RESEARCH ARTICLE

Exercise testing and adipokine levels for the evaluation of overweight and obesity in children

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Abstract

Background: Childhood obesity poses a global health threat. We investigated the association of the cardiopulmonary exercise testing indexes with adipokines levels and insulin resistance along with the beneficial effect of physical exercise on insulin resistance in children.

Material and Methods: Thirty-two obese, 21 overweight, and 30 normal-weight children participated in the current study, with mean age 11.98 (±1.95), 10.91 (±1.72), and 11.35 (±2.21) years, respectively. All children were clinically healthy. The children and their parents provided data on physical activity, while spirometry and maximal cardiopulmonary exercise testing were performed for the functional evaluation of the respiratory status of the study population.

Results: Leptin levels were significantly lower in normal-weight children compared to the obese ones ($p < 0.001$). Maximum quantity of oxygen (VO_2max) differences were statistically significant between the three groups ($p = 0.025$ for normal weight vs overweight, and $p = 0.001$ for normal vs obese children). Leptin levels were inversely related to VO_2max in obese children ($p = 0.009$, $r = -0.491$). Homeostatic model assessment of insulin resistance (HOMA-IR) was statistically significantly lower among children that were more physically active ($p = 0.042$). Leptin was significantly related to body mass index among obese children ($r = -0.582$, $p < 0.001$).

Conclusions: Leptin is significantly inversely related to VO_2max in obese children. This study, however, allows further assumptions for adipokines and childhood obesity, along with the possible role of leptin as an additional obesity index in relation with cardiopulmonary function. HIPPOKRATIA 2017, 21(3): 124-129.

Keywords: Obesity, childhood, cardiopulmonary exercise testing, leptin, adipokines

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Introduction

Obesity which is defined as abnormal or excessive fat accumulation in the adipose tissue threatens the health of the individuals[1]. Especially childhood obesity poses an evolving global health threat[1]. The obesity rate for children aged 6-11 and 12-19 years has increased since 1980 from 7 % to 20 % and from 5 % to 18 %, respectively[2,3]. However, in some western settings such as the US, Western Europe, Australia, and Japan, recent data suggest that levels of childhood obesity may have reached a plateau in the last decade[4]. This fact should not lead to complacency, as one-third of adults and almost 20 % of children and adolescents 2-19 years old are obese.

Excess body weight poses a health risk for premature morbidity and mortality. Diseases such as type 2 diabetes, dyslipidemia, hypertension, cardiovascular disease, and sleep apnea have recently become more common in children[5]. Circulating adipokines are correlated with obesity, and their levels have been related to insulin resistance[6,7]. Among them, leptin and adiponectin are the significant adipocytokines through which obesity exerts its clinical and metabolic effects[7]. Leptin which is mainly produced in the adipose tissue, acts on the hypothalamus, on receptors in the arcuate nucleus, to regulate appetite in order to be achieved energy homeostasis[8]. A decreased sensitivity to leptin has been noticed in obesity, resulting in an inability to detect satiety despite high energy stores[9]. Serum leptin levels were found elevated in obese children; besides, leptin levels decreased during the weight loss period[10].

Aerobic Capacity with all its indices as derived from the maximal exercise testing, has been used as a prognostic factor in many conditions[11-13]. Especially the maximum quantity of oxygen (VO_2max) that a person's lungs consume during maximal exercise, has been recognized as the gold standard prognostic marker in several diseases. Shazia et al showed that obese young women presented lower aerobic capacity than normal-weight ones

with lower VO_2max values in the obese group[14]. Moreover, in a systematic review and meta-analysis on exercise tolerance in adolescents, Hansen et al marked a trend for lower VO_2peak in obese adolescents than lean ones and highlighted the need for more studies in this field[15].

The child's cardiopulmonary capacity, as documented by the cardiopulmonary exercise testing (CPET) indexes, reflects the pulmonary, cardiovascular, muscle, and metabolic responses simultaneously under precise conditions of metabolic stress. The latter is also expressed via changes in adipokines levels, which might be associated with CPET indexes[16].

The aim of the present study was to investigate the associations of the cardiopulmonary exercise testing indexes with adipokines levels and insulin resistance and the impact of physical exercise on insulin resistance in children.

Material and Methods

Patients

We conducted a prospective study from 2009 until 2013 which enrolled 53 obese and overweight children who attended the Obesity clinic of the Endocrine Unit of the 3rd Department of Pediatrics of the Aristotle University of Thessaloniki at Hippokration General Hospital of Thessaloniki (overweight and obese groups) and 30 children with similar age and sex with the above two groups, who attended two different public schools in the city of Thessaloniki (normal-weight group). Normal-weight children were enrolled and participated in the study after they and their parents were informed about the study protocol, through an information leaflet that was sent to their schools. In total, 33 normal weight children accepted to participate in the study from the 123 children that were informed about it. The normal-weight group did not differ from the other groups regarding age and sex. Three normal weight children and two obese were excluded from the study. The exclusion criteria for the study were: a) chronic diseases, and b) failure to perform a cardiopulmonary exercise test.

The study was approved by the Bioethics Committee of the Medical School of Aristotle University of Thessaloniki (decision number: 91/21-12-2009), and a signed consent form by the guardian was obtained for each single child.

In the current study, 32 obese, 21 overweight, and 30 normal-weight children participated, with a mean age (± standard deviation) 11.98 ± 1.95, 10.91 ± 1.72, and 11.35 ± 2.21 years, respectively. All children were clinically healthy. The children and their parents provided data on physical activity. They were asked about in-school physical activity, as defined in their curriculum and out-of-school activity. The latter was considered to be "organized" only if the child participated in certain sports or attended a gym. Even though all "physical activity" hours were recorded, only children with "organized" physical activity, were considered as "physically active". All children reported a 2-hour per week in-school activity.

Spirometry and maximal cardiopulmonary exercise testing were performed at the Pulmonology Unit of the 3rd Department of Pediatrics.

Weight-height measurements

We used the SECA scale, model 812 (S.No 06901304) to measure the children's weight and the Holten Limited measuring rod (V/R, Veeder-Root, Crymych, Dyfed, UK) to measure their height. The growth curves for the Greek population designed by the First Paediatric Department of the University of Athens in 2000-2001, were used to determine the percentile of weight and height.

Spirometry

Spirometry was performed with an electronic volume spirometer, dry type (SensorMedics, Flow Sensor, Vmax Series, V.20-1 L, SensorMedics Corp., Yorba Linda, CA, USA). We measured the forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), and forced expiratory flow at 50 % of FVC (FEF50), using standard spirometry following the ATS/ERS guidelines[17]. No beta-2 agonists were used for 24 hours before spirometry. Data were expressed in % predicted using the normative data from the Global Lung Function Initiative software (GLI 2012, Global Lung Function Initiative Task Force, available at: http://www.lungfunction.org/).

Biochemistry

Glucose was measured by the hexokinase method (AU 2700 Analyzer, Beckman Coulter Inc., Orange County, CA, USA) and insulin by the two sites immunoassay technique (Immulite 2000 XPi Immunoassay System, Siemens Healthcare GmbH, Erlangen, Germany). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated with the following formula: HOMA-IR = Glucose (mg/dL) x insulin (mU/L)/405.

Regarding adipokines, leptin and adiponectin were measured with the "two-site" sandwich enzyme-linked immunosorbent assay (ELISA). Homocysteine was measured with electrochemical fluorescence (Centaur CP, Siemens Healthcare GmbH).

Cardiopulmonary Exercise Testing (CPET)

All participants performed a maximal cardiopulmonary exercise testing on a cycle ergometer (Ergoline, Vmax Series V.20-1, SensorMedics). Cardiac parameters were also measured (cardiograph model Corina, S.N. 101164361, Cardiosoft software V5.15). The Godfrey protocol for exercise testing was applied[18]; depending on subject's height, after baseline measurements for one minute and a warm-up period of two minutes cycling with 10 Watts (for patients <120 cm tall), 15 Watts (120-150 cm) or 20 Watts (>150 cm tall), workload was increased by 10, 15, 20 Watts, respectively every minute until volitional fatigue. Exercise testing time was kept within 8-12 min. Patient's heart rate over 85 % of maximum predicted[19] along with respiratory equivalence ratio (RER) over 1.05[20] were used as indicators of maximal

test. Maximum oxygen uptake (VO_2max), and max oxygen uptake/weight (VO_2max/kg) were measured.

Statistics

Descriptive and inferential statistics were performed. The out-of-school organized physical activity was considered as a binary variable around its median value (two hours weekly). Student's t-test and ANOVA with post-hoc Bonferroni t-test were used for the two groups and multiple comparisons, respectively. Pearson's r was used for correlations. Statistical significance was set at p =0.05. Statistical analyses were performed using the IBM SPSS Statistics for Windows (IBM SPSS, IBM Corp., Armonk, NY, USA) version 22.0 while for graphs we used the Sigma Plot 12.5 (Systat Software, London, UK).

Results

The demographic and laboratory data of the three study groups are presented in Table 1. Statistically significant differences were detected in all studied parameters, except homocysteine, as shown in Table 1. Post-hoc analysis showed that leptin levels were significantly lower in normal-weight compared to obese children (p <0.001) while the difference between overweight and obese children was marginally statistically significant (p =0.05). The VO_2max differences were also statistically significant between the three groups (p =0.025 for normal-weight vs overweight, and p =0.001 for normal-weight vs obese children). The levels of leptin and adiponectin among the three study groups are shown in Figure 1, while the VO_2max levels are showed in Figure 2. Body mass index (BMI) z-scores also differed significantly between the three groups. When adipokines and VO_2max (absolute values and adjusted for weight and height) were correlated with one another, significant correlations merged in overweight and obese children groups. Leptin was inversely related to VO_2max in obese children (r =-0.491, p =0.009) as shown in Table 2. Leptin was also related inversely to VO_2max/w (r =-0.441, p =0.021), to VO_2max/h (r =-0.420, p =0.029), and to VO_2max (r =-0.491, p =0.009) only in obese children. However, when these correlations were corrected for HOMA-IR and BMI z-scores, only BMI z-score was positively related to leptin (p =0.014 for VO_2max/w and p =0.011 for VO_2max/h regression model respectively). As for homocysteine, no statistically significant associations were detected (Table 2). Physical activity (organized or not) was not related statistically significantly to VO_2max (p >0.10 for all indices) as shown in Table 3.

Leptin was significantly higher in the more physically active group (p =0.002) (Table 3). No associations were detected between leptin, adiponectin, and HOMA-IR or between these two adipokines and the BMI, with the exception of the obese children where leptin was significantly related to BMI (r =0.582, p <0.001) and HOMA-IR positively related to FEV_1/FVC.

Discussion

The main finding of the present study was that leptin was significantly inversely related to VO_2max, adjusted for weight or height. This relation was subtle in overweight children and became evident in obese ones. Moreover, a documented, "organized" physical exercise duration of more than two hours weekly was associated with a lower HOMA-IR. Physical training may further exert its beneficial effect on health as documented by HOMA-IR index.

The VO_2max associations with adipokines' levels have been previously investigated and yielded contradictory results, mainly due to different research protocols. In the study of Unal et al, VO_2max values were significantly higher (p <0.01) and leptin levels significantly lower in trained athletes[21], while Yang et al showed that leptin levels after 24h of participating in an exercise bout of 65 %

Table 1: Demographic, biochemical and exercise test parameters of the 32 obese, 21 overweight, and 30 normal-weight children who participated in the study.

	normal-weight (n =30)	overweight (n =21)	obese (n =32)	p#
Age (years)	11.98 ± 1.95	10.91 ± 1.72	11.35 ± 2.21	0.158
Males (number, %)	16 (53 %)	9 (43 %)	18 (56 %)	
BMI (kg/m^2)	20.11 ± 3.09	25.78 ± 1.35	31.70 ± 5.37	<0.001***
BMI z score	-0.97 ± 0.48	-0.08 ± 0.21	0.85 ± 0.84	<0.001***
Weight z score	0.03 ± 1.01	-0.07 ± 1.03	0.02 ± 1.03	0.942
Height z score	0.09 ± 1.1	-0.33 ± 0.76	0.13 ± 0.92	0.209
Physical activity (hours/week)	6.35 ± 4.44	4.29 ± 2.22	3.58 ± 2.68	<0.001**
HOMA-IR	0.63 ± 0.48	1.36 ± 0.89	2.12 ± 1.44	0.002*
VO_2max (ml/kg/min)	1.24 ± 1.26	0.63 ± 0.17	0.44 ± 0.17	0.001**
Leptin (ng/ml)	14.14 ± 12.81	27.33 ± 10.59	36.77 ± 23.97	<0.001**
Adiponectin (μg/ml)	16.20 ± 6.61	12.96 ± 7.21	11.51 ± 4.38	0.011*
Homocysteine (μmol/L)	7.62 ± 1.92	7.59 ± 2.05	8.71 ± 3.73	0.229

Values are means ± standard deviation, BMI: Body mass index, HOMA-IR: Homeostatic model assessment of insulin resistance, VO_2max: maximum quantity of oxygen, #: ANOVA, ***: Statistically significant difference between Normal vs Overweight vs Obese, **: Statistically significant difference between Normal vs Overweight, Normal vs Obese, *: Statistically significant difference between Normal vs Obese.

Table 2: Adipokines correlation with physiological tests[1].

Group			Adiponectin	Homocysteine	VO_2max/w	VO_2max/h	VO_2max
Controls	Leptin	r	0.383	0.275	0.612	0.182	0.212
		p	0.349	0.509	0.107	0.666	0.615
	Adiponectin	r		0.535	-0.067	-0.280	-0.075
		p		0.172	0.875	0.502	0.860
	Homocysteine	r			0.092	-0.154	0.141
		p			0.814	0.692	0.717
	VO_2max /w	r				0.083	0.133
		p				0.831	0.734
Overweight	Leptin	r	-0.276	0.380	-0.359	-0.424	-0.393
		p	0.267	0.119	0.156	0.090	0.119
	Adiponectin	r		-0.025	0.387	0.386	0.353
		p		0.921	0.124	0.126	0.164
	Honocysteine	r			0.243	0.291	0.323
		p			0.316	0.227	0.177
	VO_2max /w	r				**0.942**	**0.820**
		p				**<0.001**	**<0.001**
Obese	Leptin	r	0.135	0.120	-0.441	**-0.420**	**-0.491**
		p	0.469	0.528	0.021	**0.029**	**0.009**
	Adiponectin	r		-0.083	0.126	0.145	0.095
		p		0.664	0.532	0.469	0.639
	Honocysteine	r			-0.202	-0.148	-0.105
		p			0.294	0.444	0.588
	VO_2max/w	r				**0.949**	**0.841**
		p				**<0.001**	**<0.001**

[1]: Significant correlations in bold, VO_2max: maximum quantity of oxygen.

Table 3: Differences in adipokines and VO_2max according to the degree of organized physical activity.

	Organized physical activity (hours/week)	Normal weight	Mean ± SD	p	Overweight	Mean ± SD	p	Obese	Mean ± SD	p
Leptin	≤ 2 h	6	5.83 ± 2.56	0.002	10	28.77 ± 13.68	0.579	24	38.00 ± 26.39	0.637
	> 2 h	24	16.41 ± 13.57		11	25.89 ± 6.82		8	33.25 ± 16.05	
Adiponectin	≤ 2 h	6	18.17 ± 4.54	0.375	10	11.95 ± 7.12	0.572	24	11.38 ± 4.47	0.782
	> 2 h	24	15.57 ± 7.11		11	13.95 ± 7.57		8	11.88 ± 4.37	
Homocysteine	≤ 2 h	6	6.88 ± 1.31	0.300	10	7.47 ± 2.79	0.812	24	9.06 ± 4.14	0.354
	> 2 h	24	7.850 ± 2.02		11	7.70 ± 1.02		8	7.62 ± 1.83	
HOMA Index	≤ 2 h	6	0.54 ± 0.43	0.560	10	2.35 ± 2.93	0.267	24	3.17 ± 4.03	0.507
	> 2h	24	0.66 ± 0.49		11	1.24 ± 0.91		8	2.18 ± 1.73	
VO_2max	≤ 2 h	6	1.39 ± 0.43	0.784	10	0.62 ± 0.16	0.875	24	0.41 ± 0.17	0.173
	> 2 h	24	1.21 ± 1.38		11	0.64 ± 1.17		8	0.51 ± 0.17	

t-test, HOMA-IR: Homeostatic model assessment of insulin resistance, VO_2max: maximum quantity of oxygen, SD: standard deviation.

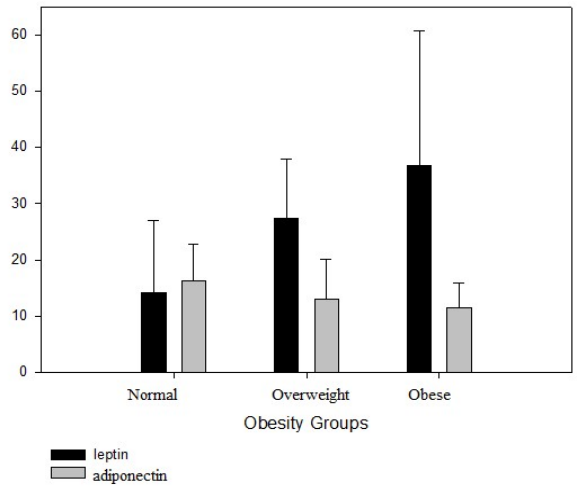

Figure 1: Leptin and adiponectin distribution in the obese, overweight, and normal-weight children. Statistical differences are evident between normal and obese group*.
*Leptin differed statistically significantly between normal-weight and overweight (p =0.05), normal-weight and obese group (p <0.001).

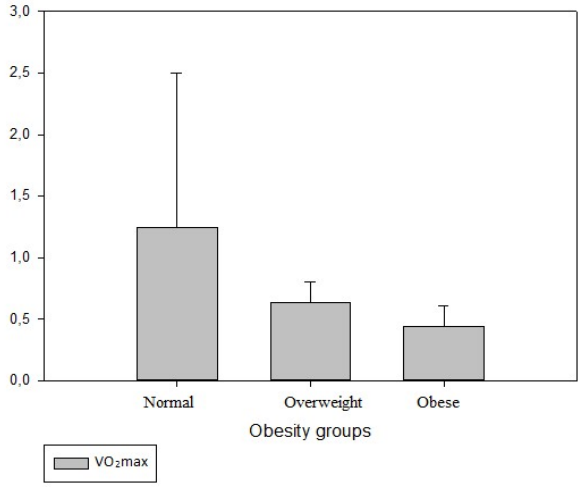

Figure 2: VO_2max differences between groups (obese, overweight, and normal-weight children)*.
*VO_2max differed statistically significantly between normal weight and overweight group (p =0.025) and between normal weight and obese group (p =0.001).

VO2max compared to 25 % were significantly higher[22]. Torjman et al did not find a change in serum leptin levels neither after short-term maximal exercise nor after four hours following long-term aerobic exercise at 50 % of VO_2max[23]. Leptin levels have been positively correlated with BMI in both genders, but negatively correlated with VO_2max only in men[24]. In the study of Paltoglou et al, baseline and post-exercise (at 70 % VO_2 max) adiponectin was greater, and leptin and high-sensitivity C-reactive protein were lower in normal-weight than in obese pre- and early pubertal boys, authors concluding that leptin and adiponectin predict negatively and positively anti-oxidation, respectively[25]. Cardiopulmonary capacity studies in children are scarce, mainly because cardiopulmonary testing in children is particularly difficult and demanding, as the child's cooperation is necessary to get maximum capacity.

In our study, leptin was significantly related to BMI only in obese children, while no associations were documented between these two adipokine levels and the insulin resistance. However, while several adipokines have been associated with obese and insulin-resistant phenotypes, a select group has been linked with insulin sensitivity, namely leptin, adiponectin. In the study of Plonka et al, the degree of body fat and the body composition (as expressed by BMI and other metabolic markers) were proportional to leptin concentration[26]. The increase of leptin levels in obese children normalized after weight loss, while leptin levels were associated with insulin resistance. In another study, all adipocytokines but TNF-a showed significant correlations (negative for adiponectin and positive for others) with clinical biomarkers of obesity and its complications: z-score for BMI, systolic and diastolic blood pressure. Adiponectin and leptin levels were also negatively and positively respectively related to central adiposity quantified by waist circumference. Leptin is the only adipokine associated with insulin resistance in children, while leptin, resistin, and IL-6 have been found to vary significantly different in obese children compared to normal weight controls[27]. The low physical activity in subjects with abdominal obesity has been independently and inversely linked to plasma leptin and insulin sensitivity.

There is also growing evidence that exercise exerts its beneficial effects partly through alterations in the adipokine profile; that is, exercise increases secretion of anti-inflammatory adipokines and reduces proinflammatory cytokines. In the present study, leptin levels were related to physical activity duration only in normal weight group, and HOMA-IR differed between the normal weight and obese children. Okazaki et al concluded that short-term and intensive exercise results in lower leptin concentration, regardless of changes in adipose tissue mass[28]. It has been found that there is a significant difference in physical activity levels between normal weight and obese/overweight adolescents.

Our results indicate that adipokines' levels may reflect cardiopulmonary capacity and they could be markers of insulin resistance. However, the small number of participants in the present study, along with the fact that information on physical exercise in the present was based on parental and children reports, do not allow generalization of our findings. The number of the children who volunteered to cardiopulmonary testing was the main sample limitation. Moreover, outcomes might be different if there was a daily recording of exercise by independent observers. A limitation of the current study is the fact that the obesity status of the participating children was determined simply by using their BMI instead of performing a dual-energy X-ray absorptiometry (DEXA) scan. Another limitation is that currently there is a newer version of HOMA-IR available (can be accessed at the University of Oxford website).

In conclusion, this is the first study to the best of our knowledge that associates the VO_2max with leptin and adiponectin in children. Innate difficulties in cardiopulmonary testing in children may account for data paucity in this age group. An interdisciplinary approach and excellent cooperation of the children is necessary. Our study allows further assumptions for adipokines and childhood obesity, along with providing information on the use of leptin as a possible additional obesity index in the future.

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Conflict of interest

The authors have no conflict of interest to report.

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