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Original article

The relationship between serum leptin and VO2max levels in pre-puberty swimmer girls: effect of acute exercise

Esin Güllü[1], Abdullah Güllü[1], Halil Düzova[2], Bilge Özgör[3], Evren Kilinç[4], Faruk Akçinar[5]

[1]Hitit University, Faculty of Sports Sciences, Corum- E-mail: gullu.esn@gmail.com; [2]İnönü University, Faculty of Medicine Department of Physiology, Malatya; [3]İnönü University, Faculty of Medicine Department of Child Health and Diseases, Malatya; [4]Mehmet Ali Aydınlar University, Faculty of Medicine, Department of Biophysics, Istanbul.
[5]İnönü University, Faculty of Sports Sciences, Malatya

Summary. *Objective*: The aim of this study was to determine the relationship between serum leptin (SL) level, which is the sensor of changes in energy intake and consumption, and maximal oxygen consumption (VO_{2max}) level in pre-puberty swimmer girls. *Methods:* Voluntary participants were divided into swimmer group (n: 16) and the control group (n: 15). Bruce protocol was used for acute exercise effect. Body composition, VO_{2max} and SL concentrations of the study group were measured before and after acute exercise. The paired-samples t-test and independent samples t-test were used for intra- and inter-group comparisons. The linear relations between the VO_{2max} and SL levels were determined by Pearson correlation coefficient. The level of significance was used at 0.05. *Results:* There was a significant difference between the SL level and test stage, test duration, HR of test-end, and VO_{2max} variables in both groups ($p<0.05$). There was a high level of negative correlation between VO_{2max} and SL levels in both groups after exercise (SG, r=-0.63; $p<0.01$, and CG, r=-0.60; $p<0.05$, respectively). *Conclusion:* Acute exercise resulted in decreased SL levels of both groups. It was concluded that regular swimming sports has a positive effect on body composition, VO_{2max}, and SL values of pre-pubertal girls.

Key words: serum leptin, VO_{2max}, body composition, acute exercise, pre-pubertal girl

Introduction

Regular participations in physical activity during adolescence lead to significant changes in hormone concentrations that may affect growth and development. For this reason, having knowledge about specific hormonal and metabolic responses during exercise of children is very important to understand the physiological benefits and potential risks of participation in sports activities on a regular basis (1). In this case, understanding metabolic responses to exercise can help to create better physical activity and nutritional recommendations for children of all ages.

Leptin is one of the hormones consisting of cytokine family and 167 amino acids with various effects on the organism (2). Leptin hormone, which acts as a sensor of changes in energy intake and consumption, secreted by adipocytes may contribute to the long-term control of energy balance and body composition by interaction with receptors in the hypothalamus (3, 4). Also, it plays an important role in the development of fetus, the onset and development of adolescence in children (5) and weight control, energy consumption and nutrient intake (6, 7), in the regulation of neuroendocrine functions (5) has a great effect. Leptin has a short-term and stimulating effect on lipid oxidation in skeletal muscle. Thus, leptin decreases lipid stores in skeletal muscle by increasing fatty acid catabolism (8).

The exercises with sufficient intensity, which may alter the balance of energy consumption, may also lead to changes in leptin levels (8). Because, the exercise is a strong stimulant for the secretion of many hormones and has been suggested to also affect serum leptin (SL) levels (9). Exercise doesn't only increase energy consumption, but also decrease the fatty mass. Since leptin hormone increases the energy consumption, it is ensured the increasing of energy consumptions during exercise, and so the fat mass also decreases. Several studies that have evaluated the effects of exercise on leptin are based on this fact (10), and so exercise can decrease SL levels (11).

Maximum oxygen consumption (VO_{2max}), which is a criterion of cardio-respiratory development, is the most reliable test to determine the maximum aerobic capacity. If someone can consume high amounts of oxygen over a unit time, this means that this person has a high aerobic capacity. That is, there is a high correlation between the maximum aerobic capacity and the ability to sustain severe effort (12). During the high-intensity exercises, fats are metabolized by hydrolysis, and so provide energy. Therefore, many studies show that fat oxidation increases significantly during exercises that are performed at the level of 85% of VO_{2max} (13, 14).

Findings showing the increasing effects of leptin on food intake and energy metabolism have led to the need to investigate the relationships between leptin levels and exercise (15). But unfortunately, despite the potential importance of pediatric exercise metabolism, a limited number of studies are currently available on this topic (1, 9). On the other hand, there are almost no studies that investigate the effect of acute exercise on SL levels of the pre-puberty girls. In conclusion, the aim of this study was to investigate the relationship between serum leptin concentrations and VO_{2max} levels of acute exercise that applied to pre-puberty girls who regularly practice swimming sports.

Materials and Methods

Ethical Considerations

Prior to the research, this study was approved by the Clinical Research Ethical Committee of the Inonu University Faculty of Medicine. The purpose and possible risks of the study were explained to the parents of the intervention group. The informed voluntary parental consent and acceptance forms prepared according to the Helsinki Declaration were read and signed by each parents. All candidates that agreed voluntarily participate in the study were included in the study after going through the required medical examinations.

The Study Group

The study group consisted of 31 healthy pre-puberty girls. The swimmer group (SG) consisted of 16 licensed swimmers who applied training for 90 minutes a day, 6-day a week at a local swimming club. The mean age of SG was 9.88±1.41 years, the height was 140.38±9.75 cm, the weight was 35.66±9.71 kg and the sport age was 26.31±10.22 months. Also, 15 healthy and sedentary girls participated in this research as control group (CG) that the mean age was 9.73±1.16 years, the height was 142.93±10.33 cm, and the weight was 41.20±10.63 kg. Both of the groups were living in the same city, and they came from similar socioeconomic backgrounds.

Study Procedures

Care was taken to ensure that both groups did not participate in any intensive exercise or activity until 48 hours before the study. In the evening before the study, the last meal and fluid consumption was asked to be terminated at 08.00 pm. Children with fasting were called to the physiology laboratory at 8.00 am with their parents and they were informed about the study. The participants were given a light and standard breakfast of 500 kcal (1.5 cheddar cheese toast, 1 egg, and 1 cup of tea with sugar) prepared earlier (16, 17). Participants were shown a movie until 10.30 am (start time of the study) and after the brief information were made again, the study started.

Concurrent Variables

The anthropometric measurements of the intervention groups were taken as shorts, t-shirts and bare feet. A stadiometer (Harpenden, Holtain Ltd. UK) with a precision of ±1 mm was used for height measurement. Body mass index (BMI), which is considered a better index for evaluating adiposity in children, was calculated using the ($Kg/m^{2.88}$) formula for corrected BMI (18). Body weight, basal metabolic rate (BMR), body fat percentage (BFP) and body fat mass (BFM) were determined by bioelectrical impedance analysis (Tanita BC-418 MA Professional, Japan). The adolescent maturity level of the subjects was determined by a pediatric specialist through the evaluation of pubic hair according to the Tanner (sexual maturity) scale ((Tanner Stage I/II/III/IV), the swimmer group was 7/4/2/3 (n=16), and control group was 4/6/1/4 (n=15), respectively) (19).

Acute Exercise Test Protocol

The Bruce test protocol, performed in a laboratory environment and on a computer-controlled treadmill (Cosmed T-150, Italy), was considered an acute exercise program. Every subject started the exercise with a 5-minute warm-up jogging at 0% slope, and subsequently the automatic protocol that was loaded in the treadmill was started. The heart rate (HR) was controlled using the (95% x 220 bpm – Age) formula (20), and it was monitored with a portable pulse meter (Polar S800i, Finland). The test was ended when the observed exhaustion against a certain workload (voluntarily fatigue) and according to the Borg scale criteria (21). The test ending stage, test duration, and ending HR values were recorded for each subject. VO_{2max} capacities of the subjects were determined using the (VO_{2max} (mL/kg/min) = 4.38 × Time (min) – 3.9) formula (22).

Collection of Blood Samples

7 ml of forearm venous blood samples were taken from every subject before and 3 minutes after the acute exercise protocol. The collected blood samples were centrifuged for obtaining serum samples, and then they were stored at -80°C for further study. The SL levels before acute exercise (SL1) and the SL levels after acute exercise (SL2) were determined by Elisa Kit (Boster Biological Technology Co. CA, USA).

Statistical Analysis

The descriptive statistics of the data were calculated and presented in the form of mean and standard deviation (X±SD) in the text. The normality of the variables was tested with the Shapiro-Wilk test and they were found to have parametric distributions (p>0.05). Thus, the independent samples t-test was used for the evaluation of the variables between groups. The paired samples t-test was used for the intra-group comparison of the VO_{2max} and the SL values. The relationship between VO_{2max} and SL levels was tested with the Pearson's simple linear correlation (r) test. The statistical analyses were conducted with the IBM SPSS 25.0 package program, and the level of significance was accepted as p<0.05.

Results

In this study, the physical, anthropometric, metabolic and physiological responses of the pre-pubertal swimmer girl group (SG; n: 16) and sedentary girl group (CG; n: 15) to acute exercise were examined. The results of obtained data before and after acute exercise of the research group are given below in tabular forms.

As a result of the research, it was observed that both groups were in the weak category when the corrected BMI values for the children were examined, and they were in stage II, according to Tanner's sexual maturity grading. For this reason, the mean of the physical, anthropometric, and sexual maturity variables of both groups were similar, since there was no significant difference between the age, height, weight, BMI, BMR, BFP and LBM values of the research group (Table 1; p>0.05).

Discussion

This study is based on the relationship between the acute exercise protocol and leptin VO_{2max} by comparing two groups of healthy pre-puberty girls (swimmers and sedentary). It was observed that there was no significant difference between the pubertal levels of the 31 girls who were taken into the study as they were in stage II of the Tanner (sexual maturity) scale. This can be explained by the fact that there are no hormonal sex-dependent changes in the early ages (23), and that the two groups were physically and anthropometrically similar. It was observed that there were a significant relationship and a direct correlation between the acute exercise (Bruce) protocol ending stages and duration. When the corrected BMI ($Kg/m^{2.88}$) results – which is considered a better index for the evaluation of adiposity of children (18) – it was determined that the both groups were healthy and non-obese.

Table 1: Descriptive statistics and inter-group comparisons of the research group

	Swimmer Group (SG, n:16)		Control Group (CG, n:15)			
Variable	**X**	**SD**	**X**	**SD**	**t**	**p**
Age (year)	9,88	1,41	9,73	1,16	0,304	0,763
Height (cm)	140,38	9,75	142,93	10,33	-0,709	0,484
Body mass (kg)	35,66	9,71	41,20	10,63	-1,517	0,140
BMI ($kg/m^{2.88}$)	13,31	2,56	14,58	2,64	-1,365	0,183
BMR (kcal)	1150	136,13	1222	147,75	-1,412	0,169
BFP (%)	23,36	6,28	26,87	6,48	-1,529	0,137
BFM (kg)	8,78	4,60	11,59	5,15	-1,607	0,119
Test ending stage	4,38	0,89	3,73	0,70	2,224	0,034*
Test duration (min)	12,88	2,67	10,31	2,05	2,977	0,006*
Ending HR (bpm)	203,44	7,47	197,53	4,79	2,636	0,014*
VO_{2max} (ml/kg/min)	52,49	11,71	40,98	9,10	3,040	0,005*
SL1 (ng/mL)	11,67	8,17	14,82	9,86	-0,971	0,339
SL2 (ng/mL)	8,53	5,55	12,20	9,22	-1,354	0,186
Sports age (months)	26,31	10,22				
Tanner stage1/2/3/4	7/4/2/3 (X±SD: 2,06±1,18)		4/6/1/4 (X±SD: 2,33±1,18)			

**p<0.05; X±SD: mean±standard deviation; SL1: SL level before acute exercise; SL2: SL level after acute exercise.*

The VO_{2max} values of the subjects after the acute exercise protocol, together with the other intra-group variables, were found to be strongly correlated with the test ending stage and test duration (p<0.01; Table 3). The HR and VO_{2max} values of the swimmer and control groups were found to be similar with results of other studies (24, 25). The inter-group comparisons revealed that the SG had better HR and $VO2_{max}$ values according to CG (p<0.05; Table 2). The inter-group comparisons revealed that the SG had better HR and VO_{2max} values (p<0.05; Table 2). This result was not a surprise. Because, swimming raises heart rate and respiratory frequency, and normally provides increased blood flow to the skeletal muscles while increasing the blood pressure (26). Thus, the chronic effect of regular training (26.31±10.22 months) is decreased HR and increased heart beat volume (27), which may have led to the significant improvement of the HR values in favor of SG. The increased VO_{2max} can depend on the frequency, intensity and the duration of the training, which can provide 5-30% increase (28, 29). Therefore, it can be said that regular swimming practices positively affect the VO_{2max} levels of the swimmer subjects.

It was observed that there was a highly linear correlation between the SL concentrations and BMI of

Table 2: Intra-group SL1 and SL2 levels of the research group

Group	Variable	X	SD	t	p
SG (n:16)	SL1 – SL2 (ng/mL)	3,14	3,66	3,43	0,004*
CG (n:15)	SL1 – SL2 (ng/mL)	2,62	4,60	2,21	0,045*

**p<0.05; SL1: SL level before acute exercise; SL2: SL level after acute exercise.*

Table 3: Intra-group correlation between VO_{2max} and other variables of the research group

Variable	SG (n:16)	CG (n:15)
Age (year)	0,04	-0,12
Height (cm)	-0,20	-0,07
Body mass (kg)	-0,45	-0,33
BMI ($kg/m^{2.88}$)	-0,42	-0,48
BMR (kcal)	-0,42	-0,274
BFP (%)	-0,43	-0,48
BFM (kg)	-0,48	-0,39
Test ending stage	0,97**	0,83**
Test duration (min)	1,00**	0,99**
Ending HR (bpm)	0,36	-0,06
SL1 (ng/mL)	-0,54*	-0,53*
SL2 (ng/mL)	-0,63**	-0,60*

***p<0.01; *p<0.05; SL1: SL level before acute exercise; SL2: SL level after acute exercise.*

the research group ($p<0.01$; Table 4). This suggests that the leptin levels were influenced by the adipose tissue that is increasing with age (23), other than the logical differences in BMIs that caused by young ages. While energy consumption increases with exercise, fat mass decreases accordingly. Since leptin increases energy consumption (10), thus, the leptin hormone can also explain the high level of correlation between the SL levels and the BMR, body fat percentage, and body fat mass variables of both groups ($p<0.01$; Table 4). In addition, it was observed that the negative moderate correlation between the test ending stage and test duration of the acute exercise and the SL levels was in the favor of the SG ($p<0.01$; $p<0.05$; Table 4). This may be the result of the CG's shorter exercise time and conclusion of the exercise in easier phases of the treadmill program (30), despite encouragement and psychological support.

The SL1 and SL2 levels were not significantly different between the two groups ($p>0.05$; Table 1). This may be because SL levels are stable among girls aged 9 to 11 (31). But also, a significant decrease was revealed in intra-group comparisons ($p<0.05$; Table 2). This may be because of the fact that chronic exercise often decreases leptin levels (32-35). Thus, the energy consumption increases with exercise while the fatty mass decreases, and subsequently, the leptin hormone contributes to the energy metabolism (32-34). This is due to key role of leptin in energy homeostasis and adipocyte secretion (36). The decreased BMI and fatty mass that results from long-term exercise can decrease leptin concentration (32-34). However, while strenuous exercises cause a decrease in leptin levels in men and women (37), a decrease in plasma leptin levels may be observed after acute exercise of 30 minutes (at 50% VO_{2max}) (38). In addition, 41 minutes or shorter exercises, if they are exhausting enough to consume, may change the concentrations of SL due to their effects on fatty acid partitioning in muscle cells (36, 39). These data support the notion that high-intensity exercises are more effective on SL levels. On the other hand, the retrieval period of blood sample after the acute exercise session (3 minutes after the protocol) (40) may have caused this decrease in SL levels. In addition, these decreases in SL levels of both groups can be attributed to circadian rhythm or hemoconcentration (41, 42). These findings suggest that exercise-related reductions in leptin may be due to changes in nutrient availability or changes in the nutrient flow in the production and secretion of leptin, the primary site of adipocytes (39).

Table 4: Intra-group correlation between SL levels and other variables of the research group

Variable	SG (n:16) SL1 (ng/mL)	SG (n:16) SL2 (ng/mL)	CG (n:15) SL1 (ng/mL)	CG (n:15) SL2 (ng/mL)
Age (year)	0,02	-0,05	0,32	0,08
Height (cm)	0,21	0,12	0,50	0,25
Body mass (kg)	0,71**	0,69**	0,85**	0,68**
BMI (kg/$m^{2.88}$)	0,77**	0,85**	0,67**	0,72**
BMR (kcal)	0,68**	0,65**	0,83**	0,63*
BFP (%)	0,74**	0,79**	0,73**	0,74**
BFM (kg)	0,77**	0,78**	0,83**	0,75**
Test ending stage	-0,51*	-0,65**	-0,26	-0,24
Test duration (min)	-0,54*	-0,63**	-0,53*	-0,62*
Ending HR (bpm)	-0,36	-0,34	-0,15	-0,22
VO_{2max} (ml/kg/min)	-0,54*	-0,63**	-0,53*	-0,60*
SL1 (ng/mL)	1	0,93**	1	0,89**

***p<0.01; *p<0.05; SL1: SL level before acute exercise; SL2: SL level after acute exercise.*

It was observed that there was a significant correlation between the SL and VO_{2max} measurements in both groups, both before and after acute exercise ($p<0.05$; Table 3). There was a moderate negative correlation between VO_{2max} and SL1 levels, and a high inverse relationship with SL2 in both groups. As similar studies had found a negative correlation between VO_{2max} and SL levels (2, 43), these results of current study were consistent with the findings of various studies in this field.

The VO_{2max} considered as a determiner for evaluation of cardiovascular fitness (44), and it is a very suitable test to reflect the type, duration and performance of exercise in a given population (45). The VO_{2max} is measured by exercise tests such as treadmill or cycle ergometer (44), and Bruce test is the most widely used of them. In this study, we observed that there were significant differences in test ending stage, test duration, ending HR and VO_{2max} values after acute exercise. This situation can be interpreted for SG and CGs as a sign of different physical performance. Because, compared to the pre-puberty swimmers, pre-pubertal sedentary girls required more oxygen and energy for an equivalent workload. Therefore, the CG working in the lesser phase of the treadmill program failed to work hard and relatively quickly exhausted despite the adequate encouragement and psychological support (30). For this reason, despite moderate changes in CG's mean leptin values, some individuals showed a large increase or decrease in leptin levels, while there were no any changes for the others (36). Several factors influence the interpretation of these results. For example, exercise training may produce changes in leptin production and/or lack of leptin, at a given time point, that leptin levels cannot be reflected by a single plasma measurement. Furthermore, there are studies indicating that leptin circulates in free form (possibly in bioactive form) or that it is due to leptin binding proteins and that the ratio of these two forms changes even among weak and obese individuals (46). Because leptin is one of the most important regulators of energy balance (37), and given the important role of fat mass in circulating leptin levels, the role of adipose tissue in the secretion of these hormones can be directly correlated with VO_{2max} (47). Thus, it can be interpreted that individuals who regularly exercise can also decrease serum leptin levels as the VO_{2max} (aerobic) levels increase (43). To date, it has been suggested that different results of the effects of exercise on leptin may be responsible for leptin changes in exercise, fluctuations in food intake, intensity, duration of exercise, and circadian rhythm. Therefore, it can be said that even acute exercises with enough intensity to affect energy balance or body fat mass may change leptin secretion (39). So, an increase in VO_{2max} can be expected as long as exercise can be sustained. Correspondingly, regular and prolonged exercises increase fat metabolism and decrease fatty mass, and also suppress SL levels (2). For these reasons, acute exercises with enough intensity to affect the energy balance or the body fat mass may justify our findings that lead to a decrease in serum leptin levels.

Conclusion

The increases in VO_{2max} during acute exercise were found directly related to BMI, BMR, BFP, BFM, and SL levels. Regular and long-term swimming exercises increased the pre-puberty girls' fat metabolism, and thus reduced the amount of body fat and suppressed serum leptin levels, too. Correspondingly, it can be said that long-term and regular swimming exercises (due to tendency to adapt to chronic exercises) cause a decrease in leptin levels. Therefore, it was observed that swimming sport performed regularly in pre-puberty period of girls had a positive effect on BMI, BFP, BFM, HR, VO_{2max} and SL values. For all these reasons, regular swimming exercises may be recommended for healthy development of pre-puberty girls.

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Correspondence:
Esin Güllü
Hitit University, Faculty of Sports Sciences, Corum
E-mail: gullu.esn@gmail.com