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The Effect of Eight-Week Combined Exercise Training Program with Sweet Almond Supplementation on Plasma Levels of Leptin and Orexin in Overweight Women

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ABSTRACT

Background: The purpose of this study was to investigate the effect of an eight-week combined exercise training program with sweet almond supplementation on plasma levels of leptin and orexin in overweight women. **Methods:** This research was a quasi-experimental study. The statistical population consisted of 60 overweight women with a body mass index (BMI) of 25-30 kg/m^2 who were randomly divided into four groups of training, training-sweet almond supplement, supplement, and control. The participants participated in three training sessions weekly and consumed sweet almond supplementation for eight consecutive weeks. The participants' blood samples were taken 24 hours before the first session and after the last session, in a 12-hour fasting state. Liptin concentrations were measured using Pishtaz Teb Inc. kits. Orexin levels were determined through enzymatic method using the Virro Inc. kit. A two-way repeated measures ANOVA was conducted to analyze the inter- and intra-group variance. The overall alpha significance level was set at $P \leq 0.05$ for all the statistical analyses. **Results:** Eight weeks of combined exercise training with sweet almond supplementation Showed a significant reduces in serum level leptin concentration and increased the plasma orexin levels. **Conclusion:** According to the results of the present study It can be used from combined training and sweet almonds to reduce appetite and lose weight in overweight women.

Keywords: *Combined exercise training; Sweet almond supplementation; Leptin; Orexin; Overweight women*

Introduction

According to the statistics, 50% of the world population is over 20 years old and they have a body mass index (BMI) of ≥ 25. Worldwide, at least 2.8 million people die each year as a result of rate of diseases is caused by overweight or obesity (Halliwill *et al.*, 2013). Obesity is a complex condition characterized by excessive accumulation of adipose tissue. According to various studies and statistics, obesity has been identified as one of the negative factors for people's health and longevity and is associated with many health issues, such as cardiovascular diseases, diabetes, blood pressure,

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and lipids. The World Health Organization (WHO) refers to the rapid increase in the prevalence of obesity as an epidemic of obesity (obesity epidemic) and associates obesity and its implications with one of the main health risks in the world today (Zar *et al.*, 2017). Recognizing the factors that can help reduce this problem is of high importance. Obesity is result of the imbalance between energy intake and energy expenditure. Sport activities play an important role in reducing and maintaining body weight (Mohammadi Moghaddam *et al.*, 2014). Weight is associated with the hormonal changes in the plasma level, which can affect the physiological function of the body. Weight gain can change the hormone orexin and leptin levels.

Orexin is an appetite-stimulating hormone produced by posterior hypothalamus that secretes into the blood. It can increase the appetite by affecting the appetite centers in the brain. Orexin production in obese individuals is generally less than the normal ones. It has an inverse relationship with BMI (King *et al.*, 2009). In a study conducted on the animals, the researchers found that although orexin-deficient animals ate less, they were heavier than the non-orexin-deficient ones. According to their findings, obesity in orexin-deficient animals may be due to the inability of brown preadipocytes to be differentiated from brown fat, which reduces the brown fat thermogenesis and leads to dampening of energy expenditure (Sellayah *et al.*, 2011). On the other hand, a study investigated the effects of orexin on sleep and wakefulness, energy homeostasis, and intestinal secretion among a newly created mouse line overexpressing human prepro-orexin gene. The results showed that orexin inhibited the animals' rapid eye movement sleep. In addition, it increasingly affected the metabolic heat production independent of uncoupling protein mediated thermogenesis in brown fat (Mäkelä, 2010). The deficiency of this hormone in the human body is largely responsible for the development of obesity, since it reduces the body's ability to burn fats and calories. Therefore, doing exercises can improve the individuals' quality of life and health (Messina *et al.*, 2014).

On the other hand, one of the important hormones that play role in obesity is leptin, which disorder can trigger the abnormal increase and decrease of the body weight. A close relationship was found between serum leptin, fat percentage, and BMI; so that elevated body fat increased leptin secretion and caused gradual resistance to leptin's activity. In recent years, the effect of leptin on arterial blood pressure and heart rate was demonstrated. Researchers believe that in obese participants, increased levels of leptin in the blood (as an indicator of leptin's resistance) affects the obesity-associated diseases, cardiovascular diseases, type 2 diabetes, insulin resistance, and high blood pressure (Keller *et al.*, 2005). In this regard, doing physical exercises can boost the quality of one's health. In physical education, combined exercise training is a combination of aerobic and weight training that increases muscle mass and reduces fat simultaneously. A lot of studies have investigated the role of aerobic exercises on leptin secretion; however, some contradictions exist in the results. For example, Khorshidi (Khorshidi *et al.*, 2012) observed that 12 weeks of aerobic training reduced serum leptin levels and increased serum testosterone, which is contrary to the results reported by Ara (Ara *et al.*, 2006) and Sari (Sari *et al.*, 2007), who found that aerobic exercise did not affect the leptin secretion.

The fruit of the almond is a drupe weighing between 20 and 80 grams. Research shows that diets consisting of almonds increase blood lipoprotein levels. Dietary consumption of almonds reduces the coronary heart diseases and has antioxidant properties. Peanuts, like other nuts, have high levels of unsaturated fats and low levels of saturated fat. Therefore, its consumption is effective in reducing fats, notably cholesterol and reduces the risk of cardiovascular diseases (Esfahlan *et al.*, 2010).

In the study by Stephens et al., consuming almond and its oil reduced the risk of cardiovascular diseases by lowering blood cholesterol levels. Almond consumption also led to a significant increase in high density lipoprotein cholesterol (HDL-c) and a significant decrease in

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total cholesterol (TC) and low density lipoprotein cholesterol (LDL-c) (Stephens *et al.*, 2010). In another study, Spiller et al. found that TC and LDL-c levels in the almond-consuming group were significantly lower than those who did not consume almonds. However, the role of almond consumption besides physical activity has been underestimated (Spiller *et al.*, 1998).

In general, considering the importance of identifying strategies for improving the physiological conditions of overweight women, controversial results over the effect of aerobic training on plasma levels of leptin and orexin, lack of research on combined exercise trainings, and absence of continuous and sufficient research on sweet almond supplementation, this study was conducted. The purpose was to investigate the effect of eight weeks of combined exercise training with sweet almond supplementation on plasma levels of leptin and orexin in overweight women.

Materials and Methods

Design and participants: This study was carried out with a quasi-experimental design in terms of the applied purpose and data collection. The statistical population of this study consisted of overweight women in Ardakan city, Yazd province, Iran. To conduct the study, 60 participants with a BMI 25-30 were randomly selected and randomly divided into four groups of training, training-sweet almond supplement, supplement, and control (n = 15 in each group). The inclusion criteria for the participation in the study were personal consent, women with a BMI 25-30, absence of movement disorders, not participating in any sport programs except the current research program, and lack of food supplements consumption that affected the research process. The exclusion criteria were the participants' withdrawal, taking drugs, smoking, and not implementing the protocols thoroughly. Training and training-sweet almond supplement groups went through the combined exercise training program during three training sessions of about 45 minutes, carried out weekly for eight consecutive weeks.

Intervention: Aerobic exercises involved 10 minutes of warm-up, 25 to 45 minutes of walking/running at 65-75% of the maximum heart rate on the treadmill, and 5 minutes of cool-down, which were performed in two sessions each week. Resistance exercises consisted of chest presses, underhand cable pulldowns, front and back biceps curls, leg presses, as well as knee bending and straightening at 55 to 65% of one repetition maximum during three-time periods with an interval of two to three minutes (**Table 1**). Each session of resistance training involved 10 minutes of warm-up and five minutes of cool-down. Prior to the exercises, participants' one repetition maximum was estimated for each seven movements using the lifted weight formula (30/ number of repetitions)+1 (Maud and Foster, 2006). With regard to the previous research, the training-sweet almond supplement and supplement groups consumed a pack of 50 grams of sweet almond as an afternoon snack three times a week for eight weeks. The blood samples were taken 24 hours before the first session and after the last session in a 12-hour fasting state. To simulate the sampling time and to control the circadian rhythm, the sampling was carried out at the beginning and end of the study at eight o'clock in the mornings. To control the daytime rhythm, sampling at the beginning and end of the study was performed at eight o'clock in the morning.

Measurements: Five ml of blood was taken from the right arm vein of the participants. Leptin concentrations were measured using human-specific kits of Pishtaz Teb Inc. Orexin levels were measured through enzymatic method using the Virro Inc. kit.

Ethical considerations: All stages of this research has been approved by Ethics Committee of Islamic Azad University Bafgh Branch with code IR.IAU.B. 1397,28-14-5/1127

Data analysis: Descriptive statistical methods including central tendency and dispersion indices were used to analyze the data. For the inferential statistics, Shapiro-Wilk test was used to test the normal distribution of data in pre-test and post-test. A two-way repeated measures ANOVA was run

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thereafter. The significance level was preset at P-value ≤ 0.05 and all statistical analyses were conducted using SPSS 22.

Results

Table 2 demonstrates the mean of age, weight, height, BMI, plasma levels of orexin and leptin in control and experimental groups before and after chronic aerobic exercise. The results showed that eight weeks of combined exercise training had a significant effect on plasma levels of leptin and orexin among the overweight women. It increased the serum orexin levels and decreased the leptin levels ($P \leq 0.05$), but eight weeks of sweet almond supplementation had no significant effect on plasma levels of leptin and orexin in overweight women; however, it leads to a slight increase in orexin and a slight decrease in leptin levels ($P \leq 0.05$). Moreover, the results indicated a significant effect on simultaneous use of combined exercise training and sweet almond supplements on plasma levels of leptin and orexin in overweight women: concurrent use of sweet almond supplements decreased the leptin levels significantly and increased the orexin levels significantly ($P \leq 0.05$, **Table 3**).

The means' comparisons showed that simultaneous use of sweet almond supplements and combined exercise training did not have a significant effect on serotonin elevation and reduction of leptin compared to the sweet almond supplement and combined exercise training groups. In other words, training and of sweet almond supplementations did not have a synergistic effect on each other ($P \leq 0.05$)

Table 1. Combined exercise training program for eight weeks

Training Repetition	1th week I[a]	1th week R[b]	2nd week I[a]	2nd week R[b]	3th week I[a]	3th week R[b]	4th week I[a]	4th week R[b]	5th week I[a]	5th week R[b]	6th week I[a]	6th week R[b]	7th week I[a]	7th week R[b]	8th week I[a]	8th week R[b]
Endurance	65	25	65	25	65	30	65	30	65	35	65	35	65	40	65	40
Strength	60	3*8	60	3*8	60	3*10	60	3*10	60	3*12	60	3*12	60	3*14	60	3*14

[a]: Intensity percentage, [b]:Repetition

Table 2. Mean (± Standard Deviation) of age, height, weight, body mass index (BMI), orexin, leptin in 4 groups

Variables	Training- Supplement	Training	Supplement	Control	P-value[a]
Age (y)	34.79 ± 6.62	33.20 ± 4.35	36.45 ± 3.51	34.85 ± 3.54	0.40
Height (cm)	175.30 ± 7.54	173.13 ± 7.24	176.2 ± 3.26	174.12 ± 4.09	0.65
Weight (kg)	85.25 ± 9.32	84.19 ± 6.41	88.57 ± 10.27	85.50 ± 8.60	0.66
BMI(kg/m^2)	27.81 ± 2.19	28.09 ± 2.35	28.60 ± 4.09	28.14 ± 2.20	0.15
Orexin (ng/ml)	11.35 ± 1.32	11.10 ± 1.28	9.25 ± 1.05	09.04 ± 1.76	0.66
Leptin (ng/ml)	1.43 ± 0.57	1.68 ± 0.97	1.99 ± 0.77	2.22 ± 0.98	0.52

[a]: Shapiro-Wilk test

Table 3. Comparison of lmean (± standard deviation) of leptin and orexin indices in pre-test and post-test in 4 groups

Variables	Groups	Pre-test	Post-test	P-value[a]	P-value[b]
Leptin (ng/ml)	Training- Supplement	2.31 ± 0.62	1.43 ± 0.91	0.001	0.008
	Training	2.20 ± 0.86	1.68 ± 0.57	0.001	
	Supplement	2.18 ± 0.94	1.99 ± 0.78	0.12	
	Control	2.22 ± 0.72	2.17 ± 0.98	0.75	
Orexin ng/ml)	Training- Supplement	9.25 ± 1.35	11.35 ± 1.28	0.001	0.06
	Training	9.15 ± 1.99	11.10 ± 1.32	0.001	
	Supplement	8.44 ± 1.87	8.95 ± 1.09	0.54	
	Control	9.02 ± 1.68	8.99 ± 1.67	0.86	

[a]: Student t-test, b: Two-way ANOVA test

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Discussion

The results indicated that eight weeks of combined exercise training with sweet almond supplementation had a significant reducing effect on the plasma levels of leptin in overweight women. Some researchers reported that aerobic, resistance, and combined physical exercises reduced blood leptin levels (Ghiasi *et al.*, 2017). In contrast, this result was not in agreement with the results of Omraii et al. (Omraii, 2016). The disagreement between the results can be attributed to the age, gender, and type of the exercise of the participants.

Research revealed that sport activities in adults led to weight loss and fat percentage reduction in a short period of time. The mechanism that regulates the changes in energy balance and leptin level is still unclear, but it is clear that this happens through a change in the expression of the ob gene in the adipose tissue. The adipose tissue can detect the energy balance and lipid content as an energy storage mechanism that regulates the *ob* gene expression. Recent studies have shown that subcutaneous fat compared to visceral fat is the main source of leptin secretion. On the other hand, the results showed that almond supplementation reduced the level of leptin; however, it was not significant and could be attributed to the high levels of oleic acid in almond because. Since this fatty acid and its Co-A decrease the phosphatidate phosphohydrolase enzyme activity, they reduce the liver triacylglycerol. The presence of lignans and fatty acids, like linoleic acid in the almond and its oil, reduce the absorption and synthesis of fat. Therefore, almond supplement consumption along with the combined exercise training can have beneficial effects on leptin.

On the other hand, the physiological pressure resulting from physical activity is one of the potential regulators of leptin secretion from adipose tissue. The changes in metabolism balance, systemic hormonal concentrations, and energy expenditure may affect plasma leptin concentration and subsequently leptin function. Besides their effective roles in weight loss, doing exercises and physical activity can trigger the hormone concentration affecting leptin such as cortisol, insulin, and sex hormones. Anaerobic exercises may affect the leptin secretion by influencing these factors, which requires further investigation (Mohammadhassani *et al.*, 2015).

The results also showed that eight weeks of combined exercise training with sweet almond supplementation had significantly increased the plasma levels of orexin in overweight women.

This is in line with the findings of Sellayah et al. (Sellayah *et al.*, 2011) and Bronsky (Bronský *et al.*, 2007). These researchers believe that physical exercises are associated with increasing the orexin levels. However, the results obtained by Mohammadhassani et al. (Mohammadhassani *et al.*, 2015) and Abdi (Abdi *et al.*, 2015) were in contrast to them. They found that doing exercises did not affect the orexin levels. The reason for contradiction of the results can be attributed to the intensity and duration of the exercises. Sellayah et al. found that obesity in participants with orexin deficiency may be due to the inability of brown preadipocytes to be differentiated from brown adipose tissue, which reduced the brown fat thermogenesis and lowered the energy expenditure (Sellayah *et al.*, 2011) . Teske et al. demonstrated a relationship between hypothalamic orexin receptors and spontaneous physical activity, which were stimulated by orexin A: obesity resistance due to the increased orexin messages is accompanied by high levels of spontaneous physical activity. According to their findings, spontaneous physical activity in lean rats was more sensitive to both orexin-1 receptor antagonist and a quick response to orexin-2 antagonist (Teske *et al.*, 2008). It is worth noting that a part of the biological function of orexin was discovered by Makela. This researcher proved that orexin messages greatly inhibited diet-induced obesity and the development of insulin resistance in rodents. Although this substance does not change the respiratory fraction (indirect index of lipid versus carbohydrate intake), it increases energy expenditure in large quantities. Furthermore, an intense orexin stimulation reduces the food intake (Mäkelä, 2010).

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So far, the effect of almond consumption on orexin levels has not been considered by any research study. The present research findings did not show any significant effect of almond consumption on orexin levels. However, research has shown that diets containing almonds increase blood lipoprotein levels. Consuming almonds mainly reduces coronary heart diseases and has antioxidant properties. Almond has a high level of unsaturated fats and a low level of saturated fat. Therefore, its consumption is effective in reducing lipids, especially cholesterol and reduces the risk of cardiovascular diseases (Ramezanpour and Khosravi, 2013).

Its role on orexin performance is ambiguous, since Hassanpur (Hassanpour, 2016) found that the effect of aerobic exercises along with sweet almond supplementation could lead to a significant difference on body composition and aerobic endurance compared to aerobic exercises. Moreover, Bronsky et al. investigated the effect of concurrent exercise and dietary restrictions on orexin and leptin levels in obese children (33 girls and 25 boys) and indicated that orexin-A levels increased during the weight loss (Bronský *et al.*, 2007). By comparing these two studies, it can be expected that almond consumption and orexin levels should have interactive effects on body composition and in fat level changes, but the results of this study did not support this assumption. The reason for this result may be attributed to the low almond consumption in the groups. Thus, further research is needed in this area.

Conclusion

The results of this study indicated that combined exercise training along with sweet almond supplementation could affect levels of serum orexin and leptin in overweight women. However, these two interventions did not reinforce each other and their simultaneous use did not increase the sole effect of combined exercise training. Accordingly, it can be recommended that simultaneous training and supplement consumption develop an effective intervention on orexin and leptin levels in overweight women.

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Authors' contribution

Afkhami F performed fieldwork and experimental work and Fattahi A and Abbasi H contributed in the study design, data collection, data handling, and manuscript preparation. All authors read and approved the final manuscript.

Conflict of interest

None

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