-060) to the nearest 0.1 kg wearing light clothes and height was measured by a portable stadiometer (Tanita® Leicester) to the nearest 0.1 cm.

Isotope dilution.

The isotope (deuterium oxide) dilution method was used to measure TBW with a stable Hydra gas isotope ratio mass spectrometer (PDZ; Europa Scientific, Northwich, Cheshire, UK). The first urine sample (baseline) was taken after an overnight fast when subjects arrived for tests in the morning and just before the administration of the tracer. The dose was 0.1 g of deuterium oxide per kilogram of body weight, diluted in 30 ml of water. Equilibration time was 4 hours for all participants, whom was permitted only minimal movement, no food or drink ingestion and were prevented from going to the water closet. After this time, the second urine sample was collected. Both samples were prepared to be analyzed by filling tubes and were kept in the freezer. The amount of deuterium enrichment in both samples were analyzed and TBW calculated by specific algorithms.

Dual-energy X-ray absorptiometry (DXA).

Whole body scan was performed using DXA (Hologic, Explorer, USA). Scans were performed on subjects following the procedures of the Hologic QDR for Windows® system from the manufacturer, and the system was calibrated before each session using the Hologic Anthropomorphic Spine Phantom. After imaging whole, the scan was analysed and the regions of interest were isolated following the protocol: the horizontal shoulder line just below participant's chin; the lines of the spine region were adjusted with the curvature and the spine was divided at the T12-L1 disc space; the horizontal line above the pelvis was located just above the iliac crest; arms were separated from the trunk with vertical lines bisecting the shoulder joints not including any arm soft tissue in the body region; legs were separated from the pelvis by positioning the angled lines of the pelvic triangle bisecting the femoral neck; the vertical line between the legs run to entirely divide the legs; the lateral leg lines were moved to include the entire thigh without crossing the hands. Each scan was reanalysed by a single investigator to ensure that markers were positioned systematically (TEM <3%). The system software provided the total and regional mass, fat and bone measures.

Bioelectrical impedance analysis (BIA).

A multifrequency bioelectrical analyzer (MediSystem-SanoCare Human Systems S.L., Spain) was used to obtain whole-body impedance values following general recommendations (Kyle et al., 2004). Four electrodes were used for each participant and were placed on right side of the body; two on hand and wrist and two more on right foot and ankle, each pair separated by a distance between 4-5 cm. Skin area for electrodes was cleaned with alcohol (propanol) before attachment. Subjects lied in supine position on a nonconductive surface during 10 minutes before the analysis, and removed all metallic objects in contact with skin. Arms were in abduction of ≈30º from trunk and legs separated by ≈45º. The analyzer was calibrated before the evaluation with external resistances. All participants attended at morning in fasting conditions and were advised not to practice vigorous physical activity the last 48 hours (Lukaski, Bolonchuk, Hall, & Siders, 1986). Previous to the analysis, they were required to void the bladder. BIA analysis was done throughout the manufacturer software. Measurements were performed by two trained technicians.

Anthropometry (ANT).

An anthropometric classical procedure was performed to measure skinfolds thickness and circumferences variables, which were assessed by a caliper (Holtain) and tape respectively. Skinfolds measures bigger than 4 cm were excluded because of the caliper limit. Triceps, subscapular, thigh and calf skinfolds, and arm, thigh and calf circumferences were obtained in accordance with international standards guidelines by three anthropometrists accredited by the ISAK. They all met the accepted limits for technical error measurements (TEM <3%) (Stewart et al., 2011).

Fat free mass by the Reference Method (FFM-REF)

First, TBW was assessed using the reference technique of deuterium dilution. Then, the specific constants for H_{FFM} were applied to estimate FFM which was calculated twice using the hydration constants proposed by Lohman (FFM-REF_L) and more recently by Wells et al. (FFM-REF_W) (Lohman, 1986; Wells et al., 2010). The equation used was as follows:

$$FFM\ (kg) = TBW / H_{FFM} \qquad \textit{Equation 9}$$

Fat free mass by the Alternative Methods

DXA. Total body lean mass reported by DXA analysis was considered as the FFM variable (FFM-DXA), which was composed by lean and bone tissues from the entire body.

BIA. Two estimations of FFM were done from BIA analysis. On one hand, the equation developed by Houtkooper et al. was used for predicting FFM-BIA (Houtkooper et al., 1992), which was validated against a multicomponent model in healthy white children and youth aged 10-19y (R^2 = 0.95, SEE = 2.1 kg; equation 10). On the other hand, TBW was predicted using the equation of Kushner et al. (Kushner et al., 1992), and after, H_{FFM} were applied to estimate FFM (FFM-BIA_d) similarly to the procedures by the reference method applying equation 9 (R^2 = 0.99, SEE = 1.24 kg; equation 11).

$$FFM\ (kg) = 0.61 * (Height^2/Resistance) + (0.25 * Weight) + 1.31$$

Equation 10

$$TBW\ (kg) = 0.593 * (Height^2/Resistance) + (0.065 * Weight) + 0.04$$

Equation 11

where height was in cm, resistance in ohms (measured at 50 kHz current), and weight in kg.

ANT. FFM-ANT was calculated using the 2C model based on previous predicted FM from anthropometric measures using the equations recommended by Slaughter et al. for children 8-18y of age as in the Study 1 (Slaughter et al., 1988). The equation including the sum of triceps + calf skinfold thicknesses was preferred, and it was applied in 427 subjects (R^2 = 0.78, SEE = 3.8 %; equation 12). The alternative equation was used instead only in cases when the sum of triceps + subscapular was higher than 35 mm (n = 88, 52 girls, 36 boys; R^2 = 0.78, SEE = 3.7 %; equation 13). After FM equations were applied, the equation 14 was used for FFM-ANT, as follows:

$$Boys, FM\ (\%) = 0.735 * (Triceps + Calf) + 1.0$$

$$Girls, FM\ (\%) = 0.610 * (Triceps + Calf) + 5.1 \qquad \textit{Equation 12}$$

$$Boys, FM\ (\%) = 0.783 * (Triceps + Subscapular) + 1.6$$

$$Girls, FM\ (\%) = 0.546 * (Triceps + Subscapular) + 9.7 \qquad \textit{Equation 13}$$

$$FFM\ (kg) = Weight - (FM/100 * Weight) \qquad \textit{Equation 14}$$

where triceps, calf, and subscapular refer to skinfold thickness in mm, FM in percentage, and weight in kg.

Skeletal muscle mass by DXA and Anthropometry

DXA. SMM was estimated using DXA-based Kim's models validated against MRI for pediatric and adult populations (Kim et al., 2004; Kim et al., 2006). The pediatric model performed better in children with Tanner stage below 5 and the adult model in those aged 16y or older due to the variation of skeletal muscle distribution with maturation level. For our sample, developmental stages were only recorded partially. Instead, we used median age at entry into maturity stage to classify the sample into pediatric and adult groups assuming that all children aged 16y and beyond had achieved stage 5 (Sun et al., 2002; Walvoord, 2010). Thus, the pediatric model was used for subjects below the age of 16y (R^2 = 0.986, SEE = 0.502 kg; equation 15) and the adult model for subjects aged ≥16y (R^2 = 0.965, SEE = 1.38 kg; equation 16). The bone and fat masses were subtracted from total mass of both the right and left arms and legs to calculate appendicular lean soft tissue (ALST). The equations used were as follows:

$$SMM\ (kg) = (0.042 * Weight) - (0.015 * Height) + [0.003 * (ALST * Height)] + 1.734 \quad \textit{Equation 15}$$

$$SMM\ (kg) = (1.18 * ALST) - (0.03 * Age) - 0.14 \quad \textit{Equation 16}$$

where weight was in kg, height in cm, ALST in kg, and age in years.

ANT. Validated age-specific models were applied to estimate SMM from anthropometric variables (Lee et al., 2000; Poortmans et al., 2005). Lee's model was developed and cross-validated against MRI in adults (R^2 = 0.91, SEE = 2.2 kg; equation 17) and the same formula was later used with adapted coefficients for a child and adolescent population by Poortmans et al. (R^2 = 0.966, SEE not reported; equation 18). The sample was again split into two groups as explained before, assuming that all children aged 16y and beyond had achieved stage 5 (Sun et al., 2002; Walvoord, 2010). So, Poortmans' model was used for participants under the age of 16y (pediatric group, SMM-ANT_P) and the equation of Lee et al. developed for adults was applied in participants aged 16y or older (young adult group, SMM-ANT_A). The equation of Poortmans et al. was also used in the young adult group as it was validated in youth 7-24y in order to compare which equation performed better in the comparisons with the adult model from DXA. The equations were as follows:

$$SMM\ (kg) = Height * [(0.00744 * CAG^2) + (0.00088 * CTG^2) + (0.00441 * CCG)^2 + (2.41 * Sex) - (0.048 * Age) + Race + 7.84 \quad \textit{Equation 17}$$

$$SMM(kg) = Height * [(0.0064 * CAG^2) + (0.0032 * CTG^2) + (0.0015 * CCG^2)] + (2.56 * Sex) + (0.136 * Age) \quad \textit{Equation 18}$$

where height was in m, CAG, CTG and CCG in cm, age in years, sex was 0 for female and 1 for male, and race was 0 for white.

Sport Participation (SP). Information about sport participation was obtained by *ad hoc* questionnaires. Participants were classified as sports participants (SP) if they were involved in organized sport or exercise practice a minimum of 3 times per week and with a duration of ≥3hour/week, and as non-sport participants (NSP), either they practiced sports less than 3 days per week or did not participate. The questionnaires were filled by the participants and revised on site by evaluators. Among those children who could not answer properly or had doubts about their sport practices, we asked for help from their parents, teachers or coaches.

Maturity Offset (MO). Maturity offset was assessed as predicted YPHV using the alternative models created by Moore et al. that included age x height interaction for predictions without sitting height (Moore et al., 2015). Subsequently, YPHV was subtracted to age to calculate predicted APHV. Additionally, MO was converted into a categorical variable where those subjects whom did not yet achieve their PHV (negative MO value) were classified as pre-PHV, and those with a value of zero or higher (positive MO value) were classified as post-PHV. The equations used for boys (R^2 = 0.896; SEE = 0.542) and girls (R^2 = 0.898; SEE = 0.528) were as follows:

$$Boys, MO = -7.999994 + [0.0036124 * (age * height)]$$

$$Girls, MO = -7.709133 + [0.0042232 * (age * height)] \qquad \textit{Equation 19}$$

where age was in years and height in cm.

Anaerobic performance assessment.

Static explosive strength.

Handgrip strength was measured on both non-dominant and dominant hands using a hand-held portable dynamometer with adjustable widths (Takei Hand Grip

Dynamometer, Digital display, Tokyo, Japan). Subjects were instructed to squeeze the dynamometer as hard as possible for 3 seconds after adjustment for hand size, without pressing the instrument against their body or bending at the elbow while standing. Muscle strength of lower limbs (LLS) was measured by a squat extension with a hand held portable dynamometer, with a range of measure of 0-250 kg (J Herrera, Barcelona). Two measurements were taken at 3 to 5-minute interval for each test and the best result was selected for analysis.

Dynamic explosive strength.

Vertical jumps (VJ) and sprint test were used to evaluate dynamic strength. All VJ were performed on a jump mat and jump height was measured (SmartJump, Fusion Sport®, United Kingdom). Subjects performed squat jump (SJ), countermovement jump (CMJ) and CMJ with free arms (CMJA) after a technical explanation and demonstration. They were allowed to try 2-3 times before the measured attempts to get familiarized with the movements and to assure that the correct technique was executed. Sprint test was carried out in a flat surface and photoelectrical cells were used to measure time spent in 30m (SmartSpeed™, Fusion Sport®, United Kingdom). Partial time from the start to first 15m was registered (T0-15m) and it was subtracted from the total time spent in 30m (T0-30m) to calculate partial time from 15 to 30m (T15-30m). Each subject was standing right behind the initial pair of photocells and the start was free of signal. They were instructed to run as fast as possible beyond the 30m point. The best result out of two attempts of every test was used for the analysis.

Statistical analysis. All variables are described as mean ± SD. Concurrent validity was firstly analyzed by differences between methods, and the effect size (ES) using the *d* coefficient of Cohen (*d*) (Thalheimer & Cook, 2002). Afterwards, a simple regression analysis between reference and alternative techniques was carried out and individual slopes and intercepts constants were compared with 45 degrees' line (concordance coefficient correlation, ρ_c) and zero intercept respectively; also, the two ρ_c components were calculated: a) accuracy between techniques was obtained from bias correction factor (Cb), and b) precision was described with Pearson's coefficient correlation (r) (Lin, 1989). An agreement analysis was conducted to confirm systematic and proportional bias by using Bland and Altman plots (Bland & Altman, 1986) and Kendall's Tau (τ) correlation coefficients. For construct validity, simple and partial correlations were carried out between body composition variables and strength values. Non-parametric tests were used for non-normally distributed variables. The statistical significance threshold was set at P value less than 0.05.

STUDY 3. Fluid Distribution and Regional Skeletal Muscle Mass Predict Anaerobic Performance in Adolescents: Results of Sprint and Isometric Strength.

Design. These studies were conducted in subsamples of the larger studies described in the previous studies and for whom we had valid data a written consent from participants and parents or tutors (see study 1 and 2). Acute or chronic diseases were the only exclusion criteria for these two analyses. In the first analysis, we selected all participants with valid data for molecular body composition assessment by anthropometry and BIA, and 30m sprint test; in order to facilitate the comprehension of the manuscript we will refer this analysis as *Intracellular water and Sprint Performance*. The second analysis was conducted in all adolescents with at least a valid test of LLS by isometric dynamometry and anthropometric assessment of SMM both total and regional (arm, thigh and calf); specific results and discussion from this analysis will be indicated as *Regional SMM and Lower Limb Strength*.

Sample.

Intracellular water and Sprint Performance.

One hundred and fifty-nine healthy adolescents between 12 and 18y-old (15.0 ± 1.5y) were recruited from local high-schools and local sport clubs. Athletes (AT) and non-athletes (NAT) were identified using an *ad hoc* questionnaire (NAT = 101, 41 boys, 60 girls, and AT = 58, 27 boys, 31 girls).

Regional SMM and Lower Limb Strength.

One hundred and sixty-six healthy adolescents between 12 and 18y-old (15.2 ± 1.7y, BMI = 21.9 ± 3.9 kg/m^2) were recruited from local high-schools and local sport clubs. Only 158 participants met the criteria for variables included in the models (NAT = 92, 38 boys, 54 girls, and AT = 66, 42 boys, 24 girls).

Procedures.

Body composition assessment. Body weight was measured using a digital scale (Tanita®, model UM-060) to the nearest 0.1 kg wearing light clothes and height was measured barefoot by a portable stadiometer (Tanita® Leicester) to the nearest 0.1 cm.

ANT. Standard procedures for anthropometry were performed to measure circumferences and skinfolds variables with a caliper (Holtain) and a tape respectively. The perimeters of the arm, thigh and calf were measured and triceps, thigh and calf skinfolds were removed to calculate corrected girths in each region: mid-upper arm (ACG), thigh (TCG), and calf (CCG) as follows:

$$Mc = C - (\pi * SKF) \qquad \textit{Equation 20}$$

where Mc was muscle circumference or corrected girth, C = limb circumference, and SKF = skinfold thickness, all measures in cm.

Body FM was calculated using the equations of Slaughter et al. (R^2 = 0.78, SEE = 3.8 %; equation 21) and then FFM was derived from body weight (equation 22) (Slaughter et al., 1988). Validated age-specific models were applied to obtain SMM based on anthropometric variables (Lee et al., 2000; Poortmans et al., 2005). As reported in the previous studies, the model of Poortmans et al. was used for participants under the age of 16y (R^2 = 0.966, SEE not reported; equation 23) and the equation of Lee et al. in participants aged 16y or older (R^2 = 0.91, SEE = 2.2 kg; equation 24) due to the variation of skeletal muscle distribution with maturation level (previously explained in Study 1 and 2 in this section). The equations applied were as follows:

$$Boys, FM\ (\%) = 0.735 * (Triceps + Calf) + 1.0$$

$$Girls, FM\ (\%) = 0.610 * (Triceps + Calf) + 5.1 \qquad \textit{Equation 21}$$

$$FFM\ (kg) = Weight - (FM/100 * Weight) \qquad \textit{Equation 22}$$

$$SMM(kg) = Height * [(0.0064 * CAG^2) + (0.0032 * CTG^2) + (0.0015 * CCG^2)] + (2.56 * Sex) + (0.136 * Age) \quad \textit{Equation 23}$$

$$SMM\ (kg) = Height * [(0.00744 * CAG^2) + (0.00088 * CTG^2) + (0.00441 * CCG)^2 + (2.41 * Sex) - (0.048 * Age) + Race + 7.84 \quad \textit{Equation 24}$$

where triceps and calf refer to skinfold thickness in mm, FM in percentage, weight in kg, height in m, age in years, CAG, CTG, and CCG in cm, sex was 0 for female and 1 for male, and race was 0 for white.

BIA. Bioelectrical impedance method (Lukaski et al., 1986) was used to obtain hydration variables with multifrequency bioimpedance device (MediSystem®, Sanocare, Spain). A detailed procedure of BIA method was described above (see Study 2). Intracellular water (ICW) was calculated based on the equation TBW = ECW + ICW (equation 25) after obtaining TBW by the age-specific equation of Kushner et al. (R^2 = 0.99, SEE = 1.24 kg; equation 26) (Kushner et al., 1992). All hydration variables were analyzed as absolute values (kg) and relative to body weight as percentage (%). The equations used were as follows:

$$ICW = TBW - ECW \quad \textit{Equation 25}$$

$$TBW\ (kg) = 0.593 * (Height^2/Resistance) + (0.065 * Weight) + 0.04 \quad \textit{Equation 26}$$

where height was in cm, resistance in ohms (measured at 50 kHz current), and weight in kg.

Sport participation (SP). Information about sports participation was obtained by an *ad hoc* questionnaire. Participants were classified as athletes (AT) if they were involved in organized and competitive sports at least ≥3times/week of training plus competition with a duration of ≥1hour/session, and those physically inactive or active but exercised <3hours/week and were not involved in regular training were classified as non-athletes (NAT). The questionnaires were filled by the participants and revised on site by evaluators. Among those children who could not answer properly or had doubts about their sport practices, we asked for help from their parents, teachers or coaches.

Maturity offset (MO). Predicted years from PHV (YPHV) was calculated using the equation of Moore et al. (2015) in the study about *Intracellular water and Sprint Performance* (equation 27). The equations used for boys (R^2 = 0.896; SEE = 0.542) and girls (R^2 = 0.898; SEE = 0.528) were as follows:

$$Boys, MO = -7.999994 + [0.0036124 * (age * height)]$$

$$Girls, MO = -7.709133 + [0.0042232 * (age * height)] \qquad \textit{Equation 27}$$

where age was in years and height in m.

For the study about *Regional SMM and Lower Limb Strength*, predicted YPHV was calculated with the sex specific equations of Mirwald et al. (2002) because sitting height was available in this subset sample (equation 28). The equations used for boys (R^2 = 0.915; SEE = 0.490) and girls (R^2 = 0.910; SEE = 0.499) were as follows:

$$Boys, MO = -29.769 + [0.0003007 * (leg\ length * sitting\ height)] - [0.01177 * (age * leg\ length)] + [0.01639 * (age * sitting\ height)] + (0.445 * (leg/height)]$$

$$Girls, MO = -16.364 + [0.0002309 * (leg\ length * sitting\ height)] + [0.006277 * (age * sitting\ height)] + (0.179 * leg/height) + [0.0009428 * (age * weight)] \qquad \textit{Equation 28}$$

where leg length, sitting height and height were in cm, age in years and weight in kg.

In both studies, predicted APHV was calculated by subtracting the value obtained from the equation to each subject's age (Mirwald, Baxter-Jones, Bailey, & Beunen, 2002; Moore et al., 2015).

Anaerobic Performance. A running exercise during 5 minutes and two submaximal trials of each test to warm-up was required to all participants. All tests were performed during the same morning and with at least 30 minutes of resting among tests, which were carried out with the following protocol: Firstly, a hand-held portable dynamometer device (J Herrera, Barcelona) was used to assess lower limb strength (LLS) from a squat position. Participants were asked to exercise progressively and keep the maximal strength during 2-3 seconds. Secondly, photoelectrical cells were used to measure time spent in 30m (SmartSpeed^{TM}, Fusion Sport®, United Kingdom). Subjects were asked to run at maximum speed until a cone situated at 40m from the start line (infrared cell beam was crossed at 30m). The start was free of signal with the subject standing right behind the initial pair of photocells. Partial time from the start to first 15m (T0-15m) and total time spent in 30m (T0-30m) were registered. Then, partial time from 15 to 30m (T15-30m) was calculated by subtracting T0-15m to the total time. The test was carried out in a flat surface free of wind influence and the best performance value out of two trials was used for the analyses.

Statistical analysis. Differences between groups were analyzed by independent sample T-test. Partial correlations were performed to determine associations between body composition variables and 30m sprint values and LL strength. The best-correlated variables with sprint or LLS performance were included in a stepwise regression analysis to determine body composition predictors of sprint performance after adjusting for age, sex and sport participation (AT = 1 or NAT = 0). A type I error of 5% was accepted in all statistical tests (P<0.05), which were conducted with SPSS® package (version 15.0; IBM Corporation, Armonk, NY, USA).

IV. RESEARCH STUDIES

STUDY 1.

The Relevance of Sport Participation, Body Size and Maturation on Total and Regional Skeletal Muscle Mass Along Childhood and Adolescence

STUDY 2.

Concurrent Validity of Methods for Estimating Fat Free Mass and Skeletal Muscle Mass in Children, Adolescents and Young Adults: Anaerobic Performance and Sports Participation Constructs

STUDY 3.

Fluid Distribution and Regional Skeletal Muscle Mass Predict Anaerobic Performance in Adolescents: Results of Sprint and Isometric Strength

STUDY 1.

The Relevance of Sport Participation, Body Size and Maturation on Total and Regional Skeletal Muscle Mass Along Childhood and Adolescence

INTRODUCTION

The acquisition of skeletal muscle mass (SMM) during childhood and adolescence must be a main concern to ensure healthy growth and improve motor development (Hands, Larkin, Parker, Straker, & Perry, 2009; Martins et al., 2004; Martins et al., 2011; Wells, 2007). More recently, a growing interest in SMM assessment has been renewed since positive associations between muscle fitness and health have been described: A relationship between cardiometabolic risk factors and muscle fitness has been reported in youth (Andersen, Riddoch, Kriemler, & Hills, 2011; Artero et al., 2011; Guinhouya & Hubert, 2011; Steene-Johannessen, Anderssen, Kolle, & Andersen, 2009); longitudinal data analysis have demonstrated that greater isometric muscle strength in youth is associated with lower levels of cardiovascular risk factors later in young adulthood (Grøntved et al., 2013) and can be used to predict adult metabolic syndrome independent of cardiorespiratory fitness (Fraser et al., 2016); higher thigh circumference have been associated to a lower risk of early morbidity and mortality in adults (Heitmann & Frederiksen, 2009); the conservation of a high SMM in adolescence has been reported to be associated with a better bone mass later in adulthood (Fricke et al., 2010; Jackowski et al., 2014); muscle area was found to be associated with bone strength index and modeling at the forearm during puberty (Fricke et al., 2008).

Considering results from these previous studies and the spurt of SMM may occur during the adolescence (Malina & Bouchard, & Bar-Or, 2004), reference data of total and regional SMM in children and adolescents must be of necessity for follow-up for healthy growth.

Early in the last century Matiegka proposed the first model to estimate total body SMM (Matiegka, 1921); however, only variables like height, weight and the body mass index (BMI) have been widely investigated during growth and developmental periods, and for which growth monitoring charts for clinical and field settings are available for age groups and ethnicities (Cole, 2012; Tanner & Whitehouse, 1976; Wright et al., 2002). Less focus has received the study of the evolution of SMM in youth apart from the first seminal growth studies from the last decades of the past century (Fomon et al., 1982; Malina, 1969a, 1969b, 1974; Malina & Johnston, 1967) or the development of the upper arm index for the assessment of nutritional status (Frisancho, 1974, 1981; Frisancho & Tracer, 1987; Guo, Roche, & Houtkooper, 1989; Gurney & Jelliffe, 1973; Johnston & Malina, 1966; VanItallie, Yang, Heymsfield, Funk, & Boileau, 1990).

Despite the significance of SMM to physiology, nutrition and performance, only few techniques as magnetic resonance imaging (MRI) and computerized tomography (CT) are currently available for direct measurements of SMM and they are highly expensive and CT impractical for children because of the radiation exposure. Due to the needs of an organ-tissue level technique to quantify SMM, whole body molecular level techniques have been used to estimate fat free mass (FFM) as a surrogate of SMM. As an alternative to FFM, a DXA-based whole-body approach to estimate SMM from appendicular lean soft tissue mass (ALST) has been used in children and adolescents (Kim et al., 2006; Quiterio, Carnero, Silva, Bright, & Sardinha, 2009). Considering the addressed need for generating reference data (Wells, 2014), some recent studies have contributed to the effort of fulfilling this lack. Thus, data from the study of Webber and Barr provided DXA-based measurements of SMM in children and adolescents (Webber & Barr, 2012). Additionally, new reference data for FFM were derived from 4C model in UK population (Wells et al., 2012). Although DXA is cheaper, safer and faster to apply than CT and MRI, it is still impractical for assessments in children and adolescents in many clinical and field settings. So, data obtained from more user friendly and simple techniques such as anthropometry or bioelectrical impedance (BIA) are required to assess total and regional SMM in most contexts (Wells & Fewtrell, 2006).

In this way, a recent dataset was published by McCarthy and colleagues providing BIA-derived reference curves to illustrate gender and age-related variation in FFM and appendicular skeletal muscle mass (aSMM) in children and adolescents (McCarthy et al., 2014). The traditional technique of anthropometry is more inexpensive and less dependent on environmental error (previous meal, hydration or temperature) than BIA. To our knowledge, there are only two validated equations to estimate total body SMM from anthropometric variables which have been specifically developed for children and adolescents (Lee et al., 2000; Poortmans et al., 2005). Regarding regional SMM, anthropometric measures can easily offer an estimation of regional indices such as muscle cross-sectional area (mCSA plus bone) through derived-equations with acceptable validity (Housh et al., 1995; Jones & Pearson, 1969) and are still used to develop new reference curves for nutritional-status assessment (Addo, Himes, & Zemel, 2017).

During the growth period, participation in regular physical activity programs plays a positive role in bone and lean mass accrual (Ara et al., 2006; Baxter-Jones et al., 2008) in addition to other health-derived benefits mentioned above (Andersen et al., 2011; Guinhouya & Hubert, 2011; Janssen & Leblanc, 2010; Strong et al., 2005). Nevertheless, there is need to consider account for biological maturity and body size, when examining its independent effect and explaining differences among adolescents (Baxter-Jones et al., 2005; Claessens, Lefevre, Beunen, & Malina, 2006). To our best knowledge, there is a lack in the availability of combined reference data that combine regional and total SMM derived from field techniques along with the analysis of sport participation and maturation. And, sometimes results are expressed in non-practical ways for informative comparison with other published data after adjusting for body size differences (Wells, 2001). Hence, we aimed to: 1) describe trends of regional and total SMM derived from anthropometry among age and sex groups in children and adolescents; 2) examine the influence of sport participation and maturity offset in the evolution of muscularity; and 3) provide reference data for young Caucasians across a wide-range of age.

METHODS

Study design. Cross-sectional data of anthropometry assessments from several studies conducted in our laboratory were collected from 2006 to 2015. Children and adolescents were from Northwestern, Center, East, and South of Spain. Data from a separate study performed in Lisbon (Portugal) were also pooled together. The volunteers were recruited from local schools and sports clubs through written and oral advertisements. Each participant and one parent were informed about the research procedures; afterwards, written consent was signed by parents of children who accepted to participate in the study. Inclusion criteria in this analysis were: range of age between 8 to 18y-old, be free of acute or chronic diseases, and not having any total or partial amputated limb.

Sample. The initial cohort was 1780 subjects from whom 1659 were in the range of age 8-18y. The sample was limited to Caucasians (n = 1559) because of the low representability of other ethnicities (n = 57, missing data n = 43). After selecting those with partial or total anthropometry assessments, a final sample of 1438 healthy participants were included in the analysis (66.8% boys and 33.2% girls, mean age = 13.63 ±2.60y; mean BMI

= 20.22 ±3.50 kg/m^2). A total of 632 participants were recruited from schools and 806 from sports clubs.

All procedures and consent forms were approved by the Ethical Committee Board of the Faculty of Medicine of University of Málaga for the studies performed in Spain and by the Faculty of Human Kinetics at University of Lisbon for the study carried out in Portugal.

Procedures.

Body composition. Body weight was measured using a digital scale (Tanita®, model UM-060) to the nearest 0.1kg wearing light clothes and height was measured by a portable stadiometer (Tanita® Leicester) to the nearest 0.1cm. An anthropometric classical procedure was performed to measure skinfolds thickness and circumferences variables, which were assessed by a caliper (Holtain) and tape respectively. Triceps, thigh and calf skinfolds, and arm, thigh and calf circumferences were obtained in accordance with international standards guidelines by four anthropometrists accredited by the ISAK. They all met the accepted limits for TEM in multicenter studies (<3%) (Stewart et al., 2011).

Total body FFM. FFM was calculated using a 2C model (equation 3) based on the equations of Slaughter et al. to estimate FM from skinfold measures (R^2 = 0.78, SEE = 3.8%; equation 1). If the the sum of triceps + subscapular was higher than 35mm, the alternative recommended equation was used (R^2 = 0.78, SEE = 3.7%; equation 2) (Slaughter et al., 1988).

Total body SMM. Validated age-specific models were used to estimate SMM from anthropometric variables (Lee et al., 2000; Poortmans et al., 2005). Briefly, Poortmans' model was utilized for participants under 16y and Lee's model for those aged 16y and older following the recommendation suggested by Kim et al. for maturation-related variation in SMM (Kim et al., 2006). Lee's model was developed and cross-validated against MRI (R^2

= 0.91, SEE = 2.2 kg; equation 5) and the same equation was later used with adapted coefficients for a child and adolescent population by Poortmans et al. (R^2 = 0.966, SEE not reported; equation 4).

Regional FFM. Middle upper arm, thigh and calf cross-sectional muscle areas (bone included) were calculated from each limb circumference and skinfold as follows: Firstly, muscle circumference was estimated by corrected limb circumference (equation 6). Then, the muscle area (Ma) was calculated (equation 7).

Sport Participation (SP). Sport participation information of each subject was obtained by *ad hoc* questionnaires. Participants were classified as sport participants (SP) if they were involved in sport practice a minimum of 3 times per week, and as non-sport participants (NSP), either they were practiced sports less than 3 days per week or did not participate. The questionnaires were filled by the participants and revised on site by evaluators. Among those children who could not answer properly or had doubts about their sport practices, we asked for help from their parents, teachers or coaches.

Maturity Offset (MO). Maturity offset was assessed by predicted years from peak height velocity (YPHV) using the alternative models created by Moore et al. that included age x height interaction for predictions without sitting height (boys, R^2 = 0.896, SEE = 0.542; girls, R^2 = 0.898, SEE = 0.528; equation 8) (Moore et al., 2015). Subsequently, those subjects whom did not yet achieve their PHV (negative MO value), were classified as pre-PHV, and those ones with a value of zero or higher (positive MO value) were classified as post-PHV.

Statistical Analyses. Subjects were grouped annually by chronological age from eight to eighteen years and thinness, overweight and obese categories were calculated following the International Obesity Task Force (IOTF) BMI cut-offs (Cole & Lobstein, 2012). For further analysis, participants were classified by school-age periods in Spain: <12-

old y in Primary School (P-S), 12-15y-old in Secondary School (S-S) and >15y-old in High School (H-S). Descriptive data are expressed as means ± SD. Simple regression analysis was used to explore the relationship between main variables with age and height, and slopes were compared for sex and SP groups. General linear model of mixed factors was used to analyze variance of FFM or SMM variables among age and school groups; interactions: age x sex, age x SP, age x sex x SP, School periods x sex x SP. Tukey's multiple comparison tests were carried out to observe which groups differed from each other. Additionally, a simple allometric model was applied in natural logarithmic form to describe regional and total muscle mass scaling relations to height.

Statistical significance was defined as $P<0.05$ (two tailed) for all tests. Statistical procedures were performed using SPSS® statistics software (version 15.0; IBM Corporation, Armonk, NY, USA) and GraphPad Prism (version 7; GraphPad Software, La Jolla, CA, USA).

RESULTS

The sample included 74.4% of normal weight participants (n = 1069; 732 boys, 337 girls), 16.2% of overweight (n = 232; 147 boys, 85 girls) and only 3.5% of obese (n = 50; 30 boys, 20 girls) with morbid obesity representing 0.7% of the total sample (n = 10; 7 boys, 3 girls). Thinness had a prevalence of 5.9% (n = 85; 51 boys, 34 girls). Sample characteristics are presented in table 3 by sex and chronological age groups. In girls, median of weight and height increased until 15 and 17 years of age respectively, whereas in boys, both variables increased up to the age of 18. Differences between sexes were not significant for age groups before 14y, except for girls whom were heavier at 11y and slightly smaller at 13y than their peers (P=0.035 and P=0.017, respectively). But when aligned by biological age groups (years from PHV), boys were significantly older, taller, heavier, had less fat and greater total and regional SMM than girls at all maturity ages, from -3y pre-PHV to +4y post-PHV (insufficient data for -4y group comparison).

Total body percentage (%) of SMM increased with age and reached the highest value at 18y in boys and at 17-18y in girls (table 4). The highest values of FFM were coincident with those for SMM in boys at age of 18y (kg and %) and in girls at 17y (kg), but peak %FFM in girls was seen at groups of 8y and 10y-old (77.5 ± 7.4 and 77.4 ± 5.6 respectively).

In all age groups, boys had greater mean values of %SMM and %FFM than girls (all P<0.01) except for %FFM at 10y and FFM in kg at 10-12y (P>0.05). Additionally, the spurt of SMM expressed in kg happened at earlier ages for boys (from 14 to 15y, Δ3.80 ± 0.42 kg; P<0.000) than for girls (from 15 to 16y, Δ2.61 ± 0.49 kg; P<0.000), however it occurred at similar ages when it was expressed as % of body weight (from 15 to 16y, Δ3.53 ± 0.45% in boys and Δ5.39 ± 0.58% in girls; both P<0.000) (table 4).

Regional muscle variables are presented as Mc instead of Ma because both showed similar correlation coefficients with body composition variables and less calculations are required for corrected girths (table 4). Overall, muscle girths increased with age similarly for boys and girls until age of 12y. After, it continued to increase and reached the highest value at late ages in both sexes but with significantly higher mean values in boys than in girls (P>0.05). The spurt of ACG, TCG and CCG was seen before the predicted age at PHV in girls (around 9-11y), whereas in boys was found after PHV (from 14 to 15y; all P<0.000).

Both sex groups increased their regional muscles of the arm and calf in ≈1 cm and of the thigh in ≈1.5 cm, with height controlled for. In boys, regional spurt of lower limbs was seen from 14-15y and (TCG, Δ1.66 ± 0.42 and CCG, Δ1.10 ± 0.28; both P=0.005), and from 13-14y for the upper-arm muscles (ACG, Δ1.10 ± 0.29; P=0.011). Although in girls the spurt of regional muscles was seen at younger ages, the amounts were similar to those seen in boys

(ACG, Δ1.25 ± 0.49 and TCG, Δ1.14 ± 0.55 cm from 10-11y; TCG, Δ1.56 ± 0.83 cm from 9-11y; all P>0.05).

Table 3. Characteristics of the sample by age and sex groups.

Age	**N**	**Sex**	**Age** *(y)*	**Weight** *(kg)*	**Height** *(cm)*	**BMI** *(kg/m²)*	**BF** *(%)*
8	25	Girls	8.3 ± 0.3	30.3 ± 6.9	131.4 ± 4.6	17.4 ± 3.3	22.5 ± 7.4
	46	Boys	8.5 ± 0.3	29.1 ± 5.1	130.6 ± 6.2	17.0 ± 2.3	14.3 ± 6.3
9	31	Girls	9.5 ± 0.4	34.8 ± 5.3	136.6 ± 5.7	18.6 ± 2.5	26.2 ± 6.1
	54	Boys	9.5 ± 0.3	34.2 ± 7.2	137.1 ± 5.7	18.1 ± 2.8	17.1 ± 7.5
10	24	Girls	10.2 ± 0.4	37.1 ± 9.8	143.2 ± 9.0	17.9 ± 2.7	22.6 ± 5.6
	65	Boys	10.5 ± 0.3	36.6 ± 7.2	141.8 ± 6.7	18.1 ± 2.7	19.6 ± 7.2
11	45	Girls	11.4 ± 0.3	44.4 ± 11.1	149.1 ± 8.5	19.8 ± 3.4	25.7 ± 6.7
	100	Boys	11.5 ± 0.3	39.8 ± 6.7	147.1 ± 6.8	18.3 ± 2.3	19.4 ± 6.8
12	38	Girls	12.5 ± 0.4	46.4 ± 11.5	153.0 ± 7.1	19.7 ± 3.5	23.8 ± 8.3
	114	Boys	12.4 ± 0.3	46.0 ± 10.5	153.8 ± 8.2	19.3 ± 3.1	20.5 ± 8.6
13	73	Girls	13.4 ± 0.3	50.6 ± 9.6	158.4 ± 7.3	20.1 ± 3.1	23.4 ± 5.9
	108	Boys	13.4 ± 0.3	50.8 ± 11.2	161.3 ± 8.5	19.4 ± 3.1	17.8 ± 8.9
14	73	Girls	14.4 ± 0.3	53.4 ± 10.0	159.9 ± 5.9	20.8 ± 3.5	25.7 ± 7.3
	119	Boys	14.4 ± 0.3	58.3 ± 14.6	166.8 ± 8.9	20.8 ± 4.1	17.8 ± 10.2
15	67	Girls	15.4 ± 0.3	58.2 ± 9.7	162.0 ± 7.4	22.2 ± 3.8	28.1 ± 7.8
	131	Boys	15.3 ± 0.3	66.5 ± 12.0	174.2 ± 9.7	21.8 ± 3.2	17.4 ± 7.9
16	52	Girls	16.4 ± 0.3	56.7 ± 11.5	162.8 ± 7.9	21.3 ± 3.5	26.2 ± 7.7
	116	Boys	16.3 ± 0.3	67.4 ± 12.2	175.1 ± 8.6	21.9 ± 3.1	15.6 ± 7.5
17	35	Girls	17.3 ± 0.3	59.1 ± 7.5	165.9 ± 8.3	21.6 ± 2.0	25.7 ± 6.4
	72	Boys	17.3 ± 0.3	70.6 ± 11.7	177.0 ± 10.6	22.5 ± 2.7	15.1 ± 6.2
18	15	Girls	18.1 ± 0.2	57.5 ± 7.8	164.5 ± 7.0	21.2 ± 2.4	25.1 ± 6.9
	35	Boys	18.2 ± 0.3	71.5 ± 7.9	177.7 ± 7.6	22.6 ± 2.0	12.4 ± 4.6

Age, subjects grouped annually by chronological age; BMI, body mass index; BF, body fat.

Table 4. Total and regional skeletal muscle mass (SMM) and fat free mass (FFM) variables by age and sex groups.

Group	N	Sex	FFM *(%)*	FFM *(kg)*	SMM *(%)*	SMM *(kg)*	ACG *(cm)*	TCG *(cm)*	CCG *(cm)*
8	25	Girls	77.5 ± 7.4	23.0 ± 3.2	30.1 ± 3.5	8.9 ± 1.3	16.6 ± 1.5	32.2 ± 2.4	23.4 ± 1.5
	46	Boys	85.7 ± 6.3	24.7 ± 2.9	40.3 ± 3.5	11.6 ± 1.4	16.5 ± 1.3	32.7 ± 3.0	23.1 ± 1.5
9	31	Girls	73.8 ± 6.1	25.7 ± 2.2	28.4 ± 2.8	9.9 ± 1.2	17.0 ± 1.2	33.3 ± 2.3	23.6 ± 1.5
	54	Boys	82.9 ± 7.5	27.9 ± 3.8	38.6 ± 3.2	13.0 ± 1.9	17.5 ± 1.8	34.3 ± 3.1	24.4 ± 2.0
10	24	Girls	77.4 ± 5.6	28.4 ± 5.5	30.4 ± 2.4	11.2 ± 2.5	17.1 ± 2.1	34.7 ± 3.4	25.5 ± 2.5
	65	Boys	80.4 ± 7.2	29.0 ± 3.9	39.7 ± 3.4	14.3 ± 2.0	17.6 ± 2.1	36.5 ± 2.9	25.3 ± 2.0
11	45	Girls	74.3 ± 6.7	32.5 ± 6.4	30.6 ± 2.8	13.4 ± 3.3	18.8 ± 2.4	37.4 ± 4.3	26.6 ± 2.7
	100	Boys	80.6 ± 6.8	31.8 ± 3.8	38.9 ± 2.9	15.3 ± 1.9	17.9 ± 1.7	37.5 ± 2.9	25.9 ± 1.9
12	38	Girls	76.2 ± 8.3	34.5 ± 4.1	31.6 ± 2.7	14.4 ± 2.6	19.1 ± 1.8	38.6 ± 3.4	27.5 ± 1.7
	114	Boys	79.5 ± 8.6	36.0 ± 5.7	38.5 ± 3.5	17.3 ± 2.6	19.2 ± 2.3	39.2 ± 3.3	27.6 ± 2.5
13	73	Girls	76.6 ± 5.9	38.3 ± 5.1	31.1 ± 2.5	15.6 ± 2.8	19.8 ± 1.8	39.2 ± 3.8	28.2 ± 2.0
	108	Boys	82.2 ± 8.9	41.1 ± 6.7	39.7 ± 3.8	19.9 ± 3.4	20.5 ± 2.5	41.5 ± 3.7	29.0 ± 2.8
14	73	Girls	74.3 ± 7.3	39.1 ± 4.5	31.0 ± 2.8	16.2 ± 2.4	20.1 ± 2.1	39.9 ± 3.4	28.5 ± 2.0
	119	Boys	82.2 ± 10.2	46.8 ± 8.0	39.8 ± 3.3	22.8 ± 4.4	22.2 ± 3.0	44.0 ± 4.6	30.7 ± 2.8
15	67	Girls	71.9 ± 7.8	41.3 ± 4.5	30.9 ± 2.9	17.5 ± 2.4	20.9 ± 2.1	41.1 ± 3.3	29.4 ± 1.9
	131	Boys	82.6 ± 7.9	54.4 ± 8.0	40.5 ± 3.5	26.7 ± 4.0	24.0 ± 2.6	47.3 ± 3.8	32.9 ± 2.5
16	52	Girls	73.8 ± 7.7	41.5 ± 6.2	36.3 ± 4.5	20.2 ± 2.8	20.6 ± 2.4	40.9 ± 4.0	29.4 ± 2.9
	116	Boys	84.4 ± 7.5	56.4 ± 8.7	43.9 ± 4.4	29.1 ± 3.8	24.8 ± 2.8	47.8 ± 4.4	33.6 ± 2.8

17	35	Girls	74.3	±	6.4	43.7	±	5.3	37.3	±	3.1	22.0	±	2.1	20.9	±	1.4	42.5	±	3.0	30.6	±	2.0
	72	Boys	84.9	±	6.2	59.4	±	9.7	43.8	±	3.2	30.5	±	4.2	25.2	±	2.3	48.4	±	4.1	33.8	±	2.9
18	15	Girls	74.9	±	6.9	42.8	±	5.3	37.2	±	3.6	21.3	±	2.4	20.8	±	2.1	42.1	±	3.9	29.5	±	2.4
	35	Boys	87.6	±	4.6	61.5	±	6.5	45.0	±	2.7	31.5	±	2.8	25.8	±	2.0	49.1	±	2.6	34.8	±	2.0

Group, subjects grouped annually by chronological age; FFM, fat free mass; SMM, skeletal muscle mass; ACG, arm corrected girth; TCG, thigh corrected girth; CCG, calf corrected girth.

Influence of sport participation (SP).

More than one-third of participants (77.2%) were involved in regular (at least 3 times per week) sports programs (n = 1081, 274 girls, 807 boys, 38 missing). Organized physical activities like running, dancing and resistance training were considered as sport in this study. General linear model analyses revealed positive interaction of SP and age for SMM (P=0.003), FFM (P=0.000) and regional muscle variables (ACG, P=0.003; TCG, P=0.000; CCG, P=0.001) but disappeared after introducing height as covariate. When sex was included in the model (age x sex x SP), significant interaction was found only for SMM (P=0.005) and ACG (P=0.022). And, if MO was introduced as second covariate (years from PHV), the interaction (age x sex x SP) turned into significant for all muscle variables except for TCG (SMM, P=0.008; ACG, P=0.013; TCG, P>0.05; CCG, P=0.001).

Maturity offset (MO).

Ages of MO ranged from 11 to 14y-old groups for the whole sample. Among girls, predicted PHV occurred at some point between 10 and 13y whereas for boys occurred at later ages, from 12 to 15y. At age of 11y, 17.8% of the girls were classified as post-PHV while at 12y almost all of them were post-PHV (73.7%). In boys 34.3% were post-PHV at 13y increasing the percentage up to 91.6% at 14y (table 5). All participants younger than 11y were pre-PHV and those aged 15y or older were post-PHV.

Table 5. Distribution of mature and non-mature participants per groups of age and sex.

	Girls		Boys	
Age group	**Pre-PHV**	**Post-PHV**	**Pre-PHV**	**Post-PHV**
11 years	37 (82.2%)	8 (17.8%)	100 (100%)	
12 years	10 (26.3%)	28 (73.7%)	114 (100%)	
13 years		73 (100%)	71 (65.7%)	37 (34.3%)
14 years		73 (100%)	10 (8.4%)	109 (91.6%)
15 years		67 (100%)		131 (100%)

PHV, peak high velocity.

Changes of SMM across school periods.

The analysis of SMM data calculated as mean ± SD showed increases in total and regional muscle from early to late school-age periods in all groups regardless sport practice (*P*<0.05). Yet, from S-S to H-S, only the SP group increased significantly their limb muscle girths excepting girls who did not increase their arm girth (*P*>0.05). Overall, boys had more muscle than girls in S-S and H-S but not in P-S, where only SP boys had more total body SMM than SP girls (mean difference = 3.00 kg, 95% confidence interval, CI = 1.46 to 4.54, *P*<0.000). Boys involved in sport practice had 5.62 kg (standard error of the difference, SED = 0.39) more of total body SMM than active girls in S-S and 8.93 kg (SED = 0.61) more in H-S (*P*<0.0001 for all). Among the group without SP, boys had ≈7 kg more of SMM than girls in S-S and in H-S (7.02 kg, SED = 0.61 and 7.71 kg, SED = 0.80, respectively; *P*<0.000 for both). Comparisons between SP and NSP with sex split revealed that only active boys in H-S had higher amounts of total body SMM than non-active ones (mean difference = 2.18 kg, 95% CI = 0.15 to 4.21, *P*<0.05) (figure 1).

Concerning regional muscularity, boys and girls had similar amounts of muscle girths in P-S yet in later school periods boys showed higher values than girls in S-S and in H-S regardless sport practice (P<0.05). Comparisons between SP and NSP revealed that both groups of girls did not differ in any limb variable at any school period (P>0.05) while SP boys had greater amounts of muscle in the lower limbs than NSP but only in H-S (48.95 ± 3.82 cm vs. 45.79 ± 4.18 cm for TCG, P<0.001; 34.17± 2.85 cm vs. 32.61 ± 2.21 cm for CCG, P<0.05). Conversely, NSP boys had a greater mean value than SP ones for the upper-arm in S-S (22.91 ± 3.07 vs. 21.38 ± 3.17 cm, P<0.001). Comparisons among school periods showed that all regional muscularity variables increased from P-S to S-S and to H-S in all groups but only in SP groups increased significantly from S-S to H-S (P<0.05), except for SP girls who did not change their muscle arm circumference (P>0.05).

When height was introduced as covariate in the regional SMM models, differences among school periods disappeared in all cases except for boys who were involved in sport practice. From P-S to S-S, SP boys increased ACG in 0.78 cm (95% CI = 0.67 to 1.40; P<0.01), TCG in 1.44 cm (95% CI = 0.48 to 2.40), and CCG in 1.16 cm (95% CI = 0.55 to 1.77); and, from S-S to H-S, they increased 2.19 cm in ACG (95% CI = 1.47 to 2.91; P<0.0001), 2.51 cm in TCG (95% CI = 1.39 to 3.63), and 1.88 cm in CCG (95% CI = 1.16 to 2.60; all P<0.0001).

Sex comparisons results also changed with height as covariate, in which case SP girls and SP boys did not differ in ACG or CCG means in S-S (P>0.05). Among sport practice groups, significant differences between SP and NSP boys in H-S disappeared for CCG, and were reduced for TCG in H-S (2.02 cm, SED = 0.59, P<0.05) and for ACG in S-S (-1.22 cm, SED = 0.32, P≤0.01).

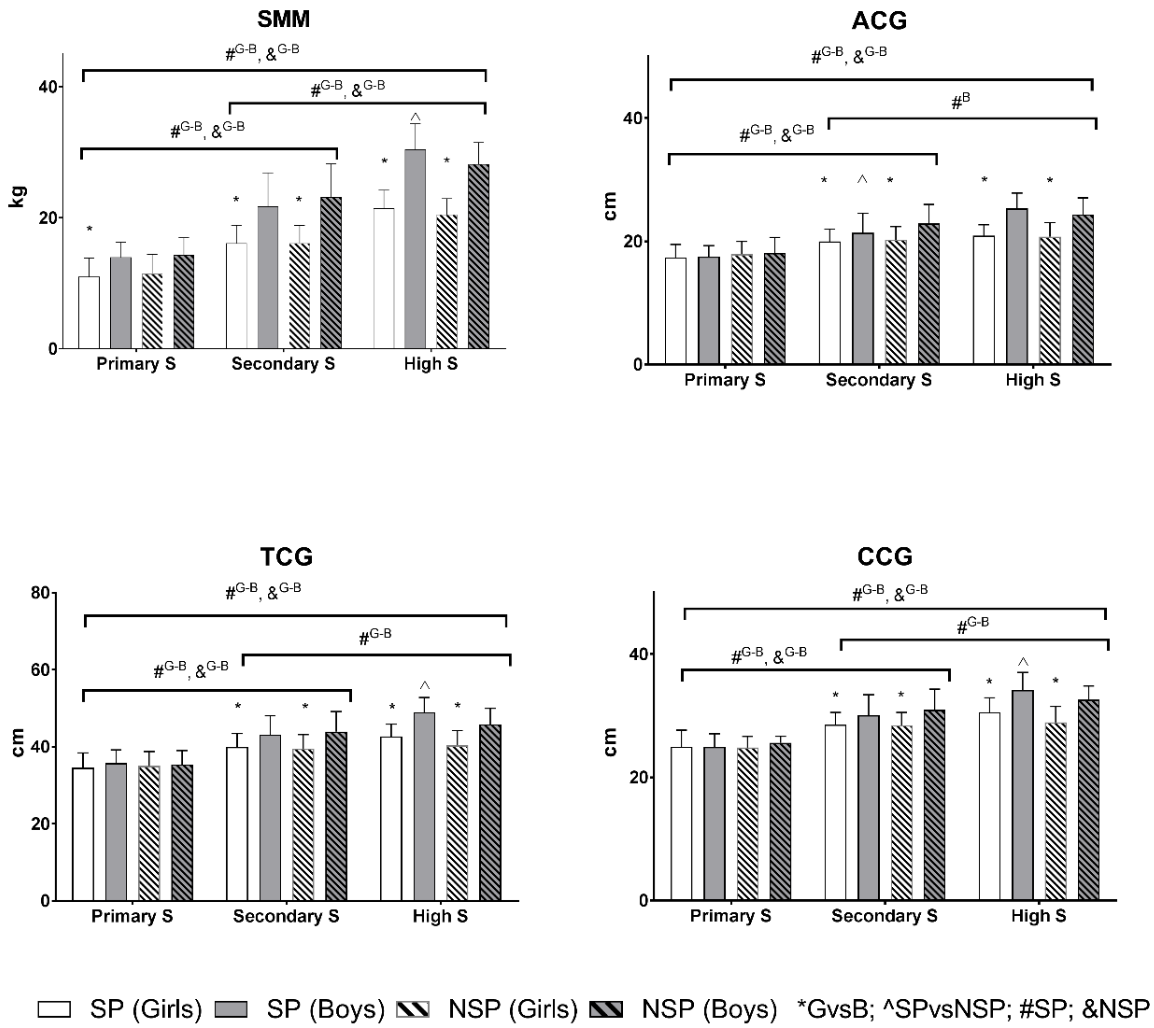

Figure 1. Comparison of total and regional muscularity across school periods. Symbols over brackets indicate *P* value <0.05 for comparisons GvsB, girls and boys; SPvsNSP, sport participant and non-sport participant; #SP, same sex and sport participant; &NSP, same sex and non-sport participant; G, girls; B, boys; S, school. ACG, arm corrected girth; TCG, thigh corrected girth; CCG, calf corrected girth.

Further comparisons were performed with height and chronological age as covariates. In this case, differences between SP and NSP groups disappeared and differences between sexes emerged for total and regional SMM in P-S. Thus, boys had ≈6 kg more of total body SMM than girls in P-S regardless sport practice (SP group, +5.93 kg, 95% CI = 4.57 to 7.29; NSP group, +5.84 kg, 95% CI = 1.85 to 9.82; $P \leq 0.001$). Relative to regional muscularity in

P-S, new sex differences were found in the SP group. Active boys had 1.45 cm more of ACG, 3.71 cm more of TCG, and 1.27 cm more of CCG than active girls. But after controlling for maturity (YPHV), SP boys only had more TCG than SP girls (2.19 cm, 95% CI = 0.38 to 4.01; $P \leq 0.01$). Comparisons among school periods after height and age were controlled for resulted in no changes for any group in TCG and CCG ($P > 0.05$); only SP boys increased their ACG in 1.43 cm from S-S to H-S (95% CI = 0.50 to 2.35; $P \leq 0.0001$) and in 1.29 cm from P-S to H-S (95% CI = 0.29 to 2.29; $P \leq 0.01$).

Evolution of regional muscularity across age by sexes and SP groups.

As expected, regional muscularity increased throughout age in all sex and sport practice groups, with lower values in girls (figure 2). Regarding sport practice, comparison of the slopes of girth and age relationship between SP and NSP was significant only for ACG in girls ($P = 0.004$), what may suggest that for girls involved in sports the arm grow at a faster rate than for those in the NSP group. Other comparisons showed that both girls and boys included in the SP group had higher amounts of muscle girths in the lower limb than those in the NSP group of the same sex, but the opposite was seen for ACG in boys (intercept differences in the regression analysis of girth and age $P < 0.0001$). Nonetheless, if height was introduced as a covariate, results showed that active boys and girls had higher muscle girths across age than their non-active peers as the slopes of girth and age relationships were significantly higher in the SP groups ($P \leq 0.001$).

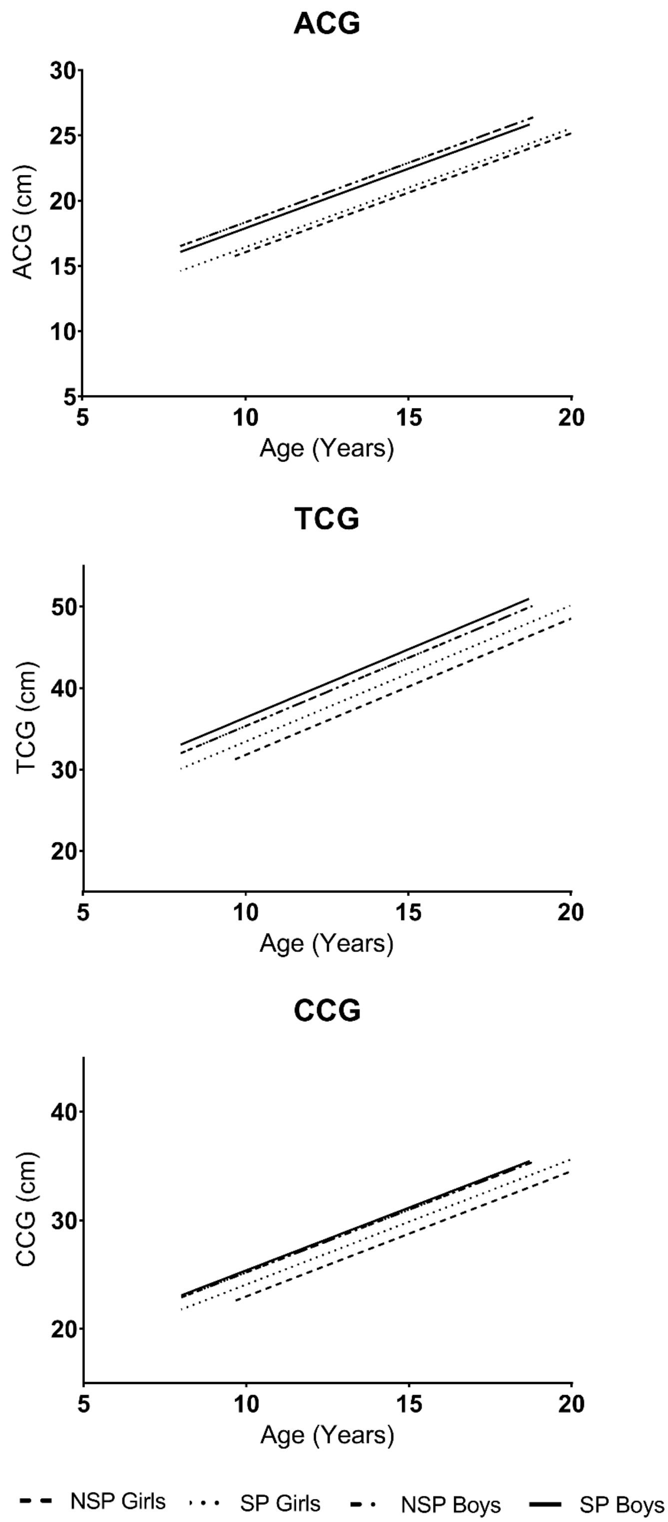

Figure 2. Regional muscularity evolution across age by sex and SP. ACG, arm corrected girth; TCG, thigh corrected girth; CCG, calf corrected girth; SP, girls and boys involved in sport participation; NSP, non-sport participation.

Body size adjustment.

Ln-ln regression analysis was performed in order to scale FFM and SMM variables to height (Ht) and find specific allometric constants for sex, maturity and SP groups. The coefficient for FFM:Ht was 2.807 for the combined sample, but sex groups differed significantly from each other as boys showed higher values than girls. Similar sex differences were found for the three regional muscle variables relations to height but not for the ratio SMM:Ht, in which case, girls showed higher values than boys (table 6). After explored the results by age group, the highest value (3.207 ± 0.299) was found in the 18y-old group and the lowest value (1.963 ± 0.245) at 9y of age (*P*=0.0012).

Sport participation analysis suggested that NSP youth had more muscle per cm of height than those who practiced sports for all variables except in the calf site, although differences were not significant (*P*>0.05). Yet, when examined SP and NSP groups by gender (table 6), SP girls showed higher slope values for all variables compared to NSP girls but only intercept value of the ratio TCG:Ht was significantly higher in the active girls (*P*=0.03). Conversely, SP boys showed lower slope values of the muscle-to-height ratios than NSP boys for all variables except for CCG:Ht but differences were not significant. However, intercept values of the regressions were significantly lower in the NSP group of boys for SMM:Ht and ACG:Ht (*P*<0.01). After controlled for chronological age (figure 3), SP boys still showed higher intercept values than NSP boys for ACG but NSP showed higher slope values for SMM:Ht compared to SP boys, while SP and NSP girls did not differ in any muscle variable (*P*>0.05).

Table 6. Slope values and standard errors of the muscle-to-height ratios by sex and sport participation.

	FFM:Ht			**SMM:Ht**			**ACG:Ht**			**TCG:Ht**			**CCG:Ht**		
Group	Mean		SE	Mean		SE	Mean		SE	Mean		SE	Mean		SE
Girls^{S}	2.55	±	0.06¥	3.14	±	0.09*	0.97	±	0.06¥	1.05	±	0.05^	0.99	±	0.04¥
Boys	2.82	±	0.03	2.95	±	0.04	1.38	±	0.03	1.21	±	0.02	1.21	±	0.02
Girls SP^{Y}	2.55	±	0.07	3.14	±	0.10	0.98	±	0.07	1.06	±	0.06*	1.02	±	0.05
Girls NSP	2.58	±	0.11	3.10	±	0.19	0.95	±	0.12	1.05	±	0.10	0.93	±	0.09
Boys SP^{Y}	2.81	±	0.03	2.91	±	0.04^	1.35	±	0.03¥	1.21	±	0.03	1.21	±	0.03
Boys NSP	2.89	±	0.15	3.14	±	0.16	1.48	±	0.12	1.26	±	0.11	1.15	±	0.10

FFM, fat free mass; ACG, arm corrected girth; TCG, thigh corrected girth; CCG, calf corrected girth; SP, sport participants; NSP, non-sport participants; SE, standard error. Slope units are: ln of Ht and girths in cm, and FFM in kg. *P<0.05, ^P≤0.01, ¥P≤0.000; ^{S}Slope differences and ^{Y}Intercept differences between sex groups.

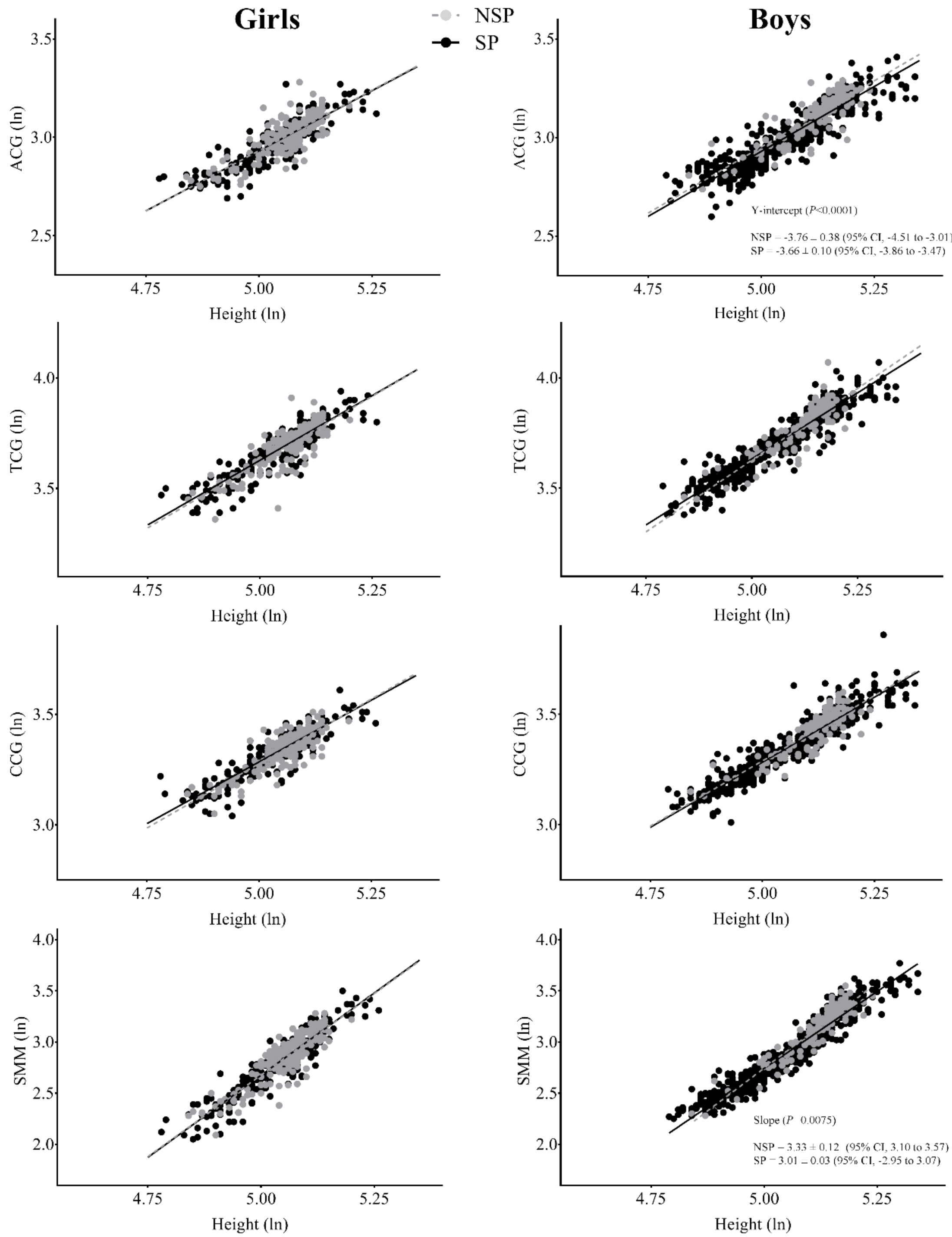

Figure 3. Ln-ln regression plots for SMM and height controlled by chronological age separated into sex and sport participation groups. ACG, arm corrected girth; TCG, thigh corrected girth; CCG, calf corrected girth; SMM, skeletal muscle mass; Ln, natural logarithm.

Further analysis was carried out between pre- and post-PHV groups within pubertal ages in boys (12-15y) and in girls (11-13y) with chronological age controlled for. The results showed that in girls, only the slope of the regression of ACG and Ht was significantly higher in the pre-PHV group (1.44 ± 0.22 vs. 0.91 ± 0.12; *P*=0.0224). In boys, a higher slope value of TCG and Ht was found in the post-PHV group (0.89 ± 0.05 vs. 1.14 ± 0.05; *P*=0.0006). The regression analysis of ACG and CCG with Ht showed that boys had higher amounts of muscle circumference per cm of height before PHV (intercept differences *P*<0.0001).

Comparisons between sport participation groups within maturity age resulted in higher slopes of TCG and Ht in active boys before PHV (0.91 ± 0.05 vs. 0.26 ± 0.20; *P*=0.0044), and in the non-active boys after PHV (1.11 ± 0.05 vs. 1.57 ± 0.24, SP vs NSP respectively; *P*=0.0235). Moreover, SP boys showed a higher intercept value after PHV for the regression of ACG and Ht (*P*=0.0114). Among girls, differences were found only in the post-PHV group for CCG with a higher slope value in the non-active group (0.78 ± 0.10 vs. 1.18 ± 0.19, SP vs NSP respectively; *P*=0.0448).

DISCUSSION

Data presented in this study show trends for total FFM and SMM in children and adolescents across a wide-range of age. Regional indicators of muscularity are also described for 13 specific age groups by sex based on anthropometric measurements. This simple and practical approach to quantify whole-body and regional FFM can be useful for physicians, physical education teachers, and coaches working with healthy pediatric and young populations. Our results may be helpful to inform of normal range of FFM, nevertheless the novel contribution of our study was to report a large dataset of muscle-to-height ratio and analyze the contribution of sport participation and maturity offset in late childhood and adolescence.

Our observations of FFM and SMM evolution with age and between genders are consistent with the literature (McCarthy et al., 2014; Webber & Barr, 2012; Wells et al., 2012). Total FFM (kg) increases with age for both girls and boys yet a non-linear age-related pattern occurs when it is expressed as relative to body weight. Specific changes by sex group occur prior to puberty, so %FFM decreases in girls and the opposite was observed in boys. This latter adaptation has been related with a major contribution of FM component in girls than in boys after this period of maturation mainly related with differences in hormonal changes. In this study, boys gained more weight from 14 to 15y but maintained the proportion of FM and FFM while girls did from 10 to 11y by increasing FM, in both cases weight increase was accompanied by growth in height. This earlier expansion of FM than FFM could be sustaining the hypothesis of a close link between the development of an energy store in the form of adipose tissue and maturation (Vizmanos & Marti-Henneberg, 2000).

Similar to FFM, SMM increases with age in a non-linear pattern although peak of SMM is attained at older ages for both sexes regardless of expressing it in absolute or relative value. These results are consistent with previous reference data obtained by BIA (McCarthy et al., 2014; Wells et al., 2012) but differed from others lean mass values derived from cross-sectional data assessed by DXA in a Spanish sample of girls between 13 to 18.5y (Vicente-Rodriguez et al., 2008). However, the sample size of this latter study was smaller than the one included in this study which may underpower the results of this latter study.

The SMM or FFM growth spurt seems to occur from 15 to 16y in girls (2.6 ± 0.48 kg gained) and from 14 to 15y in boys (3.80 ± 0.42 kg gained) for our sample. Previous studies with different body composition techniques have reported similar trends for boys but earlier ages for girls. A longitudinal study in Canadian subjects showed a peak velocity of total body lean mass at age of 12.2y in girls (5.05 ± 1.2 kg) and 13.8y in boys (8.55 ± 1.67 kg) (Rauch, Bailey, Baxter-Jones, Mirwald, & Faulkner, 2004). Reference data derived from BIA showed that boys increased the fastest aSMM up to 15y and girls did it up to 13y (McCarthy et al., 2014). For FFM derived from 4C model, growth spurt appeared to occur earlier for boys (13-14y) and girls (12-13y) from what can be advertised from the reported results (Wells et al., 2012). These small discrepancies between studies may be explained by differences in study design, age group categories, ethnicity, body composition methodology, and the component analyzed (Wells, 2014).

Relative to regional muscularity, ACG, TCG and CCG change significantly every year before and after the growth spurt period in this study (11 and 13y for girls, 12 and 15y for boys). Regarding sexual differences, boys have greater mean values than girls at the three sites at each age group except for CCG at age of 10 and 11, and for ACG at age of 11, which is in agreement with our data reporting earlier maturation in girls. Nevertheless, boys catch up girls at age of 12 and a more noticeable growth in boys than in girls occurs throughout ages 11 to 15y. Specifically, the development of ACG and TCG seems to be smoother in girls compared to boys during this time period. Our results are supported by findings of gender heterogeneity, which described marked sex differences after age of 12y in arm muscle area (Addo et al., 2017) and reported ≈1.2 times greater body circumferences in girls than in boys between 10-12y (Loos et al., 1997). Other body composition components (intramuscular fat or bone mass), maturation, body size, and intense PA (sport participation), may contribute to the observed variability across sex and age groups.

Body Composition.

The importance of FM for a healthy and normal muscle mass size has been recently highlighted in adults due to the physiological relationship between the two compartments (Bosy-Westphal & Mülller, 2015). To explore this hypothesis, we analyzed the effect of total and regional variables of FM as covariates in the prediction of regional muscularity. Our results showed that %FM was significantly associated with the size of ACG and TCG (P>0.001) but not with CCG (P=0.185). However, the results obtained for specific associated-region skinfold thicknesses showed a significant effect at the three regional sites (ACG and TCG, P>0.001; CCG, P=0.045), suggesting that calf muscles development is independent of total but not regional fat.

Regardless of the relationship with local fat, girths are also affected by bone size. The classical equations to estimate bone free mCSA must not be valid for pediatric and adolescent populations because the regression equations were derived from adult data (Heymsfield, McManus, Smith, Stevens, & Nixon, 1982). As such, simple mathematical calculations can be applied from values of limb circumferences and skinfold thicknesses to estimate regional free subcutaneous adipose tissue areas, where muscle mass is the main component (Gurney & Jelliffe, 1973; Knapik, Staab, & Harman, 1996). Even though the assumptions of concentric circles are not strictly correct, several studies have shown construct validity of this method in children and adolescents; for example, the upper arm index has been classically addressed as one of the most used health-related parameters in nutrition (Frisancho, 1974; Sen, Mondal, & Dey, 2011) and performance research (De Ste Croix, 2007).

Sport participation.

Despite the cross-sectional design of our study, our findings partially agree with published longitudinal data in the independent relationship between SP and SMM accrual during growth spurt (Ara et al., 2006; Baxter-Jones et al., 2008). Both boys and girls increased their SMM across school-age periods, and boys had significantly greater mean values than girls at every school period except when they do not practice sport in P-S.

The analysis by sex and sport participation groups suggests that after puberty, the effect of sport practice may affect total body SMM accrual and is also affected by sex because only active boys showed significantly greater SMM than non-active boys in H-S (>15y). Sex differences remained significant after controlled by body size (height) with higher amounts of total body SMM in boys than girls at every educational period but differences between SP and NSP groups of boys disappeared. Yet, only active boys increased their total SMM among school-age periods once height and age were accounting for and active girls did from P-S to H-S. For girls, increases from S-S to H-S were independent of sport practice. So, it can be interpreted that the effect of sport practice may affect total body SMM accrual throughout all school periods only in boys.

Controversial results were found for regional SMM explored across school periods by SP and sex groups. Male adolescents involved in sports had more muscle in the lower limbs than NSP adolescents in H-S (mean difference = 3.16 ± 0.68 cm, TCG, $P<0.001$; 1.56 ± 0.44 cm, CCG, $P<0.05$) but lower arm muscle girth in S-S (mean difference = -1.53 ± 0.34 cm, $P<0.001$). Yet, body size explained differences in the arm and calf muscle girths. Only SP boys had more muscle in the thigh than NSP after controlled for height. In girls, sport practice does not seem to influence SMM accrual at any school period; however, the regression analysis between individual muscle girths and age indicated that SP girls gained more arm muscle with age than NSP girls ($P<0.01$).

The evolution of regional muscularity with age suggested that sport practice does not increase the rate of lower limb muscle growth across age either in boys or in girls but the SP volunteers had higher amounts of regional muscularity, which may reveal larger circumferences (figure 2). Nonetheless, once differences in body size were accounted for, the regression analysis between individual muscle girths and age indicated that SP groups gained more limb muscle with age than NSP, what suggests faster growth in active boys and girls.

Maturity offset.

Regional muscularity at pubertal ages differed between pre- and post-PHV groups. The thigh muscles grew at a higher rate per cm of height after PHV than before PHV in boys (13-15y), and they also had higher amounts of muscle per cm of height in the upper-arm and calf after PHV (significantly different intercepts). In girls, regional muscularity grew at similar rates before and after PHV. Yet, additional analyses within sport participation groups revealed that differences between pre- and post-PHV groups were mediated by sport practice. Only the SP group of girls differed between pre- and post-PHV in ACG and TCG while muscle girths of NSP girls grew at similar rates before and after PHV. Thus, it can be interpreted that sports practice influences regional muscle-to-height growth throughout the developmental period, growing at a higher rate in the pre- than in the post-PHV group.

In boys, thigh muscle girth of the SP group was found to grow more rapidly per cm of height than NSP before reaching their PHV but not after. And, like in girls, growth rate differences between pre- and post-PHV were only present in the active group. In both genders, the rate of development of the calf seems to be independent of sport practice during growth period. All together these results suggest that, although peak muscle mass is reached after PHV, regional muscles grow at slower rates during post-PHV pubertal ages. The fact that calf site behaves in a different way from other regions could be related with a lower % of the genetic variability (64%) compared to arm and thigh (87-93%) at age of 14y (Loos et al., 1997).

Despite the limitations of using maturity prediction equations (Malina et al., 2006; Malina & Koziel, 2014a, 2014b), our findings are in line with longitudinal data on the importance of physical activity for an improved lean mass accrual during the adolescent growth period but partially disagree with our data relative to limbs (Baxter-Jones et al., 2008). Overall, these results seem to support our initial hypothesis suggesting that sport participation could positively influence skeletal muscle growth during the developmental period.

Body size adjustment.

The conventional ratio FFM:Ht may remove partially the effect of body size and permit to compare data among samples. However, allometric factors represent a more elegant and accurate way to adjust body mass data. Our findings showed differences between boys and girls in the relationship between FFM and height similar to those reported for body cell mass (2.5 for females and 3 for males) (Murphy & Davies, 2008), which may confer our data construct validity and so physiological relevance. Our results are consistent with another study that reported sex differences in FFM in early age stages of the growth period (6-7y, 8-10y, 11-13y; 14-15y only for boys) and later at 16-18y-age for males and females (Barlett, Puhl, Hodgson, & Buskirk, 1991).

The analysis of regional muscle mass scaling to height may thus help understanding and interpreting body composition measurements. On this sense, we found power values close to value of 1 unit after normalizing regional indicators by height (cm), being in all cases higher for boys than for girls, which may agree with the fact that males have more SMM not only because larger body size but for another biological reasons (hormonal).

In summary, the results of this study are unique in reporting a large dataset of total and regional muscle mass variables across gender and sport participation groups. Additionally, the current study extends previous published data focused on elucidating population differences in SMM, maturation and sport participation of children and adolescents using a simple and low-cost technique. Muscle girths data presented here may contribute to a better understanding of the phenotypes in regional development of SMM. Finally, analyses of scaling to height highlighted and confirmed that anthropometric method is useful to observe and assess the differences between sex and SP groups independent of maturation and body size. Further longitudinal studies exploring regional muscularity and sport practice are needed to corroborate our results and emphasize the physiological significance of these reference data.

STUDY 2.

Concurrent Validity of Methods for Estimating Fat Free Mass and Skeletal Muscle Mass in Children, Adolescents and Young Adults: Anaerobic Performance and Sports Participation Constructs

INTRODUCTION

During the last century pediatric body composition research was mainly aimed in providing reference data and the development of new methods for assessing fat, muscle and bone (Lohman et al., 2013). Currently, a growing interest has been focused in the relationship between health and skeletal muscle (Wolfe, 2006) and several studies have found relationships between health outcomes and muscle such as: Improved bone mass (Fricke et al., 2010; Jackowski et al., 2014), bone strength (Fricke et al., 2008). Furthermore, muscle fitness was associated with lower cardiometabolic (Steene-Johannessen et al, 2009; Artero et al, 2011; Andersen et al, 2011; Guinhouya et al, 2011) and cardiovascular risk factors (Grøntved et al., 2013), and more recently, improved vascular health was correlated with muscle strength in prepubertal children (Peplies et al., 2016).

All previous studies were conducted with sophisticated methods to assess skeletal muscle mass (SMM) or its surrogate fat free mass (FFM), such as magnetic resonance, computed-tomography or multicomponent methods, which are not available in the most part of field settings and require time consuming protocols, trained technicians, are expensive or put on risk children and adolescents. Due to this latter limitation, indirect methodologies have been created to predict SMM and FFM with mathematical models (equations) which use SMM or FFM-related variables measured by field techniques such as anthropometry and bioelectrical impedance analysis (BIA). However, the accuracy and validity of the derived equations depends on which method were validated against and if the methods selected are specific of assessed population to guarantee unbiased conclusions; however, there is no single method that is error-free (Gallagher & Song, 2003), which may affect the relationship between health outcomes or performance and SMM or FFM. So, finding the best correlated methodology for assessment SSM or FFM with biological constructs represent an important research area.

Nowadays there are highly reliable body composition techniques and models to estimated SMM or FFM in field settings at low cost. Firstly, the 2C anthropometric model developed by Slaughter et al. has been widely used to estimate FM and derived FFM by subtracting (Slaughter et al., 1988); this was one of the first specific methods to estimate FFM in children and adolescents and validated against a 4C model (gold standard or reference method to FM). On the other hand, FFM can be calculated through TBW assessments from BIA devices, which is able to provide accurate TBW estimations validated against deuterium isotope dilution technique in children (Kyle et al., 2015). Furthermore, there are also predictive equations to FFM from BIA data, which validated by multicomponent model as criterion measure (Cordain, Whicker, & Johnson, 1988; Houtkooper et al., 1992; Sun, Chumlea, Heymsfield, & Lukaski, 2003).

Regarding predictive models to estimate SMM, limited models based on accessible techniques are available to apply in children and adolescents validated with MRI analysis. To our knowledge, only Kim et al. measured SMM by MRI in a variety of sample including children, adolescents and young adults. Other available models were developed to predict SMM from DXA (Kim et al., 2004; Kim et al., 2006). However, the use of DXA is still limited for some professionals like coaches or physical education teachers. On this sense, an equation to predict SMM from a more accessible technique such as anthropometry was published for a age range sample of 7-24y (Poortmans et al., 2005), however this equation was not validated against MRI or specific for physically active children and adolescents and an indirect model was used to estimated SMM (Lee et al., 2000). Nonetheless, in our knowledge there is a lack of large cohort studies analyzing the concurrent validity of field methodologies to assess SMM or FFM in young athletes, additionally construct validity has been barely covered in the literature apart from exceptions with health variables.

Body composition constructs (components) are especially important in weight-sensitive sports but also to monitor athletic performance and training programs (Ackland et al., 2012). Moreover, physical activity during growth period was found to have an independent effect on the development of lean body mass of children and adolescents (Baxter-Jones et al., 2008). It is also a matter of fact that anaerobic performance of simple exercise must be the most important construct FFM and SMM-related since this type of exercises are highly dependent of muscle contraction. Despite these previous efforts to develop new and more accurate techniques, there is still a gap validation studies between SMM anatomy and functionality (constructs of functional body composition) (Heymsfield et al., 2014). Intervention across the entire study population necessitates instrumentation high resolution (accuracy) and reproducibility (reliability) to be able to assess functional changes. So, validity and agreement for assessments of both FFM and SMM must be main concerns in order to elucidate new health and sport performance associations. It should be pertinent to analyze if known models to estimate FFM and SMM in children and adolescents have a good association with anaerobic performance (strength) to evaluate construct validity.

Considering the previous paragraphs, the aims of this study were: 1) to analyze the validity and agreement between laboratory and field methods to estimate FFM and SMM in a large sample of children, adolescents, and young adults, and 2) to explore construct validity by the individual associations SMM or FFM and strength tests.

We firstly hypothesized that validity of FFM estimations from DXA, BIA, and anthropometry varies among techniques when compared to the reference method (deuterium oxide dilution (D_2O)); secondly, there is no agreement between estimation values of FFM and SMM estimation values assessed by DXA, BIA, and anthropometry, and the criterion method of D_2O; and finally, the relationship between FFM or SMM variables and anaerobic performance changes significantly with the technique used to assess FFM or SMM.

METHODS

Design. A series of validation studies were design using large dataset of the Biodynamic and Body Composition Laboratory in order to confirm or refuse the hypothesis formulated in the previous section. Studies were carried in children and adolescents at schools or sport clubs, where a study advertisement during physical education classes and training sessions was done to recruit participants. After informing about the research procedures, written consent was signed by all participants and by parents of subjects under age of 18. Data was collected from 2006 to 2014. Subjects were from Southeast and Northwest of Spain. Inclusion criteria in this analysis were: range of age between 6 to 21y-old, be free of acute or chronic diseases, and not having any total or partial amputated limb.

Validation studies

Concurrent Validity. External validation of FFM assessments by dual-energy X-ray absorptiometry (DXA), biological impedance analysis (BIA) and anthropometry (ANT) methodologies were carried out using deuterium oxide dilution method as reference (REF). Additionally, validity and agreement between SMM estimates by age- and sex-specific predictive equations based on anthropometry and DXA were explored.

***Construct Validity*.** Anaerobic performance assessment paradigm relied in isometric and dynamic explosive strength measurements.

Sample. A total of 531 measures of TBW from 340 volunteers (159 females and 181 males) were included in the initial analysis as reference method of FFM for the study of concurrent validity. Assessments for concurrent validity analysis for alternative FFM methods included a sample distributed as follows: FFM from DXA, n = 92; anthropometry, n = 515; and BIA, n = 487. The groups were ethnically diverse with 480 Caucasians, 15 Hispanics, 10 North-Africans, 4 Gipsies, and 6 Black-Americans in the DXA and ANT groups; and with 457 Caucasians, 12 Hispanics, 9 North-Africans, 4 Gipsies, and 6 Black-Americans in the BIA group. A total of 230 (67 girls,163 boys) had sport practice and 290 (175 girls, 115 boys) did not had it (11 missing).

Regarding SMM comparisons where predicted equations from DXA and anthropometric techniques were applied, the sample size was 225 participants (89 females and 136 males), from whom 39.6% did not practice any sport (32 girls, 28 boys) and the rest were involved in sports (54 girls, 99 boys). Sports participation variable was missed in 12 subjects. Ethnicity was composed by Caucasians (n = 203), Hispanics (n = 7) North-Africans (n = 7), Gipsies (n = 2), Black-Americans (n = 5) and Indians (n = 1).

Procedures.

Body composition assessment. Body weight was measured using a digital scale (Tanita®, model UM-060) to the nearest 0.1 kg wearing light clothes and height was measured by a portable stadiometer (Tanita® Leicester) to the nearest 0.1 cm.

Fat free mass by the Reference Method.

Isotope dilution. The isotope (deuterium oxide) dilution method was used to measure TBW in urine samples with a stable Hydra gas isotope ratio mass spectrometer (PDZ; Europa Scientific, Northwich, Cheshire, UK). The first urine sample (baseline) was taken early in the morning after an overnight fast, and the second voiding at 4 hours. The dose was 0.1 g of D_2O per kg of body weight, diluted in 30 ml of water. During the 4 hours of equilibration time, all participants were required minimal movement, no food or drink ingestion and no bladder voiding. The amount of deuterium enrichment in both samples were analyzed and TBW calculated by specific algorithms. Subsequently, the specific constants of H_{FFM} were applied for FFM estimation (equation 9) which was calculated twice using hydration constants proposed by Lohman (FFM-REF_L) and later by Wells (FFM-REF_W) (Lohman, 1986; Wells et al., 2010).

Fat free mass by the Alternative Methods.

DXA. Whole body scan was performed using DXA (Hologic, Explorer, USA) and following the manufacturer's procedures (Hologic QDR for Windows®). The system was calibrated before each session using the Hologic Anthropomorphic Spine Phantom. After completion of the scan, the image was analyzed by a trained investigator and the regions of interest (arms and legs) were isolated using the specific anatomic landmarks (TEM <3%). The system software provided the total and regional mass, fat, and bone measures. The fat mass was next subtracted from total body mass to obtain FFM (FFM-DXA).

BIA. A multifrequency bioelectrical analyzer (MediSystem-SanoCare Human Systems S.L., Spain) was used to obtain whole-body impedance values following general recommendations (Kyle et al., 2004). Four standard electrodes were placed on the right side of the body (hand and foot) and the analyzer was calibrated before the evaluation with external resistances by two trained investigators. Subjects lied in supine position on a nonconductive surface during 10 minutes before the analysis and attended at morning in fasting conditions and voided bladder; they were advised not to practice vigorous physical activity the last 48 hours (Lukaski et al., 1986). BIA analysis was done throughout the manufacturer software and two estimations of FFM were calculated. On one hand, the equation developed by Houtkooper et al. for healthy white children and youth aged 10-19y (Houtkooper et al., 1992) was used to estimate FFM (FFM-BIA; R^2 = 0.95, SEE = 2.1 kg; equation 10). On the other hand, TBW was first predicted using the equation of Kushner et al. (Kushner et al., 1992), and after, H_{FFM} constants were applied to estimate FFM similarly to the procedures by the reference method (FFM-BIA_d; R^2 = 0.99; SEE = 1.24 kg; equation 11).

ANT. An anthropometric classical procedure was performed to measure skinfolds thickness and circumferences variables, which were assessed by a caliper (Holtain) and tape respectively. Skinfolds measures bigger than 4 cm were excluded because of the caliper limit. Triceps, subscapular, thigh and calf skinfolds, and arm, thigh and calf circumferences were obtained in accordance with international standards guidelines by three anthropometrists accredited by the ISAK (TEM <3%) (Stewart et al., 2011). FFM was calculated using the 2C model (equation 14) based on previous predicted FM from anthropometric measures (FFM-ANT) applying the equations of Slaughter et al. developed for children aged 8-18y (Slaughter et al., 1988). The equation including triceps + calf was preferred and applied in 427 subjects (equation 12); the alternative equation including triceps + subscapular (equation 13) was used when skinfolds' sum was higher than 35 mm (n = 88, 52 girls, 36 boys).

Skeletal muscle mass by DXA and Anthropometry

DXA. Once the analysis of the total body scan was completed and the regions of interest (arms and legs) were isolated, the fat and bone masses were subtracted from both right and left arms and legs total masses to obtain ALST. Then, SMM was estimated using the DXA-based models developed by Kim et al. and validated against MRI (SMM-DXA). The authors found that the adult model (Kim et al., 2004) performed well in children and adolescents at Tanner stage 5 but overestimated SMM in those at Tanner stage ≤4 because of the variation of skeletal muscle distribution with maturation level. So, a pediatric specific model was developed for children with Tanner stage below 5 (Kim et al., 2006). For our sample, developmental stages were only recorded partially so, we used median age at entry into maturity stage to classify the sample into pediatric and adult groups. It was assumed that all children aged 16y and beyond had achieved stage 5 (Sun et al., 2002; Walvoord, 2010). Thus, the pediatric model was applied in subjects below the age of 16y (R^2 = 0.986, SEE = 0.502 kg; equation 15) and the adult model in subjects aged ≥16y (R^2 = 0.965, SEE = 1.38 kg; equation 16).

ANT. Validated age-specific models were applied to estimate SMM from anthropometric variables (Lee et al., 2000; Poortmans et al., 2005). Briefly, Lee's model was developed and cross-validated against MRI in adults (R^2 = 0.91, SEE = 2.2 kg; equation 18) and the same equation was later used in children and adolescents with adapted coefficients by Poortmans et al. (R^2 = 0.966, SEE not reported; equation 17). Following the same logic than for DXA maturation-related variation in SMM (Kim et al., 2006), Poortmans' model was utilized for participants under 16y (pediatric group, SMM-ANT_P) and Lee's model for those aged 16y and older (young adult group, SMM-ANT_A). Additionally, Poortmans' model was also used for participants aged 16y and older (young adult group) as it was validated in youth 7-24y in order to compare which model performed better in the comparisons with the adult model from DXA.

Sport Participation (SP). Information about sport participation was obtained by *ad hoc* questionnaires. Participants were classified as sports participants (SP) if they were involved in organized sport practice 3 or more times per week, and otherwise as non-sport participants (NSP). The questionnaires were filled by the participants and revised on site by the evaluators. Among those children who could not answer properly or had doubts about their sports practices, we asked for help from their parents, teachers or coaches.

Maturity Offset (MO). Maturity offset was assessed as YPHV using sex-specific predictive equations (boys, R^2 = 0.896; SEE = 0.542; girls, R^2 = 0.898, SEE = 0.528; equation 19) that included age x height interaction (Moore et al., 2015). Subsequently, predicted APHV was calculated by subtracting YPHV to each subject's age.

Anaerobic performance assessment.

<u>Static explosive strength</u>. Handgrip strength was measured on both non-dominant and dominant hands using a hand-held portable dynamometer with adjustable widths (Takei Hand Grip Dynamometer, Digital display, Tokyo, Japan). Subjects were instructed to squeeze the dynamometer as hard as possible for 3 seconds, without pressing the instrument against their body or bending at the elbow. Lower limbs muscle strength (LLS) was measured by a squat extension with a hand held portable dynamometer, with a range of measure of 0-250 kg (J Herrera, Barcelona, Spain). Two measurements were taken at 3 to 5-minute interval for each test and the best result was selected for analysis.

Dynamic explosive strength. Vertical jumps (VJ) and sprint test were used to evaluate dynamic strength. VJ were performed on a jump mat and jump height was measured (SmartJump, Fusion Sport®, United Kingdom). Subjects performed squat jump (SJ), countermovement jump (CMJ) and CMJ with free arms (CMJA) after a technical explanation and demonstration. They were allowed to try 2-3 times before the measured attempts to get familiarized with the movements and to assure that the correct technique was executed. Sprint test was carried out in a flat surface and photoelectrical cells were used to measure time spent in 30m (SmartSpeed™, Fusion Sport®, United Kingdom). Partial time from the start to first 15m was registered (T0-15m) and it was subtracted from the total time spent in 30m (T0-30m) to calculate partial time from 15 to 30m (T15-30m). Each subject was standing right behind the initial pair of photocells and the start was free of signal. They were instructed to run as fast as possible beyond the 30m point. The best result out of two attempts of every test was used for the analysis.

Statistical analysis. All variables are described as mean ± SD. Concurrent validity was firstly analyzed by differences between methods, and the effect size (ES) using the *d* coefficient of Cohen (*d*) (Thalheimer & Cook, 2002). Afterwards, a simple regression analysis between reference and alternative techniques was carried out and individual slopes and intercepts constants were compared with 45 degrees' line (concordance coefficient correlation, ρ_c) and zero intercept respectively; also, the two ρ_c components were calculated: a) accuracy between techniques was obtained from bias correction factor (Cb), and b) precision was described with Pearson's coefficient correlation (r) (Lin, 1989). An agreement analysis was conducted to confirm systematic and proportional bias by using Bland and

Altman plots (Bland & Altman, 1986) and Kendall's Tau (τ) correlation coefficients. For construct validity, simple and partial correlations were carried out between body composition variables and strength values. Non-parametric tests were used for non-normally distributed variables. The statistical significance threshold was set at *P* value less than 0.05.

RESULTS

Validity of FFM

The characteristics of the sample are summarized in table 7. A total of 531 measures of TBW were analyzed (287 boys; 244 girls). Two measures were excluded from the analysis because TBW from deuterium dilution was more than 72% of total body weight and was considered out of biological limits. Another measure of FFM estimated by BIA was discarded as it showed a difference of more than 30 kg compared to the reference method in a subject weighted 115 kg.

The sample was composed by 68% measurements of normal weight participants (n = 361; 186 boys, 175 girls), 18.3% of overweight (n = 97; 58 boys, 39 girls), 6% of obese and 1.9% of morbid-obese (n = 42; 31 boys, 11 girls). Thinness had a prevalence of 5.8% (n = 31; 12 boys, 19 girls).

In general, participants presented a wide range in body composition variables. Body weight and height ranged from 20.5 kg and 115.8 cm to a maximum of 132.7 kg and 186.6 cm respectively. Mean value for hydration of body weight from deuterium technique was 60.36% for the entire sample. Boys were significantly taller, heavier, and had more FFM and TBW than girls, who had significantly more fat than boys (table 7). There were more percentage of sport participants within the group of boys (58.68%) than within the group of girls (27.69%).

Table 7. Descriptive characteristics of the sample with measurements from deuterium technique.

		GIRLS		BOYS	
Variables		Mean	±SD	Mean	±SD
Age	*(years)*	14.67	2.56	14.64	2.76
Sport	*(SP/NSP)*	(67 / 175)		(163 / 115)	
Height	*(cm)*	157.43[¥]	8.98	164.15	14.37
Weight	*(kg)*	52.27[¥]	10.34	60.13	18.27
BMI	*(kg/m^2)*	20.96	3.21	21.83	4.50
FM[1]	*(%)*	25.68[¥]	5.86	20.64	9.97
FM[1]	*(kg)*	13.85[¥]	5.27	13.60	11.22
FFM-REF_L	*(kg)*	40.85[¥]	7.90	48.84	13.33
FFM-REF_W	*(kg)*	41.48[¥]	7.95	49.02	13.42
FFM-REF_L	*(%)*	78.56[¥]	6.45	82.01	6.14
TBW[2]	*(%)*	59.29[¥]	5.01	61.27	4.67
TBW[2]	*(kg)*	30.80[¥]	5.83	36.41	9.74

SD, standard deviation; Max., maximum; Min, minimum; M, male; F, female; SP, sport participant; NSP, non-sport participant; BMI, body mass index; FM%, fat mass percent; FM, fat mass; FFM, fat free mass; TBW, total body water; [1]Estimated by anthropometric equations (Slaughter, 1988); [2]Estimated by the reference method; **P*<0.000 between FFM-REF_L and FFM-REF_W.

Measures of FFM from the reference method using Wells' hydration constants of FFM (FFM-REF_W) were significantly higher than those calculated using Lohman's (mean difference = -0.38 ± 0.47 kg; rho = 0.99; *P*<0.0001). Regression analysis between the two proposed hydration constants of FFM revealed that slope was significantly different from zero (*P*<0.0001). Although the two FFM calculations (Wells and Lohman) showed high coefficients of correlations (range, *r* = 0.83 to 0.91), FFM-REF_L showed lower mean differences and effect size, and slightly higher correlation coefficients when compared with alternative methods compared to FFM-REF_W (not shown). So, subsequent analyses were performed with FFM-REF_L as the criterion measure.

Overall, all FFM measures from alternative techniques underestimated FFM measures relative to deuterium dilution for the sample pooled together. FFM from ANT showed the lowest bias (-2.29; 95% CIs, -2.77 to -1.80), and similar values were found for FFM-BIA (bias = -2.42; 95% CIs, -2.82 to -2.02). Both techniques showed little differences compared to the reference method as denoted by a small effect size. Conversely, large differences were found between FFM-DXA and FFM-REF_L (bias = -5.48; 95% CIs, bias = -6.12 to -3.88) and for FFM derived from estimating TBW by BIA (-4.83; 95% CIs, -5.27 to -4.28) as denoted by medium effect size.

The analyses by sexes (table 8) revealed that all the alternative methods correlated better with FFM-REF_L in the group of boys than in girls, and best alternative method was not coincident in both groups. Anthropometry was the alternative method that showed the lowest differences with the reference and small effect size in the group of girls, and BIA in boys (≈6% and ≈4% of underestimation respectively).

Table 8. Differences between reference and alternative methods for FFM (ALT-REF) by groups of sex.

			Differences				T-test	Effect Size	*r*
Variables		***Group***	Mean		SD	Mean (%)	*P*	Cohen's *d*	
FFM-DXA	*(kg)*	*Girls*	-7.03	±	4.66	-18.79	<0.000	1.46	0.60
FFM-BIA	*(kg)*	*Girls*	-3.14	±	4.36	-8.45	<0.000	0.44	0.83
FFM-ANT	*(kg)*	*Girls*	-2.33	±	4.41	-6.11	<0.000	0.33	0.83
FFM-BIA_d	*(kg)*	*Girls*	-6.25	±	5.11	-18.77	<0.000	0.88	0.76
FFM-DXA	*(kg)*	*Boys*	-3.80	±	5.45	-7.18	<0.000	0.42	0.87
FFM-BIA	*(kg)*	*Boys*	-1.79	±	4.51	-3.87	<0.000	0.14	0.94
FFM-ANT	*(kg)*	*Boys*	-2.25	±	6.52	-4.85	<0.000	0.18	0.87
FFM-BIA_d	*(kg)*	*Boys*	-3.49	±	5.68	-8.53	<0.000	0.27	0.91

SD, standard deviation; FFM, fat free mass; BIA, bioelectrical impedance; ANT, anthropometry; BIA_d, FFM calculated from estimation of TBW from bioelectrical impedance; *r*, Pearson's correlation coefficient; *P*, significance values for nonparametric paired sample T-test analysis.

Regarding concordance between measurements for the entire sample, low to high coefficients were found for all comparisons between FFM-REF_L and alternative techniques (range ρ_c, 0.834-0.916). The best concordance was found between FFM-BIA and the reference method (ρ_c = 0.916) and also the best bias correction factor was found for FFM-BIA. Nonetheless, FFM from the other techniques showed also values close to 1 (C_b range 0.974-0.995), which reflects high accuracy for all techniques. Precision ranged from the lowest of 0.86 for DXA to the highest value of 0.93 for BIA.

In both genders, FFM-BIA was the method that showed the best concordance results although in girls it was lower than the range seen for the combined sample (ρ_c = 0.764) while in boys it was the highest among the alternative methods (ρ_c = 0.926). It was also the more accurate and precise among the alternative methods in girls, yet FFM-ANT showed slightly lower concordance results than those found for BIA with the deuterium method (figure 4). In boys, both BIA alternative methods showed similar bias correction factor although concordance and precision were lower in the derived BIA method (figure 5).

Regression analysis between FFM-REF_L and estimations from alternative techniques confirmed FFM-BIA as the alternative technique that performed better with the reference method. The slope of the regression between FFM-REF_L and FFM-BIA was similar to the 45º line, as well as it was for FFM-ANT and FFM-DXA in the sex-combined sample (P>0.05). However, intercepts of the regression were significantly different from zero, which reflects systematic error in the three techniques (P<0.0001). The regression between FFM-REF_L and FFM-BIA_d revealed a proportional error as slope differed significantly from the identity line (P<0.001).

The regression analysis performed by sex groups showed significant differences between the slopes of FFM-DXA and FFM-BIA with the identity line in boys (P=0.0034 and

P=0.0466, respectively) while in girls, slopes of the alternative methods did not differ from the 45º line in any case (*P*>0.05). Yet, systematic error was found for the four alternative techniques in girls, and for the two other remained techniques in boys (ANT and BIA_d; all *P*<0.0001).

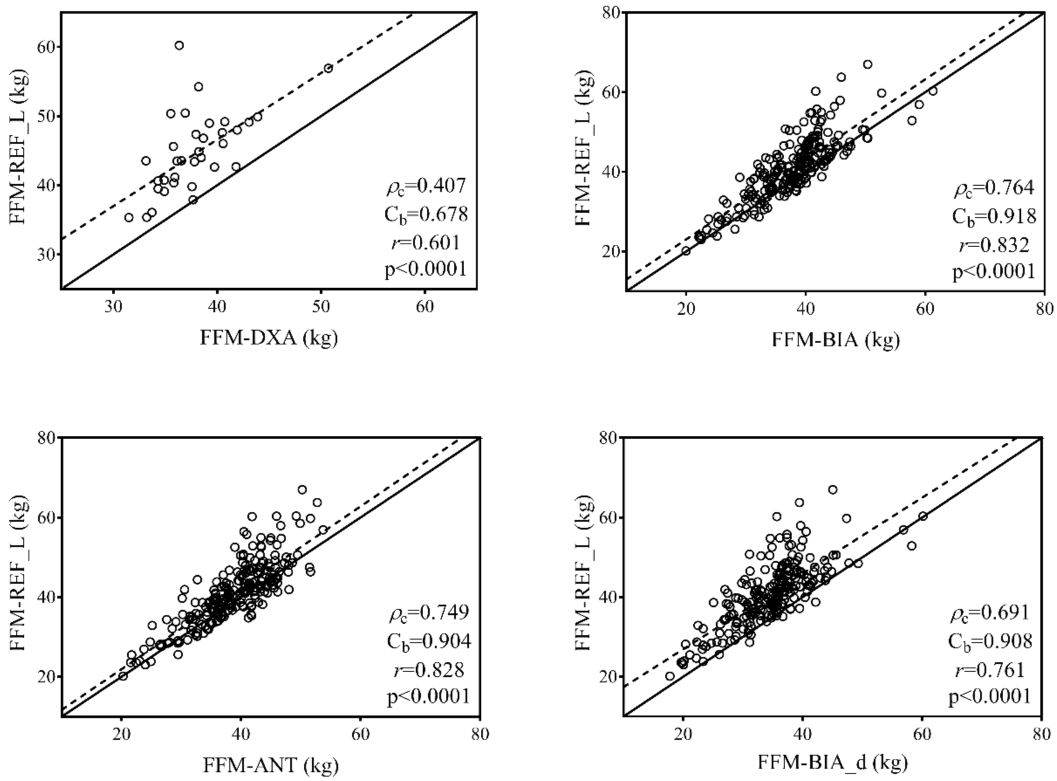

Figure 4. Scatter plot between FFM measurements by reference and alternative techniques in girls. Reproducibility (concordance correlation coefficient, ρ_c), accuracy (bias correction factor, C_b) and precision (Pearson's correlation coefficient, *r*) of FFM. Dashed line is fit line of simple linear regression between reference and alternative pairs of data; solid line is identity line (perfect reproducibility).

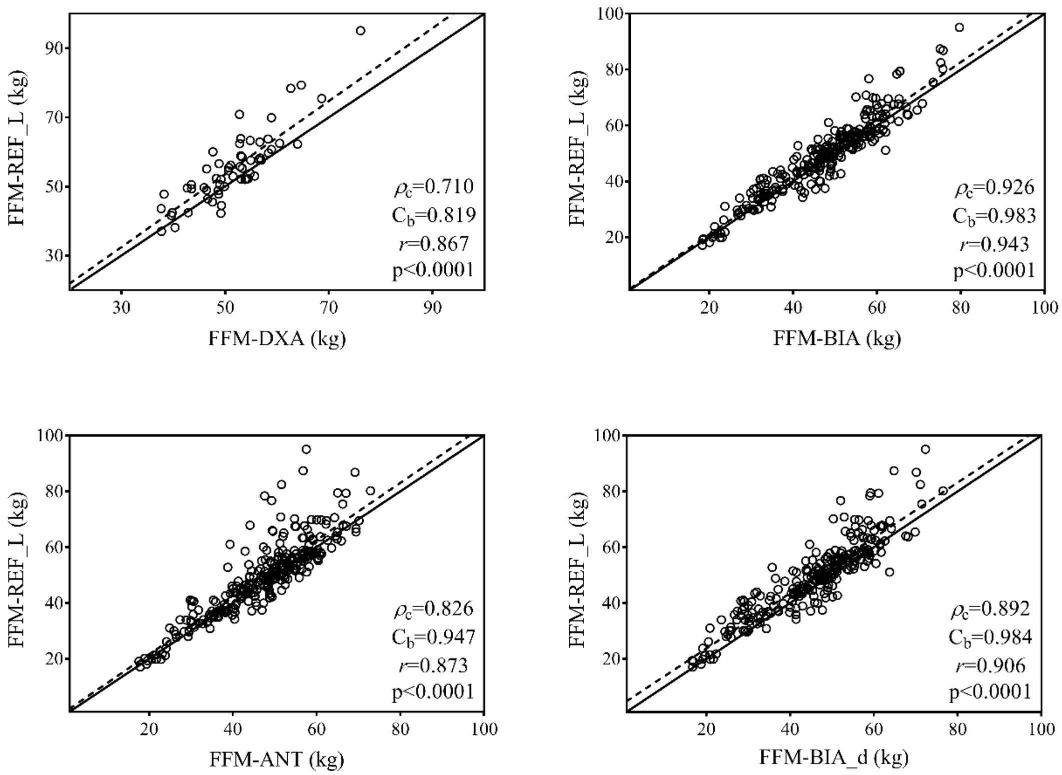

Figure 5. Scatter plot between FFM measurements by reference and alternative techniques in boys. Reproducibility (concordance correlation coefficient, ρ_c), accuracy (bias correction factor, C_b) and precision (Pearson's correlation coefficient, *r*) of FFM. Dashed line is fit line of simple linear regression between reference and alternative pairs of data; solid line is identity line (perfect reproducibility).

The agreement between the reference and each of the alternative techniques for FFM showed wide limits. For the entire sample, systematic error was found for all comparisons (P>0.0001) and a significant negative tendency was found for FFM-BIA and FFM-ANT (τ = -0.075, P=0.013 and τ = -0.112, P=0.000, respectively), which reflects a low agreement as the amount of the measures increased with the size of measurement.

For the sample split by sexes and sport participation groups (figures 6 and 7), systematic error was present in both genders for all the alternative techniques compared to the reference method regardless sport practice (P<0.01). However, proportional error was found only in the non-active groups of boys and girls, with the exception of FFM-BIA which also showed a negative tendency in the SP group of boys (τ = -0.136, P=0.017).

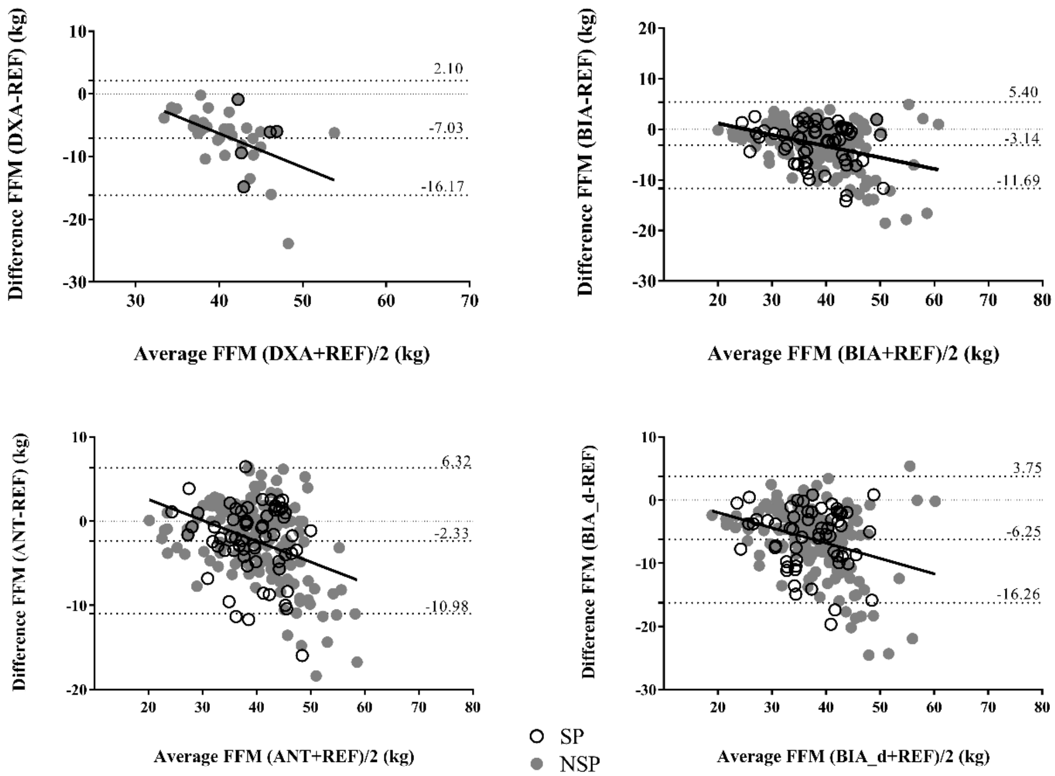

Figure 6. Agreement between FFM measurements by reference and alternative techniques in girls by groups of sport practice. Bland-Altman plot for the corresponding linear relation as the average vs. differences; the dotted horizontal lines are mean differences and 95%CIs for the sample pooled together.

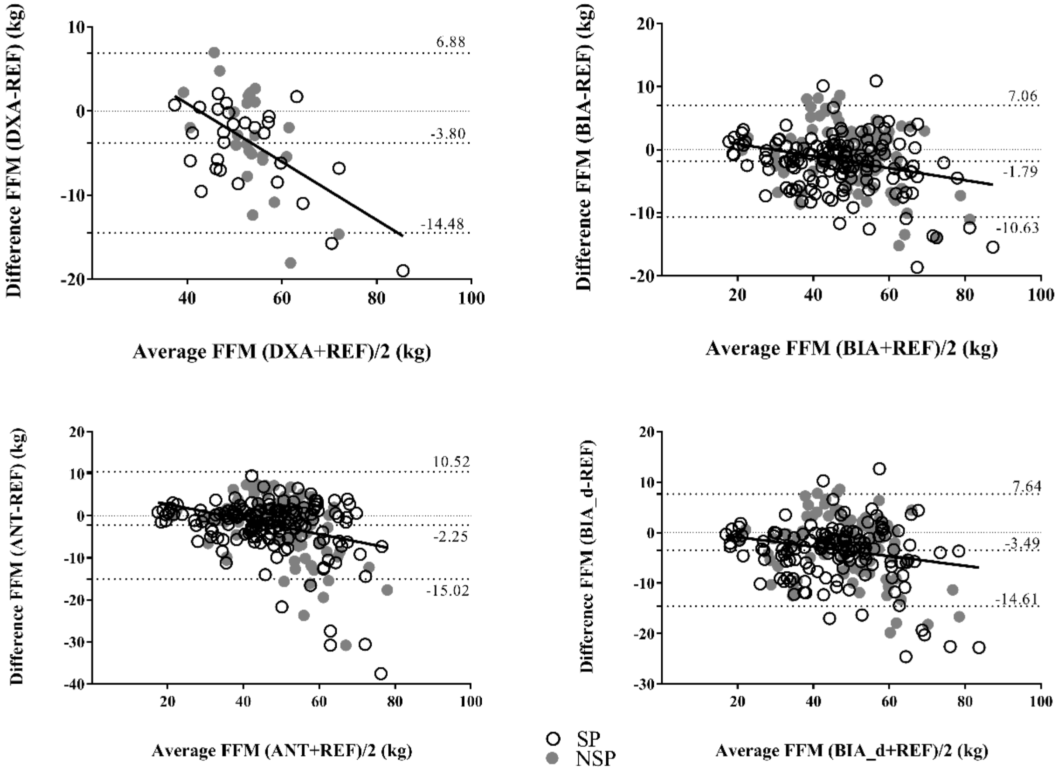

Figure 7. Agreement between FFM measurements by reference and alternative techniques in boys by groups of sport practice. Bland-Altman plot for the corresponding linear relation as the average vs. differences; the dotted horizontal lines are mean differences and 95%CIs for the sample pooled together.

A reduction of means differences was observed for all alternative techniques when only measures from normal-weight subjects were analyzed in the entire sample, but all remained significant (FFM-DXA, bias = -4.16 ± 3.91 kg; FFM-BIA, -1.74 ± 3.74 kg; FFM-ANT, -0.73 ± 3.47 kg; FFM-BIA_d, -3.72 ± 4.57 kg; *P*<0.000 in all comparisons). The ES increased from 0.51 to 0.59 for FFM-DXA but was reduced for all other variables (Coen's *d* = 0.19, FFM-BIA; *d* = 0.08, FFM-ANT; *d* = 0.38, FFM-BIA_d). Precision was very close to the previous results where all assessments were pooled together (*r* = 0.86, FFM-DXA; *r* = 0.93, FFM-BIA; r = 0.88, FFM-ANT; *r* = 0.89, FFM-BIA_d). Perfect accuracy was found for FFM-ANT (C_b = 1.0), and almost perfect for BIA and BIA_d (both C_b = 0.99). Accuracy of FFM-DXA was reduced (C_b = 0.946).

Relative to the analysis among sport participation groups of mean differences between the reference and the alternatives techniques, the ES was lower in the SP groups for genders pooled together. But comparisons with sex groups split showed different results. In girls, ES was higher in the SP groups for the four alternative techniques (table 9). Controversial results were found in boys, where the ES of comparisons between reference and FFM-DXA and FFM-ANT were lower in the active groups but higher in the NSP group for FFM-BIA and FFM-ANT (table 10). Nonetheless, FFM estimated by BIA continued to be the most accurate and precise alternative technique for both SP and NSP groups of boys and FFM from ANT in girls, yet FFM-BIA showed higher precision in the SP group of girls (0.76 vs 0.81, respectively) and similar in the NSP (0.85 vs 0.84, ANT vs BIA).

Table 9. Differences between reference and alternative methods for FFM (ALT-REF) in girls.

Variables		Group	Differences Mean		SD	Mean (%)	T-test *P*	Effect Size Cohen's *d*	*r*
FFM-DXA	*(kg)*	*NSP*	-6.96	±	4.67	-18.70	<0.000	1.42	0.64
FFM-DXA	*(kg)*	*SP*	-7.44	±	5.14	-19.27	0.063	2.48	-0.17
FFM-BIA	*(kg)*	*NSP*	-3.16	±	4.45	-8.36	<0.000	0.43	0.84
FFM-BIA	*(kg)*	*SP*	-2.96	±	4.03	-8.40	<0.000	0.47	0.81
FFM-ANT	*(kg)*	*NSP*	-2.32	±	4.43	-5.92	<0.000	0.31	0.85
FFM-ANT	*(kg)*	*SP*	-2.23	±	4.27	-6.29	<0.000	0.37	0.76
FFM-BIA_d	*(kg)*	*NSP*	-6.31	±	5.17	-18.69	<0.000	0.86	0.77
FFM-BIA_d	*(kg)*	*SP*	-5.91	±	4.80	-18.59	<0.000	0.91	0.73

NSP, non-sport participant; SP, sport participant; SD, standard deviation; FFM, fat free mass; BIA, bioelectrical impedance; ANT, anthropometry; BIA_d, FFM calculated from estimation of TBW from bioelectrical impedance; *r*, Pearson's correlation coefficient; *P*, significance values for nonparametric paired sample T-test analysis.

Table 10. Differences between reference and alternative methods for FFM (ALT-REF) in boys.

			Differences				**T-test**	**Effect Size**	***r***
Variables		**Group**	Mean		SD	Mean (%)	*P*	Cohen's *d*	
FFM-DXA	*(kg)*	*NSP*	-3.20	±	6.00	-5.98	0.014	0.45	0.75
FFM-DXA	*(kg)*	*SP*	-4.40	±	5.18	-8.40	<0.000	0.40	0.93
FFM-BIA	*(kg)*	*NSP*	-1.22	±	4.61	-2.50	0.011	0.11	0.89
FFM-BIA	*(kg)*	*SP*	-2.23	±	4.50	-4.87	<0.000	0.16	0.95
FFM-ANT	*(kg)*	*NSP*	-2.08	±	6.58	-4.32	0.017	0.20	0.82
FFM-ANT	*(kg)*	*SP*	-2.45	±	6.64	-5.34	<0.000	0.18	0.89
FFM-BIA_d	*(kg)*	*NSP*	-2.82	±	5.63	-6.42	<0.000	0.26	0.87
FFM-BIA_d	*(kg)*	*SP*	-4.02	±	5.79	-10.05	<0.000	0.29	0.92

NSP, non-sport participant; SP, sport participant; SD, standard deviation; FFM, fat free mass; BIA, bioelectrical impedance; ANT, anthropometry; BIA_d, FFM calculated from estimation of TBW from bioelectrical impedance; *r*, Pearson's correlation coefficient; *P*, significance values for nonparametric paired sample T-test analysis.

Concordance between measurements was always better in the active group than in the non-active group for all techniques in the entire sample. Overall, concordance correlation coefficients were higher in boys than in girls (figure 8 in girls and figure 9 in boys). The highest concordance was found for the alternative method of BIA in both genders regardless sport participation but better coefficients in the SP than in the NSP groups (girls, 0.785 and 0.761; boys, 0.936 and 0.885, SP and NSP). Accuracy of the alternative techniques of BIA, BIA_d, and ANT were very similar in girls, and for BIA and BIA_d in boys.

Regression analysis within sport participation groups with genders pooled together revealed that slopes were significantly different from zero when comparing the reference method with each alternative technique regardless sport practice. A systematic error of estimation was found for FFM-DXA in both groups ($P<0.05$), and also for FFM-BIA_d but only in the NSP group ($P<0.0001$). Estimates of FFM from BIA and ANT showed proportional error compared to the reference method in both groups ($P<0.0001$). The agreement analysis between the reference and each of the alternative techniques showed systematic error for all comparisons in the SP and in the NSP group ($P>0.0001$). Moreover, the previous proportional errors found in the entire sample were found to be only significant FFM-ANT in the NSP group (τ = -0.155, $P<0.001$), and for FFM-BIA in the SP group (τ = -0.095, $P<0.046$).

The four alternative techniques showed significant slope deviation from zero in both sexes ($P\leq0.001$), except for FFM-DXA in the group of active girls ($P=0.779$) but number of participants was very low (n = 5). In the SP groups were also found proportional errors between the reference method and FFM-BIA_d in girls ($P=0.0337$), and FFM-DXA in active boys ($P=0.028$). In all other comparisons, a systematic error was found between the reference and the alternative methods regardless sex and sport practice ($P\leq0.01$).

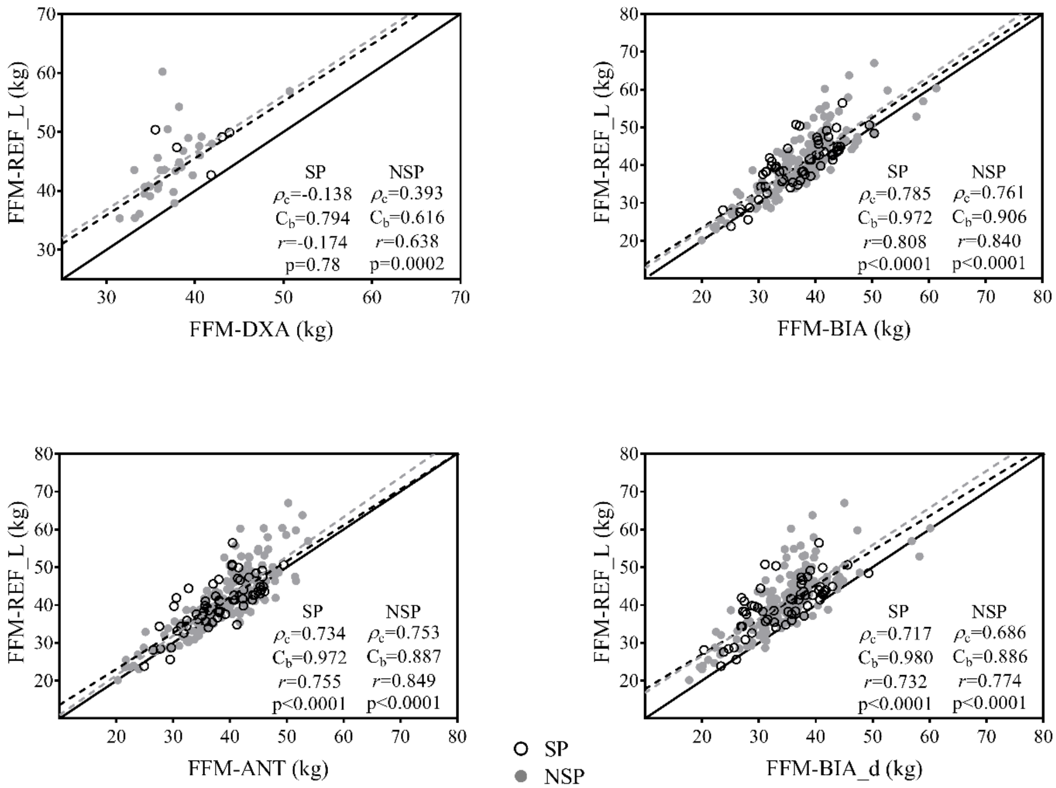

Figure 8. Scatter plot between FFM measurements by reference and alternative techniques in girls by groups of sport practice. Reproducibility (concordance correlation coefficient, ρ_c), accuracy (bias correction factor, C_b) and precision (Pearson's correlation coefficient, *r*) of FFM. Dashed line is fit line of simple linear regression between reference and alternative pairs of data; solid line is identity line (perfect reproducibility).

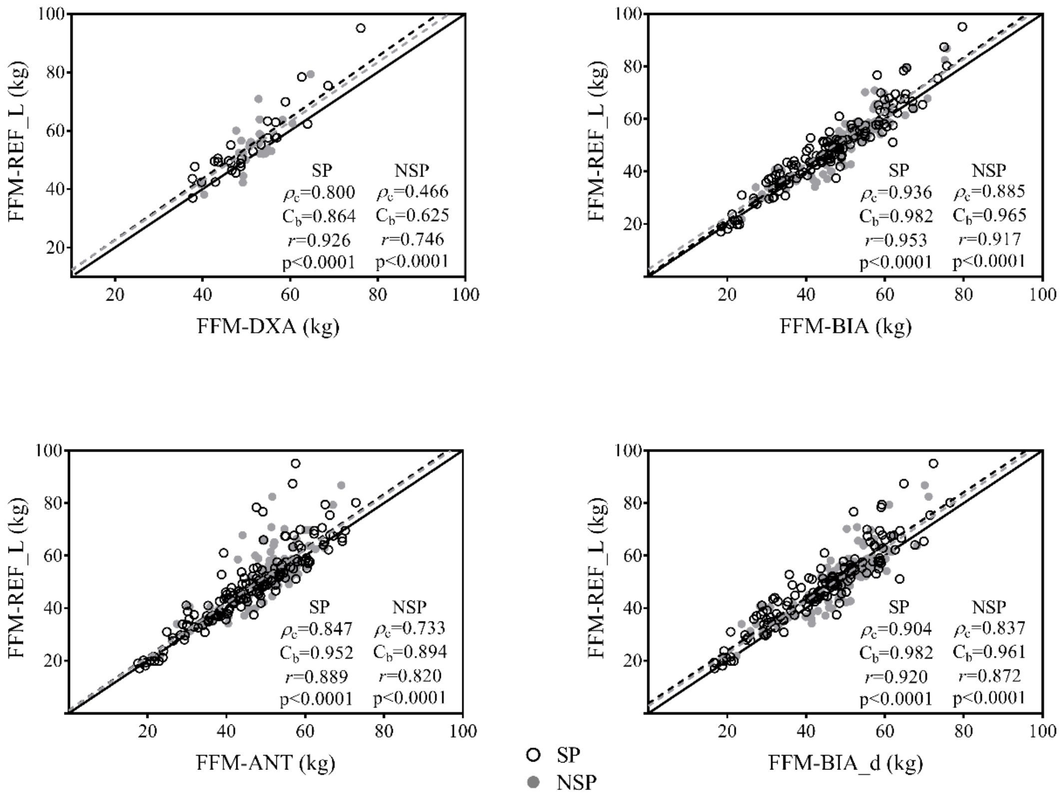

Figure 9. Scatter plot between FFM measurements by reference and alternative techniques in boys by groups of sport practice. Reproducibility (concordance correlation coefficient, ρ_c), accuracy (bias correction factor, C_b) and precision (Pearson's correlation coefficient, *r*) of FFM. Dashed line is fit line of simple linear regression between reference and alternative pairs of data; solid line is identity line (perfect reproducibility).

Validity of SMM

The characteristics of the sample included in the validity of the SMM are presented in table 11. For the entire sample, mean age and BMI were 14.65y and 20.45 kg/m^2 respectively. SMM measures from DXA were significantly lower than measures from ANT (*P*<0.000). Boys and girls differed in all variables with higher values seen in boys, but both groups had similar mean age and BMI (*P*>0.05).

Table 11. Descriptive characteristics of the sample with measurements of SMM by DXA and ANT by groups of sex.

		GIRLS		BOYS	
Variables		Mean	SD	Mean	SD
Age	*(years)*	14.76	2.39	14.58	2.83
Sport	*(SP/NSP)*	(54 / 32)		(99 / 28)	
Height	*(cm)*	158.82$^¥$	9.16	164.69	17.27
Weight	*(kg)*	51.50*	10.12	57.22	17.56
BMI	*(kg/m^2)*	20.26	2.92	20.57	3.39
FM[1]	*(%)*	28.62$^¥$	5.58	21.04	7.87
FM[1]	*(kg)*	15.04$^¥$	5.11	11.92	5.81
SMM-DXA	*(kg)*	16.31$^¥$	3.37	22.42	9.24
SMM-ANT	*(kg)*	17.67$^¥$	3.78	24.01	8.03
SMM-DXA	*(%)*	33.41$^¥$	3.41	40.27	5.34
SMM-ANT	*(%)*	34.36$^¥$	3.93	41.83	4.16

**P*<0.05; ^ *P*≤0.01; $^¥$*P*≤0.001; SD, standard deviation; Max., maximum; Min, minimum; SP, sport participant; NSP, non-sport participant; BMI, body mass index; FM, fat; SMM, skeletal muscle mass; TBW, total body water; [1]Estimated by DXA.

Prevalence of overweight and obesity was 17.3% and 2.7% (n = 39 overweight, 24 boys and 15 girls; n = 6 obese, 4 boys and 2 girls). Normal weight participants represented 72% of the total sample (n = 162, 100 boys and 62 girls) and 8% were classified as thinness (n = 18; 8 boys, 10 girls). When %FM measured by DXA as criterion of classification for overweight and obesity (Taylor, Jones, Williams, & Goulding, 2002), overweight represented 23.1% of the total sample (38 boys, 14 girls) and obesity was 6.2% of the total sample (13 boys, 1 girl).

Regarding concurrent validity, the pediatric model in the group aged <16y was explored separately from the young adult group (≥16y). Predicted SMM from DXA and ANT in the pediatric group were significantly different with a mean bias ≈8%, although effect size was low and correlation was high, which indicates good accuracy and precision for the sample pooled together as confirmed by a high concordance correlation coefficient (ρ_c = 0.935) and bias correction factor (C_b = 0.990).

The analyses performed in the young adult group (≥16y) showed lower bias when using Poortmans' model compared to when Lee's model was applied, which was confirmed by higher concordance coefficient (ρ_c = 0.928 vs. 0.862) and lower mean differences and effect size than for the adult model. Accuracy was also better for SMM-ANT_P (C_b = 0.970 vs. 0.891), yet, the adult model showed better precision. The agreement analysis between the anthropometric- and DXA-pediatric models revealed a systematic error (P<0.0001) and a significant proportional error for the sample pooled together (τ = -0.174, P<0.01).

In both groups of boys and girls aged <16y (pediatric group), SMM predicted from the anthropometric model compared to SMM from DXA showed high precision and low to moderate ES but significant mean differences, especially in boys (12.24 ± 9.36 and 2.78 ± 8.55 %, boys and girls) (table 12 and 13). Further analysis by sex in the pediatric group (figure 10) showed that concordance was slightly better in the boys who also showed better precision than girls, but the accuracy in this later group was almost perfect (girls, C_b = 0.999). Moreover, the agreement analysis confirmed the systematic error previously found in the entire sample continue to be significant both male and female (*P*>0.05) but proportional error was present only in the boys' group (τ = -0.345, *P*<0.0001).

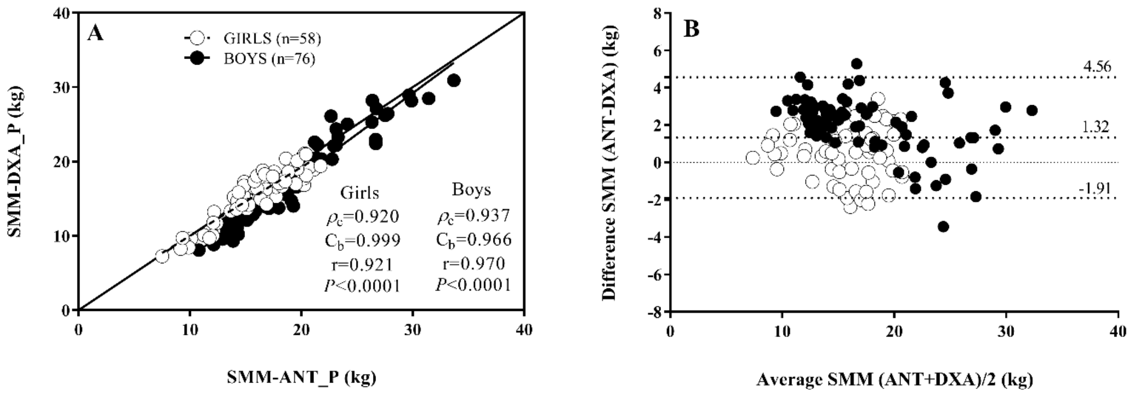

Figure 10. Scatter plot between measurements by DXA and alternative technique for the pediatric group (<16y). Reproducibility (concordance correlation coefficient, ρ_c), accuracy (bias correction factor, C_b) and precision (Spearman's correlation coefficient, rho) of SMM. Dashed line is fit line of simple linear regression between reference and alternative pairs of data; solid line is identity line (perfect reproducibility) (A). Bland-Altman plot for the corresponding linear relation plotted in panel A as the average vs. differences; the dotted horizontal lines are mean differences and 95%CIs for the sample pooled together (B).

For the analysis in the adult group (≥16y), the anthropometric pediatric model of Poortmans et al. showed similar SMM means to those predicted from DXA in the group of girls (*P*>0.05) although low precision (*r* = 0.670), and significantly underestimated SMM from DXA in boys (-2.93 ± 8.06 %, *P*=0.012). Predicted SMM using the anthropometric adult model of Lee et al. significantly overestimated SMM-DXA in both genders. Small ES was seen in all comparisons (*d* range, 0.05 to 0.36) (table 12 and 13).

Table 12. Differences between DXA and ANT estimations of SMM in girls.

Alternative	*Reference*		**Age group**	**Differences** *Mean*		*SD*	*Mean (%)*	**T-test** *P*	**Effect Size** Cohen's *d*	***r***
SMM-ANT_P	**SMM-DXA_P**	*(kg)*	*<16y*	0.46	±	1.39	2.78	0.016	0.13	0.921
SMM-ANT_P	**SMM-DXA_A**	*(kg)*	*≥16y*	-0.12	±	2.04	-1.05	0.555	0.05	0.670
SMM-ANT_A	**SMM-DXA_A**	*(kg)*	*≥16y*	3.04	±	1.49	14.84	<0.000	1.31	0.833

SD, standard deviation; SMM, skeletal muscle mass; DXA, dual X-ray energy absorptiometry; ANT, anthropometry; *P*, significance values for nonparametric paired sample T-test analysis; *r*, Pearson's coefficient of correlation.

Table 13. Differences between DXA and ANT estimations of SMM in boys.

Alternative	*Reference*		**Age group**	**Differences** *Mean*		*SD*	*Mean (%)*	**T-test** *P*	**Effect Size** Cohen's *d*	***r***
SMM-ANT_P	**SMM-DXA_P**	*(kg)*	*<16y*	1.98	±	1.54	12.24	<0.000	0.36	0.970
SMM-ANT_P	**SMM-DXA_A**	*(kg)*	*≥16y*	-0.92	±	2.71	-2.93	0.012	0.14	0.934
SMM-ANT_A	**SMM-DXA_A**	*(kg)*	*≥16y*	1.09	±	2.84	4.40	0.001	0.17	0.947

SD, standard deviation; SMM, skeletal muscle mass; DXA, dual X-ray energy absorptiometry; ANT, anthropometry; *P*, significance values for nonparametric paired sample T-test analysis; *r*, Pearson's coefficient of correlation.

As expected, significant characteristics were found between SP and NSP groups of the same sex (table 14). Non-active groups were significantly older, taller, and heavier than active groups but did not differed in body fat or %SMM predicted from ANT. Only NSP girls had significantly lower %SMM predicted from DXA than SP girls (P<0.05).

Differences between reference and alternative methods by groups of sport participation are presented in table 15 for girls and table 16 for boys. In the pediatric group (<16y), means of SMM from ANT significantly overestimated SMM-DXA in both genders except in the NSP group of girls (P>0.05), although precision was high in all groups (range, 0.91-0.98).

The analyses carried out in the adult groups (≥16y) revealed non-significant means differences in the NSP groups of boys and girls between the model of Poortmans et al. and DXA, yet, precision was lower in girls compared to boys (r = 0.65 vs 0.86). In the SP groups, only the model of Lee et al. showed similar means of SMM between ANT and DXA in boys (P>0.05) with high accuracy (r = 0.97) and no effect size (d = 0.00). This model showed higher accuracy in NSP boys than the pediatric one but significantly overestimated DXA measures (2.23 ± 1.51 kg; P<0.000). The same adult model of Lee et al. applied in girls highly overestimated DXA estimates of SMM in both SP and NSP groups (10.10 ± 7.37 and 16.78 ± 6.08 %). In the SP group of girls, the ANT pediatric model slightly underestimated DXA measures but precision was moderate (r = 0.66) (table 15 and 16).

Table 14. Descriptive characteristics of the sample with SMM measures by groups of sex and sport participation.

		GIRLS						BOYS					
		NSP (n=32)			**SP** (n=54)			**NSP** (n=28)			**SP** (n=99)		
Variables		Mean		SD	Mean		SD	Mean		SD	Mean		SD
Age	*(years)*	15.88¥	±	2.03	14.16	±	2.35	16.44¥	±	1.83	14.08	±	2.78
Height	*(cm)*	160.30*	±	9.23	157.87	±	9.32	171.67¥	±	9.83	163.50	±	18.24
Weight	*(kg)*	52.16^	±	9.55	50.82	±	10.63	64.92¥	±	12.69	55.17	±	17.80
BMI	*(kg/m^2)*	20.16	±	2.56	20.21	±	3.12	21.93^	±	3.71	20.08	±	3.00
FM[1]	*(%)*	29.00	±	5.28	28.15	±	5.62	21.89	±	7.53	20.70	±	8.13
FM[1]	*(kg)*	15.43	±	5.07	14.59	±	5.08	14.70^	±	7.04	11.06	±	5.14
SMM-ANT	*(kg)*	18.80	±	3.49	16.90	±	3.85	27.83¥	±	4.74	22.97	±	8.35
SMM-ANT	*(%)*	36.16	±	3.86	33.30	±	3.71	43.21	±	4.00	41.38	±	4.26
SMM-DXA	*(kg)*	16.46	±	3.01	16.18	±	3.66	25.45	±	4.67	21.70	±	10.01
SMM-DXA	*(%)*	32.53*	±	3.52	33.99	±	3.18	39.85	±	4.51	40.52	±	5.70

*P<0.05; ^P≤0.01; ¥P≤0.001; SD, standard deviation; SP, sport participant; NSP, non-sport participant; BMI, body mass index; FM, fat mass; [1]Estimated by DXA; SMM, skeletal muscle mass; ANT, anthropometry; DXA, dual-X ray absorptiometry; mean comparisons are between SP and NSP for the same sex group.

Table 15. Differences between alternative and reference methods for SMM in girls by groups of sport participation.

Alternative	*Reference*		**Group**	**Differences**				**T-test**	**Effect Size**	***r***
				Mean		*SD*	*Mean (%)*	*P*	Cohen's *d*	
SMM-ANT_P	**SMM-DXA_P**	*(kg)*	*NSP <16y*	0.52	±	1.56	3.29	>0.05	0.15	0.91
SMM-ANT_P	**SMM-DXA_P**	*(kg)*	*SP <16y*	0.45	±	1.39	2.69	0.04	0.13	0.92
SMM-ANT_P	**SMM-DXA_A**	*(kg)*	*NSP ≥16y*	0.15	±	1.86	0.50	>0.05	0.07	0.65
SMM-ANT_P	**SMM-DXA_A**	*(kg)*	*SP ≥16y*	-0.77	±	2.43	-4.83	0.006	0.28	0.66
SMM-ANT_A	**SMM-DXA_A**	*(kg)*	*NSP ≥16y*	3.42	±	1.25	16.78	<0.000	1.65	0.85
SMM-ANT_A	**SMM-DXA_A**	*(kg)*	*SP ≥16y*	2.10	±	1.69	10.10	0.006	0.81	0.84

NSP, non-sport participant; SP, sport participant; SD, standard deviation; SMM, skeletal muscle mass; DXA, dual X-ray energy absorptiometry; ANT, anthropometry; *P*, significance values for nonparametric paired sample T-test analysis; *r*, Pearson's coefficient of correlation.

Table 16. Differences between alternative and reference methods for SMM in boys by groups of sport participation.

Alternative	*Reference*		**Group**	**Differences**				**T-test**	**Effect Size**	***r***
				Mean		*SD*	*Mean (%)*	*P*	Cohen's *d*	
SMM-ANT_P	**SMM-DXA_P**	*(kg)*	*NSP <16y*	2.45	±	1.46	13.62	0.016	0.42	0.98
SMM-ANT_P	**SMM-DXA_P**	*(kg)*	*SP <16y*	1.87	±	1.55	11.52	<0.001	0.35	0.97
SMM-ANT_P	**SMM-DXA_A**	*(kg)*	*NSP ≥16y*	-0.13	±	2.25	-1.30	>0.05	0.04	0.86
SMM-ANT_P	**SMM-DXA_A**	*(kg)*	*SP ≥16y*	-1.78	±	2.90	-5.08	0.002	0.22	0.96
SMM-ANT_A	**SMM-DXA_A**	*(kg)*	*NSP ≥16y*	2.33	±	1.51	7.02	<0.000	0.76	0.90
SMM-ANT_A	**SMM-DXA_A**	*(kg)*	*SP ≥16y*	0.02	±	3.32	1.50	>0.05	0.00	0.97

NSP, non-sport participant; SP, sport participant; SD, standard deviation; SMM, skeletal muscle mass; DXA, dual X-ray energy absorptiometry; ANT, anthropometry; *P*, significance values for nonparametric paired sample T-test analysis; *r*, Pearson's coefficient of correlation.

Concordance between ANT and DXA measures was also explored in the young adult group (>16y) for the two anthropometric models, the pediatric and the adult one. In girls, the pediatric model of Poortmans et al. showed similar moderate concordance correlation coefficient in the SP and the NSP groups (0.635 and 0.632). Bias correction factor was high but precision was low in both groups (figure 11). A better concordance correlation coefficient was seen in active girls when the adult model of Lee et al. was applied but not in the NSP group, although precision was better in this case (r = 0.838 and 846, SP and NSP girls respectively). However, slopes differed from the identity line in all cases except in SP girls when the pediatric model was applied (P=0.055), and systematic error was seen when the adult model was applied (P<0.0001).

Among boys, although precision was similar for both anthropometric models in the the SP group, the pediatric model showed higher concordance correlation coefficient and bias correction factor than the adult one (ρ_c = 0.866 vs 0.773; C_b = 0.898 vs 0.798). Contrary, in the NSP group, the best concordance correlation coefficient was found when the adult anthropometric model was applied, which also was more accurate than the pediatric one (figure 12). Nonetheless, a proportional error was found in both anthropometric measures of SMM compared to DXA (all P<0.0001).

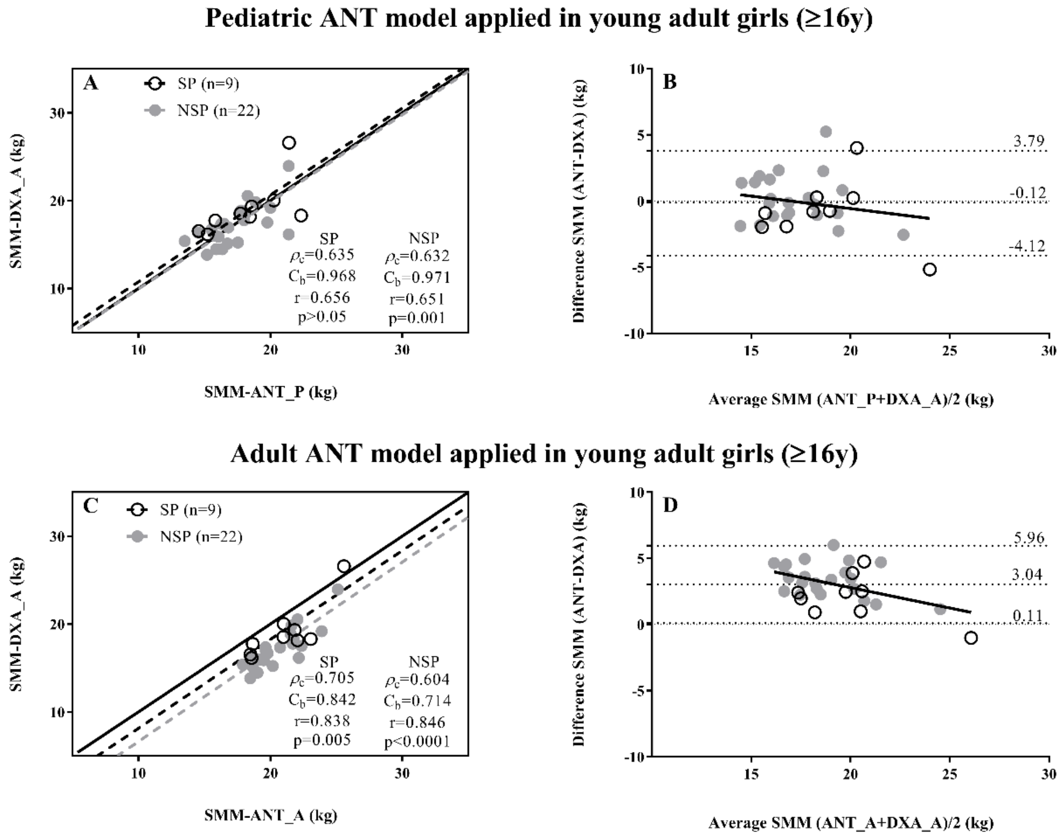

Figure 11. Scatter plot between measurements by DXA and alternative technique for young adult girls (≥16y). Reproducibility (concordance correlation coefficient, ρ_c), accuracy (bias correction factor, C_b) and precision (Pearson's correlation coefficient, r) of SMM. Dashed line is fit line of simple linear regression between reference and alternative pairs of data; solid line is identity line (perfect reproducibility) (A, C). Bland-Altman plot for the corresponding linear relation plotted in panel B and C as the average vs. differences; the dotted horizontal lines are mean differences and 95%CIs for the sample pooled together (B, D).

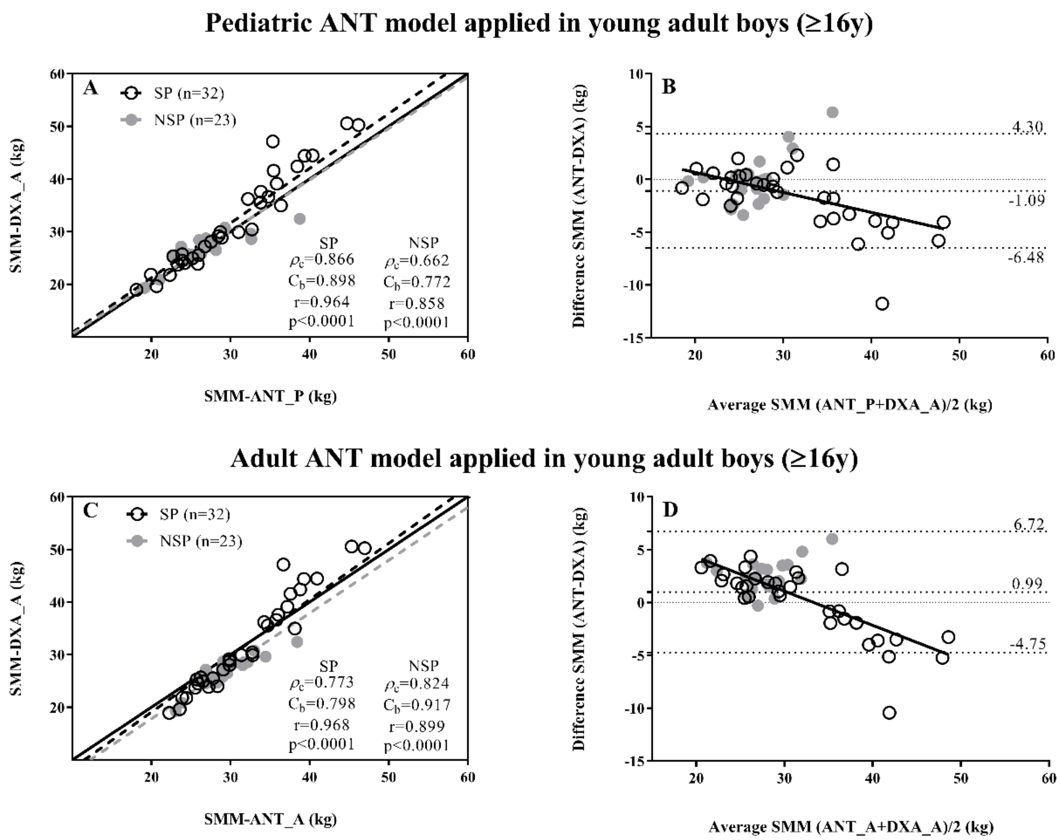

Figure 12. Scatter plot between measurements by DXA and alternative technique for young adult boys (≥16y). Reproducibility (concordance correlation coefficient, ρ_c), accuracy (bias correction factor, C_b) and precision (Pearson’s correlation coefficient, r) of SMM. Dashed line is fit line of simple linear regression between reference and alternative pairs of data; solid line is identity line (perfect reproducibility) (A, C). Bland-Altman plot for the corresponding linear relation plotted in panel B and C as the average vs. differences; the dotted horizontal lines are mean differences and 95%CIs for the sample pooled together (B, D).

Construct validity.

SMM variables selected for construct validity were SMM-DXA and SMM-ANT with combined pediatric (<16y) and adult (≥16) models in the same variable, and SMM-ANT_P where the pediatric equation was used for the sample pooled together. Positive associations were found for all FFM and SMM variables with anaerobic performance tests (*P*>0.05) except FFM-DXA and SJ (r = 228, *P*=0.051, n=36). Sprint test showed negative coefficients reflecting that the higher muscle amount the lowest time spent in the sprint. The lowest correlation coefficient was found between FFM-REF_W and SJ (τ = 0.207, n = 263) and the highest between SMM-DXA and left handgrip (τ = 0.764, n = 186). In general, strength assessed by dynamometry showed higher correlation coefficients with lean and muscle mass variables than explosive dynamic tests (table 17).

No single method to estimate FFM or SMM was the best correlated with all performance tests. Yet, SMM-DXA was the best correlated variable with left and right handgrip and LLS while FFM-BIA_d showed the best associations with CMJA and sprint. FFM-BIA_d using Lohman's values for H_{FFM} showed very similar correlation coefficients compared to FFM-BIA_d estimated by Well's constants with CMJ (τ = 0.376, n = 518, *P*<0.000, CMJA (τ = 0.435, n = 237, *P*<0.000), and sprint test (τ = -0.395, T0-15m, n = 484; τ = -0.406, T15-30m, n = 484; τ = -0.427, T0-30m, n = 494; all *P*<0.000).

Table 17. Correlation coefficients between FFM and SMM variables and anaerobic performance tests.

Variables		**Handgrip_R** *(kg)*	**Handgrip_L** *(kg)*	**LLS** *(kg)*	**SJ** *(cm)*	**CMJ** *(cm)*	**CMJA** *(cm)*	**T0-15m** *(s)*	**T15-30m** *(s)*	**T0-30m** *(s)*
FFM-REF_L	*(kg)*	0.598 (198)[1]	0.596 (194)	0.394 (450)	0.211 (263)	0.224 (455)	0.229 (192)	-0.269 (446)	-0.242 (446)	-0.299 (455)
FFM-REF_W	*(kg)*	0.596 (198)	0.593 (194)	0.391 (450)	0.207 (263)	0.219 (455)	0.220 (192)	-0.265 (446)	-0.240 (446)	-0.293 (455)
FFM-ANT	*(kg)*	0.714 (315)	0.721 (313)	0.423 (556)	0.294 (264)	0.338 (581)	0.376 (298)	-0.350 (547)	-0.375 (547)	-0.385 (557)
FFM-BIA_d	*(kg)*	0.741 (259)	0.749 (257)	0.393 (481)	0.307 (263)	0.376 (518)	0.436 (237)	-0.395 (484)	-0.407 (484)	-0.428 (494)
FFM-BIA	*(kg)*	0.726 (259)	0.730 (257)	0.384 (481)	0.267 (263)	0.335 (518)	0.396 (237)	-0.356 (484)	-0.377 (484)	-0.388 (494)
FFM-DXA	*(kg)*	0.752 (186)	0.758 (186)	0.591 (186)	0.228* (36)	0.374 (221)	0.404 (166)	-0.369 (175)	-0.393 (175)	-0.395 (183)
SMM-ANT_P	*(kg)*	0.657 (312)	0.675 (310)	0.415 (544)	0.346 (255)	0.349 (569)	0.380 (295)	-0.369 (535)	-0.366 (535)	-0.412 (545)
SMM-ANT	*(kg)*	0.673 (312)	0.688 (310)	0.425 (544)	0.335 (255)	0.358 (569)	0.383 (295)	-0.370 (535)	-0.377 (535)	-0.408 (545)
SMM-DXA	*(kg)*	0.758 (186)	0.764 (186)	0.509 (186)	0.276^ (36)	0.394 (221)	0.421 (166)	-0.376 (175)	-0.389 (175)	-0.408 (183)

*P>0.05; ^P<0.05; all other correlations P<0.000; [1]measures included in the analysis (n); R, right; L, left.

Different results were found with the sample split in sex groups. Vertical jumps were no longer significantly correlated with any of the lean mass variables (results not shown), and in girls, sprint test correlated only partially. Significant correlation coefficients ranged from 0.146 to 0.920 in boys and from -0.099 to 0.750 in girls ($P<0.05$). Best correlated methods with isometric strength were FFM-BIA_d with right handgrip, SMM-ANT_P with left handgrip and FFM-REF_W with LLS in girls' group. For boys, SMM-ANT_P correlated with handgrip and SMM-DXA with LLS (table 18).

Table 18. Correlation coefficients between FFM and SMM variables and isometric strength tests by sex groups.

		Handgrip_R *(kg)*		**Handgrip_L** *(kg)*		**LLS** *(kg)*	
Variables		**Girls**	**Boys**	**Girls**	**Boys**	**Girls**	**Boys**
FFM-REF_L	*(kg)*	0.721 (89)[1]	0.598 (109)	0.721 (87)	0.610 (107)	0.383 (209)	0.356 (241)
FFM-REF_W	*(kg)*	0.720 (89)	0.795 (109)	0.720 (87)	0.812 (107)	0.384 (209)	0.521 (241)
FFM-ANT	*(kg)*	0.706 (136)	0.760 (179)	0.727 (135)	0.761 (178)	0.188 (259)	0.501 (297)
FFM-BIA_d	*(kg)*	0.726 (110)	0.743 (149)	0.743 (109)	0.748 (148)	0.153 (230)	0.436 (251)
FFM-BIA	*(kg)*	0.721 (110)	0.728 (149)	0.727 (109)	0.735 (148)	0.151 (230)	0.425 (251)
FFM-DXA	*(kg)*	0.683 (68)	0.766 (118)	0.728 (68)	0.764 (118)	0.312 (78)	0.660 (108)
SMM-ANT_P	*(kg)*	0.703 (134)	0.918 (178)	0.750 (133)	0.920 (177)	0.287 (252)	0.659 (292)
SMM-ANT	*(kg)*	0.656 (134)	0.760 (178)	0.704 (133)	0.757 (177)	0.216 (252)	0.475 (292)
SMM-DXA	*(kg)*	0.658 (68)	0.773 (118)	0.720 (68)	0.766 (118)	0.329 (78)	0.669 (108)

All correlations $P \leq 0.001$; [1]measures included in the analysis (n); LLS, lower limb strength.

After controlling isometric strength tests for height and APHV, FFM-BIA_d correlated best with handgrip in girls (r = 0.541, right hand; r = 0.569, left hand; both P<0.000), although SMM-ANT correlated as well as with left handgrip. Similarly, in boys, the best correlation was observed among SMM-ANT and left handgrip (r = 0.638, P<0.000) although for FFM-DXA, right handgrip was the top association (r = 0.713, P<0.000). Leg strength was best associated with SMM-DXA in both groups (r = 1.000, boys; r = 0.638, girls; P<0.000), and for boys, FFM-DXA correlated equally with LLS as SMM-DXA (r = 1.000, P<0.000).

Regarding dynamic strength tests, correlations are shown in table 19. For girls, all FFM or SMM variables correlated similarly with CMJ or CMJA, while for boys SMM-DXA was the best associated with CMJ and FFM-ANT with CMJA. Sprint times were higher correlated with lean variables in boys than in girls. FFM-BIA_d was the only method that correlated with T0-15m in girls. SMM-ANT_P was the best associated variable with all sprint times in boys as well as for T15-30m and T0-30m in girls. After controlling by BMI and APHV, all correlations were significant and coefficient values increased compared to those obtained in the simple correlation analysis (range r = -0.291 to 0.858, P<0.05). Overall, SMM-ANT was the best variable associated with all tests except with T15-30m, in which case FFM-BIA_d showed the highest correlation coefficient (r = -0.536, P<0.000). The lowest associations were found between FFM-DXA with SJ and FFM-REF_W with the rest of dynamic performance tests. Within sex analysis, SMM-ANT remained the best associated variable with CMJ and CMJA in boys and girls, and also with T0-30m in boys while FFM-DXA was best correlated with T0-15m (all P<0.0001). Sprint time T15-30m was best associated with FFM-BIA_d in girls (r = -0.224, P=0.001) and with SMM-DXA in boys (r = -0.478, P<0.000) (table 20).

Table 19. Correlation coefficients between FFM and SMM variables and dynamic strength tests by sex groups.

Variables		**CMJ** *(cm)*		**CMJA** *(cm)*		**T0-15m** *(s)*		**T15-30m** *(s)*		**T0-30m** *(s)*	
		Girls	**Boys**	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**	**Boys**
FFM-REF_L	*(kg)*	-0.127 (214)	0.182^ (241)	-0.102 (86)	0.405$^{¥}$ (106)	0.036 (205)	-0.144$^{¥}$ (241)	-0.060 (205)	-0.159$^{¥}$ (241)	-0.061 (214)	-0.165$^{¥}$ (241)
FFM-REF_W	*(kg)*	-0.128 (214)	0.146* (241)	-0.105 (86)	0.370$^{¥}$ (106)	0.040 (205)	-0.236$^{¥}$ (241)	-0.058 (205)	-0.240$^{¥}$ (241)	-0.058 (214)	-0.263$^{¥}$ (241)
FFM-ANT	*(kg)*	-0.018 (266)	0.522$^{¥}$ (315)	0.012 (124)	0.709$^{¥}$ (174)	-0.035 (242)	-0.366$^{¥}$ (305)	-0.143$^{¥}$ (242)	-0.382$^{¥}$ (305)	-0.119 (252)	-0.404$^{¥}$ (305)
FFM-BIA_d	*(kg)*	0.075 (236)	0.401$^{¥}$ (282)	0.084 (94)	0.666$^{¥}$ (143)	-0.099* (225)	-0.306$^{¥}$ (259)	-0.197$^{¥}$ (225)	-0.325$^{¥}$ (259)	-0.185^ (235)	-0.339$^{¥}$ (259)
FFM-BIA	*(kg)*	-0.008 (236)	0.352$^{¥}$ (282)	0.000 (94)	0.630$^{¥}$ (143)	-0.049 (225)	-0.266$^{¥}$ (259)	-0.168$^{¥}$ (225)	-0.293$^{¥}$ (259)	-0.116 (235)	-0.296$^{¥}$ (259)
FFM-DXA	*(kg)*	-0.075 (83)	0.561$^{¥}$ (138)	0.045 (55)	0.680$^{¥}$ (111)	-0.165 (61)	-0.395$^{¥}$ (114)	-0.186* (61)	-0.443$^{¥}$ (114)	-0.229 (69)	-0.426$^{¥}$ (114)
SMM-ANT_P	*(kg)*	-0.001 (259)	0.421$^{¥}$ (310)	0.070 (122)	0.665$^{¥}$ (173)	-0.069 (235)	-0.465$^{¥}$ (300)	-0.229$^{¥}$ (235)	-0.492$^{¥}$ (300)	-0.193^ (245)	-0.505$^{¥}$ (300)
SMM-ANT	*(kg)*	-0.024 (259)	0.509$^{¥}$ (310)	0.027 (122)	0.684$^{¥}$ (173)	-0.051 (235)	-0.344$^{¥}$ (300)	-0.165$^{¥}$ (235)	-0.364$^{¥}$ (300)	-0.144* (245)	-0.374$^{¥}$ (300)
SMM-DXA	*(kg)*	-0.039 (83)	0.566$^{¥}$ (138)	0.053 (55)	0.681$^{¥}$ (111)	-0.162 (61)	-0.404$^{¥}$ (114)	-0.141 (61)	-0.450$^{¥}$ (114)	-0.237 (69)	-0.437$^{¥}$ (114)

*P<0.05; ^P≤0.01; $^{¥}P$≤0.001; measures included in each analysis (n).

Table 20. Partial correlations[1] coefficients between FFM and SMM variables and dynamic tests by sex groups.

Variables		**CMJ** *(cm)*		**CMJA** *(cm)*		**T0-15m** *(s)*		**T15-30m** *(s)*		**T0-30m** *(s)*	
		Girls	**Boys**	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**	**Boys**
FFM-REF_L	*(kg)*	0.096 (210)	-0.008 (237)	0.168 (82)	0.243^ (102)	-0.176^ (201)	-0.232¥ (237)	0.092 (201)	0.166^ (237)	-0.242¥ (210)	-0.316¥ (237)
FFM-REF_W	*(kg)*	0.097 (210)	-0.004 (237)	0.165 (82)	0.231* (102)	-0.174^ (201)	-0.235¥ (237)	0.093 (201)	0.157* (237)	-0.241¥ (210)	-0.318¥ (237)
FFM-ANT	*(kg)*	0.225¥ (262)	0.377¥ (311)	0.423¥ (120)	0.648¥ (170)	-0.337¥ (238)	-0.444¥ (301)	-0.182^ (238)	-0.287¥ (301)	-0.384¥ (248)	-0.512¥ (301)
FFM-BIA_d	*(kg)*	0.279¥ (232)	0.251¥ (278)	0.444¥ (90)	0.580¥ (139)	-0.372¥ (221)	-0.431¥ (255)	-0.224¥ (221)	-0.297¥ (255)	-0.348¥ (231)	-0.505¥ (255)
FFM-BIA	*(kg)*	0.248¥ (232)	0.210¥ (278)	0.416¥ (90)	0.619¥ (139)	-0.362¥ (221)	-0.386¥ (255)	-0.200^ (221)	-0.240¥ (255)	-0.336¥ (231)	-0.454¥ (255)
FFM-DXA	*(kg)*	0.214 (79)	0.513¥ (134)	0.404^ (51)	0.703¥ (107)	-0.572¥ (57)	-0.605¥ (110)	-0.196 (57)	-0.466¥ (110)	-0.553¥ (65)	-0.556¥ (110)
SMM-ANT_P	*(kg)*	0.282¥ (255)	0.523¥ (306)	0.481¥ (118)	0.914¥ (169)	-0.384¥ (231)	-0.504¥ (296)	-0.220¥ (231)	-0.280¥ (296)	-0.458¥ (241)	-0.601¥ (296)
SMM-ANT	*(kg)*	0.305¥ (255)	0.610¥ (306)	0.483¥ (118)	0.943¥ (169)	-0.349¥ (231)	-0.566¥ (296)	-0.220¥ (231)	-0.352¥ (296)	-0.412¥ (241)	-0.662¥ (296)
SMM-DXA	*(kg)*	0.278 (79)	0.484¥ (134)	0.458¥ (51)	0.664¥ (107)	-0.569¥ (57)	-0.593¥ (110)	-0.141 (57)	-0.478¥ (110)	-0.542¥ (65)	-0.548¥ (110)

[1]Controlled by BMI and APHV; *$P<0.05$; ^$P\leq0.01$; ¥$P\leq0.001$; measures included in each analysis (n)

Regarding jumping and sprinting, SMM-ANT was the best correlated method with VJ in both girls (*r* = 0.244, SJ; *r* = 0.305, CMJ; *r* = 0.483, CMJA; *P*<0.001) and boys (*r* = 0.249, SJ; *r* = 0.610, CMJ; *r* = 0.943, CMJA; *P*<0.001). Sprint times correlations differed between sexes, FFM-DXA was best correlated with T0-15m and T0-30m in girls (*r* = -0.572 and -0.553, respectively, *P*<0.000), and FFM-BIA_d with T15-30m (*r* = -0.553, *P*<0.000). For boys, we could not find any pattern for three different lean variables and each one correlated had the best correlation with a different sprint time variable, thus, FFM-DXA accounted most for T0-15m (*r* = -0.605, *P*<0.000), SSM-DXA for T15-30m (*r* = -0.478, *P*<0.000), and SMM-ANT for T0-30m (*r* = -0.662, *P*<0.000).

Further regression analysis was performed between muscle estimates from each method and anaerobic performance tests in order to explore the amount of strength that is explained by each method (figure 13). The slopes of the regressions were significantly different from zero in all cases (*P*≤0.01). As expected, SMM explained strength variables better than FFM except for sprinting, in which case FFM-BIA_d explained the highest amount of the time spent in 15m. The best muscle variable accounting for the higher variance in strength was SMM-DXA, explaining 86% of handgrip, 58% of LLS, 32% of CMJ, and 48% of CMJA. However, approximately 30% of the variance in 30m sprint test was explained by the other three different muscle models: SMM-ANT (32.3%), FFM-BIA_d (31.9%), and SMM-DXA (31.8%). Similarly, time spent in 15m sprint was better explained by FFM-BIA_d (26.2%) than by SMM-ANT (25.5%) and SMM-DXA (25.3%), although these differences were not statistically different.

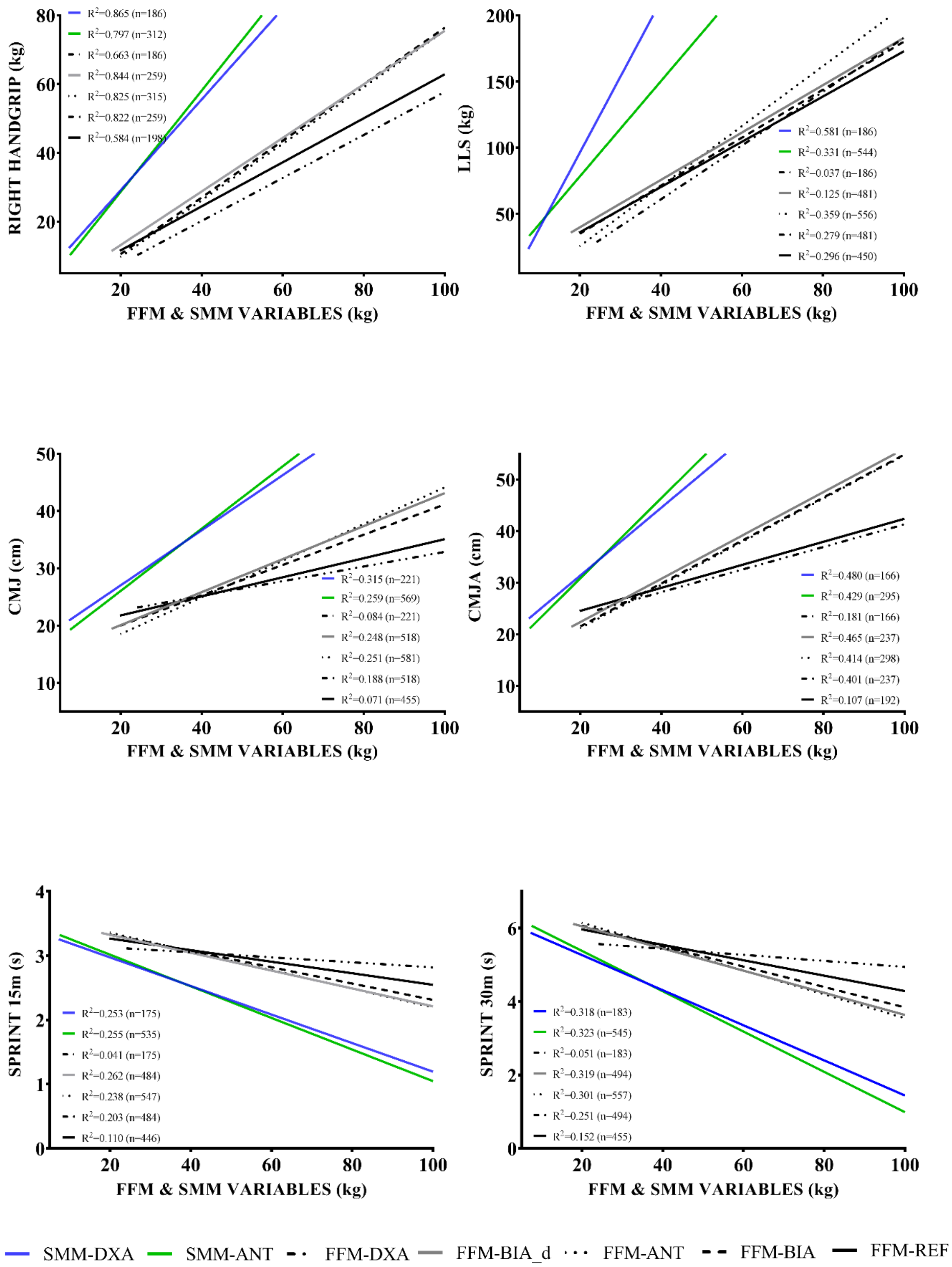

Figure 13. Regression lines between measures of fat free mass (FFM) and skeletal muscle mass (SMM) estimated by each method (see legend on bottom) and strength tests. R^2, coefficient of determination.

DISCUSSION

This study explored validity and agreement between two field techniques and one laboratory method with the reference method of deuterium oxide dilution to estimate FFM in a youth sample of a wide range of age (6-21y). Furthermore, we explored agreement between two SMM predictive models from anthropometry and DXA, together with an analysis of the associations of all selected methods with a variety of anaerobic performance tests to explore construct validity. To our knowledge, this is the first time that concurrent and construct validity are explored simultaneously in a considerable sample of Spanish young subjects, including analysis among sex, maturation, and sports participation groups.

Our results showed significant underestimation of FFM measures from all the alternative techniques compared to D_2O dilution method. FFM assessed by DXA was the poorest method associated with the reference and it is inconsistent with Wells' study that found no bias between DXA and 4C-model in males and females, although a different DXA equipment was used and (Wells et al., 2012), which joined with different sample characteristics could explain these discrepancies. On the other hand, anthropometry showed the lowest mean differences with D_2O, although barely different from those observed with BIA that had higher precision and smaller limits of agreement, so the most accurate method for FFM estimates in this population seem to be BIA applying Houtkooper et al. equation. However, it was found a significant proportional error explained by the presence of overweight and obese subjects, which disappeared after analyzed only in normal weight subjects. That can be related with higher hydration of FFM of obese children (Haroun et al., 2005); supporting the recommendation exclusion of under- and overweight participants (Lohman & Going, 2006).

Sports practice does not appear to influence general agreement between FFM measures by BIA and D_2O methods, however higher accuracy and precision were observed in the active group for all techniques. Moreover, for normal weight subjects, the equation of Houtkooper et al. did not differ from the identity line in athletes although underestimation was found. Similar results were found when using Kushner et al. equation to estimate FFM from predicted TBW in athletes, although significant bias was present in both groups. These results are inconsistent with a previous study exploring TBW in adolescent where agreement between Kushner et al. equation and D_2O method indicated no significant mean differences in boys athletes (Quiterio, Silva, et al., 2009). A possible explanation could be sample differences such as number of participants, mean age and height, as our sample was composed by more than doubled athletes whom were younger and smaller than the Portuguese. Moreover, mean predicted TBW by Kushner et al. equation was much lower in our sample while TBW from D_2O was very similar. A technological reason is still plausible since BIA devices differed between ours and Quiterio's study.

The two anthropometric models to estimate SMM overestimated DXA measures in both groups, the pediatric and the young adult group. Moreover, we found sex differences between boys and girls in the pediatric model, where concordance between methods was better in boys than in girls, and a proportional error was affecting agreement in boys but not in girls. Conversely, in the young adult group the slopes between ANT and DXA did not differed between sexes. This disagreement could be related with limitation of Poortmans' model since it was validated against DXA while Kim's used MRI as reference, and sex was found to be a predictor variable of SMM in the anthropometric models but not in the DXA-based models. Moreover, since Poortmans et al. equation was validated in a Caucasian population with age 7-24y, further analysis conducted in the young adult group using this equation revealed better agreement than Lee et al. equation despite a lower precision of the former equation.

Sport practice was explored in the young adult group because of the differences found between the slopes of the regression of ANT and DXA measures between athletes and non-athletes. Opposite to what was found for FFM, when compared to SMM by DXA, anthropometry seems to perform better in non-active subjects than in sports participants. The equation of Poortmans et al. showed no significant proportional bias in this group. A possible explanation could be that in the validation study subjects involved in structured physical activity program were not included in the study. Conversely, for the SP group showed no systematic bias (significant mean difference) with DXA, nevertheless the proportional error found it may have been related larger amounts of SMM of young athletes. These findings suggest that the amount of SMM in athletes older that 16y supports the positive influence on physical activity on lean mass accrual during adolescence (Baxter-Jones et al., 2008).

Relative to the study of construct validity, the results confirmed our hypothesis about differences within methods and its individual relationships with strength tests. As expected, SMM variables were better associated with strength than FFM variables, especially SMM from DXA, which model includes lean mass from the extremities where a large proportion of total-body SMM is present (Heymsfield et al., 1990). Differences can be also attributed to the paradigm used to estimate or measure the entity, anatomical or physiological variable. Since, the DXA- and adult anthropometric-based models were validated against MRI seems reasonable they been better associated with anaerobic performance construct than molecular components. However, some of the methods used to estimate FFM like BIA correlated better than SMM with sprinting. These results suggest that properties of the entity measured by BIA (TBW or some its compartments) may relate well with dynamic strength but not with isometric. Nevertheless, it was paradigmatic that measures of FFM from deuterium dilution were low correlated with all strength variables compared to the other FFM estimations, except in girls whom had FFM by deuterium as the best associated method with strength of lower limbs. This influence of sex in construct validity was also found in other techniques, so SMM by anthropometry was the best method for boys, while BIA was the highest correlated method with time in 15m in girls. These results may lead scientists to think that characteristics associated to the entity measured by BIA may be affected by sex (Kushner et al., 1992), which affects the associations with sprint performance.

Overall, all FFM and SMM estimation methods correlated better with dynamometry than with explosive dynamic tests, possibly due to other factors influencing movement performance like training adaptations, neural influences (Ross, Leveritt, & Riek, 2001), maturational status on muscle metabolism changes (Armstrong, Barker, & McManus, 2015), running mechanics and coordination variability (Boyer, Freedman Silvernail, & Hamill, 2016), among others.

Despite the lack of agreement found between FFM assessed by DXA and the reference deuterium oxide dilution, it was found that DXA correlated better with most strength tests even after controlling for BMI and somatic maturity (APHV) confounding factors, both SMM and FFM entities depending on the strength outcome variable. The models developed by Kim et al. to estimate SMM accounted for the higher variance in strength from handgrip and counter movement jumps. Among field techniques, anthropometry was best associated with vertical jumps in girls and boys but explained better the variance in 30m sprint with the sample pooled together.

Even though our aim was to analyze associations between methods to assess lean mass and strength variables from a general and practical approach, it is well known that performance on strength by children and adolescents are influenced by many variables like age, sex, growth, maturation, biomechanical factors, training, (Malina et al., 2004; Rowland, 2005) apart from genetic factors (Gollnick, 1982; Loos et al., 1997). Longitudinal analysis are still needed for a better understanding of age and sex associated development in strength (De Ste Croix, 2007).

Besides factors inherent to methods and technical error of measurement, another limitation may have blunted partially the effects of sport participation and maturation in our validation analysis. A more objectively way to measure physical activity level, and differentiate among levels, years and volume and intensity of training in addition to a deeper analysis by pubertal stages, would lead to a more comprehensive research and better understanding of the mechanisms explaining differences between active and non-active children and adolescents.

Despite the limitations of the study, this extensive analysis contributes to describe FFM and SMM agreement between several available and accessible techniques in a sample of Spanish youth. We believe that data presented here would be useful for comparing and interpret data, in addition to help professionals working with youth populations, like sport coaches and physical education teachers, on choosing the best-matched method to the aim pursued.

In conclusion, concurrent and construct validity of available methods to estimate FFM and SMM in children and adolescents vary among sex and sport participation groups. There was not a perfect method for all situations, which was indicated by the lack of agreement between some laboratory and field methods. Regarding construct validity, we could not find a method better associated with all constructs (strength tests) than the others. However, we found that the simple technique of BIA was adequate for FFM estimation under controlled circumstances and applying age- and sex-specific equations. Similarly, predicting SMM from anthropometry was found to be an alternative to DXA laboratory method but with some limitations related to age, sex and sport practice; thus, for NSP adolescents the pediatric model performed well for all ages, while the adult model was better in post-pubertal active youngsters. Our results evidence the need for developing specific models to estimate FFM and SMM in physically active prepubertal and pubertal youth and to be validated against reference methods. It seems that the heterogeneity of associations between constructs and methods to estimate FFM or SMM requires an analysis where subcomponents of FFM will be included in multiple regression analysis in order to weight the actual importance of each method.

STUDY 3.

Fluid Distribution and Regional Skeletal Muscle Mass Predict Anaerobic Performance in Adolescents: Results of Sprint and Isometric Strength

INTRODUCTION

The relationship between body composition and performance has been classically associated to FM and aerobic capacity, SMM and strength or hydration and endurance capacity (Ackland et al., 2012; Müller, Bosy-Westphal, Later, Haas, & Heller, 2009; Wilmore, 1983). Other components or regional body composition have received less attention in the scientific literature and its importance is commonly ignored by physical activity and health professionals, particularly in youth healthy population.

Although TBW content (hydration) has been classically associated with aerobic performance, the importance of hydration in anaerobic performance has gained attention in the last years (Kraft et al., 2012). It could be speculated that this relationship is an acute mechanism (dehydration) or associated to FFM (larger FFM higher absolute strength); suggesting that anaerobic performance is determined by whole body composition components or dehydration-related consequences (negative feed-back). Nevertheless, studies conducted in the past and current century have suggested mechanisms supporting the importance of chronic fluid distribution and regional SMM for anaerobic performance.

More than 30 years ago a Danish group (Sjøgaard & Saltin, 1982) reported a relationship between ICW and analytic exercise in adults, which has been refuted in athletes (Silva et al., 2014; Silva, Fields, Heymsfield, & Sardinha, 2010). However, the relationships between anaerobic performance assessed by sprints and hydration have not been analyzed extensively in the literature particularly among adolescents.

The analysis of the associations between SMM and strength throughout chronological age and biological maturation must be useful to describe the importance of body composition in identifying factors that predict sport performance in young athletes. Also, some SMM indicators have been using for the assessment of nutrition status since seventies like upper arm circumference or arm areas (Frisancho & Tracer, 1987; Sen et al., 2011). Additionally, it has been suggested that strength may have implications in short-time and lifelong for cardiovascular health (Fraser et al., 2016; Grøntved et al., 2013) and SMM could play a positive role in this association. So, it seems that strategies for preserving SMM might be important for children and adolescents' health and performance. But it still remains a lack of comprehensive information about the association between regional SMM development and performance throughout childhood and adolescence.

This lack of anaerobic-performance-related studies represent a paradoxical situation because children and adolescents are primarily involved in short-term high intensity exercise in almost all daily tasks, games or sports events. The analysis of these associations must be useful to describe the importance of hydration, FFM, and regional SMM for performance in healthy adolescents.

The main aim of this study was to explore two paradigms related to anaerobic performance and subcomponents of body composition in adolescents. Firstly, we aimed to analyze the association between 30 meters sprint test and molecular body composition variables in young athletes and non-athletes. Secondly, to examine the association between lower-limb strength (LLS) and regional SMM variables in young athletes and non-athletes. Finally, it was our purpose to identify molecular and cellular body composition indicators successfully predict sprint performance and LLS accounting for maturation status.

METHODS

Design. These studies were conducted in subsamples of the larger studies described in the previous chapters and for which we had valid data a written consent from participants and parents or tutors (see study 1 and 2). Acute or chronic diseases were the only exclusion criteria for these two analyses. In the first analysis, we selected all participants with valid data for molecular body composition assessment by ANT and BIA, and 30 meters sprint test; in order to facilitate the comprehension of the manuscript we will refer this analysis as *Intracellular water and Sprint Performance*. The second analysis was conducted in all adolescents with at least a valid test of LLS by isometric dynamometry and anthropometric assessment of SMM both total and regional (arm, thigh and calf); specific results and discussion from this analysis will be indicated as *Regional SMM and Lower Limb Strength.*

Sample.

Intracellular water and Sprint Performance. One hundred and fifty-eight healthy adolescents between 12 and 18y-old (15.0 ± 1.5y) were recruited from local high-schools and local sport clubs. An *ad hoc* questionnaire was used to identify those participants involved in organized and competitive sports (≥3times/week of training plus competition, duration of ≥1hour/session), classified as athletes (AT), and those physically inactive

(<3hours/week), classified as non-athletes (NAT) (AT = 58, 27 boys, 31 girls, and NAT = 100, 41 boys, 59 girls).

Regional SMM and Lower Limb Strength. One hundred and sixty-six healthy adolescents between 12 and 18y-old (15.2 ± 1.7y) were recruited from local high-schools and local sport clubs. Only 158 participants met the criteria for variables included in the models (age = 15.03 ± 1.63y; NAT = 92, 38 boys, 51 girls, and AT = 66, 42 boys, 24 girls).

Procedures.

Body Composition Assessment. Standard procedures for anthropometry were performed to measure circumferences and skinfolds variables (Stewart et al., 2011) . Body Fat (BF) was calculated using Slaughter's equation and FFM was derived from body weight. Validated age-specific models were applied to obtain Skeletal Muscle Mass (SMM) based on anthropometric variables (Poortmans' (<16 years) and Lee's models) (Lee et al., 2000; Poortmans et al., 2005). Bioelectrical impedance method (Lukaski et al., 1986) was used to obtain Total Body Water (TBW) with multifrequency bioimpedance device (MediSystem®, Sanocare, Spain). Intracellular Water (ICW) was obtained by the equation: TBW = ECW + ICW (specific models to estimate TBW and ECW are reported in study 2). All hydration variables were analyzed as absolute values (kg) and percentage (%).

Regional SMM was assessed by corrected perimeters as reported in study 1. Briefly, arm, thigh and calf perimeters measured and triceps, thigh and calf skinfolds were removed to calculated muscle circumferences in each region: arm muscle (ACG), thigh muscle (TCG) and calf muscle (CCG).

Maturity Assessment. Predicted age at peak height velocity (APHV) was calculated using the equation of Mirwald et al. (2002) converted into categorical variables as reported in study 2 (Mirwald et al., 2002).

Anaerobic Performance and Sport Participation. Participants were required to perform 5 minutes run and two submaximal trials of each test as a warm-up. All tests were performed during the same morning and with at least 30 minutes of resting among tests, which were carried out with following protocol: Firstly, a portable dynamometer device (J Herrera, Barcelona) was used to assess lower limb strength (LLS) from squat position. Participants were asked to exercise progressively and keep the maximal strength during 2-3 seconds. Secondly, photoelectrical cells were used to measure time spent in 30m (SmartSpeed™, Fusion Sport®, United Kingdom), subjects were asked to run at maximum speed until a cone situated at 40m from start line (infrared cell beam was crossed at 30m). The best performance value out of two trials was used for the analyses**.** AT and NAT were identified using an *ad hoc* questionnaire.

Statistical analysis. Differences between groups were analyzed by independent sample T-test. Partial correlations were performed to determine associations between body composition variables and 30m-sprint values and LL strength. The best-correlated variables with sprint or LLS performance were included in a stepwise regression analysis to determine body composition predictors of sprint performance after adjusting for age, sex and sport participation (AT= 1 or NAT=0). A type I error of 5% was accepted in all statistical tests ($P<0.05$), which were conducted with SPSS® package (version 15.0; IBM Corporation, Armonk, NY, USA).

RESULTS

Intracellular water and Sprint Performance

As expected, AT were faster than NAT in 30m (mean difference = 0.43 ± 0.09 s; *P*<0.001), but there were not significant differences in ICW (mean difference = 1.18 ± 0.94 %; *P*>0.05). NAT had significantly more fat than AT (mean difference = 6.11 ± 1.29; *P*<0.001). No significant differences were found between groups for SMM and TBW (*P*>0.05).

Comparisons of the sample characteristics by sex groups showed that active and non-active girls differed only in BMI, fat, and speed. Active girls had lower BMI, % of BF and were faster at 30m-sprint test (table 21). Active boys were also faster than the NSP boys and had lower fat but they also had more % of TBW and ICW (table 22).

Table 21. Comparison of the characteristics of the groups of girls.

		GIRLS AT (n=23)			GIRLS NAT (n=57)		
Variables		Mean		SD	Mean		SD
Age	*(years)*	14.64	±	1.38	14.86	±	1.59
Weight	*(kg)*	50.85	±	8.13	54.28	±	9.74
Height	*(cm)*	160.34	±	7.16	160.31	±	6.65
BMI	*(kg/m^2)*	19.68*	±	2.06	21.02	±	3.28
BF	*(%)*	21.39$^{¥}$	±	5.15	27.10	±	6.79
SMM	*(kg)*	17.14	±	3.28	17.74	±	3.39
SMM	*(%)*	33.81	±	4.20	32.82	±	3.81
TBW	*(%)*	56.42	±	3.61	54.79	±	5.43
ICW	*(%)*	13.97	±	3.43	12.72	±	6.09
ICW	*(kg)*	6.97	±	1.58	6.74	±	3.54
APHV	*(years)*	12.42	±	0.50	12.49	±	0.51
Speed 30m	*(m/s)*	5.80$^{¥}$	±	0.33	5.16	±	0.36
Time 30m	*(s)*	5.19$^{¥}$	±	0.30	5.85	±	0.43

*P<0.05; $^{¥}P\leq$0.001; AT, athletes; NAT, non-athletes; BMI, body mass index; BF, body fat; SMM, skeletal muscle mass; TBW, total body water; ICW, intracellular water; APHV, age at peak height velocity.

Table 22. Comparison of the characteristics of the groups of boys.

Variables		BOYS AT (n=24)			BOYS NAT (n=32)		
		Mean		SD	Mean		SD
Age	*(years)*	15.13	±	1.39	15.41	±	1.58
Weight	*(kg)*	58.85	±	11.45	61.81	±	13.44
Height	*(cm)*	169.91	±	9.53	167.75	±	8.79
BMI	*(kg/m²)*	20.28	±	2.98	21.82	±	4.01
BF	*(%)*	16.56*	±	7.24	21.44	±	9.23
SMM	*(kg)*	24.76	±	5.23	24.80	±	4.92
SMM	*(%)*	42.28	±	5.78	40.43	±	4.19
TBW	*(%)*	60.78*	±	3.66	58.36	±	5.04
ICW	*(%)*	19.57*	±	4.15	16.90	±	4.44
ICW	*(kg)*	11.49	±	3.14	10.07	±	2.17
APHV	*(years)*	13.82	±	0.48	14.04	±	0.43
Speed 30m	*(m/s)*	6.38$^{¥}$	±	0.46	6.00	±	0.39
Time 30m	*(s)*	4.73$^{¥}$	±	0.38	5.02	±	0.33

*P<0.05; $^{¥}P$≤0.001; AT, athletes; NAT, non-athletes; BMI, body mass index; BF, body fat; SMM, skeletal muscle mass; TBW, total body water; ICW, intracellular water; APHV, age at peak height velocity.

A significant correlation was found between ICW and 30m-sprint test after controlled by age and kg of BF (table 23). The best association for the sample pooled together was found in AT when sprint performance was expressed as speed (m/s) and ICW in kg (r = 0.766, P<0.001). With sex split, the same variables were the best correlated ones in boys (r = 0.663, P≤0.001) but different results were found in girls, in which case, the best correlation was seen between time in 30m (s) and kg of ICW (r = -0.485, P<0.05).

Table 23. Partial correlations between intracellular water (ICW) and 30m-sprint performance.

			ALL		ATHLETES		NONATHLETES	
			ICW (%)	**ICW (kg)**	**ICW (%)**	**ICW (kg)**	**ICW (%)**	**ICW (kg)**
Variables		***Group***	*r*	*r*	*r*	*r*	*r*	*r*
T 30m	*(s)*	Girls	-0.060	-0.142	-0.459*	-0.485*	-0.041	-0.107
S 30m	*(m/s)*	Girls	0.076	0.155	0.488*	0.511*	0.057	0.120
T 30m	*(s)*	Boys	-0.374^	-0.543¥	-0.422	-0.627^	-0.225	-0.394*
S 30m	*(m/s)*	Boys	0.374^	0.545¥	0.458*	0.663¥	0.233	0.403*

*P<0.05; ^P≤0.01; ¥P≤0.001; T, time; S, speed; *r*, correlation coefficient; ICW, intracellular water. Comparisons between groups of athletes and non-athletes of the same sex.

The analysis of partial correlations provided information of the highest coefficients of correlation body composition variables and sprint performance, which was ICW. Additionally, the comparison between regression lines between AT and NAT for the entire sample showed significantly different slopes and intercepts between both groups independently of the unit utilized to express ICW (% or kg, $P<0.05$ for both); AT had steeper slopes than NAT. However, results by sex and sport practice groups showed similar slopes between AT and NAT in girls and in boys ($P=0.260$ and $P=0.723$) for the regression between speed 30m and kg of ICW. Coefficient of determination ranged from the lowest of 0.03 in NSP girls to the highest of 0.66 in SP boys (figure 14).

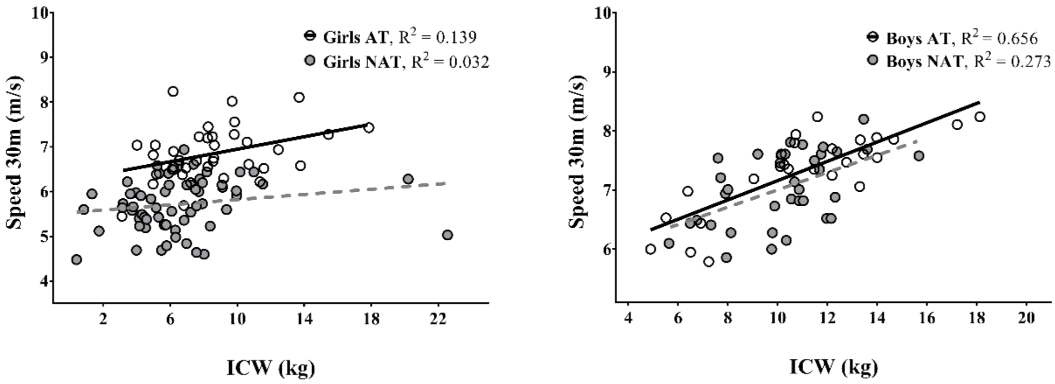

Figure 14. Association between intracellular water (ICW) and 30m-sprint performance in adolescents in girls and boys. NAT, non-athlete adolescents (grey dots and lines); AT, athlete adolescents (black dots and lines). Lines represent the best fit for simple regression analysis.

In order to analyze the impact of sport practice all participants were pooled together; %SMM, kg of ICW, and the athlete condition were significant predictors of 30m time in the initial model ($P<0.001$; $R^2 = 0.571$, adjusted $R^2 = 0.561$, SEE = 0.38 s). The second stepwise regression analysis was performed again with speed in 30m as dependent variable. We observed that SMM (kg), the athlete condition, and %ICW were identified as predictors ($P<0.001$; $R^2 = 0.620$, adjusted $R^2 = 0.612$, SEE = 0.37 m/s). The final model was: Speed 30-m (m/s) = 3.926 + (0.051*SMM) + (0.495*AT) + (0.033*ICW). Maturity offset as years from PHV was not included in any model.

Regional SMM and Lower Limb Strength.

The analysis of the participants pooled together showed that AT were stronger than NAT (mean difference = 23.83 kg, SE = 4.81, $P<0.001$) and they also had greater values of CCG (mean difference = 1.31 cm, SE = 0.49, $P<0.01$). NAT had significantly more fat and less SMM than AT (mean difference FM = 4.19 %, SE = 1.55; SMM = -2.16 %, SE = 0.84; both $P<0.01$). No significant differences were found between groups for age, weight, height, BMI, and predicted APHV ($P>0.05$). Further analysis within sex groups revealed that differences between AT and NAT in FM and LLS were present only in the girls' group, in which case, NAT girls had significantly higher amounts of fat mean difference = 4.88 ± 1.59 %, $P=0.003$) and less LLS than AT girls (mean difference = -24.54 ± 6.22 kg, $P\leq0.000$). In boys, non-significant differences were found between AT and NAT for any variable ($P>0.05$) (table 24).

The best correlation between SMM variables and LLS test after controlling by height was for total SMM in NAT (r = 0.631, $P<0.000$). In AT, any SMM variable correlated significantly with LLS ($P\geq0.05$) while the three muscle variables correlated significantly with LLS for the sample combined, and CCG showed slightly better coefficient than TCG. Similar results were seen after performed the analysis by sex groups, only non-athletic groups of boys and girls showed significant correlation between muscle variables and strength. The best coefficient was found for total boys SMM in NAT girls (r = 0.468, $P<0.000$) while boys showed similar coefficients for LLS correlated with total SMM and CCG (0.511 and 0.503, $P<0.01$) (table 25).

Table 24. Differences in body composition variables, maturation and strength between athletes (AT) and non-athletes (AT) by sex groups.

		GIRLS NAT			GIRLS AT			BOYS NAT			BOYS AT		
Variables		Mean		SD	Mean		SD	Mean		SD	Mean		SD
Age	*(years)*	15.03	±	1.69	14.58	±	1.53	15.26	±	1.68	15.07	±	1.55
Weight	*(kg)*	54.99	±	9.72	51.89	±	9.96	65.63	±	18.32	65.56	±	13.90
Height	*(cm)*	160.03	±	6.98	159.11	±	8.25	168.93	±	8.51	169.94	±	9.23
BMI	*(kg/m^2)*	21.40	±	3.20	20.38	±	2.85	22.79	±	5.24	22.57	±	3.78
FM	*(%)*	28.13^	±	6.87	23.25	±	5.51	24.25	±	13.09	21.82	±	10.53
FM	*(kg)*	15.83^	±	5.94	12.36	±	4.71	17.86	±	16.30	15.14	±	9.68
SMM	*(kg)*	17.60	±	3.20	17.25	±	4.16	25.15	±	5.65	25.71	±	5.34
SMM	*(%)*	32.23	±	4.09	33.10	±	3.29	38.94	±	4.63	39.49	±	4.17
CCG	*(cm)*	28.50	±	2.11	29.20	±	2.93	31.36	±	2.71	32.01	±	2.92
APHV	*(years)*	12.92	±	0.76	12.92	±	0.58	14.21	±	0.71	14.05	±	0.60
PHV	*(years from)*	2.11	±	1.17	1.66	±	1.29	1.05	±	1.42	1.02	±	1.41
LLS	*(kg)*	43.06$^{¥}$	±	15.38	67.60	±	28.68	75.30	±	25.23	87.42	±	32.78

^$P \leq 0.01$; $^{¥}P \leq 0.001$; BMI, body mass index; FM, fat mass; SMM, skeletal muscle mass; CCG, calf corrected girth; APHV, predicted age at peak height velocity; PHV, predicted years from peak height velocity; LLS, lower limb strength. *P* values are for comparisons between AT and NAT of the same sex.

Maturation offset expressed as PHV only correlated significantly with participants pooled together, and when APHV was used, it correlated with LLS for the combined sample and NAT. When examining sex differences in the same athletic group, maturation variables were the only ones that differed between sexes as APHV correlated better with boys and PHV with AT girls (table 25).

Stepwise regression analysis was performed with LLS as dependent variable. When participants were pooled together, variables of SMM, athlete condition and height were predictors of LLS test result (R^2 = 0.488, *P*<0.001, equation 1 including total SMM). Similar results were found for an alternative model including CCG, athlete condition, sex and height (R^2 = 0.484, *P*<0.001; equation 2 including regional SMM). Maturation status was not found a predictor of LLS neither it was expressed as APHV nor YPHV. The models were as follows:

$$LLS\ (kg) = -110.523 * \ (1.983 * SMM) + (18.266 * AT) + (0.769 * Height)$$

R^2: 0.488, adjusted R^2: 0.478, SEE: 22.34 kg *Equation 1*

$$LLS\ (kg) = -182.108 + (1.070 * Height) + (16.295 * AT) + (11.208 * Sex) + (1.979 * CCG)$$

R^2: 0.484, adjusted R^2: 0.471, SEE: 22.49 kg *Equation 2*

where SMM was in kg, Height and CCG in cm, AT coded as athlete = 1, non-athlete = 0, and Sex coded as male = 1, female = 0.

Table 25. Partial correlations between SMM and LLS by sex and athlete condition.

			ALL			NON-ATHLETES			ATHLETES		
Variables			**All**	**Girls**	**Boys**	**All**	**Girls**	**Boys**	**All**	**Girls**	**Boys**
SMM	*(kg)*	*r*	0.334	0.110	0.256	0.631	0.468	0.511	0.025	-0.206	0.108
		P	0.000	0.342	0.023	0.000	0.000	0.001	0.841	0.346	0.501
TCG	*(cm)*	*r*	0.280	0.152	0.218	0.480	0.357	0.424	-0.011	-0.159	0.040
		P	0.000	0.187	0.053	0.000	0.009	0.009	0.932	0.468	0.803
CCG	*(cm)*	*r*	0.288	0.238	0.216	0.502	0.329	0.503	0.011	0.021	0.022
		P	0.000	0.037	0.055	0.000	0.016	0.002	0.930	0.926	0.890
APHV	*(y)*	*r*		0.037	0.105		0.144	-0.094		-0.099	0.353
		P		0.748	0.355		0.304	0.581		0.654	0.024
PHV	*(y)*	*r*		-0.166	0.222		0.291	0.472		-0.568	0.110
		P		0.149	0.050		0.035	0.003		0.005	0.494

SMM, skeletal muscle mass; TCG, tight corrected circumference; CCG, calf corrected circumference; APHV, age at peak height velocity; LLS, lower limbs strength; *r*, correlation coefficient; *P* values are from correlations adjusted to height.

DISCUSSION

The main findings of these two analyses were that either water distribution and regional SMM were significant predictors of anaerobic performance, which confirm our initial hypotheses.

Intracellular water and Sprint Performance.

Our results were in accordance with studies conducted in adults, and confirm that sprint performance in adolescents was partially dependent on muscle mass (Perez-Gomez et al., 2008). The main finding was that ICW was a significant predictor of sprint in adolescents. The classical study by Sjøgaard and Saltin (1982) suggested that muscles with large fibers have a smaller ECW space than muscle with small fibers (Sjøgaard & Saltin, 1982). So, an association between fiber size and ICW could be plausible histochemical mechanism explaining this relationship. In our knowledge, there are not studies where this topic has been broadly explored, however it is well stablished that type II fibers (fast-twitch) have larger areas than type I.

The relationship between sprint performance and ICW could have been affect by other factors, which modify either ICW or sprint capacity. Training status must be an important determinant of anaerobic performance, so AT show better results in the sprint test than NAT, however both groups presented a significant correlation between ICW and sprint performance. Although the association values were always higher in the AT group, this difference cannot be attributed to larger SMM or ICW in AT since the multiple regression analysis indicated ICW was a significant predictor of sprint after including SMM, nonetheless the athletic status was also a significant predictor. An absolute (kg) larger ICW component in AT may not be the reason because the slopes of the regression between % ICW and sprint continue to be significantly different between groups. These results may suggest that sport practice may improve the relationship between ICW and sprint performance independently of whole body composition and percentage of intracellular hydration.

Our data are in agreement with studies that showed training level as a factor associated with jump performance in children and adolescents (Keiner, Sander, Wirth, & Schmidtbleicher, 2013). Another study with adult judo athletes reported significant associations between handgrip strength (Silva, Fields, Heymsfield, & Sardinha, 2011) and upper limbs power (Silva et al., 2010) with ICW hydration determined by isotope dilution (D_2O). This relationship between acute hydration and anaerobic performance has been confirmed in adults (Kraft et al., 2012), nevertheless mechanistic studies need to be conducted to demonstrated an independent molecular effect of ICW.

Regional SMM and Lower Limb Strength.

In accordance with other studies, our results confirm that LLS performance in adolescents was partially dependent on SMM (Hoshikawa et al., 2013; Pitcher et al., 2012). The main finding in this study was that a simple measure as CCG contributed significantly for estimating LLS as SMM did, which may have important implications for assessing in field settings.

As expected, the multiple regression analysis indicated that sport practice played a role in LLS estimation. These data are in accordance with other study where training level was shown to be related with acyclic exercise in adolescents (Keiner et al., 2013). In our results, maturation represented as predicted age at PHV was not highly significantly correlated with LLS. These results are similar to those in the study of De Ste Croix et a. (De Ste Croix, Armstrong, Welsman, & Sharpe, 2002), where it was found that age and sexual maturation did not contribute to peak knee strength after controlled for body mass and stature. Also, Baxter-Jones and coworkers (Baxter-Jones et al., 2008) reported that daily physical activity has a significant independent influence on the growth of lean body mass during adolescence after controlled for biological maturity and stature. In summary, it seems that the influence of maturity in strength might be mediated by its repercussion in lean mass without any additional leverage. Finally, it is important to indicate that these data add new information to the body of knowledge in young athlete performance, since a single measurement as CCG was significantly associated with LLS although TCG represents a much larger SMM proportion and was also implicated in the muscle mass recruited during the test. This information could be useful for talent identification models and highlights the importance of regional body composition for healthy physical fitness.

This study has some limitations which may have confounded and increased the variability of our results. Firstly, the assessment of fluid distribution by BIA is highly correlated with isotope dilution technique although it may be affected by many external factors, which we could not control, for example external temperature, menstrual cycle in girls or nutrition status. Sprint tests were partially carried out in outdoor facilities and the environment temperature could affect the test performance. Regarding regional SMM analysis, our model of assessment is not validated in adolescents and this may introduce a bias in our results. Finally, our dynamometer had a low level of individualization and is possible it could have limited results in short participants.

In conclusion, our findings support the hypothesis that subcomponents of FFM such as ICW and regional SMM are independent predictors of sprint and lower limbs isometric strength respectively. These results must be useful to explore the mechanisms of sprint and strength exercise performances in adolescents. However, more research with different samples is necessary to confirm our results. It is important to note that the associations found were independent of SMM, TBW or FFM and may justify the necessity of a more comprehensive body composition assessment to understand changes in performance, mainly with young athletes involve in intensive sport practice.

V. CONCLUSIONS

CONCLUSIONS

STUDY 1. The Relevance of Sport Participation, Body Size and Maturation on Total and Regional Skeletal Muscle Mass Along Childhood and Adolescence

1. New reference values for FFM and SMM derived from anthropometry are presented for a wide-age range of children and adolescents.
2. Sport participations seems to influence differences between total and regional SMM school periods after puberty.

STUDY 2. Concurrent Validity of Methods for Estimating Fat Free Mass and Skeletal Muscle Mass in Children, Adolescents and Young Adults: Anaerobic Performance and Sports Participation Constructs

1. Although there is no available method to estimate FFM and SMM that fits well for all groups, it seems that BIA performs better in both sports participants and non-sport participants' children, adolescents, and young adults.
2. The best method associated with strength was FFM assessed by DXA after controlling for body size and maturity. However, BIA correlated better with some strength tests.

STUDY 3. Fluid Distribution and Regional Skeletal Muscle Mass Predict Anaerobic Performance in Adolescents: Results of Sprint and Isometric Strength.

1. Intracellular water is a predictor of the anaerobic performance of a cyclic exercise as sprint, independent from TBW and SMM.
2. A simple measure of regional muscularity can predict lower limbs strength independent of total body SMM.

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APPENDIX

HOJA DE INFORMACIÓN PARA EL POSIBLE PARTICIPANTE

1. Información General: Mediante el presente documento se pretende informar a los padres/madres de los atletas menores de edad vinculados con el Club de Atletismo Nerja.com-Cueva de Nerja-UMA acerca del desarrollo de una investigación científica.

"INDICADORES DE MASA MUSCULAR ESQUELÉTICA Y RENDIMIENTO ANAERÓBICO EN LA NIÑEZ Y LA ADOLESCENCIA"

- Investigador: Lorena Correas Gómez, licenciada en Ciencias de la Actividad Física y el Deporte, Máster en Investigación en Actividad Física y el Deporte y Entrenadora Nacional de Atletismo.
- Estudios: Programa Oficial de Postgrado "Práctica Deportiva: Innovación y aplicación, Universidad de Málaga (UMA). Programa distinguido con Mención de Calidad por el Ministerio de Educación.
- Posibles participantes: Atletas entre 10 y 18 años.
- Metodología a emplear:
  - Antropometría: Mediciones de talla, peso, pliegues de grasa y perímetros.
  - Bioimpedancia eléctrica: Mediciones de agua corporal, masa grasa y masa libre de grasa.
  - Batería de tests de condición física: Evaluación de la resistencia, velocidad, fuerza isométrica y capacidad de salto.
  - Datos de maduración: Mediante identificación con dibujos.
  - Cuestionarios: Para recoger información sobre la actividad física y alimentaria realizada mediante encuestas validadas y diario de entrenamiento.

2. Objetivo: El objetivo principal de este estudio es valorar la composición corporal y el estado de condición física de los atletas entre 10 y 18 años que entrenan atletismo con el fin de definir modelos antropométricos aplicados al rendimiento deportivo en la niñez y la adolescencia.

3. Beneficios derivados del estudio: Proporcionar un informe individual para el conocimiento del estado de composición corporal y de la condición física, relacionado con la salud. Regalo de un vale para una prueba en la Universidad de Málaga para conocer la densidad mineral ósea.

4. El estudio se realizará en dos momentos/días en las instalaciones más cercanas al lugar de entrenamiento: Todas las pruebas en jornada de mañana; las pruebas de condición física se realizan en un día diferente.

5. No se conocen acontecimientos y efectos adversos derivados del estudio.

6. El estudio tiene un carácter totalmente **voluntario** en su participación, así como la posibilidad de retirarse del estudio en cualquier momento.

7. Todo el tratamiento de los datos tendrá riguroso celo y sólo el/los responsable/s del estudio conocerán la base de datos relacional de nombres y códigos siendo este método el utilizado para mantener la confidencialidad, garantizándose el anonimato de los participantes.

8. Cada una de las pruebas y valoraciones se llevarán a cabo y serán supervisadas por **especialistas** en Ciencias de la Actividad Física y el Deporte (profesores/entrenadores/colaboradores entrenados).

9. El/los investigador/es responsable/s del estudio mantendrá informado al sujeto, a quien se le entregará un **informe final** personalizado con los resultados del estudio.

Los padres podrán consultar cualquier duda y tema relacionado con el estudio el mismo día de las pruebas o ponerse en contacto con el investigador principal.

UNIVERSIDAD DE MÁLAGA
LABORATORIO DE BIODINÁMICA Y COMPOSICIÓN CORPORAL

GOBIERNO DE ESPAÑA — MINISTERIO DE CIENCIA E INNOVACIÓN

Proyecto GEOS

Documento Informativo para posibles participantes

1. **Objetivo:**
   - Estudiar la composición corporal (sobrepeso y obesidad) de escolares.

2. **Metodología Empleada:**
   - Antropometría: Mediciones de talla, peso, pliegues de grasa y perímetros corporales.
   - Impedancia Bioeléctrica: Estimaciones de masa magra.
   - Cuestionarios: Recogida de datos de alimentación, prácticas de actividad física, estilo de vida, salud y maduración.

3. **Beneficios derivados del estudio:**
   - Conocimiento con elevada precisión de la composición corporal y de sus asociaciones con la salud de los escolares.

4. El estudio se realizará durante una mañana.
5. Todos los tests y evaluaciones han sido utilizados en poblaciones semejantes en diversos estudios españoles y en otros países, no conociéndose efectos adversos resultantes de los mismos.
6. La participación en el estudio tiene un carácter TOTALMENTE voluntario, así como la posibilidad de retirarse del mismo en cualquier momento sin dar ninguna explicación.
7. Todo el tratamiento de datos tendrá un riguroso celo y solamente el investigador principal del proyecto tendrá acceso a la base de datos de nombres de los participantes. Para efectos de tratamiento estadístico los participantes aparecerán identificados con un código numérico, garantizándose el anonimato en todo momento.
8. Cada una de las pruebas y valoraciones serán supervisadas por especialistas en fisiología del ejercicio (médicos deportivos y licenciados en ciencias de la actividad física).
9. El investigador responsable del estudio mantendrá informado al participante y a sus tutores, y se les entregará un informe final personalizado con los resultados del estudio.

La participación no supondrá alteración alguna del desarrollo normal de los entrenamientos, no implicando más pérdida para el participante que la duración de las pruebas durante el día del estudio

Los padres o tutores podrán consultar cualquier duda o tema relacionado con el estudio con la investigadora del proyecto Lorena Correas Gómez

TODOS LOS PROCEDIMIENTOS UTILIZADOS ESTÁN ACORDES CON LA DECLARACIÓN DE HELSINKI PARA ESTUDIOS CON HUMANOS

Facultad de Ciencias de la Educación
Departamento de Didáctica de la Expresión Musical, Plástica y Corporal
Campus de Teatinos, s/n. 29071-Málaga Tlf.: 952 13 24 51

CONSENTIMIENTO INFORMADO

Yo (nombre y apellidos)____ con DNI nº _____________ en condición de padre, madre o tutor del deportista (nombre y apellidos)___________________________________ confirmo lo siguiente:

- He leído la hoja de información que se me ha entregado.
- He podido hacer preguntas sobre el estudio.
- He recibido suficiente información sobre el estudio.
- Comprendo que la participación es voluntaria.
- Comprendo que mi hijo/a puede retirarse del estudio:
  - Cuando quiera.
  - Avisando con antelación.
  - Sin tener que dar explicaciones.
  - Sin que ello repercuta en sus cuidados médicos.

Presto libremente mi conformidad para la participación en el estudio:

“Indicadores de masa muscular esquelética y rendimiento anaeróbico en la niñez y la adolescencia"

En _____________, a ____ de __________________ de 201_.

Firma del padre/madre/tutor: ____________________________________

Firma del participante: ____

Contacto: Lorena Correas Gómez

lcorreas.cafd@gmail.com

LABORATORIO DE BIODINÁMICA
Y COMPOSICIÓN CORPORAL

Proyecto GEOS

Consentimiento Informado para la Participación en el Estudio

Yo, (nombre y apellidos del padre/madre) ____
con DNI nº:________________, autorizo a mi hijo/a ___________________________________
del curso _________a la participación en el proyecto GEOS en el centro educativo _________________________________. Además:

- He leído la hoja de información que se ha entregado.
- He recibido suficiente información sobre el estudio.
- Podré resolver todas las dudas sobre el estudio con el Dr. Elvis Álvarez Carnero investigador principal del proyecto (Universidad de Málaga), y con el Profesor _______________, docente en el centro educativo _____________________________________ durante la reunión informativa que se celebrará el día ____ de _____________ del 201_ a las ____: _____ horas.

Comprendo que su participación es voluntaria.
Comprendo que el niño puede o puedo retirarse/lo del estudio:

1. *Cuando quiera.*
2. *Sin tener que dar explicaciones.*
3. *Sin que esto repercuta en el entrenamiento habitual y la competición.*

Doy la conformidad para que mi hijo/a participe en el estudio GEOS:
"Gasto Energético, Obesidad y Salud Infantil: ¿Es la Intervención Escolar de los Profesores de Educación Física un Arma Real en la Guerra contra la Obesidad?""

En ___________, ________ de _________________ del 201_

Firma del Participante Firma del padre, madre o tutor

Facultad de Ciencias de la Educación
Departamento de Didáctica de la Expresión Musical, Plástica y Corporal
Campus de Teatinos, s/n. 29071-Málaga Tlf.: 952 13 26 27

NORMAS DE ACTUACIÓN PARA ATLETAS PARTICIPANTES EN EL ESTUDIO:

"Indicadores de masa muscular esquelética y rendimiento anaeróbico en la niñez y la adolescencia"

- Para acudir al día de las pruebas lo harás en ayunad, es decir, sin desayunar nada, ni comida ni bebida. Esto es muy importante porque si no se así los resultados no son válidos. No traigas bocadillo ni zumo porque durante la mañana se te dará una vez finalizadas las pruebas.
- Debes acudir al lugar el día y hora en el que has sido citado, a ser posible en coche. En caso de hacerlo andando, lo harás lentamente y con el mínimo esfuerzo.
- La puntualidad es muy importante ya que las exploraciones empiezan a las 9:00 horas y los especialistas tienen un orden de actuación preciso.
- El día de las pruebas, del que serás avisado con anterioridad, tendrás que asistir en ropa deportiva para tu comodidad. Como ropa interior traerás un bañador o ropa corta de competición.

TU CITA: _________ día ________ de ______________a las______horas

LUGAR:

____

GRACIAS POR TU COLABORACIÓN

Nom. ______________ ______________________ **Sexo (M/F)** ________
nombre apellidos

F.Nto. [/ /] **ID evaluador** [JRAC | | LCG |]
día mes año

Hora [:] **Fecha de evaluación** [/ /] **Código** ________
día mes año

Altura del banco ______ cm **Edad decimal** ______________________

	Básicos	**Medición 1**	**Medición 2**	**Medición 3**	**Media**
1	Masa corporal	.	.	.	.
2	Estatura	.	.	.	.
3	Talla sentado	.	.	.	.
	Pliegues				
4	Tríceps	.	.	.	.
5	Antebrazo				
6	Muslo	.	.	.	.
7	Pantorrilla med.	.	.	.	.
	Circunferencias				
8	Brazo relajado	.	.	.	.
9	Antebrazo	.	.	.	.
10	Cintura mín.	.	.	.	.
11	Cintura máx. (crestas)	.	.	.	.
12	Muslo medio (troc-tib.)	.	.	.	.
13	Muslo (ref. pliegue)	.	.	.	.
14	Pantorrilla máx.	.	.	.	.
	Longitudes				
15	Muslo	.	.	.	.
16	Pierna	.	.	.	.

17	**BIA**	**Z**	**R**	**Xc**	**Ángulo de fase**	**GC**
	1					
	10					**MM**
	25					
	50					**ACT**
	75					
	100					**AEC**
	150					

18	**Índice de maduración (Tanner)**
	Estadios
Vello	
Mamas/Genital	

OBSERVARCIONES: ______________________

CONDICIÓN FÍSICA

Fecha __ __ / __ __ / __ __ **Hora** ___ : __ **Temperatura** ______

SALTO

	T' VUELO	ALTURA
SJ Intento 1		
SJ Intento 2		
CMJ Intento 1		
CMJ Intento 2		

VELOCIDAD

	0-15m	15-30m	0-30m
Tiempo Intento 1			
Tiempo Intento 2			

DINAMOMETRÍA TREN INFERIOR

Diestro Zurdo	Intento 1	Intento 2
Piernas		

DINAMOMETRÍA MANUAL

Diestro Zurdo	Intento 1	Intento 2
DCHA		
IZDA		

RESISTENCIA

FC REPOSO	Nº PALIERS	FC RECUPERACIÓN	1’	2’	3’	4’	5’

UNIVERSIDAD DE MÁLAGA

Cuestionario de Práctica Deportiva

Nombre:_______________________________Edad:_______ Sexo(M/F):________

1. ¿Practicas deporte? _____ ¿Cuál o cuáles?

   Deporte 1: ________________________. ¿Federado?_______.

   Deporte 2: ________________________. ¿Federado?_______.

   Deporte 3: ________________________. ¿Federado?_______.

2. ¿A qué edad comenzaste a realizar deporte competitivo?___________________.

   ¿Cuántos años llevas federado?____.

   ¿Qué nivel de competición practicas? Provincial_____ Autonómico_____ Nacional_____ Internacional_____

3. Normalmente, ¿cuántos días entrenas y compites por semana en cada uno de los deportes? (Escribe debajo del nombre del deporte en el día de la semana que corresponda y los minutos que entrenas/compites cada uno de los días).

Día de la semana	Deporte 1	Deporte 2	Deporte 3
Lunes			
Martes			
Miércoles			
Jueves			
Viernes			
Sábado			
Domingo			

4. ¿En algún deporte entrenas más de una vez al día? Escribe una cruz, en la tabla anterior, en aquellos deportes en los que entrenes más de una vez al día.

5. Normalmente, faltas al entrenamiento:
   a. A menudo.
   b. Algunas veces.
   c. Excepcionalmente/raramente.
   d. Nunca/casi nunca.