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Adiposity, aerobic fitness, muscle fitness and markers of inflammation in children

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ABSTRACT

Purpose: The purpose of this study was to describe levels of inflammation markers in Norwegian children and to examine the associations of adiposity, aerobic fitness and muscle fitness with markers of inflammation. **Methods:** In 2005–2006, 1467 9-year-olds were randomly selected from all regions in Norway. The participation rate was 89%. The inflammatory markers evaluated included C-reactive protein (CRP), leptin, adiponectin, plasminogen activator inhibitor-1, tumor necrosis factor- α (TNF-α), hepatocyte growth factor, resistin and interleukin-6. We assessed muscular strength by measuring explosive, isometric and endurance strength. Aerobic fitness was measured directly during a maximal cycle ergometer test. Adiposity was expressed as waist circumference (WC). **Results:** The girls had significantly higher levels of CRP, leptin, adiponectin and resistin and lower levels of TNF-α compared to the boys. We observed a graded association of CRP and leptin levels across quintiles of WC, aerobic fitness and muscle fitness (P ≤0.001 for all participants). The regression analyses revealed that WC, aerobic fitness and muscle fitness were independently associated with the CRP (WC β= 0.158, P < 0.001; aerobic fitness β= -0.190, P < 0.001; muscle fitness β= -0.158, P < 0.002) after adjustments for gender, age, Tanner pubertal stage and the other independent variables. The same pattern was observed for leptin levels (WC β= 0.406, P < 0.001; aerobic fitness β= –0.298, P < 0.001; muscle fitness β= –0.064, P < 0.036). **Conclusions:** These data represent a reference material with respect to inflammatory markers. Our results show that adiposity, aerobic and muscle fitness were independently associated with the CRP and leptin levels.

Key words: Obesity, physical fitness, inflammation and youth

Introduction

Paragraph Number 1 Adipose tissue serves as an endocrine organ that secretes several inflammatory adipocytokines, chemokines and growth factors (7, 39), including tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), adiponectin, leptin, plasminogen activator inhibitor-1 (PAI-1), resistin (22, 28) and hepatocyte growth factor (HGF) (24). These inflammatory markers are important in the regulation of biological functions, such as appetite, energy balance, insulin sensitivity, lipid metabolism and blood pressure (4).

Paragraph Number 2 C-reactive protein (CRP) is a non-specific marker of inflammation that has been shown to be a powerful predictor for the development of type 2 diabetes and **cardiovascular disease** (CVD) in adults and has been shown to be useful in the early detection of CVD risk in youth (12). In fact, CRP levels have been associated with early arterial changes in both healthy (16) and obese individuals (17). One adipocytokine, leptin, has been associated with impaired vascular function (31) and insulin resistance in children (32, 36). Moreover, leptin has been shown to predict the development of metabolic syndrome in adults (9).

Paragraph Number 3 Population-representative data regarding inflammatory markers are not well developed in children, and studies have often been limited by small, non-representative samples. Thus, representative data are needed to describe the levels of inflammatory cytokines and establish reference values in the population.

Paragraph Number 4 Regular physical activity in children is associated with potential benefits for many health outcomes and is important for the healthy growth and development of children (37). Moreover, several studies have shown that aerobic fitness is a strong predictor for the clustering of CVD risk factors in children (1, 8, 15, 25). Similarly, recent

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findings have also shown an independent inverse association between muscle strength (2) and muscle fitness (10, 33) with the clustering of CVD risk factors.

Paragraph Number 5 However, the relationships between, adiposity, aerobic fitness, muscle fitness and inflammatory markers have been less studied, although there are studies that show that CRP levels are inversely associated with aerobic fitness and muscle strength in both adults (19) and children (6, 14, 21, 23, 30). Isasi et al. (23) found that aerobic fitness was inversely associated with CRP levels in boys and girls aged 6-24 years and that the association was more pronounced in boys than in girls. Furthermore, Ruiz et al. (29) observed that low grade inflammation (CRP and complement factor C3) was negatively associated with muscle strength in adolescents, and Hosick et al. and Martinez-Gomez et al. recently showed that aerobic fitness (13) and muscle fitness (20) were related to leptin. Nevertheless, we are not aware of any study that has evaluated the independent associations of obesity, directly measured aerobic fitness and muscle fitness with markers of inflammation in a national representative sample of children.

Paragraph Number 6 Therefore, the aims of this study were i) to describe the levels of markers of inflammation in Norwegian children and ii) to examine the associations of adiposity, aerobic fitness and muscle fitness with markers of inflammation.

Methods

Paragraph Number 7 This study is part of the "Physical Activity among Norwegian Children Study" conducted in 2005 and 2006 (18, 34). **Statistics Norway selected the cohort by cluster sampling with schools as the primary unit. In the selection of schools, population density and geography were taken into account. When a school agreed to participate, we invited all fourth graders into the study.** We recruited a cohort of 9 year-old students (in 4th grade) from a total of 40 elementary schools across all regions in Norway to participate in this

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cross-sectional study. The study was carried out in accordance with the Helsinki Declaration and was approved by the Regional Committee for Medical Research Ethics and the Norwegian Social Science Data Services. Each participant's parent or guardian provided written informed consent, and all of the subjects agreed to participate.

Participants

Paragraph Number 8 Of the 1467 subjects invited to participate in the study, 1306 accepted, for an overall participation rate of 89%. In the current report, only those participants with a valid blood sample are included. Twenty-four participants were excluded due to CRP values > 10 mg/L. In addition, 231 participants were excluded from the blood analyses for failing to provide a consent form for the blood sample, hemolyzed blood samples, insufficient samples, or for eating or being absent on the day of blood sampling. There were no differences in the mean age, sex distribution, body mass index (BMI), body weight or aerobic fitness level between the participants with or without blood samples. In the analyses, only participants with complete measurements for all parameters (inflammatory markers, aerobic fitness, muscle fitness and WC) were included; therefore, another 215 participants were excluded. Complete data were available for 836 subjects, and these subjects formed the basis for the association analyses in the present investigation. No differences were found between the participants with complete data and those who were excluded with respect to sex distribution, BMI, body weight, WC, aerobic fitness and the levels of CRP, PAI-1, IL-6 and TNF-α. Higher levels of HGF (p=0.038) and lower muscle fitness (p=0.001) were found among those with incomplete data.

Measures

Paragraph Number 9 Measures of anthropometry, pubertal stage, aerobic and muscle fitness and a fasting blood sample were obtained in the school setting by trained research assistants.

Body height (nearest mm) was measured using wall-mounted tapes, and body weight (nearest 0.1 kg) was measured in lightweight clothing using an electronic scale (SECA 770 GmbH, Hamburg, Germany). BMI was calculated as the weight (kg) divided by the height squared (m^2). Waist circumference (WC) was measured with a metal anthropometric tape midway between the lower rib and the iliac crest at the end of a normal expiration. The intra-class (within an observer) and inter-class (between observers) correlation coefficients for WC measurements were 0.93 and 0.94, respectively. The identification of pubertal status was assessed by trained personnel according to the Tanner classification system (38); analyses in the current study were based on breast development in the girls and genitalia development in the boys.

Paragraph Number 10 After fasting overnight, venous serum blood samples were collected between 8:00 and 10:00 AM. The samples were centrifuged for 10 minutes at 2500 x *g* and separated within 30 minutes. CRP was measured using a Hitachi 917 automatic analyzer (Hitachi, Tokyo, Japan) with a highly sensitive latex-enhanced turbidimetric assay from Roche Diagnostics, Mannheim, Germany (range 0.1–20 mg/l, intra-assay coefficient of variation <2% at 0.5 mg/l). The serum levels of various adipokines were quantified using the Human Serum Adipokine panel A (adiponectin, resistin and PAI-1 [total]) and panel B (TNF-α, IL-6, HGF and leptin) kits (Linco Research, Inc. St. Charles, MI) and the Luminex-100 system (Luminex Corporation, Austin, TX, USA). The samples were analyzed, and the acquired fluorescence data were evaluated using STarStation software (Version 2.0; Applied Cytometry Systems, Sheffield, UK).

Paragraph Number 11 Aerobic fitness (VO_{2peak} (ml·min-1·kg-1)) was directly assessed during a progressive cycle test until exhaustion using an electronically braked cycle ergometer (Ergomedic 839E; Monark, Varberg, Sweden). The test is described in detail elsewhere (30). The criteria for maximal exhaustion were met based on the subjective judgment by the tester,

who looked for signs of intense effort in the subject (e.g., facial flushing or difficulties in maintaining pedal frequency), and when the heart rate was ≥185 beats per minute or the respiratory exchange ratio was ≥ 0.99.

Paragraph Number 12 Muscle fitness was measured by explosive strength, isometric strength and endurance strength. These tests are described in detail elsewhere (20). In short, upper limb strength was assessed by a handgrip strength test using a hand dynamometer (Baseline® Hydraulic Hand Dynamometer, Elmsford, NY, USA) with the subject squeezing the dynamometer, **with the dominant hand,** at maximum isometric effort for approximately 2–3 seconds,. Explosive strength in the lower body was assessed with a standing broad jump. Abdominal muscular endurance was measured by a sit-up test in which the total number of correctly performed sit-ups was counted within 30 seconds. Lastly, the endurance of the trunk extensor muscles was measured by a modified Biering-Sørensen test.

Paragraph Number 13 To account for the differences in body size, the peak handgrip was adjusted for body weight (kg). **To create a measure of overall muscle fitness we developed a muscle fitness score including test of explosive strength, isometric strength and muscle endurance. A similar approach has previously been used by our group (33) and others (10, 29).** Each of the variables was standardized as follows: standardized value = (value − mean)/SD. The muscle fitness score was computed by combining the standardized values of the handgrip strength, standing broad jump, sit-ups and the Biering-Sørensen test. The muscle fitness score was calculated as the mean of the four standardized scores by sex.

Statistical analyses

Paragraph Number 14 The data were analyzed using SPSS statistical software, v. 19.0 (SPSS Inc., Chicago, IL, USA), and the values are expressed as the mean (SD) unless otherwise stated. We assessed the differences in the anthropometric data using a one-way analysis of

variance (ANOVA), and the distribution of each variable was tested for a Gaussian distribution. CRP, leptin, resistin, TNF-α, IL-6 PAI-1 and HGF values were transformed using the natural logarithm for all of the analyses. A general linear model was used to compare the groups, adjusting for pubertal stages. A partial correlation, adjusted for pubertal stages, was used to examine the bivariate associations of WC, aerobic fitness and muscle fitness with inflammatory markers. We found no interaction for sex on the main exposures, and, consequently, further analyses were performed for boys and girls together. ANOVA with the Bonferoni post hoc test was used to assess the differences in leptin and CRP levels across quintiles of aerobic fitness, muscle fitness and WC. Three separate multiple regression models were used to determine the association of aerobic fitness, muscle fitness and WC (independent variables) with inflammatory markers (outcome variable) and pubertal stage. A final model was tested by additionally adjusting for the other independent variables to test the independent and joint associations of WC, aerobic fitness and muscle fitness with inflammatory markers.

Results

Paragraph Number 15 Table 1 shows the anthropometric characteristics of the study population. There was a great variation in BMI (ranging from 12.5 to 30.5 kg/m^2) and **according to the age adjusted cutoffs described by Cole et al (5),** the prevalence of overweight (including obesity) was 19.6% and 15.4 % for girls and boys, respectively. Moreover, the distribution of aerobic fitness in the population showed great diversity, with levels ranging from 24.3 to 67.8 **VO$_{2peak}$** ml·min-1·kg-1. Finally, the majority of the population (74 % of the girls and 92% of the boys) was classified as pre-pubertal, with the remainder in early puberty (data not shown).

Paragraph Number 16 Mean values (SD), medians and 95 % confidence intervals (CI) for the inflammatory markers are given in Table 1. With respect to gender differences, girls had significantly higher levels of CRP (0.15 mg/l), leptin (2.52 ng/ml), adiponectin (0.06 μg/ml) and resistin (3.6 pg/ml), and lower levels of TNF-α (0.71 pg/ml), compared to the boys. Moreover, as seen in table 1 several inflammatory markers have a large SD, expressing the variability of these markers in the study population. There is no convincing rationale in children to describe specific levels where risk for future disease is elevated, therefore we display **deciles** in table 2, to give a more detailed description of the levels of inflammatory markers in the population. For all variables levels there were substantial differences between the least favorable decile compared to the most favorable decile. **All means and medians presented in table 1 and 2 are produced before transformation.**

Associations between adiposity, aerobic fitness, muscle fitness and inflammatory markers

Paragraph Number 17 The correlation between inflammatory markers, WC, aerobic fitness and muscle fitness are summarized in Table 3. The partial correlations for boys and girls, controlling for pubertal stage, revealed that WC, aerobic fitness and muscle fitness were highly correlated. The CRP level was positively correlated with WC and negatively correlated with the aerobic fitness and muscle fitness. Moreover, CRP had a moderately positive correlation with IL-6 and leptin, but it had a weak correlation with HGF, TNF-α (boys only) and resistin. Furthermore, leptin had a strong positive correlation with WC and a negative correlation with the aerobic fitness and muscle fitness levels. **TNF-α were not correlated with either WC or aerobic fitness, but was weakly correlated with muscle fitness. In girls only, IL-6 was weakly correlated with WC and muscle fitness. Lastly, HGF correlated**

weakly with WC (girls only) and muscle fitness (boys only) and resistin was weakly correlated with aerobic fitness in boys.

Paragraph Number 18 Figures 1 and 2 show the graded associations of the CRP and leptin levels, **respectively** across quintiles of WC, aerobic fitness and muscle fitness. A strong graded association was observed across quintiles (p ≤0.001 for all), with leptin and CRP levels increasing from low (Q1) to high (Q5) for WC and decreasing from low (Q1) to high (Q5) for aerobic fitness and muscle fitness. The same patterns were found when divided by sex (p ≤0.001 for all). For both CRP and leptin, the levels in the least favorable quintile were significantly different from the two most favorable quintiles (p ≤0.001 for all). The levels of CRP and leptin were approximately 2 times and 6 times higher, respectively, in the most unfavorable quintile when compared to the most favorable quintile.

Paragraph Number 19 The regression analyses with CRP and leptin (outcome variables) and WC, aerobic fitness and muscle fitness (independent variables adjusted for pubertal stage) are shown in Table 4. The results revealed that WC was positively associated with both leptin and CRP and that aerobic fitness and muscle fitness were inversely associated with both leptin and CRP (p ≤0.001 for all) (Model 1-4). For CRP level, additional adjustments for the independent variables equally attenuated the associations; however, they did remain significant, showing that these variables were independently associated with CRP (p ≤0.002 for all). **A similar pattern was observed for leptin showing, equally attenuated, but still significant associations for each independent variable after additional adjustment in model 4. For the other inflammatory markers the final regression model (model 4) revealed that HGF was, for boys only, significant associated with all three independent variables (β = 0.132, 0.183 and - 0.174, for WC, aerobic fitness and muscle fitness respectively). TNF-α was correlated with muscle fitness (β = - 0.149) and resistin was, for**

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boys only, associated with aerobic fitness (β = - 0.168). No associations were found for IL-6 and PAI-1.

Discussion

Paragraph Number 20 This study presents national reference data on selected inflammatory markers in children. Moreover, the study demonstrates that WC, aerobic fitness and muscle fitness are independently associated with CRP and leptin levels after adjusting for confounding factors.

Paragraph Number 21 In general, girls had higher levels of leptin, adiponectin and resistin and lower levels of TNF-α in comparison to boys. It is difficult to explain the differences in inflammatory markers by sex; however, one possible explanation is that inflammatory reactions are driven by the hormonal status. Regardless, the fact that these sex differences are already present in prepubertal children indicates that other explanations should be investigated (4). The sex-related differences for adiponectin and leptin found in the present study are in concordance with previous studies (3, 27). Bottner et al.(3) investigated sex-related differences in adiponectin levels during puberty and found that a decreased adiponectin level in boys was inversely associated with testosterone levels. Similarly, greater serum leptin concentrations among girls is a consistent finding in the literature (11, 13) and is strongly related to the amount of adipose tissue in children. Furthermore, we know that adipose tissue is a source of inflammatory cytokines (7), and, because females biologically have more fat tissue than boys, one might assume that additional fat could potentially lead to an excess production of adipokines in females. Nonetheless, based on the present data and existing literature, it is difficult to explore the potential clinical relevance of the observed sex-related differences within the context of inflammatory markers. These findings suggest that the influence of physical and puberty development should be considered when comparing levels

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of inflammatory markers in different populations (3) and when ultimately using these markers in clinical settings. Moreover, **all inflammatory markers differed considerably between the least and most favorable decile**. However, it is again difficult to explore the clinical relevance of these differences. On the other hand, there is growing evidence suggesting cut points for CRP in early detection of CVD risk. Applying cut-off values of CRP concentration (41) to distinguish between **low (<1mg/l) , moderate (1 to 3 mg/l) and high (>3mg/l) risk for future CVD, showed that, 83.3 % and 11.8 % and 4.9 % of the present population had values in these categories respectively.**

Associations between adiposity, aerobic fitness, muscle fitness and inflammatory markers

Paragraph Number 22 This study demonstrates that WC, aerobic fitness and muscle fitness are independently associated with CRP and leptin levels. Moreover, we observed lower levels of these markers among the children who had high fitness levels and higher levels among overweight children (data not shown). There was a clear graded association of WC, aerobic fitness and muscle fitness with the leptin and CRP levels. **For the other inflammation markers associations were weak (HGF, TNF-α and resistin) or not significant (IL-6 and PAI-1).**

Paragraph Number 23 The strong and positive association between adiposity and CRP in the present study is a consistent finding in the pediatric literature. We have showed earlier that individuals with a high WC have significantly higher CRP levels compared to a randomly selected control group (35). Moreover, several studies show that CRP is related to adiposity, independent of sex, age and pubertal status (6, 26, 40); indeed, BMI, WC and skinfold thickness have emerged as the main predictors for CRP levels in these studies.

Paragraph Number 24 However, there are other potentially important predictors. For example, recent studies have shown that both aerobic fitness and muscle fitness are potential predictors for CRP levels (14, 21, 23). In agreement with the present study, previous reports have demonstrated inverse associations between aerobic fitness and CRP levels among children. In most of these studies, however, the significant association of aerobic fitness was lost after adjusting for adiposity in the model. **Conversely**, the association between CRP and aerobic fitness persisted after adjusting for WC in the present study. There are some methodological differences that could partly explain the discrepancies in the findings of these studies. Firstly, except for the study by Cook et al.(6), the other studies have included relatively small populations (14, 21, 23). Secondly, with the exception of the Parret et al. study (23), our study is the only one that used directly measured oxygen consumption as a measure of aerobic fitness; the others estimated aerobic fitness based on a maximum cycle ergometer test (14, 30) or used the 20 meter shuttle run test (21). The direct measurement of oxygen consumption is considered the best physiological measure of aerobic fitness and might provide more accurate values compared to other methods. Consequently, the use of a valid and accurate method to assess aerobic fitness could explain the strong and independent relationship with CRP observed in the present study.

Paragraph Number 25 The association between CRP and muscle strength has not been extensively described in children. Ruiz et al. (29) found that the CRP level was negatively associated with muscle strength in a cross-sectional study of Spanish adolescents. However, after controlling for potential confounders, including aerobic fitness, body fat and fat-free mass, the association was only significant for those defined as overweight. In contrast, we observed a negative association between the CRP and muscle strength, even after controlling for other predictor variables. Some potential reasons for the discrepancies could be the difference in the sample sizes and the subjects' age between the two studies.

Paragraph Number 26 Although few studies have examined the relationship between leptin and aerobic and muscle fitness, the studies by Hosick et al. (13) and Martinez-Gomez et al. (20) have shown that leptin levels are associated with aerobic (13, 20) and muscle fitness (20) in children and adolescents. Hosick et al. (13) concluded that the relationship between leptin and aerobic fitness was dependent on weight status. Similarly, Martinez-Gomez reported that WC had the strongest correlation with leptin; however, Martinez-Gomez (20) showed that both aerobic and muscle fitness were inversely associated with leptin after controlling for WC. Our findings are in agreement with these studies. In the regression analyses, we observed that WC was the strongest predictor of leptin levels, and the relationship with muscle fitness was attenuated the most after adjusting for the other "predictor" variables. Nevertheless, the associations in the final regression model were significant, indicating that muscle fitness, aerobic fitness and WC have an independent association with leptin levels.

Paragraph Number 27 We have shown previously that both aerobic and muscle fitness are independently and negatively associated with metabolic risks in youth (33). Moreover, we know that CRP levels have been associated with early arterial changes in both healthy (16) and obese individuals (17), and leptin has been related to insulin resistance (35) and has been found to affect vessel walls (31). Consequently, one can assume that these two markers may have significant impact on the development of type 2 diabetes and CVD. As adiposity, muscle fitness and aerobic fitness are modifiable, our findings highlight the possible contribution these can make toward improving health in young people. Hence, the achievement of a healthy body composition and adequate aerobic and muscle fitness levels could be important in the prevention of type 2 diabetes and CVD. Therefore, we recommend the promotion of regular participation in physical activity that affects both aerobic capacity and muscle strength as the appropriate approach to public health. However, the present findings need to be further explored in future interventional and prospective studies.

Strengths and limitations

Paragraph Number 28 The main strengths of this study include i) the recruitment of a large, nationally representative sample of children and ii) the investigation of a number of inflammatory markers and the availability of objective measures of body composition, aerobic fitness and muscle fitness.

Paragraph Number 29 Our results should be interpreted with the understanding of some limitations. First, one weakness is the cross-sectional nature of the study. The exposures and outcomes were measured within the same time frame; thus, the results suffer from the lack of validity for a causal relationship. Second, any observational study may be subject to measurement bias and confounding. However, the measured variables were characterized by a high degree of measurement precision, as they were relatively stable over time and were obtained under standardized and controlled conditions. Third, the muscle fitness was measured by explosive strength, isometric strength and endurance strength only. Other tests could have been chosen, but the Eurofit test battery has been widely used for children and adolescents throughout Europe, and the tests are simple, practical and reliable.

Conclusions

Paragraph Number 30 In conclusion, this study presents national reference data on selected inflammatory markers in children. The study demonstrate that **9-year old** girls have higher levels of leptin, adiponectin and resistin and lower levels of TNF-α compared to boys. In addition, adiposity, aerobic and muscle fitness were independently associated with the CRP and leptin levels. Increasing aerobic fitness and muscle strength could be an appropriate strategy to achieve favorable changes in CRP and leptin levels. Future interventional and prospective studies examining the role of adiposity, aerobic and muscle fitness on cytokines are warranted.

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Table 1. Mean values (SD), medians and 95 % confidence intervals (CI) for population characteristics and inflammatory markers.

	Girls			Boys			
	Mean (SD)	Median	95% CI	Mean (SD)	Median	95% CI	P value*
Number (range)	427-495			471-556			
BMI (kg/m^2)	17.5 (2.7)	16.9	17.3-17.8	17.3 (2.5)	16.7	17.1-17.4	**0.043**
Waist circumference (cm)	63.2 (7.8)	61.8	62.6-63.9	62.1 (7.0)	61.0	61.5-62.6	**0.008**
VO$_{2peak}$ (ml·min^{-1}·kg^{-1})	42.9 (6.7)	43.0	42.3-43.5	48.2 (7.1)	48.3	47.6-48.7	**<0.001**
CRP (mg/l)	0.76 (1.23)	0.33	0.65-0.87	0.61 (1.02)	0.23	0.52-0.69	**<0.001**
TNF-α (pg/ml)	5.90 (2.37)	5.76	5.68-6.11	6.61 (4.54)	6.31	6.22-7.04	**0.017**
Leptin (ng/ml)	6.78 (8.26)	3.92	6.04-7.51	4.26 (4.23)	1.97	3.73-4.78	**<0.001**
Adiponektin (µg/ml)	4.15 (0.58)	4.27	4.01-4.20	4.09 (0.63)	4.20	4.04-4.15	**0.032**
PAI-1 (ng/ml)	78.8 (108.8)	64.7	69.2-88.4	80.7 (74.9)	69.1	74.6-86.8	0.229
IL-6 (pg/ml)	2.77 (7.62)	1.19	2.05-3.20	4.87 (22.7)	1.31	2.81-6.92	0.101
Resistin (pg/ml)	61.6 (43.0)	51.3	58.8-66.4	58.0 (40.2)	48.3	54.7-61.4	**0.021**
HGF (ng/ml)	0.69 (0.40)	0.61	0.66-0.73	0.76 (0.54)	0.67	0.71-0.80	0.234

BMI, body mass index; CRP, c-reactive protein; HGF, hepatic growth factor; IL-6, interleukin-6; PAI-1, plasminogen activator inhibitor-1; tumor necrosis factor-α, TNF-α; VO2peak (ml·min-1·kg-1) = aerobic fitness. *; P-value girls compared with boys. **Table displays untransformed means and medians.**

Table 2. Inflammatory markers in deciles

	Deciles									
	1	2	3	4	5	6	7	8	9	10
CRP (mg/l)	0.09	0.12	0.14	0.18	0.24	0.32	0.44	0.67	1.16	3.41
TNF-α (pg/ml)	2.17	4.14	4.80	5.34	5.75	6.28	6.79	7.37	8.19	11.94
Leptin (ng/ml)	0.47	0.89	1.30	1.79	2.32	3.19	4.45	6.67	10.37	23.34
Adiponektin (µg/ml)	2.86	3.50	3.78	3.99	4.15	4.29	4.43	4.56	4.70	4.94
PAI-1 (ng/ml)	26.7	37.7	45.6	53.2	61.4	71.3	81.2	94.6	116.1	210.4
IL-6 (pg/ml)	0.09	0.30	0.52	0.74	1.06	1.49	2.18	3.16	5.21	23.88
Resistin (pg/ml)	22.7	31.6	36.5	42.1	47.6	52.7	59.9	69.3	88.0	151.9
HGF (ng/ml)	0.18	0.35	0.45	0.52	0.60	0.68	0.79	0.91	1.11	1.70

CRP, c-reactive protein; HGF, hepatic growth factor; IL-6, interleukin-6; PAI-1, plasminogen activator inhibitor-1; tumor necrosis factor-α, TNF-α. **Table displays untransformed data.**

Table 3. Partial correlation (r) adjusted for pubertal stage between WC, VO_{2peak}, muscle fitness and inflammatory markers.

	Waist		VO_{2peak}		MF		CRP		Leptin		PAI-1		Adiponektin		HGF	
	Girls	*Boys*	*Girls*	*Boys*	*Girls*	*Boys*	*Girls*	*Boys*	*Girls*	*Boys*	*Girls*	*Boys*	*Girls*	*Boys*	*Girls*	*Boys*
Waist																
VO_{2peak}	-0.575**	-0.589**														
MF	-0.508**	-0.402**	0.530**	0.533**												
CRP	0.316**	0.289**	-0.298**	-0.310**	-0.284**	-0.268**										
Leptin	0.620**	0.597**	-0.418**	-0.570**	-0.372**	-0.427**	0.269**	0.369**								
PAI-1	0.044	0.007	-0.099*	-0.105*	-0.088	-0.039	0.095	0.093	0.090	0.084						
Adiponektin	0.241**	0.023	0.109*	-0.101*	-0.070	-0.078	-0.028	-0.014	-0.105*	0.026	0.395**	0,344**				
HGF	0.111*	0.090	-0.072	0.013	-0.091	-0.130*	0.111*	0.155*	0.169*	0.107*	0.089	0.065	-0.162*	0.194**		
IL-6	0.116*	0.021	-0.057	-0.031	-0.118*	-0.012	0.284**	0.192**	0.112*	0.079	0.023	-0.060	-0.049	-0.036	0.157*	0.162*
TNF-α	0.064	0.047	-0.016	-0.004	-0.108*	-0.188**	0.082	0.118*	0.122*	0.022	0.110*	0.107*	0.045	0.027	0.265**	0.365**
Resistin	-0.047	0.021	-0.036	-0.105*	-0.002	-0.025	0.143*	0.100*	0.017	0.028	0.502**	0.638**	0.467**	0.490**	0.023	0.027

CRP, c-reactive protein; HGF; hepatic growth factor; IL-6, interleukin-6; MF; muscle fitness; PAI-1, plasminogen activator inhibitor-1; TNF-α, tumor necrosis factor-α

*$P < 0.01$; **$P < 0.05$

Table 4. Associations between WC, aerobic fitness and muscle fitness with inflammatory markers

Predictor variable	CRP			Leptin		
	β	*P* Value	R^2	β	*P* Value	R^2
1 VO$_{2peak}$ (ml·min^{-1}·kg^{-1})	-0.338	**<0.001**	0.114	-0.555	**<0.001**	0.349
2 Muscle fitness	-0.284	**<0.001**	0.087	-0.388	**<0.001**	0.214
3 Waist (cm)	0.322	**<0.001**	0.101	0.602	**<0.001**	0.396
4 VO$_{2peak}$ (ml·min^{-1}·kg^{-1})	-0.190	**<0.001**		-0.298	**<0.001**	
Muscle fitness	-0.122	0.002		-0.064	0.036	
Waist (cm)	0.158	**<0.001**	0.147	0.406	**<0.001**	0.465

Data are standardized coefficients. Model 1-3 is adjusted for puberty. Model 4 is adjusted for all covariates plus WC, aerobic fitness and muscle fitness. R^2 values are displayed for each model
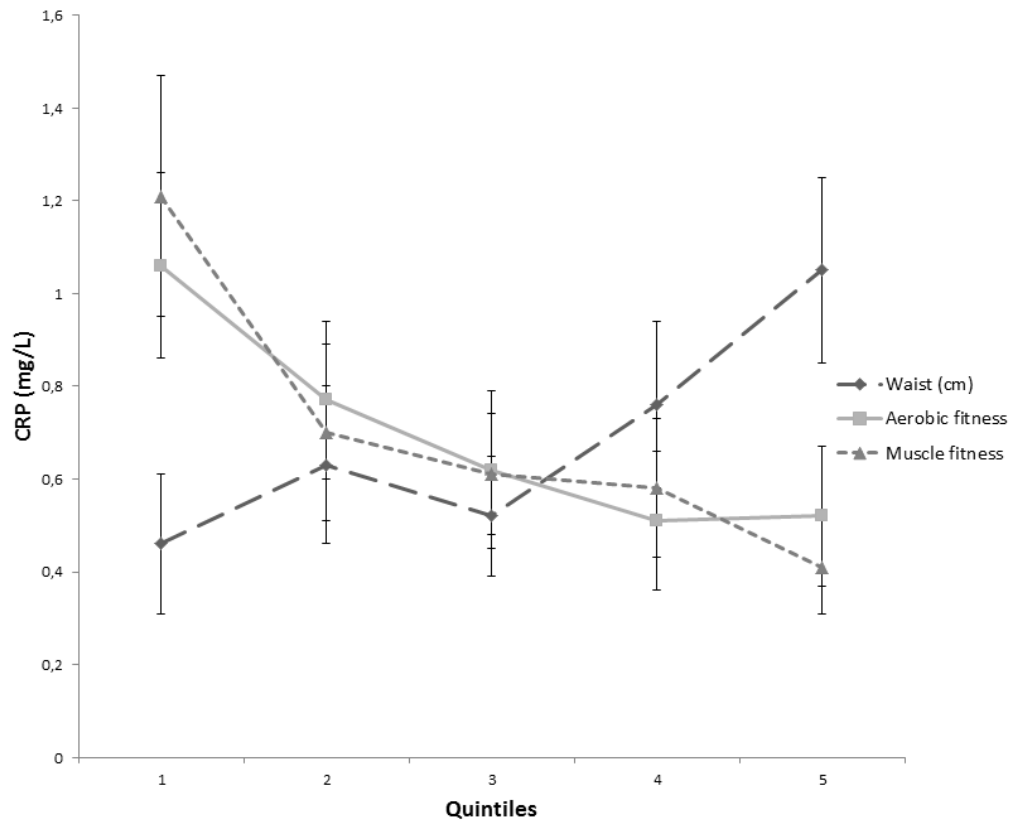
Figure 1. CRP levels in quintiles of WC, aerobic fitness and muscle fitness. A main effect of all predictor variables were observed across quintiles (p for all ≥0.001) with CRP levels increasing from low (Q1) to high (Q5) WC and decreasing from low (Q1) to high (Q5) aerobic fitness or muscle fitness. CRP; C-reactive protein, WC; waist circumference. **Figure displays untransformed CRP levels**.
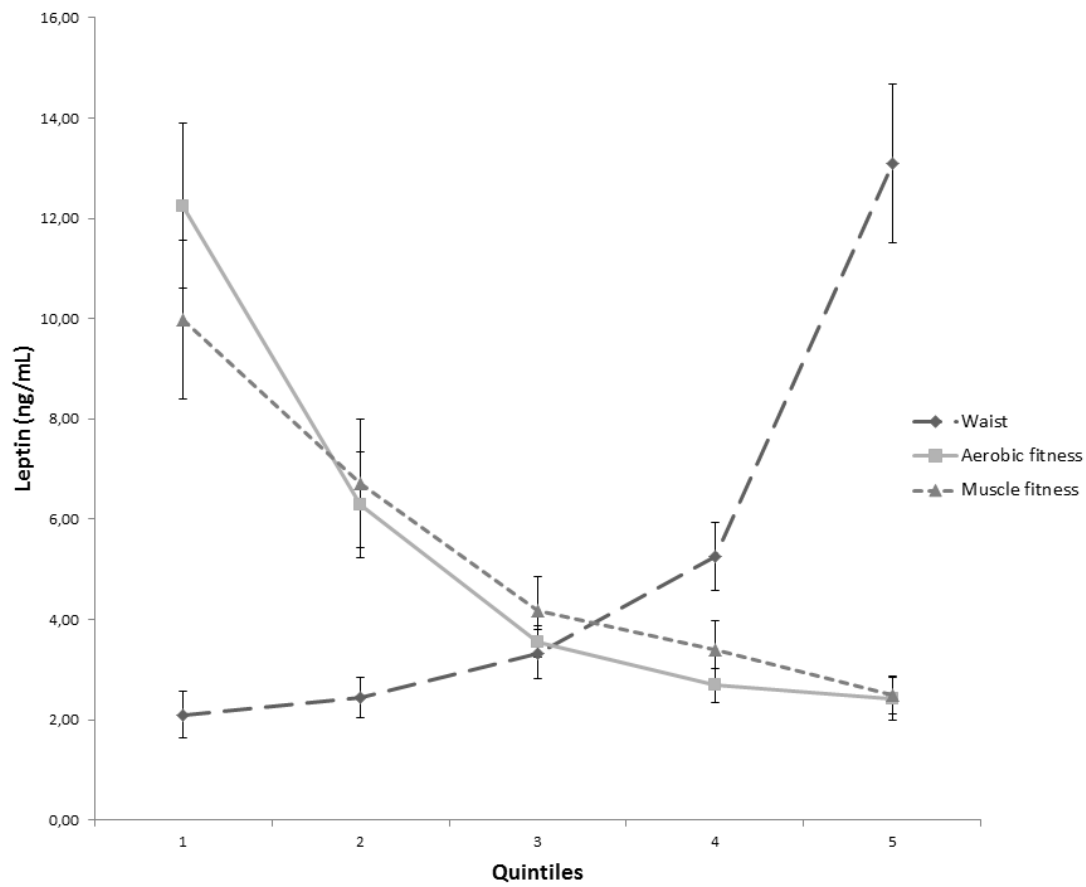
Figure 2. Leptin levels in quintiles of WC, aerobic fitness and muscle fitness. A main effect of all predictor variables were observed across quintiles (p for all ≥0.001) with leptin levels increasing from low (Q1) to high (Q5) WC and decreasing from low (Q1) to high (Q5) aerobic fitness or muscle fitness. WC; waist circumference. **Figure displays untransformed leptin levels**.