The associations between peak $O_2$ consumption and leptin in 10- to 12-year-old boys
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Summary

The aim of this study was to assess the associations of circulating levels of leptin with the peak $O_2$ consumption (VO$_{peak}$) in 10- to 12-year-old boys of different BMI selected by Cole et al. (BMJ, 320,2000,1–6): total group ($n = 248$), normal ($n = 190$), overweight ($n = 34$) and obese ($n = 24$). We hypothesized that there is a close relationship in overweight and obese subgroups of boys with relative VO$_{peak}$ kg$^{-1}$ (ml min$^{-1}$ kg$^{-1}$) and leptin. Most of the subjects were Tanner stage 2. Peak $O_2$ consumption was measured directly using an increasing incremental protocol until volitional exhaustion on an electronically braked cycle ergometer. The expired gas was sampled continuously breath-by-breath mode for the measurement of oxygen consumption (MetaMax, Germany). Blood samples were obtained after an overnight fast from an antecubital vein for leptin measurements. Peak $O_2$ consumption ($l$ min$^{-1}$) was higher or lower (ml min$^{-1}$ kg$^{-1}$) in overweight and obese groups, compared with normal BMI group. Leptin was higher in overweight and obese groups, compared with normal BMI groups. Peak $O_2$ consumption ($l$ min$^{-1}$) correlated significantly with leptin only in total group ($n = 248, r = 0.196$). Contrary, relative VO$_{peak}$ kg$^{-1}$ correlated significantly and negatively with leptin. The relationship was highest on the total group ($r = -0.674$). We can conclude that leptin first of all correlated negatively with relative peak $O_2$ consumption. Absolute VO$_{peak}$ correlated with leptin only in total group.

Introduction

Leptin is an adipocyte derived 16-kDa hormone, produced by white adipose cells, that, among other functions, has the capacity to regulate appetite, acting on the central nervous system receptors probably through its action on the neuropeptide Y (Kraemer et al., 2002). It is a major regulator in the organism of energy uptake and homeostasis (Kraemer et al., 2002). Leptin expression is greater in subcutaneous than in visceral adipose tissue (Moro et al., 1998).

In mice, it was proven that leptin deficiency predisposed to worsening neuro mechanical upper airway function and administration of leptin in leptin-deficient mice increased minute ventilation, suggesting that its central effect in the leptin-deficient mice model may be attributable to a generalised increase in ventilator drive. Further, both obesity and leptin deficiency were associated with elevations in passive air resistance in respiratory tract and with marked decreases in active pharyngeal neuromuscular responses (Polotsky et al., 2012). Inflammatory processes mediated by other adipokynes can contribute to worsening the respiratory functions in obese (Alesandrova, 2012).

While leptin is being produced mainly by subcutaneous fat tissue, hexogen leptin in deficient subjects may stimulate oxidative phosphorylation, mitochondrial biogenesis and insulin signalling, the net effects of which may result in improvements in aerobic exercise capacity and metabolic homeostasis (Miller et al., 2001). Contrary, hyperleptinemia in obese may reflect resistance to leptin at a cellular level and thus a decline in aerobic capacity (Franks et al., 2007).

Some studies exist considering energy expenditure at rest rather than peak $O_2$ consumption (VO$_{peak}$) in association with leptin levels (Nagy et al., 1997; Salbe et al., 1997; Bishop, 1999), as higher energy expenditure at rest is known to be positively related to VO$_{peak}$ (Bishop, 1999). These studies can be helpful in understanding the leptin/VO$_{peak}$ association. In prepubertal children, Nagy et al. (1997) did not find any direct effect of leptin and no indirect effect of fat mass (through leptin) on any measure of energy expenditure at rest. While in contrast, Salbe et al. (1997) found in a sample of 123 five-year-old Pima Indian children, a significant correlation between rest energy expenditure measured by the doubly labelled water method and leptin levels. These differences can be explained with the presence of females in the Salbe...
et al. (1997) study, who are known to have higher levels of leptin than males. However, probably the difference in physical activity, body composition, eating habits, etc. may influence the relationship between energy expenditure and leptin.

Studies on the relationship between leptin and VO_{2peak} in adults show a positive correlation (Ostlund et al., 1996; Miller et al., 2001). Very limited studies exist on the relationships between leptin and VO_{2peak} in children and adolescents (Roemmich et al., 1998). Roemmich et al. (1998) in a sample of 16 prepubertal and 13 pubertal normal and overweight boys did not find this relationship. Also, leptin levels are known to increase with puberty in the Roemmich et al. (1998) study.

The relationship between leptin and VO_{2peak} is not completely clear in children of different BMI, because the existing previous studies produced conflicting results, failing to found an association in normal subjects (Roemmich et al., 1998; Hosick et al., 2010) or were conducted with normal and overweight children, but not with obese children (Hosick et al., 2010).

We hypothesized that there is a negative relationship in overweight and obese subgroups of boys with relative VO_{2peak} (ml min^{-1} kg^{-1}) and leptin.

The aim of this study was to assess the associations of circulation levels of leptin with the peak O_{2} consumption in 10–12-year-old boys with different BMI.

**Material and methods**

Subjects of this study were a cross sectional sample of 248 healthy boys (age 10–12 years) from different schools from the city of Tartu and surroundings (Estonia). They all were included into the routine physical education classes at school. This study is a part of a larger longitudinal project investigating the metabolic syndrome risk factors in boys during pubertal development. Almost all boys in particular class whose parents agreed were tested. This study was approved by the Medical Ethics Committee of the University of Tartu (Estonia). The subjects were considered as a whole group and also divided according to Cole et al. (2000) into three body mass index (BMI, kg m^{-2}) subgroups as normal (<19.8–21.9, n = 190), overweight (≥19.8–21.9 and ≥24–26.8, n = 34) and obese (<24–26.8, n = 24).

Body height was measured using a Martin metal anthropometer to the nearest 0.1 cm with a standard technique. Body mass was measured with minimal clothing to the nearest 0.05 kg using a medical electronic scale (AKD instruments, Abingdon, UK), and BMI was calculated as body mass (kg) divided by height squared (m^{2}). Pubertal development of the participants was assessed based on self-assessment using an illustrated questionnaire of pubertal stages according to Tanner classification method (Tanner, 1962).

Peak O_{2} consumption was measured directly using an incremental exercise test protocol until volitional exhaustion on an electronically braced cycle ergometer (Corival V3, Lode, Netherlands). Initial work rate was 60 W and increments 25 W after every 3 min until volitional exhaustion. Pedalling rate was set 70 rpm. Subjects were verbally encouraged to produce the maximal effort. The expired gas during cycle ergometer test was sampled continuously in breath-by-breath mode for the measurement of O_{2} consumption using a portable open circuit spirometry system (MetaMax 3B Cortex, Leipzig, Germany). All data were calculated by means of computer analysis using standard software (MetaMax-Analysis 3-21, Cortex, Leipzig, Germany). Peak oxygen uptake (VO_{2peak} 1 min) was measured, and VO_{2peak} per kilogram of body mass was calculated. VO_{2peak} consumption values were considered acceptable when two of the following three criteria were met (Petterson et al., 2001): (i) VO_{2} plateau defined as a failure of oxygen uptake to increase by greater than 2.0 ml kg^{-1} min^{-1} with increased test load, (ii) Heart rate ≥95% from the predicted individual maximum (formula 220–age) and/or (iii) respiratory exchange ratio ≥1.05.

To determine the concentration of leptin, blood samples were obtained after an overnight fast from an antecubital vein with the participant in the sitting position between 8:00 and 9:00 a.m. The blood serum was separated and frozen at −80°C for later analysis. Leptin concentrations were determined by an Elisa sandwich method using a kit from Medignost (GmbH, Reutlingen, Germany). The intra- and inter-assay CV-s were less than 10%.

Statistical analysis was performed with SPSS 18.0. Normal distribution of data was controlled, and data which were not normally distributed were log-transformed. Descriptive statistics (mean ± SD) were calculated. Differences between groups were analysed using ANOVA (LSD post hoc). Partial correlation was used to find relationships between leptin concentration and peak O_{2} consumption controlling for age and pubertal status. Stepwise regression analysis was performed to find out which parameter of peak oxygen consumption (l min^{-1} or ml min^{-1} kg^{-1}) affects leptin concentration most after controlling for age and pubertal status. Leptin was inserted in the model as dependent parameter, and VO_{2peak} and VO_{2peak} kg^{-1} as independent parameters. The level of significance was at P<0.05 for all statistical analysis.

**Results**

Mean anthropometric parameters, Tanner stages, leptin and peak O_{2} consumption in different groups are presented in Table 1. There were not any significant (P>0.05) differences between groups in mean age, Tanner stage and body height. Body mass and BMI were higher in overweight and obese groups compared with normal BMI group. Peak O_{2} consumption was significantly higher (l min^{-1}) or significantly lower (ml min^{-1} kg^{-1}) in overweight and obese groups, respectively (Table 1). Leptin was significantly higher (P<0.05) in overweight and obese groups compared with normal BMI group. All the subjects were about Tanner stage 2 (Table 1).
**Table 1** Mean (± SD) anthropometrical parameters, Tanner’s stage, peak O\(_2\) consumption and blood leptin concentration in boys.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total group (n = 248)</th>
<th>Normal BMI group (n = 190)</th>
<th>Overweight (n = 34)</th>
<th>Obese (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>11.18 ± 0.65</td>
<td>11.13 ± 0.65</td>
<td>11.12 ± 0.56</td>
<td>11.36 ± 0.73</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>149.5 ± 7.7</td>
<td>148.6 ± 7.5</td>
<td>151.6 ± 6.4*</td>
<td>155.1 ± 8.4*</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>43.3 ± 11.5</td>
<td>38.1 ± 5.9</td>
<td>52.5 ± 5.7*</td>
<td>69.4 ± 11.6*</td>
</tr>
<tr>
<td>BMI (kg m(^{-2}))</td>
<td>19.2 ± 4.3</td>
<td>17.1 ± 1.6</td>
<td>22.7 ± 1.5*</td>
<td>29.2 ± 3.2*</td>
</tr>
<tr>
<td>Leptin (ng ml(^{-1}))</td>
<td>7.63 ± 10.92</td>
<td>3.38 ± 4.10</td>
<td>13.71 ± 8.14*</td>
<td>32.61 ± 13.91*</td>
</tr>
<tr>
<td>VO(_{2})peak (l min(^{-1}))</td>
<td>1.96 ± 0.33</td>
<td>1.91 ± 0.32</td>
<td>2.08 ± 0.29*</td>
<td>2.21 ± 0.31*</td>
</tr>
<tr>
<td>VO(_{2})peak kg(^{-1}) (ml kg(^{-1}) min(^{-1}))</td>
<td>47.02 ± 8.34</td>
<td>50.05 ± 6.20</td>
<td>40.50 ± 5.72*</td>
<td>32.38 ± 4.30*</td>
</tr>
<tr>
<td>Tanner stage (1/2/3/4/5)</td>
<td>2.0 ± 0.652/</td>
<td>1.9 ± 0.746/</td>
<td>2.2 ± 0.64/</td>
<td>2.1 ± 0.52/</td>
</tr>
<tr>
<td></td>
<td>154/40/2/0</td>
<td>115/27/2/0</td>
<td>21/9/0/0</td>
<td>18/4/0/0</td>
</tr>
</tbody>
</table>

BMI, body mass index.
*Significantly different from normal group; P<0.05.
#Significantly different from overweight group; P<0.05.

Partial correlations were age and Tanner stage was eliminated between leptin and VO\(_{2}\)peak are presented in Table 2. It is interesting to note that the absolute peak O\(_2\) consumption (l min\(^{-1}\)) correlated significantly with leptin only in total group. In different BMI subgroups, the relationship was no longer significant (P>0.05). Contrary, relative VO\(_{2}\)peak (ml min\(^{-1}\) kg\(^{-1}\)) correlated highly with leptin. The negative relationships were the highest in the total group (Table 2, r = 0.674). In all subgroups, this relationship was significant too (P<0.05).

In Table 3, the results of the regression analysis are presented. The relationship between leptin and VO\(_{2}\)peak (both l min\(^{-1}\) and ml min\(^{-1}\) kg\(^{-1}\)) was very high only in the total group (n = 248; 53.0%, R\(^2\) × 100, Table 3) the group consists boys with different BMI. In normal weight, overweight and obese group, only VO\(_{2}\)peak has important relationship with leptin (7-3%, 35-8% and 24-2%, respectively, R\(^2\) × 100).

**Table 2** Partial correlations (controlling for age and Tanner stage) between leptin and peak O\(_2\) consumption in boys.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total group (n = 248)</th>
<th>Normal BMI group (n = 190)</th>
<th>Overweight (n = 34)</th>
<th>Obese (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO(_{2})peak (l min(^{-1}))</td>
<td>0.196**</td>
<td>-0.017</td>
<td>-0.155</td>
<td>-0.144</td>
</tr>
<tr>
<td>VO(_{2})peak kg(^{-1}) (ml kg(^{-1}) min(^{-1}))</td>
<td>-0.674***</td>
<td>-0.247**</td>
<td>-0.464**</td>
<td>-0.468*</td>
</tr>
</tbody>
</table>

BMI, body mass index.
P<0.05.
**P<0.01.
***P<0.001.

**Table 3** Regression analysis with VO\(_{2}\)peak (l min\(^{-1}\)) and VO\(_{2}\)peak kg\(^{-1}\) (ml kg\(^{-1}\) min\(^{-1}\)) as independent variables and leptin as the dependent variable after controlling for age and pubertal status.

<table>
<thead>
<tr>
<th>Model</th>
<th>R(^2) × 100</th>
<th>(\beta)</th>
<th>P</th>
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<tbody>
<tr>
<td>Total group (n = 248)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO(_{2})peak (l min(^{-1}))</td>
<td>53.0</td>
<td>0.314</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VO(_{2})peak kg(^{-1}) (ml kg(^{-1}) min(^{-1}))</td>
<td>-0.705</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Normal BMI group (n = 190)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO(_{2})peak (ml kg(^{-1}) min(^{-1}))</td>
<td>7.3</td>
<td>-0.247</td>
<td>0.001</td>
</tr>
<tr>
<td>Overweight (n = 34)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO(_{2})peak (ml kg(^{-1}) min(^{-1}))</td>
<td>35.8</td>
<td>-0.422</td>
<td>0.007</td>
</tr>
<tr>
<td>Obese (n = 24)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO(_{2})peak (ml kg(^{-1}) min(^{-1}))</td>
<td>24.2</td>
<td>-0.487</td>
<td>0.028</td>
</tr>
</tbody>
</table>

BMI, body mass index.

**Discussion**

Our results indicate that leptin was significantly related to relative VO\(_{2}\)peak (ml min\(^{-1}\) kg\(^{-1}\)) in all groups. The negative relationships in obese groups were higher than in normal BMI group. However, the relationships were strongest in total group (53.0%, R\(^2\) × 100). The relationship with peak O\(_2\) consumption (l min\(^{-1}\)) was not significant, except in total group.

The mean values of VO\(_{2}\)peak kg\(^{-1}\) were relatively high in the subjects of the current study. For example, in our total group, the mean value was higher than recommended cut-offs (in 8 to 11-year-old boys 43.6 ml min\(^{-1}\) kg\(^{-1}\)) by Adegbuyoye (2011) and 47.02 ± 8.34 ml min\(^{-1}\) kg\(^{-1}\) (Table 1). Our mean results are higher in obese group compared with slightly higher BMI (32.9 ± 4.8 kg m\(^{-2}\)) obese children by Andreacci et al. (2005). On the other side, cardiorespiratory fitness is regarded as important marker of boys health, because its effect on association with obesity (Ortega, 2008). One of the reasons of relatively high VO\(_{2}\)peak kg\(^{-1}\) is the fact that our boys were selected (healthy, some of them taking part on the sport club activities).
The mean leptin levels in our study were very different (Table 1) between groups. This is typical in other studies too. There are a very few studies about the relationships between leptin and energy expenditure. In animal models, injection of leptin in mice resulted in increases in O$_2$ consumption (Pelley and mounter et al., 1995). Contrary, Nagy et al. (1997) data do not support the hypothesis that leptin concentration (independent of fat mass) is related to measure of energy expenditure in children (Nagy et al., 1997). Finally, in boys, the high rates of fat utilization decline during maturation (Stephens et al., 2006). Very few studies have investigated the relation of leptin in peak O$_2$ consumption. In our study, there were highly significant relationships between leptin and VO$_{2\text{peak}}$ kg$^{-1}$ in all measured groups (Table 2). Only one study by Roemmich et al. (1998) confirmed these results in prepubescent boys and girls. Recently, in two studies indicated that indirectly measured VO$_{2\text{max}}$ (20 m shuttle run) were independently and jointly associated with lower concentrations in leptin in adolescence (Jiménez-Pavón et al., 2012; Martínez-Gomez et al., 2012). One explanation is that, for example, lean boys had a low leptin concentration. On the other side, lean boys had a relatively high VO$_{2\text{peak}}$ kg$^{-1}$ too. Absolute VO$_{2\text{peak}}$ (I min$^{-1}$) and VO$_{2\text{peak}}$ kg$^{-1}$ showed a significant correlation with leptin, we can explain with the fact that leptin increases total energy expenditure.

One of the limitations of our study is that we have not measured more completely the participant physical activity (we asked only the participation in physical education classes) and subjects dietary intake. It will be interesting to select the boys by groups using different leptin values, not differences in BMI. Finally, it will be more correct to select boys to the different groups using body fat mass.

We can conclude that leptin first of all correlated negatively with relative peak O$_2$ consumption. Absolute VO$_{2\text{peak}}$ correlated with leptin only in total group.

Acknowledgement

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Conflict of interest

The authors have no conflicts of interest.

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