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PEDIATRIC HIGHLIGHT

Associations of physical activity, cardiorespiratory fitness and fatness with low-grade inflammation in adolescents: the AFINOS Study

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Objective: To examine the independent associations of objectively measured physical activity (PA), cardiorespiratory fitness (CRF) and fatness with low-grade inflammatory markers in adolescents.
Design: Cross-sectional study in Spain.
Subjects: A sample of 192 adolescents aged 13–17 years.
Measurements: PA was assessed with an accelerometer for 7 days. A 20-m shuttle-run test was used to assess CRF. Skinfold thicknesses at six sites and WCs were measured. BMI was calculated from measured height and weight. C-reactive protein (CRP), interleukin-6 (IL-6) and complement factors C3 and C4 were assayed. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from glucose and insulin. Regression analysis adjusted for potential confounders and HOMA-IR was used to determine the associations between PA, CRF and fatness with low-grade inflammatory markers.
Results: Total PA, vigorous PA and MVPA were positively associated with CRF ($r = 0.25$–0.48), whereas vigorous PA was negatively associated with skinfolds ($r = -0.27$). CRF was inversely associated with fatness, ($r = -0.30$ to -0.48). CRF and fatness were inversely and positively associated with HOMA-IR ($r = -0.16$ and 0.21, respectively). PA variables were not independently associated with inflammatory markers. CRF and fatness were inversely and positively associated with CRP, C3 and C4, respectively. Only body fat explained a relevant amount of the variance of the model in CRP (4%) and C4 (19%), whereas CRP and body fat jointly explained the variance in C3 (25%). All these observations were independent of HOMA-IR.
Conclusions: These findings support the key role of CRF and fatness on low-grade inflammation, as well as the possible indirect role of habitual PA through CRF and body fat in adolescents.
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Introduction

Cardiovascular diseases (CVDs) are the principal cause of death in the developed countries.[1] Nowadays, it is well documented that genesis of CVD occurs in early ages—even though the clinical symptoms are not observed until adulthood.[2] Until recently, the traditional CVD risk factors (for example, hypertension, high cholesterol, and so on) were the most common markers to predict morbidity and mortality.[1] However, atherogenesis involves an inflammatory process[3] in which there is an increase of inflammatory cytokines and acute-phase reactant levels within the arterial wall. Thus, inflammatory proteins and cytokines have been proposed as new emerging CVD risk factors.[4] Similarly, other studies have shown that elevated concentrations of adipokines and chemokines seem to have a key role in the pathogenesis of type-2 diabetes that leads to a low-grade inflammation status.[5]

C-reactive protein (CRP) has been highlighted as one of the most powerful predictors of CVD risk in adults[6] and

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children.[7] Interleukin-6 (IL-6) is a proinflammatory cytokine whose main effect is the induction of hepatic CRP levels. In addition, IL-6 also has a mediator role between obesity, inflammation, insulin resistance and CVD.[8] C3 and C4 complement factors are other inflammatory markers that have an important role in acute tissue injury,[9] and substantial associations have been found with traditional CVD risk factors[10] in adults. These novel inflammatory markers have, therefore, a potential interest for pediatric CVD risk factor control and future preventive strategies.[11]

In adults, physical activity (PA) and cardiorespiratory fitness (CRF) have shown an inverse relationship with all-cause mortality and several chronic diseases, including CVD.[12] Conversely, fatness is a positive independent risk factor in CVD development.[13] Among children and adolescents, PA, CRF and fatness have been associated with traditional CVD risk factors.[14,15] As CVD risk factors usually track from childhood to adulthood,[16] an active lifestyle beginning in early ages has been the focus of public health strategies to prevent CVD risk factors. The mechanisms through which PA, CRF and fatness influence traditional CVD risks have been not entirely clarified and the new inflammatory markers could have a role in this etiology; however, there is limited and mixed evidence in youth regarding the association of PA, CRF, adiposity and inflammatory makers.[11,17]

Previous studies examining the associations between PA, CRF and fatness with low-grade inflammation in children and adolescents show several limitations. PA has often been assessed by subjective methods (for example, questionnaires), whereas objective instruments such as accelerometers can measure PA more accurately.[18] To date, there is a lack of knowledge about the associations between objectively measured PA and inflammatory markers in adolescents. Furthermore, no adjustments have been used in several studies to evaluate whether associations of PA, CRF and fatness are independent of each other in predicting inflammatory markers. Insulin resistance may also have an important role in low-grade inflammation,[5] and PA, CRF and fatness levels have been also associated with insulin resistance during adolescence.[19] Thus, it is necessary to know whether insulin resistance mediates the associations of PA, CRF and fatness on inflammatory markers. The aim of this study was to examine the independent associations of objectively measured PA, fitness and fatness on inflammatory markers (CRP, IL-6, C3 and C4) in adolescents.

Materials and methods

Design and subjects

The AFINOS (La Actividad Física como Agente Preventivo del Desarrollo de Sobrepeso, Obesidad, Alergias, Infecciones y Factores de Riesgo Cardiovascular en Adolescentes: Physical Activity as a Preventive Agent of the Development of Overweight, Obesity, Infections, Allergies and Factors of Cardiovascular Risk in Adolescents) Study is a surveillance study in the region of Madrid (Spain) where health status and lifestyle indicators were assessed by questionnaire in a representative adolescent sample aged 13–17 years ($n \sim 2000$). A set of measurements including anthropometry, objectively measured PA, physical fitness and blood analysis were performed in a sub-sample of 232 adolescents. In this sub-sample, 195 adolescents (99 boys and 96 girls) had a complete set of anthropometry, PA, CRF and inflammatory markers. To minimize the confounder of an ongoing infection, three adolescents (1 boy and 2 girls) with CRP $>10\,\mathrm{mg\,l^{-1}}$ were excluded. Hence, 98 adolescent boys and 94 adolescent girls ($n=192$) were included in this study. Total data collection in the sub-sample lasted for 4 months between November 2007 and February 2008.

Before participation in the AFINOS Study, all volunteer adolescents and their parents/guardians were informed about the characteristics of the study and the adolescents gave assent, and parents or guardians also provided signed written consent. The AFINOS Study was approved by the Ethics Committee of Puerta de Hierro Hospital (Madrid, Spain) and the Bioethics Committee from the Spanish National Research Council.

Physical examination

Weight (kg) and height (m) were obtained by standardized procedures without shoes and in underwear. Body mass index (BMI) was calculated as the square of the weight/height ratio ($\mathrm{kg\,m^{-2}}$). Skinfold thicknesses were measured on the left side of the body to the nearest 0.1 mm using a skinfold caliper (Caliper Holtain; Holtain Ltd, Wales, UK) at the following six sites: triceps, biceps, subscapular, suprailiac, thigh and calf. Body circumferences were measured with a non-elastic tape to the nearest 1 mm in the following five sites: biceps, contract biceps, waist, hip and calf. The complete set of anthropometric measurements was performed twice, but not consecutively. This anthropometric protocol for adolescents has been previously standardized in the AVENA (Alimentación y Valoración del Estado Nutricional en Adolescentes; Food and Assessment of the Nutritional Status of Spanish Adolescents) study.[20] The sum of six skinfolds (Sum6) was used as an indicator of total body fat and the waist circumference (WC) as an indicator of abdominal body fat. Weight status was categorized by BMI according to the age and sex cut-offs proposed by the International Obesity Task Force (www.iotf.org). Pubertal status (I to V) was determined on the basis of self-report according to Tanner and Whitehouse,[21] for breast development and pubic hair in adolescent girls, and pubic hair in adolescent boys.

Measurement of PA

The adolescents wore the ActiGraph (ActiGraph GT1M; ActiGraph, Pensacola, FL, USA) accelerometer. The ActiGraph

GT1M is small ($3.8 \times 3.7 \times 1.8$ cm), lightweight (27 g), compact (polycarbonate plastic) and detects vertical accelerations ranging in magnitude from 0.05 to 2.00 *g*, with a frequency response of 0.25–2.50 Hz. The ActiGraph (previously known as MTI and CSA) has been widely validated in laboratory settings and under free-living conditions in youth.[22] Activity counts are summed over a user-specified interval of time called an 'epoch'. The accelerometer protocols used in the AFINOS Study have been described in detail elsewhere.[23] Briefly, the adolescents wore the accelerometer using a 15-s epoch, at the lower back, for seven consecutive days and was only removed during sleeping and water-based activities. Data were processed by the JAVA software developed to analyze the output from the ActiGraph. The software excluded bouts of 10 continuous minutes of zeros from the analysis output, considering these periods as non-wearing time. An inclusion criterion for this study was an activity monitor recording of at least 10 h per day, for 4 days, one of which had to be a weekend day. The ratio of the raw variable obtained by the ActiGraph accelerometer (total counts) to the wearing time was considered as total PA in counts per minute (c.p.m.). Moreover, Freedson's age-specific cut-off points[22] for moderate PA and vigorous PA were used to estimate the time (min day^{-1}) spent in these PA intensities. The time spent in moderate-to-vigorous PA (MVPA) was computed by summing the moderate and vigorous periods.

Measurement of CRF

CRF was assessed by a 20-m shuttle-run test. The progressive 20-m shuttle-run test is one of the most widely used field tests to assess CRF among children and adolescents, and has been described in detail elsewhere.[24] In brief, participants ran as long as possible back and forth across a 20-m space at a specified audio signal protocol that increased by 0.5 km h^{-1} each minute or stage. The test was finished when the participants failed to reach the end lines concurrent with the audio signals on two consecutive occasions. The last lap completed was considered the individual CRF level for being the raw variable obtained. For comparative purposes with previous studies, maximal oxygen consumption (VO_{2max}, ml kg^{-1} min^{-1}) was also estimated by the Leger equation.[24]

Blood sampling

After overnight fasting for 10 h, blood samples were collected between 0800 and 0900 hours. For all participants, blood was extracted by venipuncture from the antecubital vein (16 ml). After 1 h of collection, the blood was centrifuged and aliquots of sera were stored at −80 °C. The C3 and C4 complement factors, and CRP, were measured in the serum by immunoturbidimetry (Olympus AU2700 Analyzer; Olympus UK Ltd, Watford, UK). IL-6 concentrations were assessed using the High Sensitivity Human Cytokine MILLIPLEX MAP kit (Millipore Corp., Billerica, MA, USA), and collected by a Luminex-100 system Version 2.3 (Luminex, Austin, TX, USA). Glucose and insulin levels were also measured by an enzymatic assay using an Olympus AU2700 Analyzer. Quality control of the assays was assured by the Regional Health Authority, as compulsory for all clinical laboratories in Spain. The homeostasis model assessment of insulin resistance (HOMA-IR) was used as a surrogate index for the assessment of insulin resistance.[25] The HOMA-IR was calculated by dividing by 22.5 the product of glucose (mmol l^{-1}) and insulin (μU ml^{-1}).

Statistical analysis

The descriptive characteristics are presented as mean ± (s.d.), unless otherwise stated. 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, total PA, MVPA, CRF, insulin and body fat variables (BMI, Sum6, WC). Square roots were applied to C3, C4, IL-6 and vigorous PA. Differences between sexes were determined by analysis of variance and χ^2-test for proportions was used for categorical data. The interaction factors for sex (sex × main exposures) were checked to determine whether sex modified the associations of PA, CRF and fatness with inflammatory markers. As no significant interaction was found for sex, all analyses were performed with boys and girls together to increase the statistical power. Partial correlations, controlling for age, sex and pubertal status, were used to analyze the relationships between PA (total PA, moderate PA, vigorous PA, MVPA), CRF and body fat (BMI, Sum6, WC). Partial correlations controlling for the same potential confounders were also used to analyze the relationships between PA, CRF, fatness and insulin resistance (glucose, insulin, HOMA-IR), and low-grade inflammatory markers (CRP, IL-6, C3, C4). Separate models by linear regression analyses were used to determine the associations of PA, CRF and body fat (predictor variables) with inflammatory markers (outcome variables) controlling for age, sex, pubertal status and HOMA-IR. A final model was tested with all the predictor variables to examine the independent and joint associations between the predictor and the dependent variables. All statistical analyses were performed using the Statistical Package for the Social Sciences for Windows, v.15.0 (SPSS Inc., Chicago, IL, USA). The level of significance for all analyses was set at $P<0.05$.

Results

The descriptive characteristics of the sample are shown in Table 1. Adolescent boys were taller and heavier than girls, although there were no significant differences in BMI between the sexes. Adolescent girls had higher total body fat, whereas boys had higher abdominal body fat. Approximately 19% were overweight (16%) or obese (3%) with no significant differences between the sexes. The levels of PA and

CRF were significantly greater in boys than in girls, with the exception of moderate PA. There were no differences between the sexes regarding any of the inflammatory markers. In addition, glucose was significantly higher in boys.

Table 2 shows the partial correlations between PA, CRF and fatness. The PA variables were significantly related to each other, as were the fatness variables. Total PA, vigorous PA and MVPA were significantly correlated with CRF ($r=0.340$, 0.477 and 0.249, respectively; all $P\leqslant0.001$). Only vigorous PA was inversely correlated with total body fat ($r=-0.268$; $P<0.001$). By contrast, CRF was inversely correlated with BMI, Sum6 and WC ($r=-0.347$, -0.477 and -0.299, respectively; all $P<0.001$).

Table 1 Description of the study adolescence population

	All (n = *192*)	*Boys* (n = *98*)	*Girls* (n = *94*)
Age (years)	14.8 (1.3)	14.7 (1.2)	15.0 (1.3)
Weight (kg)	60.7 (12.0)	63.8 (13.5)	57.5 (9.2)**
Height (m)	1.7 (0.1)	1.7 (0.1)	1.6 (0.1)**
Pubertal status (I/II/III/IV/V)	1/16/36/100/39	1/4/24/40/29	0/12/12/60/10
Body mass index (kg m^{-2})[a]	21.7 (3.5)	21.8 (3.8)	21.7 (3.1)
Overweight/obesity (%)	16/3	15/2	16/4
Sum of six skinfolds (mm)[a]	33.5 (12.1)	29.3 (12.1)	37.8 (10.6)**
Waist circumference (cm)[a]	73.4 (9.4)	75.2 (9.7)	71.5 (8.6)*
Waist-to-hip ratio (cm)[a]	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)
Total PA (c.p.m.)[a]	499 (159)	564 (167)	431 (117)**
Moderate PA (min day^{-1})	61.6 (20.4)	67.8 (21.4)	55.0 (17.2)**
Vigorous PA (min day^{-1})[b]	12.2 (10.8)	17.9 (11.6)	6.4 (5.7)**
MVPA (min day^{-1})[a]	73.8 (27.4)	85.7 (28.6)	61.4 (19.5)**
CRF (laps)[a]	45 (23)	58 (23)	31 (13)**
CRF (ml kg^{-1} min^{-1})	40.9 (4.9)	44.6 (5.1)	36.4 (4.1)**
C-reactive protein (mg l^{-1})[a]	0.7 (1.3)	0.8 (1.5)	0.6 (0.9)
Interleukin-6 (pg ml^{-1})[b]	16.6 (28.6)	17.7 (27.9)	15.4 (29.4)
C3 (g l^{-1})[b]	1.2 (0.2)	1.2 (0.2)	1.1 (0.2)
C4 (g l^{-1})[b]	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)
Glucose (mmol l^{-1})	5.2 (0.4)	5.3 (0.4)	5.1 (1.7)**
Insulin (mU dl^{-1})[a]	14.2 (9.9)	13.1 (9.2)	13.5 (6.1)
HOMA-IR	3.3 (2.4)	3.3 (3.0)	3.2 (1.7)

Abbreviations: CRF, cardiorespiratory fitness; HOMA-IR, homeostasis model assessment of insulin resistance; PA, physical activity. Data are mean ± s.d. [a]Values were natural log-transformed before analysis, but the non-transformed values are presented in the table. [b]Values were square-root-transformed before analysis, but the non-transformed values are presented in the table; *$P<0.01$, **$P<0.001$: denote statistical significant differences between the sexes.

Partial correlations between PA, CRF, fatness and insulin resistance and low-grade inflammatory markers are shown in Table 3. PA levels were not significantly correlated with insulin resistance and low-grade inflammatory markers, but a borderline and inverse correlation was found between vigorous PA and C3 ($r=-0.142$, $P=0.053$). CRF was inversely correlated with both insulin and HOMA-IR, as well as with CRP, C3 and C4. Only Sum6 was related to fasting glucose, whereas BMI, Sum6 and WC were correlated with insulin and HOMA-IR. Conversely, glucose was correlated with both complement factors ($r=0.277$ for C3 and $r=0.257$ for C4; both $P<0.001$) after controlling for age, sex and pubertal status. There were no significant correlations between insulin and inflammatory markers, whereas HOMA-IR was only significantly associated with C3 ($r=0.165$; $P=0.024$). CRP and complement factors were positively associated each other, but IL-6 was only significantly associated with CRP levels ($r=0.179$; $P=0.015$).

Multiple regression analyses with inflammatory markers as the dependent variables and PA, CRF and body fat as predictor variables adjusted for age, sex, pubertal status and HOMA-IR are shown in Table 4. Total PA was not significantly associated with inflammatory markers (model-1). Further analyses using moderate PA, vigorous PA and MVPA instead of total PA showed similar results (results not shown). Both CRF (model-2) and total body fat (model-3) were inversely and positively associated with CRP, C3 and C4, respectively. Similarly, BMI and WC were also significantly associated with CRP, C3 and C4 (results not shown). To examine the independent association between total body fat and abdominal body fat to explain the variance in CRP, C3 and C4, we included two combinations between both types of variables into the model (Sum6 + WC and BMI + WC) after adjustment for the same potential confounders. These analyses showed that (1) only abdominal body fat explained the variance in CRP; (2) both total and abdominal body fat variables explained the variance in C3 and (3) only total body fat variables explained the variance in C4. A final model including jointly PA, CRF and fatness in the model was tested (model-4). This analysis showed that CRF and body fat independently explain the variance in C3, and

Table 2 Partial correlations between PA, fitness and fatness in adolescents ($n=192$)

	MPA	*VPA*	*MVPA*	*CRF*	*BMI*	*Sum6*	*WC*
Total PA (c.p.m.)[a]	0.774*	0.729*	0.908*	0.340*	−0.030	−0.063	0.004
Moderate PA (min day^{-1})	—	0.370*	0.919*	0.078	−0.004	0.069	0.052
Vigorous PA (min day^{-1})[b]		—	0.613*	0.477*	−0.112	−0.268*	−0.081
MVPA (min day^{-1})[a]			—	0.249*	−0.012	−0.014	0.027
CRF (laps)[a]				—	−0.347*	−0.477*	−0.299*
Body mass index (kg m^{-2})[a]					—	0.760*	0.798*
Sum of 6 skinfolds (mm)[a]						—	0.698*
Waist circumference (cm)[a]							—

Abbreviations: BMI, body mass index; CRF, cardiorespiratory fitness; MPA, moderate PA; MVPA, moderate-to-vigorous PA; PA, physical activity; VPA, vigorous PA; WC, waist circumference. Data were adjusted for age, sex and pubertal status. [a]Values were natural log-transformed before analysis. [b]Values were square-root-transformed before analysis. *$P\leqslant0.001$.

Table 3 Partial correlations between PA, fitness, fatness and insulin resistance and low-grade inflammatory markers in adolescents ($n=192$)

	Glucose	*Insulin*	*HOMA-IR*	*CRP*	*IL-6*	*C3*	*C4*
Total PA (c.p.m.)[a]	−0.048	−0.037	−0.043	0.036	0.002	−0.005	0.044
Moderate PA (min day^{-1})	0.043	0.038	0.042	0.075	0.029	0.118	0.104
Vigorous PA (min day^{-1})[b]	−0.042	−0.043	−0.047	−0.060	−0.061	−0.142	−0.084
MVPA (min day^{-1})[a]	0.012	0.019	0.021	0.016	−0.018	0.051	0.078
CRF (laps)[a]	−0.125	−0.151*	−0.158*	−0.148*	0.019	−0.323***	−0.250***
Body mass index (kg m^{-2})[a]	0.123	0.162*	0.173*	0.250***	−0.014	0.454***	0.337***
Sum of six skinfolds (mm)[a]	0.214**	0.190**	0.211**	0.236***	0.050	0.474***	0.404***
Waist circumference (cm)[a]	0.129	0.186*	0.197**	0.301***	0.031	0.467***	0.318***
Glucose (mmol l^{-1})	—	0.207**	0.353***	0.100	−0.031	0.277***	0.257***
Insulin (mU dl^{-1})[a]		—	0.988***	0.049	0.124	0.126	0.103
HOMA-IR			—	0.063	0.112	0.165*	0.139
C-reactive protein (mg l^{-1})[a]				—	0.179*	0.379***	0.331***
Interleukin-6 (pg ml^{-1})[b]					—	0.035	0.012
C3 (g l^{-1})[b]						—	0.635***
C4 (g l^{-1})[b]							—

Abbreviations: BMI, body mass index; CRF, cardiorespiratory fitness; CRP,C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; IL, interleukin; MVPA, moderate-to-vigorous PA; PA, physical activity. Data were adjusted for age, sex and pubertal status. [a]Values were natural log-transformed before analysis. [b]Values were square-root-transformed before analysis. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Table 4 Associations between PA, fitness and fatness with inflammatory markers in adolescents ($n=192$)

Model	*Predictor variables*	*Outcomes*											
		C-reactive protein (mg l^{-1})[a]			*Interleukin-6 (pg ml^{-1})*[b]			*C3 (g l^{-1})*[b]			*C4 (g l^{-1})*[b]		
		β	*P*	R^2	β	*P*	R^2	β	*P*	R^2	β	*P*	R^2
1	Total PA (c.p.m.)[a]	0.037	0.648	0.009	0.016	0.846	0.012	−0.023	0.772	0.025	0.037	0.475	0.051
2	CRF (laps)[a]	−0.199	0.033	0.014	0.041	0.436	0.011	−0.427	<0.001	0.137	−0.312	<0.001	0.110
3	Body fat (mm)[a]	0.241	0.002	0.040	−0.016	0.842	0.005	0.491	<0.001	0.232	0.250	<0.001	0.113
4	Total PA (c.p.m.)[a]	0.085	0.314		0.006	0.948		0.070	0.352		0.108	0.167	
	CRF (laps)[a]	−0.121	0.277		0.045	0.691		−0.227	0.022		−0.163	0.113	
	Body fat (mm)[a]	0.198	0.027	0.038	−0.004	0.964	0.016	0.403	<0.001	0.245	0.337	<0.001	0.187

Abbreviations: CRF, cardiorespiratory fitness; PA, physical activity. Data were adjusted for age, sex, pubertal status and homeostasis model assessment of insulin resistance. [a]Values were natural log-transformed before analysis. [b]Values were square-root-transformed before analysis.

only body fat in CRP and C4. This association of CRF and body fat with C3 remained significant after including the CRF × body fat interaction term in the model. The results in the final model did not vary using other PA and body fat variables.

Discussion

As far as to the knowledge of the authors, this is the first study to associate objectively measured PA (that is, accelerometers) with low-grade inflammation (serum CRP, C3, C4, IL-6) in adolescents. An inverse and borderline significant association between vigorous PA and C3 was found. Controlling for CRF and body fat, however, this borderline association did disappear. No other direct associations were present between PA and inflammation, but PA was positively associated with CRF and negatively associated with body fat. Hence, although the plausible anti-inflammatory role of PA in adolescents needs further investigation, our results seem to indicate that PA may have an indirect role through CRF and body fat health determinants.

Our data are in consonance to a study of pre-pubertal 9-year-old Swedish children,[26] which used objectively measured PA using an accelerometer, showing no associations between objectively measured PA and serum levels of CRP, C3, C4 or fibrinogen. This study by Ruiz *et al.*[26] is the only other study that has used accelerometers for objective measurement of PA when examining the associations between PA and inflammation in youth, but in a younger age group than the one studied in this study.

In general, there is limited evidence in adolescents regarding the association between PA and inflammatory makers, and those studies that have examined this association have often assessed PA by subjective methods (for example, survey). Self-reports are commonly used in PA epidemiology research because they are inexpensive and do not need too much time to evaluate these behaviors (that is, 10–15 min). Nevertheless, self-reports are subjective and rely on memory and are only moderately correlated in children and adolescents ($r\sim0.3$) when compared with criterion measures to assess PA.[18]

Previous studies using self-reports for assessing PA in youth have shown equivocal findings.[17] Thus, whereas several

studies have found significant and inverse associations between PA and inflammatory markers, other studies have failed to detect significant differences between active and inactive youth.[11,17] In addition, several of these studies did not control for body fat or CRF in their analyses,[27–29] or if they did so, the associations became attenuated or non-significant.[30,31]

We also showed that CRF was inversely associated with CRP, C3 and C4. Nevertheless, these associations did not remain statistically significant after controlling for body fat in the analyses with CRP and C4, and were attenuated but remained significant with C3. Furthermore, body fat indicators were positively associated with CRP and complement factors. Studies examining associations of CRF and fatness with inflammatory markers in young people have shown similar results that support the central role of CRF and fatness on low-grade inflammation.[17,32,33] However, to our knowledge, we present the first data examining whether insulin resistance might mediate the associations of CRF and fatness with low-grade inflammation.

A recent body of evidence links obesity, inflammation and insulin resistance.[5,8] As CRF and body fat are strongly associated ($r \sim 0.5$ in this study) and several studies have shown a protective effect of CRF on insulin resistance in youth,[19] whether CRF and body fat have a direct role on low-grade inflammation or an indirect role through insulin resistance is unknown. Thus, despite the observation that both CRF and body fat indicators were, respectively, inversely and positively associated with insulin resistance in this study, our findings suggest that both CRF and body fat are associated with low-grade inflammation in adolescents, independent of the insulin resistance levels. This result is important as insulin resistance is associated with early-onset type-2 diabetes.[34] Notwithstanding, these novel findings should be confirmed or disproved in future interventional and prospective studies.

Hence, preliminary evidence seems to indicate that achievement of a healthy weight during adolescence may be the most effective strategy to prevent chronic low-grade inflammation and future cardiovascular and metabolic diseases. Whereas genetic and early programming features have been associated with low-grade inflammation in youth,[35,36] an active lifestyle and a desirable CRF may attenuate its effects. This has important implications for public health, as specific strategies should be developed for adolescents due to the well-known decline in PA levels during this crucial life period. Likewise, other types of PA related to muscular fitness (that is, resistance training) might be taken into consideration during adolescence because high levels of muscular fitness have showed negative associations with inflammatory proteins.[37] Therefore, understanding the interrelationships between PA, fitness and fatness may be the main way to prevent low-grade inflammation in these ages.

This study has limitations. These findings may not be generalizable due to the relatively small sample size. Our cross-sectional design cannot establish the causal direction between the variables studied. Although adolescents with CRP levels $>10\,mg\,l^{-1}$ were not included, we cannot be sure that elevated levels of inflammatory markers were due to an emerging infection. Similarly, one blood sample may not be enough to determine chronic low-grade inflammation. Finally, accelerometers also have inherent limitations such as diversity of cut-off points and epoch length to define and capture PA intensities, underestimate PA with horizontal movements (bicycling, dancing, martial arts) or static activities (for example, resistance training), and water-based PA cannot be captured.[23] Finally, more precise quantification of body fatness (for example, dual-energy X-ray absorptiometry, magnetic resonance imaging) may also provide insight into the role of total and abdominal adiposity on low-grade inflammatory markers.

In summary, CRF and fatness are inversely and positively associated with inflammatory markers (CRP, C3, C4) in healthy adolescents, respectively, after adjustments for potential confounders including HOMA-IR. However, CRF and fatness jointly were only associated with C3, whereas fatness was independently associated with CRP and C4. Even though objectively assessed PA was not associated with inflammatory markers, PA may have a relevant indirect role by improving CRF and reducing body fat during adolescence. The mechanisms by which CRF and fatness mediate inflammation require further longitudinal and experimental studies of youth to clarify.

Conflict of interest

The authors declare no conflict of interest.

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