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The effects of endurance training on the relationships body composition plasma ghrelin and leptin levels

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Abstract

The present research was conducted over 35 voluntary students studying at Collage of Physical Education and Sports of Selcuk University. The students were at 18-24 years age group and were composed of 18 women (10 experimental and 8 control) and 17 men (9 experimental and 8 control). A special diet program was not applied to experimental students. Experimental groups were subjected to 60 minutes cycling exercises in 4 days of the week for 8 weeks at 50-70% of previously determined heart rate. Control groups were prevented from any special sportive activities except for their practical courses. Ghrelin levels were measured by Enzyme Immuno Assay (EIA) and leptin levels were measured by ELISA method. Effects of gender on body weights, percent body fat (PBF) and maximal aerobic capacities (VO_{2max}) of the men and women students measured at the beginning and end of the tests were found to be significant (P<0.001). Exercises significantly increased only VO_{2max} levels of men and women experimental groups (P<0.001). Pre- and post-experimental leptin and ghrelin levels were also significantly affected by the gender (P<0.001), however, exercises did not significantly affect the levels of these hormones. It was concluded that endurance trainings did not have significant effects on body weights, PBF and body mass index (BMI) of both men and women experimental groups, but significant increases were observed in VO_{2max} levels of entire experimental students with applied exercise programs and gender was found to be significant with regard to body weight, PBF and VO_{2max} levels of the students. Additionally, leptin and ghrelin levels were not also significantly affected by the applied exercise program, but the effects of gender on leptin and ghrelin levels were not also significantly affected by the applied exercise program, but the effects of gender on leptin and ghrelin levels were found to be highly significant.

Keywords: Endurance training, ghrelin, leptin.

INTRODUCTION

Rapidly developing technology results in a slowdown in physical activity in daily routines. Such a slowdown brings together various health problems, obesity and related complications. Proper physical exercises may be developed to overcome these health problems. However, significant changes are observed in body compositions and bio-chemical parameters based on type, rate and duration of these exercises and such changes can be observed through the changes in leptin and ghrelin hormone levels with effective roles on metabolism (2,31).

Ghrelin, discovered by Kojima et al. (16), is the first and only hormone determined to increase appetite of humans when supplied peripherally. Ghrelin is a peptide-formed hormone. It is dominantly produced in stomach and released to blood circulation and stimulates hunger center of the brain (15,35). Ghrelin increases energy intake and regulates eating behavior and mass balance. It has a negative interaction with leptin hormone (24).

Leptin is produced in adipose tissue cells and inhibits fat storage through transferring information to brain about fat storages of adipose tissues (36,12). It is known that regular exercises dissolve fats from adipose tissue and strongly effect fatty acid and glucose metabolism (21). The outcomes indicating increasing effects of leptin on nutrient intake and energy metabolism have brought the need for research to investigate the relationships between leptin levels and exercises (34). Although there are several researches investigating the effects of endurance trainings on body composition, the number of research investigating the relationships between endurance trainings and ghrelin-leptin hormone levels of different genders and the effects of endurance trainings on body composition of them are highly insufficient. Thus, the present research was conducted to investigate the effects of cyclic

exercise programs on leptin and ghrelin hormone levels of men and women.

MATERIAL & METHOD

Selection of Research Group and Training Program

A total of 35 voluntary students studying at Collage of Physical Education and Sports of Selcuk University participated in this research. Non-smoker and not regularly exercising students were at 18-24 years age group and they were composed of 18 women (10 experimental and 8 control) and 17 men (9 experimental and 8 control). Participants were informed about the research and a written consent of them were taken to present their voluntary participation into this research. Then they were randomly separated into research groups. Approval of Ethics Committee of Collage of Physical Education and Sports of Selcuk University was also received before the initiation of research program.

Experimental groups were subjected to 60 minutes cycling exercises in 4 days of the week for 8 weeks at 50-70% of previously determined heart rate. Target Heart Rates (HR) of experimental students were calculated by using Karvonen method.

- HR = (% Exercise intensity × Heart rate reserve) + restingHR
- Heart rate reserve = maxHR restingHR (26).

Experimental groups were subjected to 10 minutes warm-up and cool-down exercises before and after each exercise program. The program was implemented at 50% intensity during the initial 3 weeks, at 60% intensity between the 4th and 6th weeks and at 70% intensity during the last 2 weeks. Control groups were not included into any exercise programs during the 8 weeks research period except the practical courses they took.

Measurements and Tests

Body composition

Heights of participants (cm) was measured with a Seca mechanical balance with a height meter (±1 mm) in a position with bare-foot stepping horizontal over the base, conjoined heels, stretched knees and up-straight body. Body weights of participants (kg) were also measured with the same device (±100 g) with shorts and T-shirts of men and women students are on. Body mass index (BMI) was calculated by dividing body weight (kg) to the square of height (m) (1). Percent body fat (PBF) was determined with a Holtain skinfold caliper under 10 g/sq mm pressure by taking the thickness of triceps, biceps, subscapularis and surailiac skin folds and by calculating the body intensities.

- Men = 1.1631 0.0630*Log (biseps + triceps + subscapularis + suprailiac)
- Women= 1.1599 0.0717*Log (biseps + triceps + subscapularis + suprailiac)

% Fat = (4.95 / Body sensity - 4.50)* 100 (4).

Maximal aerobic capacity

Following sufficient warm-up, participant students were subjected to incremental cyclic ergometry at 60 cycles per minute and 50 watt (W) loading. The load was increased 25 W in each 3 minutes. The tests were terminated when the participants reached to maximal heart rate (maxHR = 220 - age) or when the respiratory exchange ratio exceeded 1.1 or in cases where the participants were not able to reach 60 cyclic rate per minute or in case where they were so exhausted to complete the test. Oxygen consumption during the exercises was monitored with an indirect calorimeter (Cosmed K4, Italy) and VO_{2max} values were determined.

Body composition and aerobic capacity measurements of entire participants were performed before the training program and at the end of eighth week.

Blood analyses

Blood samples were taken from the participants into anticoagulant vacutainer blood sampling tubes at the beginning of experiments, at the end of 4th and 8th weeks at 8 o'clock 24 hours after the last exercise. Blood samples were stored in an ice-bath and quickly transferred to laboratory. Then, the samples were centrifuged at + 4°C and 3000 rpm for 20 minutes to have blood plasma. The plasma samples were then separated into 3 pools and preserved in a deep-freezer at -20 °C until the analyses. A special diet program was not applied to experimental and control groups and participants were asked to come blood sampling without eating anything 12 hours ahead of the blood sampling.

Plasma ghrelin and leptin levels of the blood samples were measured. Ghrelin levels were measured by Enzyme Immuno Assay (EIA) method and plasma leptin levels were measured by Enzyme-Linked Immuno Sorbent Assay (ELISA) method.

Statistical Analysis

Arithmetic means and standard error means of all parameters were calculated. Following the normal distribution and variance homogeneity evaluations of the data, Bonferroni corrections were performed and variance analysis was carried out according to repeated measures experimental design with three factors (time-gender-group; 3x2x2) to investigate the effects of aerobic exercises on plasma ghrelin and leptin levels. In case of any significance in investigated parameters, the differences among the groups were tested by Tukey's multiple range tests through separate single-factor variance analysis for three measurement times. Effects of training and genders on body composition were tested again with variance analysis in accordance with repeated measures experimental design with three factors (time-gender-group; 2x2x2). Significant differences between training-control groups or ender groups were tested by independent groups t-tests; the changes in body composition variables before and after the training programs were tested by dependent groups t-test. The relationships between plasma ghrelin-leptin levels and body composition variables evaluated were through Pearson correlation coefficient (r).

RESULTS

Body Compositions and Maximal Aerobic Capacity Levels

Current results revealed significant effects of gender on body weights, PBF and VO_{2max} (P<0.001). At the end of experimental program, while significant increases were observed in body weights of men groups, decreases were observed in body weights of control groups (P<0.05). PBF of women groups were significantly lower than the control groups both before and after the experiments (P<0.05). The endurance trainings significantly increased only the VO_{2max} values of both men and women (P<0.001; Table 1).

Plasma Ghrelin Hormone Levels

Plasma ghrelin hormone levels exhibited significant differences with regard to genders before the experiments (P<0.01) and the value was 4.68 ng/ml in women control group and 2.82 ng/ml in men control group. Similar difference was also observed in experimental groups and the value was 4.39 ng/ml in women experimental group and 3.37 ng/ml in men experimental group. Such differences continue throughout the experiments.

Variables	Gender	Grup	Pre- Experiment	Post- Experiment	Т	Gr	G	TxGr	TxG	GrxG	TxGrxG
Body	Women	Control	59.0±2.0&	59.1±2.2 ^{&}	0.53	1.37	38.81***	13.57**	2.19	0.57	2.70
Weight		Experimental	54.6±0.7*	55.6±1.1 ^{&}							
_	Men	Control	70.9±2.9	69.6±3.0¥							
		Experimental	68.9±2.2	69.9±2.2¥							
BMI	Women	Control	22.0±0.6	22.0±0.7	0.23	1.59	0.73	3.30	1.22	0.05	2,18
		Experimental	21.0±0.3	21.1±0.4							
	Men	Control	22.6±0.8	22.2±0.8							
		Experimental	21.6±0.7	21.8±0.7							
PBF	Women	Control	24.1±1.0 ^{&}	24.4±0.9 ^{&}	3.42	6.39*	163.3***	1.74	0.54	0.88	1.54
		Experimental	22.7±0.7*	23.1±0.7&							
	Men	Control	14.0±1.3#	13.8±1.1							
		Experimental	10.6±0.7	11.2±0.8							
VO _{2max}	Women	Control	35.5±2.1*	35.1±2.3 ^{&}	61.45***	4.58*	65.38***	59.78***	0.01	0.17	0.73
		Experimental	33.4±1.1 ^{&}	43.2±1.2¥#&							
	Men	Control	48.2±2.5	48.8±2.1							
		Experimental	48.5±1.3	57.2±2.0¥ #							

Tablo 1. Body compositions and VO_{2max} levels of men and women students (Mean±SD).

T= Time, Gr=group, G=Gender, TxGr=Time-Group interaction, TxG=Time-Gender interaction, GrxG=Group-Gender interaction, TxGrxG=Time-Group-Gender interaction, TxGrxG=Time-Gender inter

P*<0.05, **P*<0.01, *P*<0.001; Significant effects of factors or interactions according to repeated measures with three factors.

**P*<0.05; Significant differences in pre and post-experiment variables of men and women of control or experimental groups.

* P<0.05; Significant differences between control and experimental groups of men and women.

& P<0.05; Significant differences between men and women of control or experimental groups.

Tablo 2. Ghrelin levels of women and men students (ng/ml).

Measurement	Gender	Group	Mean ± SD	T	Gr	G	TxGr	TxG	GrxG	TxGrxG
I.	Women	Control	4.68 ± 0.68^a	1.70	0.22	10.43**	0.49	0.20	0.64	0.04
		Experimental	$4.39\pm0.34^{\rm a,b}$							
	Men	Control	$2.82 \pm 0.33^{b,c}$							
		Experimental	$3.37 \pm 0.30^{a,b,c}$							
II.	Women	Control	4.78 ± 0.72							
		Experimental	4.12 ± 0.42							
	Men	Control	3.10 ± 0.23							
		Experimental	3.13 ± 0.42							
III.	Women	Control	5.28 ± 1.05							
		Experimental	4.61 ± 0.79							
	Men	Control	3.67 ± 0.33							
		Experimental	3.52 ± 0.32							

I: Beginning of experiments, II: 4th week of experiments, III: 8th week of experiments T= Time, Gr=group, G=Gender, TxGr=Time-Group interaction, TxG=Time-Group-Gender interaction, TxGrxG=Time-Group-Gender interaction, "P<0.01; Significant effects of gender based on variance analysis according to repeated measures with three factors. ^{a,b,c} P<0.05; Significant differences between the groups in I. Measurement based on single-way variance analysis/Tukey's test; the difference between the groups with different letters in the same column are significant.

Tablo 3. Leptin levels of women and men students (ng/ml).

Measurement	Gender	Group	Mean ± SD	Т	Gr	G	TxGr	TxG	GrxG	TxGrxG
I.	Women	Control	16.13 ± 3.27 a							
		Experimental	$8.95 \pm 1.4^{\mathrm{b,c}}$							
	Men	Control	2.78 ± 0.74 b							
		Experimental	0.84 ± 0.35 b,d							
II.	Women	Control	14.24 ± 3.03 a							
		Experimental	8.32 ± 0.75^{b}							
	Men	Control	1.6 ± 0.46 c							
		Experimental	0.97 ± 0.48 c							
III.	Women	Control	17.69 ± 5.22 a	2.87	3.68*	36.55***	0.43	3.06	1.50	0.19
		Experimental	$12.79 \pm 2.76^{a,b}$							
	Men	Control	2.04 ± 1.18 c,b							
		Experimental	0.63 ± 0.26 ^{c,d}							

I: Beginning of experiments, II: 4th week of experiments, III: 8th week of experiments T= Time, Gr=group, G=Gender, TxGr=Time-Group interaction, TxG=Time-Gender interaction, GrxG=Group-Gender interaction, TxGrxG=Time-Group-Gender interaction, ""P<0.001; Significant effects of gender based on variance analysis according to repeated measures with three factors. ^{abc.d} P<0.05; Significant difference between the groups in I., II. And III. Measurements based on single-way variance analysis/Tukey's test; the difference between the groups with different letters in the same column is significant. * P<0.05; Significant effects of group factors based on variance analysis according to repeated measures with three factors.

Significant differences were not observed in plasma ghrelin hormone levels of both men and women control and experimental groups at the 4th and 8th weeks of the experimental period (Table 2).

Plasma Leptin Hormone Levels

The leptin hormone levels of women control group at the beginning of the experiments (16.13 ng/ml) did not significantly change until the end of the experiments, but the leptin levels of women experimental group at the beginning of the experiments (8.95 ng/ml) increased to 12.79 ng/ml at 8th week of the experiments. However, such an increase was not found to be significant. Contrary to women experimental group, there was a slight increase in leptin levels of men experimental group at the 4th week with the training program, but such an increase turn into a slight decrease at 8th week. Leptin levels of men and women control groups were significantly higher than the experimental

groups at the beginning, 4^{th} and 8^{th} week of the experiments (P<0.05), leptin levels of women control and experimental groups were also significantly higher than the leptin levels of men (P<0.001; Table 3).

Relationships between Pre- and Post-Experimental Body Composition Levels and Measured Hormone Levels

The relationships between pre-experimental body composition levels of women and men students and measured hormones revealed a significant negative correlation (p<0.05) between PBF and ghrelin levels of women control groups. A significant relationships of body weight was observed with BMI and plasma leptin levels (P<0.05) and highly significant relationships was observed with PBF (P<0.01). With regard to relationship between the hormones, a significant negative relationship was observed between plasma leptin and ghrelin levels (P<0.05).

A significant relationship was observed between PBF and leptin levels of men control groups (P<0.05). On the other hand, a significant relationship was not observed between plasma leptin and ghrelin levels. In experimental groups of women and men students, a significant relationship was not observed neither between body composition parameters and the hormones nor between the hormones (Table 4).

The relationships between post-experimental body composition levels of women and men students and measured hormones revealed highly significant relationships of plasma levels of women control group with their body weights and BMI (P<0.01) and significant relationships with PBF (P<0.05). Similar relationships were also observed in men control groups. In experimental groups, while there was a significant relationship between plasma ghrelin levels and PBF of women (P<0.05), significant relationship was observed between plasma leptin level and PBF of men (P<0.05). With regard to relationship between the hormones, significant relationships were only observed between plasma leptin and ghrelin levels of women experimental group (P<0.05; Table 4).

DISCUSSION

Endurance training significantly increased maximal oxygen consumption capacities of both

men and women. Gender had significant impacts on body weight, percent body fat (PBF) and maximal oxygen consumption capacities. Endurance training did not have significant effects on ghrelin and leptin levels, but gender had significant effects also on ghrelin and leptin levels.

Body weights and PBF of especially men experimental group significantly increased with endurance training. The values decreased in control groups and body weights and PBF of women control and experimental groups did not exhibited significant changes. However, exercise programs significantly increased the VO_{2max} levels of men and women. Previous research also reported positive impacts of exercise programs on body composition (33) and increased VO_{2max} values (30,9,29).

Appetizing ghrelin hormone is primarily produced in stomach and increasing ghrelin levels were reported during the long-lasting energy consuming conditions such as diets and exercises (8). Leidy et al (19) indicated that chronic exercises performed in 5 days of the week for 3 months significantly increased the ghrelin levels of young women and reported a significant negative correlation between ghrelin levels and body weights. Similarly, decreasing ghrelin levels with decreasing body weights were also reported for obese women without a special diet program and performing 45 minutes walking and cycling exercises in 5 days of the week for 12 months (6).

Crours	R		Pre-exp		Post-experiments				
Groups	K	BMI	PBF	Leptin	Ghrelin	BMI	PBF	Leptin	Ghrelin
Women control	Body weight	0.89**	0.82*	0.83*	-0.52	0.90**	0.88**	0.88**	0.18
	BMI		0.74*	0.80*	-0.59		0.77*	0.83**	0.29
	PBF			0.88**	-0.77*			0.71*	-0.04
	Leptin				-0.72*				0.29
Women experimental	Body weight	-0.12	-0.11	-0.58	0.50	0.24	0.03	-0.24	-0.05
	BMI		0.36	0.02	-0.23		0.45	0.19	0.45
	PBF			0.45	-0.27			0.41	0.68*
	Leptin				-0.48				0.69*
Men control	Body weight	0.96***	0.76*	0.63	-0.07	0.95***	0.82*	0.87**	0.25
	BMI		0.86**	0.66	-0.23		0.88**	0.83*	0.27
	PBF			0.75*	-0.41			0.81*	0.35
	Ghrelin				-0.47				0.00
Men experimental	Body weight	0.95***	0.41	0.19	-0.40	0.94***	0.45	0.16	0.06
	BMI		0.55	0.30	-0.19		0.66	0.41	0.01
	PBF			0.63	0.35			0.72*	0.31
	Ghrelin				-0.09				-0.17

Table 4. Relationships between pre- and post-experimental body composition levels and measured hormone levels.

BMI: Body Mass Index, PBF: Percent Body Fat. * P<0.05, ** P<0.01: Significant relationships based on Pearson correlation analysis.

Contrary to that study, Ebal et al. (5) reported significant decreases in ghrelin levels and body weights of rats subjected to 2 hours weekly exercises for 5 weeks. Similarly, Hsu et al. (11) reported significant decreases in blood ghrelin levels of rats subjected to 20 minutes swimming exercises. Contrary to studies indicating changes in plasma ghrelin release with certain intensity and duration exercises, the present results indicated that cycling exercises applied during the 8 weeks of research program did not significantly affect the plasma ghrelin hormone levels of control and experimental men and women participants. Complying with the findings of Schuttle et al. (32), a negative correlation was observed between pre-experimental PBF and ghrelin levels of women control group. However, at the end of 8th week, significant relationship between PBF and ghrelin levels was observed only in experimental women group. Such contradictory results may be due to physical conditions of the participants since they were composed of mediumactive individuals and their body mass index values were within normal limits.

Exercises programs may decrease body fat through increased energy consumption and leptin hormone may increase energy consumption (37). Dirlewanger et al. (3) observed that blood leptin levels did not change with 30 minutes exercises twice in a day for 3 days. Similarly, Houmard et al. (10) indicated unchanged blood leptin levels with 60 minutes 75% exercises for 6 days. Ozçelik et al. (25) investigated the acute and chronic effects of aerobic training program together with hypo-caloric (1200-1400 kcal) diet program on PBF and blood leptin levels of obese women patients and observed that exercise and diet programs did not have an acute impact on leptin levels but serum leptin levels decreased with decreasing PBF in long-duration exercise programs. These findings were supported by later studies and it was pointed out that acute exercises did not change blood leptin levels (23,28,18). Perusse et al. (27) investigated the relationships between chronic exercises and leptin levels and reported that cyclic exercises applied for 20 weeks did not significantly change blood leptin levels of sedentary women. In another study, decreasing leptin levels were observed in obese children with 4 months exercise program, but leptin levels of the children increased again 4 months after the exercises (7). Okazaki et al. (22), on the other hand, reported that 12-months exercise and a diet program decreased BMI, PBF and blood leptin levels of obese and non-obese sedentary mid-aged women. The researches investigating the relationships between body weight and leptin levels revealed significant relationships between basal leptin levels and age of the patients and reported significantly higher plasma leptin levels for young and old obese women than for slim ones. These researches also indicated a positive relationship between body weights and leptin levels (13). The aerobic exercise programs applied to overweight and obese men and women significantly reduced body weights and leptin levels (17,20,14). In the present research, cycling exercise program did not significantly affect plasma leptin levels of both control and experimental groups of men and women participants. The studies reporting decreasing blood leptin levels with exercise programs were all carried over obese men (20), obese children (7), obese sedentary women (22) and obese young women (17). All these target groups have potential to have decreased leptin levels. Therefore, it was thought that composition of research group affected the outcomes of the present research since the participants were medium-active normal-weight young men and women.

The present study has some limitations; initially young and healthy participants of the study desing may not have changing leptin and ghrelin levels. Performance of such studies over obese individuals may change the results. Limiting exercise program with 8 weeks, non-applying any additional diet program and not allowing the participant to eat anything 12 hours before the blood sampling and most of all improper control of these parameters might have differentiate the results of the present study.

As to conclude, the cyclic training program did not affect the body composition, plasma ghrelin and leptin levels of men and women participants, but increased maximal oxygen consumption of them. It was also concluded that these hormone levels significantly affected by the gender.

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