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Negative correlation between serum IL-6 level and cardiorespiratory fitness in 10- to 11-year-old boys with increased BMI

Abstract

Background/Aims: Some markers of inflammation have been found to be associated with cardiorespiratory fitness levels, but only few studies have studied this in overweight children. The aim of this study was to investigate associations between markers of inflammation and the fitness levels measured by peak oxygen consumption (VO$_{2_{peak}}$ and VO$_{2_{peak}}$/kg) in boys with increased body mass index (BMI) and with normal BMI.

Participants/Methods: Subjects were 38 boys with BMI above 85th percentile (OWB) and 38 boys with normal BMI (NWB) at the age of 10 to 11 years. Serum concentrations of IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFNγ, TNF-α, IL-1α, IL-1β, MCP-1, EGF, CRP and associations with measured cardiorespiratory fitness levels were studied. High-sensitive chips were used to measure 13 markers of inflammation.

Results: Mean VO$_{2_{peak}}$ was significantly higher (2.1±0.3 vs. 1.8±0.3 L/min; p<0.05) and mean VO$_{2_{peak}}$/kg significantly lower (33.7±4.7 vs. 48.9±6.4 mL/min/kg; p<0.05) in OWB than in NWB group. Out of 13 measured biochemical markers IL-6 correlated with VO$_{2_{peak}}$/kg (r = –0.37; p<0.05) and TNF-α with VO$_{2_{peak}}$ (r = 0.41; p<0.01) in OWB. BMI and IL-6 together explained 44.5% of the variability of VO$_{2_{peak}}$/kg in the OWB group.

Conclusions: Overweight boys had lower cardiorespiratory fitness level measured by VO$_{2_{peak}}$/kg and this was negatively correlated with serum IL-6 level. Measurement of serum IL-6 level in overweight boys may help to identify subjects who need specific exercise formats to achieve maximal beneficial health effects and to reduce their risk for the development of type 2 diabetes and atherosclerosis later in life.

Keywords: inflammation; obesity; oxygen consumption; prepubertal boys.

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Introduction

Different studies have reported that childhood obesity is associated with risk factors for cardiovascular diseases and atherosclerotic processes (1, 2). In addition, inflammatory process plays an important role in the pathogenesis of atherosclerosis (3). According to our previous study (4), many biochemical markers of low-grade inflammatory processes are already involved in the development of obesity in childhood, as well as in the early phase of atherosclerosis (5). Ridker et al. (6) found that inflammatory factors including CRP and IL-6 are important risk factors for atherosclerosis that could predict future cardiovascular diseases. High-level inflammatory markers are predictors for other diseases like the development of type 2 diabetes and obesity in adults (7). Some investigators have found that greater cardiorespiratory fitness is associated with a lower risk of different cardiovascular diseases in children and adolescents (8, 9).

Cardiorespiratory fitness shows the ability of a subject to perform aerobic exercise, and peak oxygen consumption (VO$_{2_{peak}}$) is an accurate measure of aerobic capability to characterize cardiovascular fitness (10). Many studies have shown that cardiorespiratory fitness level in children is best determined by VO$_{2_{peak}}$ (10, 11). The absolute VO$_{2_{peak}}$ (L/min) increases steadily with age in normal-weight and also in obese children and adolescents (12). In contrast to the normal-weight group, no age differences were seen in VO$_{2_{peak}}$ expressed to their body mass, i.e., relative
Subjects and methods

This cross-sectional study included 76 Estonian schoolboys aged 10 to 11 years who were recruited from different local schools in Tartu City (n=59) and Tartu County (n=17) where physical-education lessons were held twice a week. The subjects were divided into two groups: 38 boys with BMI at least above the 85th percentile, without any additional chronic diseases [overweight boys (OWB)], and another 38 boys with BMI <85th percentile [normal-weight boys (NWB)]. Majority (n=36) of the boys in the OWB group were obese, i.e., their BMI was >95th percentile. The OWB group was further divided into three subgroups: (1) the low OWB group, consisting of 14 boys with BMI <25.5 (23.97±0.88); (2) the middle OWB group, 13 boys with BMI between 25.5 and 29.1 (26.98±1.04); and (3) the high OWB group, 11 boys with BMI >29.1 (32.02±2.53). Thereby every boy in the OWB group was matched by a boy from the NWB by age and their everyday physical activity level. All boys were on their ordinary everyday diet. Pubertal development was assessed by self-report, using an illustrated questionnaire according to the Tanner classification. All studied boys were recruited as part of a larger study. The study was approved by the Medical Ethics Committee of the University of Tartu (Estonia), and parents signed an informed consent form.

Anthropometry and body composition

The body height was measured to the nearest 0.1 cm using a Martin metal anthropometer, and body mass (medical electronic scale, A & D Instruments, Ltd., UK) was measured to the nearest 0.05 kg. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m²).

Maximal exercise testing

Cardiorespiratory fitness was determined by a stepwise incremental exercise test until volitional exhaustion performed on an electrically braked bicycle ergometer (Corival V3, Lode, Netherlands). Initial work rate was 50 W and was incremented by 25 W after every 3 min until volitional exhaustion. Pedaling frequency was set to 60–70 rpm. Subjects were verbally encouraged to produce maximal effort. Respiratory gas exchange variables were measured throughout the test using breath-by-breath mode with data being recorded in 10-s intervals. During all tests, the subjects breathed through a facemask. Oxygen uptake (VO₂), carbon dioxide output, and minute ventilation were continuously measured using a portable open-air spirometry system (MetaMax I, Cortex, Leipzig, Germany). The analyzer was calibrated with gases of known concentration before the test according to the manufacturer’s guidelines. All data were calculated by means of computer analysis using standard software (MetaMax-Analyysis 3.21, Cortex). VO₂peak (L/min) was measured and VO₂peak per kilogram of body mass (VO₂peak/kg) was calculated.

Blood analysis

Blood samples were obtained from a vein of the subjects after an overnight fast and before breakfast, between 8 a.m. and 9 a.m. The blood serum was separated and then frozen at −20°C for later analysis. Markers of inflammation, IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFNγ, TNFα, IL-1α, IL-1β, MCP-1, EGF, and CRP, using Evidence® Biochip Technology (Randox Laboratories Ltd., UK), were measured from fasting serum samples and high-sensitive chips were used. There were no detection limits or sensitivity problems with any measured
markers of inflammation. The Cytokine and Growth Factors High-Sensitivity Array of the Biochip system was used for simultaneous quantitative detection of multiple, related cytokine immunoassays (in parallel) from a single sample, and no the inflammation markers were below detection limits. For all measured markers, intra-assay precision was 5.1–8.5% and inter-assay precision was more than 5.8% and <9.9%.

**Statistical analysis**

Statistical analysis was performed using SPSS for Windows v. 15.0 (SPSS, Chicago, IL, USA). To calculate the mean values and standard deviations, standard statistical methods were used. The Shapiro-Wilk test was used, and non-normally distributed variables were log transformed. Differences between the OWB and NWB groups were measured by an unpaired, independent two-tailed t-test. Relationships between inflammation markers and VO$_{2\text{peak}}$ and VO$_{2\text{peak}/kg}$ were assessed by Pearson correlation analysis. Stepwise multiple regression analysis was performed to determine the independent effect of the 13 inflammation parameters and BMI on VO$_{2\text{peak}}$ and VO$_{2\text{peak}/kg}$. The level of significance was set at a p-value of <0.05.

**Results**

The clinical characteristics of the subjects are presented in Table 1. As expected, mean VO$_{2\text{peak}}$ was significantly higher and VO$_{2\text{peak}/kg}$ significantly lower in OWB than in NWB. Significantly higher serum IL-6 (1.1±0.6 vs. 0.8±0.3 pg/mL, p<0.001), IL-8 (9.5±3.6 vs. 7.7±2.9 pg/mL, p<0.05), IFN$\gamma$ (2.2±1.2 vs. 1.5±1.0 pg/mL, p<0.05), MCP1 (212.8±58.0 vs. 165.8±67.9 pg/mL, p<0.005), and CRP (2.3±2.1 vs. 1.0±0.2 mg/L, p<0.001) levels were observed in the OWB group compared to the NWB group (4).

The Pearson correlations between 13 inflammatory parameters and VO$_{2\text{peak}}$ showed that, in the OWB group, only serum TNF$\alpha$ concentration correlated positively with VO$_{2\text{peak}}$, while IL-6 correlated negatively (p<0.05) with VO$_{2\text{peak}/kg}$ (Table 2; Figure 1). In the total group, there were more significant correlations (Table 2).

The stepwise multiple regression analysis showed that BMI and IL-6 together explained 44.5% of the variability of VO$_{2\text{peak}}$ and TNF$\alpha$ 13.8% of the variability of VO$_{2\text{peak}}$ in the OWB group (Table 3). The stepwise multiple regression analysis in the NWB group did not identify any significant determinants of VO$_{2\text{peak}}$ or VO$_{2\text{peak}/kg}$.

**Discussion**

The results of our study showed that absolute VO$_{2\text{peak}}$ was significantly higher and relative VO$_{2\text{peak}}$ per kilogram body

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**Table 1** Clinical characteristics of the subjects.

<table>
<thead>
<tr>
<th></th>
<th>NWB (n=38)</th>
<th>OWB (n=38)</th>
<th>Total (n=76)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>11.0±0.8</td>
<td>11.2±0.7</td>
<td>11.1±0.7</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>146.0±7.5$^*$</td>
<td>153.2±7.8</td>
<td>149.5±8.4</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>36.8±5.6$^*$</td>
<td>64.5±12.0</td>
<td>50.3±16.7</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>17.2±1.7$^*$</td>
<td>27.3±3.6</td>
<td>22.1±5.8</td>
</tr>
<tr>
<td>VO$_{2\text{peak}, \text{L/min}}$</td>
<td>1.8±0.3$^*$</td>
<td>2.1±0.3</td>
<td>2.0±0.4</td>
</tr>
<tr>
<td>VO$_{2\text{peak}, kg, mL/(min kg)}$</td>
<td>48.9±6.4$^*$</td>
<td>33.7±4.7</td>
<td>41.1±9.4</td>
</tr>
</tbody>
</table>

Values are shown as mean±SD. $^*$p<0.05.

**Table 2** Pearson correlation coefficients between serum biochemical markers and VO$_{2\text{peak}}$ and VO$_{2\text{peak}/kg}$ in the OWB group (n=38), NWB group (n=38), and total group (n=76).

<table>
<thead>
<tr>
<th></th>
<th>NWB</th>
<th>OWB</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2 (pg/mL)</td>
<td>0.016</td>
<td>0.062</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>-0.149</td>
<td>0.170</td>
<td>0.242$^*$</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>0.046</td>
<td>0.105</td>
<td>0.242$^*$</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>-0.075</td>
<td>0.163</td>
<td>0.181</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>-0.103</td>
<td>0.205</td>
<td>0.103</td>
</tr>
<tr>
<td>VEGF (pg/mL)</td>
<td>-0.093</td>
<td>-0.141</td>
<td>-0.066</td>
</tr>
<tr>
<td>IFN$\gamma$ (pg/mL)</td>
<td>0.028</td>
<td>0.261</td>
<td>0.264$^*$</td>
</tr>
<tr>
<td>TNF$\alpha$ (pg/mL)</td>
<td>-0.163</td>
<td>0.405$^*$</td>
<td>0.146</td>
</tr>
<tr>
<td>IL-1$\alpha$ (pg/mL)</td>
<td>-0.203</td>
<td>0.171</td>
<td>0.172</td>
</tr>
<tr>
<td>IL-1$\beta$ (pg/mL)</td>
<td>-0.156</td>
<td>0.232</td>
<td>0.131</td>
</tr>
<tr>
<td>MCP1 (pg/mL)</td>
<td>-0.064</td>
<td>0.054</td>
<td>0.147</td>
</tr>
<tr>
<td>EGF (pg/mL)</td>
<td>0.055</td>
<td>0.028</td>
<td>0.102</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.169</td>
<td>0.221</td>
<td>0.368$^b$</td>
</tr>
</tbody>
</table>

$^*$p<0.05. $^b$p<0.01.
mass significantly lower in the OWB group than in the NWB group. We also found that cardiorespiratory fitness characterized by VO\textsubscript{2peak} (mL/min/kg) was negatively correlated with serum IL-6 concentration, while in the OWB group VO\textsubscript{2peak} (L/min) was positively correlated with serum TNF\textalpha level.

The low-grade systemic inflammation was associated with the body composition values, especially with the amount of fat mass in obese children (16). Proinflammatory cytokines, such as IL-6 and TNF\textalpha, were also associated with obesity characteristics (17). In our study, we found that, higher IL-6 was associated with lower cardiorespiratory fitness expressed as VO\textsubscript{2peak}/kg in the OWB group (see Figure 1). This is similar to the study by Gaeini et al. (10) where IL-6 was negatively correlated with VO\textsubscript{2peak}/kg in a group of boys with normal and increased BMI, whereas no such correlation was found within the OWB group. This was probably due to the fact that the number of overweight subjects (aged 11–14 years) in that study was quite small: only 10 boys. Kullo et al. (15) found, similarly to our study, that serum IL-6 concentration was negatively associated with VO\textsubscript{2peak}/kg in a group of men (BMI from 22.4 to 44.0 kg/m\textsuperscript{2}). Pischon et al. (18) found that healthy men and women with lower physical activity level had higher levels of IL-6 and CRP. In the Kullo et al. (15) study, reduced cardiorespiratory fitness was correlated with increased IL-6 and CRP. In addition, Gaeini et al. (10) suggested that emphasis on physical activity and cardiovascular fitness in children is an important factor for prevention of atherosclerosis in adulthood. It is known that IL-6 may be a predictor for the development of type 2 diabetes (7). In a multiple regression analysis, 44.5% of the variability of VO\textsubscript{2peak}/kg in OWB was determined by BMI and IL-6 together. These results suggest that increased serum IL-6 concentration may be a useful biochemical marker for identifying subjects who need specific exercise formats to achieve maximal beneficial health effects from exercise and, in the long-term, can reduce their risk for the development of type 2 diabetes and atherosclerosis. The exercises that suit the overweight children with high serum IL-6 concentrations and therefore with low cardiorespiratory fitness levels are regular aerobic exercises like walking and jogging (19). In addition, strength training with relatively low intensity is also recommended in overweight and obese subjects (20). Whereas exercises that usually require high cardiorespiratory fitness level such as high-intensity exercises are not suitable for overweight children (21).

Another finding of our study was that serum TNF\textalpha concentration was positively correlated with absolute VO\textsubscript{2peak} (L/min) in overweight boys. These results are similar to the study by Nemeth et al. (11), who found that, in 12- to 14-year-old Hispanic and Asian-American normal- and overweight children, cardiorespiratory fitness measured by VO\textsubscript{2peak} was positively correlated with TNF\textalpha. In both studies, TNF\textalpha

Table 3. Results of stepwise multiple regression analysis (see Statistical analysis section)

<table>
<thead>
<tr>
<th>Group</th>
<th>NW</th>
<th>OW</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO\textsubscript{2peak}/kg</td>
<td>BMI</td>
<td>38%</td>
</tr>
<tr>
<td></td>
<td>BMI, IL-6</td>
<td>44.5%</td>
</tr>
<tr>
<td>VO\textsubscript{2peak}</td>
<td>TNF\textalpha</td>
<td>13.8%</td>
</tr>
</tbody>
</table>

R\textsuperscript{2}×100 is shown describing the percentage of variability of VO\textsubscript{2peak}/kg or VO\textsubscript{2peak} as explained by dependent variables.
level was higher in subjects with higher body fat and with higher muscle mass. Muscle mass has been found to be the major determinant of cardiorespiratory fitness (11). In other words, subjects with increased BMI and muscle mass have also higher absolute cardiorespiratory fitness values and higher serum TNFα level. In addition, TNFα is produced by adipose tissue and is associated with the development of obesity complications such as diabetes and atherosclerosis (22, 23). Thus, children with higher BMI and bigger muscle mass have a tendency to have higher serum TNFα levels and this, in turn, results in higher absolute VO2peak and lower relative VO2peak/kg per body mass (11). In the long-term, overweight subjects have increased risk of developing diabetes and atherosclerosis and therefore the increased levels of TNFα may play a role in the pathogenesis of these complications (11, 22, 23).

In our study, cardiorespiratory fitness was measured by absolute VO2peak and relative VO2peak/kg per body mass. The results of our study showed that VO2peak was significantly higher and VO2peak/kg significantly lower in the OWB group. These results are similar to the studies by Eliakim et al. (14), Gaeini et al. (10), and Rosa et al. (13), where a lower VO2peak/kg was found in overweight children compared to the children with normal weight.

Of the 13 biochemical markers measured in our study, only IL-6 was significantly correlated with cardiorespiratory fitness relative to body mass as assessed by VO2peak/kg in the OWB group. No significant correlations were found between other inflammation markers and VO2peak/kg in the OWB group. To our best knowledge, no studies have previously looked at the associations between such a complex of biochemical markers and cardiorespiratory fitness in overweight children. Eliakim et al. (14) studied the association between serum IL-6 concentration and VO2peak in children aged 8 to 17 years, but did not find a significant correlation between these parameters. Therefore, it is likely that other measured markers of inflammation in this study are not associated with cardiorespiratory fitness, at least in children.

In summary, overweight boys had lower cardiorespiratory fitness level as measured by VO2peak/kg and this was negatively correlated with serum IL-6 level. Measurement of serum IL-6 level in overweight children may help to identify subjects who need specific aerobic exercise formats and levels to achieve maximal beneficial health effects and to reduce their risk for the development of type 2 diabetes and atherosclerosis later in life.

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Conflict of interest statement

Disclosure statement: The authors have nothing to disclose.

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