Pediatric RESEARCH

CLINICAL RESEARCH ARTICLE

Independent and combined associations of physical fitness components with inflammatory biomarkers in children and adolescents

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BACKGROUND: We aimed to examine the independent and combined associations of cardiorespiratory fitness, muscular fitness, and motor ability with single and clustered inflammatory biomarkers in children and adolescents.
METHODS: This study included 503 children and adolescents. Cardiorespiratory fitness, upper- and lower-muscular fitness, and motor ability were assessed using field-based tests. Fasting blood samples were obtained to determine the levels of a set of inflammatory biomarkers. Global physical fitness and clustered inflammatory biomarker scores were computed. Associations between physical fitness and inflammatory biomarkers were analyzed through linear regression. Differences in inflammatory biomarker levels between physical fitness tertiles were tested.
RESULTS: Global physical fitness was inversely associated with single and clustered inflammatory biomarkers in children ($p < 0.05$); and with C-reactive protein, complement factor C4, leptin, and clustered inflammatory biomarkers in adolescents ($p < 0.025$). Cardiorespiratory fitness and upper-muscular fitness were negatively and independently associated with several single and clustered inflammatory biomarkers in children and adolescents ($p < 0.05$). Differences were found between the lowest and the highest tertiles of global physical fitness in clustered inflammatory biomarker levels ($p < 0.010$).
CONCLUSION: Physical fitness was negatively associated with single and clustered inflammatory biomarkers, independently of body mass index. Increasing physical fitness levels in youth might contribute to reduce the cardiovascular risk.

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INTRODUCTION

Cardiovascular disease (CVD) is one of the main causes of global mortality and disease-related morbidity.[1] Among the main conventional risk factors for CVD, obesity, hypertension, insulin resistance, and dyslipidemia are included.[2] Furthermore, since obesity is considered as an independent CVD risk factor and the adipose tissue has been identified as an endocrine organ, it is important to note the existence of new CVD risk factors.[3] It is now known that adipocytes secrete a diverse group of proteins called inflammatory biomarkers[3] (i.e., C-reactive protein (CRP), complement factors C3 (C3) and C4 (C4), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), leptin, and adiponectin), which have shown to take part in different biological functions, including immunity, insulin sensitivity, appetite, angiogenesis, lipid metabolism, vascular function, and inflammation.[3] Since inflammatory cells recruitment precedes cardiovascular impairment and CVD, these inflammatory biomarkers might be more useful than conventional risk factors in predicting future cardiovascular events.[4]

Cardiorespiratory fitness and muscular fitness levels in adulthood have shown to be negatively associated with all-cause and CVD mortality.[5,6] In addition, cardiorespiratory fitness and muscular fitness have also been associated with traditional CVD risk factors such as triglycerides, low- and high-density lipoprotein cholesterol, glucose, and adiposity, in childhood and adolescence.[7] The mechanisms through which cardiorespiratory fitness, muscular fitness, and adiposity influence traditional CVD risks have not been entirely clarified and inflammatory biomarkers could have a role in this etiology.[8]

Several studies have shown an inverse association between cardiorespiratory and muscular fitness with individual inflammatory biomarkers in both children and adolescents. Most of them were focused on CRP,[9,10] and only a few studies have examined the association between cardiorespiratory and muscular fitness with the rest of the aforementioned inflammatory biomarkers: C3/C4, IL-6, leptin, TNF-α, and adiponectin.[8,11–14] Importantly, recent evidence shows that clustering of inflammatory biomarkers (i.e., CRP, C3, C4, and leptin) seems to explain a significant part of the metabolic risk factors in adolescents with high inflammatory status and low muscular strength.[15] Similarly, clustering of CVD risk factors seems to be a much stronger measure of cardiovascular health in children and adolescents than single risk factors.[16]

In addition, although the components of physical fitness with documented potential for improving health are cardiorespiratory and muscular fitness and motor ability,[7] there are no previous studies investigating the influence of motor ability on

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inflammatory biomarkers in children and adolescents. Furthermore, to the best of our knowledge, no previous study has investigated the independent and combined influence of cardiorespiratory fitness, muscular fitness, and motor ability on inflammatory biomarkers. Because these components are so associated with one another, it is important to differentiate which ones are more relevant in relation with inflammatory biomarkers.[17]

Therefore, the aim of the present study was to examine the independent and combined associations of cardiorespiratory fitness, muscular fitness, and motor ability with single and clustered inflammatory biomarkers (i.e., CRP, C3, C4, IL-6, leptin, TNF-α, and adiponectin) in children and adolescents.

METHODS

Participants

Participants selected for this study were enrolled in the UP&DOWN study.[18] Data from the present study were collected from September 2011 to June 2012. Children (6–11.9 years) and adolescents (12–17.9 years) were recruited from schools in Cádiz and Madrid, respectively. The present study includes 503 participants (230 children; 273 adolescents) with complete data on blood sampling. Parents and school supervisors were informed by letter about the nature and purpose of the study, and written informed consent was provided. The study protocols were approved by the Ethics Committee of the Hospital Puerta de Hierro (Madrid, Spain), the Bioethics Committee of the National Research Council (Madrid, Spain), and the Committee for Research Involving Human Subjects at the University of Cádiz (Cádiz, Spain).

Measurements

Tanner stage. After a brief visual observation of the explicative drawings, participants self-classified in one of the five stages of pubertal development according to Tanner & Whitehouse.[19] To do this, breast development in girls and genital development in boys were used.

Anthropometric data. Harmonization and standardization of body composition indices used to assess body composition in the UP&DOWN study were strictly controlled.[18] Anthropometric data included body weight and height. Weight was measured with an electronic scale (Type SECA 861; range, 0.05–130 kg; precision, 0.05 kg), and height was measured in the Frankfort plane with a telescopic stature-measuring instrument (Type SECA 225; range, 60–200 cm; precision, 1 mm). The measurements were carried out twice, but not consecutively, and the mean value of the two measurements was used in the analyses. Body mass index (BMI) was calculated as weight/height squared (kg/m^2).

Physical fitness. Physical fitness was assessed following the assessing levels of physical activity (ALPHA) health-related fitness test battery for children and adolescents.[20] All tests were performed in a single session.

Cardiorespiratory fitness. We used the 20-m shuttle run test as a measure of cardiorespiratory fitness. Participants were required to run between two lines 20 m apart, while keeping pace with audio signals emitted from a prerecorded CD. The initial speed is 8.5 km/h and is increased by 0.5 km/h/min (1 min equals one stage). The test finishes when the participant fails to reach the end lines concurrent with the audio signals on two consecutive occasions, or when the participant stops because of fatigue. The test was performed once and the last completed stage or half-stage at which the subject dropped out was scored.[20]

Muscular fitness. Muscular fitness was assessed based on maximum handgrip strength (upper-limb maximal strength) and the standing long jump (lower-limb explosive strength) tests. A digital hand dynamometer with an adjustable grip was used (TKK 5101 Grip D; Takey, Tokyo, Japan) for the handgrip strength test.[21] The grip-span of the dynamometer was adjusted according to the hand size for determining the maximum handgrip strength using the equations specifically developed for children[22] and adolescents.[21] The test was performed twice, and the maximum score for each hand was recorded in kilograms. The average of the scores achieved by left and right hands was calculated, and it was divided by body weight (hereinafter referred to as relative handgrip strength). The standing long jump test was performed from a starting position behind a line, standing with the feet approximately shoulder width apart. The test was performed twice and the best score was recorded in centimeters.

Motor ability. Motor ability was assessed with the 4×10-m shuttle run test of speed of movement, agility, and coordination. The test was performed twice, and the fastest time was recorded in seconds. Since a lower score (seconds) in this test means a higher performance, it was inverted by multiplying it by -1.

Global physical fitness. To generate the global physical fitness variable, 20-m shuttle run, relative handgrip strength, standing long jump, and motor ability scores were standardized by age and sex groups as follows: *z*-standardized value = (value−mean)/standard deviation (SD). Then, the sum of these standardized scores was calculated.

Inflammatory biomarkers. A fasting blood sample was obtained from the cubital vein early in the morning at the schools. A volume of 13.5 ml of blood were drawn from each subject, and 3.5 ml of them (anticoagulated blood in EDTA) were analyzed to obtain hemogram data. The remainder blood (dried gel and sodium citrate) was centrifuged; serum and plasma were removed and then frozen at −80 °C to be analyzed later.

The following inflammatory biomarkers were analyzed by turbidimetry (Olympus AU2700 Analyzer; Olympus UK Ltd., Watford, UK): CRP and serum complement factor C3 and C4. The coefficients of variation (inter-assay precision) were <2% for all proteins (1.90% for CRP, 1.39% for C3, and 1.19% for C4). Detection limits (sensitivity) for the analyses were 0.007 mg/L for CRP, 0.01 g/L for C3, and 0.002 g/L for C4. IL-6, leptin, TNF-α, and adiponectin were quantified by multiple analyte profiling (xMAP) technology (xMAP Technology; Luminex, Austin, TX) using a kit (5 + 1) plex: 171-A7003M Bio-Plex Pro Human Diabetes Adiponectin Assay; YB0000002Y Bio-Plex Human Diabetes 3-Plex Assay; 171D50001 Bio-Plex Human Cytokine Stds; 171B5006M Bio-Plex Human IL-6 set; 171B5026M Bio-Plex Human TNF-alpha set. All inflammatory biomarker analyses were performed at the same time at the Spanish National Research Council.

A clustered inflammatory biomarker score was computed by summing the age- and sex-standardized scores [*z*-standardized value = (value–mean)/SD] from CRP, C3, C4, IL-6, leptin, TNF-α, and adiponectin.

Statistical analysis

Data analysis was performed using the Statistical Package for the Social Sciences for Windows, v.22.0 (SPSS Inc., Chicago, IL). The level of significance for all analyses was set at $p < 0.05$. Descriptive data are shown as mean and SD unless otherwise indicated. Differences between age groups were tested by one-way analysis of variance (ANOVA) for continuous variables and the χ^2 test for nominal variables. Preliminary analyses showed significant interactions between age groups and physical fitness variables (all $p<0.01$), but not between sex and physical fitness variables (all $p > 0.1$); therefore, all analyses were performed separately for children and adolescents, independently of sex.

Table 1. Descriptive characteristics of the population sample

	All		Children		Adolescents		*P*
	n = 503		*n* = 230		*n* = 273		
	Mean	SD	Mean	SD	Mean	SD	
Age (years)	11.3	3.4	8.1	1.5	14.1	1.6	**<0.001**
Tanner stage	2.5	1.2	1.4	0.5	3.4	0.8	**<0.001**
Weight (kg)	43.8	16.6	30.7	9.6	54.8	12.8	**<0.001**
Height (cm)	146.1	19	129.1	11.1	160.4	10.5	**<0.001**
Body mass index (kg/m^2)	19.7	3.7	18.1	3.2	21.1	3.5	**<0.001**
20-m shuttle run (stage)	4.4	2.6	2.9	1.7	5.7	2.6	**<0.001**
Handgrip strength (kg)	19.6	9.4	12.1	3.7	25.8	8.2	**<0.001**
Standing long jump (cm)	137.2	37	111.6	22.2	159	33	**<0.001**
4 × 10-m shuttle run (s)	13.3	1.9	14.6	1.8	12.3	12	**<0.001**
C-reactive protein (mg/dL)[a]	12.2	31.8	16.3	40.2	8.3	20.2	**0.025**
Complement factor C3 (mg/dL)[a]	93.5	29.9	92.3	33.4	92.7	24.9	0.888
Complement factor C4 (mg/dL)[a]	20.3	9.8	19.5	10.3	20.3	7.8	0.341
Interleukin-6 (pg/mL)[a]	38.5	50.8	41	44.3	33.6	52.5	0.097
Leptin (ng/mL)	9.1	8.3	8.9	8.9	9.5	7.9	0.455
Tumor necrosis factor α (pg/mL)[a]	75.6	62.4	66.2	69.3	83.2	55.2	**0.003**
Adiponectin (μg/mL)[a]	14.0	7.4	14.7	7	13.4	7.8	0.057

Results are shown as mean ± SD (standard deviation)
[a]Values were naturally log-transformed before analysis, but non-transformed values are presented
Age group differences are shown in bold ($p < 0.05$)

To achieve normality in the residuals, all the variables were checked for normality of distribution before the analysis and transformations were performed. Natural logarithm was applied to CRP, C3, C4, IL-6, TNF-α, and adiponectin.

Partial correlations adjusted for sex, Tanner stage, and BMI were used to analyze the relationships between cardiorespiratory fitness, muscular fitness, motor ability, global physical fitness index, and inflammatory biomarkers. The association of cardiorespiratory fitness, muscular fitness (relative handgrip strength and standing long jump tests), motor ability, and global physical fitness (predictor variables) with single and clustered inflammatory biomarkers (outcomes) were analyzed by the linear regression analysis. All analyses were controlled by sex, Tanner stage, and BMI. In order to study the independent association of cardiorespiratory fitness, upper- and lower-muscular fitness, and motor ability with inflammatory biomarkers, further adjustments by the remaining physical fitness components were performed.

Finally, global physical fitness was grouped into three categories (low, medium, and high) according to tertiles. Analysis of covariance (ANCOVA) with Bonferroni's post hoc multiple comparison tests was used to assess the differences of inflammatory biomarker levels across global physical fitness groups, adjusted by sex, Tanner stage, and BMI.

RESULTS

Table 1 presents descriptive characteristics of the study sample. Adolescents were heavier and taller than children and had higher levels of BMI, 20-m shuttle run, handgrip strength, standing long jump, 4 × 10-m shuttle run (all $p < 0.001$), and TNF-α ($p = 0.05$) than children. Whereas, children had significantly higher levels of CRP ($p = 0.025$) than adolescents.

Supplemental Table S1 (online) presents the partial correlation between inflammatory biomarkers and physical fitness tests. Overall, all fitness components and global physical fitness were negatively correlated with inflammatory biomarkers ($p < 0.05$).

Table 2 shows the associations between physical fitness and inflammatory biomarkers in children and adolescents after adjusting for sex, Tanner stage, and BMI. Overall, fitness components were negatively associated with all inflammatory biomarkers and clustered inflammatory biomarkers (all $p < 0.05$) in children, but only with leptin and clustered inflammatory biomarkers (all $p < 0.001$) in adolescents. Moreover, relative handgrip strength ($p = 0.003$) and the standing long jump ($p = 0.029$) test were inversely associated with CRP, while the 20-m shuttle run ($p < 0.001$), relative handgrip strength ($p = 0.025$), and the standing long jump tests ($p = 0.025$) were inversely associated with IL-6 and C4, in adolescents, respectively. There was no association between physical fitness components with C3, TNF-α, and adiponectin in adolescents.

Independent associations between physical fitness tests and inflammatory biomarkers in children and adolescents are shown in Table 3. The 20-m shuttle run test was independently associated with C3 and C4 in children ($\beta = -0.055$ and $\beta = -0.073$, respectively; $p < 0.05$) and adolescents ($\beta = -0.023$ and $\beta = -0.041$, respectively; $p < 0.05$). Relative handgrip strength showed an independent association with CRP and IL-6 in both age groups ($\beta = -0.175$ and $\beta = -0.066$ for children and $\beta = -0.058$ and $\beta = -0.020$ for adolescents, respectively; all $p < 0.05$). Moreover, the 20-m shuttle run test and relative handgrip strength were independently associated with leptin in children ($\beta = -0.086$; $p = 0.015$ and $\beta = -0.053$; $p = 0.009$, respectively), and the standing long jump test was independently associated with leptin in adolescents ($\beta = -0.008$; $p < 0.002$). Finally, the 20-m shuttle run test and relative handgrip strength were independently associated with the clustered inflammatory biomarkers in children ($\beta = -0.423$ and $\beta = -0.203$, respectively; $p < 0.05$) and adolescents ($\beta = -0.205$ and $\beta = -0.108$, respectively; $p < 0.05$).

Table 4 shows the associations between global physical fitness in children and adolescents and inflammatory biomarkers. Global physical fitness was inversely associated with all inflammatory biomarkers and clustered inflammatory biomarkers in children (all

Table 2. Associations between physical fitness and inflammatory biomarkers in children and adolescents

Predictor variables	Children				Adolescents			
	Adjusted R^2	β	SE	P	Adjusted R^2	β	SE	P
		($n = 154$)		**CRP (mg/dL)**		**($n = 161$)**		
20-m shuttle run (stage)	0.061	−0.024	0.108	0.825	0.048	−0.111	0.059	0.062
Handgrip strength/weight	0.135	−0.172	0.053	**0.002**	0.067	−0.061	0.020	**0.003**
Standing long jump (cm)	0.094	−0.013	0.007	0.073	0.041	−0.011	0.005	**0.029**
4 × 10-m shuttle run (s^{-1})	0.085	−0.130	0.095	0.176	0.011	−0.016	0.138	0.905
		($n = 230$)		**C3 (mg/dL)**		**($n = 273$)**		
20-m shuttle run (stage)	0.125	−0.071	0.017	**<0.001**	0.071	−0.017	0.009	0.057
Handgrip strength/weight	0.078	−0.024	0.010	**0.017**	0.057	−0.003	0.003	0.381
Standing long jump (cm)	0.080	−0.003	0.001	**0.013**	0.066	−0.001	0.001	0.110
4 × 10-m shuttle run (s^{-1})	0.098	−0.054	0.016	**0.001**	0.060	−0.002	0.019	0.921
		($n = 230$)		**C4 (mg/dL)**		**($n = 273$)**		
20-m shuttle run (stage)	0.110	−0.101	0.024	**<0.001**	0.092	−0.040	0.012	**0.001**
Handgrip strength/weight	0.061	−0.035	0.014	**0.015**	0.062	−0.008	0.004	0.065
Standing long jump (cm)	0.062	−0.005	0.002	**0.013**	0.071	−0.002	0.001	**0.025**
4 × 10-m shuttle run (s^{-1})	0.088	−0.084	0.024	**<0.001**	0.061	−0.034	0.025	0.175
		($n = 230$)		**IL-6 (pg/mL)**		**($n = 273$)**		
20-m shuttle run test (stage)	0.099	−0.121	0.041	**0.003**	0.003	−0.002	0.021	0.922
Handgrip strength/weight	0.143	−0.098	0.023	**<0.001**	0.024	−0.015	0.007	**0.025**
Standing long jump (cm)	0.108	−0.009	0.003	**0.004**	0.013	−0.002	0.002	0.126
4 × 10-m shuttle run (s^{-1})	0.121	−0.136	0.039	**0.001**	0.006	−0.018	0.043	0.677
		($n = 230$)		**Leptin (ng/mL)**		**($n = 273$)**		
20-m shuttle run (stage)	0.560	−0.108	0.029	**<0.001**	0.535	−0.114	0.022	**<0.001**
Handgrip strength/weight	0.565	−0.069	0.016	**<0.001**	0.479	−0.036	0.008	**<0.001**
Standing long jump (cm)	0.541	−0.005	0.002	**0.025**	0.526	−0.012	0.002	**<0.001**
4 × 10-m shuttle run (s^{-1})	0.541	−0.063	0.029	**0.029**	0.514	−0.250	0.046	**<0.001**
		($n = 217$)		**TNF-α (pg/mL)**		**($n = 257$)**		
20-m shuttle run (stage)	0.016	−0.062	0.028	**0.030**	−0.002	−0.013	0.013	0.324
Handgrip strength/weight	0.014	−0.035	0.017	**0.036**	−0.007	−0.001	0.004	0.852
Standing long jump (cm)	0.013	−0.004	0.002	**0.044**	−0.005	0.001	0.001	0.475
4 × 10-m shuttle run (s^{-1})	0.010	−0.052	0.027	0.061	−0.003	0.018	0.026	0.491
		($n = 230$)		**Adiponectin (μg/mL)**		**($n = 273$)**		
20-m shuttle run (stage)	0.048	−0.056	0.020	**0.006**	0.046	−0.014	0.017	0.421
Handgrip strength/weight	0.022	−0.016	0.012	0.182	0.048	0.000	0.006	0.969
Standing long jump (cm)	0.026	−0.003	0.002	0.102	0.067	−0.003	0.001	0.052
4 × 10-m shuttle run (s^{-1})	0.036	−0.046	0.020	**0.022**	0.057	−0.054	0.035	0.120
		($n = 217$)		**Clustered inflammatory biomarkers**		**($n = 257$)**		
20-m shuttle run (stage)	0.275	−0.684	0.133	**<0.001**	0.159	−0.303	0.065	**<0.001**
Handgrip strength/weight	0.260	−0.370	0.082	**<0.001**	0.167	−0.125	0.124	**<0.001**
Standing long jump (cm)	0.242	−0.039	0.010	**<0.001**	0.158	−0.025	0.005	**<0.001**
4 × 10-m shuttle run (s^{-1})	0.255	−0.568	0.131	**<0.001**	0.115	−0.404	0.141	**<0.001**

CRP C-reactive protein, C3 complement factor C3, C4 complement factor C4, IL-6 interleukin-6, TNF-α tumor necrosis factor, SE standard error
Significant results are in bold ($p < 0.05$)
β indicates estimated standardized regression coefficient
All analyses were controlled for sex, Tanner stage, and BMI

$p < 0.05$). However, it was only inversely associated with CRP, C4, leptin, and clustered inflammatory biomarkers in adolescents (all $p < 0.05$).

Figure 1 shows the differences on inflammatory biomarker levels (i.e., CRP; C3; C4; and leptin) and clustered inflammatory biomarkers with global physical fitness groups (low, medium, and high). Significant differences were found between the lower and the higher global physical fitness groups with CRP ($p = 0.016$), C3 ($p < 0.001$), C4 ($p = 0.001$), leptin ($p < 0.001$), and clustered inflammatory biomarker ($p = 0.015$) levels in children. Moreover, significant differences were reported between medium and high global physical fitness groups with C3 ($p < 0.011$), leptin ($p < 0.001$), and clustered inflammatory biomarker ($p = 0.010$) levels in children. In adolescents, there were significant differences between low and high and low and medium global physical fitness groups with C3 (both $p < 0.05$), leptin (both $p < 0.001$), and

Table 3. Independent associations between physical fitness tests and inflammatory biomarkers in children and adolescents

Predictor variables	Children				Adolescents			
	Adjusted R^2	β	SE	P	Adjusted R^2	β	SE	P
		(n = 154)		**CRP (mg/dL)**		**(n = 161)**		
	0.111				0.092			
20-m shuttle run (stage)	–	0.146	0.127	0.252	–	−0.078	0.076	0.306
Handgrip strength/weight	0.124	−0.175	0.064	**0.007**	0.105	−0.058	0.026	**0.027**
Standing long jump (cm)	–	−0.006	0.010	0.573	–	−0.007	0.008	0.355
4 × 10-m shuttle run (s^{-1})	–	−0.003	0.132	0.979	–	0.381	0.198	0.056
		(n = 230)		**C3 (mg/dL)**		**(n = 273)**		
	0.121				0.067			
20-m shuttle run (stage)	0.122	−0.055	0.021	**0.008**	0.045	−0.023	0.011	**0.048**
Handgrip strength/weight	–	−0.008	0.012	0.498	–	−0.001	0.004	0.828
Standing long jump (cm)	–	0.001	0.002	0.743	–	−0.001	0.001	0.613
4 × 10-m shuttle run (s^{-1})	–	−0.024	0.023	0.293	–	0.034	0.028	0.219
		(n = 230)		**C4 (mg/dL)**		**(n = 273)**		
	0.113				0.083			
20-m shuttle run (stage)	0.126	−0.073	0.030	**0.017**	0.083	−0.041	0.015	**0.008**
Handgrip strength/weight	–	−0.013	0.017	0.431	–	−0.003	0.005	0.640
Standing long jump (cm)	–	0.001	0.003	0.617	–	0.000	0.002	0.939
4 × 10-m shuttle run (s^{-1})	–	−0.049	0.033	0.144	–	0.018	0.038	0.631
		(n = 230)		**IL-6 (pg/mL)**		**(n = 273)**		
	0.127				0.017			
20-m shuttle run (stage)	–	−0.038	0.050	0.445	–	0.019	0.026	0.473
Handgrip strength/weight	0.127	−0.066	0.028	**0.020**	0.017	−0.020	0.009	**0.036**
Standing long jump (cm)	–	0.000	0.004	0.935	–	−0.002	0.003	0.554
4 × 10-m shuttle run (s^{-1})	–	−0.055	0.055	0.315	–	0.048	0.064	0.452
		(n = 230)		**Leptin (ng/mL)**		**(n = 273)**		
	0.568				0.576			
20-m shuttle run (stage)	0.568	−0.086	0.035	**0.015**	–	−0.048	0.026	0.073
Handgrip strength/weight	0.568	−0.053	0.020	**0.009**	–	−0.014	0.009	0.150
Standing long jump (cm)	–	0.000	0.003	0.951	0.576	−0.008	0.003	**0.002**
4 × 10-m shuttle run (s^{-1})	–	−0.028	0.019	0.478	–	0.021	0.065	0.753
		(n = 217)		**TNF-α (pg/mL)**		**(n = 257)**		
	0.013				−0.017			
20-m shuttle run (stage)	–	−0.033	0.035	0.347	–	0.010	0.017	0.545
Handgrip strength/weight	–	−0.020	0.020	0.335	–	−0.006	0.006	0.322
Standing long jump (cm)	–	−0.002	0.003	0.611	–	0.001	0.002	0.536
4 × 10-m shuttle run (s^{-1})	–	−0.006	0.039	0.878	–	0.004	0.040	0.913
		(n = 230)		**Adiponectin (μg/mL)**		**(n = 273)**		
	0.038				0.061			
20-m shuttle run (stage)	–	−0.049	0.025	0.056	–	0.006	0.022	0.788
Handgrip strength/weight	–	0.006	0.014	0.686	–	0.012	0.008	0.129
Standing long jump (cm)	–	0.000	0.002	0.990	–	−0.003	0.002	0.118
4 × 10-m shuttle run (s^{-1})	–	−0.022	0.028	0.438	–	−0.046	0.052	0.373
		(n = 217)		**Clustered inflammatory biomarkers**		**(n = 257)**		
	0.292				0.203			
20-m shuttle run (stage)	0.292	−0.423	0.168	**0.013**	0.203	−0.205	0.092	**0.027**
Handgrip strength/weight	0.292	−0.203	0.100	**0.045**	0.203	−0.108	0.034	**0.002**
Standing long jump (cm)	–	−0.001	0.015	0.935	–	−0.008	0.009	0.347
4 × 10-m shuttle run (s^{-1})	–	−0.168	0.187	0.370	–	0.378	0.221	0.088

CRP C-reactive protein, C3 complement factor C3, C4 complement factor C4, IL-6 interleukin-6, TNF-α tumor necrosis factor alpha, SE standard error
Significant results are in bold ($p < 0.05$)
β indicates estimated standardized regression coefficient
All analyses were controlled for sex, Tanner stage, and BMI. Further adjustments by the remaining physical fitness tests were performed

Table 4. Associations between global physical fitness and inflammatory biomarkers in children and adolescents

Dependent variables	Global physical fitness									
	Children					Adolescents				
	n	Adjusted R^2	*β*	SE	*P*	*n*	Adjusted R^2	*β*	SE	*P*
CRP (mg/dL)	154	0.110	−0.490	0.200	**0.016**	161	0.044	−0.400	0.141	**0.021**
C3 (mg/dL)	230	0.101	−0.121	0.036	**0.001**	273	0.065	−0.032	0.024	0.184
C4 (mg/dL)	230	0.088	−0.182	0.052	**0.001**	273	0.069	−0.072	0.032	**0.026**
IL-6 (pg/mL)	230	0.139	−0.346	0.084	**<0.001**	273	0.014	−0.086	0.055	0.118
Leptin (ng/mL)	230	0.552	−0.200	0.061	**0.001**	273	0.535	−0.374	0.058	**<0.001**
TNF-α (pg/mL)	217	0.020	−0.141	0.059	**0.018**	257	−0.010	0.010	0.034	0.769
Adiponectin (μg/mL)	230	0.033	−0.092	0.043	**0.035**	273	0.059	0.046	0.065	0.155
Clustered inflammatory markers	217	0.248	−0.197	0.046	**<0.001**	257	0.156	−0.110	0.024	**<0.001**

CRP C-reactive protein, C3 complement factor C3, C4 complement factor C4, IL-6 interleukin-6, TNF-α tumor necrosis factor alpha, SE standard error
Significant results are in bold ($p < 0.05$)
β indicates estimated standardized regression coefficient
All analyses were controlled for sex, Tanner stage, and BMI

clustered inflammatory biomarker ($p = 0.009$ and $p = 0.044$, respectively) levels. There was also a significant difference between medium and high global physical fitness groups with leptin ($p < 0.001$) levels. There were not significant differences across global physical fitness groups with CRP and C4 in adolescents.

DISCUSSION

The main finding in this study was that global physical fitness was inversely associated with single and clustered inflammatory biomarkers, after adjusting for potential confounders, including BMI in children and adolescents. Likewise, CRF and upper-muscular fitness were independently associated with single and clustered inflammatory biomarkers. Conversely, motor ability was not independently associated with inflammatory biomarkers. In spite of the observational nature of our study, these results contribute to the current knowledge by suggesting that cardiorespiratory and muscular fitness may have a beneficial influence on the inflammatory profile in children and adolescents. Of note, children and adolescents with a high global physical fitness level exhibited the lowest levels of inflammatory biomarkers.

In the present study, we found an inverse association of the global physical fitness (including cardiorespiratory fitness, muscular fitness, and motor ability) with all single inflammatory biomarkers and the clustered inflammatory biomarkers in children. Moreover, global fitness was inversely associated with CRP, C4, leptin, and the clustered inflammatory biomarkers in adolescents. Given the fact that the origins of CVD can be found in some cases at early years[23] and inflammatory biomarkers track from childhood to adulthood,[24] it would be relevant to increase global physical fitness levels during childhood and adolescence in order to improve the inflammatory profile and to reduce the risk of CVD during adulthood. Therefore, developing health and educational policies that encourage global physical fitness during childhood should be a priority. To our knowledge, this is the first study that analyzes the effect of global physical fitness with single and clustered inflammatory biomarkers in children and adolescents.

A higher number of single inflammatory biomarkers were associated with global physical fitness in children than in adolescents; furthermore, the associations between global physical fitness and single and clustered inflammatory biomarkers were stronger in children. We have previously observed[25] that the association between vigorous physical activity and traditional CVD risk factors (i.e., a cluster including waist circumference, systolic blood pressure, high-density lipoprotein cholesterol, triglycerides, and glucose) was dependent on physical fitness (including CRF and upper- and lower-MF) in children; however, in adolescents, the association between moderate-to-vigorous and vigorous physical activity and CVD risk factors was independent of fitness. These results seem to indicate that physical activity could exert an important role in the prevention of CVD risk in adolescents rather than in children, where physical fitness seems to be the predominant determinant of the cardiovascular profile.

The scientific literature has reported that both cardiorespiratory[10,11,26] and muscular fitness[10,12,15,26] are inversely associated with inflammatory biomarkers (i.e., CRP, C3, C4, IL-6, leptin, and adiponectin) in children and adolescents. However, little is known about the relationship between motor ability and inflammatory biomarkers. Previously, one study has shown an inverse correlation between motor ability and IL-6.[27] Our results support these statements, since we found that several single and clustered inflammatory biomarkers were inversely associated with cardiorespiratory fitness, muscular fitness, and motor ability. It is important to highlight that these associations were independent of BMI levels. Other studies have also found an adiposity-independent association between CRF and MF with different inflammatory biomarkers, using BMI,[12] waist circumference,[10,26] or body fat percentage[11,15] as covariates.

The relationship between adiposity (BMI or skinfolds) with inflammatory biomarkers has been well established.[28,29] On the other hand, it has been shown that physical fitness may have an important role preventing increases in adiposity in children and adolescents.[7,30] According to different studies, despite the evident crucial role of adiposity, overweight and obese children and adolescents may improve the inflammatory profile if they maintain adequate levels of physical fitness.[31,32]

We analyzed the independent association of cardiorespiratory fitness, muscular fitness, and motor ability with inflammatory biomarkers. Our findings confirmed that cardiorespiratory fitness and upper-muscular fitness were independently associated with single and clustered inflammatory biomarkers after adjusting for additional confounders, whereas motor ability seems to be dependent on cardiorespiratory and muscular fitness levels. Recently, Artero et al.[31] found that muscular fitness was negatively associated with single and clustered inflammatory biomarkers (i.e., CRP, C3, C4, and leptin), even when they performed additional adjustments for cardiorespiratory fitness.

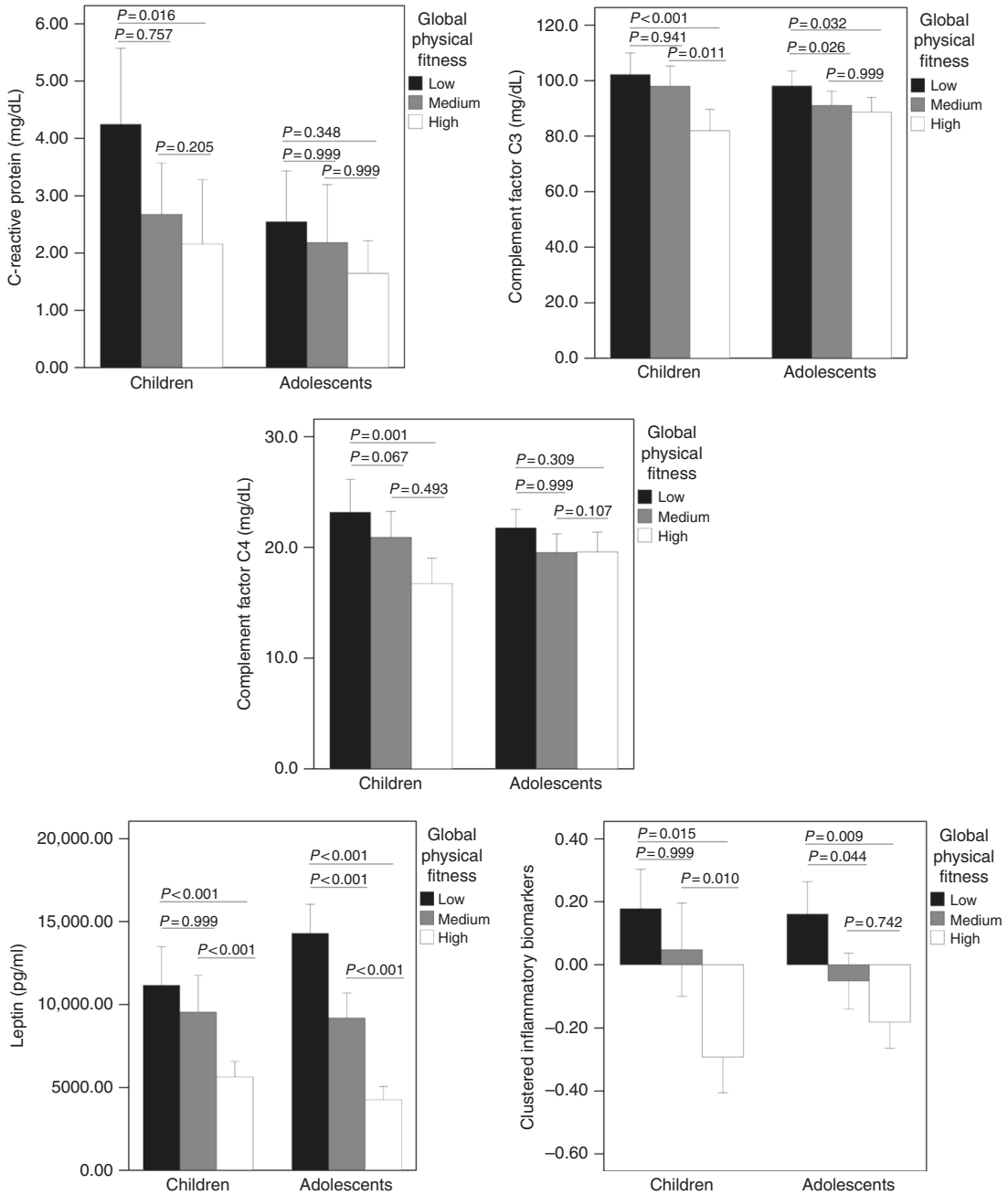

Fig. 1 Differences across global physical fitness groups on C-reactive protein, C3-protein, C4-protein, leptin, and clustered inflammatory biomarker levels. Error bars represent 95% CIs. Analyses were adjusted by sex, pubertal development, and BMI

Similarly, Steene-Johannessen et al.[10] reported that independently of adiposity and cardiorespiratory fitness, higher muscular strength was associated with lower levels of inflammatory biomarkers such as CRP, leptin, and TNF-α. These results suggest that we should emphasize in cardiorespiratory and muscular fitness training in order to improve the inflammatory profile in children and adolescents.

The rationale for the lack of associations between IL-6, TNF-α, and physical fitness among children and adolescents in the present study remains to be determined. Sobieska et al. found that IL-6[27] correlated positively with muscular fitness and negatively with motor ability. Hosick et al.[33] found that higher levels of cardiorespiratory fitness were associated with lower levels of IL-6. However, different studies have found no significant associations of cardiorespiratory and muscular fitness with IL-6.[10,34] It has been suggested that IL-6 may have a beneficial effect causing increased insulin sensitivity of the muscles and handling to the production of anti-inflammatory substances.[32] IL-6 could be generated in the muscles themselves, elevating glucose levels through the stimulation of insulin production.[35] On the other hand, IL-6 is produced to a greater extent in adipose tissue and it has been used to link obesity with insulin resistance and diabetes, as well as atherosclerosis.[28,29] Nevertheless, elevated levels of IL-6 associated with obesity are reduced if obesity is accompanied by elevated levels of cardiorespiratory fitness.[36] Likewise, TNF-α plays an important role in the synthesis and secretion of inflammatory markers from adipose tissue as it is an autocrine/paracrine regulator of fat cell size and function, thereby influencing insulin

resistance and metabolic disorders.[37] However, TNF-α seems to be dependent on physical fitness but not adiposity.[36] Besides, Steene-Johannessen et al.[10] found a weak inverse association between cardiorespiratory and muscular fitness with TNF-α. Oppositely, Nemet et al.[34] found that TNF-α was paradoxically positively correlated with cardiorespiratory fitness. Although everything points to a positive trend between the development of physical fitness and improvements in the inflammatory profile, more studies are needed in the population of children and adolescents to clarify the associations between IL-6, TNF-α, and physical fitness.

Finally, we examined the differences across the global physical fitness levels with single inflammatory biomarkers (i.e., CRP, C3, C4, and leptin) and the clustered inflammatory biomarkers. We observed significant differences in CRP, C3, C4, leptin, and the clustered inflammatory biomarkers among the different global physical fitness levels in children. In adolescents, we found significant differences in C3, leptin, and the clustered inflammatory biomarkers between the different physical fitness levels. Children and adolescents at the highest tertile of the global physical fitness level presented a significantly lower amount of inflammatory biomarkers. From a public health perspective, promoting physical activity that mainly involves cardiorespiratory and upper-muscular fitness tasks during early years may be important for the future health of children and adolescents.

This study presents limitations including its cross-sectional design. This kind of design does not allow us to set any conclusions about the direction of the associations. Moreover, the study sample consisted of a single ethnic group (white Caucasian). In addition, the study has several strengths. First, the use of a wide range of inflammatory biomarkers to assess inflammation. Second, we analyze the independent and combined effect of physical fitness on inflammatory biomarkers including a cluster. Finally, the complete and standardized assessment of health-related physical fitness (i.e., cardiorespiratory fitness, muscular fitness, motor ability, and body composition), using the ALPHA health-related fitness test battery, which is composed of valid, reliable, safe, simple, and practical tests has been widely used in children and adolescents.[20]

CONCLUSIONS

In conclusion, this study shows that physical fitness, mainly cardiorespiratory fitness and upper-muscular fitness, are negatively associated with single and clustered inflammatory biomarkers independently of BMI. Increasing physical fitness levels might contribute to achieve a favorable inflammatory profile in children and adolescents and to reduce the risk of CVD. Our results are of importance because the consequences of childhood and adolescence inflammation levels tend to track into adulthood. Future interventional and prospective studies examining the role of physical fitness on inflammatory biomarkers are clearly needed.

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AUTHOR CONTRIBUTIONS

A.D.-A. contributed to acquire the data and the conception and design of the study, had full access to all the study data, performed statistical analyses, and drafted the original manuscript. A.P.-B., J.C.-C., R.I.-G., I.E.-C. and S.G.-M. contributed to acquire the data, made critical revision of the initial manuscript, and approved the final manuscript as submitted. AM handled funding, made critical revision of the initial manuscript, and approved the final manuscript as submitted. J.C.-P. is the guarantor of this work, had full access to all the study data, handled funding, contributed to acquire the data and the conception and design of the study, made critical revision of the initial manuscript, and approved the final manuscript as submitted. All authors have read and approved the final version of this manuscript, and agree with the order of presentation of the authors.

ADDITIONAL INFORMATION

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