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ORIGINAL ARTICLE

Assessment of acute aerobic exercise in the morning versus evening on asprosin, spexin, lipocalin-2, and insulin level in overweight/obese versus normal weight adult men

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ABSTRACT

The aim of this study was to examine the acute effect of aerobic exercise when performed in the morning and evening on obesity-related hormones of asprosin, spexin, lipocalin-2, and insulin in normal weight (NW) and overweight/obese (OW/OO) adults. A total of 20 adult male individuals (10 NW and 10 OW/OO) volunteered their participation. Both groups were subjected to an aerobic exercise protocol in moderate intensity (heart rate reserve of 55–59%) for 30 min at two different time periods of the day (morning: 08:00–10:00 h, evening: 20.00–22.00 h) at least 3 d apart. BeBis analysis revealed the OW/OO group consumed significantly less energy (1781.59 ± 410.71 kcal) as compared with NW group (2380.28 ± 445.50 kcal) before the evening exercise (about 3 d) (p <.05). As compared with the NW group, basal serum asprosin, insulin, and lipocalin-2 hormone levels were higher in the OW/OO group, and serum spexin level was lower in OW/OO group (p <.05). Body temperature significantly increased after morning and evening aerobic exercise in both groups. The increase in body temperature was significantly higher after the evening exercise in the OW/OO group compared to the NW group (p <.05). Significant decrease in serum asprosin lipocalin-2, and insulin levels was observed in both groups after exercise (p <.05). Evening aerobic exercise more greatly decreased serum asprosin, lipocalin-2, and insulin level in the OW/OO group as compared with the NW group (p <.05). In conclusion, it is thought that negative energy balance caused by psychological energy restriction and evening aerobic exercise, which leads to a further increase in body temperature, triggers greater decrease of orexigenic signals (suppression of appetite), and is more effective in the development of adipose tissue inflammation and insulin sensitivity, especially in OW/OO group.

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Introduction

Obesity is a noncommunicable disease characterized by excessive adipose tissue that leads to significant increase in metabolic disorders, and associated comorbidity (Ilacqua et al. 2019). Today, obesity is the 5th highest ranking global public health problem, and is responsible for 4.8% of deaths worldwide (Wilson et al. 2018). The latest WHO report indicates ~13% of the world's adult population, or more than 650 million adults (11% of men and 15% of women), were obese in 2016 ([WHO] World Health Organization 2018). As in other countries, obesity is increasing in Turkey. While the prevalence of obesity was 8.6% in 1975, it increased by approximately fourfold in 2016, to 32.1%. According to the WHO (2017), among the European countries Turkey has the highest prevalence of obesity in adults >18 y of age. Therefore, it is extremely important to conduct more research to understand the pathophysiology of obesity and develop treatment for the adult population.

Obesity is a complex multifactorial health problem involving interaction between factors of genetics, behavior, environment, will power, self-control, appetite regulation, and energy metabolism (Haghshenas et al. 2014; Serter 2003). The physiology of obesity is based on increase in body weight as a result of positive energy balance (WHO 1997). Energy balance control, appetite control, and possible mechanisms of obesity are regulated by the hypothalamus, especially the arcuate nucleus, which is the key site of feedback control of appetite and food intake (Konturek et al. 2005; Suzuki et al. 2010). Appetite control is a complex process that involves communication between the arcuate nucleus of the hypothalamus, gastrointestinal system, and adipose tissue (Stensel 2010). Adipose tissue plays an important role in regulating metabolic activity, systemic energy balance, appetite, satiety, thermogenesis, inflammation, lipid metabolism, and glucose homeostasis by

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communicating with peripheral organs and brain through adipokines (Luo and Liu 2016; Ramanjaneya et al. 2010; Scheja and Heeren 2019). Therefore, understanding of adipose tissue biology and pathology is of great importance for the prevention and treatment of obesity and obesity-related diseases (Rosen and Spiegelman 2006). Recent studies demonstrated the hormones of asprosin, spexin, lipocalin-2, and insulin that are secreted from adipose tissue play a key role in regulating appetite, satiety, and inflammation. .

Asprosin is a fasting-induced glycogenic polypeptide that controls hepatic glucose production and insulin sensitivity (Kajimura 2017), and it also stimulates hunger via the hormone grelin (Duerrschmid et al. 2017). Spexin is a potent, natural, and satiety-inducing peptide that plays an important role in regulating food intake, energy metabolism, and body weight (Walewski et al. 2014; Zheng et al. 2017). Lipocalin-2 is an adipokine that acts as an antagonist in the homeostatic regulation of the inflammation in adipose tissue (Zhang et al. 2008). Recently, lipocalin-2 hormone has been shown to create a feeling of satiety (Mera et al. 2019; Mosialou et al. 2017). Insulin is one of the most important hormones involved in glucose homeostasis; its level of secretion occurs in proportion to the level of fat stored in adipose tissue (Begg and Woods 2012; Porcari et al. 2015).

Regular physical activity or exercise is the best nonpharmacological treatment approach for obesity, as it results in negative energy balance and weight loss (Golbidi and Laher 2014; Hazell et al. 2016; Mika et al. 2019). Many studies demonstrate acute exercise reduces insulin resistance (Bashiri et al. 2014; Golbidi and Laher 2014), suppresses the feeling of subjective hunger (Thackray et al. 2016), decreases the level of ghrelin (Larsen et al. 2017), and exerts anti-inflammatory effect evident by reduced biomarkers indicative of adipose tissue inflammation in obesity (Gleeson et al. 2011). In addition to exercise, the circadian clock system plays a role in the regulation of energy metabolism, adipose tissue biology, and beneficial effects of exercise. The central clock (Suprachiasmatic Nucleus = SCN) is a key homeostatic regulator that controls many genomic and physiological responses in almost all cells mediated through peripheral clocks in tissues and organs. The SCN and peripheral clocks are synchronized by both photic and nonphotic stimuli, such as light, sleep, food intake, temperature, and exercise (Lopez-Minguez et al. 2016; Savikj et al. 2019). Therefore, optimizing stimuli that have an impact on circadian rhythm are extremely important to improve circadian clock health, and to ensure the circadian clocks work in a coordinated manner (Lopez-Minguez et al. 2016). In particular, timing exercise to coincide with greatest physiological and molecular response contributes additionally to the protection of chronobiological homeostasis, and the management of metabolic diseases, such as obesity (Gabriel and Zierath 2019; Lewis et al. 2018). Previous studies showed exercise at right time of the day caused phase shift in the circadian rhythm of SCN (Hamaguchi et al. 2015; Lewis et al. 2018), thereby alleviating the negative consequences of circadian misalignment in humans (Bilski et al. 2016; Schroeder et al. 2012), activating metabolic pathways and systemic energy homeostasis to greater extent in skeletal muscle (Sato et al. 2019), and better suppressing hunger due to the shifts in energy balance.

Only a limited number of studies have compared the effects of exercise performed in the morning and evening on subjective feeling of hunger (Alizadeh et al. 2015; Irandoust and Taheri 2018), appetite-related hormones (Algul et al. 2017; Larsen et al. 2019), blood glucose, insulin sensitivity, and hypoglycemia risks (Basse et al. 2018; Gomez et al. 2015; Savikj et al. 2019), and their results have been inconsistent and contradictory. The number of publisher studies examining the effect of exercise on asprosin (Ko et al. 2019; Schumann et al. 2017; Wiecek et al. 2018) and spexin (Fathi et al. 2016) is limited; moreover, no studies have examined the effect of exercise performed in different time periods of the day on asprosin, spexin, lipocalin-2, and insulin levels in relation to energy intake of both normal weight and overweight/obese individuals. The purpose of this study was to investigate the role of exercise in both normal weight and obese individuals when performed at different circadian times (morning versus evening) on energy regulation, appetite, and the involved regulatory hormones

Methods

Determination of the sample group

The criteria sampling method was used to determine the sample group of this study. Accordingly, participants were selected using specified inclusion criteria relative to the purpose of the research (Patton 2002).

Inclusion criteria

Inclusion criteria were: age 30–45 y, normal weight (NW) or overweight (OW)/obese (OO) weight, absence of any injury, systemic disorder, or chronic disease, no-regular use of medications, nonsmoker, and nonfrequent alcohol consumer.

Participants

A total of 20 adult males – 10 NW and 10 OW/OO according to the WHO (1997) body mass index classification – between 30 and 45 y of aged, working in different

academic and administrative units at the Mugla Sitki Kocman University (MSKU), and meeting all inclusion criteria voluntarily participated.

Ethical concerns

The study was approved by the Human Research Ethics Committee of MSKU (Decision no: 1, Protocol no: 170078, Date: 02.01.2018) and was conducted in accordance with international ethical standards for human biological rhythm research (Portaluppi et al. 2010)

Sample size calculation

Sample size was based on the standardized effect size (SES) calculated using the mean and standard deviation values of similar studies reported in the literature (Algul et al. 2016, 2017; Hulley et al. 2013).

(1) Average effect size (ES) = (ES1)+(ES2)/2 = (187.9–172.5) + (144.2–134)/2 = 12.80
(2) Average standard deviation (SD) = (5.8 + 12)/2 = 8.9
(3) SES = ES/SD = 12.80/8.9 = 1.43

*The standardized effect size was placed in the G*Power (3.1.9.4) analysis program [Two-sided, $\alpha = 0.05$, Power $(1-\beta) = 0.95$, Effect size = 1.43]. Accordingly, the minimum sample number per group (NW and OW/OO) was determined found to be 9.

Study variables

Body weight and height were measured by the Seca brand measurement tool (0.01 kg, and 0.01 cm sensitivity) (Gunay et al. 2013).

Body mass index (BMI) was calculated as body weight in kilograms/height in meters squared. [WHO] World Health Organization (1997) classifies person of BMI of <18.5 kg/m^2 as "underweight", 18.5–24.9 kg/m^2 as "normal weight", 25–29.9 kg/m^2 as "overweight", 30–34.9 kg/m^2 as "obese class I", 35–39.9 kg/m^2 as "obese class II", and ≥40 kg/m^2 as "obese class III". In this study, participants with BMI of 18.5–24.9 formed the NW group, and participants with BMI of 25–29.9 or 30–39.9 formed the OW/OO group.

Body fat percentage was ascertained by foot-to-foot Bioelectrical Impedance Analysis (BIA, Tanita TBF-401A device). Values were calculated automatically by equations pre-programmed by the manufacturer (Dixon et al. 2008). Participants were invited to the laboratory at 10:00 h and asked to press four contact electrons mounted on the platform surface with their bare feet and stand motionless and upright until results were visible on the screen.

Physical activity level was determined by the International Physical Activity Questionnaire (short form) developed by Craig et al. (2003), but using the Turkish version shown valid and reliable by Ozturk (2005). It includes questions about physical activities (how many days a week and how many mins) performed ≥10 mins the past week (Craig et al. 2003). The total physical activity score in units of MET-min/week was calculated for each participant by recommended formulas. Physical activity level of participants was classified as low (<600 MET-min/week), medium (601–3000 MET-min/week), and high (> 3000 MET-min/week) (IPAQ 2005; Ozturk 2005)

Circadian chronotype was determined by Morningness-Eveningness questionnaire form, a Likert scale type question consisting of 19 questions, with 4 options as possible answers. Each response option is clearly schematized. A timetable, divided into a 7 h timeframe of 15 min sub-categories was used to answer questions 1, 2, and 10. Five different circadian type classifications are possible according to the total score for 19 questions: "absolutely morning type" (70–86 points), "near morning type" (59–69 points), "intermediate type" (42–58 points), "near evening type" (31–41 points), "absolutely the evening type" (16–30 points). Validity of the original questionnaire and classification of circadian type were tested with changes in body temperature (Punduk et al. 2005).

Sleep quality was assessed by self-report 8-item Epworth Sleep Quality that queries the general sleepiness level of the individual in eight different daily life situations. The probability of individuals' falling asleep is graded between 0 and 3, with score of 0 representing it never happens, score of 1 representing it occasionally happens, score of 2 representing it happens in medium frequency, and score of 3 it happens very often. The sum of the answers to the 8 questions gives the sleep quality (Izci et al. 2008).

Body temperature of the participants was measured by the IR900 pistol type fever meter before and immediately after aerobic exercise.

Determination of energy intake was achieved over a 7d span through individual 24 h backward food consumption records obtained from participants before morning exercise (4 consecutive days) and between morning and evening exercise (~3 consecutive days). Participants were instructed by a dietitian about how to keep food consumption records by noting in detail on provided forms everything they ate or drank. Reminder messages were sent daily to secure compliance. The food consumption records were checked before starting the second exercise session. Food and macronutrient consumption was

derived from records using standard weights so as to determine the size, amount, and quantity of intakes (Merdol Kutluay 2003; Rakicioglu et al. 2012). The computer-based package program of the Nutrition Information System (BeBis 8.1) was utilized to calculate total energy intakes of participants, and macronutrient intake amounts.

Collection and processing of blood samples were done by the healthcare staff in the gymnasium environment where the exercise sessions were conducted. Prior to the study, the medical staff were trained by the medical biochemistry specialist for proper blood collection procedure, separation of blood in specified amounts, and centrifugation technique. Venous blood samples of the participants before and after the exercise were taken from the antecubital vein by the medical staff, and placed in 8 ml red cap biochemical tubes. The blood samples were kept at room temperature for 30 min, then centrifuged at 1000xg for 15 min, and separated by automatic pipette into 5 different serum eppendorf containers; 4 of them containing ≥300 µl of serum in, and one of them ≥2000 µl of serum. Then, the blood samples were refrigerated in the laboratory of the Department of Medical Biochemistry of the Faculty of Medicine, M.S.K.U. Serum samples reserved for spexin, asprosin, and lipocalin-2 were stored at −20°C until analysis, and serums for insulin analysis was stored at +4°C.

Biochemical analysis for serum asprosin, spexin, and lipocalin-2 levels was achieved by the Elisa method using the Multiskan Go (Thermoscientific) microplate reader. Analyzes were made by the "blind method" without knowledge of participants' BMI to reduce bias. All serum samples were double analyzed. Human Asprosin Elisa Kit (E-EL-H2266-Elabscience Biotechnology, kit measurement range 0.31–20 ng/mL; within-run CV<10%), Human Spexin Elisa Kit (E-EL-H5607-Elabscience Biotechnology, measuring range 78.13–5000 pg/mL; within-run CV<10%), Human Lipocalin-2 Elisa Kit (E-EL-H0096-Elabscience Biotechnology, measurement range 0.16–10 ng/mL; within-run CV<10%) were used. Insulin was determined using the sandwich principle of the Electrochemiluminescence Immunological Test. The reference range was 2.6–24.9 µU/mL.

Data collection

Measurements were performed during nine exercise sessions distributed over 8d in the MSKU Sports Hall (July-August, 2018). Five participants were invited to each exercise session (Table 1). In the first session, height and weight were assessed, and body fat percentage calculated. The volunteers completed the study questionnaires, and food consumption records were distributed with detailed explanation on their use. They were reminded to continue their normal food programs, avoid excessive fat intake, not to perform high-intensity exercise, not to use any caffeine, stimulants, alcohol, and nutritional supplements, such as additional vitamins and antioxidants, during the study period (~7d) (Algul et al. 2017). Exercise protocols were applied at subsequent sessions to the participants at different times of the day (Table 2)

Exercise sessions

A randomized, counter-balanced research design (Mitchell and Jolley 2013) was used to order the exercise sessions of participants to minimize learning and sequence effects. The 10 participants in each of the NW and OW/OO groups were randomly divided into four groups according to BMI classification and order of exercise sessions. One group of 10 participants had the sequence of the morning exercise session followed ≥3d later the evening exercise session and the other 10 participants the reversed order of the evening exercise sessions (Table 1)

Aerobic exercise protocol

Each participant in the four groups were subjected to the aerobic exercise protocol in the morning (08:00–-10:00 h), and evening (20:00–22:00 h) separated by ≥3d intervals. The morning exercise sessions was applied to the participants on an empty stomach (no food consumption for ≥10 h). Before the evening exercise sessions, participants were asked to take light food ≥3 h beforehand (Algul et al. 2017; Ozcelik et al. 2017). The aerobic exercise protocol lasted 50 min in total, including 10 min of warm-up, 30 min of aerobic

Table 1. The days of the four groups to participate in two different time periods of the day.

Monday	Wednesday		Monday	Tuesday	Wednesday	Thursday	Friday
Preliminary interview	1. Session	Break 5d	2. Session 1. Group morning = 5 participants 3. Session 3. Group evening = 5 participants	4. Session 2. Group morning = 5 participants 5. Session 4. Group evening = 5 participants		6. Session 1. Group evening = 5 participants 7. Session 3. Group morning = 5 participants	8. Session 2. Group evening = 5 participants 9. Session 4. Group morning = 5 participants

Table 2. The same experimental procedure sequence, and time intervals were used in eight sessions in the morning and evening hours.

Familiarization session		2., 3., 4., 5., 6., 7., 8., 9. Session		
1. session		Before aerobic exercise	Aerobic exercise	After aerobic exercise
Anthropometric measurements • Height and body weight • Bioelectrical impedance analysis • Information about food consumption records • Informing about the points to be followed during the study Application of scales • International PhysicalActivity Questionnaire (Short Form) • Morningness-Eveningness Questionnaire • The Epworth Sleepiness Scale (10:00–12:00 h)	3d of rest	• The participants came to the gym (07:45–07:50 h) • Wearing polar watches and determining the resting heart rate • Body temperature measurement • Determining the target heart rate of each participant • Taking blood samples (08:00–08:30 h or 20:00–20:30 h) • Warm-up and stretching exercises for 10 min (08:00–08:40 h/20:00–20:40 h) • The centrifugation of blood samples taken before exercise, and blood separation after centrifugation	30 min aerobic exercise (08:40–09:25 h/ 20:40–21:25 h)	• Taking blood samples immediately after exercise • Body temperature measurement (09:10–09:30 h/21:10–21:30 h) • 10 min cooling exercises (09:30–09:40 h./21:00–21:15 h) • Centrifugation of blood samples taken before exercise, and blood separation after centrifugation • Transport of blood samples to the laboratory on the cold chain

exercise, and 10 min of cooling (ACSM 2018). The target heart rate (HR) of each participant was calculated by the Karvonen formula before the exercises (Karvonen et al. 1957). Participants were warned not to exceed the target HR during exercise sessions. After the participants warmed up for 10 min, they were subjected to 30 min of moderate intensity aerobic exercise equivalent to 55–59% of their HR reserve as recommended by the American College of Sports Medicine ([ACSM] American College of Sports Medicine 2018) for obese and sedentary adults. The session was completed after 10 min of cooling exercises. The HR and energy expenditure during the exercise were determined using the RS400 polar watch.

Statistical analysis

Data are reported as mean ± SD. Data analysis was performed by the SPSS (version 18.0) program. The Shapiro-Wilk test indicated the data were normally distributed. Independent Sample t test was used to compare the energy consumption during exercise, and resting HR of the NW and OW/OO groups according to exercise time, and basal asprosin, spexin, lipocalin-2, and insulin mean values of BMI groups. Comparison of the energy intake of the NW and OW/OO group before exercise and between exercise days was carried out with Independent Sample t test. Mixed Design Split ANOVA was utilized to compare the body temperature, asprosin, spexin, lipocalin-2, and insulin mean values in BMI groups (NW and OW/OO) according to before and between the two individual exercise times. Variances were found to be homogeneous for BT, asprosin, spexin, lipocalin-2, and insulin. Therefore, sphericity assumed values were taken into account. Significance level was evaluated according to $p < .05$, $p < .1$, and $p < .001$.

Results

The mean of age, BMI, and body fat percentage of the NW group were, respectively, 37.70 ± 4.66 y, 23.64 ± .54 kg/m^2, 18.06 ± 1.60, and the mean age, BMI, and body fat percentage of the OW/OO group were, respectively, 37.10 ± 4.33 y, 30.01 ± 3.45 kg/m^2, and 30.08 ± 3.18.

According to the Epworth Sleep Quality Scale, all participants in the NW and OW/OO group were found to have a good sleep quality. According to the Morningness-Eveningness Questionnaire, eight participants of NW group were an intermediate type and 2 a near morning type; in the OW/OO group, 1 participant was a morning type, 2 a near morning type, and 7 an intermediate type.

Table 3. Energy intake of the NW and OW/OO groups during the study.

	BMI Groups	Protein (g)	%	Fat (g)	%	CHO (g)	%	Energy (kcal)	t	p
BME	NW	81.19	14.5	107.16	40.6	252.84	45	2316.49 ± 523.46	1.797	p =.089
	OW/OO	67.81	14.4	77.17	35.4	220.12	44.9	1857.94 ± 614.01		
BEE	NW	89.14	16	107.09	42	239.55	42.3	2380.28 ± 445.50	3.124	p <.01
	OW/OO	67.31	15.5	88.37	43.4	176.86	41.2	1781.59 ± 410.71		
EED	NW	46.57	15.7	60.13	42.3	130.20	42.1	1225.21 ± 391.27	2.067	p =.053
	OW/OO	34.71	15.3	35.75	34.9	99.18	41.2	864.22 ± 389.89		

BMI: Body Mass Index; NW: Normal Weight, OW/OO: Overweight/Obese; BME: Before Morning Exercise, BEE: Before Evening Exercise, EESD: Evening Exercise Day; CHO: Carbohydrate

The International Physical Activity Questionnaire (short form) rated for the NW group 2 participants highly active and 8 participants moderately active; in the OW/OO group, it rates 1 participant low active and 9 participants were moderately active.

Participants of the NW and OW/OO group consumed, respectively, an average of 2316.49 ± 523.46 kcal and 1857.94 ± 614.01 kcal energy before the morning exercise (4 consecutive days). No statistically significant difference was found in the comparison of the daily energy intake of NW and OW/OO group before morning exercise ($p > .05$). The same participants of the NW and OW/OO group consumed, respectively, an average of 2380.28 ± 445.50 kcal and 1781.59 ± 410.71 kcal daily energy between the morning and evening exercise or evening exercise and morning exercise (~3d duration). It was determined that the OW/OO group consumed significantly less energy as compared with NW group before as well as between the two time-of-day exercise tests ($p < .01$). Finally, participants of the NW and OW/OO groups consumed, respectively, an average of 1225.21 ± 391.27 kcal and 864.22 ± 389.89 kcal daily energy the day of the evening exercise (in the morning until 20:00 h). When the energy intakes on the evening exercise day of the NW and OW OO group were compared, there was no statistically significant difference ($p > .05$), but the OO/OW group consumed less energy.

As shown in Table 4, No statistically significant exercise time difference was detected in comparison of energy consumption during exercise, and resting heart rate in both BMI groups ($p > .05$)

Statistically significant difference were found between the NW and OW/OO groups' basal (before-morning exercise session) serum asprosin [$t(18) = -2.709$, $p < .05$], serum spexin [$t(18) = 2.749$, $p < .05$], and serum insulin [$t(18) = -2.900$, $p < .05$] hormone values. Before the evening exercise session, statistically significant difference between NW and OW/OO groups was found in basal (before-evening exercise session) serum asprosin [$(t(18) = -2.747$, $p < .001$], serum spexin [$t(18) = 2.631$, $p < .05$], serum lipocalin-2 [$(t(18) = -2.261$, $p < .05$], and serum insulin [$(t(18) = -2.683$, $p < .05$] values

A statistically significant difference was found in the comparison of body temperature pretest and posttest mean values independent of exercise time. There was a significant increase in body temperature in the posttest compared to the pretest in both BMI groups [NW group: $F(1,18) = 85.278$, $p < .001$, partial eta squared: .826 versus OW/OO group: $F(1,18) = 276.253$, $p < .001$, partial eta squared: .939]. Exercise time had a significant effect on the body temperature mean value without discriminating pretest and posttest. In both BMI groups, a higher increase in body temperature was detected in the evening aerobic exercise compared to the morning aerobic exercise [NW group: $F(1,18) = 4.875$, $p < .05$, partial eta squared: .213 versus OW/OO group: [$F(1,18) = 20.035$, $p < .001$, partial eta squared: .527]. Exercise Time* pretest and posttest interaction did not have a significant effect on body temperature in both BMI groups ($p > .05$)

Significant increase in body temperature was found in the posttest compared to the pretest after both morning and evening aerobic exercise, regardless of the BMI

Table 4. Comparison of NW and OW/OO group's energy consumption during exercise (EC), and resting heart rate (RHR) measured before exercises according to exercise time.

BMI Groups	Variables	Exercise Time	N	M ± S.D.	t	p
NW	EC (kcal)	Morning	10	419.50 ± 55.01	−1.073	p =.311
		Evening	10	451.20 ± 110.67		
	RHR (beat/min)	Morning	10	70.00 ± 8.64	−.423	p =.683
		Evening	10	71.00 ± 8.91		
OW/OO	EC (kcal)	Morning	10	441.00 ± 137.36	−.650	p =.532
		Evening	10	472.50 ± 66.82		
	RHR (beat/min)	Morning	10	70.80 ± 3.45	−1.186	p =.266
		Evening	10	73.90 ± 7.85		

BMI: Body Mass Index; NW: Normal Weight, OW/OO: Overweight/Obese

groups. [Morning: F(1,18) = 79.237, $p < .001$, partial eta squared: .815 versus Evening: F(1,18) = 557.357, $p < .001$, partial eta squared: .969]. BMI Groups* pretest_posttest interaction showed a statistically significant effect on body temperature. Compared to the NW group, the body temperature showed significantly greater increase after the evening aerobic exercise in the OW/OO group [F (1,18) = 5.357, $p < .05$, partial eta squared: .229].

Statistically significant difference was found in the between mean values of serum asprosin before and after aerobic exercise in both BMI groups independent of exercise time. Serum asprosin level decreased postexercise compared to preexercise in both BMI groups [NW: F(1,18) = 20.101, $p < .001$, partial eta squared:.528 versus OW/OO: F(1,18) = 27.999, $p < .001$, partial eta squared: .609]. For both BMI groups, the mean values of serum asprosin did not differ significantly according to exercise time without discriminating pretest and posttest ($p > .05$). The Exercise Time*pretest_posttest interaction on serum asprosin was not statistically significant [NW group: F(1,18) = 1.054, $p > .05$, partial eta squared: .055 versus OW/OO group: Exercise Time*pretest_posttest: F(1,18) = .733, $p > .05$, partial eta squared: .039].

Statistically significant difference was found in the comparison of serum asprosin mean values before and after morning and evening aerobic exercise, regardless of the BMI group. There was a significant decrease in serum asprosin level after both aerobic exercise times [Morning: F(1,18) = 22.291, $p < .001$, partial eta squared: .553 versus F(1,18) = 25.502, $p < .001$, partial eta squared: .581]. There was a significant difference in comparing serum asprosin mean values of BMI groups in both exercise times without any pretest and posttest distinctions. Compared to the NW group, serum asprosin level was significantly higher in the OW/OO group [Morning: F(1,18) = 12.216, $p < .01$, partial eta squared: .404 versus Evening: F(1,18) = 21.202, $p < .001$, partial eta squared: .541]. In addition, the Groups*pretest_posttest interaction was found to have a significant effect on serum asprosin level. After evening aerobic exercise, there was significant greater decrease in serum asprosin level in the OW/OO group as compared with the NW group [Evening: F(1,18) = 5.548, $p < .05$, partial eta squared: .236].

Morning and evening aerobic exercise had no statistically significant effect on serum spexin level in both BMI groups ($p > .05$).

Statistically significant difference was found in the comparison of serum spexin mean values of the NW and OW/OO groups without discriminating pretest and posttest. Serum spexin level was significantly lower in OW/OO group compared to NW group [Morning: F (1,18) = 12.387, $p < .01$, partial eta squared: .408 versus Evening: F(1,18) = 5.375, $p < .05$, partial eta squared: .230]

A statistically significant difference was detected in the comparison of the pretest and posttest of serum lipocalin-2 mean values without any exercise time distinction in the OW/OO group [F(1,18) = 6.979, $p < .05$, partial eta squared: .279]. It was observed that the serum lipocalin-2 mean value decreased significantly after aerobic exercise in the OW/OO group. Exercise time and Exercise Time*pretest_posttest interaction had no statistically significant effect on serum lipocalin-2 level ($p > .05$)

BMI Groups* pretest_posttest interaction showed a statistically significant effect on the serum lipocalin-2 level in the evening time. Compared to the NW group, serum lipocalin-2 mean value of the OW/OO group decreased significantly after evening aerobic exercise [Evening: F(1,18) = 14.151, $p < .01$, partial eta squared: .440].

A statistically significant difference was found in the comparison of serum insulin pretest and posttest mean values without any exercise time distinction in both BMI groups. Serum insulin decreased after aerobic exercise in both BMI groups [NW group: F(1,18) = 116.039, $p < .001$, partial eta squared: .866 versus OW/OO group: F (1,18) = 59.569, $p < .001$, partial eta squared: .768]. Moreover, Exercise Time and Exercise Time*pretest_posttest interaction did not significantly have an impact on serum insulin in both BMI groups ($p > .05$).

There was a significant decrease in serum insulin level after both morning and evening aerobic exercise without discriminating BMI groups [Morning: F(1,18) = 43.736, $p < .001$, partial eta squared: .708 versus Evening: F (1,18) = 160.945, $p < .001$, partial eta squared: .899]. Statistically significant difference was found in comparing serum insulin mean values of BMI groups in both exercise times without discriminating pretest and posttest. Serum insulin was found to be significantly higher in the OW/OO group than in the NW group [Morning: F (1,18) = 11.299, $p < .01$, partial eta squared: .386 versus Evening: F(1,18) = 7.571, $p < .05$, partial eta squared: .296]. BMI Groups*pretest_posttest interaction did not have a significant effect on serum insulin in the two exercise time. However, although not significant, serum insulin decreased more in the OW/OO group than in the NW group, in particular, after evening aerobic exercise [Morning: F(1,18) = 2.312, $p > .05$, partial eta squared: .114] versus [Evening: F(1,18) = 4.172, $p > .05$, partial eta squared: .188].

Discussion

The aim of this study was to compare the basal values of serum asprosin, spexin, lipocalin-2, and insulin between the NW and OW/OO groups, and also to examine the

effects on these hormones of aerobic exercise performed by participants of the two groups in morning versus evening.

According to Bioelectrical Impedance Analysis, the body fat percentage (BF%) of the OW/OO group (30.08 ± 3.18) was significantly higher than that of the NW group (18.06 ± 1.60) in this study. The BMI is commonly used as a measure of overweightness and obesity for epidemiological studies, but BMI cannot distinguish between fat and lean mass, and underestimates BF%, especially in the overweight category (Gomez-Ambrosi et al. 2012). BF% ranges are defined by the American Exercise Council as 22–25% for overweight, and 26–31% for obesity in adult men (Marcus 2013). Previous studies also indicated that the cutoff point for total body fat as >25% to identify obesity in adult men (Okorodudu et al. 2010; Romero-Corral et al. 2008). Therefore, in this study, the NW and OW/OO groups categorized according to BMI classification also match the ranges or cutoff points specified above for the body fat percentage.

Before starting the exercise, periphial and core temperature tend to rise due to increase of cathecholamine levels. Along with muscular activation, there is a heat production of around 1°C every 5 min during aerobic exercise. Then, thermoregulation sweat mechanisms are activated try to create heat regulation by increasing fluid loss. (Tucker 2008). We found a significant increase in body temperature after morning and evening aerobic exercise in both BMI groups (Table 6, 7). Miller et al. (2020) stated that core body temperature increased by about 0.4°C after aerobic exercise between 20:45 and 21:00 h in healthy males. Another study indicated that core body temperature increased by 0.6°C after vigorous intensity treadmill walking exercise (Sandroff et al. 2016). Compared to the NW group, the body temperature in the OW/OO group showed significantly greater increase after the evening exercise. The body temperature of the NW group increased by 0.46°C, while that of the OW/OO group rose by 0.56 after evening exercise (Table 7). Eijsvogels et al. (2011) stated that the heat responses to the exercise showed a parallel course with the body mass index. As body mass increased, core temperature also increased more in exercise. Our study results support this.

We found the basal serum asprosin level of the OW/OO group to be higher in the evening and morning compared to the NW group (Table 5). Previous studies reported the level of asprosin is higher in obese than normal weight individuals (Alan et al. 2019; Wang et al. 2019). Ugur and Aydin (2019) found asprosin to be increased approximately twofold to threefold in obese I and II classes and increased approximately fourfold in obese class III. Romere et al. (2016) demonstrated asprosin is secreted not only from adipose tissue, but also from the lungs, heart, and gastrointestinal tract. Wang et al. (2019) suggested the explanation for high asprosin levels in obese individuals might be its release from other organs, and also Duerrschmid et al. (2017) and Romere et al. (2016) pointed out that asprosin is an orexigenic hormone that acts centrally and triggers appetite. Thus, the high level of asprosin in obese individuals might create a feeling of hunger, and trigger more food intake, suggesting obesity might develop due to increased body weight and body fat.

In this study, a significant reduction in serum asprosin level was detected in both BMI groups after aerobic exercise in both the morning and evening (Table 8, 9). Only a limited number of published studies have examined the acute effect of exercise on asprosin. Schumann et al. (2017) reported in obese individuals that anaerobic exercise had no immediate effect on asprosin level. Wiecek et al. (2018) showed significant decrease in asprosin level of women measured 3 min after anaerobic exercise. Blood glucose plays a suppressive role on asprosin via negative feedback (Romere et al. 2016), and Wiecek et al. (2018) suggested decrease in asprosin level measured 3 min after exercise might be related to blood glucose level. These latter investigators found asprosin level low 3 min postexercise when blood sugar concentration was high, and that asprosin level was highest 30 min postexercise when the blood sugar concentration was lowest. We did not assess blood glucose level in our study. This is a limitation of our research. In future studies, assessment of changes in insulin and glucagon-related pathways plus cAMP is recommended to identify mechanisms underlying the beneficial effect of aerobic exercise on hepatic asprosin-dependent pathways (Ko et al. 2019).

We found evening aerobic exercise more greatly reduced serum asprosin level in the OW/OO group as compared with the NW group (Table 9). To our knowledge, no studies have examined the circadian or time-of-day effect of exercise on asprosin level. Previous studies have showed that aerobic exercise performed by obese women in the morning (08:00–10:00 h) versus other times of the day more greatly decreased subjective appetite sensations (Alizadeh et al. 2015; Irandoust and Taheri 2018). Larsen et al. (2019) emphasized high-intensity interval exercises performed in the early to mid-afternoon (14:00–16:00 h) compared to in the morning stimulated more reduction in oxygenic signals, thereby stimulating greater decrease in ghrelin hormone.

Table 5. Comparison of basal asprosin, spexin, lipocalin-2, and insulin mean values measured before both morning and evening exercise according to BMI groups.

Exercise Time	Hormones	BMI Groups	N	M ± S.D.	t	*p*
Morning	Serum Asprosin (ng/mL)	NW	10	.70 ±.17		
		OW/OO	10	.97 ±.26	−2.709	***p* <.05**
	Serum Spexin (pg/mL)	NW	10	261.93 ± 92.57	2.749	***p* <.05**
		OW/OO	10	168.47 ± 54.81		
	Serum Lipocalin-2 (ng/mL)	NW	10	108.39 ± 35.33	−1.103	*p* =.285
		OW/OO	10	126.79 ± 39.18		
	Serum İnsulin (µU/mL)	NW	10	8.58 ± 3.34	−2.900	***p* <.05**
		OW/OO	10	16.01 ± 7.38		
Evening	Serum Asprosin (ng/mL)	NW	10	.66 ±.12	−2.747	***p* <.001**
		OW/OO	10	1.11 ±.22		
	Serum Spexin (pg/mL)	NW	10	279.08 ± 104.91	2.631	***p* <.05**
		OW/OO	10	179.44 ± 57.77		
	Serum Lipocalin-2 (ng/mL)	NW	10	101.76 ± 24.36	−2.261	***p* <.05**
		OW/OO	10	124.21 ± 19.80		
	Serum İnsulin (µU/mL)	NW	10	7.79 ± 2.30	−2.683	***p* <.05**
		OW/OO	10	12.41 ± 4.93		

BMI: Body Mass Index; NW: Normal Weight; OW/OO: Overweight/Obese

Table 6. Comparison of body temperature (BT) mean values in BMI groups (NW and OW/OO) according to exercise time.

					Pretest_posttest		Exercise Time		Exercise Time * pretest_posttest	
BMI Groups	Exercise Time	Hormone	Measurement	M ± S.D.	F	*P*	*F*	*P*	F	*P*
NW	Morning exercise	BT (°C)	Pre test	35.70 ±.61	85.278	***p* <0.001**	4.875	***p* <0.05**	0.227	*p* =0.640
			Post test	36.21 ±.78						
	Evening exercise	BT (°C)	Pre test	36.29 ±.42						
			Post test	36.75 ±.43						
OW/OO	Morning exercise	BT (°C)	Pre test	35.59 ±.37	276.253	***p* <0.001**	20.035	***p* <0.001**	0.379	*p* =0.546
			Post test	36.11 ±.39						
	Evening exercise	BT (°C)	Pre test	36.37 ±.39						
			Post test	36.93 ±.44						

BMI: Body Mass Index; NW: Normal Weight; OW/OO: Overweight/Obese; pretest_posttest: Within Subject Effects; Exercise Time: Between Subject Effects

Table 7. Comparison of body temperature (BT) mean values in two exercise time (morning and evening exercise) according to BMI groups.

					Pretest_posttest		BMI Groups		BMI Groups* pretest_posttest	
Exercise Time	BMI Groups	Hormone	Measurement	M ± S.D.	F	*P*	*F*	*P*	F	*P*
Morning	NW	BT (°C)	Pre test	35.70 ±.61	79.237	***p* <0.001**	0.178	*p* =0.678	.007	*p* =0.925
			Post test	36.21 ±.78						
	OW/OO	BT (°C)	Pre test	35.59 ±.37						
			Post test	36.11 ±.39						
Evening	NW	BT (°C)	Pre test	36.29 ±.42	557.357	***p* <0.001**	0.476	*p* =0.499	5.357	***p* <0.05**
			Post test	36.75 ±.43						
	OW/OO	BT (°C)	Pre test	36.37 ±.39						
			Post test	36.93 ±.44						

BMI: Body Mass Index; NW: Normal Weight; OW/OO: Overweight/Obese; pretest_posttest: Within Subject Effects; BMI Groups: Between Subject Effects

In humans, spexin gene expression was found to be downregulated 14.9-fold in obese omental and subcutaneous fat (Walewski et al. 2014). Before-exercise basal serum spexin levels of our OW/OO subjects in both the evening and morning exercise were lower than in NW subjects (Table 5). Recent studies indicate obese individuals have low spexin values (Kolodziejski et al. 2018; Kumar et al. 2016; Lin et al. 2018; Walewski et al. 2014), and spexin might have a potential role in obesity. However, more research is needed to identify the reasons for the observed low levels of this hormone in obese individuals, and to develop a spexin-based anti-obesity drug (Lv et al. 2019).

Morning and evening aerobic exercise when applied to the NW and OW/OO group exerted no statistically significant effect on serum spexin level ($p > .05$) (Table 10,11). Only one study previously examined the effect of exercise on spexin hormone, finding no significant impact on its level (Fathi 2016). The exercise intensity used in both this and the Fathi et al. (2016) study was "moderate" according to ACSM (2018). Therefore, performing the exercise at a higher intensity or in accord

Table 8. Comparison of asprosin mean values in BMI groups (NW and OW/OO) according to exercise time.

					Pretest_posttest		Exercise Time		Exercise Time * pretest_posttest	
BMI Groups	Exercise Time	Hormone	Measurement	M ± S.D.	F	*P*	*F*	*P*	F	*P*
NW	Morning exercise	Serum Asprosin (ng/mL)	Pre test	.70 ±.17	20.101	***p* <0.001**	0.103	*p* =0.752	1.054	*p* =0.318
			Post test	.52 ±.07						
	Evening exercise	Serum Asprosin (ng/mL)	Pre test	.66 ±.12						
			Post test	.54 ±.11						
OW/OO	Morning exercise	Serum Asprosin (ng/mL)	Pre test	.97 ±.26	27.999	***p* <0.001**	0.969	*p* =0.338	.733	*p* =0.403
			Post test	.75 ±.17						
	Evening exercise	Serum Asprosin (ng/mL)	Pre test	1.11 ±.22						
			Post test	.80 ±.27						

BMI: Body Mass Index; NW: Normal Weight; OW/OO: Overweight/Obese; pretest_posttest: Within Subject Effects; Exercise Time: Between Subject Effects

Table 9. Comparison of asprosin mean values in two exercise time (morning and evening exercise) according to BMI groups.

					Pretest_posttest		BMI Groups		BMI Groups* pretest_posttest	
Exercise Time	BMI Groups	Hormone	Measurement	M ± S.D.	F	*P*	*F*	*P*	F	*P*
Morning	NW	Serum Asprosin (ng/mL)	Pre test	.70 ±.17	22.291	***p* <0.001**	12.216	***p* <0.01**	0.292	*p* =0.595
			Post test	.52 ±.07						
	OW/OO	Serum Asprosin (ng/mL)	Pre test	.97 ±.26						
			Post test	.75 ±.17						
Evening	NW	Serum Asprosin (ng/mL)	Pre test	.66 ±.12	25.502	***p* <0.001**	21.202	***p* <0.001**	5.548	***p* <0.05**
			Post test	.54 ±.11						
	OW/OO	Serum Asprosin (ng/mL)	Pre test	1.11 ±.22						
			Post test	.80 ±.27						

BMI: Body Mass Index; NW: Normal Weight; OW/OO: Overweight/Obese; pretest_posttest: Within Subject Effects; BMI Groups: Between Subject Effects

Table 10. Comparison of spexin mean values in BMI groups (NW and OW/OO) according to exercise time.

					Pretest_posttest		Exercise Time		Exercise Time * pretest_posttest	
BMI Groups	Exercise Time	Hormone	Measurement	M ± S.D.	F	*P*	*F*	*P*	F	*P*
NW	Morning exercise	Serum Spexin (pg/mL)	Pre test	261.93 ± 92.57	.663	*p* =0.426	0.011	*p* =0.917	0.396	*p* =0.537
			Post test	255.99 ± 58.13						
	Evening exercise	Serum Spexin (pg/mL)	Pre test	279.08 ± 104.91						
			Post test	232.62 ± 122.67						
OW/OO	Morning exercise	Serum Spexin (pg/mL)	Pre test	168.47 ± 54.81	1.905	*p* =0.184	1.139	*p* =0.300	0.689	*p* =0.417
			Post test	176.03 ± 37.24						
	Evening exercise	Serum Spexin (pg/mL)	Pre test	179.44 ± 57.77						
			Post test	209.81 ± 69.57						

BMI: Body Mass Index; NW: Normal Weight; OW/OO: Overweight/Obese; pretest_posttest: Within Subject Effects; Exercise Time: Between Subject Effects

Table 11. Comparison of spexin mean values in two exercise time (morning and evening exercise) according to BMI groups.

					Pretest_posttest		BMI Groups		BMI Groups* pretest_posttest	
Exercise Time	BMI Groups	Hormone	Measurement	M ± S.D.	F	*P*	*F*	*P*	F	*P*
Morning	NW	Serum Spexin (pg/mL)	Pre test	261.93 ± 92.57	.003	*p* =0.956	12.387	***p* <0.01**	0.217	*p* =0.647
			Post test	255.99 ± 58.13						
	OW/OO	Serum Spexin (pg/mL)	Pre test	168.47 ± 54.81						
			Post test	176.03 ± 37.24						
Evening	NW	Serum Spexin (pg/mL)	Pre test	279.08 ± 104.91	.064	*p* =0.804	5.375	***p* <0.05**	1.454	*p* =0.243
			Post test	232.62 ± 122.67						
	OW/OO	Serum Spexin (pg/mL)	Pre test	179.44 ± 57.77						
			Post test	209.81 ± 69.57						

BMI: Body Mass Index; NW: Normal Weight; OW/OO: Overweight/Obese; pretest_posttest: Within Subject Effects; BMI Groups: Between Subject Effects

with changes in long-term lifestyle, such as weight reduction, and increasing physical activity level (Al-Daghri et al. 2019) can have a significant effect on spexin hormone level.

The before-exercise basal serum lipocalin-2 level of the OW/OO group, compared to NW group, was statistically higher only in the evening exercise (Table 5). Previous studies identified close relationship between Lipocalin-2

hormone, obesity, and BMI, lipocalin-2 being high in obese individuals (Huang et al. 2012; Mohammadi and Reddy 2014; Wang et al. 2007; Zaki et al. 2015). Obesity is a pro-inflammatory condition with augmentation of multiple markers related to inflammation (Hotamisligil 2006). Overproduction of proinflammatory factors, such as IL-6 and TNF-α, in obesity was found to trigger release of lipocalin-2, which is effective in the homeostatic regulation of autocrine or paracrine responses. Given the anti-inflammatory role of lipocalin-2, augmented lipocalin-2 in obesity might be a protective mechanism against excessive proliferation of inflammation (Zhang et al. 2008).

We found significant decrease in serum lipocalin-2 level only in the evening exercise by the OW/OO group (Table 12,13). The results of published investigations are inconsistent regarding the effect of exercise on lipocalin-2. Intensity of the exercise plays a major role in these contradictory results, as some studies reported decreased lipocalin-2 level after aerobic exercise (Di Rosa et al. 2019; Khademi et al. 2019; Mohammadi and Reddy 2014), while other studies reported increased lipocalin-2 level after anaerobic exercise (Damirchi et al. 2011). As suggested by the results of this study, reduction in lipocalin-2 are linked with other phenomena, like reduction of inflammatory markers, such as interleukin-6, and TNF-α, although not C-reactive protein (Moghadasi and Domieh 2014; Mohammadi and Reddy 2014). These inflammatory markers of adipose tissue were not measured in our study, and, therefore, we are unable determine if acute aerobic exercise had any effect on them.

Basal serum insulin level measured in the morning and evening was higher in the OW/OO group than in the NW group (Table 5). The published literature indicates insulin levels are higher in OW/OO individuals than in NW individuals (Achachluie and Abbaszadegan 2018; Amor et al. 2018; Gonzalez-Cantero et al. 2018; Meyer-Gerspach et al. 2016; Short et al. 2018). For example, Short et al. (2018) found OW/OO individuals had 66% higher fasting insulin levels, and 9% lower insulin sensitivity than NW individuals. Basal insulin level is higher in obese individuals and tends to reflect body adiposity. This is explained by decreased sensitivity of liver, muscle, and adipose tissue to insulin in obese individuals, resulting in high levels of insulin being secreted from the pancreas (Berryman et al. 2013). Mehran et al. (2012) claimed insulin level should be maintained low in order to provide energy consumption in white adipose tissue with uncoupling protein-3 (UCP) expression.

We found statistically significant reductions in serum insulin level following aerobic exercise in both the morning and evening sessions and in both the NW and OW/OO groups (Table 14,15). Studies conducted on obese individuals reported acute exercise reduces insulin level and improves insulin sensitivity (Numao

Table 12. Comparison of lipocalin-2 mean values in BMI groups (NW and OW/OO) according to exercise time.

					Pretest_posttest		Exercise Time		Exercise Time * pretest_posttest	
BMI Groups	Exercise Time	Hormone	Measurement	M ± S.D.	F	*P*	*F*	*P*	F	*P*
NW	Morning exercise	Serum Lipocalin-2 (ng/mL)	Pre test	108.39 ± 35.33	2.332	*p* =0.144	0.124	*p* =0.729	0.193	*p* =0.666
			Post test	116.43 ± 18.12						
	Evening exercise	Serum Lipocalin-2 (ng/mL)	Pre test	101.76 ± 24.36						
			Post test	116.30 ± 27.70						
OW/OO	Morning exercise	Serum Lipocalin-2 (ng/mL)	Pre test	126.79 ± 39.18	6.979	***p* <0.05**	1.267	*p* =0.275	0.867	*p* =0.364
			Post test	113.67 ± 24.14						
	Evening exercise	Serum Lipocalin-2 (ng/mL)	Pre test	124.21 ± 19.80						
			Post test	96.82 ± 12.45						

BMI: Body Mass Index; NW: Normal Weight; OW/OO: Overweight/Obese; pretest_posttest: Within Subject Effects; Exercise Time: Between Subject Effects

Table 13. Comparison of lipocalin-2 mean values in two exercise time (morning and evening exercise) according to BMI groups.

					Pretest_posttest		BMI Groups		BMI Groups* pretest_posttest	
Exercise Time	BMI Groups	Hormone	Measurement	M ± S.D.	F	*P*	*F*	*P*	F	*P*
Morning	NW	Serum Lipocalin-2 (ng/mL)	Pretest	108.39 ± 35.33	.078	*p* =0.783	0.597	*p* =0.450	1.358	*p* =0.259
			Posttest	116.43 ± 18.12						
	OW/OO	Serum Lipocalin-2 (ng/mL)	Pretest	126.79 ± 39.18						
			Posttest	113.67 ± 24.14						
Evening	NW	Serum Lipocalin-2 (ng/mL)	Pretest	101.76 ± 24.36	1.330	*p* =0.264	0.034	*p* =0.856	14.151	***p* <0.01**
			Posttest	116.30 ± 27.70						
	OW/OO	Serum Lipocalin-2 (ng/mL)	Pretest	124.21 ± 19.80						
			Posttest	96.82 ± 12.45						

BMI: Body Mass Index; NW: Normal Weight; OW/OO: Overweight/Obese; pretest_posttest: Within Subject Effects; BMI Groups: Between Subject Effects

Table 14. Comparison of insulin mean values in BMI groups (NW and OW/OO) according to exercise time.

					Pretest_posttest		Exercise Time		Exercise Time * pretest_posttest	
BMI Groups	Exercise Time	Hormone	Measurement	M ± S.D.	F	*P*	*F*	*P*	F	*P*
NW	Morning exercise	Serum Insulin (μU/mL)	Pretest	8.58 ± 3.34	116.039	***p* <0.001**	1.184	*p* =0.291	0.090	*p* =0.767
			Posttest	3.26 ± 1.81						
	Evening exercise	Serum Insulin (μU/mL)	Pretest	7.79 ± 2.30						
			Posttest	2.16 ±.59						
OW/OO	Morning exercise	Serum Insulin (μU/mL)	Pretest	16.01 ± 7.38	59.569	***p* <0.001**	2.669	*p* =0.120	0.114	*p* =0.739
			Posttest	7.52 ± 3.64						
	Evening exercise	Serum Insulin (μU/mL)	Pretest	12.41 ± 4.93						
			Posttest	4.63 ± 2.95						

BMI: Body Mass Index; NW: Normal Weight; OW/OO: Overweight/Obese; pretest_posttest: Within Subject Effects; Exercise Time: Between Subject Effects

Table 15. Comparison of insulin mean values in two exercise time (morning and evening exercise) according to BMI groups.

					Pretest_posttest		BMI Groups		BMI Groups* pretest_posttest	
Exercise Time	BMI Groups	Hormone	Measurement	M ± S.D.	F	*P*	*F*	*P*	F	*P*
Morning	NW	Serum Insulin (μU/mL)	Pretest	8.58 ± 3.34	43.736	***p* <0.001**	11.299	***p* <0.01**	2.312	*p* =0.146
			Posttest	3.26 ± 1.81						
	OW/OO	Serum Insulin (μU/mL)	Pretest	16.01 ± 7.38						
			Posttest	7.52 ± 3.64						
Evening	NW	Serum Insulin (μU/mL)	Pretest	7.79 ± 2.30	160.945	***p* <0.001**	7.571	***p* <0.05**	4.172	*p* =0.056
			Posttest	2.16 ±.59						
	OW/OO	Serum Insulin (μU/mL)	Pretest	12.41 ± 4.93						
			Posttest	4.63 ± 2.95						

BMI: Body Mass Index; NW: Normal Weight; OW/OO: Overweight/Obese; pretest_posttest: Within Subject Effects; BMI Groups: Between Subject Effects

et al. 2011; Saunders et al. 2012). Short et al. (2018) found acute moderate aerobic exercise performed by NW and OW/OO individuals improved insulin sensitivity for at least 17 h. Yaribeygi et al. (2019) emphasized the role of some molecular mechanisms by which aerobic exercise increases insulin sensitivity, i.e. upregulation of glucose transporter protein (GLUT-4) and increased GLUT-4 density in insulin-dependent cell membranes. Aerobic exercise decreased inflammation by reducing adipokines, and it improved beta cell functions through regulation of RS-1 phosphorylation that plays an important role in improving insulin signal delivery. Furthermore, increased capillarization due to aerobic exercise-induced angiogenesis stimulates higher glucose uptake by myocytes (Yaribeygi et al. 2019).

Although BMI Groups*pretest_posttest interaction did not have a statistically significant effect on serum insulin level, we additionally found evening exercise triggered greatest reduction of serum insulin level, especially in the OW/OO group (Table 15). Few studies have examined the effects of exercise performed in the morning versus afternoon or evening hours on glucose metabolism parameters. Savikj et al. (2019) found high-intensity interval exercises performed at 16:00 h were more effective in reducing blood glucose level than those performed at 08:00 h. According to Gomez et al. (2015), afternoon exercise performed at 16:00 h compared with the morning exercise performed at 07:00 h had a lower risk of hypoglycemia, plus improved next-day metabolic control. Basse et al. (2018) found exercise did not affect the circadian rhythm of insulin sensitivity such that the greater reduction of insulin level by evening compared to morning exercises can be attributed at least in part to insulin circadian rhythmicity. Boden et al. (1996a), Boden et al. (1996b), Carrasco-Benso et al. (2016), and Saad et al. (2012) suggested that insulin sensitivity is higher in the evening than other times of the 24 h. Circadian rhythms in insulin release and sensitivity may be due to circadian rhythms in blood glucose (La Fleur et al. 2001). Therefore, measuring blood glucose level in subsequent studies may provide clearer information about the decrease in insulin level.

Compared to the NW group, the OW/OO group had a greater increase in body temperature after evening exercise. This may have been triggered by more extensive reductions in serum asprosin, lipocalin-2, and insulin (not significant) levels after evening exercise in the OW/OO group. The increasing body temperature after exercise was more effective on appetite-related hormones, and as a result, food intake decreased. Jeong et al. (2018) observed that small rise in the body temperature within the physiological range (37°C– 38°C) after exercise

decreased food intake via TRPV1-like thermoreceptors in anorexigenic POMC-expressing neurons in the arcuate nucleus of the hypothalamus. In addition, although the OW/OO group did not have any restrictions about eating and drinking during the study (about 7d in duration), they dieted because of their psychological perceptions about the importance of the study. The daily energy intake by the OW/OO group (1781.59 ± 410.71 kcal) before the evening exercise was significantly lower than that of the NW group (2380.28 ± 445.50 kcal), causing a negative energy balance. Hubert et al. (1998) defined this situation as an "acute energy deficit." Therefore, the negative energy balance appears to trigger greater reductions in asprosin, lipocalin-2, and insulin levels in the OW/OO group. In addition, some studies suggest that exercise+food restriction (Johnson et al. 2016; Vitola et al. 2009) plus psychological perception can manage the effect of exercise on appetite and appetite-related hormones. King (1999) emphasized an individual's strong will of controlling food intake (for example, dietary restriction, nutritional-related cognitions, attitude about exercise) can psychologically manage the effect of exercise on appetite, and also psychological status of the individual can have a very strong effect on food intake after exercise

Conclusion

In this study, basal serum asprosin, lipocalin-2, and insulin level were found higher to be in the OW/OO group, while serum spexin level was lower compared to the normal weight group. This demonstrates asprosin, spexin, lipocalin-2, and insulin should be considered important both in the development and treatment of obesity. Aerobic exercise of "moderate intensity" triggered decrease in asprosin, lipocalin-2, and insulin both in our NW and OW/OO groups, although this exercise intensity was insufficient to increase spexin level. Moderate aerobic exercise applied to the OW/OO group in the evening hours led to more significant reductions in serum asprosin, lipocalin-2, and insulin level. We hypothesize the negative energy balance caused by psychological energy restriction and evening aerobic exercise, which leads to a further increase in body temperature triggers, more greatly decreases orexigenic signals (suppression of appetite) and effectively induces development of adipose tissue inflammation and insulin sensitivity, especially in OW/OO group.

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Declaration of interest

The authors report no conflict of interest.

References

Achachluie FK, Abbaszadegan M. 2018. Relationship between serum chemerin levels and insulin resistance index and cardio–respiratory function in non–active obese and lean men. Adv Obes Weight Manag Control. 8:160–164. doi:10.15406/aowmc.2018.08.00234.

[ACSM] American College of Sports Medicine. 2018. ACSM's guidelines for exercise testing and prescription. Philadelphia (PA): Wolters Kluwer Health.

Alan M, Gurlek B, Yilmaz A, Aksit M, Aslanipour B, Gulhan I, Mehmet C, Taner CE. 2019. Asprosin: a novel peptide hormone related to insulin resistance in women with polycystic ovary syndrome. Gynecol Endocrinol. 35(3):220–223. doi:10.1080/09513590.2018.1512967

Al-Daghri NM, Wani K, Yakout SM, Al-Hazmi H, Amer OE, Hussain SD, Sabico S, Ansari MGA, Al-Musharaf S, Alenad AM, et al. 2019. Favorable changes in fasting glucose in a 6-month self-monitored lifestyle modification programme inversely affects spexin levels in females with prediabetes. Sci Rep. 9(1):9454. doi:10.1038/s41598-019-46006-0

Algul S, Ozcelik O. 2016. Akut aerobik egzersizin nesfatin-1 uzerine etkilerinin belirlenmesi. FU Sag Bil Tip Derg. 30:05–08.

Algul S, Ozdenk C, Ozcelik O. 2017. Variations in leptin, nesfatin-1 and irisin levels induced by aerobic exercise in young trained and untrained male subjects. Biol Sport. 34:339–344. doi:10.5114/biolsport.2017.69821.

Alizadeh Z, Mostafaee M, Mazaheri R, Younespour S. 2015. Acute effect of morning and afternoon aerobic exercise on appetite of overweight women. Asian J Sports Med. 6:e24222. doi:10.5812/asjsm.6(2)20156.24222.

Amor M, Itariu BK, Moreno-Viedma V, Keindl M, Jürets A, Prager G, Langer F, Grablowitz V, Zeyda M, Stulnig TM. 2018. Serum myostatin is upregulated in obesity and correlates with ınsulin resistance in humans. Exp Clin Endocrinol Diabetes. 127:550–556. doi:10.1055/a-0641-5546.

Bashiri J, Rahbaran A, Gholami F, Ahmadizad S, Nikoukheslat S, Moradi A. 2014. The effect of acute exercise on serum vaspin level and its relation to insulin sensitivity in overweight elderly men. Zahedan J Res Med Sci. 16:16–19.

Basse AL, Dalbram E, Larsson L, Gerhart-Hines Z, Zierath JR, Treebak JT. 2018. Skeletal muscle insulin sensitivity show circadian rhythmicity which is independent of exercise training status. Front Physiol. 9:1198. doi:10.3389/fphys.2018.01198.

Begg DP, Woods SC. 2012. The central insulin system and energy balance. In: Joost HG, editor. Appetite control. Berlin (Ger): Springer; p. 111–129.

Berryman DE, Davy BM, List EO. 2013. Control of energy balance. In: Martha H, Stipanuk MH, Caudill ME, editors. Biochemical, physiological, and molecular aspects of human nutrition. Cambridge (MA): Elsevier; p. 501–518.

Bilski J, Jaworek J, Pokorski J, Nitecki J, Nitecka E, Pokorska J, Mazur-Bialy A, Szklarczyk J. 2016. Effects of time of day and the wingate test on appetite perceptions, food intake and plasma levels of adipokines. J Physiol Pharmacol. 67:667–676. PMID: 28011947

Boden G, Chen X, Urbain JL. 1996a. Evidence for a circadian rhythm of insulin sensitivity in patients with NIDDM caused by cyclic changes in hepatic glucose production. Diabetes. 45:1044–1050. doi:10.2337/diab.45.8.1044.

Boden G, Ruiz J, Urbain JL, Chen X. 1996b. Evidence for a circadian rhythm of insulin secretion. Am J Physiol Endocrinol Metab. 271:246–252. doi:10.1152/ajpendo.1996.271.2.E246.

Carrasco-Benso MP, Rivero-Gutierrez B, Lopez-Minguez J, Anzola A, Diez-Noguera A, Madrid JA, Lujan JA, Martinez-Augustin O, Scheer FA. 2016. Human adipose tissue expresses intrinsic circadian rhythm in insulin sensitivity. Faseb J. 30:3117–3123. doi:10.1096/fj.201600269RR.

Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, et al. 2003. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 35:1381–1395. doi:10.1249/01.MSS.0000078924.61453.FB.

Damirchi A, Rahmani-Nia F, Mehrabani J. 2011. Lipocalin-2: response to a short-term treadmill protocol in obese and normal-weight men. J Hum Sport Exerc. 6:59–66. doi:10.4100/jhse.2011.61.07.

Di Rosa M, Castrogiovanni P, Trovato FM, Malatino L, Ravalli S, Imbesi R, Szychlinska MA, Musumeci G. 2019. Adapted moderate training exercise decreases the expression of Ngal in the rat kidney: an immunohistochemical study. Appl Sci. 9:1041. doi:10.3390/app9061041.

Dixon CB, Andreacci JL, Ledezma C. 2008. Effect of aerobic exercise on percent body fat using leg-to-leg and segmental bioelectrical impedance analysis in adults. Int J Body Comp Res. 6:27–34. Doi:10.1519/JSC.0b013e3181b86735.

Duerrschmid C, He Y, Wang C, Li C, Bournat JC, Romere C, Saha PK, Lee ME, Phillips KJ, Jain M, et al. 2017. Asprosin is a centrally acting orexigenic hormone. Nat Med. 23:1444–1453. doi:10.1038/nm.4432

Eijsvogels TMH, Veltmeijer MTW, Schreuder THA, Poelkens F, Thijssen DHJ, Hopman MTE. 2011. The impact of obesity on physiological responses during prolonged exercise. Int J Obes. 35:1404–1412. Doi:10.1038/ijo.2010.277.

Fathi R. 2016. The effect of single session of interval aerobic exercise on serum spexin levels in active young men. J Sport Physiol Phys Activ. 10:37–46.

Gabriel BM, Zierath JR. 2019. Circadian rhythms and exercise re-setting the clock in metabolic disease. Nat Rev Endocrinol. 15:197–206. doi:10.1038/s41574-018-0150-x.

Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS, Nimmo MA. 2011. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. Nat Rev Immunol. 11:607–615. doi:10.1038/nri3041.

Golbidi S, Laher I. 2014. Exercise induced adipokine changes and the metabolic syndrome. J Diabetes Res. 18:726861. doi:10.1155/2014/726861

Gomez AM, Gomez C, Aschner P, Veloza A, Munoz O, Rubio C, Santiago V. 2015. Effects of performing morning versus afternoon exercise on glycemic control and hypoglycemia frequency in type 1 diabetes patients on sensor-augmented insulin pump therapy. J Diabetes Sci Technol. 9:619–624. doi:10.1177/1932296814566233.

Gomez-Ambrosi J, Silva C, Galofre JC, Escalada J, Santos S, Millan D, Vila N, Ibanez P, Gil MJ, Valenti V, et al. 2012. Body mass index classification misses subjects with increased cardiometabolic risk factors related to elevated adiposity. Int J Obes. 36:286–294. doi:10.1038/ijo.2011.100.

Gonzalez-Cantero J, Martin-Rodriguez JL, Gonzalez-Cantero A, Arrebola JP, Gonzalez-Calvin JL. 2018. Insulin resistance in lean and overweight non-diabetic Caucasian adults: study of its relationship with liver triglyceride content, waist circumference and BMI. PLoS One. 13:e0192663. doi:10.1371/journal.pone.0192663.

Gunay M, Tamer K, Cicioglu I. 2013. Spor fizyolojisi ve performans olcumu. Ankara (TR): Gazi Kitapevi.

Haghshenas R, Jafari M, Ravasi A, Kordi M, Gilani N, Shariatzadeh M, Hedayati M, Rahimi M. 2014. The effect of eight weeks endurance training and high-fat diet on appetite-regulating hormones in rat plasma. Iran J Basic Med Sci. 17:237–243. doi:10.22038/IJBMS.2014.2580.

Hamaguchi Y, Tahara Y, Hitosugi M, Shibata S. 2015. Impairment of circadian rhythms in peripheral clocks by constant light is partially reversed by scheduled feeding or exercise. J Biol Rhythms. 30:533–542. doi:10.1177/0748730415609727.

Hazell TJ, Islam H, Townsend LK, Schmale MS, Copeland JL. 2016. Effects of exercise intensity on plasma concentrations of appetite-regulating hormones: potential mechanisms. Appetite. 98:80–88. doi:10.1016/j.appet.2015.12.016.

Hotamisligil GS. 2006. Inflammation and metabolic disorders. Nature. 444:860–867. doi:10.1038/nature05485.

Huang Y, Yang Z, Ye Z, Li Q, Wen J, Tao X, Chen L, He M, Wang X, Lu B, et al. 2012. Lipocalin-2, glucose metabolism and chronic low-grade systemic inflammation in Chinese people. Cardiovasc Diabetol. 11:22292925. doi:10.1186/1475-2840-11-11.

Hubert P, King NA, Blundell JE. 1998. Uncoupling the effects of energy expenditure and energy intake: appetite response to short-term energy deficit induced by meal omission and physical activity. Appetite. 31:9–19. Doi:10.1006/appe.1997.0148.

Hulley SB, Cummings SR, Browner WS, Grady DG, Newman TB. 2013. Designing clinical research. Philadelphia (PA): Lippincott Williams & Wilkins.

Ilacqua A, Emerenziani GP, Guidetti L, Baldari C. 2019. The role of physical activity in adult obesity. In: Watson RR, Diego S, eds. CA: Elsevier. p. 123–128.

[IPAQ] International Physical Activity Questionnaire. 2005. Guidelines for data processing and analysis of the international physical activity questionnaire short

and long forms [blog]. Ipaq. [accessed 2019 April 26]. http://www.ipaq.ki.se
Irandoust K, Taheri M. 2018. Effects of different daytime exercises on the quality of sleep and appetite of obese women. Int Arch Health Sci. 5:111–114. doi:10.4103/iahs.iahs_41_18.
Izci B, Ardic S, Firat H, Sahin A, Altinors M, Karacan I. 2008. Reliability and validity studies of the Turkish version of the Epworth Sleepiness Scale. Sleep Breath. 12:161–168. Doi:10.1007/s11325-007-0145-7.
Jeong JH, Lee DK, Liu SM, Jr SC C, Schwartz GJ, Jo YH. 2018. Activation of temperature-sensitive TRPV1-like receptors in ARC POMC neurons reduces food intake. PLoS Biol. 16:1–24. doi:10.1371/journal.pbio.2004399.
Johnson ML, Distelmaier K, Lanza IR, Irving BA, Robinson MM, Konopka AR, Shulman GI, Nair KS. 2016. Mechanism by which caloric restriction improves insulin sensitivity in sedentary obese adults. Diabetes. 65:74–84. doi:10.2337/db15-0675.
Kajimura S. 2017. Adipose tissue in 2016: advances in the understanding of adipose tissue biology. Nat Rev Endocrinol. 13:69–70. doi:10.1038/nrendo.2016.211.
Karvonen M, Kentala E, Mustala O. 1957. The effect of training on heart rate: a longitudinal study. Ann Med Exp Biol Fenn. 35:307–315. PMID: 13470504
Khademi Y, Hosseini SA, Dana F, Hamidi A, Azadmanesh M, Pirouz M. 2019. Effect of high intensity interval training with flaxseed on interleukin-1 beta and lipocalin-2 gene expressions in the heart tissue of rats. J Arch Mil Med. 6: e83888. doi:10.5812/jamm.83888.
King NA. 1999. What processes are involved in the appetite response to moderate increases in exercise-induced energy expenditure? Proc Nutr Soc. 58:107–113. doi:10.1079/pns19990015.
Ko JR, Seo DY, Kim TN, Park SH, Kwak HB, Ko KS, Rhee BD, Han J. 2019. Aerobic exercise training decreases hepatic asprosin in diabetic rat. J Clin Med. 8:666. doi:10.3390/jcm8050666.
Kolodziejski PA, Pruszynska-Oszmalek E, Korek E, Sassek M, Szczepankiewicz D, Kaczmarek P, Nogowski L, Mackowiak P, Nowak KW, Krauss H, et al. 2018. Serum levels of spexin and kisspeptin negatively correlate with obesity and insulin resistance in women. Physiol Rep. 67:45–56. doi:10.33549/physiolres.933467.
Konturek PC, Konturek JW, Czesnikiewicz-Guzik M, Brzozowski T, Sito E, Konturek SJ. 2005. Neuro-hormonal control of food intake: basic mechanisms and clinical implications. J Physiol Pharmacol. 56:5–25. PMID: 16340035
Kumar S, Hossain J, Nader N, Aguirre R, Sriram S, Balagopal PB. 2016. Decreased circulating levels of spexin in obese children. J Clin Endocrinol Metab. 101:2931–2936. doi:10.1210/jc.2016-1177.
La Fleur SE, Kalsbeek A, Wortel J, Fekkes ML, Buijs RM. 2001. A daily rhythm in glucose tolerance: a role for the suprachiasmatic nucleus. Diabetes. 50:1237–1243. doi:10.2337/diabetes.50.6.1237.
Larsen P, Marino F, Melehan K, Guelfi KJ, Duffield R, Skein M. 2019. Evening high-intensity interval exercise does not disrupt sleep or alter energy intake despite changes in acylated ghrelin in middle-aged men. Exp Physiol. 104:826–836. doi:10.1113/EP087455.
Larsen PS, Donges CE, Guelfi KJ, Smith GC, Adams DR, Duffield R. 2017. Effects of aerobic, strength or combined exercise on perceived appetite and appetite-related hormones in inactive middle-aged men. Int J Sport Nutr Exerc Metab. 27:389–398. doi:10.1123/ijsnem.2017-0144.
Lewis P, Korf HW, Kuffer L, Groß JV, Erren TC. 2018. Exercise time cues (zeitgebers) for human circadian systems can foster health and improve performance: a systematic review. BMJ Open Sport Exerc Med. 4:e000443. doi:10.1136/bmjsem-2018-000443.
Lin C, Huang T, Zhao L, Zhong LLD, Lam WC, Fan B, Bian ZX. 2018. Circulating spexin levels negatively correlate with age, BMI, fasting glucose, and triglycerides in healthy adult women. J Endocr Soc. 2:409–419. doi:10.1210/js.2018-00020.
Lopez-Minguez J, Gomez-Abellan P, Garaulet M. 2016. Circadian rhythms, food timing and obesity. Proc Nutr Soc. 75:501–511. doi:10.1017/S0029665116000628.
Luo L, Liu M. 2016. Adipose tissue in control of metabolism. J Endocrinol. 231:77–99. doi:10.1530/JOE-16-0211.
Lv SY, Zhou YC, Zhang XM, Chen WD, Wang YD. 2019. Emerging roles of npq/spexin in physiology and pathology. Front Pharmacol. 10:457. doi:10.3389/fphar.2019.00457.
Marcus JB. 2013. Culinary nutrition: the science and practice of healthy cooking. Illinois (IL): Academic Press; p. 438.
Mehran AE, Templeman NM, Brigidi GS, Lim GE, Chu KY, Hu X, Botezelli JD, Asadi A, Hoffman BG, Kieffer TJ, et al. 2012. Hyperinsulinemia drives diet-induced obesity independently of brain insulin production. Cell Metab. 16:723–737. doi:10.1016/j.cmet.2012.10.019.
Mera P, Ferron M, Mosialou I. 2019. Regulation of energy metabolism by bone-derived hormones. Cold Spring Harb Perspect Biol. 8:a031666. doi:10.1101/cshperspect.a031666.
Merdol Kutluay T. 2003. Toplu beslenme yapilan kurumlar icin standart yemek tarifeleri. Ankara (TR): Hatipoglu Yayinevi.
Meyer-Gerspach AC, Cajacob L, Riva D, Herzog R, Drewe J, Beglinger C, Wölnerhanssen BK. 2016. Mechanisms regulating insulin response to intragastric glucose in lean and non-diabetic obese subjects: a randomized, double-blind, parallel-group trial. PLoS One. 11:e0150803. doi:10.1371/journal.pone.0150803.
Mika A, Macaluso F, Barone R, Di Felice V, Sledzinski T. 2019. Effect of exercise on fatty acid metabolism and adipokine secretion in adipose tissue. Front Physiol. 10:26. doi:10.3389/fphys.2019.00026.
Miller DJ, Sargent C, Roach GD, Scanlan AT, Vincent GE, Lastella M. 2020. Moderate-intensity exercise performed in the evening does not impair sleep in healthy males. Eur J Sport Sci. 20:80–89. Doi:10.1080/17461391.2019.1611934.
Mitchell ML, Jolley JM. 2013. Research design explained. Belmont (CA): Cengage Learning.
Moghadasi M, Domieh AM. 2014. Effects of resistance versus endurance training on plasma lipocalin-2 in young men. Asian J Sports Med. 5:108–114. PMID: 25834704
Mohammadi A, Reddy PV. 2014. Impact of aerobic exercise training on insulin resistance and plasma lipocalin 2 levels in obese young men. Biomed Pharmacol J. 7:45–52. doi:10.13005/bpj/451.
Mosialou I, Shikhel S, Liu JM, Maurizi A, Luo N, He Z, Huang Y, Zong H, Friedman RA, Barasch J, et al. 2017.

MC4R-dependent suppression of appetite by bone-derived lipocalin 2. Nature. 543:385–390. doi:10.1038/nature21697.
Numao S, Katayama Y, Hayashi Y, Matsuo T, Tanaka K. 2011. Influence of acute aerobic exercise on adiponectin oligomer concentrations in middle-aged abdominally obese men. Metab Clin Exp. 60:186–194. doi:10.1016/j.metabol.2009.12.011.
Okorodudu DO, Jumean MF, Montori VM, Romero-Corral A, Somers VK, Erwin PJ, Lopez-Jimenez F. 2010. Diagnostic performance of body mass index to identify obesity as defined by body adiposity: a systematic review and meta-analysis. Int J Obes. 34:791–799. doi:10.1038/ijo.2010.5.
Ozcelik O, Algul S, Deniz M, Baydas F, Tan F. 2017. Sabah ve gece yapilan aerobik egzersizin antrenmanli deneklerde serum irisin duzeyine etkilerinin belirlenmesi. Genel Tip Derg. 27:1–5.
Ozturk M 2005. Universitede egitim-ogretim goren ogrencilerde uluslararasi fiziksel aktivite anketinin gecerliligi ve guvenirligi ve fiziksel aktivite duzeylerinin belirlenmesi [dissertation]. Ankara (TR): Hacettepe Universitesi.
Patton MQ. 2002. Qualitative evaluation and research methods. London (UK): Sage.
Porcari J, Bryant C, Comana F. 2015. Exercise physiology. Philadelphia (PA): Davis Company.
Portaluppi F, Smolensky MH, Touitou Y. 2010. Ethics and methods for biological rhythm research on animals and human beings. Chronobiol Int. 27:1911–1929. doi:10.3109/07420528.2010.516381.
Punduk Z, Gur H, Ercan G. 2005. Sabahcil- aksamcil anketi Turkce uyarlamasinda guvenirlik calismasi. Turk Psikiyatri Derg. 16:40–45.
Rakicioglu N, Acar Tek N, Ayaz A, Pekcan G. 2012. Yemek ve besin fotograf katalogu: olcu ve miktarlar. Ankara (TR): Hatipoglu Yayinevi.
Ramanjaneya M, Chen J, Brown JE, Tripathi G, Hallschmid M, Patel S, Kern W, Hillhouse EW, Lehnert H, Tan BK, et al. 2010. Identification of nesfatin-1 in human and murine adipose tissue: a novel depot-specific adipokine with increased levels in obesity. Endocrinology. 151:3169–3180. doi:10.1210/en.2009-1358.
Romere C, Duerrschmid C, Bournat J, Constable P, Jain M, Xia F, Saha PK, Del Solar M, Zhu B, York B, et al. 2016. Asprosin, a fasting-induced glucogenic protein hormone. Cell. 165:566–579. doi:10.1016/j.cell.2016.02.063.
Romero-Corral A, Somers VK, Sierra-Johnson J, Thomas RJ, Bailey KR, Collazo-Clavell ML, Allison TG, Korinek J, Batsis JA, Lopez-Jimenez F. 2008. Accuracy of body mass index in diagnosing obesity in the adult general population. Int J Obes. 32:959–966. doi:10.1038/ijo.2008.11.
Rosen ED, Spiegelman BM. 2006. Adipocytes as regulators of energy balance and glucose homeostasis. Nature. 444:847–853. doi:10.1038/nature05483.
Saad A, Dalla Man C, Nandy DK, Levine JA, Bharucha AE, Rizza RA, Basu R, Carter RE, Cobelli C, Kudva YC, et al. 2012. Diurnal pattern to insulin secretion and insulin action in healthy individuals. Diabetes. 61:2691–2700. doi:10.2337/db11-1478.
Sandroff BM, Motl RW, Davis SL. 2016. Effects of vigorous walking exercise on core body temperature and inhibitory control in thermosensitive persons with multiple sclerosis. Neurodegener Dis Manag. 6:13–21. doi:10.2217/nmt.15.69.
Sato S, Basse AL, Schönke M, Chen S, Samad M, Altintas A, Laker RC, Dalbram E, Barrds R, Baldi P, et al. 2019. Time of exercise specifies the impact on muscle metabolic pathways and systemic energy homeostasis. Cell Metab. 30:92–110. Doi:10.1016/j.cmet.2019.03.013.
Saunders TJ, Palombella A, McGuire KA, Janiszewski PM, Despres JP, Ross R. 2012. Acute exercise increases adiponectin levels in abdominally obese men. J Nutr Metab. 2012:148729. doi:10.1155/2012/148729.
Savikj M, Gabriel BM, Alm PS, Smith J, Caidahl K, Björnholm M, Fritz T, Krook A, Zierath JR, Wallberg-Henriksson H. 2019. Afternoon exercise is more efficacious than morning exercise at improving blood glucose levels in individuals with type 2 diabetes: a randomised crossover trial. Diabetologia. 62:233–237. doi:10.1007/s00125-018-4767-z.
Scheja L, Heeren J. 2019. The endocrine function of adipose tissues in health and cardiometabolic disease. Nat Rev Endocrinol. 15:507–524. doi:10.1038/s41574-019-0230-6.
Schroeder AM, Truong D, Loh DH, Jordan MC, Roos KP, Colwell CS. 2012. Voluntary scheduled exercise alters diurnal rhythms of behaviour, physiology and gene expression in wild-type and vasoactive intestinal peptide-deficient mice. J Physiol. 590:6213–6226. doi:10.1113/jphysiol.2012.233676.
Schumann U, Qiu S, Enders K, Bosnyak E, Laszlo R, Machus K, Trajer E, Jaganathan S, Zugel M, Steinacker JM. 2017. Asprosin, a newly identified fasting-induced hormone is not elevated in obesity and is insensitive to acute exercise. Med Sci Sports Exerc. 49:1023. doi:10.1249/01.MSS.0000519807.24061.CA.
Serter R. 2003. Obezite atlasi. Ankara (TR): Karakter Color Basimevi.
Short KR, Pratt LV, Teague AM. 2018. A single exercise session increases insulin sensitivity in normal weight and overweight/obese adolescents. Pediatr Diabetes. 19:1050–1057. doi:10.1111/pedi.12684.
Stensel D. 2010. Exercise, appetite and appetite-regulating hormones: implications for food intake and weight control. Ann Nutr Metab. 57:36–42. doi:10.1159/000322702.
Suzuki K, Simpson KA, Minnion JS, Shillito JC, Bloom SR. 2010. The role of gut hormones and the hypothalamus in appetite regulation. Endocr J. 57:359–372. doi:10.1507/endocrj.k10e-077.
Thackray A, Deighton K, King J, Stensel D. 2016. Exercise, appetite and weight control: are there differences between men and women? Nutrients. 8:583. doi:10.3390/nu8090583.
Tucker R. 2008. Thermoregulation, fatigue and exercise modality. In: Marino FE, editor. Medicine and sport science. Basel (SW): Karger; p. 26–38.
Ugur K, Aydin S. 2019. Saliva and blood asprosin hormone concentration associated with obesity. Int J Endocrinol. 2019:2521096. doi:10.1155/2019/2521096.
Vitola BE, Deivanayagam S, Stein RI, Mohammed BS, Magkos F, Kirk EP, Klein S. 2009. Weight loss reduces liver fat and improves hepatic and skeletal muscle insulin sensitivity in obese adolescents. Obesity. 17:1744–1748. doi:10.1038/oby.2009.171.
Walewski JL, Ge F, Loddell H, Levin N, Schwartz GJ, Vasselli JR, Pomp A, Dakin G, Berk PD. 2014. Spexin is

a novel human peptide that reduces adipocyte uptake of long chain fatty acids and causes weight loss in rodents with diet-induced obesity. Obesity. 22:1643–1652. doi:10.1002/oby.20725.

Wang CY, Lin TA, Liu KH, Liao CH, Liu YY, Wu VC, Wen MS, Yeh TS. 2019. Serum asprosin levels and bariatric surgery outcomes in obese adults. Int J Obes. 43:1019–1025. doi:10.1038/s41366-018-0248-1.

Wang Y, Lam KS, Kraegen EW, Sweeney G, Zhang J, Tso AW, Chow WS, Wat NM, Xu JY, Hoo RL. 2007. Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance, and hyperglycemia in humans. Clin Chem. 53:34–41. doi:10.1373/clinchem.2006.075614.

[WHO] World Health Organization. 1997. Preventing and managing the global epidemic of obesity. Geneva (SUI): WHO Library Cataloguing-in-Publication Data. Report No: 894.

[WHO] World Health Organization. 2017. Prevalence of obesity among adults, ages 18+, 1975-2016 [blog]. Gamap Server. [accessed 2019 Feb 14]. gamapserver.who.int/gho/interactive_charts/ncd/risk_factors/obesity/atlas.html

[WHO] World Health Organization. 2018. Obesity and overweight [blog]. Who Int. [accessed 2019 Feb 11]. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

Wiecek M, Szymura J, Maciejczyk M, Kantorowicz M, Szygula Z. 2018. Acute anaerobic exercise affects the secretion of asprosin, irisin and other cytokines-a comparison between sexes. Front Physiol. 9:1782. doi:10.3389/fphys.2018.01782

Wilson LF, Baade PD, Green AC, Jordan SJ, Kendall BJ, Neale RE, Olsen CM, Youlden DR, Webb PM, Whiteman DC. 2018. The impact of changing the prevalence of overweight/obesity and physical inactivity in Australia: an estimate of the proportion of potentially avoidable cancers 2013–2037. Int J Cancer. 144:2088–2098. doi:10.1002/ijc.31943.

Yaribeygi H, Atkin SL, Simental-Mendia LE, Sahebkar A. 2019. Molecular mechanisms by which aerobic exercise induces insulin sensitivity. J Cell Physiol. 234:12385–12392. doi:10.1002/jcp.28066

Zaki ME, El-Bassyouni H, Youness E, Mohamed N. 2015. Lipocalin-2 is an inflammatory biomarker associated with metabolic abnormalities in Egyptian obese children. J Appl Pharm Sci. 5:07–12. doi:10.7324/JAPS.2015.50502.

Zhang J, Wu Y, Zhang Y, LeRoith D, Bernlohr DA, Chen X. 2008. The role of lipocalin-2 in the regulation of inflammation in adipocytes and macrophages. Mol Endocrinol. 22:1416–1426. doi:10.1210/me.2007-0420.

Zheng B, Li S, Liu Y, Li Y, Chen H, Tang H, Liu X, Lin H, Zhang Y, Cheng CHK. 2017. Spexin suppress food intake in zebrafish: evidence from gene knockout study. Sci Rep. 7:14643. doi:10.1038/s41598-017-15138-6.