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Associations of serum leptin, ghrelin and peptide YY levels with physical activity and cardiorespiratory fitness in adolescent boys with different BMI values

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ABSTRACT: The aim of this study was to investigate the differences in associations of serum acylated and des-acylated ghrelin, peptide YY (PYY) and leptin levels with physical activity (PA) and cardiorespiratory fitness (CReF) in adolescent boys (mean age of 14.0 years) with overweight (OWB; n=55) and with normal weight (NWB; n=154). Methods: Total PA was measured by 7-day accelerometry (counts/min) and CReF by peak oxygen consumption (VO_2peak/kg). Results: No differences were found in serum PYY, acylated ghrelin or des-acyl ghrelin levels, whereas mean leptin (11.6±10.6 vs. 2.0±2.7 ng/ml; $p<0.05$) and insulin (18.1±8.7 vs. 11.0±6.2 mU/l; $p<0.05$) levels were significantly higher in OWB compared to NWB. Mean CReF was significantly lower in OWB compared to NWB (39.7±8.7 vs. 50.5±6.8 ml/min/kg; $p<0.05$). Leptin was negatively correlated with CReF in both groups ($r=-0.43$; $p<0.05$), des-acylated ghrelin with CReF only in OWB ($r=-0.36$; $p<0.05$). In OWB leptin was negatively correlated with total PA ($r=-0.32$; $p<0.05$) and positively with sedentary time of PA ($r=0.35$; $p<0.05$). In NWB 28.1% of the variability of CReF was determined by leptin and insulin resistance index (HOMA-IR), whereas in OWB 71.9% was determined by trunk FM and BMI. Conclusions: Leptin concentration was inversely associated with CReF in adolescent boys independently of BMI in both groups, while des-acylated ghrelin was associated with CReF only in OWB. Low PA in OWB was associated with high serum leptin level.

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ABBREVIATIONS

BMI	body mass index	MVPA	moderate-to-vigorous physical activity
CReF	cardiorespiratory fitness	NWB	normal weight boys
DXA	dual-energy X-ray absorptiometry	OWB	overweight boys
FFM	fat-free mass	PA	physical activity
FM	fat mass	PYY	peptide YY
HOMA-IR	homeostasis model assessment-estimated insulin resistance	TB	total body
		VO_2peak	peak oxygen consumption
MPA	moderate physical activity	VPA	vigorous physical activity

INTRODUCTION

Higher physical activity (PA) attenuates the health risks of obesity and has positive effects on body mass reduction [1, 2]. The results of our recent longitudinal study showed that PA, especially vigorous PA (VPA), is an important factor for predicting overweight in boys during puberty [3]. Specifically, at least 60 min per day of moderate-to-vigorous PA (MVPA) with at least 15 min of VPA is desired to reduce the risk of developing overweight in later puberty [3]. Furthermore, it is also known that energy expenditure influences the control of appetite and energy intake [1] and the regulation of body composition is influenced by regular PA, which plays an important role in energy homeostasis [4]. The specific effects of PA level on weight loss also involve changes in appetite, which are partly mediated by many circulating hormones such as ghrelin, leptin and insulin [4].

Ghrelin, a very powerful stimulator of appetite, can increase body fat by increasing caloric intake [5]. Ghrelin circulates in the blood in acylated and non-acylated forms [6]. Acylated ghrelin is associated with the regulation of growth hormone secretion, cardiac performance, cell proliferation and adipogenesis, and affects appetite, food-intake and energy balance [7-9], whereas des-acylated ghrelin has been found to be associated with adipogenesis [6] and insulin sensitivity [10]. Another appetite hormone, peptide YY (PYY), is an appetite suppressant gut hormone secreted from the enteroendocrine cells of the ileum and colon [11-12]. Peptide YY acts as an appetite suppressant and inhibits food intake in humans [13]. Adult obese individuals secrete less PYY than non-obese individuals [14], while in children the serum PYY level was found to be similar between overweight and normal weight participants [15].

Leptin regulates energy homeostasis by stimulating satiety, thereby informing the brain about the body's energy stores [16]. In addition, an increase in fat-free mass (FFM) through PA will increase the demand for energy, and this will involve an increase in basal hunger. Moreover, a decrease in fat mass (FM) will lead to greater postprandial inhibitory control of appetite, partly through an increase in insulin and leptin sensitivity [1]. As leptin is directly associated with total body (TB) FM, [16] higher leptin levels have been observed in obese children and adolescents [17-19].

It has been suggested that different appetite hormones are influenced by physical exercise in children during growth [20]. For example, Broom et al. [21] found that acylated ghrelin and PYY may regulate appetite during and after PA, while Martins et al. [22] found that PYY was elevated during moderate intensity aerobic exercise in

TABLE 1. Main characteristics of the subjects and the main results. Mean ± SD are shown. Significant ($p < 0.05$) difference between groups is shown with *. NWB – normal weight boys; OWB – overweight boys; PA – physical activity; FM – fat mass; FFM – fat-free mass.

Group	NWB (n=154)	OWB (n=55)
Age (yrs)	14.0±0.7	14.0±0.8
Body height (cm)	168.4±9.1	172.0±7.8*
Body mass (kg)	53.6±8.8	80.0±17.8*
BMI (kg/m^2)	18.8±1.9	26.8±4.5*
Tanner stage (1|2|3|4|5)	4.08±0.78 (0|0|40|61|53)	4.13±0.74 (0|0|11|25|19)
Total body FM (kg)	8.1±3.4	25.8±12.3*
Total body FFM (kg)	42.9±8.0	50.0±9.8*
Trunk FM (kg)	3.1±1.4	11.3±5.6*
VO_2peak/l (l/min)	2.7±0.6	3.1±0.5*
VO_2peak/kg (ml/min kg)	50.5±6.8	39.7±8.7*
Sedentary time (min/day)	569.7±95.1	564.6±89.6
Moderate PA (min/day)	36.6±15.0	41.2±16.3
Vigorous PA (min/day)	20.9±16.8	15.7±11.9*
Moderate-vigorous PA (min/day)	57.5±27.7	56.9±22.1
Total PA (counts/min)	410.5±170.0	394.9±141.7
Testosterone (nmol/l)	13.9±6.1	9.7±5.3*
Leptin (ng/ml)	2.0±2.7	11.6±10.6*
Insulin (mU/l)	11.0±6.2	18.1±8.7*
Glucose (mmol/l)	5.1±0.4	5.2±1.9
HOMA-IR	2.5±1.4	4.1±2.1*
Acylated ghrelin (pg/ml)	550.4±851.1	660.4±1124.5
Des-acylated ghrelin (pg/ml)	312.1±182.9	299.8±209.0
Peptide YY (pg/ml)	93.3±56.8	94.4±49.7

TABLE 2. Partial correlation coefficients between different variables in NWB (n=154) and OWB (n=55) groups controlled for BMI, age and Tanner stage. Statistically significant correlations are shown with * ($p < 0.05$) and ** ($p < 0.01$).

	Acylated ghrelin (pg/ml)		Des-acylated ghrelin (pg/ml)		PYY (pg/ml)		Leptin (ng/ml)	
	NWB	OWB	NWB	OWB	NWB	OWB	NWB	OWB
VO_2peak (l/min)	-0.143	-0.317*	-0.228	-0.352*	0.016	0.122	-0.495**	-0.507**
VO_2peak/kg (ml/min/kg)	-0.123	-0.272	-0.193	-0.360*	-0.020	0.095	-0.429**	-0.444*
Sedentary time(min/day)	-0.068	0.044	-0.091	0.025	-0.058	0.010	0.122	0.348*
MPA (min/day)	0.014	0.060	0.011	0.011	-0.177	0.134	0.138	-0.192
VPA (min/day)	0.039	-0.143	-0.046	-0.104	-0.071	0.017	-0.122	-0.154
MVPA (min/day)	0.030	-0.023	-0.021	-0.044	-0.134	0.120	0.000	-0.239
Total PA (counts/min)	0.040	-0.062	-0.026	-0.078	-0.103	0.081	-0.055	-0.322*

adults. In adolescents, Jiménez-Pavon et al. [16] found that leptin was negatively associated with VPA and MVPA levels, whereas Martinez-Gomez et al. [20] found that leptin is associated only with VPA, but not with MVPA in adolescents. However, to our best knowledge, the possible associations of acylated ghrelin, des-acylated ghrelin and PYY with objectively measured different PA levels in adolescent boys with different BMI have not been studied.

The daily level of PA is strongly associated with cardiorespiratory fitness (CReF) in children and adolescents [23-24]. Overweight boys (OWB) have lower CReF as directly measured by VO_2peak/kg in comparison with normal weight boys (NWB) [25]. It was found that a higher level of CReF was a significant predictor of decrease in body FM from childhood to adolescence [26]. Pomerants et al. [27] found that VO_2peak/kg was negatively associated with total ghrelin and leptin concentrations in boys at different pubertal stages. In addition, other studies have also found that leptin is negatively associated with CReF in adolescents [16, 20]. Although there have been studies examining the associations between these hormones and PA in adults, to our best of knowledge, very little is known about the relationships of ghrelin isoforms and PYY concentration with directly measured CReF in overweight adolescents in comparison with normal weight adolescent boys. Accordingly, the aim of this study was to investigate the differences in associations of serum acylated and des-acylated ghrelin, PYY and leptin levels with PA and CReF in adolescent boys with different BMI values.

SUBJECTS AND METHODS

Participants

This study included 209 Estonian boys aged between 12 and 16 years. In this study we focused only on boys, to eliminate the possible impact of sex hormones on these associations due to the menstrual cycles in girls. They were recruited from local schools, where physical education lessons were held twice a week. The participants were divided into two groups according to their body mass index (BMI). The group of overweight boys (OWB) included 18 boys with BMI between the 85th and 95th percentile and 37 boys with BMI above the 95th percentile of Estonian BMI charts [28], making a total of 55 boys. The group of normal weight boys (NWB) included 154 boys with BMI below the 85th percentile. All participants and their parents completed a questionnaire about their current acute or chronic illnesses, and only boys who reported themselves as healthy and without any acute or chronic illnesses were recruited. All parents and children signed an informed consent form. The Medical Ethics Committee of the University of Tartu (Estonia) approved this study.

Anthropometry and pubertal development

Body height was measured to the nearest 0.1 cm using a Martin metal anthropometer, and body mass was measured to the nearest 0.05 kg with a medical electronic scale (A&D Instruments Ltd., Abingdon, UK). Body mass index (BMI) was also calculated (kg/m^2). Pubertal development was assessed by self-report using an illustrated questionnaire according to the Tanner classification method (Tanner 1962) that has been previously validated [29] and used in our previous studies in boys [27, 30-31]. The boys were given photographs, figures and descriptions of genitalia and public hair development stages, and asked to choose the one that most accurately reflected their appearance. If there was a disagreement between the stage of genitalia and pubic hair, then the final decision was made according to the degree of genitalia development [29].

Body composition

Total body (TB) fat mass (FM), fat-free mass (FFM) and trunk FM were measured by dual-energy X-ray absorptiometry (DXA; DPX-IQ; Lunar Corporation, Madison, Wisc., USA) using proprietary software,

TABLE 3. Results of stepwise multiple regression analysis with cardiorespiratory fitness and PA characteristics as dependent variables and acylated ghrelin, des-acylated ghrelin, PYY, leptin, testosterone, BMI, age, Tanner stage, TB FM, TB FFM, trunk FM, HOMA-IR, and insulin as independent variables.

Dependent variable	Independent variable	NWB	OWB
VO_2peak	FFM	72.8%	52.3%
	FFM, Leptin	-	60.9%
VO_2peak/kg	Leptin	16.9%	-
	Leptin, HOMA-IR	28.1%	-
	Trunk FM	-	68.5%
	Trunk FM, BMI	-	71.9%
Sedentary time	Testosterone	12.1%	-
Moderate PA	Testosterone	10.8%	-
	Testosterone, PYY	15.3%	-
	Testosterone, PYY, HOMA-IR	20.2%	-
Vigorous PA	-	-	-
Moderate-vigorous PA (min/day)	Testosterone	8.7%	-
Total PA (counts/min)	Testosterone	11.4%	-

R^2 x 100 is shown describing the percentage of variability of dependent variables the different characteristics can explain.

version 3.6. Participants were scanned in light clothing while lying flat on their backs with their arms by their sides. The fast scan mode and standard subject positioning were used for TB measurements, and a single examiner evaluated all DXA measurements and results. Analysis was performed using an extended analysis option, and a single examiner evaluated all DXA measurements and results. Intra-subject variations for body composition measurements were less than 2%.

Cardiorespiratory fitness

Cardiorespiratory fitness (CReF) was determined by a stepwise incremental exercise test until volitional exhaustion using an electrically braked bicycle ergometer (Corival V3, Lode, Netherlands) [25]. The initial work rate was 50 W and was increased by 25 W after every 3 min until volitional exhaustion. Pedalling frequency was set to 60-70 rpm. Participants were verbally encouraged to make a maximal effort. Respiratory gas exchange variables were measured throughout the test using the breath-by-breath mode with data being stored in 10 s intervals. All subjects breathed through a facemask during the test. A portable open-air spirometry system (MetaMax I, Cortex, Leipzig, Germany) was used to continuously measure oxygen consumption (VO_2), carbon dioxide output and minute ventilation. The analyser 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-Analysis 3.21, Cortex, Leipzig, Germany). Finally, VO_2peak (l/min) was measured and VO_2peak per kilogram of body mass (VO_2peak/kg) was calculated.

Physical activity

Physical activity (PA) was measured with the ActiGraph accelerometer (model GT1M ActiGraph; Monrovia, CA, USA). The accelerometer is small (3.8 x 3.7 x 1.8 cm), lightweight (27 g), and has a uniaxial monitor designed to detect and measure vertical accelerations ranging from 0.05 to 2.00 G with a frequency response of 0.25-2.50 Hz. All participants were asked to wear the accelerometer on the right hip for seven consecutive days during the wake up time. The accelerometer data were analysed using the activity counts of 15 s epochs [3]. For the analyses of accelerometer data, all night activity (24:00-6:00 hours), and all sequences of 10 min or more of consecutive zero counts, were excluded from each individual's recording [3, 32].

Physical activity was included for further analyses if the subject had accumulated a minimum of eight hours of wear time data per day for at least two weekdays and one weekend day [3, 32]. The number of physically active children was 47 (85%) in OWB and 132 (85%) in NWB. The total amount of PA (total PA) was expressed as the total number of counts divided by the registered time (counts/min) [3, 32]. Moderate PA (MPA) and vigorous PA (VPA) were determined as the time spent above cut-off points of 2000 and

4000 counts/min respectively and expressed as minutes per day. In addition, moderate-vigorous PA (MVPA) was also calculated as the sum of MPA and VPA, similar to our previous studies [3, 32]. The time spent in sedentary time was determined as the time spent below 100 counts per minute and expressed as minutes per day [3, 32].

Blood analysis

Venous blood samples were obtained from a vein before breakfast between 8 a.m. and 9 a.m. after an overnight fast. The blood serum was separated and then immediately frozen at -80°C for further analysis. Leptin was determined by radioimmunoassay (Mediagnost GmbH, Reutlingen, Germany). Serum acylated ghrelin was analysed using the human acylated ghrelin ELISA commercial kit (Human Acylated Ghrelin Enzyme Immunoassay Kit, Bertin Pharma, Montigny-le-Bretonneux, France). As acylated ghrelin is unstable and sensitive to de-acylation, samples used for the measurement of acylated ghrelin were made immediately after the serum was thawed for the first time on the day of analysis. While running ELISA kits, all work was completed on ice [11, 33]. Des-acyl ghrelin was measured using the human unacylated ghrelin ELISA commercial kit (Human Unacylated Ghrelin Immunoassay Kit, Bertin Pharma, Montigny-le-Bretonneux, France). Peptide YY was also determined using a commercial ELISA kit (Millipore, Millipore Corporation, Billierca, MA, USA). Testosterone and insulin were analysed using Immulite 2000 (DPC, Los Angeles, CA, USA). Glucose was measured in serum with a commercial kit (Boehringer, Mannheim, Germany). The insulin resistance index was calculated using homeostasis model assessment (HOMA-IR): fasting insulin (mU/l) x fasting glucose (mmol/l)/22.5 [34].

Statistical analysis

All statistical analyses were performed using SPSS, version 20.0 (SPSS Inc, Chicago. IL, USA). Standard statistical methods were used to calculate means and standard derivations. Evaluation of normality was performed with the Shapiro-Wilk method, and variables not normally distributed were log-transformed. An unpaired independent two-tailed t test was used to determine differences between groups. Relationships between measured values were evaluated by partial correlation analysis controlled for BMI, age and Tanner stage. Stepwise multiple regression analysis was performed to clarify the determinants of the variability of cardiorespiratory fitness and PA using acylated ghrelin, des-acylated ghrelin, PYY, leptin, testosterone, BMI, age, Tanner stage, TB FM, TB FFM, trunk FM, insulin and HOMA-IR as possible factors. The level of significance was set at $P < 0.05$.

RESULTS

Overweight adolescent boys were taller and heavier with higher BMI, FM and FFM levels than normal weight adolescents ($p < 0.05$) (Table 1). The Tanner stages were similar in both groups (G3-G5). Mean VO_2peak/kg was significantly lower and VO_2peak significantly higher in OWB than in NWB (Table 1). In addition, VPA was significantly lower in OWB compared to NWB. OWB showed higher levels of leptin, insulin and HOMA-IR, and lower testosterone concentration when compared with NWB (Table 1). There were no significant differences in acylated ghrelin, des-acylated ghrelin or PYY levels between studied groups.

In both groups, leptin was negatively correlated with VO_2peak ($r=-0.50$ to -0.51) and VO_2peak/kg ($r=-0.43$ to -0.44) after controlling for BMI, age and Tanner stage (Table 2). In OWB, leptin was negatively correlated with total PA ($r=-0.32$; $p<0.05$) and positively with sedentary time ($r=0.35$; $p<0.05$). In OWB serum acylated ghrelin concentration was negatively correlated with VO_2peak ($r=-0.32$; $p<0.05$), whereas des-acylated ghrelin was correlated with both VO_2peak ($r=-0.35$; $p<0.05$) and VO_2peak/kg ($r=-0.36$; $p<0.05$) when controlling for BMI, age and Tanner stage (Table 2).

The stepwise multiple regression analysis showed that in NWB 28.1% of the variability of VO_2peak/kg was determined by leptin and HOMA-IR, whereas in OWB 71.9% of its variability was determined by trunk FM and BMI. In the NWB group 11.4% of the variability of total PA was determined by serum testosterone level (Table 3).

DISCUSSION

In this study, the differences in associations between circulating levels of acylated ghrelin, des-acylated ghrelin, PYY and leptin with CReF and different types of PA in overweight and normal weight adolescent boys were investigated. The adolescent boys with obesity were taller but in the same pubertal stage as NWB, similar to the study by Widman et al. [35]. In this study we found that mean serum concentrations of acylated ghrelin, des-acylated ghrelin and PYY were not different between OWB and NWB groups. As expected, serum leptin and insulin concentrations were higher and testosterone concentrations lower in OWB compared with NWB. In addition, we found that leptin was negatively correlated with total PA and positively with sedentary time.

Puberty is a period with relatively rapid height and weight gain that is accompanied by a decline in PA levels together with an increase in sedentary time [3]. In our study OWB had a significantly lower VPA level in comparison with NWB, while other PA modes were not different between the groups. Other studies have also found significantly lower VPA in overweight adolescent boys and girls in comparison with normal weight children [36-37]. Therefore, we could suggest that overweight children should do more VPA, which may help to keep the weight gain under control. Future longitudinal studies are needed to clarify the role of VPA and other modes of PA in weight control in overweight children.

Significant correlations between PA levels and hormones in this study were found in the OWB group only. Leptin was negatively correlated with total PA and positively with sedentary time. These correlations were expected and are most likely due to the negative impact of increased weight and FM on PA described in previous studies [38-39]. It is also known that PA increases insulin sensitiv-

ity and decreases insulin release, which in turn reduces leptin level [20]. Many studies have found that VPA particularly is negatively associated with leptin level in adolescents [16, 20]. Whether this relationship is more due to lifestyle or genetic factors is not known. It is more likely that there are interactions between lifestyle and genetic factors that together influence the relationship between leptin and PA [40]. Raj and Kumar [41] reported that interactions between genetic, neuroendocrine, metabolic, psychological, environmental and socio- cultural factors are clearly evident in childhood obesity. Although in this study we did not find any significant correlations with VPA, OWB did significantly less VPA compared to NWB. We have recently shown that at least 60 min per day of MVPA with at least 15 min of VPA should reduce the risk of developing overweight in puberty [3]. Therefore, it is very important for obese children to increase total PA and reduce sedentary time.

Similarly to other studies, CReF assessed by direct measurement of VO_2peak per kg was significantly lower in OWB compared to NWB [25, 35]. We found that VO_2peak/kg was negatively correlated with leptin in both groups, similarly to previous studies [16, 20]. We also found that leptin and HOMA-IR together explained 28.1% of the variability of VO_2peak/kg in NWB. Thus, in NWB leptin influences CReF independently of FM. The results were the same after controlling for PA, indicating that the association between CReF and leptin is independent in adolescents, similar to the study by Jiménez-Pavon et al. [16]. Quite a different picture was seen in OWB, whose VO_2peak/kg was determined by FM characteristics, namely by trunk FM and BMI, which together explained 72% of the variability of CReF. It is known that in children and adolescents CReF decreases progressively as BMI increases [42-43].

We have previously observed that serum total ghrelin concentration in boys at different pubertal stages is negatively correlated with VO_2peak/kg [26]. In this study we found that in OWB, but not in NWB, serum des-acylated ghrelin concentration was also negatively correlated with VO_2peak/kg. Cederberg et al. [44] demonstrated in young healthy men that an increase in des-acylated ghrelin level during 6 months of intense exercise training was associated with reduced weight, TB FM, TB fat % and waist circumference, but not with FFM. Inverse associations of changes in des-acylated ghrelin with changes in waist circumference and fat % were independent of weight at baseline, and changes in weight and exercise performance. Furthermore, it has been shown that ghrelin can be an indicator of fat oxidation during a weight reduction programme in obese children and adolescents [45]. Douglas et al. [46] did not find any correlations between acylated ghrelin and CReF in young men. To our best knowledge, no studies have examined the associations between des-acylated ghrelin and VO_2peak/kg in overweight adolescents. From our study we could suggest that only des-acylated ghrelin has a significant impact on CReF, at least in this study population. However, as our study was cross-sectional, further longitudinal studies are necessary to clarify the causal relationship between des-acylated ghrelin and CReF.

We did not find any significant relationships between PA and serum PYY and ghrelin isoforms levels. To our best knowledge, no studies have been done to study the associations between PA and serum ghrelin isoforms and PYY in adolescents. However, an important determinant of total PA and its subtypes in NWB was serum testosterone level. Eleven percent of the variability of total PA was determined by testosterone and 20% of the variability of MPA by testosterone together with PYY and HOMA-IR. Mackelvie et al. [47] reported that des-acylated ghrelin changes correlated significantly with changes in testosterone concentrations in adolescent boys with different BMI values. As in our study serum testosterone was an important determinant of total PA and its subtypes, we could suggest that testosterone may play an important role in the level and type of PA.

There are some limitations in our study that should be considered. Firstly, our cross-sectional design will not show a direct causative impact of our findings. Another potential limitation could be the sample size of the studied subjects. A larger number of subjects would raise the statistical power to discover further significant associations. However, the number of subjects in our study was quite similar to previous similar studies in this field [15, 35]. Finally, our results are limited to a specific group of Caucasian male adolescents with a specific age range. Accordingly, additional investigations in children and adolescents with specific sex, age, pubertal stage and ethnicities are needed to further clarify these associations. Although our study has some limitations, this is the first study to our best of knowledge in adolescent boys with different BMI values to investigate the associations between ghrelin isoforms and PYY with PA measured by accelerometry and CReF parameters directly assessed.

CONCLUSIONS

Serum leptin concentration is inversely associated with CReF in adolescent boys independently of their BMI in both groups. Low PA is associated with high serum leptin level, and low CReF level is related to high serum des-acylated ghrelin level in OWB. Longitudinal studies are needed to confirm our findings and to find causal relationships.

Conflict of interests: the authors declared no conflict of interests regarding the publication of this manuscript.

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