

The effect of different term swimming exercise in rats on serum leptin levels

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Summary. The aim of this study was to investigate the effect of serum leptin in rats performing various duration swimming exercises. In the study, 30 healthy albino wistar male rats with an average weight of 180-220 grams were divided into 5 groups including; control, water exercises, 15, 30 and 60 minutes swimming groups. Animals were swim-exercised for 90 days. At the end of 90 days, after performed urethane anesthesia, blood samples were taken by intracardiac way. Collected blood was analysed according to procedures. Data were analyzed by using SPSS 15. One-way anova and tukey multiple comparison test were performed in the study. The homogeneity of the variances was examined by Levene's statistic. Results showed that the leptin levels were as follows: control (1480,00 pg/ml), water exercise (705,83 pg/ml), 15 (602,33 pg/ml), 30 (396,67 pg/ml) and 60 (435,83 pg/ml). Statistically significant difference was found between control and 30 minutes swimming group ($p=0,012$) and control and 60 minutes swimming group ($p=0,017$) ($p<0,05$). It is determined that 3 months of different terms (15, 30, 60 minute) of swimming exercise reduces the levels of leptin.

Keywords: swimming exercise, rat, leptin

Introduction

Biochemical parameters differ in terms of the type, intensity, period and continuity of the exercise. The effect mechanism of recently discovered leptin hormone which has significant roles in energy homeostasis and the association between leptin and exercise have been assessed with various acute and chronic exercise practices (1-18). In these studies, the dominant thought is that short-term (<12 weeks) acute exercises do not influence leptin levels or have a very little effect (1, 6, 7, 9, 13-15), while long term (>12 weeks) and long term chronic exercises influence serum leptin levels (4, 5, 8, 10, 12, 16, 18). However, there are studies which show that leptin levels decrease as a result of acute exercises (2, 3, 11, 17).

Several investigators reported that exercise may result in reductions depending on the duration and

calorie expenditure whereas others have reported no change in leptin concentrations. Exercise doesn't generate decrease in leptin concentrations; Torjman et al. (1999), Weltman et al. (2000), Kraemer et al. (2002). Besides, exercise that generated decrease in leptin concentrations; Zafeiridis et al. (2003). In general, the decrease in leptin concentration after a long-term exercise (≥ 60 min) has been attributed to diurnal reduction in circulating leptin and hormonal changes induced by exercise. Exercises of very long duration that generated a sufficient energy imbalance (kilocalorie intake versus kilocalorie expenditure) suppress the amplitude of the diurnal rhythm of leptin (21).

Information regarding the response of serum leptin to a single bout of resistance exercise is limited. In contrast to continuous running of moderate intensity, heavy resistance exercise is a potent nonoxidative stimulus that produces differential neural, metabolic,

and neuroendocrine responses (9). Exercise is another physiological stress that may alter leptin secretion. In rats, ob mRNA levels were found to be decreased immediately and 3 h after a prolonged exercise bout to exhaustion (22). Significant reductions in serum leptin levels were observed after exercise training, but these were explained by changes in fat mass. One study performed in animals has shown that acute exercise in rats was associated with a 30% reduction of the expression of the ob gene in the adipose tissue (23).

According to some authors, low leptin concentration was probably due to weight reduction. Thus, short-term training (< 12 weeks) and long-term training (\geq 12 weeks) have disparate findings concerning leptin concentration. The reduction of leptin has been attributed to alteration in energy balance, improvements in insulin sensitivity, alteration in lipid metabolism and lipid concentration and unknown factors (21). Within this framework, a significant relationship is thought to exist between serum leptin level and type of exercise. Further, the characteristics of a sport affect the serum leptin levels of an athlete who does that sport (24). Physical activity is important for long-term regulation of body weight, partly because it increases the resting metabolic rate. Weight reduction after physical exercise is correlated with reductions in plasma leptin concentrations in obese middle-aged women. However, results regarding the effects of exercise on plasma leptin concentrations, independent of fat mass, are conflicting (25).

Leptin levels and body mass index have a positive relationship (26, 27). Leptin has a critical role on regulation of body weight and also body fat mass. Given the key role of leptin on regulation of body weight and prevention of obesity, it seemed that leptin levels were decreased during the elevation of body weight. But most obese humans have higher circulations of leptin. It has been indicated that obesity might induce state of leptin resistance (28). Moreover, Leptin is one of a growing number of adipocytokines that play an important role in the regulation of body weight by coordinating metabolism, feeding behavior, energy balance, fertility and neuroendocrine responses. Originally thought to be exclusively produced by adipocytes, it is now evident that leptin is expressed, in lesser amounts, in many fetal and adult tissues

including: placenta stomach, mammary gland, skeletal muscle, pancreas, and bone (29).

Leptin, which is the product of obesity gene (30), regulates weight and food intake in both animals and people, suppresses hunger (31) and decreases food intake (32), increases energy use and prevents fat accumulation (25, 9, 33, 34, 35). The most important effect of leptin on energy expenditure is providing increase in thermogenesis (17). In addition, leptin has been found to have a great number of metabolic and endocrine functions (36, 37, 38, 39) and also have a role in obesity, bone development, wound healing (40), fetus development (41), start and development of adolescence in children (37), hypertension and heart diseases (42, 16), and the regulation of gastrointestinal functions and glucose metabolism (42, 33, 43).

The association of leptin, which is found to have a great number of functions for human metabolism, with exercise is still not clear. We can say the effects of physical exercise on leptin are still being discussed and currently controversial. When studies conducted so far are examined, the effect of different durations of chronic exercise on leptin has not been researched yet, to the best of our knowledge. Thus, the purpose of this study is to show the effects of long and different durations of swimming exercise (15, 30, 60 minutes for 3 months) on the leptin levels of rats.

Materials and Methods

Adult male Wistar rats weighing 180–220 gr. were used throughout this study after at least 1 week of acclimatization. All described procedures were approved by the local ethics committee. Animals were housed in groups of 5 and were allowed free access to food and water, except for the short time that the animals were removed from their cages for the experiments. All animals were kept in a temperature controlled ($22\pm 1^\circ\text{C}$) environment on a 12-h light/dark cycle.

Experimental Design

Rats were assigned to the following experiments and groups: (Group 1) control group; (Group 2) 15 minutes-trained for 90 days; (Group 3) 30 minutes-trained for 90 days; (Group 4) 60 minutes-trained for

90 days (Group 5) adapted to the water. Each animal group was composed of six rats.

The adaptation to the water proceeded during experimental period. The purpose of the adaptation to water was to reduce stress without promoting a physical training adaptation (44, 45).

Exercise training program

The swimming was performed in water at a temperature of 32-33°C between 11.00-13.00 A.M. Training period lasted 90 days and consisted of 15, 30, and 60 minutes of daily sessions for seven days/week without workload. Exercise was performed by swimming in three training glass tanks (length 100 cm, width 50 cm, depth 50 cm) containing tap water.

At the end of 90 days, after performed urethane (1.25 g/kg) anesthesia, blood samples were taken by intracardiac way. Blood samples were collected into EDTA bottles for hormonal assay.

Analyzes were made in Samsun Veterinary Control Institute Biology Laboratory. In leptin analysis; Sigma Rat Leptin Elisa Kit was used. All reagents and samples were taken to room temperature (18-25°C) before use.

All standards and samples were repeated twice. 100 ml of each standard and samples were added into appropriate wells. Well were covered and incubated for 2.5 hours at room temperature with gentle shaking by Heidolph Tiramax 1000 orbital shaker. The solution was discarded and washed 4 times with 1x Wash Solution. Each well was washed and filled with 1x Wash Buffer (300 ml) using a multichannel pipette. At each step removal of liquid was completed for good performance. Biotinylated antibody was prepared and added 100 ml of 1x prepared to each well and was incubated for 1 hour at room temperature with gentle shaking. The solution was discarded and washed 4 times with 1x wash solution. Each well was washed and filled with 1x Wash Buffer (300 ml) using a multichannel pipette. 100 ml of prepared Streptavidin solution was added to each well and incubated for 45 minutes at room temperature with gentle shaking. The solution was discarded and washed 4 times with 1x Wash Solution. Each well was washed and filled with 1x Wash Buffer (300 ml) using a multichannel pipette. 100 ml of TMB One-Step Substrate Reagent (Item H) to each well was added and incubated for 30 minutes at room

temperature in the dark with gentle shaking. 50 ml of Stop Solution was added to each well and was read at 450 nm immediately. The mean absorbance of each data was calculated. On the sigma plot software, the standards were plotted on the curve and the curve was drawn at these points. By using this standard curve, the leptin concentrations of the samples were calculated in pg/ml.

Data were analyzed using SPSS 15 (Statistical Package for the Social Sciences) package and the error rate was determined as 5%. One-way anova was used to determine the differences between the groups, and the Tukey's Multiple Comparison Test was used to determine which groups the difference was in when the difference was found. The homogeneity of the variances was examined by Levene's statistic.

Results

Serum leptin levels of rats which were trained with swimming exercises of different durations (15, 30, 60 minutes for 3 months) are given in the tables below respectively.

In the study, the highest average leptin values were found in the control group, while the lowest average leptin values were found in the 30 minutes swimming group.

Statistically significant difference was found between control and 30 minutes swimming group ($p=0,012$) and control and 60 minutes swimming group ($p=0,017$) ($p<0,05$). Differences were found between groups ($p=0,010$).

Discussion

In the present study, statistically significant difference was found between control and 30 minutes swimming group and control and 60 minutes swimming group when rats which were trained for 15, 30 and 60 minutes of swimming for three months were examined.

No statistically significant differences were found between the leptin levels of the control and water adaptation group. Short exercises during the one-week

Table 1. Post-swimming exercise serum leptin values and One Way Anova results

Groups	n	\bar{x} pg/ml	sd	F	p
Control	6	1480,00	870,05		
Water adaptation	6	705,83	513,98		
15 Minutes of swimming	6	602,33	491,48	4,179	0.010
30 Minutes of swimming	6	396,67	269,48		
60 Minutes of swimming	6	435,83	242,54		

$p < 0,05$

Table 2. Post-swimming exercise serum leptin values and paired comparisons

Groups	n	\bar{x} pg/ml	ss	Control	Water adaptation	15 min. swimming	30 min. swimming	60 min. swimming
				P	P	P	P	P
Control	6	1480,00	870,05		0,114	0,057	0,012	0,017
Water adaptation	6	705,83	513,98			0,997	0,846	0,900
15 Minutes of swimming	6	602,33	491,48				0,960	0,981
30 Minutes of swimming	6	396,67	269,48					1,000
60 Minutes of swimming	6	435,83	242,54					

$p < 0,05$

long process of water adaptation were not found to create stress-induced leptin changes. Although not significant, decreases in leptin levels of the water adaptation group when compared with the control group decreased leptin levels of one-week long water exercises; however, this decrease was not sufficient and supports our thought that stress-induced leptin decreases are not experienced.

Statistically significant difference was found between the control group and the 30 minute swimming group in terms of leptin levels. In their study they applied a training of 9 weeks treadmill exercise-every day for the first two weeks and twice a day for the following 7 weeks, which started with 30 minutes and got 2 minutes longer every day, Zhao et al. (2011) found a significant decrease in the leptin levels of rats (n=16) (35). In another study, Kondo et al. (2006) found decrease in leptin levels as a result of exercises they applied on 8 obese women for 7 months-4-5 days a week and 30-60 minutes a day (12). In a study conducted on women between the ages of 40 and

49 who were in premenopausal period and who were not doing regular exercise (n=40), Gözlükaya (2008) found significant changes in the leptin hormone levels of the quick tempo group as a result of 54-minute walking exercise which started as 30 minutes a day and increased 3 minutes a week for five days a week in 10 weeks (46). Another study showed that 30 min of swimming exercise caused a 30% reduction in leptin levels in lean but not obese (fa/fa) Zucker rats (22). Zafeiridis et al. (2003) controlled the effects of maximum strength, muscular hypertrophy and resistance exercise protocols on serum leptin concentrations. Leptin concentrations significantly decreased 30-minute into recovery after exercise protocols compared with the respective baseline values. While decreases are found in leptin levels in all studies as a result of exercises, data from investigations examining single exercise bouts suggest that serum leptin concentrations are unaltered by short duration (41 minutes or less), non-exhaustive exercise, but may be affected by short duration, exhaustive exercise (47). Weltman et al. (2000) found that 30 min

of exercise at various intensities and caloric expenditure (from 150 ± 11 to 529 ± 45 kcals) in 7 healthy young men did not cause modifications in leptin levels during the exercise and during the recovery (3.5 hours) (20). Exercise programs preferred in studies have been planned as 9, 10 and 12 weeks, for periods of 30 minutes and more. According to the results of this study, it can be said that at least 9-10 week-long regular exercise programs with unit periods of 30 minutes and more can cause significant changes in leptin levels.

In the present study, statistically significant difference was found between the control group and the 60-minute swimming group in terms of leptin levels. Benatti et al. (2008) applied swimming exercises on rats ($n=18$) which lasted 40 minutes for 5 days in the first week and continued as 5 days a week and 60 minutes a day for the following 8 weeks. They found that 9-week long swimming exercises decreased the serum leptin levels of rats irrespective of their body fat mass. In their study they applied a diet for 4 months and an hour long moderate exercise 3-4 days a week on obese men ($n=15$) (48). Pasman et al. (1998) found with blood samples taken from the study group at months 0, 2, 4, 10 and 16 that exercise decreased plasma leptin levels irrespective of body fat change. In their study they examined the association between diet, exercise and obesity (4). Murakami et al. (2007) found that as a result of 60-minute long aerobic exercise for three days a week during 12 weeks decreased leptin levels (49). Moazami et al. (2013) found that 24-week long aerobic exercise (three days a week, for 60 minutes) decreased serum leptin levels in obese women ($n=15$) (50). While the results of the aforementioned studies are similar to the results of our study, the study period of our study is 12 weeks, as in Murakami et al. (2007)'s study; however, the frequency of their exercise is different from our study. The fact that the results are similar in all these studies although different exercises were applied in different intensities and frequencies shows that the type, content, intensity or frequency of exercise is not effective on the change in leptin levels and the change can occur as a result of regular and long-term exercises. Benatti et al. (2008)'s results showed that 9 week-long exercise can cause significant decreases in leptin levels and this shows that 9 week and longer regular exercises which last longer than 30 minutes can be enough.

In the present study, no statistically significant difference was found when the results between control group and 15-minute swimming group and water adaptation group and 15-minute swimming group measurement results were compared. Akbarpour (2013) applied a running exercise program on obese men ($n=16$) for 12 weeks as three days a week. Following 10 minute long warm up, the running period was 15 min for the first session, and every two sessions 1.5 min was added to the running period in a stepwise manner until the running period reached 30 min. The running exercise program was found to decrease serum leptin levels significantly ($p=0,003$); there were decreases in the leptin levels in interval measures conducted at the end of 6th week; however, the decrease was not found to be significant (51). This was thought to occur as a result of insufficient training period and intensity. These two studies show the significance of both unit exercise duration and long-term chronic exercises to have significant decreases in leptin. In our study, it was found that 15-minute long exercises decreased leptin levels; however, this decrease was not significant, while 30 and 60-minute long exercises caused significant decrease. According to these results, it can be said that to obtain significant decreases in leptin levels, unit exercise durations are as important as long-term exercises and 15-minute long exercises are not sufficient. The fact that the average leptin levels of the 15-minute swimming group were higher than both 30 and 60-minute swimming group supports this thought.

As a conclusion, while swimming exercises for 30 and 60 minutes during 12 weeks decreased the serum leptin levels of rats significantly, this significant decreasing was not found in 15-minute exercises. There are no experimental animal studies in literature conducted with parameters similar to ours in literature. Studies conducted in this field with different procedures and different approaches will give healthier information.

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