# Associations between Spanish children's physical activity and physical fitness with lean body mass

## Abstract

The aim of the present study is to investigate the associations between physical activity (PA) and physical fitness (PF) with lean body mass (LBM) and to assess whether PA mediates the association between PF and LBM. 279 children (150 boys) aged 7.5±0.3 years participated in the study. PA was assessed by accelerometry and PF with handgrip and the standing long jump test. Total lean soft tissue mass index (TLSTMI), muscle cross-sectional area index (MCSAI), and fat-free mass index (FFMI) were evaluated using dual-energy X-ray absorptiometry, peripheral quantitative computed tomography, and bioimpedance analysis, respectively.

Total (β=0.247) and vigorous PA (β=0.143) were associated with TLSTMI in girls. In boys, total (β=0.337), light (β=0.290), vigorous (β=0.200), and moderate-vigorous PA (β=0.189) were associated with TLSTMI. Only total PA was associated with FFMI (β=0.299). Handgrip strength does not mediate the relationship between total PA and TLSTMI. Positive associations were found between handgrip strength and TLSTMI, MCSAI, and FFMI in both girls and boys.

In children, there is a positive association between total and vigorous PA with TLSTMI. Handgrip strength does not mediate the relationship between total PA and TLSTMI. It was associated with TLSTMI, MCSAI, and FFMI.

**Key words:** lean body mass, muscle cross-sectional area, physical activity, physical fitness, schoolchildren

## 1. Introduction

Lean body mass (LBM) is mainly constituted by muscle mass, internal organ non-adipose components, and extracellular fluid (Kuriyan, 2018). In recent years, LBM has been considered to play an essential role in growth maintenance, normal development, and systemic glucose metabolism in children (Liu et al., 2019). It has also been associated with the risk of cardiovascular disease (S. Kim & Valdez, 2015), affecting bone health (bone mineral density and structure in both sexes during childhood) (Dorsey et al., 2010; Sioen et al., 2016), and cognitive development (Scheurer et al., 2018), among others. Studies on children and adolescents with low lean mass showed a higher cardiometabolic risk (S. Kim & Valdez, 2015), related to significantly higher waist circumference, blood pressure, triglycerides, and total cholesterol/high-density lipoprotein cholesterol values (Burrows et al., 2017; Gracia-Marco et al., 2016). Other studies have shown an increased risk of metabolic syndrome (Burrows et al., 2017; J. H. Kim & Park, 2016; K. Kim et al., 2016). In this regard, the literature revealed that those presenting a phenotype combining low lean mass and obesity had the most unfavorable cardiometabolic risk profile (Burrows et al., 2017). Therefore, low levels of lean mass in children and adolescents may represent a public health problem and a burden on the health system for future stages in life.

Currently, it is accepted that several factors can influence the development of lean mass/muscle mass throughout the life cycle, including fetal programming (Isganaitis, 2019; Labayen et al., 2006; Larqué et al., 2019), early nutritional status (Singhal et al., 2003), age, gender (Wells, 2000), hormones (Veldhuis et al., 2005), diet, and physical activity and exercise (Kulkarni et al., 2014; Westerterp et al., 2021)

Physical activity and exercise play an essential role in the development of both the size of muscle fibers and the recruitment of motor units, thus, developing strength (Dotan et al., 2012) and metabolic adaptations (Boisseau & Delamarche, 2000).

A cross-sectional study, in preschool-age children between 5 and 6, showed that a level of MVPA below the World Health Organization recommendation was significantly associated with a lower content of muscle mass and FFM (0.8%

and 1.19%, respectively); these differences were more evident in boys than in girls (Wyszyńska et al., 2020).

A longitudinal study (Leppänen et al., 2016, 2017) in 4.5-year-old children demonstrated that children with higher and moderate to vigorous physical activity (MVPA) levels had a higher fat-free mass index (FFMI) after a 12-month follow-up. Similar results have been reported in preschool children (Henriksson et al., 2016) and adolescents (Baxter-Jones et al., 2008; Ramires et al., 2016).

The relationship between physical activity levels, physical fitness, and body composition with fat mass has been widely studied in children and adolescents (Henriksson et al., 2016; Santos et al., 2019). Regarding its relationship with LBM, Baxter-Jones et al. (Baxter-Jones et al., 2008) reported that daily physical activity had a significant independent influence on LBM development during adolescence (after controlling for biological maturity and stature). In addition, studies in children have shown that higher fat-free mass (FFM) values are associated with better cardiorespiratory fitness, improved upper and lower body muscle strength (Fraser et al., 2020), motor fitness (Henriksson et al., 2016), and a healthier cardiovascular profile later in life, with a lower risk of premature death (Ruiz et al., 2009). Thus, it seems reasonable to hypothesize that physical activity or physical fitness might play a crucial role in the association between fitness-lean or physical activity-lean in this age group.

Given the available evidence, it is apparent that studies examining the relationship between physical activity levels, physical fitness, and body composition, specifically LBM in children between 6 and 12 years of age, are limited (Hao et al., 2019). Furthermore, most of the available studies have used statistical methods such as analysis of covariance, multiple linear regression, or logistic regression to adjust for confounding factors, statistical methods that cannot distinguish the effect of mediating variables.
Furthermore, there are important differences between DXA, pQCT, and BIA. On the one hand, DXA is an accurate device for assessing body composition of the whole body and regions/segments (Laskey, 1996). At the same time, pQCT

allows assessment at the appendicular level (limb muscle cross-sectional area)(Frank-Wilson et al., 2015). Still, they are not available to everyone due to their high price and radiation exposure (Guglielmi et al., 2016). On the other hand, BIA is an effective alternative to assess body composition of the whole body, low price in the absence of radiation (Orsso et al., 2019). Therefore, it will be important to use all three devices to assess LBM. In addition, the normalization of body size should also be considered, since most of the evidence available to date uses absolute or relative measures of LBM (kg and%), which makes it difficult to make comparisons between individuals or populations (Wells et al., 2002).

Therefore, in this study, we hypothesized that children with higher physical activity or physical fitness would have higher LBM values, and physical activity or physical fitness would be a mediator between the fitness-lean or physical activity-lean associations. Its purposes are to (1) investigate the associations between objectively assessed physical activity, physical fitness, and LBM measured by dual-energy X-ray absorptiometry (DXA), bioimpedance analysis (BIA), and peripheral quantitative computed tomography (pQCT); and (2) evaluate whether these associations were mediated by total physical activity or physical fitness or with handgrip strength in a Spanish cohort of children.

## 2. Material and Methods

### *2.1 Study participants*

This longitudinal observational study evaluated a representative cohort of children born in Spain between 2009-2010. The initial sample consisted of 1602 newborns, followed every month during the first year and then every year until they turned 6.

In 2016 and 2017, the recruited families (n=952) from the baseline examination were invited to participate in this follow-up study. They were invited to participate in an additional body composition assessment in our laboratory. From the 415 children who participated in this follow-up assessment, 136 were excluded from the study for the following reasons: lack of data on body composition and physical fitness (n=90), or lack of valid accelerometer data (n=46). Finally, 279 children (150 boys and 129 girls), between 6 and 8 years, with complete DXA, pQCT, BIA, physical activity, and physical fitness examination data were included in this study.

### *2.2 Ethics statement*

This study was conducted following the Declaration of Helsinki (Fortaleza 2013 review) ethical guidelines. In 2009, it was approved by the Ethics Committee in Clinical Research. In 2016, it was again approved by the same committee for the follow-up presented in this manuscript.

All participants were evaluated after parents' signed the informed consent and the verbal assent of the children.

### *2.3 Body composition*

#### *2.3.1 Weight, height, and bioimpedance analysis (BIA):*

The weight and bioelectrical impedance analysis were measured using a TANITA BC 418 MA electronic scale (Tanita Europe BV, Amsterdam, Netherlands) with a 0.1 kg precision and 0-200 kg range. Following the manufacturer's instructions (Tanita Corporation - Japan, 2002), the children were asked to stand barefoot and with as little clothing as possible on the weighing platform, touching the electrodes, in a stable position, without bending the knees, and with both hands grabbing the handles.

A SECA® 225 portable stadiometer (SECA® 225, Hamburg, Germany) with a precision of 0.1 cm and a range of 70-220 cm was used to determine the height. The body mass index (BMI) was calculated as the weight divided by the squared height ($kg/m^2$). The AnthroPlus software from the World Health Organization (WHO) (World Health Organization, 2006) was used to calculate

BMI age and gender-specific *z*-scores. The FFMI was calculated relating the FFM in kilograms divided by the squared height in meters.

### *2.3.2 Dual-energy X-ray absorptiometry (DXA):*

Dual-energy X-ray absorptiometry was used to determine the LBM (kg), using the DXA QDR-Explorer™ 4500 (Hologic Inc., Bedford, Massachusetts, USA). All measurements we performed following manufacturer's instructions (National Health and Nutrition Examination Survey (NHANES), 2012). The participants were measured lightly clothed and without metal objects or jewelry. An examination of the whole body was performed. The participants were placed supine, with arms along the body, without touching the trunk (Crabtree et al., 2007). Analyzes and regions of interest (ROI) were determined using Pediatric Hologic Corp. software version 12.4. The whole-body scan intra-measures coefficient variation of LBM in our laboratory was 1.9.

This study used the sum value of the fat, lean, and bone masses (Lean soft tissue mass LSTM) = LBM - bone mineral content (BMC). The total LSTM index (TLSTMI) and the appendicular LSTM index (ALSTMI) were calculated by dividing both the LSTM (Kg) and the appendicular LSTM (Kg) by the height squared.

In this study, the DXA and BIA analyses were carried out between 4.30 pm and 6.30 pm, 2 -4 hours after the end of the previous meal.

### *2.3.3 Peripheral quantitative computed tomography (pQCT):*

Stratec XCT 2000 L (Stratec Medizintechnik, Pforzheim, Germany) was used to measure the muscle cross-sectional area (MCSA) at 66% of the total length of the left tibia (González-Agüero et al., 2013), a method that has proven to be valid for estimating the lean mass (Córdoba-Rodríguez et al., 2021).

All the measurements were carried out after the calibration of the equipment. The total length of the tibia was determined using a segmometer, measuring the distance from the cleft of the knee’s medial joint to the tibia’s (leg) Sphyrion tibial medial malleolus (Roggen et al., 2015).

For the definition of the reference line in the distal tibia, an exploratory scout view was performed, and the reference line was placed at the medial end of the distal epiphysis. The intra-measures coefficient of variation for MCSA using pQCT was 1.69, as previously reported (Gómez-Bruton et al., 2014).

The muscle cross-sectional area index (MCSAI) was calculated by dividing the muscle cross-sectional area (MCSA) by the squared height.

The pQCT and DXA images were evaluated visually by a technician to identify motion artifacts. The images showing movement were subjected to a new exploration or excluded from the data analysis. Moreover, an external evaluator reviewed all the images to endorse the image quality.

### *2.4 Physical activity*

Physical activity was evaluated using the ActiGraph wGT3x-BT triaxial accelerometer (ActiGraph, Ft. Walton Beach, USA). Parents/caregivers and children were asked to place the accelerometer under the child's clothing at the level of the right iliac crest, during their waking day, for seven consecutive days and to complete a diary to assess the minutes and reasons why accelerometers were not being used (Bammann et al., 2011).

Additionally, children and parents / caregivers were advised not to use accelerometers in water activities.

All the accelerometers were programmed to record data every 15 seconds (epoch). Twenty minutes or more of consecutive zero counts were defined as unused time.

For the information processing, a day in which the child used the accelerometer for at least 8 hours was considered a valid day. A minimum of 3 days was required, including at least one weekend day, as was previously used by other authors in similar population groups (Moliner-Urdiales et al., 2010a)

Actilife software version 6.0 (ActiGraph, Pensacola, FL., USA) was used for data processing. The cut-off points determined by Evenson were used as a reference to define the time dedicated to sedentary and different intensity physical activity (sedentary: 0–100, light: 101–2295, moderate: 2296–4011, vigorous: 4012, and more accelerometer counts per minute [cpm]) (Evenson et al., 2008).

The absolute amount of time in each intensity category determined from the accelerometer data was used for the analyzes, taking as a reference that the World Health Organization (WHO)(World Health Organization, 2020) frequently uses the min/day metric to issue their physical activity recommendations.

Most of the children had complete actigraphic data of 7 days (n=126, 42.2%) or 6-5 days (n=118, 42.4%) of. The average follow-up included 6.2 days of recordings.

### *2.5 Upper limb strength - Handgrip strength test:*

A TKK-5401 (Takei Scientific Instruments Co., Ltd., Niigata, Japan) digital handgrip dynamometer with a precision of 0.1kg and adjustable grip was used according to the size of the hand derived from the following gender-specific equations (España-Romero et al., 2008):

Boys: $Y = X/4 + 0.44$ cm

Girls: $Y= 0.3X - 0.52$ cm

Where Y = optimal grip and X = size of the wide-open hand, measured from the tip of the thumb to the tip of the little finger.

During the measurement, participants stood, elbow extended, avoiding any bodily contact with the dynamometer, except with the measured hand. The children were asked to press as hard as possible for 3 to 5 seconds with each hand; two attempts were made with each hand, three minutes of rest interval between each of them. The dynamometer display was aligned to face the examiner, providing blind measurements to the children. The final score was calculated as the average of the best attempt obtained for the left and right hands in kg (De Miguel-Etayo et al., 2014).

### *2.6 Lower limb strength*. *Standing long jump test*

The long jump test was used to assess the explosive strength of the lower extremities. The children were asked to stand, feet slightly apart, in front of a starting line. Then, from a standing position, a forward jump was made using the arms' impulse, being careful not to step on the starting line and pushing with both feet at the same time. The measurement was made from the impulse line to the heel closest to the start line and recorded in cm (López-Gil et al., 2020). Two attempts were made, recording the highest value obtained.

### *2.7 Covariates*

We recorded the following variables for all the children:

#### *2.7.1 Parental education*

*Highest level of education:* the parents were asked to report their highest achieved level of education when they came to our lab in 2016-2017 (no studies; basic-primary studies; intermediate studies [including modules vocational training and secondary studies]; higher studies; and university degrees). These were later coded according to the International Standard Classification of Education (ISCED-2011) (Statistics, 2012) and categorized again into low (0-2), medium (3-4), and high (5-8) educational levels (Unesco, 1997).

#### *2.7.2 Anthropometric data of the child at birth:*

At the beginning of the study, in the first visit (15 days after birth), the pediatric and nursing staff conducting the *Programa de Salud Infantil* (Child Health Program) collected the birth weight variable.

#### *2.7.3 Food frequency questionnaire and diet quality index (DQI):*

A previously validated, semi-quantitative food frequency questionnaire was used to assess the dietary intake (Mouratidou et al., 2019), including 37 foods and beverages. The responding parents were asked to classify each food's consumption frequency using a 6-Point Likert scale ranging from never to every day (never/less than once a month, 1 to 3 times per month, 1 day/week, 2 to 4 days/week, 5 to 6 days/week, and every day) and serving size.

The DQI was subsequently calculated. This index mainly assesses the components of dietary diversity, dietary quality, and dietary balance. Dietary diversity evaluated the daily consumption of at least one food serving from the eight recommended food groups. This component's score ranged from 0 to 9 for at least one serving from a recommended food group. Diet quality expressed whether the children made optimal decisions on the food quality within a food group. Dietary balance was calculated from the difference between the food intake's suitability and its excess in the diet (Huybrechts et al., 2010; Vyncke et al., 2013). These three components of the DQI were presented in percentages. The categories' scores were summed and divided by 3 to compute the overall DQI, resulting in scores ranging from −33% to 100%. The components' mean were used to calculate the DQI, with the highest scores reflecting greater compliance with the diet (Iglesia et al., 2020).

### *2.8 Statistical analysis*

Statistical analyses were carried out using IBM SPSS Statistics® software, version 25 (IBM Corp., Armonk, NY, USA). The variables studied were presented as means (M) ±, standard deviations (SD), and median and interquartile intervals (25th and 75th) in the case of non-normally distributed variables. The distribution of variables was checked and verified using the Kolmogorov-Smirnov test. The Student's t-test or the Mann-Whitney U test were used to identify sex differences between each of the studied variables. Because we observed a significant interaction effect between gender and physical activity and physical fitness, all the analyses were performed separately for boys and girls.

Multiple linear regression (Forced Entry) was used to study the association between physical activity (total, light, moderate, vigorous, or MVPA) or physical fitness (handgrip strength, standing long jump) with the body composition outcomes, which included TLSTMI, MCSAI, and FFMI. These relationships were analyzed in individual regression models (one for each physical activity level or physical fitness variable) using educational level, DQI, and birth weight as covariates. The assumptions of independence of errors were verified for all the multiple regression models using the Durbin-Watson test. Equally, collinearity diagnosis was carried out through the variance inflation factor (VIF).

For the significant models, we used a mediation analysis according to the procedure proposed by Baron and Kenny (Baron & Kenny, 1986) using the macro PROCESS developed by Hayes (Hayes, 2013) with a bootstrap threshold of 10,000 and model 4 for Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM Corporation, New York, USA). The purpose of this model was to examine the total effect (c) and the individual direct effects (a, b, c´) reflected by the non-standardized regression coefficient (β), as well as the statistical significance of the relationship between each model's independent and dependent variables. The model also examined the indirect effect obtained from the coefficients' product (a × b). It indicated the change in TLSTMI by the change in vigorous physical activity and the change in TLSTMI by the handgrip strength change, mediated by the proposed mediator (handgrip strength or physical activity, respectively). If zero was not included in the estimate's 95% confidence interval (CI), we concluded that the indirect effect (IE) was statistically significant, as shown in Figure 1.

## 3. Results

### *3.1 Descriptive statistics*

The characteristics of participants ranked by gender are shown in **Table 1.** Boys showed higher levels of TLSTM (kg), TLSTMI (kg/$m^2$), ALSTMI (kg/$m^2$), MCSA ($mm^2$), MCSAI ($mm^2$/$m^2$), FFM (Kg), FFMI (kg/$m^2$), total, light, moderate, vigorous, and MVPA, handgrip strength and standing long jump than girls (all $p<0.05$).

### *3.2 Association between physical activity or physical fitness with LBM outcomes*

Adjusted associations between physical activity and TLSTMI, MCSAI, and FFMI for both boys and girls are shown in **Table 2.**

In girls, we observed that for Model 1 (physical activity [min/day]), there was an association between total physical activity and TLSTMI (β=0.272, p=0.004). In Model 2, after adding the DQI, the parent's educational level, and age, an association was found between total physical activity (β=0.275, p=0.004) and vigorous physical activity (β=0.159, p=0.001) with TLSTMI. Finally, once the birth weight was added to create Model 3, slight decreases were found for the significant association between total physical activity (β=0.247, p=0.010) and vigorous physical activity (β=0.143, p=0.001) with TLSTMI.

In boys, Model 1 showed a positive association between total physical activity (β=0.335, p=<0.001), light (β=0.287, p=0.001), and vigorous physical activity (β=0.203, p=0.020) with TLSTMI by DXA. After adding the DQI, the parents' educational level, and the age to create Model 2, slight increases were found in the significant associations between total physical activity (β=0.371, p=<0.001), light physical activity (β=0.302, p=0.001), vigorous (β=0.224, p=0.013). An association was found between MVPA (β=0.189, p=0.038) with TLSTMI (Table 2). Once birth weight was added to create Model 3, slight decreases were observed in the associations between total physical activity (β=0.337, p<0.001), light (β=0.290, p=0.001) and vigorous physical activity (β=0.200, p=0.023) with TLSTMI (Table 2). The association between MVPA and TLSTMI in this model disappeared.

No associations were found between physical activity and MCSAI for girls or boys. Regarding FFMI assessed with bioimpedance, no associations were found between FFMI and physical activity in girls. For boys, total physical activity significantly predicted FFMI.

The adjusted associations between handgrip strength and TLSTMI, MCSAI, and FFMI both in boys and girls are shown in **Table 3.** For both genders, handgrip strength was a significant predictor for all the models. For girls, in Model 1, handgrip strength was positively associated with TLSTMI, MCSAI, and FFMI ($\beta=0.363$, $p<0.001$; $\beta=0.262$, $p=0.002$; $\beta=0.236$, $p=0.004$, respectively). In Model 2, significant associations remained for TLSTMI, MCSAI, and FFMI ($\beta=0.350$, $p<0.001$; $\beta=0.282$, $p=0.001$; $\beta=0.218$, $p=0.008$, respectively). However, they decreased slightly for TLSTMI and FFMI. Finally, once birth weight was added to Model 3, the significant associations again decreased slightly, but remained significant ($\beta=0.314$, $p<0.001$; $\beta=0.267$, $p=0.002$; $\beta=0.196$, $p=0.017$, respectively).

In boys, in Model 1 handgrip strength was positively associated with TLSTMI, MCSAI, and FFMI ($\beta=0.478$, $p<0.001$; $\beta=0.273$, $p<0.001$; $\beta=0.289$, $p<0.001$, respectively). In Model 2, significant associations were maintained for TLSTMI, MCSAI, and FFMI ($\beta=0.464$, $p<0.001$; $\beta=0.287$, $p<0.001$; $\beta=0.307$, $p<0.001$, respectively) but decreased slightly for TLSTMI and increased for MCSAI and BIA. Finally, once birth weight was added to Model 3, the significant associations again decreased slightly for TLSTMI and increased for MCSAI and BIA ($\beta=0.425$, $p<0.001$; $\beta=0.301$, $p<0.001$; $\beta=0.325$, $p=0.008$, respectively).

There was no association between standing long jump and TLSTMI, MCSAI, and FFMI in both girls and boys (Table 3). This analysis was also performed adjusting standing long jump by weight, obtaining similar results.

### *3.3 Mediation analysis*

An analysis of the handgrip's strength mediation effect on the association of total physical activity with TLSTMI values indicated an association between total physical activity and handgrip strength ($\beta_1=0.0057$, 95% CI: 0.0000 to 0.0114, p 0.0484*) in the first regression equation (a). In the second equation (b), handgrip strength was positively associated with TLSTMI ($\beta_3=0.2188$, 95% CI: 0.1637 to 0.2739, $p<0.001$). The third equation (c´) showed a positive relationship between total physical activity and TLSTMI ($\beta_{dir}$=0.0073, 95% CI: 0.0045 to 0.0101 p<0.001*). The relationship between total physical activity and TLSTMI was not statistically significant when including handgrip strength in the model (a * b), indicating that handgrip strength does not mediate in this relationship ($\beta_{ind}$=0.0013, 95% CI: 0.0000 a 0.0026) (Figure 1).

## 4. Discussion

Our results indicate that this sample of children accumulated around 110 min of MVPA. Studies available in the population of the United States (Trost et al., 2002) and Europe (Riddoch et al., 2004), where physical activity data were objectively measured, suggest that young children (schoolchildren) accumulate more than 100 min of MVPA per day. Our results are comparable with these findings. This could be because children are more active at these ages (possibly through organized activities (physical exercise and youth sports) and not organized (recess and unstructured activity (Wickel & Eisenmann, 2007)**,** and active transport (Carver et al., 2011))), to later decrease the physical activity in adolescence, especially in girls.

Our main findings indicate that there is an association between total physical activity and vigorous physical activity with TLSTMI in girls, while for boys, the associations were found between total, light, vigorous, and MVPA with TLSTMI. One possible explanation may be the role of certain hormones (growth hormone, IGF-1, and gender steroids, such as testosterone and estradiol), which play a fundamental role in developing skeletal muscle during infancy, childhood, and adolescence, depending on gender (Veldhuis et al., 2005).

Additionally, our results indicate that upper limb strength (handgrip strength) does not mediate the relationship between total physical activity and TLSTMI. As far as we know, this is the first time that these results are shown in a broad sample of young children using high precision objective methods.

In agreement with our results, a study involving 283 Chinese adolescent girls also found a significant positive association between total physical activity level assessed by questionnaire and LBM ($p<0.001$), suggesting that higher physical activity levels may reflect a higher LBM (Foo et al., 2007). Similarly, a study by Jiménez-Pavón study using accelerometers found that total physical activity was positively associated with FFM ($p<0.05$) in 2,200 (1016 male, 1184 females) European adolescents of both sexes (Jiménez-Pavón et al., 2013). In the same line, Deheeger et al. (Deheeger et al., 1997) found that total physical activity was positively associated with the percentage of FFM ($r=0.23$; p 0.03) in ten-year-old children ($n=86$). Rennie et al. also showed that the level of physical activity was positively associated with the lean mass index (LMI) in a study carried out with 100 children aged 6 to 8 (Rennie et al., 2005). Our study is in the same line as previously published studies, suggesting that total physical activity positively affects non-adipose tissue in both female and male children and adolescents.

Regarding vigorous physical activity and MVPA, a study by Jiménez-Pavón found positive associations of vigorous physical activity with FFM and muscle mass (all $p<0.05$) in both sexes (Jiménez-Pavón et al., 2013). In this study, skinfold thickness and bioimpedance were used to evaluate body composition, accelerometry for physical activity. Similarly, a study by Hao et al. found a positive association between MVPA and SMMI ($\beta=0.20$, $p<0.001$). It involved 640 adolescents and concerned fat-free soft tissue mass assessed by DXA, subsequently determining the skeletal muscle mass index (SMMI) (Hao et al., 2019). Additionally, in a longitudinal study conducted in Pelotas (Brazil) (Ramires et al., 2016), a consistent moderate and vigorous physical activity practice during adolescence was associated with a greater lean mass index in both sexes.

Conversely, studies such as the one developed by Moliner-Urdiales et al. (Moliner-Urdiales et al., 2010a) in a sample of 363 Spanish adolescents aged 12.5 to 17.5 did not observe an association between FFM and physical activity levels. Heelan et al. (Heelan & Eisenmann, 2006), in 4- to 7-year-old children, observed, only in girls, a negative correlation between MVPA and FFM ($r=-0.39$, $p<0.05$).

Different factors could explain our contrasting results. They include the individuals' age ranges included in each study (preschoolers (Leppänen et al., 2016), adolescents (Foo et al., 2007; Hao et al., 2019; Jiménez-Pavón et al., 2013), and schoolchildren (Deheeger et al., 1997; Rennie et al., 2005)) and their maturation stage. Other factors include the methods for assessing body composition (DXA (Foo et al., 2007; Hao et al., 2019; Heelan & Eisenmann, 2006), anthropometry (Deheeger et al., 1997; Jiménez-Pavón et al., 2013), BIA (Jiménez-Pavón et al., 2013), isotopic dilution (Rennie et al., 2005), and air displacement plethysmography (Leppänen et al., 2016)) and physical activity (questionnaires (Deheeger et al., 1997; Foo et al., 2007; Ramires et al., 2016; Rennie et al., 2005) and accelerometers (Jiménez-Pavón et al., 2013)). They also include the different types of devices for assessing physical activity ( uniaxial (Hao et al., 2019; Heelan & Eisenmann, 2006; Moliner-Urdiales et al., 2010b) or triaxial devices (Jiménez-Pavón et al., 2013; Leppänen et al., 2016), as well as the different cutoff points to define physical activity intensities, varying sampling intervals (epochs) (10s (Leppänen et al., 2016), 15 s (Jiménez-Pavón et al., 2013) and 1min (Hao et al., 2019; Heelan & Eisenmann, 2006)), and sporadic or episodes of accumulated physical activity data. Moreover, the presence of FFM (sum of lean mass and bone mass) or fat-free soft tissue mass (FFM - bone mineral content) and the use of absolute values (kg (Foo et al., 2007; Jiménez-Pavón et al., 2013)) or relative values (% (Deheeger et al., 1997) or index (Hao et al., 2019; Leppänen et al., 2016; Ramires et al., 2016; Rennie et al., 2005)) to express the value of the FFM/LBM can make it difficult to compare individuals of different sizes appropriately because FFM varies with height, weight, age. The percentage of FFM automatically decreases in proportion to the increase in the % of body fat. The last possible affecting factors are the covariates taken into account in the analyzes (age and maturation stage, the mother's BMI and educational level, the father's BMI and educational level, and the age at the time of measurement and time of use awake).

Some of these factors were also reported in a systematic review carried out by Poitrasl et al. (Poitras et al., 2016). Their purpose was to examine the relationships between objectively measured physical activity and relevant indicators (body composition, cardiometabolic biomarkers, and physical fitness, among others) in 5- to 17-year-old children and adolescents.

Our findings can support emerging evidence suggesting that different physical activity intensities, including light physical activity, may significantly affect health outcomes. They are in line with Corson's findings in 1,731 adolescents aged 12 to 19, evaluated during the National Health and Nutrition Examination Survey 2003/2004 and 2005/2006 (Carson et al., 2013)—reinforcing the claim that some activity is better than none, but that "more is better" (Tremblay et al., 2011). However, we also found that the assessment method could influence results. No significant associations were found when evaluating the associations using the variables of the lean component determined with BIA or pQCT. An explanation for this could be the fact that, although BIA can provide information on the whole-body FFM status (like DXA), alterations in body water, such as dehydration, can influence it, increasing resistance to electricity, and subsequently underestimating the FFM (or overestimating body fat). Another influence could be the time of the day. In this study, the DXA and BIA analyses were carried out between 4.30 pm and 6.30 pm to ensure that no child was fasting, influencing the results. Other influences could include food consumption and recent activity (exercise) (Heymsfield et al., 2015).

On the other hand, pQCT quantifies the cross-sectional area of the peripheral (appendicular)/limb muscle (MCSA) (Frank-Wilson et al., 2015) and not the whole body, which could explain why we found no associations between physical activity and MCSAI. MCSA can also be influenced by the anthropometric characteristics of the subjects (body and limb size); therefore, we used MCSAI. Meanwhile, DXA provides information on the LSTM (Bazzocchi et al., 2016) of the whole body or regional/segmental zones (Laskey, 1996), with the advantages of its high precision and reproducibility (Guglielmi et al., 2016).

Our main findings also suggest a positive association between upper body muscle strength (handgrip strength) and TLSTMI, MCSAI, and FFMI in both boys and girls, after controlling for gender, education level, DQI, and birth weight. The results were consistent regardless of the methodology used to assess body FFM; however, the associations were weaker when BIA was used to measure LSTM markers. Similar results were found in the MINISTOP (mobile device-based intervention aimed at stopping obesity in pre-schoolers) study (Henriksson et al., 2016), where PREFIT (physical fitness test battery in preschool children) was used to measure physical fitness (Ortega et al., 2015) in 303 4-year-old children. Its associations showed a higher FFMI in participants with better upper body muscle strength (handgrip strength) ($\beta=0.39$, $p<0.001$).

Anthropometric variables such as body height, body mass, and BMI, which can influence grip strength, may explain these findings. In the case of body height, it is directly correlated with grip strength; this could partially explain its close relation to LBM (Jürimäe et al., 2009). Both the MINISTOP study and the present study made the respective height adjustment.

The present study found no association between standing long jump and TLSTMI, MCSAI, and FFMI. However, another study by Vicente-Rodriguez et al. (Vicente-Rodriguez et al., 2004) showed an increase in the FFM of 28 children (soccer players) followed for three years. They found no significant differences in muscle strength after evaluation through vertical jumps and maximal isometric force (MIF) during leg extension. Conversely, Henriksson et al.(Henriksson et al., 2016) observed that higher FFMI was associated with improved lower-body muscular strength ($\beta=0.22$, $p<0.001$). This disparity could be explained by the different populations included in each of the studies (preschoolers, schoolchildren, and soccer players), as well as their maturation stage (preschoolers, schoolchildren, and pre-puberty), the body composition evaluation methods (DXA and air displacement plethysmography) and the measurement methodology for the forces generated during vertical jumps (force plate versus standing long jump test). The lack of association between lower limb strength and FFM may also be because this test is considered weight-dependent, requiring propulsion or body elevation. Therefore, children and adolescents with higher LBM may not display better performance in these tests because they likely weigh differently. These results could suggest that higher performance in some physical fitness tests may occur in underweight individuals because of the lower load that they must move.

Finally, the relationship between physical activity and physical fitness (handgrip strength) and TLSTMI in our study is mainly consistent with the existing evidence showing positive associations independent of physical activity and physical fitness in TLSTMI. Our research shows that the association of total physical activity with TLSTMI is not mediated by physical fitness (handgrip strength). Therefore, future actions seeking to improve TLSTM in boys and girls should encourage activities that enhance physical activity levels and physical fitness.

### *4.1 Limitations and Strengths*

Within the limitations of the present study, there is the fact that the data cannot be extrapolated to other populations. Studies with different age groups and different ethnic groups should be developed in the future. Furthermore, due to its cross-sectional design, the observed associations cannot be interpreted as causal relationships.

Despite these limitations, the strengths of our study are many. To date, studies on a homogeneous sample with such young children are limited. The use of accelerometers allowed the objective assessment of physical activity, including both weekdays and weekend days. LSTM and FFM were measured using two different techniques, DXA and BIA, the former, a robust method accepted as the gold standard method. MCSA was measured by pQCT, a lower-cost method previously validated against DXA in this population. The DXA and pQCT measurements and the respective image analysis were performed by two trained researchers in each of the techniques. The TLSTM, ALSTM, MCSA, and FFM results were adjusted for the height of the subjects. Different tests were used to assess muscle strength (handgrip strength and standing long jump), controlling for several confounders, including gender, age, birth weight, DQI, and parents’ education level. Furthermore, it appears to be the first study evaluating handgrip strength as a potential mediator of the relationship between physical activity and TLSTMI in children.

## 5. Conclusions

The present study suggests positive associations between total and vigorous physical activity and TLSTMI in boys and girls aged 6 to 8. Its results indicate that upper limb strength (handgrip strength) does not mediate the relationship between total physical activity and TLSTMI.

The results also suggest an association between handgrip strength and TLSTMI, MCSAI, and FFMI in 6- to 8-year-old children. More studies are needed in the future to clarify the relationships between physical activity and physical fitness with TLSTM, MCSA, and FFM better, taking into account other confounding factors such as genetics factors, as well as physical activity intensities. To date, most of the available studies focus on the relationship at higher intensities of physical activity (i.e., MVPA and vigorous physical activity).

Public policies should encourage participation in all physical activities, including light physical activity. As we have shown, it can be an effective substitute for sedentary activities and expand the focus beyond the MVPA as a strategy to promote our children’s health. Likewise, exercises that improve upper extremity strength and lean mass should be encouraged, which, as mentioned above, is an important tissue in children.

## Disclosure Statement

The authors report no conflict of interest.

## Bibliography

Bammann, K., Sioen, I., Huybrechts, I., Casajús, J. A., Vicente-Rodríguez, G., Cuthill, R., Konstabel, K., Tubić, B., Wawro, N., Rayson, M., Westerterp, K., Mårild, S., Pitsiladis, Y. P., Reilly, J. J., Moreno, L. A., & De Henauw, S.

(2011). The IDEFICS validation study on field methods for assessing physical activity and body composition in children: Design and data collection. *International Journal of Obesity*, *35*, S79–S87. https://doi.org/10.1038/ijo.2011.38

Baron, R. M., & Kenny, D. A. (1986). The moderator–mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. *Journal of Personality and Social Psychology*, *51*(6), 1173. https://doi.org/https://doi.org/10.1037/0022-3514.51.6.1173

Baxter-Jones, A. D. G., Eisenmann, J. C., Mirwald, R. L., Faulkner, R. A., & Bailey, D. A. (2008). The influence of physical activity on lean mass accrual during adolescence: a longitudinal analysis. *Journal of Applied Physiology*, *105*(2), 734–741. https://doi.org/https://doi.org/10.1152/japplphysiol.00869.2007

Bazzocchi, A., Ponti, F., Albisinni, U., Battista, G., & Guglielmi, G. (2016). DXA: Technical aspects and application. *European Journal of Radiology*, *85*(8), 1481–1492. https://doi.org/10.1016/j.ejrad.2016.04.004

Boisseau, N., & Delamarche, P. (2000). Metabolic and hormonal responses to exercise in children and adolescents. *Sports Medicine*, *30*(6), 405–422. https://doi.org/10.2165/00007256-200030060-00003

Burrows, R., Correa-Burrows, P., Reyes, M., Blanco, E., Albala, C., & Gahagan, S. (2017). Low muscle mass is associated with cardiometabolic risk regardless of nutritional status in adolescents: A cross-sectional study in a Chilean birth cohort. *Pediatric Diabetes*, *18*(8), 895–902. https://doi.org/10.1111/pedi.12505

Carson, V., Ridgers, N. D., Howard, B. J., Winkler, E. A. H., Healy, G. N., Owen, N., Dunstan, D. W., & Salmon, J. (2013). Light-Intensity Physical Activity and Cardiometabolic Biomarkers in US Adolescents. *PLoS ONE*, *8*(8). https://doi.org/10.1371/journal.pone.0071417

Carver, A., Timperio, A. F., Hesketh, K. D., Ridgers, N. D., Salmon, J. L., & Crawford, D. A. (2011). How is active transport associated with children’s and adolescents’ physical activity over time? *International Journal of Behavioral Nutrition and Physical Activity*, *8*, 1–6.

https://doi.org/10.1186/1479-5868-8-126

Córdoba-Rodríguez, D. P., Iglesia, I., Gomez-Bruton, A., Miguel-Berges, M. L., Flores-Barrantes, P., Casajús, J. A., Moreno, L. A., & Rodríguez, G. (2021). Quantitative peripheral computed tomography to measure muscle area and assess lean soft tissue mass in children. *Annals of Human Biology*, 1–27. https://doi.org/10.1080/03014460.2021.1877352

Crabtree, N. J., Leonard, M. B., & Zemel, B. S. (2007). *Dual-Energy X-Ray Absorptiometry BT - Bone Densitometry in Growing Patients: Guidelines for Clinical Practice* (A. J. Sawyer, L. K. Bachrach, & E. B. Fung (eds.); pp. 41–57). Humana Press. https://doi.org/10.1007/978-1-59745-211-3_3

De Miguel-Etayo, P., Gracia-Marco, L., Ortega, F. B., Intemann, T., Foraita, R., Lissner, L., Oja, L., Barba, G., Michels, N., Tornaritis, M., Molnár, D., Pitsiladis, Y., Ahrens, W., & Moreno, L. A. (2014). Physical fitness reference standards in European children: The IDEFICS study. *International Journal of Obesity*, *38*(September), S57–S66. https://doi.org/10.1038/ijo.2014.136

Deheeger, M., Rolland-Cachera, M. F., & Fontvieille, A. M. (1997). Physical activity and body composition in 10 year old French children: Linkages with nutritional intake? *International Journal of Obesity*, *21*(5), 372–379. https://doi.org/10.1038/sj.ijo.0800415

Dorsey, K. B., Thornton, J. C., Heymsfield, S. B., & Gallagher, D. (2010). Greater lean tissue and skeletal muscle mass are associated with higher bone mineral content in children. *Nutrition and Metabolism*, *7*, 1–11. https://doi.org/10.1186/1743-7075-7-41

Dotan, R., Mitchell, C., Cohen, R., Klentrou, P., Gabriel, D., & Falk, B. (2012). Child-adult differences in muscle activation - A review. *Pediatric Exercise Science*, *24*(1), 2–21. https://doi.org/10.1123/pes.24.1.2

España-Romero, V., Artero, E. G., Santaliestra-Pasias, A. M., Gutierrez, A., Castillo, M. J., & Ruiz, J. R. (2008). Hand Span Influences Optimal Grip Span in Boys and Girls Aged 6 to 12 Years. *Journal of Hand Surgery*, *33*(3), 378–384. https://doi.org/10.1016/j.jhsa.2007.11.013

Evenson, K. R., Catellier, D. J., Gill, K., Ondrak, K. S., & McMurray, R. G.

(2008). Calibration of two objective measures of physical activity for children. *Journal of Sports Sciences*, *26*(14), 1557–1565. https://doi.org/10.1080/02640410802334196

Foo, L. H., Zhang, Q., Zhu, K., Ma, G., Greenfield, H., & Fraser, D. R. (2007). *Influence of body composition , muscle strength , diet and physical activity on total body and forearm bone mass in Chinese adolescent girls*. 1281–1287. https://doi.org/10.1017/S0007114507787421

Frank-Wilson, A. W., Johnston, J. D., Olszynski, W. P., & Kontulainen, S. A. (2015). Measurement of muscle and fat in postmenopausal women: precision of previously reported pQCT imaging methods. *Bone*, *75*, 49–54. https://doi.org/10.1016/J.BONE.2015.01.016

Fraser, B. J., Blizzard, L., Cleland, V., Schmidt, M. D., Smith, K. J., Gall, S. L., Dwyer, T., Venn, A. J., & Magnussen, C. G. (2020). Factors associated with muscular fitness phenotypes in Australian children: A cross-sectional study. *Journal of Sports Sciences*, *38*(1), 38–45. https://doi.org/10.1080/02640414.2019.1679575

Gómez-Bruton, A., Gonzalez-Agüero, A., Casajús, J. A., & Rodríguez, G. V. (2014). Swimming training repercussion on metabolic and structural bone development; benefits of the incorporation of whole body vibration or pilometric training; the RENACIMIENTO project. *Nutricion Hospitalaria*, *30*(2), 399–409. https://doi.org/10.3305/nh.2014.30.2.7603

González-Agüero, A., Vicente-Rodríguez, G., Gómez-Cabello, A., & Casajús, J. A. (2013). Cortical and trabecular bone at the radius and tibia in male and female adolescents with Down syndrome: a peripheral quantitative computed tomography (pQCT) study. *Osteoporosis International : A Journal Established as Result of Cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*, *24*(3), 1035—1044. https://doi.org/10.1007/s00198-012-2041-7

Gracia-Marco, L., Moreno, L. A., Ruiz, J. R., Ortega, F. B., de Moraes, A. C. F., Gottrand, F., Roccaldo, R., Marcos, A., Gómez-Martínez, S., Dallongeville, J., Kafatos, A., Molnar, D., Bueno, G., de Henauw, S., Widhalm, K., & Wells, J. C. (2016). Body Composition Indices and Single and Clustered

Cardiovascular Disease Risk Factors in Adolescents: Providing Clinical-Based Cut-Points. *Progress in Cardiovascular Diseases*, *58*(5), 555–564. https://doi.org/10.1016/j.pcad.2015.11.002

Guglielmi, G., Ponti, F., Agostini, M., Amadori, M., Battista, G., & Bazzocchi, A. (2016). The role of DXA in sarcopenia. *Aging Clin Exp Res*, *28*(6), 1047–1060. https://doi.org/10.1007/s40520-016-0589-3

Hao, G., Pollock, N. K., Harris, R. A., Gutin, B., Su, S., & Wang, X. (2019). Associations between muscle mass, physical activity and dietary behaviour in adolescents. *Pediatric Obesity*, *14*(3), 1–8. https://doi.org/10.1111/ijpo.12471

Hayes, A. F. (2013). Mediation, moderation, and conditional process analysis. Introduction to mediation, moderation, and conditional process analysis: A regression-based approach. *New York: Guilford Publications*, 1–20.

Heelan, K. A., & Eisenmann, J. C. (2006). Physical activity, media time, and body composition in young children. *Journal of Physical Activity and Health*, *3*(2), 200–209. https://doi.org/10.1123/jpah.3.2.200

Henriksson, P., Cadenas-Sanchez, C., Leppänen, M. H., Nyström, C. D., Ortega, F. B., Pomeroy, J., Ruiz, J. R., & Löf, M. (2016). Associations of fat mass and fat-free mass with physical fitness in 4-year-old children: Results from the MINISTOP trial. *Nutrients*, *8*(8), 1–11. https://doi.org/10.3390/nu8080473

Heymsfield, S. B., Gonzalez, M. C., Lu, J., Jia, G., & Zheng, J. (2015). Skeletal muscle mass and quality: Evolution of modern measurement concepts in the context of sarcopenia. *Proceedings of the Nutrition Society*, *74*(4), 355–366. https://doi.org/10.1017/S0029665115000129

Huybrechts, I., Vereecken, C., De Bacquer, D., Vandevijvere, S., Van Oyen, H., Maes, L., Vanhauwaert, E., Temme, L., De Backer, G., & De Henauw, S. (2010). Reproducibility and validity of a diet quality index for children assessed using a FFQ. *British Journal of Nutrition*, *104*(1), 135–144. https://doi.org/10.1017/S0007114510000231

Iglesia, I., Intemann, T., De Miguel-Etayo, P., Pala, V., Hebestreit, A., Wolters, M., Russo, P., Veidebaum, T., Papoutsou, S., Nagy, P., Eiben, G., Rise, P., De Henauw, S., & Moreno, L. A. (2020). Dairy consumption at snack meal occasions and the overall quality of diet during childhood. Prospective and cross-sectional analyses from the idefics/i.family cohort. *Nutrients*, *12*(3), 1–20. https://doi.org/10.3390/nu12030642

Isganaitis, E. (2019). Developmental Programming of Body Composition: Update on Evidence and Mechanisms. *Current Diabetes Reports*, *19*(8), 1–20. https://doi.org/10.1007/s11892-019-1170-1

Jiménez-Pavón, D., Fernández-Vázquez, A., Alexy, U., Pedrero, R., Cuenca-García, M., Polito, A., Vanhelst, J., Manios, Y., Kafatos, A., Molnar, D., Sjöström, M., & Moreno, L. A. (2013). Association of objectively measured physical activity with body components in European adolescents. *BMC Public Health*, *13*(1). https://doi.org/10.1186/1471-2458-13-667

Jürimäe, T., Hurbo, T., & Jürimäe, J. (2009). Relationship of handgrip strength with anthropometric and body composition variables in prepubertal children. *HOMO- Journal of Comparative Human Biology*, *60*(3), 225–238. https://doi.org/10.1016/j.jchb.2008.05.004

Kim, J. H., & Park, Y. S. (2016). Low muscle mass is associated with metabolic syndrome in Korean adolescents: the Korea National Health and Nutrition Examination Survey 2009-2011. *Nutrition Research*, *36*(12), 1423–1428. https://doi.org/10.1016/j.nutres.2016.09.013

Kim, K., Hong, S., & Kim, E. Y. (2016). Reference values of skeletal muscle mass for Korean children and adolescents using data from the Korean national health and nutrition examination survey 2009-2011. *PLoS ONE*, *11*(4), 1–10. https://doi.org/10.1371/journal.pone.0153383

Kim, S., & Valdez, R. (2015). Metabolic risk factors in U.S. youth with low relative muscle mass. *Obesity Research and Clinical Practice*, *9*(2), 125–132. https://doi.org/10.1016/j.orcp.2014.05.002

Kulkarni, B., Hills, A. P., & Byrne, N. M. (2014). Nutritional influences over the life course on lean body mass of individuals in developing countries. *Nutrition Reviews*, *72*(3), 190–204. https://doi.org/10.1111/nure.12097

Kuriyan, R. (2018). Body composition techniques. *Indian Journal of Medical Research*, *148*(5), 648–658. https://doi.org/10.4103/ijmr.IJMR_1777_18

Labayen, I., Moreno, L. A., Blay, M. G., Blay, V. A., Mesana, M. I., González-Gross, M., Bueno, G., Sarría, A., & Bueno, M. (2006). Early programming of body composition and fat distribution in adolescents. *Journal of Nutrition*, *136*(1), 147–152. https://doi.org/10.1093/jn/136.1.147

Larqué, E., Labayen, I., Flodmark, C. E., Lissau, I., Czernin, S., Moreno, L. A., Pietrobelli, A., & Widhalm, K. (2019). From conception to infancy — early risk factors for childhood obesity. *Nature Reviews Endocrinology*, *15*(8), 456–478. https://doi.org/10.1038/s41574-019-0219-1

Laskey, M. A. (1996). Dual-energy X-ray absorptiometry and body composition. *Nutrition*, *12*(1), 45–51. https://doi.org/10.1097/01.mco.0000165010.31826.3d

Leppänen, M., Henriksson, P., Delisle Nyström, C., Henriksson, H., Ortega, F. B., Pomeroy, J., Ruiz, J. R., Cadenas-Sanchez, C., & Löf, M. (2017). Longitudinal physical activity, body composition, and physical fitness in preschoolers. In *Medicine and Science in Sports and Exercise* (Vol. 49, Issue 10). https://doi.org/10.1249/MSS.0000000000001313

Leppänen, M., Nyström, C., Henriksson, P., Pomeroy, J., Ruiz, J. R., Ortega, F., Cadenas-Sánchez, C., & Löf, M. (2016). Physical activity intensity, sedentary behavior, body composition and physical fitness in 4-year-old children: Results from the ministop trial. *International Journal of Obesity*, *40*(7), 1126–1133. https://doi.org/10.1038/ijo.2016.54

Liu, J., Yan, Y., Xi, B., Huang, G., & Mi, J. (2019). Skeletal muscle reference for Chinese children and adolescents. *Journal of Cachexia, Sarcopenia and Muscle*, *10*(1), 155–164. https://doi.org/10.1002/jcsm.12361

López-Gil, J. F., Brazo-Sayavera, J., García-Hermoso, A., & Yuste Lucas, J. L. (2020). Adherence to Mediterranean Diet Related with Physical Fitness and Physical Activity in Schoolchildren Aged 6–13. *Nutrients*, *12*(2), 567. https://doi.org/10.3390/nu12020567

Moliner-Urdiales, D., Ortega, F. B., Vicente-Rodriguez, G., Rey-Lopez, J. P., Gracia-Marco, L., Widhalm, K., Sjöström, M., Moreno, L. A., Castillo, M. J., & Ruiz, J. R. (2010a). Association of physical activity with muscular strength and fat-free mass in adolescents: The HELENA study. *European*

*Journal of Applied Physiology*, *109*(6), 1119–1127. https://doi.org/10.1007/s00421-010-1457-z

Moliner-Urdiales, D., Ortega, F. B., Vicente-Rodriguez, G., Rey-Lopez, J. P., Gracia-Marco, L., Widhalm, K., Sjöström, M., Moreno, L. A., Castillo, M. J., & Ruiz, J. R. (2010b). Association of physical activity with muscular strength and fat-free mass in adolescents: The HELENA study. *European Journal of Applied Physiology*, *109*(6), 1119–1127. https://doi.org/10.1007/s00421-010-1457-z

Mouratidou, T., Mesana Graffe, M. I., Huybrechts, I., De Decker, E., De Craemer, M., Androutsos, O., Manios, Y., Galcheva, S., Lateva, M., Gurzkowska, B., Kułaga, Z., Birnbaum, J., Koletzko, B., & Moreno, L. A. (2019). Reproducibility and relative validity of a semiquantitative food frequency questionnaire in European preschoolers: The ToyBox study. *Nutrition*, *65*, 60–67. https://doi.org/10.1016/j.nut.2019.03.003

National Health and Nutrition Examination Survey (NHANES). (2012). *Body Composition Procedures Manual*. *July*, 1–125.

Orsso, C. E., Tibaes, J. R. B., Oliveira, C. L. P., Rubin, D. A., Field, C. J., Heymsfield, S. B., Prado, C. M., & Haqq, A. M. (2019). Low muscle mass and strength in pediatrics patients: Why should we care? *Clinical Nutrition*, *38*(5), 2002–2015. https://doi.org/10.1016/j.clnu.2019.04.012

Ortega, F. B., Cadenas-Sánchez, C., Sánchez-Delgado, G., Mora-González, J., Martínez-Téllez, B., Artero, E. G., Castro-Piñero, J., Labayen, I., Chillón, P., Löf, M., & Ruiz, J. R. (2015). Systematic Review and Proposal of a Field-Based Physical Fitness-Test Battery in Preschool Children: The PREFIT Battery. *Sports Medicine*, *45*(4), 533–555. https://doi.org/10.1007/s40279-014-0281-8

Poitras, V. J., Gray, C. E., Borghese, M. M., Carson, V., Chaput, J.-P., Janssen, I., Katzmarzyk, P. T., Pate, R. R., Connor Gorber, S., & Kho, M. E. (2016). Systematic review of the relationships between objectively measured physical activity and health indicators in school-aged children and youth. *Applied Physiology, Nutrition, and Metabolism*, *41*(6), S197–S239. https://doi.org/10.1139/apnm-2015-0663

Ramires, V. V., Dumith, S. C., Wehrmeister, F. C., Hallal, P. C., Menezes, A. M. B., & Gonçalves, H. (2016). Physical activity throughout adolescence and body composition at 18 years: 1993 Pelotas (Brazil) birth cohort study. *International Journal of Behavioral Nutrition and Physical Activity*, *13*(1), 1–13. https://doi.org/10.1186/s12966-016-0430-6

Rennie, K. L., Livingstone, M. B. E., Wells, J. C. K., Mcgloin, A., Coward, W. A., & Prentice, A. M. (2005). *Association of physical activity with body-composition indexes in children aged 6 – 8 y at varied risk of obesity 1 – 3*. 13–20. https://doi.org/https://doi.org/10.1093/ajcn/82.1.13

Riddoch, C. J., Andersen, L. B., Wedderkopp, N., Harro, M., Klasson-Heggebø, L., Sardinha, L. B., Cooper, A. R., & Ekelund, U. (2004). Physical Activity Levels and Patterns of 9- and 15-yr-Old European Children. *Medicine and Science in Sports and Exercise*, *36*(1), 86–92. https://doi.org/10.1249/01.MSS.0000106174.43932.92

Roggen, I., Roelants, M., Sioen, I., Vandewalle, S., De Henauw, S., Goemaere, S., Kaufman, J.-M., & De Schepper, J. (2015). Pediatric Reference Values for Tibial Trabecular Bone Mineral Density and Bone Geometry Parameters Using Peripheral Quantitative Computed Tomography. *Calcified Tissue International*, *96*(6), 527–533. https://doi.org/10.1007/s00223-015-9988-2

Ruiz, J. R., Castro-Piñero, J., Artero, E. G., Ortega, F. B., Sjöström, M., Suni, J., & Castillo, M. J. (2009). Predictive validity of health-related fitness in youth: a systematic review. *British Journal of Sports Medicine*, *43*(12), 909 LP – 923. https://doi.org/10.1136/bjsm.2008.056499

Santos, D. A., Magalhaes, J. P., Judice, P. B., Correia, I. R., Minderico, C. S., Ekelund, U., & Sardinha, L. B. (2019). Fitness Mediates Activity and Sedentary Patterns Associations with Adiposity in Youth. *Medicine & Science in Sports & Exercise*, *51*(2), 323–329. https://doi.org/10.1249/MSS.0000000000001785

Scheurer, J. M., Zhang, L., Plummer, E. A., Hultgren, S. A., Demerath, E. W., & Ramel, S. E. (2018). Body Composition Changes from Infancy to 4 Years and Associations with Early Childhood Cognition in Preterm and Full-Term Children. *Neonatology*, *114*(2), 169–176.

https://doi.org/10.1159/000487915

Singhal, A., Wells, J., Cole, T. J., Fewtrell, M., & Lucas, A. (2003). Programming of lean body mass: A link between birth weight, obesity, and cardiovascular disease? *American Journal of Clinical Nutrition*, *77*(3), 726–730. https://doi.org/10.1093/ajcn/77.3.726

Sioen, I., Lust, E., De Henauw, S., Moreno, L. A., & Jiménez-Pavón, D. (2016). Associations Between Body Composition and Bone Health in Children and Adolescents: A Systematic Review. *Calcified Tissue International*, *99*(6), 557–577. https://doi.org/10.1007/s00223-016-0183-x

Statistics, U. I. for. (2012). *International standard classification of education: ISCED 2011*. UNESCO Institute for Statistics Montreal.

Tanita Corporation - Japan. (2002). *Body Composition Analyzer BC-418 Instruction Manual*. 1–43.

Tremblay, M. S., Warburton, D. E. R., Janssen, I., Paterson, D. H., Latimer, A. E., Rhodes, R. E., Kho, M. E., Hicks, A., LeBlanc, A. G., Zehr, L., Murumets, K., & Duggan, M. (2011). New Canadian physical activity guidelines. *Applied Physiology, Nutrition and Metabolism*, *36*(1), 36–46. https://doi.org/10.1139/H11-009

Trost, S. G., Pate, R. R., Sallis, J. F., Freedson, P. S., Taylor, W. C., Dowda, M., & Sirard, J. (2002). Age and gender differences in objectively measured physical activity in youth. *Medicine and Science in Sports and Exercise*, *34*(2), 350–355. https://doi.org/10.1097/00005768-200202000-00025

Unesco. (1997). *International Standard Classification of Education-ISCED 1997: November 1997*. Unesco.

Veldhuis, J. D., Roemmich, J. N., Richmond, E. J., Rogol, A. D., Lovejoy, J. C., Sheffield-Moore, M., Mauras, N., & Bowers, C. Y. (2005). Endocrine control of body composition in infancy, childhood, and puberty. *Endocrine Reviews*, *26*(1), 114–146. https://doi.org/10.1210/er.2003-0038

Vicente-Rodriguez, G., Ara, I., Perez-Gomez, J., Serrano-Sanchez, J. A., Dorado, C., & Calbet, J. A. L. (2004). High Femoral Bone Mineral Density Accretion in Prepubertal Soccer Players. *Medicine & Science in Sports &*

*Exercise*, *36*(10). https://journals.lww.com/acsm-msse/Fulltext/2004/10000/High_Femoral_Bone_Mineral_Density_Accretion_in.19.aspx

Vyncke, K., Cruz Fernandez, E., Fajó-Pascual, M., Cuenca-García, M., De Keyzer, W., Gonzalez-Gross, M., Moreno, L. A., Beghin, L., Breidenassel, C., Kersting, M., Albers, U., Diethelm, K., Mouratidou, T., Grammatikaki, E., De Vriendt, T., Marcos, A., Bammann, K., Börnhorst, C., Leclercq, C., … Huybrechts, I. (2013). Validation of the Diet Quality Index for Adolescents by comparison with biomarkers, nutrient and food intakes: The HELENA study. *British Journal of Nutrition*, *109*(11), 2067–2078. https://doi.org/10.1017/S000711451200414X

Wells, J. C. K. (2000). A Hattori chart analysis of body mass index in infants and children. *International Journal of Obesity*, *24*(3), 325–329. https://doi.org/10.1038/sj.ijo.0801132

Wells, J. C. K., Cole, T. J., & team, A. study. (2002). Adjustment of fat-free mass and fat mass for height in children aged 8 y. *International Journal Of Obesity*, *26*, 947. https://doi.org/10.1038/sj.ijo.0802027

Westerterp, K. R., Yamada, Y., Sagayama, H., Ainslie, P. N., Andersen, L. F., & Anderson, L. J. (2021). *Physical activity and fat-free mass during growth and in later life*. 1–7.

Wickel, E. E., & Eisenmann, J. C. (2007). Contribution of youth sport to total daily physical activity among 6- to 12-yr-old boys. *Medicine and Science in Sports and Exercise*, *39*(9), 1493–1500. https://doi.org/10.1249/mss.0b013e318093f56a

World Health Organization. (2006). *WHO child growth standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development*.

World Health Organization. (2020). WHO Guidelines on physical activity and sedentary behaviour. In *World Health Organization*. https://apps.who.int/iris/bitstream/handle/10665/325147/WHO-NMH-PND-2019.4-eng.pdf?sequence=1&isAllowed=y%0Ahttp://www.who.int/iris/handle/1066

5/311664%0Ahttps://apps.who.int/iris/handle/10665/325147

Wyszyńska, J., Matłosz, P., Szybisty, A., Lenik, P., Dereń, K., Mazur, A., & Herbert, J. (2020). Obesity and Body Composition in Preschool Children with Different Levels of Actigraphy-Derived Physical Activity—A Cross-Sectional Study. *Journal of Clinical Medicine*, *9*(4), 1210. https://doi.org/10.3390/jcm9041210

**Table 1. Main characteristics of the participating children (n= 279).**

| Characteristics | All n= 279 (M±SD) | Girls n= 129 (M±SD) | Boys n= 150 (M±SD) | P-value |
|---|---|---|---|---|
| Age (y) | 7.5 ± 0.3 | 7.6 ± 0.4 | 7.5 ± 0.3 | 0.41 |
| **Anthropometric measurements** | | | | |
| Height (cm) | 126.1 ± 5.9 | 125.6 ± 5.6 | 125.5 ± 6.0 | 0.12 |
| Weight (kg)[a] | 26.4 (23.6 - 30.0) | 26.2 (23.6 - 30.0) | 26.7 (23.7 - 30.8) | 0.59 |
| BMI (kg/m$^2$)[a] | 16.6 (15.4 - 18.3) | 16.7 (15.5 - 18.3) | 16.6 (15.4 - 18.5) | 0.74 |
| BMI z-score† | 0.84 ± 1.2 | 0.76 ± 1.1 | 0.87 ± 1.3 | 0.48 |
| **BIA** | | | | |
| FFM (kg)[a] | 20.5 (18.7 - 22.5) | 19.9 (18.5 - 21.8) | 20.8 (19.1 - 23.4) | <0.01* |
| FFMI (kg/m$^2$) | 13.1 ± 1.4 | 12.7 (12.1 - 13.4) | 13.2 (12.4 - 14.2) | <0.01* |
| **DXA** | | | | |
| TLSTM (kg)[a] | 18.1 (16.6 – 20.0) | 17.5 (16.2 - 18.7) | 18.9 (17.0 - 21.0) | <0.01* |
| TLSTMI (kg/m$^2$) | 11.6 ± 1.1 | 11.2 ± 1.0 | 11.9 ± 1.1 | <0.01* |
| ALSTMI (Kg/m$^2$) | 4.7± 0.5 | 4.5± 0.5 | 4.8± 0.5 | <0.01* |
| **pQCT** | | | | |
| Tibia length (mm) | 274.3 ± 18.1 | 275.8 ±17.3 | 273.1 ±18.8 | 0.14 |

| | | | | |
|---|---|---|---|---|
| MCSA (mm$^2$) | 3275.3 ± 496.0 | 3136.5 ± 473.2 | 3389.1 ± 488.3 | <0.01* |
| MCSAI (mm$^2$ /m$^2$) | 2057.1 ± 263.1 | 1996.3 ± 257.6 | 2112.1 ± 256.5 | <0.01* |
| **Physical activity** | | | | |
| Light (min/day) | 41.3 ± 11.1 | 37.0 ± 9.9 | 45.0 ± 10.8 | <0.01* |
| Moderate (min/day)[a] | 29.2 (21.1 - 36.2) | 24.3 (18.1 - 32.1) | 31.9 (24.9 - 41.1) | <0.01* |
| Vigorous (min/day)[a] | 69.0 (55.6 - 85.0) | 59.8 (50.0 - 75.7) | 77.5 (64.2 - 92.6) | <0.01* |
| MVPA (min/day)[a] | 97.3 (77.4 - 121.4) | 85.9 (67.8 – 108.4) | 110.8 (88.0 -134.0) | <0.01* |
| Total PA (min/day) | 269.6 (240.3 – 299.7) | 254.0 (231.3 – 285.2) | 282.5 (245.9 – 312.7) | <0.01* |
| **Physical fitness** | | | | |
| Handgrip strength (kg) | 10.5 ± 2.2 | 10.1 ± 2.0 | 10.9 ± 2.1 | <0.01* |
| Standing long jump (cm) | 102.7 ± 17.8 | 98.9 ± 17.0 | 107.6 ± 17.7 | <0.01* |

Abbreviation: ALSTMI= Appendicular lean soft tissue mass index; BIA= Bioelectrical impedance; BMI= Body mass index; MCSA= Muscle cross-sectional area; DXA= Dual-energy X-ray absorptiometry; FFM= Fat-free mass; FFMI= Fat-free mass index; MVPA= Moderate-vigorous physical activity; pQCT= quantitative peripheral computed tomography; TLSTM= Total lean soft tissue mass; TLSTMI= Total lean soft tissue mass index

Normally distributed variables are shown as mean ± SD (Student t-test)

[a] Non-normally distributed variables are shown as median and interquartile intervals (25th and 75th, U Mann–Whitney)

[†] BMI z-scores were calculated according to the World Health Organization (WHO)

* Significant differences by gender

Significance was set at 0.05 level.

## Table 2. Associations between physical activity and total lean soft tissue mass index, muscle cross-sectional area index, and fat-free mass index (n= 279)

| Predictors<br>Physical activity | Girls | | | | | | | | | | Boys | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | | Light | | Moderate | | Vigorous | | MVPA | | Total | | Light | | Moderate | | Vigorous | | MVPA | |
| | β | *p* | β | *p* | β | *p* | β | *p* | β | *p* | β | *p* | β | *p* | β | *p* | β | *p* | β | *p* |
| **TLSTMI by DXA** | | | | | | | | | | | | | | | | | | | | |
| *Model 1* | .272 | **.004*** | .154 | .109 | .123 | .201 | .151 | .117 | .144 | .137 | .335 | **.000*** | .287 | **.001*** | .093 | .289 | .203 | **.020*** | .166 | .058 |
| *Model 2* | .275 | **.004*** | .170 | .078 | .120 | .219 | .159 | **.001*** | .148 | .130 | .371 | **.000*** | .302 | **.001*** | .116 | .206 | .224 | **.013*** | .189 | **.038*** |
| *Model 3* | .247 | **.010*** | .151 | .111 | .111 | .248 | .143 | **.001*** | .134 | .162 | .337 | **.000*** | .290 | **.001*** | .081 | .367 | .200 | **.023*** | .160 | .072 |
| **MCSAI by pQCT** | | | | | | | | | | | | | | | | | | | | |
| *Model 1* | .148 | .131 | .112 | .253 | -.003 | .974 | .057 | .564 | .036 | .717 | .091 | .314 | .074 | .410 | .052 | .560 | .068 | .447 | .064 | .480 |
| *Model 2* | .124 | .223 | .093 | .359 | -.034 | .742 | .030 | .768 | .007 | .944 | .114 | .219 | .083 | .364 | .053 | .569 | .074 | .426 | .068 | .468 |
| *Model 3* | .097 | .347 | .077 | .448 | -.040 | .692 | .018 | .863 | -.004 | .972 | .110 | .245 | .081 | .381 | .049 | .607 | .070 | .454 | .063 | .500 |
| **FFMI by BIA** | | | | | | | | | | | | | | | | | | | | |
| *Model 1* | .125 | .194 | .132 | .173 | -.047 | .626 | .042 | .664 | .010 | .918 | .260 | **.003*** | .161 | .064 | -.010 | .908 | .078 | .373 | .047 | .594 |
| *Model 2* | .185 | .053 | .149 | .122 | -.060 | .539 | .045 | .642 | .007 | .939 | .308 | **.000*** | .175 | .050 | -.026 | .781 | .078 | .389 | .042 | .648 |
| *Model 3* | .149 | .097 | .137 | .154 | -.066 | .496 | .035 | .717 | -.001 | .988 | .299 | **.001*** | .175 | .051 | -.026 | .780 | .079 | .388 | .042 | .647 |

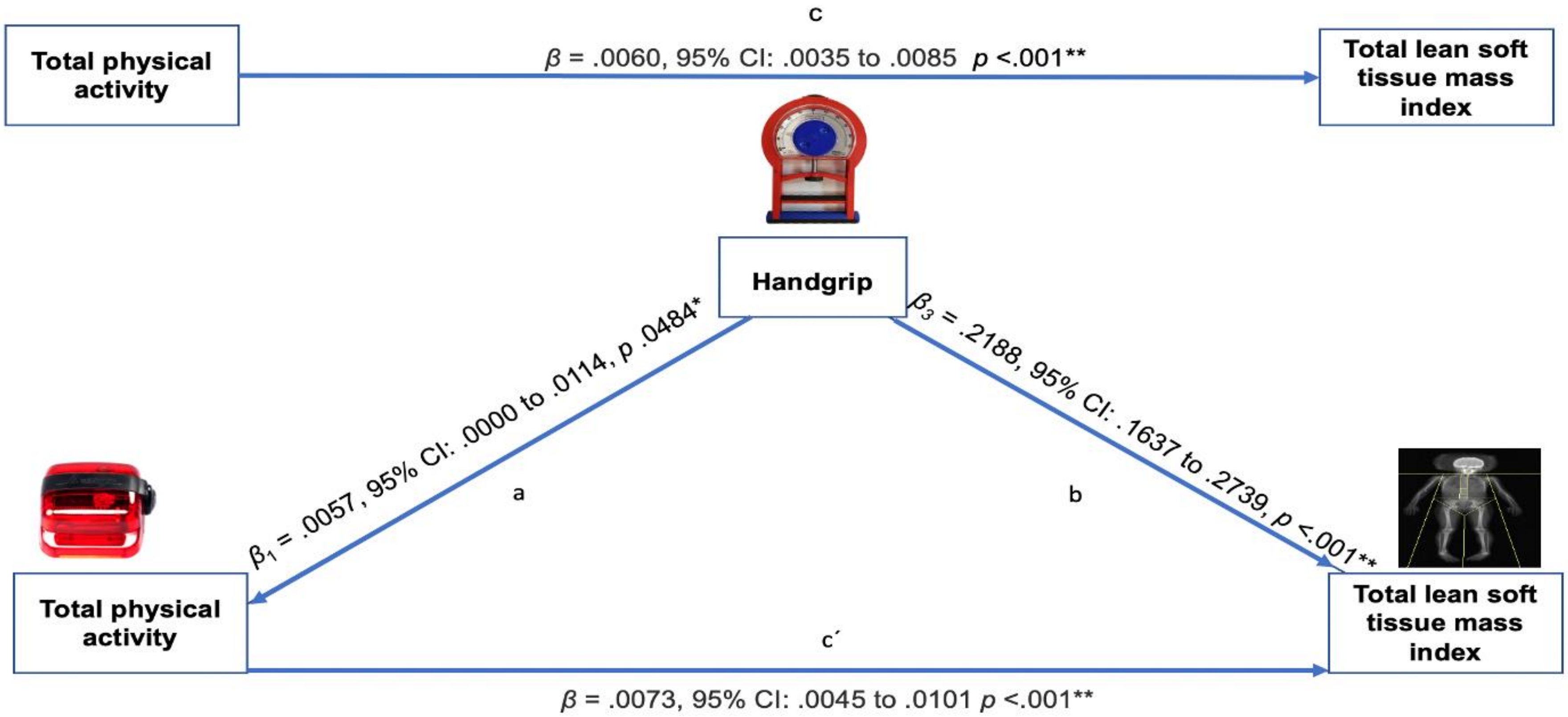
β: standardized regression coefficient
Abbreviation: BIA= Bioimpedance analysis; DXA= Dual-energy X-ray absorptiometry; FFMI= Fat-free mass index; MCSAI= Muscle cross-sectional area index; pQCT= Peripheral quantitative computed tomography; TLSTMI= Total lean soft tissue mass index.
*Model 1* Physical activity (basic model without adjustments)
*Model 2 Model 1* + diet quality index, parents’ highest education level and age.
*Model 3 Model 2* + birth weight
*Significance was set at 0.05 level.

## Table 3. Associations between physical fitness and total lean soft tissue mass index, muscle cross-sectional area index, and fat-free mass index (n= 279)

| | | Girls | | | | Boys | | | |
|---|---|---|---|---|---|---|---|---|---|
| Predictors<br>Physical fitness | | Handgrip strength | | Standing long jump | | Handgrip strength | | Standing long jump | |
| | | β | *p* | β | *p* | β | *p* | β | *p* |
| **TLSTMI by DXA** | | | | | | | | | |
| *Model 1* | | .363 | **.000*** | .055 | .512 | .478 | **.000*** | -.019 | .839 |
| *Model 2* | | .350 | **.000*** | .070 | .403 | .464 | **.000*** | -.013 | .893 |
| *Model 3* | | .314 | **.000*** | .034 | .672 | .425 | **.000*** | -.036 | .690 |
| **MCSAI by pQCT** | | | | | | | | | |
| *Model 1* | | .262 | **.002*** | .056 | .520 | .273 | **.000*** | -.064 | .508 |
| *Model 2* | | .282 | **.001*** | .074 | .404 | .287 | **.000*** | -.060 | .540 |
| *Model 3* | | .267 | **.002*** | .051 | .561 | .301 | **.000*** | -.059 | .547 |
| **FFMI by BIA** | | | | | | | | | |
| *Model 1* | | .236 | **.004*** | -.082 | .332 | .289 | **.000*** | -.084 | .367 |
| *Model 2* | | .218 | **.008*** | -.065 | .430 | .307 | **.000*** | -.083 | .367 |
| *Model 3* | | .196 | **.017*** | -.088 | .281 | .325 | **.000*** | -.082 | .384 |

β: standardized regression coefficient
Abbreviation: BIA= Bioimpedance analysis, DXA= Dual-energy X-ray absorptiometry, FFMI= Fat-free mass index, MCSAI= Muscle cross-sectional area index, pQCT= Peripheral quantitative computed tomography and TLSTMI= Total lean soft tissue mass index.
*Model 1* physical fitness (basic model without adjustments)
*Model 2 Model 1* + diet quality index, parents' highest education level and age.
*Model 3 Model 2* + birth weight
*Significance was set at 0.05 level.

**Figure 1. Serial multiple mediation model of the association between total physical activity and total lean soft tissue mass index, using handgrip strength as mediator, controlling for gender.**



***Indirect effect* $\beta$ = .0013; SE= .0007 95% CI ( .0000 to .0026)**

p ≤ 0.05*; p≤ 0.001**

# Associations between Spanish children's physical activity and physical fitness with lean body mass: The CALINA Study

Diana Paola Córdoba-Rodríguez [a], Iris Iglesia [b,c,d,*], Alejandro Gómez-Bruton [b,e,f,*], María L Miguel-Berges[b], Paloma Flores-Barrantes[b], José Antonio Casajús[b,c,e,f], Luis A. Moreno[b,c,f], Gerardo Rodríguez[b,c,d,g]

[a] Departamento de Nutrición y Bioquímica, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá DC, 110231,Colombia.

[b] Growth, Exercise, Nutrition and Development (GENUD) Research Group, Universidad de Zaragoza, Instituto Agroalimentario de Aragón (IA2), Zaragoza, 50001, España.

[c] Instituto de Investigación Sanitaria Aragón (IIS Aragón), Zaragoza, 50009, España

[d] Red de Salud Materno Infantil y del Desarrollo (SAMID), Instituto de Salud Carlos III, Madrid, 28029, España.

[e] Facultad de Ciencias de la Salud y del Deporte (FCSD), Departamento de Fisiatría y Enfermería, Universidad de Zaragoza, Zaragoza, 50009, España.

[f] Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CIBERObn), Instituto de Salud Carlos III Madrid, 28029, España.

[g] Área de Pediatría, Universidad de Zaragoza, Zaragoza, 50009, España.

*Corresponding author: Alejandro Gomez-Bruton, Growth, Exercise, Nutrition and Development (GENUD) Research Group, Universidad de Zaragoza.

E-mail: bruton@unizar.es Phone: (+34) 627340760 https://orcid.org/0000-0002-0520-1640

Diana Paola Córdoba-Rodríguez: d.cordoba@javeriana.edu.co https://orcid.org/0000-0002-7034-8796

Iris Iglesia: iglesia@unizar.es https://orcid.org/0000-0002-2219-3646

María Luisa Miguel-Berges: mlmiguel@unizar.es https://orcid.org/0000-0002-2411-9538

Paloma Flores-Barrantes: pfloba@unizar.es https://orcid.org/0000-0002-2135-900X

José Antonio Casajús: joseant@unizar.es https://orcid.org/0000-0002-7215-6931

Luis A. Moreno: lmoreno@unizar.es https://orcid.org/0000-0003-0454-653X
Gerardo Rodríguez-Martínez: gerard@unizar.es https://orcid.org/0000-0002-7985-9912

## Abstract

The aim of the present study is to investigate the associations between physical activity (PA) and physical fitness (PF) with lean body mass (LBM) and to assess whether PA mediates the association between PF and LBM. 279 children (150 boys) aged 7.5±0.3 years participated in the study. PA was assessed by accelerometry and PF with handgrip and the standing long jump test. Total lean soft tissue mass index (TLSTMI), muscle cross-sectional area index (MCSAI), and fat-free mass index (FFMI) were evaluated using dual-energy X-ray absorptiometry, peripheral quantitative computed tomography, and bioimpedance analysis, respectively.

Total (β=0.247) and vigorous PA (β=0.143) were associated with TLSTMI in girls. In boys, total (β=0.337), light (β=0.290), vigorous (β=0.200), and moderate-vigorous PA (β=0.189) were associated with TLSTMI. Only total PA was associated with FFMI (β=0.299). Handgrip strength does not mediate the relationship between total PA and TLSTMI. Positive associations were found between handgrip strength and TLSTMI, MCSAI, and FFMI in both girls and boys.

In children, there is a positive association between total and vigorous PA with TLSTMI. Handgrip strength does not mediate the relationship between total PA and TLSTMI. It was associated with TLSTMI, MCSAI, and FFMI.

**Key words:** lean body mass, muscle cross-sectional area, physical activity, physical fitness, schoolchildren

## 1. Introduction

Lean body mass (LBM) is mainly constituted by muscle mass, internal organ non-adipose components, and extracellular fluid (Kuriyan, 2018). In recent years, LBM has been considered to play an essential role in growth maintenance, normal development, and systemic glucose metabolism in children (Liu et al., 2019). It has also been associated with the risk of cardiovascular disease (S. Kim & Valdez, 2015), affecting bone health (bone mineral density and structure in both sexes during childhood) (Dorsey et al., 2010; Sioen et al., 2016), and cognitive development (Scheurer et al., 2018), among others. Studies on children and adolescents with low lean mass showed a higher cardiometabolic risk (S. Kim & Valdez, 2015), related to significantly higher waist circumference, blood pressure, triglycerides, and total cholesterol/high-density lipoprotein cholesterol values (Burrows et al., 2017; Luis Gracia-Marco et al., 2016). Other studies have shown an increased risk of metabolic syndrome (Burrows et al., 2017; J. H. Kim & Park, 2016; K. Kim et al., 2016). In this regard, the literature revealed that those presenting a phenotype combining low lean mass and obesity had the most unfavorable cardiometabolic risk profile (Burrows et al., 2017). Therefore, low levels of lean mass in children and adolescents may represent a public health problem and a burden on the health system for future stages in life.

Currently, it is accepted that several factors can influence the development of lean mass/muscle mass throughout the life cycle, including fetal programming (Isganaitis, 2019; Labayen et al., 2006; Larqué et al., 2019), early nutritional status (Singhal et al., 2003), age, gender (Wells, 2000), hormones (Veldhuis et al., 2005), diet, and physical activity and exercise (Kulkarni et al., 2014; Westerterp et al., 2021)

Physical activity and exercise play an essential role in the development of both the size of muscle fibers and the recruitment of motor units, thus, developing strength (Dotan et al., 2012) and metabolic adaptations (Boisseau & Delamarche, 2000).

A cross-sectional study, in preschool-age children between 5 and 6, showed that a level of MVPA below the World Health Organization recommendation was significantly associated with a lower content of muscle mass and FFM (0.8% and 1.19%, respectively); these differences were more evident in boys than in girls (Wyszyńska et al., 2020).

A longitudinal study (Leppänen et al., 2016, 2017) in 4.5-year-old children demonstrated that children with higher and moderate to vigorous physical activity (MVPA) levels had a higher fat-free mass index (FFMI) after a 12-month follow-up. Similar results have been reported in preschool children (Henriksson et al., 2016) and adolescents (Baxter-Jones et al., 2008; Ramires et al., 2016).

The relationship between physical activity levels, physical fitness, and body composition with fat mass has been widely studied in children and adolescents (Henriksson et al., 2016; Santos et al., 2019). Regarding its relationship with LBM, Baxter-Jones et al. (Baxter-Jones et al., 2008) reported that daily physical activity had a significant independent influence on LBM development during adolescence (after controlling for biological maturity and stature). In addition, studies in children have shown that higher fat-free mass (FFM) values are associated with better cardiorespiratory fitness, improved upper and lower body muscle strength (Fraser et al., 2020), motor fitness (Henriksson et al., 2016), and a healthier cardiovascular profile later in life, with a lower risk of premature death (Ruiz et al., 2009). Thus, it seems reasonable to hypothesize that physical activity or physical fitness might play a crucial role in the association between fitness-lean or physical activity-lean in this age group.

Given the available evidence, it is apparent that studies examining the relationship between physical activity levels, physical fitness, and body composition, specifically LBM in children between 6 and 12 years of age, are limited (Hao et al., 2019). Furthermore, most of the available studies have used statistical methods such as analysis of covariance, multiple linear regression, or logistic regression to adjust for confounding factors, statistical methods that cannot distinguish the effect of mediating variables.
Furthermore, there are important differences between DXA, pQCT, and BIA. On the one hand, DXA is an accurate device for assessing body composition of the whole body and regions/segments (Laskey, 1996). At the same time, pQCT allows assessment at the appendicular level (limb muscle cross-sectional area)(Frank-Wilson et al., 2015). Still, they are not available to everyone due to their high price and radiation exposure (Guglielmi et al., 2016). On the other

hand, BIA is an effective alternative to assess body composition of the whole body, low price in the absence of radiation (Orsso et al., 2019). Therefore, it will be important to use all three devices to assess LBM. In addition, the normalization of body size should also be considered, since most of the evidence available to date uses absolute or relative measures of LBM (kg and%), which makes it difficult to make comparisons between individuals or populations (Wells et al., 2002).

Therefore, in this study, we hypothesized that children with higher physical activity or physical fitness would have higher LBM values, and physical activity or physical fitness would be a mediator between the fitness-lean or physical activity-lean associations. Its purposes are to (1) investigate the associations between objectively assessed physical activity, physical fitness, and LBM measured by dual-energy X-ray absorptiometry (DXA), bioimpedance analysis (BIA), and peripheral quantitative computed tomography (pQCT); and (2) evaluate whether these associations were mediated by total physical activity or physical fitness or with handgrip strength in a Spanish cohort of children.

## 2. Material and Methods

### *2.1 Study participants*

This study's participants were involved in the CALINA study (Growth and Feeding during Breastfeeding and Early Childhood in Children from Aragón). This longitudinal observational study evaluated a representative cohort of children born in Aragon (Spain) between 2009-2010. The initial sample consisted of 1602 newborns (Oves Suárez et al., 2014), followed every month during the first year and then every year until they turned 6.

In 2016 and 2017, the recruited families (n=952) from the baseline examination were invited to participate in this follow-up study in Zaragoza, the biggest city in Aragón (Spain). They were invited to participate in an additional body composition assessment in our laboratory at the University of Zaragoza. From the 415 children who participated in this follow-up assessment, 136 were excluded from the study for the following reasons: lack of data on body composition and physical fitness (n=90), or lack of valid accelerometer data (n=46). Finally, 279 children (150 boys and 129 girls), between 6 and 8 years, with complete DXA, pQCT, BIA, physical activity, and physical fitness examination data were included in this study.

### *2.2 Ethics statement*

This study was conducted following the Declaration of Helsinki (Fortaleza 2013 review) ethical guidelines. In 2009, it was approved by the Ethics Committee in Clinical Research of the Government of Aragon (ref. PI ICS108/0088, Spain). In 2016, it was again approved by the same committee for the follow-up presented in this manuscript (Ref. CPPI13/00105, Spain).

All participants were evaluated after parents' signed the informed consent and the verbal assent of the children.

### *2.3 Body composition*

#### *2.3.1 Weight, height, and bioimpedance analysis (BIA):*

The weight and bioelectrical impedance analysis were measured using a TANITA BC 418 MA electronic scale (Tanita Europe BV, Amsterdam, Netherlands) with a 0.1 kg precision and 0-200 kg range. Following the manufacturer's instructions (Tanita Corporation - Japan, 2002), the children were asked to stand barefoot and with as little clothing as possible on the weighing platform, touching the electrodes, in a stable position, without bending the knees, and with both hands grabbing the handles.

A SECA® 225 portable stadiometer (SECA® 225, Hamburg, Germany) with a precision of 0.1 cm and a range of 70-220 cm was used to determine the height. The body mass index (BMI) was calculated as the weight divided by the squared height (kg/m$^2$). The AnthroPlus software from the World Health Organization (WHO) (World Health Organization, 2006) was used to calculate

BMI age and gender-specific *z*-scores. The FFMI was calculated relating the FFM in kilograms divided by the squared height in meters.

### *2.3.2 Dual-energy X-ray absorptiometry (DXA):*

Dual-energy X-ray absorptiometry was used to determine the LBM (kg), using the DXA QDR-Explorer™ 4500 (Hologic Inc., Bedford, Massachusetts, USA). All measurements we performed following manufacturer's instructions (National Health and Nutrition Examination Survey (NHANES), 2012). The participants were measured lightly clothed and without metal objects or jewelry. An examination of the whole body was performed. The participants were placed supine, with arms along the body, without touching the trunk (Crabtree et al., 2007). Analyzes and regions of interest (ROI) were determined using Pediatric Hologic Corp. software version 12.4. The whole-body scan intra-measures coefficient variation of LBM in our laboratory was 1.9; a result elsewhere described (L Gracia-Marco et al., 2012).

This study used the sum value of the fat, lean, and bone masses (Lean soft tissue mass LSTM) = LBM - bone mineral content (BMC). The total LSTM index (TLSTMI) and the appendicular LSTM index (ALSTMI) were calculated by dividing both the LSTM (Kg) and the appendicular LSTM (Kg) by the height squared.

In this study, the DXA and BIA analyses were carried out between 4.30 pm and 6.30 pm, 2 -4 hours after the end of the previous meal.

### *2.3.3 Peripheral quantitative computed tomography (pQCT):*

Stratec XCT 2000 L (Stratec Medizintechnik, Pforzheim, Germany) was used to measure the muscle cross-sectional area (MCSA) at 66% of the total length of the left tibia (González-Agüero et al., 2013), a method that has proven to be valid for estimating the lean mass (Córdoba-Rodríguez et al., 2021).

All the measurements were carried out after the calibration of the equipment. The total length of the tibia was determined using a segmometer, measuring the distance from the cleft of the knee's medial joint to the tibia's (leg) Sphyrion tibial medial malleolus (Roggen et al., 2015).

For the definition of the reference line in the distal tibia, an exploratory scout view was performed, and the reference line was placed at the medial end of the distal epiphysis. The intra-measures coefficient of variation for MCSA using pQCT was 1.69, as previously reported (Gómez-Bruton et al., 2014).

The muscle cross-sectional area index (MCSAI) was calculated by dividing the muscle cross-sectional area (MCSA) by the squared height.

The pQCT and DXA images were evaluated visually by a technician to identify motion artifacts. The images showing movement were subjected to a new exploration or excluded from the data analysis. Moreover, an external evaluator reviewed all the images to endorse the image quality.

### *2.4 Physical activity*

Physical activity was evaluated using the ActiGraph wGT3x-BT triaxial accelerometer (ActiGraph, Ft. Walton Beach, USA). Parents/caregivers and children were asked to place the accelerometer under the child's clothing at the level of the right iliac crest, during their waking day, for seven consecutive days and to complete a diary to assess the minutes and reasons why accelerometers were not being used (Bammann et al., 2011).

Additionally, children and parents / caregivers were advised not to use accelerometers in water activities.

All the accelerometers were programmed to record data every 15 seconds (epoch). Twenty minutes or more of consecutive zero counts were defined as unused time.

For the information processing, a day in which the child used the accelerometer for at least 8 hours was considered a valid day. A minimum of 3 days was required, including at least one weekend day, as was previously used by other authors in similar population groups (Moliner-Urdiales et al., 2010a)

Actilife software version 6.0 (ActiGraph, Pensacola, FL., USA) was used for data processing. The cut-off points determined by Evenson were used as a reference to define the time dedicated to sedentary and different intensity physical activity (sedentary: 0–100, light: 101–2295, moderate: 2296–4011, vigorous: 4012, and more accelerometer counts per minute [cpm]) (Evenson et al., 2008).

The absolute amount of time in each intensity category determined from the accelerometer data was used for the analyzes, taking as a reference that the World Health Organization (WHO)(World Health Organization, 2020) frequently uses the min/day metric to issue their physical activity recommendations.

Most of the children had complete actigraphic data of 7 days (n=126, 42.2%) or 6-5 days (n=118, 42.4%) of. The average follow-up included 6.2 days of recordings.

### *2.5 Upper limb strength - Handgrip strength test:*

A TKK-5401 (Takei Scientific Instruments Co., Ltd., Niigata, Japan) digital handgrip dynamometer with a precision of 0.1kg and adjustable grip was used according to the size of the hand derived from the following gender-specific equations (España-Romero et al., 2008):

Boys: $Y = X/4 + 0.44$ cm

Girls: $Y= 0.3X - 0.52$ cm

Where Y = optimal grip and X = size of the wide-open hand, measured from the tip of the thumb to the tip of the little finger.

During the measurement, participants stood, elbow extended, avoiding any bodily contact with the dynamometer, except with the measured hand. The children were asked to press as hard as possible for 3 to 5 seconds with each hand; two attempts were made with each hand, three minutes of rest interval between each of them. The dynamometer display was aligned to face the examiner, providing blind measurements to the children. The final score was calculated as the average of the best attempt obtained for the left and right hands in kg (De Miguel-Etayo et al., 2014).

### 2.6 *Lower limb strength*. *Standing long jump test*

The long jump test was used to assess the explosive strength of the lower extremities. The children were asked to stand, feet slightly apart, in front of a starting line. Then, from a standing position, a forward jump was made using the arms' impulse, being careful not to step on the starting line and pushing with both feet at the same time. The measurement was made from the impulse line to the heel closest to the start line and recorded in cm (López-Gil et al., 2020). Two attempts were made, recording the highest value obtained.

### *2.7 Covariates*

We recorded the following variables for all the children:

#### *2.7.1 Parental education*

*Highest level of education:* the parents were asked to report their highest achieved level of education when they came to our lab in 2016-2017 (no studies; basic-primary studies; intermediate studies [including modules vocational training and secondary studies]; higher studies; and university degrees). These were later coded according to the International Standard Classification of Education (ISCED-2011) (Statistics, 2012) and categorized again into low (0-2), medium (3-4), and high (5-8) educational levels (Unesco, 1997).

#### *2.7.2 Anthropometric data of the child at birth:*

At the beginning of the CALINA study, in the first visit (15 days after birth), the pediatric and nursing staff conducting the *Programa de Salud Infantil* (Child Health Program) collected the birth weight variable.

#### *2.7.3 Food frequency questionnaire and diet quality index (DQI):*

A previously validated, semi-quantitative food frequency questionnaire was used to assess the dietary intake (Mouratidou et al., 2019), including 37 foods and beverages. The responding parents were asked to classify each food's consumption frequency using a 6-Point Likert scale ranging from never to every day (never/less than once a month, 1 to 3 times per month, 1 day/week, 2 to 4 days/week, 5 to 6 days/week, and every day) and serving size.

The DQI was subsequently calculated. This index mainly assesses the components of dietary diversity, dietary quality, and dietary balance. Dietary diversity evaluated the daily consumption of at least one food serving from the eight recommended food groups. This component's score ranged from 0 to 9 for at least one serving from a recommended food group. Diet quality expressed whether the children made optimal decisions on the food quality within a food group. Dietary balance was calculated from the difference between the food intake's suitability and its excess in the diet (Huybrechts et al., 2010; Vyncke et al., 2013). These three components of the DQI were presented in percentages. The categories' scores were summed and divided by 3 to compute the overall DQI, resulting in scores ranging from −33% to 100%. The components' mean were used to calculate the DQI, with the highest scores reflecting greater compliance with the diet (Iglesia et al., 2020).

### *2.8 Statistical analysis*

Statistical analyses were carried out using IBM SPSS Statistics® software, version 25 (IBM Corp., Armonk, NY, USA). The variables studied were presented as means (M) ±, standard deviations (SD), and median and interquartile intervals (25th and 75th) in the case of non-normally distributed variables. The distribution of variables was checked and verified using the Kolmogorov-Smirnov test. The Student's t-test or the Mann-Whitney U test were used to identify sex differences between each of the studied variables. Because we observed a significant interaction effect between gender and physical activity and physical fitness, all the analyses were performed separately for boys and girls.

Multiple linear regression (Forced Entry) was used to study the association between physical activity (total, light, moderate, vigorous, or MVPA) or physical fitness (handgrip strength, standing long jump) with the body composition outcomes, which included TLSTMI, MCSAI, and FFMI. These relationships were analyzed in individual regression models (one for each physical activity level or physical fitness variable) using educational level, DQI, and birth weight as covariates. The assumptions of independence of errors were verified for all the multiple regression models using the Durbin-Watson test. Equally, collinearity diagnosis was carried out through the variance inflation factor (VIF).

For the significant models, we used a mediation analysis according to the procedure proposed by Baron and Kenny (Baron & Kenny, 1986) using the macro PROCESS developed by Hayes (Hayes, 2013) with a bootstrap threshold of 10,000 and model 4 for Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM Corporation, New York, USA). The purpose of this model was to examine the total effect (c) and the individual direct effects (a, b, c´) reflected by the non-standardized regression coefficient (β), as well as the statistical significance of the relationship between each model's independent and dependent variables. The model also examined the indirect effect obtained from the coefficients' product (a × b). It indicated the change in TLSTMI by the change in vigorous physical activity and the change in TLSTMI by the handgrip strength change, mediated by the proposed mediator (handgrip strength or physical activity, respectively). If zero was not included in the estimate's 95% confidence interval (CI), we concluded that the indirect effect (IE) was statistically significant, as shown in Figure 1.

## 3. Results

### 3.1 Descriptive statistics

The characteristics of participants ranked by gender are shown in **Table 1.** Boys showed higher levels of TLSTM (kg), TLSTMI (kg/m$^2$), ALSTMI (kg/m$^2$), MCSA (mm$^2$), MCSAI (mm$^2$/m$^2$), FFM (Kg), FFMI (kg/m$^2$), total, light, moderate, vigorous, and MVPA, handgrip strength and standing long jump than girls (all $p<0.05$).

### *3.2 Association between physical activity or physical fitness with LBM outcomes*

Adjusted associations between physical activity and TLSTMI, MCSAI, and FFMI for both boys and girls are shown in **Table 2.**

In girls, we observed that for Model 1 (physical activity [min/day]), there was an association between total physical activity and TLSTMI (β=0.272, p=0.004). In Model 2, after adding the DQI, the parent's educational level, and age, an association was found between total physical activity (β=0.275, p=0.004) and vigorous physical activity (β=0.159, p=0.001) with TLSTMI. Finally, once the birth weight was added to create Model 3, slight decreases were found for the significant association between total physical activity (β=0.247, p=0.010) and vigorous physical activity (β=0.143, p=0.001) with TLSTMI.

In boys, Model 1 showed a positive association between total physical activity (β=0.335, p=<0.001), light (β=0.287, p=0.001), and vigorous physical activity (β=0.203, p=0.020) with TLSTMI by DXA. After adding the DQI, the parents' educational level, and the age to create Model 2, slight increases were found in the significant associations between total physical activity (β=0.371, p=<0.001), light physical activity (β=0.302, p=0.001), vigorous (β=0.224, p=0.013). An association was found between MVPA (β=0.189, p=0.038) with TLSTMI (Table 2). Once birth weight was added to create Model 3, slight decreases were observed in the associations between total physical activity (β=0.337, p<0.001), light (β=0.290, p=0.001) and vigorous physical activity (β=0.200, p=0.023) with TLSTMI (Table 2). The association between MVPA and TLSTMI in this model disappeared.

No associations were found between physical activity and MCSAI for girls or boys. Regarding FFMI assessed with bioimpedance, no associations were found between FFMI and physical activity in girls. For boys, total physical activity significantly predicted FFMI.

The adjusted associations between handgrip strength and TLSTMI, MCSAI, and FFMI both in boys and girls are shown in **Table 3.** For both genders, handgrip strength was a significant predictor for all the models. For girls, in Model 1, handgrip strength was positively associated with TLSTMI, MCSAI, and FFMI ($\beta=0.363$, $p<0.001$; $\beta=0.262$, $p=0.002$; $\beta=0.236$, $p=0.004$, respectively). In Model 2, significant associations remained for TLSTMI, MCSAI, and FFMI ($\beta=0.350$, $p<0.001$; $\beta=0.282$, $p=0.001$; $\beta=0.218$, $p=0.008$, respectively). However, they decreased slightly for TLSTMI and FFMI. Finally, once birth weight was added to Model 3, the significant associations again decreased slightly, but remained significant ($\beta=0.314$, $p<0.001$; $\beta=0.267$, $p=0.002$; $\beta=0.196$, $p=0.017$, respectively).

In boys, in Model 1 handgrip strength was positively associated with TLSTMI, MCSAI, and FFMI ($\beta=0.478$, $p<0.001$; $\beta=0.273$, $p<0.001$; $\beta=0.289$, $p<0.001$, respectively). In Model 2, significant associations were maintained for TLSTMI, MCSAI, and FFMI ($\beta=0.464$, $p<0.001$; $\beta=0.287$, $p<0.001$; $\beta=0.307$, $p<0.001$, respectively) but decreased slightly for TLSTMI and increased for MCSAI and BIA. Finally, once birth weight was added to Model 3, the significant associations again decreased slightly for TLSTMI and increased for MCSAI and BIA ($\beta=0.425$, $p<0.001$; $\beta=0.301$, $p<0.001$; $\beta=0.325$, $p=0.008$, respectively).

There was no association between standing long jump and TLSTMI, MCSAI, and FFMI in both girls and boys (Table 3). This analysis was also performed adjusting standing long jump by weight, obtaining similar results.

### *3.3 Mediation analysis*

An analysis of the handgrip's strength mediation effect on the association of total physical activity with TLSTMI values indicated an association between total physical activity and handgrip strength ($\beta_1=0.0057$, 95% CI: 0.0000 to 0.0114, p 0.0484*) in the first regression equation (a). In the second equation (b), handgrip strength was positively associated with TLSTMI ($\beta_3=0.2188$, 95% CI: 0.1637 to 0.2739, $p<0.001$). The third equation (c´) showed a positive relationship between total physical activity and TLSTMI ($\beta_{dir}$=0.0073, 95% CI: 0.0045 to 0.0101 p<0.001*). The relationship between total physical activity and TLSTMI was not statistically significant when including handgrip strength in the model (a * b), indicating that handgrip strength does not mediate in this relationship ($\beta_{ind}$=0.0013, 95% CI: 0.0000 a 0.0026) (Figure 1).

## 4. Discussion

Our results indicate that this sample of children accumulated around 110 min of MVPA. Studies available in the population of the United States (Trost et al., 2002) and Europe (Riddoch et al., 2004), where physical activity data were objectively measured, suggest that young children (schoolchildren) accumulate more than 100 min of MVPA per day. Our results are comparable with these findings. This could be because children are more active at these ages (possibly through organized activities (physical exercise and youth sports) and not organized (recess and unstructured activity (Wickel & Eisenmann, 2007), and active transport (Carver et al., 2011))), to later decrease the physical activity in adolescence, especially in girls.

Our main findings indicate that there is an association between total physical activity and vigorous physical activity with TLSTMI in girls, while for boys, the associations were found between total, light, vigorous, and MVPA with TLSTMI. One possible explanation may be the role of certain hormones (growth hormone, IGF-1, and gender steroids, such as testosterone and estradiol), which play a fundamental role in developing skeletal muscle during infancy, childhood, and adolescence, depending on gender (Veldhuis et al., 2005).

Additionally, our results indicate that upper limb strength (handgrip strength) does not mediate the relationship between total physical activity and TLSTMI. As far as we know, this is the first time that these results are shown in a broad sample of young children using high precision objective methods.

In agreement with our results, a study involving 283 Chinese adolescent girls also found a significant positive association between total physical activity level assessed by questionnaire and LBM ($p<0.001$), suggesting that higher physical activity levels may reflect a higher LBM (Foo et al., 2007). Similarly, a study by Jiménez-Pavón study using accelerometers found that total physical activity was positively associated with FFM ($p<0.05$) in 2,200 (1016 male, 1184 females) European adolescents of both sexes (Jiménez-Pavón et al., 2013). In the same line, Deheeger et al. (Deheeger et al., 1997) found that total physical activity was positively associated with the percentage of FFM ($r=0.23$; p 0.03) in ten-year-old children ($n=86$). Rennie et al. also showed that the level of physical activity was positively associated with the lean mass index (LMI) in a study carried out with 100 children aged 6 to 8 (Rennie et al., 2005). Our study is in the same line as previously published studies, suggesting that total physical activity positively affects non-adipose tissue in both female and male children and adolescents.

Regarding vigorous physical activity and MVPA, a study by Jiménez-Pavón found positive associations of vigorous physical activity with FFM and muscle mass (all $p<0.05$) in both sexes (Jiménez-Pavón et al., 2013). In this study, skinfold thickness and bioimpedance were used to evaluate body composition, accelerometry for physical activity. Similarly, a study by Hao et al. found a positive association between MVPA and SMMI ($\beta=0.20$, $p<0.001$). It involved 640 adolescents and concerned fat-free soft tissue mass assessed by DXA, subsequently determining the skeletal muscle mass index (SMMI) (Hao et al., 2019). Additionally, in a longitudinal study conducted in Pelotas (Brazil) (Ramires et al., 2016), a consistent moderate and vigorous physical activity practice during adolescence was associated with a greater lean mass index in both sexes.

Conversely, studies such as the one developed by Moliner-Urdiales et al. (Moliner-Urdiales et al., 2010a) in a sample of 363 Spanish adolescents aged 12.5 to 17.5 did not observe an association between FFM and physical activity levels. Heelan et al. (Heelan & Eisenmann, 2006), in 4- to 7-year-old children, observed, only in girls, a negative correlation between MVPA and FFM ($r=-0.39$, $p<0.05$).

Different factors could explain our contrasting results. They include the individuals' age ranges included in each study (preschoolers (Leppänen et al., 2016), adolescents (Foo et al., 2007; Hao et al., 2019; Jiménez-Pavón et al., 2013), and schoolchildren (Deheeger et al., 1997; Rennie et al., 2005)) and their maturation stage. Other factors include the methods for assessing body composition (DXA (Foo et al., 2007; Hao et al., 2019; Heelan & Eisenmann, 2006), anthropometry (Deheeger et al., 1997; Jiménez-Pavón et al., 2013), BIA (Jiménez-Pavón et al., 2013), isotopic dilution (Rennie et al., 2005), and air displacement plethysmography (Leppänen et al., 2016)) and physical activity (questionnaires (Deheeger et al., 1997; Foo et al., 2007; Ramires et al., 2016; Rennie et al., 2005) and accelerometers (Jiménez-Pavón et al., 2013)). They also include the different types of devices for assessing physical activity ( uniaxial (Hao et al., 2019; Heelan & Eisenmann, 2006; Moliner-Urdiales et al., 2010b) or triaxial devices (Jiménez-Pavón et al., 2013; Leppänen et al., 2016), as well as the different cutoff points to define physical activity intensities, varying sampling intervals (epochs) (10s (Leppänen et al., 2016), 15 s (Jiménez-Pavón et al., 2013) and 1min (Hao et al., 2019; Heelan & Eisenmann, 2006)), and sporadic or episodes of accumulated physical activity data. Moreover, the presence of FFM (sum of lean mass and bone mass) or fat-free soft tissue mass (FFM - bone mineral content) and the use of absolute values (kg (Foo et al., 2007; Jiménez-Pavón et al., 2013)) or relative values (% (Deheeger et al., 1997) or index (Hao et al., 2019; Leppänen et al., 2016; Ramires et al., 2016; Rennie et al., 2005)) to express the value of the FFM/LBM can make it difficult to compare individuals of different sizes appropriately because FFM varies with height, weight, age. The percentage of FFM automatically decreases in proportion to the increase in the % of body fat. The last possible affecting factors are the covariates taken into account in the analyzes (age and maturation stage, the mother's BMI and educational level, the father's BMI and educational level, and the age at the time of measurement and time of use awake).

Some of these factors were also reported in a systematic review carried out by Poitrasl et al. (Poitras et al., 2016). Their purpose was to examine the relationships between objectively measured physical activity and relevant indicators (body composition, cardiometabolic biomarkers, and physical fitness, among others) in 5- to 17-year-old children and adolescents.

Our findings can support emerging evidence suggesting that different physical activity intensities, including light physical activity, may significantly affect health outcomes. They are in line with Corson’s findings in 1,731 adolescents aged 12 to 19, evaluated during the National Health and Nutrition Examination Survey 2003/2004 and 2005/2006 (Carson et al., 2013)—reinforcing the claim that some activity is better than none, but that “more is better” (Tremblay et al., 2011). However, we also found that the assessment method could influence results. No significant associations were found when evaluating the associations using the variables of the lean component determined with BIA or pQCT. An explanation for this could be the fact that, although BIA can provide information on the whole-body FFM status (like DXA), alterations in body water, such as dehydration, can influence it, increasing resistance to electricity, and subsequently underestimating the FFM (or overestimating body fat). Another influence could be the time of the day. In this study, the DXA and BIA analyses were carried out between 4.30 pm and 6.30 pm to ensure that no child was fasting, influencing the results. Other influences could include food consumption and recent activity (exercise) (Heymsfield et al., 2015).

On the other hand, pQCT quantifies the cross-sectional area of the peripheral (appendicular)/limb muscle (MCSA)(Frank-Wilson et al., 2015) and not the whole body, which could explain why we found no associations between physical activity and MCSAI. MCSA can also be influenced by the anthropometric characteristics of the subjects (body and limb size); therefore, we used MCSAI. Meanwhile, DXA provides information on the LSTM (Bazzocchi et al., 2016) of the whole body or regional/segmental zones (Laskey, 1996), with the advantages of its high precision and reproducibility(Guglielmi et al., 2016).

Our main findings also suggest a positive association between upper body muscle strength (handgrip strength) and TLSTMI, MCSAI, and FFMI in both boys and girls, after controlling for gender, education level, DQI, and birth weight. The results were consistent regardless of the methodology used to assess body FFM; however, the associations were weaker when BIA was used to measure LSTM markers. Similar results were found in the MINISTOP (mobile device-based intervention aimed at stopping obesity in pre-schoolers) study (Henriksson et al., 2016), where PREFIT (physical fitness test battery in preschool children) was used to measure physical fitness (Ortega et al., 2015) in 303 4-year-old children. Its associations showed a higher FFMI in participants with better upper body muscle strength (handgrip strength) (β=0.39, p<0.001).

Anthropometric variables such as body height, body mass, and BMI, which can influence grip strength, may explain these findings. In the case of body height, it is directly correlated with grip strength; this could partially explain its close relation to LBM (Jürimäe et al., 2009). Both the MINISTOP study and the present study made the respective height adjustment.

The present study found no association between standing long jump and TLSTMI, MCSAI, and FFMI. However, another study by Vicente-Rodriguez et al. (Vicente-Rodriguez et al., 2004) showed an increase in the FFM of 28 children (soccer players) followed for three years. They found no significant differences in muscle strength after evaluation through vertical jumps and maximal isometric force (MIF) during leg extension. Conversely, Henriksson et al.(Henriksson et al., 2016) observed that higher FFMI was associated with improved lower-body muscular strength (β=0.22, p<0.001). This disparity could be explained by the different populations included in each of the studies (preschoolers, schoolchildren, and soccer players), as well as their maturation stage (preschoolers, schoolchildren, and pre-puberty), the body composition evaluation methods (DXA and air displacement plethysmography) and the measurement methodology for the forces generated during vertical jumps (force plate versus standing long jump test). The lack of association between lower limb strength and FFM may also be because this test is considered weight-dependent, requiring propulsion or body elevation. Therefore, children and adolescents with higher LBM may not display better performance in these tests because they likely weigh differently. These results could suggest that higher performance in some physical fitness tests may occur in underweight individuals because of the lower load that they must move.

Finally, the relationship between physical activity and physical fitness (handgrip strength) and TLSTMI in our study is mainly consistent with the existing evidence showing positive associations independent of physical activity and physical fitness in TLSTMI. Our research shows that the association of total physical activity with TLSTMI is not mediated by physical fitness (handgrip strength). Therefore, future actions seeking to improve TLSTM in boys and girls should encourage activities that enhance physical activity levels and physical fitness.

### *4.1 Limitations and Strengths*

Within the limitations of the present study, there is the fact that the data cannot be extrapolated to other populations. Studies with different age groups and different ethnic groups should be developed in the future. Furthermore, due to its cross-sectional design, the observed associations cannot be interpreted as causal relationships.

Despite these limitations, the strengths of our study are many. To date, studies on a homogeneous sample with such young children are limited. The use of accelerometers allowed the objective assessment of physical activity, including both weekdays and weekend days. LSTM and FFM were measured using two different techniques, DXA and BIA, the former, a robust method accepted as the gold standard method. MCSA was measured by pQCT, a lower-cost method previously validated against DXA in this population. The DXA and pQCT measurements and the respective image analysis were performed by two trained researchers in each of the techniques. The TLSTM, ALSTM, MCSA, and FFM results were adjusted for the height of the subjects. Different tests were used to assess muscle strength (handgrip strength and standing long jump), controlling for several confounders, including gender, age, birth weight, DQI, and parents’ education level. Furthermore, it appears to be the first study evaluating handgrip strength as a potential mediator of the relationship between physical activity and TLSTMI in children.

## 5. Conclusions

The present study suggests positive associations between total and vigorous physical activity and TLSTMI in boys and girls aged 6 to 8. Its results indicate that upper limb strength (handgrip strength) does not mediate the relationship between total physical activity and TLSTMI.

The results also suggest an association between handgrip strength and TLSTMI, MCSAI, and FFMI in 6- to 8-year-old children. More studies are needed in the future to clarify the relationships between physical activity and physical fitness with TLSTM, MCSA, and FFM better, taking into account other confounding factors such as genetics factors, as well as physical activity intensities. To date, most of the available studies focus on the relationship at higher intensities of physical activity (i.e., MVPA and vigorous physical activity).

Public policies should encourage participation in all physical activities, including light physical activity. As we have shown, it can be an effective substitute for sedentary activities and expand the focus beyond the MVPA as a strategy to promote our children’s health. Likewise, exercises that improve upper extremity strength and lean mass should be encouraged, which, as mentioned above, is an important tissue in children.

## Acknowledgments

The authors would like to thank the parents and children who participated in this research.

The CALINA study was supported by three grants from the Carlos III Health Institute: the PI08/0559 Aragon Health Sciences Institute for the Growth and Feeding in Infants from Aragon (CALINA) project, the PI13/02359 Environmental factors influencing early development of obesity during childhood and body composition programming, and the RD16/0022 Maternal, Child Health, and Development Network (Retic SAMID) RETICS, funded by the PN

I+D+I 2008-2011 (Spain), ISCIII- Sub-Directorate General for Research Assessment and Promotion, and the European Regional Development Fund (ERDF). Additionally, we thank the Carolina Foundation Ph.D. Grants for supporting first author in producing this manuscript.

## Authors' Contributions

DPCR analyzed the data and drafted the manuscript. IIA conducted the data collection and drafted the manuscript. AGB drafted the manuscript. MLMB conducted the data collection. PFB conducted the data collection. JACS and LMA drafted the manuscript. GR conceived the study and participated in its design and coordination and helped draft the manuscript.

All the authors have read and approved this manuscript’s final version. They agree with the authors’ order of presentation.

## Disclosure Statement

The authors report no conflict of interest.

## Bibliography

Bammann, K., Sioen, I., Huybrechts, I., Casajús, J. A., Vicente-Rodríguez, G., Cuthill, R., Konstabel, K., Tubić, B., Wawro, N., Rayson, M., Westerterp, K., Mårild, S., Pitsiladis, Y. P., Reilly, J. J., Moreno, L. A., & De Henauw, S. (2011). The IDEFICS validation study on field methods for assessing physical activity and body composition in children: Design and data collection. *International Journal of Obesity*, *35*, S79–S87. https://doi.org/10.1038/ijo.2011.38

Baron, R. M., & Kenny, D. A. (1986). The moderator–mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. *Journal of Personality and Social Psychology*, *51*(6), 1173. https://doi.org/https://doi.org/10.1037/0022-3514.51.6.1173

Baxter-Jones, A. D. G., Eisenmann, J. C., Mirwald, R. L., Faulkner, R. A., & Bailey, D. A. (2008). The influence of physical activity on lean mass accrual during adolescence: a longitudinal analysis. *Journal of Applied Physiology*, *105*(2), 734–741. https://doi.org/https://doi.org/10.1152/japplphysiol.00869.2007

Bazzocchi, A., Ponti, F., Albisinni, U., Battista, G., & Guglielmi, G. (2016). DXA: Technical aspects and application. *European Journal of Radiology*, *85*(8), 1481–1492. https://doi.org/10.1016/j.ejrad.2016.04.004

Boisseau, N., & Delamarche, P. (2000). Metabolic and hormonal responses to exercise in children and adolescents. *Sports Medicine*, *30*(6), 405–422. https://doi.org/10.2165/00007256-200030060-00003

Burrows, R., Correa-Burrows, P., Reyes, M., Blanco, E., Albala, C., & Gahagan, S. (2017). Low muscle mass is associated with cardiometabolic risk regardless of nutritional status in adolescents: A cross-sectional study in a Chilean birth cohort. *Pediatric Diabetes*, *18*(8), 895–902. https://doi.org/10.1111/pedi.12505

Carson, V., Ridgers, N. D., Howard, B. J., Winkler, E. A. H., Healy, G. N., Owen, N., Dunstan, D. W., & Salmon, J. (2013). Light-Intensity Physical Activity and Cardiometabolic Biomarkers in US Adolescents. *PLoS ONE*, *8*(8). https://doi.org/10.1371/journal.pone.0071417

Carver, A., Timperio, A. F., Hesketh, K. D., Ridgers, N. D., Salmon, J. L., & Crawford, D. A. (2011). How is active transport associated with children's and adolescents' physical activity over time? *International Journal of Behavioral Nutrition and Physical Activity*, *8*, 1–6. https://doi.org/10.1186/1479-5868-8-126

Córdoba-Rodríguez, D. P., Iglesia, I., Gomez-Bruton, A., Miguel-Berges, M. L., Flores-Barrantes, P., Casajús, J. A., Moreno, L. A., & Rodríguez, G. (2021). Quantitative peripheral computed tomography to measure muscle area and assess lean soft tissue mass in children. *Annals of Human Biology*, 1–27. https://doi.org/10.1080/03014460.2021.1877352

Crabtree, N. J., Leonard, M. B., & Zemel, B. S. (2007). *Dual-Energy X-Ray Absorptiometry BT  - Bone Densitometry in Growing Patients: Guidelines for Clinical Practice* (A. J. Sawyer, L. K. Bachrach, & E. B. Fung (eds.); pp. 41–57). Humana Press. https://doi.org/10.1007/978-1-59745-211-3_3

De Miguel-Etayo, P., Gracia-Marco, L., Ortega, F. B., Intemann, T., Foraita, R., Lissner, L., Oja, L., Barba, G., Michels, N., Tornaritis, M., Molnár, D., Pitsiladis, Y., Ahrens, W., & Moreno, L. A. (2014). Physical fitness reference standards in European children: The IDEFICS study. *International Journal of Obesity*, *38*(September), S57–S66. https://doi.org/10.1038/ijo.2014.136

Deheeger, M., Rolland-Cachera, M. F., & Fontvieille, A. M. (1997). Physical activity and body composition in 10 year old French children: Linkages with nutritional intake? *International Journal of Obesity*, *21*(5), 372–379. https://doi.org/10.1038/sj.ijo.0800415

Dorsey, K. B., Thornton, J. C., Heymsfield, S. B., & Gallagher, D. (2010). Greater lean tissue and skeletal muscle mass are associated with higher bone mineral content in children. *Nutrition and Metabolism*, *7*, 1–11. https://doi.org/10.1186/1743-7075-7-41

Dotan, R., Mitchell, C., Cohen, R., Klentrou, P., Gabriel, D., & Falk, B. (2012). Child-adult differences in muscle activation - A review. *Pediatric Exercise Science*, *24*(1), 2–21. https://doi.org/10.1123/pes.24.1.2

España-Romero, V., Artero, E. G., Santaliestra-Pasias, A. M., Gutierrez, A., Castillo, M. J., & Ruiz, J. R. (2008). Hand Span Influences Optimal Grip Span in Boys and Girls Aged 6 to 12 Years. *Journal of Hand Surgery*, *33*(3), 378–384. https://doi.org/10.1016/j.jhsa.2007.11.013

Evenson, K. R., Catellier, D. J., Gill, K., Ondrak, K. S., & McMurray, R. G. (2008). Calibration of two objective measures of physical activity for children. *Journal of Sports Sciences*, *26*(14), 1557–1565. https://doi.org/10.1080/02640410802334196

Foo, L. H., Zhang, Q., Zhu, K., Ma, G., Greenfield, H., & Fraser, D. R. (2007). *Influence of body composition , muscle strength , diet and physical activity on total body and forearm bone mass in Chinese adolescent girls*. 1281–1287. https://doi.org/10.1017/S0007114507787421

Frank-Wilson, A. W., Johnston, J. D., Olszynski, W. P., & Kontulainen, S. A. (2015). Measurement of muscle and fat in postmenopausal women: precision of previously reported pQCT imaging methods. *Bone*, *75*, 49–54.

https://doi.org/10.1016/J.BONE.2015.01.016

Fraser, B. J., Blizzard, L., Cleland, V., Schmidt, M. D., Smith, K. J., Gall, S. L., Dwyer, T., Venn, A. J., & Magnussen, C. G. (2020). Factors associated with muscular fitness phenotypes in Australian children: A cross-sectional study. *Journal of Sports Sciences*, *38*(1), 38–45. https://doi.org/10.1080/02640414.2019.1679575

Gómez-Bruton, A., Gonzalez-Agüero, A., Casajús, J. A., & Rodríguez, G. V. (2014). Swimming training repercussion on metabolic and structural bone development; benefits of the incorporation of whole body vibration or pilometric training; the RENACIMIENTO project. *Nutricion Hospitalaria*, *30*(2), 399–409. https://doi.org/10.3305/nh.2014.30.2.7603

González-Agüero, A., Vicente-Rodríguez, G., Gómez-Cabello, A., & Casajús, J. A. (2013). Cortical and trabecular bone at the radius and tibia in male and female adolescents with Down syndrome: a peripheral quantitative computed tomography (pQCT) study. *Osteoporosis International : A Journal Established as Result of Cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*, *24*(3), 1035—1044. https://doi.org/10.1007/s00198-012-2041-7

Gracia-Marco, L, Ortega, F. B., Jiménez-Pavón, D., Rodríguez, G., Castillo, M. J., Vicente-Rodríguez, G., & Moreno, L. A. (2012). Adiposity and bone health in Spanish adolescents. The HELENA study. *Osteoporosis International*, *23*(3), 937–947. https://doi.org/10.1007/s00198-011-1649-3

Gracia-Marco, Luis, Moreno, L. A., Ruiz, J. R., Ortega, F. B., de Moraes, A. C. F., Gottrand, F., Roccaldo, R., Marcos, A., Gómez-Martínez, S., Dallongeville, J., Kafatos, A., Molnar, D., Bueno, G., de Henauw, S., Widhalm, K., & Wells, J. C. (2016). Body Composition Indices and Single and Clustered Cardiovascular Disease Risk Factors in Adolescents: Providing Clinical-Based Cut-Points. *Progress in Cardiovascular Diseases*, *58*(5), 555–564. https://doi.org/10.1016/j.pcad.2015.11.002

Guglielmi, G., Ponti, F., Agostini, M., Amadori, M., Battista, G., & Bazzocchi, A. (2016). The role of DXA in sarcopenia. *Aging Clin Exp Res*, *28*(6), 1047–1060. https://doi.org/10.1007/s40520-016-0589-3

Hao, G., Pollock, N. K., Harris, R. A., Gutin, B., Su, S., & Wang, X. (2019). Associations between muscle mass, physical activity and dietary behaviour in adolescents. *Pediatric Obesity*, *14*(3), 1–8. https://doi.org/10.1111/ijpo.12471

Hayes, A. F. (2013). Mediation, moderation, and conditional process analysis. Introduction to mediation, moderation, and conditional process analysis: A regression-based approach. *New York: Guilford Publications*, 1–20.

Heelan, K. A., & Eisenmann, J. C. (2006). Physical activity, media time, and body composition in young children. *Journal of Physical Activity and Health*, *3*(2), 200–209. https://doi.org/10.1123/jpah.3.2.200

Henriksson, P., Cadenas-Sanchez, C., Leppänen, M. H., Nyström, C. D., Ortega, F. B., Pomeroy, J., Ruiz, J. R., & Löf, M. (2016). Associations of fat mass and fat-free mass with physical fitness in 4-year-old children: Results from the MINISTOP trial. *Nutrients*, *8*(8), 1–11. https://doi.org/10.3390/nu8080473

Heymsfield, S. B., Gonzalez, M. C., Lu, J., Jia, G., & Zheng, J. (2015). Skeletal muscle mass and quality: Evolution of modern measurement concepts in the context of sarcopenia. *Proceedings of the Nutrition Society*, *74*(4), 355–366. https://doi.org/10.1017/S0029665115000129

Huybrechts, I., Vereecken, C., De Bacquer, D., Vandevijvere, S., Van Oyen, H., Maes, L., Vanhauwaert, E., Temme, L., De Backer, G., & De Henauw, S. (2010). Reproducibility and validity of a diet quality index for children assessed using a FFQ. *British Journal of Nutrition*, *104*(1), 135–144. https://doi.org/10.1017/S0007114510000231

Iglesia, I., Intemann, T., De Miguel-Etayo, P., Pala, V., Hebestreit, A., Wolters, M., Russo, P., Veidebaum, T., Papoutsou, S., Nagy, P., Eiben, G., Rise, P., De Henauw, S., & Moreno, L. A. (2020). Dairy consumption at snack meal occasions and the overall quality of diet during childhood. Prospective and cross-sectional analyses from the idefics/i.family cohort. *Nutrients*, *12*(3), 1–20. https://doi.org/10.3390/nu12030642

Isganaitis, E. (2019). Developmental Programming of Body Composition: Update on Evidence and Mechanisms. *Current Diabetes Reports*, *19*(8), 1–

20. https://doi.org/10.1007/s11892-019-1170-1

Jiménez-Pavón, D., Fernández-Vázquez, A., Alexy, U., Pedrero, R., Cuenca-García, M., Polito, A., Vanhelst, J., Manios, Y., Kafatos, A., Molnar, D., Sjöström, M., & Moreno, L. A. (2013). Association of objectively measured physical activity with body components in European adolescents. *BMC Public Health*, *13*(1). https://doi.org/10.1186/1471-2458-13-667

Jürimäe, T., Hurbo, T., & Jürimäe, J. (2009). Relationship of handgrip strength with anthropometric and body composition variables in prepubertal children. *HOMO- Journal of Comparative Human Biology*, *60*(3), 225–238. https://doi.org/10.1016/j.jchb.2008.05.004

Kim, J. H., & Park, Y. S. (2016). Low muscle mass is associated with metabolic syndrome in Korean adolescents: the Korea National Health and Nutrition Examination Survey 2009-2011. *Nutrition Research*, *36*(12), 1423–1428. https://doi.org/10.1016/j.nutres.2016.09.013

Kim, K., Hong, S., & Kim, E. Y. (2016). Reference values of skeletal muscle mass for Korean children and adolescents using data from the Korean national health and nutrition examination survey 2009-2011. *PLoS ONE*, *11*(4), 1–10. https://doi.org/10.1371/journal.pone.0153383

Kim, S., & Valdez, R. (2015). Metabolic risk factors in U.S. youth with low relative muscle mass. *Obesity Research and Clinical Practice*, *9*(2), 125–132. https://doi.org/10.1016/j.orcp.2014.05.002

Kulkarni, B., Hills, A. P., & Byrne, N. M. (2014). Nutritional influences over the life course on lean body mass of individuals in developing countries. *Nutrition Reviews*, *72*(3), 190–204. https://doi.org/10.1111/nure.12097

Kuriyan, R. (2018). Body composition techniques. *Indian Journal of Medical Research*, *148*(5), 648–658. https://doi.org/10.4103/ijmr.IJMR_1777_18

Labayen, I., Moreno, L. A., Blay, M. G., Blay, V. A., Mesana, M. I., González-Gross, M., Bueno, G., Sarría, A., & Bueno, M. (2006). Early programming of body composition and fat distribution in adolescents. *Journal of Nutrition*, *136*(1), 147–152. https://doi.org/10.1093/jn/136.1.147

Larqué, E., Labayen, I., Flodmark, C. E., Lissau, I., Czernin, S., Moreno, L. A., Pietrobelli, A., & Widhalm, K. (2019). From conception to infancy — early risk factors for childhood obesity. *Nature Reviews Endocrinology*, *15*(8), 456–478. https://doi.org/10.1038/s41574-019-0219-1

Laskey, M. A. (1996). Dual-energy X-ray absorptiometry and body composition. *Nutrition*, *12*(1), 45–51. https://doi.org/10.1097/01.mco.0000165010.31826.3d

Leppänen, M., Henriksson, P., Delisle Nyström, C., Henriksson, H., Ortega, F. B., Pomeroy, J., Ruiz, J. R., Cadenas-Sanchez, C., & Löf, M. (2017). Longitudinal physical activity, body composition, and physical fitness in preschoolers. In *Medicine and Science in Sports and Exercise* (Vol. 49, Issue 10). https://doi.org/10.1249/MSS.0000000000001313

Leppänen, M., Nyström, C., Henriksson, P., Pomeroy, J., Ruiz, J. R., Ortega, F., Cadenas-Sánchez, C., & Löf, M. (2016). Physical activity intensity, sedentary behavior, body composition and physical fitness in 4-year-old children: Results from the ministop trial. *International Journal of Obesity*, *40*(7), 1126–1133. https://doi.org/10.1038/ijo.2016.54

Liu, J., Yan, Y., Xi, B., Huang, G., & Mi, J. (2019). Skeletal muscle reference for Chinese children and adolescents. *Journal of Cachexia, Sarcopenia and Muscle*, *10*(1), 155–164. https://doi.org/10.1002/jcsm.12361

López-Gil, J. F., Brazo-Sayavera, J., García-Hermoso, A., & Yuste Lucas, J. L. (2020). Adherence to Mediterranean Diet Related with Physical Fitness and Physical Activity in Schoolchildren Aged 6–13. *Nutrients*, *12*(2), 567. https://doi.org/10.3390/nu12020567

Moliner-Urdiales, D., Ortega, F. B., Vicente-Rodriguez, G., Rey-Lopez, J. P., Gracia-Marco, L., Widhalm, K., Sjöström, M., Moreno, L. A., Castillo, M. J., & Ruiz, J. R. (2010a). Association of physical activity with muscular strength and fat-free mass in adolescents: The HELENA study. *European Journal of Applied Physiology*, *109*(6), 1119–1127. https://doi.org/10.1007/s00421-010-1457-z

Moliner-Urdiales, D., Ortega, F. B., Vicente-Rodriguez, G., Rey-Lopez, J. P., Gracia-Marco, L., Widhalm, K., Sjöström, M., Moreno, L. A., Castillo, M. J., & Ruiz, J. R. (2010b). Association of physical activity with muscular strength and fat-free mass in adolescents: The HELENA study. *European Journal of Applied Physiology*, *109*(6), 1119–1127. https://doi.org/10.1007/s00421-010-1457-z

Mouratidou, T., Mesana Graffe, M. I., Huybrechts, I., De Decker, E., De Craemer, M., Androutsos, O., Manios, Y., Galcheva, S., Lateva, M., Gurzkowska, B., Kułaga, Z., Birnbaum, J., Koletzko, B., & Moreno, L. A. (2019). Reproducibility and relative validity of a semiquantitative food frequency questionnaire in European preschoolers: The ToyBox study. *Nutrition*, *65*, 60–67. https://doi.org/10.1016/j.nut.2019.03.003

National Health and Nutrition Examination Survey (NHANES). (2012). *Body Composition Procedures Manual*. *July*, 1–125.

Orsso, C. E., Tibaes, J. R. B., Oliveira, C. L. P., Rubin, D. A., Field, C. J., Heymsfield, S. B., Prado, C. M., & Haqq, A. M. (2019). Low muscle mass and strength in pediatrics patients: Why should we care? *Clinical Nutrition*, *38*(5), 2002–2015. https://doi.org/10.1016/j.clnu.2019.04.012

Ortega, F. B., Cadenas-Sánchez, C., Sánchez-Delgado, G., Mora-González, J., Martínez-Téllez, B., Artero, E. G., Castro-Piñero, J., Labayen, I., Chillón, P., Löf, M., & Ruiz, J. R. (2015). Systematic Review and Proposal of a Field-Based Physical Fitness-Test Battery in Preschool Children: The PREFIT Battery. *Sports Medicine*, *45*(4), 533–555. https://doi.org/10.1007/s40279-014-0281-8

Oves Suárez, B., Escartín Madurga, L., Samper Villagrasa, M. P., Cuadrón Andrés, L., Álvarez Sauras, M. L., Lasarte Velillas, J. J., Moreno Aznar, L. A., & Rodríguez Martínez, G. (2014). Inmigración y factores asociados con la lactancia materna. Estudio CALINA. *Anales de Pediatría*, *81*, 32–38. https://doi.org/10.1016/j.anpedi.2013.09.008

Poitras, V. J., Gray, C. E., Borghese, M. M., Carson, V., Chaput, J.-P., Janssen, I., Katzmarzyk, P. T., Pate, R. R., Connor Gorber, S., & Kho, M. E. (2016). Systematic review of the relationships between objectively measured physical activity and health indicators in school-aged children and youth. *Applied Physiology, Nutrition, and Metabolism*, *41*(6), S197–S239. https://doi.org/10.1139/apnm-2015-0663

Ramires, V. V., Dumith, S. C., Wehrmeister, F. C., Hallal, P. C., Menezes, A. M. B., & Gonçalves, H. (2016). Physical activity throughout adolescence and body composition at 18 years: 1993 Pelotas (Brazil) birth cohort study. *International Journal of Behavioral Nutrition and Physical Activity*, *13*(1), 1–13. https://doi.org/10.1186/s12966-016-0430-6

Rennie, K. L., Livingstone, M. B. E., Wells, J. C. K., Mcgloin, A., Coward, W. A., & Prentice, A. M. (2005). *Association of physical activity with body-composition indexes in children aged 6 – 8 y at varied risk of obesity 1 – 3*. 13–20. https://doi.org/https://doi.org/10.1093/ajcn/82.1.13

Riddoch, C. J., Andersen, L. B., Wedderkopp, N., Harro, M., Klasson-Heggebø, L., Sardinha, L. B., Cooper, A. R., & Ekelund, U. (2004). Physical Activity Levels and Patterns of 9- and 15-yr-Old European Children. *Medicine and Science in Sports and Exercise*, *36*(1), 86–92. https://doi.org/10.1249/01.MSS.0000106174.43932.92

Roggen, I., Roelants, M., Sioen, I., Vandewalle, S., De Henauw, S., Goemaere, S., Kaufman, J.-M., & De Schepper, J. (2015). Pediatric Reference Values for Tibial Trabecular Bone Mineral Density and Bone Geometry Parameters Using Peripheral Quantitative Computed Tomography. *Calcified Tissue International*, *96*(6), 527–533. https://doi.org/10.1007/s00223-015-9988-2

Ruiz, J. R., Castro-Piñero, J., Artero, E. G., Ortega, F. B., Sjöström, M., Suni, J., & Castillo, M. J. (2009). Predictive validity of health-related fitness in youth: a systematic review. *British Journal of Sports Medicine*, *43*(12), 909 LP – 923. https://doi.org/10.1136/bjsm.2008.056499

Santos, D. A., Magalhaes, J. P., Judice, P. B., Correia, I. R., Minderico, C. S., Ekelund, U., & Sardinha, L. B. (2019). Fitness Mediates Activity and Sedentary Patterns Associations with Adiposity in Youth. *Medicine & Science in Sports & Exercise*, *51*(2), 323–329. https://doi.org/10.1249/MSS.0000000000001785

Scheurer, J. M., Zhang, L., Plummer, E. A., Hultgren, S. A., Demerath, E. W., & Ramel, S. E. (2018). Body Composition Changes from Infancy to 4 Years and Associations with Early Childhood Cognition in Preterm and Full-Term Children. *Neonatology*, *114*(2), 169–176.

https://doi.org/10.1159/000487915

Singhal, A., Wells, J., Cole, T. J., Fewtrell, M., & Lucas, A. (2003). Programming of lean body mass: A link between birth weight, obesity, and cardiovascular disease? *American Journal of Clinical Nutrition*, *77*(3), 726–730. https://doi.org/10.1093/ajcn/77.3.726

Sioen, I., Lust, E., De Henauw, S., Moreno, L. A., & Jiménez-Pavón, D. (2016). Associations Between Body Composition and Bone Health in Children and Adolescents: A Systematic Review. *Calcified Tissue International*, *99*(6), 557–577. https://doi.org/10.1007/s00223-016-0183-x

Statistics, U. I. for. (2012). *International standard classification of education: ISCED 2011*. UNESCO Institute for Statistics Montreal.

Tanita Corporation - Japan. (2002). *Body Composition Analyzer BC-418 Instruction Manual*. 1–43.

Tremblay, M. S., Warburton, D. E. R., Janssen, I., Paterson, D. H., Latimer, A. E., Rhodes, R. E., Kho, M. E., Hicks, A., LeBlanc, A. G., Zehr, L., Murumets, K., & Duggan, M. (2011). New Canadian physical activity guidelines. *Applied Physiology, Nutrition and Metabolism*, *36*(1), 36–46. https://doi.org/10.1139/H11-009

Trost, S. G., Pate, R. R., Sallis, J. F., Freedson, P. S., Taylor, W. C., Dowda, M., & Sirard, J. (2002). Age and gender differences in objectively measured physical activity in youth. *Medicine and Science in Sports and Exercise*, *34*(2), 350–355. https://doi.org/10.1097/00005768-200202000-00025

Unesco. (1997). *International Standard Classification of Education-ISCED 1997: November 1997*. Unesco.

Veldhuis, J. D., Roemmich, J. N., Richmond, E. J., Rogol, A. D., Lovejoy, J. C., Sheffield-Moore, M., Mauras, N., & Bowers, C. Y. (2005). Endocrine control of body composition in infancy, childhood, and puberty. *Endocrine Reviews*, *26*(1), 114–146. https://doi.org/10.1210/er.2003-0038

Vicente-Rodriguez, G., Ara, I., Perez-Gomez, J., Serrano-Sanchez, J. A., Dorado, C., & Calbet, J. A. L. (2004). High Femoral Bone Mineral Density Accretion in Prepubertal Soccer Players. *Medicine & Science in Sports &*

*Exercise*, *36*(10). https://journals.lww.com/acsm-msse/Fulltext/2004/10000/High_Femoral_Bone_Mineral_Density_Accretion_in.19.aspx

Vyncke, K., Cruz Fernandez, E., Fajó-Pascual, M., Cuenca-García, M., De Keyzer, W., Gonzalez-Gross, M., Moreno, L. A., Beghin, L., Breidenassel, C., Kersting, M., Albers, U., Diethelm, K., Mouratidou, T., Grammatikaki, E., De Vriendt, T., Marcos, A., Bammann, K., Börnhorst, C., Leclercq, C., … Huybrechts, I. (2013). Validation of the Diet Quality Index for Adolescents by comparison with biomarkers, nutrient and food intakes: The HELENA study. *British Journal of Nutrition*, *109*(11), 2067–2078. https://doi.org/10.1017/S000711451200414X

Wells, J. C. K. (2000). A Hattori chart analysis of body mass index in infants and children. *International Journal of Obesity*, *24*(3), 325–329. https://doi.org/10.1038/sj.ijo.0801132

Wells, J. C. K., Cole, T. J., & team, A. study. (2002). Adjustment of fat-free mass and fat mass for height in children aged 8 y. *International Journal Of Obesity*, *26*, 947. https://doi.org/10.1038/sj.ijo.0802027

Westerterp, K. R., Yamada, Y., Sagayama, H., Ainslie, P. N., Andersen, L. F., & Anderson, L. J. (2021). *Physical activity and fat-free mass during growth and in later life*. 1–7.

Wickel, E. E., & Eisenmann, J. C. (2007). Contribution of youth sport to total daily physical activity among 6- to 12-yr-old boys. *Medicine and Science in Sports and Exercise*, *39*(9), 1493–1500. https://doi.org/10.1249/mss.0b013e318093f56a

World Health Organization. (2006). *WHO child growth standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development*.

World Health Organization. (2020). WHO Guidelines on physical activity and sedentary behaviour. In *World Health Organization*. https://apps.who.int/iris/bitstream/handle/10665/325147/WHO-NMH-PND-2019.4-eng.pdf?sequence=1&isAllowed=y%0Ahttp://www.who.int/iris/handle/1066

5/311664%0Ahttps://apps.who.int/iris/handle/10665/325147

Wyszyńska, J., Matłosz, P., Szybisty, A., Lenik, P., Dereń, K., Mazur, A., & Herbert, J. (2020). Obesity and Body Composition in Preschool Children with Different Levels of Actigraphy-Derived Physical Activity—A Cross-Sectional Study. *Journal of Clinical Medicine*, *9*(4), 1210. https://doi.org/10.3390/jcm9041210

**Table 1. Main characteristics of the participating children (n= 279).**

| Characteristics | All n= 279 (M±SD) | Girls n= 129 (M±SD) | Boys n= 150 (M±SD) | P-value |
|---|---|---|---|---|
| Age (y) | 7.5 ± 0.3 | 7.6 ± 0.4 | 7.5 ± 0.3 | 0.41 |
| **Anthropometric measurements** | | | | |
| Height (cm) | 126.1 ± 5.9 | 125.6 ± 5.6 | 125.5 ± 6.0 | 0.12 |
| Weight (kg)[a] | 26.4 (23.6 - 30.0) | 26.2 (23.6 - 30.0) | 26.7 (23.7 - 30.8) | 0.59 |
| BMI (kg/m$^2$)[a] | 16.6 (15.4 - 18.3) | 16.7 (15.5 - 18.3) | 16.6 (15.4 - 18.5) | 0.74 |
| BMI z-score[†] | 0.84 ± 1.2 | 0.76 ± 1.1 | 0.87 ± 1.3 | 0.48 |
| **BIA** | | | | |
| FFM (kg)[a] | 20.5 (18.7 - 22.5) | 19.9 (18.5 - 21.8) | 20.8 (19.1 - 23.4) | <0.01* |
| FFMI (kg/m$^2$) | 13.1 ± 1.4 | 12.7 (12.1 - 13.4) | 13.2 (12.4 - 14.2) | <0.01* |
| **DXA** | | | | |
| TLSTM (kg)[a] | 18.1 (16.6 – 20.0) | 17.5 (16.2 - 18.7) | 18.9 (17.0 - 21.0) | <0.01* |
| TLSTMI (kg/m$^2$) | 11.6 ± 1.1 | 11.2 ± 1.0 | 11.9 ± 1.1 | <0.01* |
| ALSTMI (Kg/m$^2$) | 4.7± 0.5 | 4.5± 0.5 | 4.8± 0.5 | <0.01* |
| **pQCT** | | | | |
| Tibia length (mm) | 274.3 ± 18.1 | 275.8 ±17.3 | 273.1 ±18.8 | 0.14 |

| | | | | |
|---|---|---|---|---|
| MCSA (mm$^2$) | 3275.3 ± 496.0 | 3136.5 ± 473.2 | 3389.1 ± 488.3 | <0.01* |
| MCSAI (mm$^2$ /m$^2$) | 2057.1 ± 263.1 | 1996.3 ± 257.6 | 2112.1 ± 256.5 | <0.01* |
| **Physical activity** | | | | |
| Light (min/day) | 41.3 ± 11.1 | 37.0 ± 9.9 | 45.0 ± 10.8 | <0.01* |
| Moderate (min/day)[a] | 29.2 (21.1 - 36.2) | 24.3 (18.1 - 32.1) | 31.9 (24.9 - 41.1) | <0.01* |
| Vigorous (min/day)[a] | 69.0 (55.6 - 85.0) | 59.8 (50.0 - 75.7) | 77.5 (64.2 - 92.6) | <0.01* |
| MVPA (min/day)[a] | 97.3 (77.4 - 121.4) | 85.9 (67.8 – 108.4) | 110.8 (88.0 -134.0) | <0.01* |
| Total PA (min/day) | 269.6 (240.3 – 299.7) | 254.0 (231.3 – 285.2) | 282.5 (245.9 – 312.7) | <0.01* |
| **Physical fitness** | | | | |
| Handgrip strength (kg) | 10.5 ± 2.2 | 10.1 ± 2.0 | 10.9 ± 2.1 | <0.01* |
| Standing long jump (cm) | 102.7 ± 17.8 | 98.9 ± 17.0 | 107.6 ± 17.7 | <0.01* |

Abbreviation: ALSTMI= Appendicular lean soft tissue mass index; BIA= Bioelectrical impedance; BMI= Body mass index; MCSA= Muscle cross-sectional area; DXA= Dual-energy X-ray absorptiometry; FFM= Fat-free mass; FFMI= Fat-free mass index; MVPA= Moderate-vigorous physical activity; pQCT= quantitative peripheral computed tomography; TLSTM= Total lean soft tissue mass; TLSTMI= Total lean soft tissue mass index

Normally distributed variables are shown as mean ± SD (Student t-test)

[a] Non-normally distributed variables are shown as median and interquartile intervals (25th and 75th, U Mann–Whitney)

[†] BMI z-scores were calculated according to the World Health Organization (WHO)

* Significant differences by gender

Significance was set at 0.05 level.

## Table 2. Associations between physical activity and total lean soft tissue mass index, muscle cross-sectional area index, and fat-free mass index (n= 279)

| Predictors | Girls | | | | | | | | | | Boys | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Physical activity | Total | | Light | | Moderate | | Vigorous | | MVPA | | Total | | Light | | Moderate | | Vigorous | | MVPA | |
| | β | *p* | β | *p* | β | *p* | β | *p* | β | *p* | β | *p* | β | *p* | β | *p* | β | *p* | β | *p* |
| **TLSTMI by DXA** | | | | | | | | | | | | | | | | | | | | |
| *Model 1* | .272 | **.004*** | .154 | .109 | .123 | .201 | .151 | .117 | .144 | .137 | .335 | **.000*** | .287 | **.001*** | .093 | .289 | .203 | **.020*** | .166 | .058 |
| *Model 2* | .275 | **.004*** | .170 | .078 | .120 | .219 | .159 | **.001*** | .148 | .130 | .371 | **.000*** | .302 | **.001*** | .116 | .206 | .224 | **.013*** | .189 | **.038*** |
| *Model 3* | .247 | **.010*** | .151 | .111 | .111 | .248 | .143 | **.001*** | .134 | .162 | .337 | **.000*** | .290 | **.001*** | .081 | .367 | .200 | **.023*** | .160 | .072 |
| **MCSAI by pQCT** | | | | | | | | | | | | | | | | | | | | |
| *Model 1* | .148 | .131 | .112 | .253 | -.003 | .974 | .057 | .564 | .036 | .717 | .091 | .314 | .074 | .410 | .052 | .560 | .068 | .447 | .064 | .480 |
| *Model 2* | .124 | .223 | .093 | .359 | -.034 | .742 | .030 | .768 | .007 | .944 | .114 | .219 | .083 | .364 | .053 | .569 | .074 | .426 | .068 | .468 |
| *Model 3* | .097 | .347 | .077 | .448 | -.040 | .692 | .018 | .863 | -.004 | .972 | .110 | .245 | .081 | .381 | .049 | .607 | .070 | .454 | .063 | .500 |
| **FFMI by BIA** | | | | | | | | | | | | | | | | | | | | |
| *Model 1* | .125 | .194 | .132 | .173 | -.047 | .626 | .042 | .664 | .010 | .918 | .260 | **.003*** | .161 | .064 | -.010 | .908 | .078 | .373 | .047 | .594 |
| *Model 2* | .185 | .053 | .149 | .122 | -.060 | .539 | .045 | .642 | .007 | .939 | .308 | **.000*** | .175 | .050 | -.026 | .781 | .078 | .389 | .042 | .648 |
| *Model 3* | .149 | .097 | .137 | .154 | -.066 | .496 | .035 | .717 | -.001 | .988 | .299 | **.001*** | .175 | .051 | -.026 | .780 | .079 | .388 | .042 | .647 |

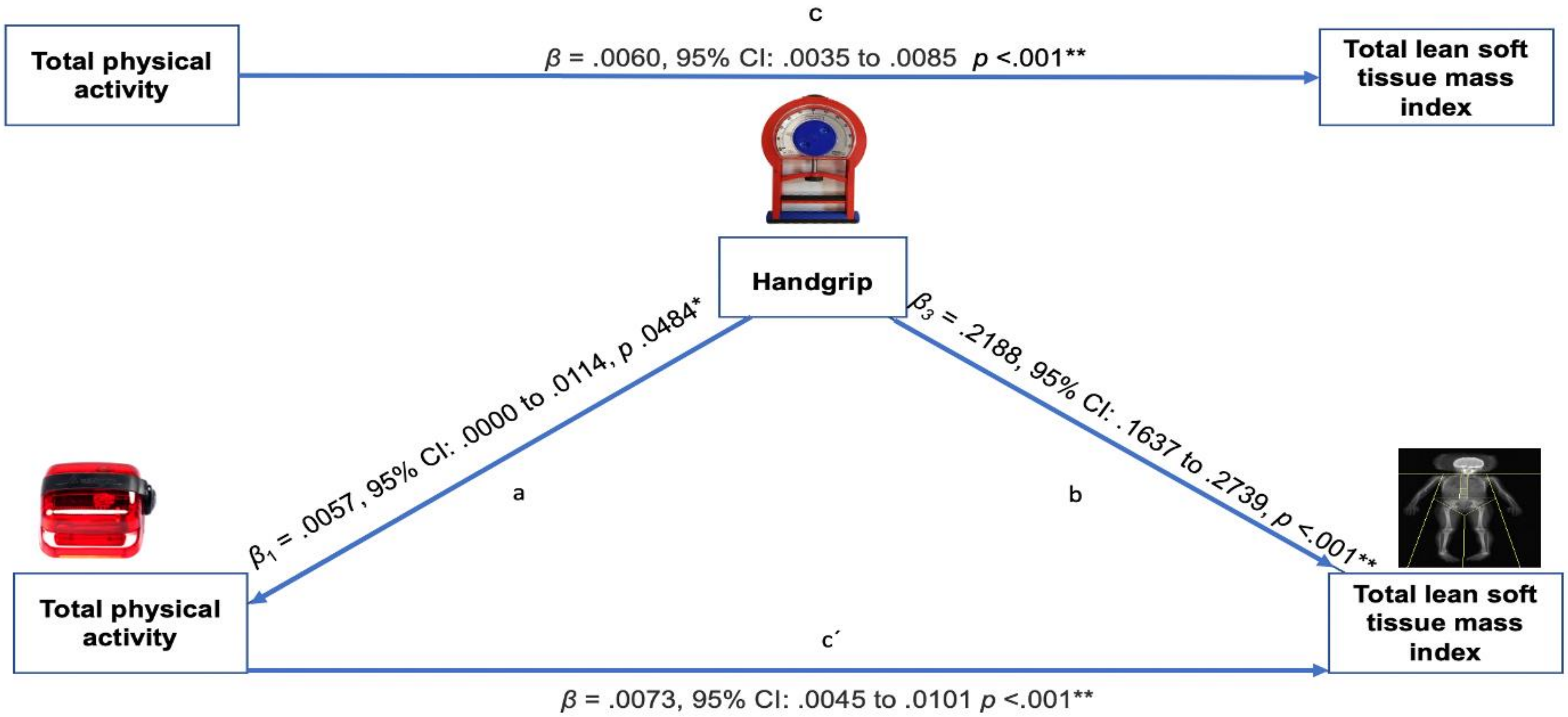
β: standardized regression coefficient
Abbreviation: BIA= Bioimpedance analysis; DXA= Dual-energy X-ray absorptiometry; FFMI= Fat-free mass index; MCSAI= Muscle cross-sectional area index; pQCT= Peripheral quantitative computed tomography; TLSTMI= Total lean soft tissue mass index.
*Model 1* Physical activity (basic model without adjustments)
*Model 2 Model 1* + diet quality index, parents' highest education level and age.
*Model 3 Model 2* + birth weight
*Significance was set at 0.05 level.

## Table 3. Associations between physical fitness and total lean soft tissue mass index, muscle cross-sectional area index, and fat-free mass index (n= 279)

| | | Girls | | | | Boys | | | |
|---|---|---|---|---|---|---|---|---|---|
| Predictors<br>Physical fitness | | Handgrip strength | | Standing long jump | | Handgrip strength | | Standing long jump | |
| | | β | *p* | β | *p* | β | *p* | β | *p* |
| **TLSTMI by DXA** | | | | | | | | | |
| *Model 1* | | .363 | **.000*** | .055 | .512 | .478 | **.000*** | -.019 | .839 |
| *Model 2* | | .350 | **.000*** | .070 | .403 | .464 | **.000*** | -.013 | .893 |
| *Model 3* | | .314 | **.000*** | .034 | .672 | .425 | **.000*** | -.036 | .690 |
| **MCSAI by pQCT** | | | | | | | | | |
| *Model 1* | | .262 | **.002*** | .056 | .520 | .273 | **.000*** | -.064 | .508 |
| *Model 2* | | .282 | **.001*** | .074 | .404 | .287 | **.000*** | -.060 | .540 |
| *Model 3* | | .267 | **.002*** | .051 | .561 | .301 | **.000*** | -.059 | .547 |
| **FFMI by BIA** | | | | | | | | | |
| *Model 1* | | .236 | **.004*** | -.082 | .332 | .289 | **.000*** | -.084 | .367 |
| *Model 2* | | .218 | **.008*** | -.065 | .430 | .307 | **.000*** | -.083 | .367 |
| *Model 3* | | .196 | **.017*** | -.088 | .281 | .325 | **.000*** | -.082 | .384 |

β: standardized regression coefficient
Abbreviation: BIA= Bioimpedance analysis, DXA= Dual-energy X-ray absorptiometry, FFMI= Fat-free mass index, MCSAI= Muscle cross-sectional area index, pQCT= Peripheral quantitative computed tomography and TLSTMI= Total lean soft tissue mass index.
*Model 1* physical fitness (basic model without adjustments)
*Model 2 Model 1* + diet quality index, parents' highest education level and age.
*Model 3 Model 2* + birth weight
*Significance was set at 0.05 level.

**Figure 1. Serial multiple mediation model of the association between total physical activity and total lean soft tissue mass index, using handgrip strength as mediator, controlling for gender.**



***Indirect effect*** **$\beta$ = .0013; SE= .0007 95% CI ( .0000 to .0026)**

$p \leq 0.05$*; $p \leq 0.001$**