Objectively-measured and self-reported physical activity and fitness in relation to inflammatory markers in European adolescents: The HELENA Study

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INTRODUCTION

While clinical symptoms are not observed until adulthood, atherosclerotic lesions may form during younger years [1]. There is strong evidence that inflammation plays a key role in

atherosclerosis development [2]. Atherogenesis involves an inflammatory process that leads to plaque instability within the arterial wall [2]. It also increases blood levels of inflammatory acute-phase reactant proteins (e.g. C-reactive protein [CRP] and complement factors) and cytokines (e.g. interleukin-6 [IL-6] and tumor necrosis factor-alpha [TNF- α]), which provide useful markers of low-grade inflammation [2].

Several lifestyle-related determinants have been investigated for their anti-inflammatory functions during adolescence [3]. Physical activity (PA) and fitness may be important protective factors for low-grade inflammation [3]. However, evidence is scarce and results are mixed regarding the association of PA and fitness with inflammation in adolescents [4]. In addition, previous studies of inflammation in this age group had some critical methodological limitations [4]. Samples were small and homogeneous [4,5] and most investigations used self-reporting to assess PA. The accuracy of self-reporting to measure PA is poor compared with objective means, particularly in younger people [4,5].

Addressing these limitations, the HELENA (HEalthy Lifestyle in Europe by Nutrition in Adolescents) study [6,7] assessed PA and fitness using both objective and self-reporting methods, in a relatively large heterogeneous sample of adolescents from nine European countries, to examine the associations of PA and fitness with inflammatory markers in adolescents.

METHODS

Design and participants

The HELENA Cross-Sectional Study (CSS) is a multi-center study aimed at obtaining reliable and comparable data on nutrition and other health indicators such as PA, physical fitness, body composition, cardiovascular disease risk factors, vitamins and mineral status, immunological biomarkers, and genetic markers in European adolescents [6,7]. HELENA-CSS data collection was undertaken during two school periods between October 2006 and December 2007 in 10 cities from nine European countries: Vienna (Austria), Ghent (Belgium), Lille (France), Dortmund (Germany), Athens and Heraklion (Greece), Pécs (Hungary), Rome (Italy), Zaragoza (Spain), and Stockholm (Sweden). The total sample consisted of 3528 adolescents, of whom 1089 participants gave blood samples. Of the participants with blood parameters, a total of 1041 adolescents had at least one of the inflammatory markers selected in the current study. To minimize the confounder of ongoing infections and chronic inflammatory diseases, 16 adolescents (including 2 girls) with CRP >10 mg/l were excluded. After these subjects were excluded, 476 boys and 549 girls ($n=1025$) participated in this study.

All participants were recruited from schools and met the following criteria established for subjects of the HELENA-CSS [6]: they were (i) aged between 12.5 and 17.5; (ii) free of any acute infection for at least one week prior; (iii) not participating simultaneously in additional clinical trials; and (iv) able to provide information on weight and height. Adolescents and their parents or guardians were provided with information about the HELENA-CSS and all provided a written informed consent. Ethics committees from each country approved the HELENA-CSS protocol and good clinical practices were conducted according to ethical guidelines (8).

Anthropometry

The anthropometric protocols followed in the HELENA-CSS study are described in detail by Nagy et al. [9]. Briefly, body height was measured to the nearest 0.1 cm with a stadiometer (SECA 225) while standing barefoot. Body weight was determined to the nearest 100 g using a balance scale (SECA 861) with subjects in their underwear. Body mass index (BMI) was calculated as body mass (kg) divided by height (m) squared. BMI Z-score was also calculated for each BMI measure with reference to sex- and age-specific limits provided by the International Obesity Task Force (www.iaso.org/iotf/). Waist circumference (WC) was measured in triplicate to the nearest 1 mm at the midpoint between the superior iliac spine and the costal edge in the midaxillary line, using an anthropometric non-elastic tape (SECA 200).

Assessment of objectively-measured and self-reported PA

Patterns of PA were objectively assessed using the ActiGraph GT1M (ActiGraphTM, Pensacola, FL, USA), and descriptive data and procedures were detailed in a previous report from the HELENA-CSS [10]. The accelerometer is a small and lightweight uniaxial monitor designed to detect accelerations ranging in magnitude from 0.05 to $2.00 \times g$ with a frequency response of 0.25–2.50 Hz. The ActiGraph has been widely calibrated for young people in laboratory and free-living conditions. Adolescents wore the accelerometer positioned at the lower back for seven consecutive days. The accelerometer was worn by subjects while awake and only removed during water-based activities. The interval of time called ‘epoch’ was set at 15 sec according to a consensus of recommendations for assessing PA in youth. Non-wearing time was defined by bouts of at least 20 min of zero outputs. At least three days of valid recording and a minimum of 8 h/d were necessary data to be included in the study. The PA variables included in this study were counts per minute (cpm) as a measure of overall PA, and time spent at moderate, vigorous and moderate-to-vigorous PA (MVPA) intensities (min/d). The time spent in moderate and vigorous PA was calculated based upon cutpoints of 2000 and

4000 cpm respectively [11,12], whereas the time spent in MVPA was calculated as the combined moderate and vigorous times.

Patterns of PA were also self-reported using the International Physical Activity Questionnaire for Adolescents (IPAQ-A) [13,14]. The IPAQ-A, which is available in several languages (French, Flemish, German, Greek, Hungarian, Italian, Spanish, Swedish and English) covers the following four domains of PA: (i) school-related PA, including activity during physical education and recess; (ii) transportation; (iii) housework; and (iv) extracurricular PA. In each of the domains, the number of days per week and the time periods each day spent walking, in moderate PA and in vigorous PA, were recorded. The data were cleaned and truncated following the guidelines provided by the IPAQ group (www.ipaq.ki.se) [13,14]. Consequently, variables obtained by the IPAQ-A for this study were the times spent (min/wk) in moderate PA, vigorous PA, MVPA, and overall PA (MVPA + walking intensities).

Assessment of objectively-measured and self-reported physical fitness

Physical fitness was assessed using standardized field-based fitness tests for youth [15]. Cardiorespiratory fitness was assessed by the 20-m shuttle-run test. Participants were required to run between two lines 20 m apart, while keeping pace with audio signals emitted from a pre-recorded CD. The initial speed was 8.5 km/h, which was increased by 0.5 km/h each minute (1 min = 1 stage). The test was finished when the participant failed to reach the end lines concurrently with the audio signals on two consecutive occasions. The last stage completed was taken as the participant's CRF. Muscular fitness was assessed using the handgrip strength (maximum handgrip strength, kg) and the standing long jump (lower limb explosive strength, cm) tests. A single muscular fitness score was computed from the two muscular tests. The individual score of each test was standardized as follows: Z-standardized

value = (value - mean)/SD. The muscular fitness score was calculated as the mean of the two standardized scores. Motor fitness was assessed with the 4 × 10 m shuttle-run test of speed-of-movement, agility and coordination (measured in seconds). A score of overall fitness was also computed as the average of the three physical fitness components studied after transformation into Z-standardized values. Since the standardized motor fitness score is inversely related to high physical fitness, it was first multiplied by -1. These physical fitness tests have been shown to be valid and reliable in young people [16].

These three specific fitness components, and overall fitness, were also assessed using four single-response items included in the International Fitness Scale (IFIS) [17]. The four 5-point Likert-scale items asked the participant to compare their perceived overall fitness, cardiorespiratory fitness, muscular strength and speed/agility (motor fitness) with their friends' physical fitness (very poor, poor, average, good and very good). IFIS was originally written in English and was culturally adapted and translated into all languages involved in the HELENA study. The IFIS scale has shown that (i) it is able to correctly rank adolescents according to their actual physical fitness levels; (ii) adolescents reporting a good/very good overall fitness, cardiorespiratory fitness or speed/agility have a healthier cardiovascular profile; and (iii) IFIS is a reliable method to be used in adolescents [17].

Inflammatory markers

Blood samples were collected in the early morning after overnight fasting. In all cases, 30 ml of blood was extracted by venipuncture from the antecubital vein by a qualified nurse. The stability of samples during transport and storage in the HELENA study has been reported previously [18]. Five key biomarkers involved in low-grade inflammation were selected for this study [19]. CRP was measured in serum by immunoturbidimetry (AU2700 biochemistry analyzer; Olympus, Watford, UK). C3 and C4 serum complements were analyzed by

nephelometry (Behring Diagnostics, California, USA). The CVs (inter-assay precision) were 1.9% for CRP, 1.4% for C3, and 1.2% for C4. Serum cytokines IL-6 and TNF- α were determined using the High Sensitivity Human Cytokine MILLIPLEXTM MAP kit (Millipore Corp., Billerica, MA, USA) and collected by flow cytometry (Luminex-100 v.2.3, Luminex Corporation, Austin, TX, USA). The intra- and inter-assay precision CVs were: 3.5% and 4.5% respectively, for IL-6; and 3.5% and 3.8%, respectively, for TNF- α . Detection limits (sensitivity) for all the analyses were 0.007 mg/l for CRP, 0.01 g/l for C3, 0.002 g/l for C4, 0.1 pg/ml for IL-6, and 0.05 pg/ml for TNF- α . Undetectable values were recorded as the specific detection limit.

Dietary intake

Total dietary intake data (kcal/d) and intake (g/d) of major nutrients (carbohydrates, fat and proteins) were obtained using a dietary assessment tool called HELENA-DIAT, which is based on a self-administered, computerized 24-hr dietary recall named YANA-C (Young Adolescents' Nutrition Assessment on Computer) [20,21]. Two computerized 24-h recalls, were performed, on two non-consecutive days, within two weeks. The YANA-C was completed by the participants in the school's computer room or in a classroom with computers.

Statistical analyses

Descriptive characteristics are presented as mean (SD) and percentages for continuous and categorical variables, respectively. All variables were checked for normality of distribution before analysis and transformations were performed to achieve normality in the residuals. A natural logarithm transformation was applied to CRP data. Square roots were applied to C3, C4, IL-6 and vigorous PA in both objective and self-reported assessments.

Differences by sex were determined by analysis of variance (ANOVA). Sex-interactions (sex $\times$ main exposures) were tested to determine whether sex modified the associations of PA or fitness with inflammatory markers. Since no significant interactions were found for sex (all $P>0.2$), all analyses were performed with boys and girls together. Partial correlations, controlling for age, sex and city (dummy variable) were used to analyze the relationships between objective and self-reported PA (overall PA, moderate PA, vigorous PA and MVPA), objective and self-reported fitness (overall fitness, cardiorespiratory fitness, muscular fitness and motor fitness) and body fat (BMI and WC). For comparative purposes, partial correlations between inflammatory markers and body fat, controlling for age, sex and city, were calculated (**Table 1 supplementary file**). Separate models by linear regression analyses were used to determine the associations of objective and self-reported PA and fitness (exposure variables) with inflammatory markers (outcome variables) controlling for age, sex, city (main covariates) and then by body fat (as obesity is considered to be a chronic low-grade inflammatory status [16]) and by dietary intake variables. The associations of PA with low-grade inflammation markers were additionally controlled for fitness measurements. Analyses were conducted using the software package Predictive Analytics version 18.0 for Macintosh (SPSS Inc., Chicago, IL, USA). The level of significance was set at $P<0.05$ for all analyses. The statistical analyses were adjusted by a weighting factor to balance the sample according to gender and age distribution, and to guarantee true representation of each of the stratified groups.

RESULTS

The descriptive characteristics of the sample are shown in **Table 1**. Adolescent boys were taller and heavier than girls and had higher levels of WC. There were no differences between the sexes in CRP, C3 and C4, but adolescent boys had higher levels of IL-6 and TNF- α than girls. In both objective and self-reported measurements, the levels of PA and fitness were significantly greater in boys than girls.

Table 2 shows the partial correlations between PA, fitness and body fat (BMI and WC) using different measurements. Patterns of objectively-measured overall PA, vigorous PA and MVPA were positively correlated with all objective and self-reported indicators of fitness, with the exception of self-reported muscular fitness. Conversely, objectively-measured moderate PA was not significantly related to any measures of fitness. Patterns of self-reported PA were also positively correlated with all objective and self-reported indicators, except overall PA and objective muscular fitness. Vigorous PA was negatively correlated with body fat variables, however, other forms of PA were not. On the other hand, objective and self-reported measures of overall fitness, cardiorespiratory fitness and motor fitness were negatively correlated with body fat. Muscular fitness indicators were not related to WC, whereas objective and self-reported muscular fitness were negatively and positively correlated with BMI, respectively. BMI Z-score was significantly associated with all fitness (objective and self-reported) variables (all $P < 0.05$), but not with any other variables.

Multiple regression analyses with inflammatory markers as dependent variables and objective and self-reported PA and fitness measurements as predictor variables adjusted for age, sex, city and BMI are shown in **Table 2 supplementary file** and **Table 3**. Regarding patterns of PA, only objectively-measured vigorous PA was significantly associated with C3, independent of confounders such as BMI (**Table 2 supplementary file**). The negative association between vigorous PA and C3 did not remain significant after including any of the objective fitness indicators as a covariate in the model (data not shown). Regarding levels of

fitness, all the objective measures of fitness were negatively associated with CRP, C3 and C4, independent of the main covariates and BMI (**Table 3**). Several measures of self-reported fitness were associated with CRP, C3 and C4, but only motor fitness remained significantly associated with C3 and C4 independent of BMI. While all significant associations of PA and fitness with CRP, C3 and C4 in model 1 were attenuated when BMI was included in the model, all remained significant. When measured and self-reported fitness were included simultaneously in the model with CRP, C3 and C4 as outcome variables, only objective fitness showed significant associations (data not shown). Levels of IL-6 were not significantly associated with fitness indicators, but motor fitness was negatively associated with TNF- α after controlling for the main covariates and BMI (**Table 3**). All analyses were repeated using WC and the findings were consistent with those using BMI (**Table 3 supplementary file**). Finally, when using BMI Z-score instead of BMI and WC, as well as total energy intake or intake of main nutrients as covariates in the models, the principal results did not substantially change (data not shown).

DISCUSSION

These data, taken from a relatively large and heterogeneous sample of European adolescents, indicate that PA (vigorous) as measured by accelerometry was inversely associated with C3, irrespective of potential confounders including adiposity indices. However, this association became non-significant after adjusting for fitness measures. Furthermore, objective fitness was inversely associated with three of the five inflammatory markers (CRP, C3 and C4), irrespective of potential confounders and adiposity. Since the interrelationship between PA, fitness and body fat is well-known [11, 12], these results suggest that PA has an indirect role in inflammation through fitness and body fat in adolescence. These findings have public health and clinical implications, as adolescence is a period of life characterized by a greater decrease in PA [22, 23]. Also, it becomes important to consider that strategies based on enhancing PA levels in adolescents, to obtain a positive effect on inflammation, may be successful when the intervention improves fitness or body fat outcomes [4]. This may still be the case even though associations to inflammatory markers by enhanced PA levels were not significant.

The associations between PA and inflammatory markers in youth have been mainly examined using self-reported PA and data generated using objective measures is limited [4]. Using both objective and self-reported PA measures of small and homogenous samples has given mixed results with null or inverse associations [4]. Nevertheless, studies using objective measures of PA are in agreement with the current findings. Previously, we examined, for the first time, the associations between objectively-measured PA (accelerometry) and inflammatory markers (CRP, C3, C4 and IL-6) in 192 Spanish adolescents [5]. In this study we found an inversely and marginally significant association between vigorous PA and C3 that disappeared after controlling for potential covariates such as fitness and body fat [5]. Similarly, PA levels measured by accelerometry were not associated with inflammatory markers (CRP, C3, C4 and fibrinogen) in 142 Swedish children [24].

The relationship of objective and self-reported measures on the associations between PA and inflammatory factors was low and there were only differences between objective and self-reported vigorous PA in relation to C3. Nevertheless, other self-reporting tools to assess PA in youth might have a greater influence on these associations. This was shown to be the case by Atienza et al. [25], who examined the associations of self-reported and objectively-measured (using accelerometers) MVPA with CRP in a nationally representative sample of US adults from the National Health and Nutritional Examination Survey (NHANES) 2003-2006. Interestingly, objective and subjective MVPA were independently associated with CRP when both measures were included in the model, but objectively-measured MVPA displayed a stronger association than self-reported MVPA [25]. In addition, Atienza et al. speculated that self-reported PA might serve as a marker for muscular strength as engagement in resistance training activities cannot be accurately captured by accelerometry [25]. Although physical fitness was unfortunately not included as a confounding factor in this study, these results from adults [25] and from adolescents in our current study indicate an age-specific association between PA and inflammation. As inflammatory processes are aggravated with age [2], any type of PA may have a positive impact on lessening inflammation independent of fitness or body fat levels during adulthood. However, improvements of fitness may be important to fight against low-grade inflammation in youth [3,4]. For public health and clinical purposes, this age-specific hypothesis must be examined in future longitudinal and intervention studies.

In the current study, we observed that measured overall fitness, as well as three components of fitness (cardiorespiratory, muscular and motor fitness), were modestly related to some inflammatory markers (CRP, C3 and C4), independent of proxy measures of body fat. Our results highlight the ‘fat but fit’ idea in youth to prevent future cardiovascular diseases [26]. Thus, fitness may have an anti-inflammatory function to counteract obesity-

induced inflammatory diseases. To our knowledge, no study investigating the influence of overall fitness or motor fitness on low-grade inflammation in adolescents has been undertaken to date. Previous studies that examined the associations between fitness and inflammatory markers also had limitations [4]. Indeed, most studies in youth solely used objective measurements of cardiorespiratory fitness (e.g. sub-maximum cycloergometer and treadmill tests) to examine the associations with low-grade inflammation [4]. These studies had mixed results but, in general, the significant associations were attenuated or non-significant after adjusting for body fat [4]. Regarding muscular fitness, Ruiz et al. [27] found that muscle strength was negatively associated with the inflammatory proteins CRP and prealbumin in overweight adolescents.

Our study used both objective and self-reported measures of fitness in youth. Borodulin et al. [28] found that both self-reported overall physical fitness and measured cardiorespiratory fitness were inversely related to CRP levels, irrespective of body fat, in 3803 Finnish adults. We found that self-reported fitness variables showed significant associations with CRP, C3 and C4 markers, but these relationships were non-significant after body fat and objectively-measured fitness were included in the model. These results suggest that adolescents' self-reported fitness is associated with their measured fitness and body fat levels [17]. On the other hand, the significant associations between self-reported motor fitness and TNF- α remained significant after controlling for the proxy measure of body fat and objectively-measured motor fitness. Although this association cannot be explained in the current study, other factors may mediate the association between self-reported fitness and inflammation. For example, because self-reported fitness is a subjective measure, it might also be influenced by psychological health (e.g. stress, anxiety, well-being, self-esteem and academic performance). In this line, there is certain evidence regarding the influence of stress, cognitive performance and body image on inflammatory activity [29-31].

Despite the fact that our study has important strengths, some limitations also deserve attention. Our findings are limited due to its cross-sectional design and causal directionality cannot be inferred. Longitudinal and clinical trials may provide new insights regarding the associations of PA, fitness and body fat with inflammation in youth. Moreover, we obtained blood samples from the participants at only one time point and this might not accurately reproduce a long-term inflammatory status. Finally, more precise measurements of body fat (e.g. magnetic resonance imaging or dual energy X-ray absorptiometry) might be valuable to examine the independent relationships between PA, fitness and low-grade inflammation.

In conclusion, higher PA during adolescence plays an indirect role lessening low-grade inflammation by enhancing fitness. Fitness and body fat must be considered as important determinants in public health strategies to fight against an inflammatory condition in youth. Longitudinal and intervention studies are warranted.

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AUTHORS' CONTRIBUTION

- a) Substantial contributions to conception and design (KW, YM, DM, FG, LAM, MS and AM), or acquisition of data (SMG, JRR, LED, FBO, MCG, TDV, IH, CB, MP, SM and MF), or analysis and interpretation of data (DMG, JRR and FBO).
- b) Drafting the article (DMG, JRR and FBO) or revising it critically for important intellectual content (SGM, LED, KW, MCG, YM, TDV, DM, IH, CB, FG, MP, SM, MF, LAM, MS, and AM)
- c) Final approval of the version to be published (all authors).

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Table 1. Physical characteristics of the study sample

	<i>N</i>	All	<i>n</i>	Boys	<i>n</i>	Girls
Age, yr	1025	14.8 (1.2)	476	14.8 (1.2)	549	14.7 (1.2)
Weight, kg	1025	58.6 (12.5)	476	61.9 (14.2)	549	55.7 (10.0)*
Height, m	1025	165.5 (9.3)	476	169.7 (10.0)	549	161.8 (7.0)*
Body mass index, kg/m ²	1025	21.3 (3.6)	476	21.4 (3.9)	549	21.2 (3.3)
Body mass index, Z-score	1025	0.5 (1.1)	476	0.6 (1.2)	549	0.4 (1.1)*
Waist circumference, cm	1010	72.4 (8.7)	467	74.5 (9.2)	543	70.6 (7.8)*
Total energy intake, kcal/d	670	2276 (1036)	312	2675 (1155)	357	1926 (767)*
Total carbohydrates intake, g/d	670	276 (131)	312	323 (151)	357	234 (92)*
Total fat intake, g/d	670	91 (51)	312	107 (57)	357	77 (40)*
Total protein intake, g/d	670	87 (42)	312	103 (45)	357	73 (34)*
European cities, % of total sample	1025		476		549	
Athens, Greece		7.4		6.7		8.0
Dortmund, Germany		11.4		13.9		9.3
Gent, Belgium		10.5		11.6		9.7
Heraklion, Greece		9.2		9.7		8.7
Lille, France		8.4		7.6		9.1
Pecs, Hungary		13.0		10.9		14.8
Roma, Italy		9.6		10.1		9.1
Stockholm, Sweden		9.7		10.3		9.1
Vienna, Austria		10.8		9.9		11.7
Zaragoza, Spain		10.0		9.5		10.6
Inflammatory markers						
CRP, mg/l ^a	997	0.8 (1.2)	461	0.9 (1.2)	536	0.8 (1.2)
C3, g/l ^b	997	1.1 (0.2)	461	1.1 (0.2)	536	1.1 (0.2)
C4, g/l ^b	988	0.2 (0.1)	458	0.2 (0.1)	530	0.2 (0.1)
IL-6, pg/ml ^b	962	22.4 (33.2)	452	27.0 (40.5)	510	18.3 (24.5)*
TNF- α , pg/ml ^b	962	6.7 (11.2)	453	7.9 (15.8)	509	5.5 (3.4)*
Objectively measured PA (Accelerometry)						
Overall PA, cpm	682	440 (157)	301	506 (170)	381	388 (124)*
Moderate PA, min/d	682	41 (15)	301	45 (16)	381	38 (13)*
Vigorous PA, min/d ^b	682	19 (14)	301	25 (16)	381	14 (11)*
MVPA, min/d	682	60 (25)	301	70 (26)	381	52 (21)*
Self-reported PA (IPAQ-A)						
Overall PA, min/wk	865	1155 (834)	391	1282 (878)	474	1050 (783)*
Moderate PA, min/wk	865	487 (391)	391	532 (407)	474	449 (375)*
Vigorous PA, min/wk ^b	865	239 (303)	391	328 (331)	474	165 (255)*
MVPA, min/wk	865	726 (600)	391	860 (644)	474	614 (538)*
Objectively measured fitness (HELENA tests)						
Overall fitness, Z-score	755	0 (0.8)	362	0.6 (0.7)	393	-0.5 (0.6)*
Cardiorespiratory fitness, stage	779	5 (3)	373	6 (3)	406	3 (2)*
Muscular fitness, Z-score	941	0 (0.9)	432	0.6 (0.9)	509	-0.5 (0.5)*
Motor fitness, sec	920	12.2 (1.3)	428	11.5 (1.1)	492	12.8 (1.2)*
Self-reported fitness (IFIS scale) ^c						
Overall fitness, score	957	3.7 (0.9)	445	4.0 (0.9)	512	3.5 (0.9)*
Cardiorespiratory fitness, score	955	3.5 (0.9)	443	3.8 (0.9)	512	3.3 (0.9)*
Muscular fitness, score	957	3.5 (0.9)	445	3.8 (0.8)	512	3.3 (0.8)*
Motor fitness, score	956	3.7 (0.9)	445	3.9 (0.9)	511	3.5 (0.9)*

Values are mean (SD) or %. CRP: C-reactive protein. C3: complement factor. C4: complement factor C4. IL-6: Interleukin-6. TNF- α : tumor necrosis factor- α . PA: physical Activity. MVPA: moderate-to-vigorous PA. IPAQ-A: International PA Questionnaire for Adolescents. IFIS: International Fitness Scale. ^a Values were natural log-transformed before analysis, but non-transformed values are presented in the table. ^b Values were square root transformed before analysis, but non-transformed values are presented in the table. ^c Likert scales ranging from 1 to 5. * $P < 0.05$ denotes statistical significance between genders.

Table 2. Partial correlations between physical activity (PA), fitness and fatness in European adolescents ($n=1025$)

	Overall PA		Moderate PA		Vigorous PA ^d		MVPA		Fatness	
	Objective (cpm)	Self-reported (min/wk)	Objective (min/d)	Self-reported (min/wk)	Objective (min/d)	Self-reported (min/wk)	Objective (min/d)	Self-reported (min/wk)	BMI (kg/m ²)	WC (cm)
Overall fitness										
Objective (Z-score) ^a	0.162***	0.102**	0.035	0.139***	0.221***	0.219***	0.148***	0.202***	-0.362***	-0.322***
Self-reported (score) ^b	0.131***	0.201***	0.058	0.157***	0.162***	0.238***	0.128***	0.223***	-0.178***	-0.202***
Cardiorespiratory fitness										
Objective (stage)	0.129**	0.082*	0.038	0.143***	0.174***	0.183***	0.123**	0.188***	-0.385***	-0.337***
Self-reported (score) ^b	0.110**	0.182***	0.043	0.140***	0.144***	0.202***	0.108**	0.194***	-0.161***	-0.177***
Muscular fitness										
Objective (Z-score) ^c	0.103**	0.112***	-0.029	0.101**	0.171***	0.178***	0.080*	0.156***	-0.073*	-0.056
Self-reported (score) ^b	0.066	0.141***	0.015	0.100**	0.074	0.150***	0.051	0.141***	0.093**	0.045
Motor fitness										
Objective (sec $\times$ -1)	0.133***	0.048	0.049	0.078*	0.166***	0.162***	0.125***	0.133***	-0.336***	-0.301***
Self-reported (score) ^b	0.078*	0.165***	0.037	0.107**	0.107**	0.155***	0.084*	0.148***	-0.232***	-0.238***
Fatness										
Body mass index (kg/m ²)	-0.016	0.027	0.033	-0.028	-0.088*	0.015	-0.030	-0.011	—	0.883***
Waist circumference (cm)	-0.028	-0.014	0.017	-0.043	-0.085*	-0.006	-0.038	-0.032	—	—

Analyses were adjusted for age, sex and city. MVPA: moderate-to-vigorous PA. ^a Z-score computed from 20-m shuttle-run, handgrip strength, standing broad jump, and 4x10-m shuttle-run tests. ^b Likert scales ranging from 1 to 5. ^c Z-score computed from handgrip strength and standing broad jump tests.

^d Values were square root transformed before analysis. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ denotes statistical significance.

Table 3. Associations of objectively measured and self-reported fitness with inflammatory markers in European adolescents ($n=1025$)

	Overall fitness				Cardiorespiratory fitness				Muscular fitness				Motor fitness			
	Objective (Z-score) ^c		Self-reported (score) ^d		Objective (stage)		Self-reported (score) ^d		Objective (Z-score) ^e		Self-reported (score) ^d		Objective (sec $\times$ -1)		Self-reported (score) ^d	
	β	<i>P</i>	β	<i>P</i>	β	<i>P</i>	β	<i>P</i>	β	<i>P</i>	β	<i>P</i>	β	<i>P</i>	β	<i>P</i>
<i>Model 1</i>																
CRP (mg/l) ^a	-0.218	<0.001	-0.038	0.269	-0.188	<0.001	-0.062	0.072	-0.180	<0.001	-0.007	0.842	-0.150	<0.001	-0.099	0.003
C3 (g/l) ^b	-0.346	<0.001	-0.117	0.001	-0.259	<0.001	-0.093	0.006	-0.255	<0.001	0.001	0.980	-0.236	<0.001	-0.148	<0.001
C4 (g/l) ^b	-0.286	<0.001	-0.088	0.009	-0.216	<0.001	-0.069	0.043	-0.177	<0.001	-0.020	0.543	-0.225	<0.001	-0.135	<0.001
IL-6 (pg/ml) ^b	-0.016	0.765	-0.024	0.480	0.025	0.617	-0.054	0.122	-0.028	0.556	-0.010	0.765	-0.046	0.264	-0.013	0.705
TNF- α (pg/ml)	-0.023	0.654	-0.041	0.235	-0.020	0.680	-0.012	0.733	-0.037	0.436	-0.036	0.386	-0.038	0.360	-0.070	0.036
<i>Model 1 + BMI</i>																
CRP (mg/l) ^a	-0.152	0.004	0.004	0.918	-0.124	0.011	-0.023	0.502	-0.134	0.004	-0.019	0.576	-0.088	0.034	-0.053	0.120
C3 (g/l) ^b	-0.232	<0.001	-0.055	0.091	-0.145	0.002	-0.032	0.330	-0.179	<0.001	-0.017	0.596	-0.140	<0.001	-0.075	0.022
C4 (g/l) ^b	-0.209	<0.001	-0.041	0.225	-0.140	0.003	-0.024	0.482	-0.120	0.008	-0.036	0.276	-0.154	<0.001	-0.082	0.014
IL-6 (pg/ml) ^b	-0.007	0.902	-0.022	0.528	0.032	0.524	-0.053	0.139	-0.024	0.614	-0.011	0.745	-0.043	0.323	-0.010	0.781
TNF- α (pg/ml)	-0.030	0.591	-0.039	0.264	-0.025	0.627	-0.009	0.790	-0.036	0.488	-0.037	0.273	-0.045	0.302	-0.071	0.041

β : standardized coefficients. Model 1: adjusted for age, sex and city. CRP: C-reactive protein. C3: complement factor. C4: complement factor C4. IL-6: Interleukin-6. TNF- α : tumor necrosis factor- α . BMI: body mass index. ^a Values were natural log-transformed before analysis. ^b Values were square root transformed before analysis. ^c Z-score computed from 20-m shuttle-run, handgrip strength, standing broad jump, and 4x10-m shuttle-run tests. ^d Likert scales ranging from 1 to 5. ^e Z-score computed from handgrip strength, and standing broad jump tests.