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Abstract

This study aimed to evaluate the associations between muscular fitness and inflammatory biomarkers and to investigate the relationship between muscular fitness and selected clustered inflammatory biomarkers in adolescents. This is a cross-sectional analysis with 529 adolescents (267 girls) aged 12-18 years. Handgrip strength and standing long jump tests assessed MF. Continuous scores of clustered inflammatory biomarkers (sum of Z-scores of C-reactive protein [CRP], C3, C4, fibrinogen, and leptin); metabolic risk factor (MRF) score (sum of Z-scores of SBP, triglycerides, ratio total cholesterol [TC]/HDL, HOMA-IR, and waist circumference [WC]) were computed. Regression analyses showed an inverse association between muscular fitness score ($\beta=-.204$; P

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Muscular Fitness, metabolic and inflammatory biomarkers in adolescents: results from LabMed Physical Activity Study

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ABSTRACT

This study aimed to evaluate the associations between muscular fitness and inflammatory biomarkers, and to investigate the relationship between muscular fitness and selected clustered inflammatory biomarkers in adolescents. This is a cross-sectional analysis with 529 adolescents (267 girls) aged 12-18 years. Handgrip strength and standing long jump tests assessed MF. Continuous scores of clustered inflammatory biomarkers (sum of Z-scores of c-reactive protein, C3, C4, fibrinogen and leptin); Metabolic Risk Factor (MRF) score (sum of Z-scores of SBP, triglycerides, ratio total cholesterol/HDL, HOMA-IR and waist circumference) were computed. Regression analyses, showed an inverse association between muscular fitness score ($\beta=-0.204$; $p<0.021$) and clustered score of inflammatory biomarkers, adjusted for age, sex, pubertal stage, socioeconomic status, adherence to the Mediterranean diet, cardiorespiratory fitness (CRF), MRF score and body fat. Analysis of covariance showed that adolescents with an adverse inflammatory profile with low levels of muscular fitness, exhibit the poorest MRF score ($F_{(3,525)}=6,461$ $p<0,001$), adjusted for age, sex, pubertal stage, socioeconomic status, adherence to the Mediterranean diet, CRF and body fatness. The inflammatory state seems to explain a significant part of the highest MRF score and in adolescents with high inflammatory status and low muscular strength.

KEYWORDS: Inflammation, Cardiometabolic Risk, Strength, Youth.

Introduction

Cardiovascular diseases lead mortality rates in developed countries (Gersh et al. 2010). Individuals with cardiovascular disease usually become symptomatic only in adulthood, but the underlying process of cardiovascular diseases, atherosclerosis, often has its onset in childhood and adolescence with an inflammatory process (McGill H.C. et al. 2000). Evidence indicates that inflammatory biomarkers might be one mechanism through which unhealthy lifestyles are linked to metabolic and cardiovascular disease (Balagopal et al. 2011).

The low-grade inflammation process is controlled by a complex system involving several inflammatory biomarkers produced by different and diverse cell types in the immune system and adipocytes. Complement factors C3 and C4 are acute phase proteins and important components of the complement pathways of the immune system with pro-inflammatory effects that associate with increased levels of cardiovascular risk factors (Engström, Hedblad, Janzon et al., 2007). Fibrinogen, a main coagulation protein (synthesized by the liver) and precursor of fibrin, is an essential component of the blood coagulation system and is known to play a vital role in a number of pathophysiological processes within the body including inflammation, atherogenesis and thrombogenesis (Thomas and Williams 2008; Balagopal et al. 2011). C-reactive protein is an acute-phase protein also synthesized in the liver, its altered levels induce endothelial dysfunction, accelerate progression of atherosclerosis and increase the risk of cardiovascular disease (Hansson 2005; Thomas and Williams 2008; Balagopal et al. 2011). Adipokines, such as leptin, an hormone produced mainly by adipose tissue plays a crucial role on control of body weight in healthy subjects with regulation of food intake and homeostasis (Sáinz et al. 2015). This adipokine directly regulates the

production of several cytokines, displays both pro and anti-inflammatory properties and both a deficiency of leptin and an excess of leptin results in the dysfunction of multiple organs (Waelpu et al. 2006). However, high levels of leptin are associated with pro-inflammatory, impaired vascular function, insulin resistance, obesity (Hansson 2005) and recently have been suggested to be included in the metabolic syndrome definition (Andersen et al. 2015). All these biomarkers are important in the regulation of biological function and have been associated with chronic low-grade inflammation (Balagopal et al. 2011).

The inflammatory status is influenced by several factors. Physical fitness during childhood and adolescence is also an important determinant of current and future health status (Ortega, Ruiz, Castillo et al., 2008; Ruiz et al., 2009). Previous research has focused on the relationships between cardiorespiratory fitness and health outcomes, namely on cardiometabolic risk factors. Presently, there is a growing interest on the health benefits of muscular fitness (Smith et al. 2014). Studies on muscular fitness have demonstrated an inverse association with cardiovascular disease and cardiometabolic risk factors (Artero et al., 2011; Mota et al., 2010; Smith et al., 2014). In addition, recently it has been shown that muscular strength is inversely and independently associated with all-cause mortality, even after adjusting for several potential confounders (Ortega, Silventoinen, Tynelius et al., 2012; Volaklis, Halle, & Meisinger, 2015)

In youth, the relationships between muscular fitness and inflammatory biomarkers have been less studied (Smith et al. 2014). Some studies have shown an inverse association between muscular fitness and some inflammatory biomarkers (Artero et al., 2013; Martinez-Gomez et al., 2012; Ruiz et al., 2008; Steene-Johannessen, Kolloe, Andersen et al., 2013). However, these associations were not

always independent of other potential confounders such as pubertal status, socioeconomic status and dietary intake. In addition, they did not take into account the combined association of muscular fitness and low-grade inflammation on the adolescents' overall metabolic risk profile. It is further known that skeletal muscle expresses and releases several ~~cytokines and~~ myokines into the circulation in response to muscle contractions, acting as an endocrine organ (Peake et al. 2015). Low-grade inflammation leads to several metabolic disorders, given that skeletal muscle is the largest organ in the human body, it seems important to try understand the interplay between muscular fitness, low-grade inflammation and metabolic risk.

Therefore, the present study aimed (i) to evaluate the associations between, muscular fitness and inflammatory biomarkers adjusted for potential confounders such as age, sex, pubertal status, socioeconomic status, dietary patterns, cardiorespiratory fitness, body fat and a clustered metabolic risk factor score in adolescents; and (ii) to investigate the combined effect of muscular fitness and inflammation on a metabolic risk factor score. We hypothesized that muscular fit adolescents will present an improved metabolic and inflammatory profile across potential confounders.

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. Detailed description of sampling and recruitment approaches, data collection, analysis strategies have been described elsewhere (Agostinis-Sobrinho et al. 2016). In short, baseline data was collected in the fall of 2011, for 1,229 apparently healthy adolescents, i.e., participants without any medical diagnose of physical or mental impairment, aged 12 to-18 years. Of the 1229 adolescents that agreed to participate in the LabMed study, 534 accepted to undergo blood collection. five individuals were excluded due to hs-CRP values >10 mg/L, which may be indicative of acute inflammation or illness. Thus, leaving 529 adolescents (267 girls, 262 boys, mean age 14.3 ± 1.7 years) as the final sample for the present report. Power analysis was calculated *post hoc* and it was higher than 0.8 for multiple regression analysis and ANCOVA.

The LabMed Physical Activity Study was conducted in accordance with the Helsinki Declaration for Human Studies and approved by the Portuguese Data Protection Authority (#1112434/2011) and the Portuguese Ministry of Science and Education (0246200001/2011). All participants were informed of the study’s goals, and written informed consent was obtained from participating adolescents and their parents or legal guardians.

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 (Seca 213, Hamburg, Germany). Body weight was measured to the nearest 0.10 kg with the participant lightly dressed using a portable electronic weight scale (Tanita Inner Scan BC 532, Tokyo, Japan) (Lohman et al. 1991). Body mass index (BMI) was calculated from the ratio of body weight (kg) to body height (m²).

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 (Lohman et al. 1991).

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 hours. 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 (Talma et al. 2013)

Blood Pressure

Blood pressure was measured using a Dynamap vital signs monitors (model BP 8800, Critikon, Inc., Tampa, Florida). Trained nurses took measurements, and all adolescents were required to sit and rest for at least 5 min prior to the first blood pressure measurement. Participants were in a seated, relaxed position with their feet resting flat on the ground. Two measurements in the non-dominant arm were taken,

after five and 10 min of rest. The mean of these two measurements was considered. If the two measurements differed by two mmHg or more, a third measure was taken (McCrinkle 2010).

Pubertal stage

Participants self-assessed their pubertal stage of secondary sex characteristics (breast and pubic hair development for girls, genital and pubic hair development for boys; ranging from stage I to V), according to the criteria of Tanner and Whitehouse (Tanner and Whitehouse 1976).

Socioeconomic Status

The socioeconomic status was assessed with the Family Affluence Scale (Currie et al. 2008), developed specifically to measure children and adolescents socio-economic status in the context of the Health Behaviour in School-Aged Children Study.

Blood Sampling

Blood samples were obtained from each subject early in the morning, following a 10-hour overnight fast by venipuncture from the antecubital vein. The samples were stored in sterile blood collection tubes in refrigerated conditions (4° to 8°C) for no longer than 4 hours during the morning of collection and then sent to an analytical laboratory for testing according to standardized procedures, as follow: (i) hs-C-Reactive Protein, latex enhanced immunoturbidimetric assay (Siemens Advia 1600/1800

Erlangen, Germany); (ii) HDL-Cholesterol, Precipitation of the Apolipoprotein B containing lipoproteins with dextran-magnesium-chloride (Siemens Advia 1600/1800 Erlangen, Germany); (iii) Glucose, Hexokinase method (Siemens Advia 1600/1800 Erlangen, Germany); (iv) Insulin, Chemiluminescence immunoassay (Siemens ACS Centaur System, Erlangen, Germany); (v) IL-6 and Interferon- α , Chemiluminescence immunoassay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA); (vi) Fibrinogen, Clauss method (Siemens BCS System, Erlangen, Germany); (vii) Complement factors C3 and C4, Immunoturbidimetric assay (Siemens Advia 1600/1800, Erlangen, German); (viii) Albumin, Colorimetric method - Bromcresol green assay (BCG) (Siemens Advia 2120i, Erlangen, Germany); (ix) Total cholesterol (TC) CHOD-POD enzymatic method (Siemens Advia 1600/1800); (x) Triglycerides, enzyme glycerol phosphate oxidase method (GPO) (Siemens Advia 1600/1800 Erlangen, Germany). Leptin CRP, C3, C4, IL-6 and Interferon- α were determined in serum and fibrinogen was determined in plasma.

Adherence to the Mediterranean Diet

To assess the degree of adherence to the Mediterranean diet, the KIDMED index (Mediterranean Diet Quality Index for children and adolescents) was used (Serra-Majem et al. 2004). 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 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). A continuum variable was computed to perform the statistical analyses.

Cardiorespiratory Fitness

Cardiorespiratory fitness (CRF) was assessed with the 20-metre Shuttle Run Test (20 m SRT) (Leger et al. 1988). 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

Upper body isometric strength (handgrip strength test) was assessed using a handgrip dynamometer, (T.K.K. 5001, Grip-A, Takei, Japan), adjusted by sex and hand size for each adolescent. The participants were instructed to stand with their arms completely extended, squeezing gradually and continuously the handgrip up to the maximum of their strength, for at least 2 seconds, performing the test twice alternating with both hands. A 90 sec period rest was given between trials. The best score for each hand was recorded in kilograms (Ruiz et al. 2011). The handgrip score (kg) was calculated as the average of the left and right and then expressed per kilogram of body weight (Artero et al. 2013; Steene-Johannessen et al. 2013).

Standing long jump test

Lower body explosive strength (standing long jump test) was performed in an indoor wood floor gymnasium and the adolescents were instructed to jump from 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 (Ruiz et al. 2011).

Data management

The homeostatic model assessment (HOMA-IR), calculated as the product of basal glucose (mmol/L) and insulin (μ IU/mL) levels divided by 22.5, was used as a proxy measure of insulin resistance (Matthews et al. 1985). A continuous score representing a composite Metabolic Risk Factor (MRF) profile was derived by summing the standardized values [Z -score = (participant's value - mean value of the sample) / standard deviation)] by age and sex, of triglycerides, systolic blood pressure, ratio total cholesterol/HDL-cholesterol, HOMA-IR and waist circumference as already proposed for adolescents (Bugge et al. 2012; Andersen et al. 2015).

Based on our preliminary correlation analysis (described in the statistical section), we computed a continuous score of clustered inflammatory biomarkers by summing the Z -scores by age and sex from the inflammatory biomarkers that were significantly correlated with the MRF score and muscular fitness (C-Reactive Protein, C3, C4, fibrinogen and leptin). High risk group (the first tertile) and Low risk group (second and third tertiles).

The results of the handgrip strength and standing long jump tests were transformed into standardized values (Z-scores) by age and sex. Then the sum of the Z-Scores of the two tests was performed to create the muscular fitness score. Participants were divided into two groups: Low Fitness group (1st tertile) and High Fitness group (2nd and 3rd tertiles). Then, according of the clustered inflammatory biomarkers score groups (High Risk and Low Risk) and muscular fitness group (Low and High), four exclusive groups were created.

Statistical Analysis

Descriptive data are presented as means and standard deviations. All variables were checked for normality. CRP, Fibrinogen, C3, C4, IL6, Leptin, Interferon- α and Albumin values were transformed using the natural logarithm. Independent Two-tailed *t*-Tests for continuous variables and Chi-square for categorical variables were used to examine sex differences.

Partial correlation, adjusted for age, sex and pubertal status was used as preliminary analysis to examine the associations between each inflammatory biomarker with fitness variables and MRF score (see Table, S 1, Pearson partial correlations between, Muscular Fitness, MRF score and Inflammatory Biomarkers).

Linear regression models were performed to determine the associations between the clustered inflammatory biomarkers score (as the dependent variable) and handgrip/weight or standing long jump or muscular fitness score (as predictor variables). We performed four different models; Model 1: Unadjusted model; Model 2: Model 1 additionally adjusted for age, sex, pubertal stage, adherence the Mediterranean diet and socio-economic status; Model 3: Model 2 additionally adjusted for MRF score

and CRF; Model 4: Model 3 additionally adjusted for Body fat. Unstandardized regression coefficients were used to express the β in the linear regression analyses.

Analysis of covariance (ANCOVA) with Bonferroni post-hoc multiple comparison tests were used to assess the differences between mean values of MRF score across groups of clustered inflammatory biomarkers score (High vs Low) stratified according with different levels of muscular fitness (Low and High). Covariates included were age, sex, pubertal stage, adherence to a Mediterranean dietary pattern (KIDMED index), socioeconomic status, CRF and body fatness.

Data analysis was performed using the Statistical Package for the Social Sciences for Windows (Version 21.0 SPSS Inc., Chicago, IL). A p value < 0.05 denoted statistical significance. Power analysis was calculated with G*Power (version 3.1.9.2, Dusseldorf, Germany).

Results

Descriptive characteristics of the participants are presented in **Table 1**. Boys were heavier and taller than girls and showed higher levels of C-reactive protein, systolic blood pressure, muscular fitness and cardiorespiratory fitness ($p < 0.05$ for all). Girls presented higher levels of leptin, fibrinogen, triglycerides and HDL-cholesterol ($p < 0.05$ for all).

INSERT table 1 here

Regression analysis (**Table 2**), showed a significant inverse association between muscular fitness score (unstandardized $\beta = -0.204$; $p < 0.021$), and handgrip strength

(unstandardized $\beta=-0,339$; $p<0.019$) with clustered score of inflammatory biomarkers, after adjustments for age, sex, pubertal stage, socioeconomic status, adherence to the Mediterranean diet, MRF score, CRF and BF% (models 4). The standing long jump test, was negatively associated with clustered score of inflammatory biomarkers ($\beta=-0.361$; $p<0.020$) when the analysis was adjusted for age, sex, pubertal stage, socioeconomic status, adherence to the Mediterranean diet and socio-economic status, MRF score and CRF (model 3); however, when the BF% was included (model 4) the results did not remain significant.

INSERT table 2 here

As shown in figure 1, the group with low muscular fitness and high clustered inflammatory biomarkers score had on average a higher MRF score (1.01, 95%CI: 0.43 – 1.58) than all other groups ($F_{(3,525)}=6.461p<0,001$).

INSERT Figure 1 here

Discussion

Our study showed that muscular fitness was inversely associated with clustered inflammatory biomarker score after adjustments for several confounders. Moreover, we observed the poorest metabolic risk profile in adolescents with low muscular fitness and high inflammatory biomarkers score. Our results indicate that the inflammatory state seems to explain a significant part of the highest MRF score and in adolescents with high inflammatory status and low muscular strength.

The relationship between inflammatory biomarkers and muscular fitness has been explored only in a few studies in adolescents (Smith et al. 2014). Previous studies have found negative associations between muscular fitness and some inflammatory biomarkers such as C3, C4, CRP, and Leptin in adolescents (Artero et al., 2013; Martinez-Gomez et al., 2012; Ruiz et al., 2008). (Martinez-Gomez et al. 2012), (The HELENA Study) has showed an inverse association between muscular fitness and CRP, C3, C4 independent of age, sex and BMI. Although, in a most recent study, on the same sample (The HELENA study), Artero et al. 2013, have showed a negative association between muscular fitness and CRP, C3, C4, leptin and white blood cells and for a selected clustered score of these inflammatory biomarkers, adjusted for age, sex, pubertal stage, CRF, HOMA-IR, but not for sum of skinfolds. In this matter, it is important to notice that in these studies the authors did not consider the confounding effect of adherence to an anti-inflammatory diet, nor the socio-economic status of the participants as in our study. We are not aware of any study that has analyzed the relationship between MF and inflammatory biomarkers independent of socio-economic status and adherence to a Mediterranean diet.

In this study we showed that C3, C4, fibrinogen, CRP and leptin were associated with MRF score. Whether the adiposity-related inflammatory state is linked to the development of cardiovascular disease in adolescents remains to be completely understood (Buchan et al. 2015). Obesity often is the most visible indicator of a metabolic condition, and therefore research has focused on the investigation of the relationship between adiposity and cardiometabolic health, with consequently preventive strategies then devised around weight loss. However, this approach does not take into consideration the overall metabolic health profile. The clustering of metabolic risk factors in the same individual, assessed by summing standardized values of individual metabolic risk factors, as a continuous variable, has been demonstrated to be a good method to assess overall metabolic risk in apparently healthy adolescents (Bugge et al. 2012). Importantly of this discussion, in the present investigation we included not only body fat, but also a selected cluster of metabolic risk factors as a confounding variable in our regression analysis, and the association between muscular fitness and clustered inflammatory biomarkers score, still remained significant. We have included Body fat percentage as covariate in our models due to its better discriminatory power for overall adiposity to identifying adverse levels of inflammatory biomarkers in adolescents, in both sexes, in this sample (Oliveira-Santos et al. 2016). In addition, in the present study, we matched the clustered score of inflammatory biomarkers to the muscular fitness groups, which allowed us to compare differences in the MRF score of the two groups of clustered of inflammatory biomarkers status by levels of muscular fitness. Indeed, recently (Roberts et al. 2015) have shown that only BMI or weight status per se may be poor surrogates for cardiovascular risk identification and that muscular fitness may be more critical to identify those at risk of metabolic and cardiovascular disorders. Roberts and colleagues findings suggest that muscular fitness

is a better predictor of oxidative stress and inflammation than weight status, in adults (Roberts et al. 2015). The skeletal muscle is a highly energetic tissue, that contributes substantially to basal metabolic rate (Volaklis et al. 2015). In this line, muscular fitness improvements may increase muscle mass and consequently become a strategic asset for the prevention of metabolic disorders.

Our results do support the current physical activity guidelines for children and adolescents that recommend regular engagement in muscle-strengthening activities due to its health-related benefits, including prevention in CVD and metabolic risk factors (W.H.O 2010). The interrelationship between inflammatory biomarkers and MRF has been described in youth (McGill H.C. et al. 2000; Hansson 2005; Bugge et al. 2012), however, our results built upon previous research by showing that adolescents with an adverse inflammatory profile and a low muscle strength may be at increased the risk of metabolic dysfunction.

There is accumulating evidence supporting that muscular strength is an emerging predictor for CVD mortality, independently of traditional risk factors such as body fat, smoking, hypertension, cardiorespiratory fitness (Volaklis et al. 2015); however, evidence about the role of muscular fitness on metabolic health of different inflammatory phenotypes in adolescents is scarce.

There is growing evidence that the systemic low-grade inflammation is closely connected to the muscle atrophy and weakness. Some studies have suggested that high levels of inflammatory biomarkers in old adults increase the risk of muscle mass and strength loss (Schaap et al. 2006, 2009). However, the causal pathway linking inflammation to subsequent loss of muscular fitness has not been fully explained, but it has been suggested that low-grade inflammation may cause a decline of physical functioning through its catabolic effects on skeletal muscle in elderly (Artero et al.

2012b) Nevertheless, there is a lack of scientific evidence in adolescents that inflammation may lead to the low muscle strength.

The strengths of our study include the consideration of important confounding variables in our analysis. Dietary intake can be a predictor of metabolic health. Previous studies reported that the adherence to a Mediterranean Dietary pattern may have a dual effect on the prevention of CVD, by improving classical CVD risk factors and also by having an intense anti-inflammatory effect (Casas et al. 2014). In our study, the inclusion of dietary intake as a confounder variable did not change our results. In addition, adolescence is a period of natural changes in several metabolic systems such as body composition and sex hormones, which may confound the results (Thomas and Williams 2008). However, in our study, the muscular fitness score, inflammatory biomarkers clustered score and MRF score were standardized by age and sex (Artero et al. 2013), and all the analyses were controlled for pubertal stage. The muscular fitness tests used in our study were based on previous studies (Agostinis-Sobrinho et al., 2016; Artero et al., 2012; Castro-Piñero et al., 2010), which have demonstrated good criterion-related validity, suggesting to be appropriated measures of muscular fitness in youth.

Some limitations of our study should be taken into consideration. **First, it is known that chronic stress is involved in metabolic alterations and we did not control our analysis for any stress psychological parameters.** Second, our cross-sectional design does not allow us to establish causality. Third, we use a single fasting baseline measurement of inflammatory biomarkers, and it is possible that this does not accurately reflect the state of low-grade inflammation. Nonetheless, we measured several inflammatory biomarkers, which provided us with a more comprehensive assessment of the inflammatory status of the adolescents, since we did not rely on only a single marker. Apart from that, all the inflammatory biomarkers included in the

clustered score were directly correlated with MRF score. In addition, a composite continuum score of inflammatory biomarkers is becoming recognized and has already been proposed in pediatric research (Artero et al., 2013; Buchan et al., 2015).

Perspective

We showed that muscular fitness is associated with the clustering of inflammatory biomarkers in adolescents after adjustments for age, sex, pubertal stage, socioeconomic status, adherence to the Mediterranean diet, CRF, MRF score and body fat. Adolescents with an adverse inflammatory profile with low levels of muscular fitness, exhibit the poorest MRF score. Although the cross-sectional design of our study, our findings suggest that a chronic inflammatory state in the presence of high metabolic risk may be attenuated by higher levels of muscular fitness. These results have a public health and clinical implications since the adolescence have been reported as period of life with several metabolic changes and a greater decrease in physical activity (Thomas and Williams 2008). The improvement of the metabolic profile together with increment of muscular fitness, may be an effective strategy for reduction in low-grade systemic inflammation and improving the health trajectory of children and adolescents.

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The authors have no conflicts of interest relevant to this article to disclose.

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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)*
Height (cm)	160.27(±9.59)	157.7(±6.68)	162.9(±11.3)*
BMI (kg m ²)	21.31(±3.84)	21.41(±3.96)	21.20(±3.73)*
Waist circumference	73.1(±10.2)	72(±10.2)	74.2 (±10.2)
Pubertal stage-A %			
II	7.8	3	12.6
III	32.3	29.2	35.5
IV	46.7	54.3	38.9
V	13.2	13.5	13
Pubertal stage-B %			
II	7	2.2	11.9
III	21.7	24.0	19.5
IV	49.5	46.8	52.3
V	21.7	27	16.4
Albumin (g/dL)	4.67(±0.010)	4.64(±0.21)	4.70(±0.20)
C3 (mg/dL)	117.44(±0.67)	118.24 (± 15.90)	116.63 (± 15.96)
C4 (mg/dL)	20.80(±0.26)	21.04 (± 6.34)	20.54 (± 6.05)
CRP mg/L	0.95(±1.88)	0.77 (±2.17)	1.13(±2.12)*
Fibrinogen (mg/dL)	264 (± 43.2)	268.13 (± 41.72)	259.77 (± 44.43)*
HDL-Cholesterol(mg/dL)	54.39(±11.95)	57.31(±12.08)	51.31(±10.99)*
IL6 (ng/L)	3.80(±5.08)	3.70(±4.49)	3.91(±5.47)
Insulin resistance (HOMA-IR)	3.45(±5.38)	3.51(±1.83)	3.39(±7.41)
Interferon α (ng/L)	10.42(±12.52)	10.40(±14.80)	10.43(±9.68)
Leptin (ng/mL)	4.12 (± 4.93)	6.21 (± 5.6)	1.98 (± 2.85)*
Ratio Total cholesterol/HDL	2.89(±0.60)	2.84(±0.58)	2.94(±0.61)
Triglycerides (mg/dL)	67.69(±32.31)	71.35(±32.67)	63.96(±31.57)*
Systolic Blood Pressure mm Hg	119.20(±12.65)	118.00(±11.61)	120.46(±13.55)*
MRF score	-0.060(±3.61)	-0.053(±3.47)	-0.064(±3.76)
Handgrip (kg)	27.21(±8.41)	23.35(±4.70)	31.14(±9.49)*
Handgrip/body weight	0.50(±0.11)	0.45(±0.08)	0.55(±0.012)*
Standing long jump (cm)	160.43(±32)	144.1(±23.4)	177.1(±31)*
20 m SRT (Nr. laps)	44.90(±1.12)	31.87(±15.25)	58.15(±26.63)*
Socioeconomic Status (FAS)	6.40(±1.70)	6.43(±1.73)	6.36(±1.68)
KIDMED Index	7.11(±2.05)	7.19(±1.98)	7.02(±2.13)

*Significantly different from girls (p<0.05) - Independent Two-tailed *t*-Tests for continuous variable and chi-square for categorical variables.

BMI: body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; CRP, C - reactive protein; FAS, Family Affluence Scale; MRF: Metabolic Risk Factor; SRT: shuttle run test; KIDMED Index, adherence to the Mediterranean index.

Table S 1. Pearson partial correlations (r, adjusted for age, sex and pubertal stage) between Muscular Fitness, MRF and Inflammatory Biomarkers

	Leptin ^a	C3 ^a	C4 ^a	C-reactive protein ^a	Fibrinogen ^a	IL-6 ^a	Albumin ^a	Interferon- α ^a
Muscular Fitness Score	-0.465*	-0.242*	-0.118*	-0.230*	-0.174*	0.016	0.040	0.064
Handgrip/Body Weight	-0.462*	-0.245*	-0.140*	-0.243*	-0.172*	0.017	0.016	0.046
Standing Long Jump	-0.341*	-0.173*	-0.064	-0.154*	-0.129*	0.009	0.053	0.065
MRF clustered score	0.442*	0.462*	0.174*	0.265*	0.307*	-0.058	0.092	-0.049

C3: complement factor C3; C4: complement factor C4; IL-6: interleukin-6; MRF: metabolic risk factor score. MRF score was computed as the sum of the following z-scores by age and sex: **waist circumference** + systolic blood pressure + triglycerides + ratio total cholesterol/HDL-cholesterol + HOMA-IR.

^a Values were natural log-transformed before analysis.

* $p < 0.001$.

Table 2. Unstandardized regression coefficients examining the association of muscular fitness with clustered inflammatory biomarkers score.

Dependent Variable	Clustered Score of Inflammatory Biomarkers*											
	Models 1			Models 2			Models 3			Models 4		
	R ²	β	P value	R ²	β	P value	R ²	β	P value	R ²	β	P value
Muscular Fitness Score	0.138	-0.705	<0.001	0.130	-0.700	<0.001	0.290	-0.349	<0.001	0.320	-0.204	0.021
Handgrip/Weight	0.136	-1.209	<0.001	0.123	-1.203	<0.001	0.291	-0.585	<0.001	0.320	-0.339	0.019
Standing Long Jump	0.068	-0.869	<0.001	0.060	-0.863	<0.001	0.274	-0.335	0.020	0.315	-0.170	0.251

* Clustered

ore of Inflammatory Biomarkers (dependent variable) was computed as the sum of the following z-scores by age and sex: CRP (C - reactive protein) + Complement factor C3 + Complement factor C4 + Leptin + Fibrinogen.

Model 1: Unadjusted model

Model 2: Model 1 additionally adjusted for age, sex, pubertal stage, adherence the Mediterranean diet and socio-economic status

Model 3: Model 2 additionally adjusted for MRF score and CRF

Model 4: Model 3 additionally adjusted for Body fat

