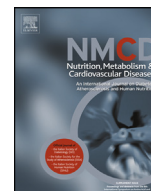


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## Association between serum adiponectin levels and muscular fitness in Portuguese adolescents: LabMed Physical Activity Study



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### KEYWORDS

Adiponectin;  
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**Abstract** *Background and aim:* Paradoxically, recent investigations have showed that adiponectin levels are inversely associated with muscle strength. However, to date, there is a lack of knowledge on the relationship between muscular fitness (MF) and adiponectin levels in adolescents. We aimed to examine the independent associations between MF and adiponectin levels in adolescents, controlling for several potential confounders.

*Methods and results:* This is a cross-sectional analysis with 529 Portuguese adolescents aged 12–18 years. A MF score was computed as the mean of the handgrip strength and standing long jump standardized values by age and gender. We measured fasting glucose, insulin, HDL-cholesterol, C-reactive protein and adiponectin. Linear regression analysis showed a significant inverse association between adiponectin (Z-score by age and sex) and MF score, after adjustments for age, sex, pubertal stage, socioeconomic status, adherence to the Mediterranean diet, body mass index, HOMA-IR, HDL-cholesterol, C-reactive protein and cardiorespiratory fitness (unstandardized  $\beta = -0.176$ ;  $p < 0.005$ ). Analysis of covariance showed a significant difference between the Low MF/Non-overweight group and the High MF/Non-overweight Group ( $p < 0.05$ ) and between the Low MF/Non-overweight and High MF/Overweight Group ( $p < 0.05$ ) ( $F_{(5, 523)} = 2.262$ ,  $p = 0.047$ ).

*Conclusion:* Adiponectin circulating levels are inversely and independently associated with MF. In non-overweight adolescents, those with high levels of MF presented lower levels of adiponectin compared to those with Low MF. Likewise, overweight adolescents with High MF presented lower levels of serum adiponectin than non-overweight adolescents with Low MF.

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### Introduction

Individuals with cardiovascular disease (CVD) usually become symptomatic only in adulthood, but the underlying process of CVD, atherosclerosis, has its onset during childhood and adolescence with an inflammatory process [1]. Several biomarkers of CVD have emerged in the last

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years. Inflammatory biomarkers such as adiponectin, Tumor Necrosis Factor Alfa (TNF $\alpha$ ), C-reactive protein (CRP) and Interleukin 6 (IL-6) have been associated with CVD risk in adolescents [2].

Adiponectin is an abundant protein in blood plasma and it is known as an insulin-sensitizing, anti-inflammatory, and anti-atherogenic adipokine [3], considered a “good adipokine” because unlike all other adipokines, its circulating levels are decreased in obese subjects [4]. Adiponectin concentrations have been negatively associated with insulin resistance [5], CRP [6] and adiposity [7], positively correlated with HDL-cholesterol [6,8] and it is considered an independent CVD risk factor [9]. It was originally assumed that adiponectin was exclusively synthesized and expressed by adipocytes [10]. However, recently it was suggested that adiponectin is also secreted and expressed by cardiomyocytes, bone-forming cells, pituitary cells and skeletal muscle [10].

Likewise, research has begun to demonstrate that high levels of adiponectin may play a paradoxical role in the pathogenesis of CVD. Indeed, investigations have shown that high levels of adiponectin are associated with increased risk of CVD and mortality in older men [11]. Although, this adiponectin paradox remains poorly understood [12].

Physical fitness is an important determinant of current and future health status during childhood and adolescence [13]. In children and adolescents research has focused on the relationship between cardiorespiratory fitness and health outcomes, but there is a growing interest on the relationship between muscular fitness (MF) and its health-related benefits [14]. MF has been inversely associated with CVD, cardiometabolic risk factors [15] and with low-grade chronic inflammation [16]. In addition, the altered muscle metabolism may play a key-role on the genesis and prevention of many common pathologic conditions [17].

Paradoxically, recent investigations have showed that adiponectin levels are inversely associated with muscle strength [11,18,19] in adults and in adolescents [20]. Research on the associations between MF and adiponectin levels has mainly focused on adults and elderly or in small samples of adolescents. Moreover, studies have often overlooked potential confounders in their analysis. For instance, an inverse association between MF and adiponectin levels was found in adolescents, after adjustments for age, gender, pubertal status and waist circumference [20].

To date, there is a lack of knowledge on the relationship between MF and adiponectin levels in adolescents. Furthermore, we are not aware of any study that has evaluated the association between adiponectin and MF independently of cardiorespiratory fitness, and other important potential confounders such as adiposity, insulin resistance (HOMA-IR), HDL-cholesterol, dietary patterns, pubertal status and socioeconomic status, and also if such relationships vary according to adolescents' weight status. Therefore, we aimed to examine the independent associations of MF and serum adiponectin levels in adolescents, controlling our analysis for the above-mentioned covariates.

## Methods

### Study design and sample

The current report is part of the “Longitudinal Analysis of Biomarkers and Environmental Determinants of Physical activity (LabMed Physical Activity Study)”, a school-based prospective cohort study carried out in four Portuguese cities from the North Region (Barcelos, Vila Nova de Gaia, Ílhavo and Braga), which aimed to evaluate the independent and combined associations of dietary intake and fitness levels on cardiometabolic risk factors.

Selection of schools was based on pragmatic, budgetary and logistical reasons. Thus, a total of 5 schools were randomly selected within that which had previous collaboration agreements established with our research centre and fitted the above-mentioned criteria. The study participants' recruitment was conducted at the selected schools. The pupils belonging to the 7th and 10th grades classes were invited to participate in the study ( $n = 1678$ ). The power calculation for that study was based on the exposure of combined healthy diet and physical activity pattern with a prevalence of 14% [21]. A sample of 754 would provide 80% power to detect 15% difference between exposed and unexposed at 5% significance. Taking into account an expected dropout rate of about 20% at each time-point, the sample size was increased to 1086.

Baseline data was collected in the fall of 2011, for 1229 apparently healthy adolescents, i.e., participants without any medical diagnose of physical or mental impairment, aged 12 to 14 years (7th grade) and 15 to 18 years (10th grade). From this sample, 534 agreed to undergo blood sampling. However, five of them were later excluded from the analysis due to (hsCRP) values  $> 10$  mg/L, which may be indicative of acute inflammation or illness, leaving 529 adolescents (267 girls, 262 boys, mean age  $14.3 \pm 1.7$  years) as the final sample for the present study. For this study power analysis was calculated *post hoc* (for  $\alpha = 0.05$ ) and it was higher than 0.8 for Multiple Regression Analysis and ANCOVA.

### Ethical and legal requirements

The study was conducted in accordance with the World Medical Association's Helsinki Declaration for Human Studies [22]. The Portuguese Data Protection Authority (#1112434/2011), the Portuguese Ministry of Science and Education (0246200001/2011) and the Ethics Committee of the Faculty of Sport, University of Porto, approved the study. All participants in this study were informed of the study's goals, and written informed consent was obtained from participating adolescents and their parents or guardians. Considering potential refusals to participate in the study due to blood analysis a “layered consent” was permitted. This allowed the participants to consent some parts of the study protocol and not others. For example, an adolescent could perform physical fitness assessments and refuse to undergo blood sampling. All adolescents, from who parental and individual consents were received, were

enrolled in the study. Throughout the study no exclusion criteria was applied to avoid discriminations. However, for the present analysis only apparently healthy adolescents were considered.

## Measures

### Anthropometrics

Body height was measured to the nearest 0.1 cm in bare or stocking feet with the adolescent standing upright against a portable stadiometer (Seca213, Hamburg, Germany). Body weight was measured to the nearest 0.10 kg, lightly dressed, with no shoes, using a portable electronic weight scale (Tanita Inner Scan BC532, Tokyo, Japan) [23]. Body mass index (BMI) was calculated from the ratio of body weight (kg) to body height (m<sup>2</sup>). Participants were classified as underweight, normal weight, overweight and obese according to Cole's cut-offs [24,25].

Waist Circumference (WC) measurements were taken in a standing position, to the nearest 0.1 cm, with a tape measure midway between the lower rib margin and the anterior superior iliac spine at the end of normal expiration [23].

Body fat percentage (BF%) was measured by bioelectrical impedance with a frequency current of 50 kHz (Tanita Inner Scan BC 532, Tokyo, Japan). Participants were asked to fast overnight for at least 10 h. After the assessors manually introduced the age, sex and height into the scale system, the participants stood on the scale with light clothes and bared foot [26].

Skinfolds thickness (sum of skinfolds); Triceps and subscapular skinfolds were measured with a skinfold calliper, with a constant pressure of 10 g/mm<sup>2</sup> (Harpenden Skinfold Caliper Model HSB-BI, UK) according to standard procedures [23].

### Pubertal stage

Participants self-assessed their pubertal stage of secondary sex characteristics (breast and pubic hair development in girls and genital and pubic hair development in boys ranging from stage I to V), according to the criteria of Tanner and Whitehouse [27].

### Socioeconomic status

Adolescents' socioeconomic status was assessed with the Family Affluence Scale [28], developed specifically to measure children and adolescents socio-economic status in the context of the Health Behavior in School-Aged Children Study.

### Blood sampling

Blood samples were obtained from each subject early in the morning, following a 10-h overnight fast by venipuncture from the antecubital vein. The samples were stored in sterile blood collection tubes in refrigerated conditions (4°–8 °C), and then sent to an analytical laboratory for testing according to standardized procedures, as

follow: (i) hs-CRP, latex enhanced immunoturbidimetric assay (Siemens ADVIA 1800, Erlangen, Germany); (ii) HDL-Cholesterol, Precipitation of the Apolipoprotein B containing lipoproteins with dextran-magnesium-chloride (Siemens Advia 1600/1800 Erlangen, Germany); (iii) Adiponectin, ELISA (Plate Reader); (iv) Glucose, Hexokinase method (Siemens Advia 1600/1800 Erlangen, Germany); (v) Insulin, Chemiluminescence immunoassay (Siemens ACS Centaur System, Erlangen, Germany). The homeostatic model assessment (HOMA) was calculated as the product of basal glucose and insulin levels divided by 22.5, and was used as a proxy measure of insulin resistance [29]. For all these variables standardized values (Z-scores) [(participant's value – mean value of the sample)/SD] by age and sex were constructed.

### KIDMED questionnaire

To assess the degree of adherence to the Mediterranean diet the KIDMED index (Mediterranean Diet Quality Index for children and adolescents) was used [30]. The index is based on a 16-questions self-administered, which sustain the principles of the Mediterranean dietary patterns, as well as, those that undermine it. The final results of index varied between 0 and 12 points. The questions that have one negative connotation in relation to Mediterranean diet were equal to (–1), the questions that constitute positive aspect were equal to (+1). Participants were classified into the follow three levels: (1) ≥8, optimal Mediterranean diet; (2) 4–7, improvement needed to adjust intake to Mediterranean patterns; (3) ≤3, very low diet quality.

### Cardiorespiratory fitness

Cardiorespiratory fitness was assessed with the 20-m Shuttle Run Test (20 m SRT) [31]. This test requires participants to run back and forth between two lines set 20 m apart. Running speed started at 8.5 km/h and increased by 0.5 km/h each minute, reaching 18.0 km/h at minute 20. Each level was announced on a tape player. The participants were instructed to keep up with the pace until exhausted. The test was finished when the participant failed to reach the end lines concurrent with the audio signals on two consecutive occasions. Otherwise, the test ended when the subject stopped because of fatigue. The participants received verbal encouragements from the investigators to achieve maximum performance, to keep running as long as possible. Number of shuttles performed by each participant was recorded.

### Muscular fitness

#### Handgrip strength

The handgrip strength (upper body isometric strength), was assessed using a handgrip dynamometer, (T.K.K. 5001, Grip-A produced by Takei, Japan), adjusted by sex and hand size for each adolescent. The participants were instructed to stand with their arm completely extended squeezing gradually and continuously the handgrip for at

least 2 s, performing the test twice alternating with both hands. A 90 s period rest was given between trials. The best score for each hand was recorded in kilograms [32]. The handgrip score (kg) was calculated as the average of the left and right hands and then expressed per kilogram of body weight [16].

### Standing long jump test

The standing long jump test (lower body explosive strength) was performed in an indoor wood floor gymnasium and the adolescents were instructed to jump the starting line and to push off vigorously and jump as far forward as possible landing on both feet and staying upright. The test was done twice, and the best attempt was recorded. The standing jump score was determined by the distance between the last heel-mark and the take-off line [32].

The results of the handgrip strength/body weight and standing long jump tests were transformed to standardized values (Z-scores) [(participant's value – mean value of the sample)/SD] by age and sex [15]. Then the sum of Z-Scores was performed to create the MF score. Participants were divided in three groups (by tertiles of MF score, by age and sex): Low MF, Medium MF and High MF.

### Statistics analysis

Data analysis was performed with the Statistical Package for the Social Sciences for Windows (Version 21.0 SPSS Inc., Chicago, IL). Descriptive data are presented as means and standard deviation. Independent Two-tailed *t*-Tests for continuous variables and Chi-square for categorical variables, respectively, were used to examine sex differences. Since no significant interaction was observed between sexes (e.g., sex x MF score), all the statistical analysis were performed with both sexes together in order to increase statistical power.

Partial correlations adjusted for sex, age and pubertal stage were performed to examine bivariate correlations between adiponectin and all other variables. Linear regression models were performed to determine the associations between adiponectin (Z-scores by age and sex) and MF score, adjusted for age, sex, pubertal stage, socioeconomic status, adherence to the Mediterranean diet, BMI, HOMA-IR, HDL-cholesterol, C-reactive protein and cardiorespiratory fitness. Unstandardized regression coefficients were used to express the  $\beta$  coefficients of the regression analyses.

Analysis of covariance (ANCOVA) with Bonferroni post-hoc multiple comparison tests were used to assess the differences of adiponectin levels across groups with different levels of MF by weight status. For this analysis, the adolescents were categorized as non-overweight (including underweight and normal weight) and overweight (including overweight and obese) and MF in Low MF, Medium MF and High MF. Covariates included were age, sex, pubertal stage, socioeconomic status, adherence to the Mediterranean diet, HOMA-IR, HDL-cholesterol, C-

reactive protein and cardiorespiratory fitness. A *P* value less than 0.05 was regarded as significant.

### Results

Descriptive characteristics of the participants are presented in Table 1. Boys were heavier and taller than girls and showed higher levels of MF and cardiorespiratory fitness ( $p < 0.001$  for all). Girls presented higher levels of adiponectin and HDL-cholesterol ( $p < 0.001$ ).

Table 2 shows partial correlations between adiponectin and all other variables. BMI was the measure of adiposity

**Table 1** Participants' characteristics.

Characteristics	Mean (SD)		
	All (n = 529)	Girls (n = 267)	Boys (n = 262)
Age (year)	14.33 (±1.73)	14.27 (±1.71)	14.39 (±1.74)
Weight (kg)	55.15 (±12.81)	53.44 (±11.18)	56.89 (±14.10) <sup>a</sup>
Height (cm)	160.27 (±9.59)	157.7 (±6.68)	162.9 (±11.3) <sup>a</sup>
BMI (kg/m <sup>2</sup> )	21.31 (±3.84)	21.41 (±3.96)	21.20 (±3.73)
Pubertal stage-A %			
I	0.4	0	0.8
II	7.4	3	11.8
III	32.3	29.2	35.5
IV	46.7	54.3	38.9
V	13.2	13.5	13
Pubertal stage-B %			
I	0.2	0	0.4
II	6.8	2.2	11.5
III	21.7	24.0	19.5
IV	49.5	46.8	52.3
V	21.7	27	16.4
Adiponectin (mg/L)	11.61 (±5.45)	12.90 (±5.75)	10.29 (±4.80) <sup>a</sup>
CRP (mg/L)	0.95 (±1.88)	0.77 (±2.17)	1.13 (±2.12) <sup>a</sup>
Insulin resistance (HOMA-IR)	3.45 (±5.38)	3.51 (±1.83)	3.39 (±7.41)
HDL-C (mg/dL)	54.39 (±11.95)	57.31 (±12.08)	51.31 (±10.99) <sup>a</sup>
Handgrip strength (kg)	27.21 (±8.41)	23.35 (±4.70)	31.14 (±9.49) <sup>a</sup>
Handgrip strength/body weight	0.50 (±0.11)	0.45 (±0.08)	0.55 (±0.12) <sup>a</sup>
Standing long jump (cm)	160.43 (±32)	144.1 (±23.4)	177.1 (±31) <sup>a</sup>
20 m SRT (Nr. laps)	44.90 (±1.12)	31.87 (±15.25)	58.15 (±26.63) <sup>a</sup>
KIDMED Index	7.11 (±2.05)	7.19 (±1.98)	7.02 (±2.13)
Socioeconomic Status (Family Affluence Scale)	6.40 (±1.70)	6.43 (±1.73)	6.36 (±1.68)

<sup>a</sup> Significantly different from girls,  $p < 0.001$  (Independent Two-tailed *t*-Tests for continuous variables or Chi-square for categorical variables); BMI, body mass index; CRP, C-Reactive Protein; KIDMED, adherence to the Mediterranean index; HDL-C, high density lipoprotein cholesterol. SRT: shuttle run test; Pubertal stage-A – breast development in girls; genital development in boys. Pubertal stage-B – pubic hair development.

that showed the highest correlation with adiponectin. Adiponectin was negatively correlated with handgrip/body weight, standing long jump and MF score ( $p < 0.05$  for all).

Regression analyses (Table 3), showed a significant inverse association between adiponectin and MF score (unstandardized  $\beta = -0.176$ ;  $p < 0.005$ ), after adjustments for age, sex, pubertal stage, socioeconomic status, adherence to the Mediterranean diet, body mass index, HOMA-IR, HDL-cholesterol, C-reactive protein and cardiorespiratory fitness.

ANCOVA (Fig. 1) adjusted for age, sex, pubertal stage, socioeconomic status, adherence to the Mediterranean diet, HOMA-IR, HDL-cholesterol, C-reactive protein and cardiorespiratory fitness showed a significant difference between the Low MF/Non-overweight group and the High MF/Non-overweight group ( $p < 0.05$ ) and between the Low MF/Non-overweight and High MF/Overweight group ( $p < 0.05$ ) ( $F_{(5, 523)} = 2.262$ ,  $p = 0.047$ ).

**Discussion**

Our results showed that adiponectin levels are negatively associated with MF even after considering potential confounders such as age, sex, pubertal stage, and socioeconomic status, adherence to the Mediterranean diet, body mass index, HOMA-IR, HDL-cholesterol, C-reactive protein and cardiorespiratory fitness. We also found significant differences of adiponectin levels according to the different levels in MF in non-overweight adolescents group. Our results suggest that MF status may play a role on the levels of serum adiponectin in adolescents independently of several confounders.

The current physical activity guidelines for children and adolescents recommend regular engagement in muscle-strengthening activities due to its health-related benefits, including prevention in CVD and metabolic risk factors [33]. Adiponectin has been proposed to be a cytokine with protective properties, but surrounded by controversy [34,35]. Recently, it has emerged an interestingly facet showing that adiponectin is also a myokine, being produced and released by skeletal muscle [10]. However, the effect or the role of adiponectin on muscle skeletal strength is still unclear, and little is known regarding the

**Table 3** Unstandardized regression coefficients examining the association of adiponectin levels and muscular fitness.

Muscular fitness (score)	Adiponectin (Z-score by age and sex)		
	$\beta$	(95%CI)	P-value R <sup>2</sup>
Model 1	-0.182	(-0.291;-0.073)	<0.001 0.019
Model 2	-0.186	(-0.293;-0.078)	<0.001 0.040
Model 3	-0.201	(-0.309;-0.093)	0.005 0.046
Model 4	-0.201	(-0.307;-0.096)	0.004 0.094
Model 5	-0.208	(-0.313;-0.103)	<0.001 0.103
Model 6	-0.176	(-0.289;-0.063)	0.004 0.108

$\beta$ : Unstandardized coefficients.  
 Model 1 – Unadjusted model.  
 Model 2 – Adjusted for age, gender, pubertal stage, socioeconomic status, KIDMED index and body mass index.  
 Model 3 – Model 2 plus adjustment for HOMA-IR.  
 Model 4 – Model 3 plus adjustment for HDL-Cholesterol.  
 Model 5 – Model 4 plus adjustment for C - reactive protein.  
 Model 6 – Model 5 plus adjustment for cardiorespiratory fitness (fully adjusted model).

pathways of this relationship [10]. Therefore, the reason of the inverse association between levels of serum adiponectin and MF cannot be explained according to the results found in the present report. However, some hypotheses can be raised concerning the possible mechanisms involved in this paradoxical relationship. First, adiponectin directly stimulates autophagic flux, however, the excessive activation of muscle autophagy, may have adverse consequences which could play a key role on muscle fibers and thus, affecting muscle function [36]. Second, adiponectin seems to have an effect on the type and size of muscle cells as demonstrated by Krause and colleagues in a mice model [37] and confirmed by an epidemiological study in an elderly population which reported that adiponectin levels are associated with low proportion of muscle fiber type IIB [38]. Third, it is possible that the type of adiponectin receptor or its isoforms, which are not yet fully understood [34], may play a key role in skeletal muscle function.

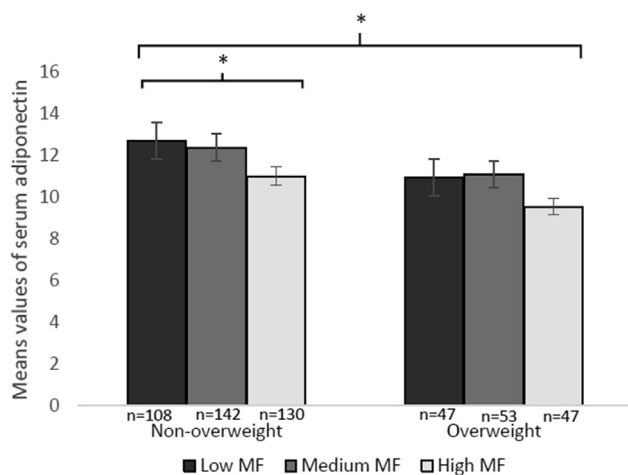
Our results are in agreement with a recent small cross-sectional study with 198 Spanish adolescents (AFINOS study) [20], in which the results showed an inverse association between adiponectin and MF after controlling for age, sex, pubertal stage and waist circumference. Furthermore, our results extend this evidence by including several

**Table 2** Partial correlations (r) between serum adiponectin, muscular fitness, cardiorespiratory fitness, KIDMED index and metabolic risk factors.

	Adiponectin <sup>b</sup>	BMI <sup>a</sup>	BF% <sup>a</sup>	WC <sup>a</sup>	Sum of skinfolds <sup>a</sup>	C-reactive protein <sup>a</sup>	HOMA <sup>a</sup>	HDL-c <sup>a</sup>	KIDMED <sup>a</sup>	20m SRT <sup>a</sup>
Adiponectin	—	-0.173**	-0.129*	-0.158**	-0.119*	-0.170**	-0.137*	0.263**	0.013	-0.014
Handgrip/Body weight	-0.115*	-0.534**	-0.549**	-0.504**	-0.551**	-0.170**	-0.298**	0.135**	-0.021	0.386**
Standing long jump	-0.145**	-0.314**	-0.385**	-0.363**	-0.419**	-0.077	-0.251**	0.097*	0.055	0.490**
Muscular Fitness (score)	-0.154**	-0.495**	-0.543**	-0.508**	-0.566**	-0.144**	-0.317**	0.136**	0.020	0.507**

Partial correlation <sup>a</sup>(r, adjusted for age, sex, pubertal stage); <sup>b</sup>(r, adjusted for age, sex, pubertal stage and BMI). BMI: body mass index; BF: body fat; WC: waist circumference; HOMA-IR: homeostasis model assessment of insulin resistance; SRT: shuttle run test (number of laps); KIDMED Index: adherence to the Mediterranean index.

For all variables standardized values (Z-scores) [(participant's value – mean value of the sample)/SD] by age and sex were constructed. \* $p < 0.03$ ; \*\* $p < 0.001$ .



**Figure 1** Means value of serum adiponectin stratified in tertiles of muscular fitness (low, medium and high) in non-overweight and overweight. \*Significantly different from the low MF/Non-overweight group ( $p < 0.05$ ).

other potential confounders, such as insulin resistance and CRP; given that, it was reported that adiponectin has an inverse relationship with markers of inflammation such as CRP [6] and insulin resistance [7] in adolescents and that CRP and insulin resistance were also negatively associated with MF in previous studies [16]. Moreover, it is known that insulin sensitivity is influenced by dietary factors and adiponectin is recognized as having insulin sensitizing properties [7,5]. Previous studies reported that daily intake of fish or omega-3 supplementation increased adiponectin levels as well as a low calorie-diet [39]. An adherence to a Mediterranean dietary pattern has also shown to be associated with adiponectin concentrations in diabetic women [40]. Importantly, in our study the association of adiponectin with MF remained significant even after the models were adjusted for CRP, HOMA-IR and adherence to a Mediterranean diet. Furthermore, our findings are consistent with the results from the most recent study in Japanese adults reporting an inverse association between circulating adiponectin levels and skeletal muscle strength after adjustment for age, physical activity, nutrition intake, CRP, BMI, metabolic syndrome and other lifestyle related potential confounders [18].

The HDL-cholesterol and BMI have been proposed in several studies to be components of metabolic syndrome definition. Recently, investigations demonstrated a positive correlation between HDL-cholesterol and adiponectin levels [6,8]. However, none of these two studies have included a fitness variable as a potential cofounder. In our report, the associations between adiponectin and MF remained even after HDL-cholesterol and BMI were included as covariates in the regression models. We have included BMI as covariate in our models due to its higher correlation with adiponectin levels than other adiposity variables such as waist circumference, body fat percentage estimated by bioelectrical impedance and skinfolds thickness (as shown in Table 2). In this line of thought, a recent review observed that currently, most of the knowledge on

the roles of adiponectin is driven from studies with obese subjects or in subjects with metabolic syndrome, a pathological host milieu; and, to clarify the context-dependent and homeostasis roles of adiponectin studies in both pathological and normal physiological states are needed [34]. Once, it seems clear that circulating adiponectin levels are reduced in obese subjects, several studies, as well as our results have demonstrated this. But it is also known that adiponectin is also segregated by the muscle cells. And, our analysis of covariance showed significant differences between Low MF and High MF in non-overweight participants, which reinforce the idea that high adiponectin levels and MF are associated, independently of the obesity status. Nonetheless, based on the present data available literature, it is difficult to explore the potential clinical relevancy of the observed differences between groups in apparently healthy adolescents. However, high levels of adiponectin and low BMI have significantly been associated with increased all-cause and cardiovascular mortality in an elderly cohort population [11].

The MF tests used in our study were based on previous studies which have shown good criterion-related validity [15,13]. It is difficult to distinguish the separate influences of adiposity and MF on adiponectin levels in a cross-sectional observational study. Access the body adiposity and lean mass profiles by more accurate tests could provide a better view regarding the interplay between MF and adiponectin levels.

In addition to MF, another important component of physical fitness is cardiorespiratory fitness, which has been referenced as powerful marker of health outcomes [13]. Some studies have showed a negative relationship between cardiorespiratory fitness and circulating adiponectin levels [20] even after adjusting for fatness [2]. In our study, we have included cardiorespiratory fitness, as a potential confounder, which attenuated the results; however, they remained significant. We are not aware of any study that has analyzed the relationship between MF and levels of serum adiponectin independent of cardiorespiratory fitness in adolescents.

Several limitations of our study should be taken into consideration. First, our cross-sectional design does not allow us to establish causality. Second, the measured serum adiponectin levels result from its release from several adipose and non-adipose tissues [34], so it should be questioned to what extent skeletal muscle may contribute to these levels, and thus, to provide a wider insight on the relationship between MF and serum adiponectin levels in adolescents. Nonetheless, it has been shown that high circulating adiponectin concentration may be an indicator of decreased physical performance, especially muscle strength, in older adults [41].

In conclusion, this cross-sectional study in Portuguese adolescents demonstrated that adiponectin circulating levels are inversely and independently associated with MF. In non-overweight adolescents, those with high levels of MF presented lower levels of serum adiponectin compared to those with low MF. Likewise, overweight adolescents

with High MF presented lower levels of serum adiponectin than non-overweight adolescents with Low MF.

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## References

- [1] McGill Jr HC, McMahan CA, Herderick EE, Malcom GT, Tracy RE, Jack P. Origin of atherosclerosis in childhood and adolescence. *Am J Clin Nutr* 2000;72:1307S–15S.
- [2] Bugge A, El-Naaman B, McMurray RG, Froberg K, Nielsen CH, Müller K, et al. Inflammatory markers and clustered cardiovascular disease risk factors in danish adolescents. *Horm Res Paediatr* 2012;78:288–96.
- [3] Parker-Duffen JL, Walsh K. Cardiometabolic effects of adiponectin. In: *Best practice and research: clinical endocrinology and metabolism*, 28; 2014. p. 81–91.
- [4] Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol* 2011;11:85–97.
- [5] Fisman EZ, Tenenbaum A. Adiponectin: a manifold therapeutic target for metabolic syndrome, diabetes, and coronary disease? *Cardiovasc Diabetol* 2014;13:103.
- [6] Stakos DA, Papaioannou HI, Angelidou I, Mantadakis E, Paraskakis E, Tsigalou C, et al. Plasma leptin and adiponectin concentrations correlate with cardiometabolic risk and systemic inflammation in healthy, non-obese children. *J Pediatr Endocrinol Metab* 2014;27:221–8.
- [7] Herder C, Schneitler S, Rathmann W, Haastert B, Schneitler H, Winkler H, et al. Low-grade inflammation, obesity, and insulin resistance in adolescents. *J Clin Endocrinol Metab* 2007;92:4569–74.
- [8] Riestra P, García-Anguaita A, Lasunción MA, Cano B, de Oya M, Garcés C. Relationship of adiponectin with metabolic syndrome components in pubertal children. *Atherosclerosis* 2011;216:467–70.
- [9] Ai M, Otokozaawa S, Asztalos BF, White CC, Cupples LA, Nakajima K, et al. Adiponectin: an independent risk factor for coronary heart disease in men in the Framingham offspring Study. *Atherosclerosis* 2011;217:543–8.
- [10] Liu Y, Sweeney G. Adiponectin action in skeletal muscle. In: *Best practice and research: clinical endocrinology and metabolism*, 28; 2014. p. 33–41.
- [11] Choi SH, Ku EJ, Hong ES, Lim S, Kim KW, Moon JH, et al. High serum adiponectin concentration and low body mass index are significantly associated with increased all-cause and cardiovascular mortality in an elderly cohort, 'adiponectin paradox': the Korean Longitudinal Study on Health and Aging (KLoSHA). *Int J Cardiol* 2015;183:91–7.
- [12] Kizer JR. Adiponectin, cardiovascular disease, and mortality: parsing the dual prognostic implications of a complex adipokine. *Metabolism* 2014;63:1079–83.
- [13] Ruiz JR, Castro-Piñero J, Artero EG, Ortega FB, Sjöström M, Suni J, et al. Predictive validity of health-related fitness in youth: a systematic review. *Br J Sports Med* 2009;43:909–23.
- [14] Smith JJ, Eather N, Morgan PJ, Plotnikoff RC, Faigenbaum AD, Lubans DR. The health benefits of muscular fitness for children and adolescents: a systematic review and meta-analysis. *Sport Med* 2014;44:1209–23.
- [15] Artero EG, Ruiz JR, Ortega FB, España-Romero V, Vicente-Rodríguez G, Molnar D, et al. Muscular and cardiorespiratory fitness are independently associated with metabolic risk in adolescents: the HELENA study. *Pediatr Diabetes* 2011;12:704–12.
- [16] Artero EG, España-Romero V, Jiménez-Pavón D, Martínez-Gómez D, Warnberg J, Gómez-Martínez S, et al. Muscular fitness, fatness and inflammatory biomarkers in adolescents. *Pediatr Obes* 2013;9:391–400.
- [17] Wolfe RR. The underappreciated role of muscle in health and disease. *Am J Clin Nutr* 2006;84:475–82.
- [18] Huang C, Niu K, Momma H, Kobayashi Y, Guan L, Nagatomi R. Inverse association between circulating adiponectin levels and skeletal muscle strength in Japanese men and women. *Nutr Metab Cardiovasc Dis* 2013;24:42–9.
- [19] Bucci L, Yani SL, Fabbri C, Bijlsma AY, Maier AB, Meskers CG, et al. Circulating levels of adipokines and IGF-1 are associated with skeletal muscle strength of young and old healthy subjects. *Biogerontology* 2013;14:261–72.
- [20] Martínez-Gómez D, Eisenmann JC, Gómez-Martínez S, Veses A, Romeo J, Veiga OL, et al. Associations of physical activity and fitness with adipocytokines in adolescents: the AFINOS study. *Nutr Metab Cardiovasc Dis* 2012;22:252–9.
- [21] Huh J, Riggs NR, Spruijt-Metz D, Chou C-P, Huang Z, Pentz M. Identifying patterns of eating and physical activity in children: a latent class analysis of obesity risk. *Obes Silver Spring* 2011;19:652–8.
- [22] World, M. A. G. A. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *J Int bioéthique = Int J Bioeth* 2004;15:124.
- [23] Lohman TG, Roche AF, Martorell R. *Anthropometric standardization reference manual*. Champaign, IL: Human Kinetics Book; 1991.
- [24] Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* 2000;320:1240–3.
- [25] Cole TJ, Flegal KM, Jackson AA. Body mass index cut offs to define thinness in children and adolescents: international survey. *Bmj* 2007;335(7612):194.
- [26] Talma H, Chinapaw MJM, Bakker B, HiraSing RA, Terwee CB, Altenburg TM. Bioelectrical impedance analysis to estimate body composition in children and adolescents: a systematic review and evidence appraisal of validity, responsiveness, reliability and measurement error. *Obes Rev* 2013;14:895–905.
- [27] Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* 1976;51:170–9.
- [28] Currie C, Molcho M, Boyce W, Holstein B, Torsheim T, Richter M. Researching health inequalities in adolescents: the development of the Health Behaviour in School-Aged Children (HBSC) family affluence scale. *Soc Sci Med* 2008;66:1429–36.
- [29] Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetol* 1985;28:412–9.
- [30] Serra-Majem L, Ribas L, Ngo J, Ortega RM, García A, Pérez-Rodrigo C, et al. Food, youth and the Mediterranean diet in Spain. Development of KIDMED, mediterranean diet quality index in children and adolescents. *Public Health Nutr* 2004;7:931–5.
- [31] Leger LA, Mercier D, Gadoury C, Lambert J. The multistage 20 metre shuttle run test for aerobic fitness. *J Sport Sci* 1988;6:93–101.
- [32] Ruiz JR, Castro-Piñero J, España-Romero V, Artero EG, Ortega FB, Cuenca MM, et al. Field-based fitness assessment in young people: the ALPHA health-related fitness test battery for children and adolescents. *Br J Sports Med* 2010;45:518–24.
- [33] World Health Organization. "Global recommendations on physical activity for health"; 2010. p. 8–10.

- [34] Esmaili S, Xu A, George J. The multifaceted and controversial immunometabolic actions of adiponectin. *Trends Endocrinol Metab* 2014;25:444–51.
- [35] Ghoshal K. Adiponectin: probe of the molecular paradigm associating diabetes and obesity. *World J Diabetes* 2015;6:151–66.
- [36] Xu A, Sweeney G. Emerging role of autophagy in mediating widespread actions of ADIPOQ/adiponectin. *Autophagy* 2015;11:723–4.
- [37] Krause MP, Liu Y, Vu V, Chan L, Xu A, Riddell MC, et al. Adiponectin is expressed by skeletal muscle fibers and influences muscle phenotype and function. *Am J Physiol Cell Physiol* 2008;295:C203–12.
- [38] Ingelsson E, Ärnlöv J, Zethelius B, Vasan RS, Flyvbjerg A, Frydryk J, et al. Associations of serum adiponectin with skeletal muscle morphology and insulin sensitivity. *J Clin Endocrinol Metab* 2009;94:953–7.
- [39] Silva FM, De Almeida JC, Feoli AM. Effect of diet on adiponectin levels in blood. *Nutr Rev* 2011;69:599–612.
- [40] Mantzoros CS, Williams CJ, Manson JE, Meigs JB, Hu FB. Adherence to the Mediterranean dietary pattern is positively associated with plasma adiponectin concentrations in diabetic women. *Am J Clin Nutr* 2006;84:328–35.
- [41] Huang C, Tomata Y, Kakizaki M, Sugawara Y, Hozawa A, Momma H, et al. High circulating adiponectin levels predict decreased muscle strength among older adults aged 70 years and over: a prospective cohort study. *Nutr Metab Cardiovasc Dis* 2015;25:594–601.