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The Effect of Intermittent Fasting and Exercise on Body Composition, Antioxidant Capacity, and Blood Parameters in Obese Rats

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HIGHLIGHTS

- The combination of intermittent fasting and exercise provides weight loss, reduces fat cell size, and reduces oxidative stress.
- Combination of intermittent fasting and exercise; despite the increase in PON1 with exercise alone, it leads to a higher decrease than the decrease seen with intermittent fasting alone.

Abstract: This study aimed to examine the effects of exercise, intermittent fasting, and combining two methods on body weight, adipose tissue, lipids, and glucoregulator and antioxidant parameters. 90 day-old male rats were randomly divided into 5 groups of 6 rats each. The groups consist of rats with normal body weight-sedentary (C), obese-sedentary (O), obese-intermittent fasting only (IF), obese-exercise (swimming) only (E), and obese-intermittent fasting together with exercise (IFE). For 8 weeks (1. phase), 1 group was fed with standard pellets (2.7 cal/g). The other 4 groups were fed with a high-fat diet (4.496 cal/g) to develop obesity. Then for 8 weeks (2. phase), all rats feeding standard pellets were exercised and/or done intermittent fasting. There was no significant difference in body weight at the beginning of the study. The change in body weight was significantly higher in C than IFE, and E. Fasting blood glucose was higher in IF and IFE. Oxidative stress index (OSI) was lower in IF and IFE. Also, paraoxonase 1 (PON1) decreased in IFE. The fat cells' diameters of IF or E were lower than O but higher than C. The fat cell diameters of IFE were smaller than C. In conclusion, a combination of intermittent fasting and exercise in obese individuals caused reduced body weight, fat cell size, and oxidative stress. Besides, it caused increased fasting blood glucose, different effects on blood lipids, and decreased PON1. Thus intermittent fasting can be suggested by considering its positive and negative aspects and keeping it under follow-up.

Keywords: Intermittent fasting; Exercise; Obesity; Antioxidant capacity; PON1.

INTRODUCTION

Intermittent fasting (IF) is an approach that requires focusing only on fasting days to reduce energy expenditure and is potentially more straightforward to implement than calorie restriction [1]. It can be used as an alternative for obese individuals who have difficulty applying calorie-restricted diets for a long time [2]. Intermittent fasting can reduce body fat distribution and help improve biochemical parameters [3, 4]. Intermittent fasting shows tissue-specific redox changes that are improving in the heart, worsening in the brain, and ineffective in skeletal muscles [5]. However, the decreased body weight caused by intermittent fasting generally leads to decreased lipid peroxidation and oxidative stress [6]. However, long-term intermittent fasting has been found to increase oxidant release and oxidative protein modifications in skeletal muscle and adipose tissue [7]. One of the methods used in the treatment of obesity is exercise. Exercise induces browning of subcutaneous white adipocytes, improving energy balance and reducing visceral obesity-related disorders [8]. Similarly, it is thought that intermittent fasting activates white adipose tissues by turning them into brown adipose tissues [9]. Intermittent fasting shows a survival response to stress similar to exercise in cells, particularly in the mitochondria [10]. Combining both applications' stress may produce more robust and beneficial effects for metabolic health [11]. Paraoxonase 1 (PON1) and arylesterase (ARES) are among the parameters that show the oxidant status. The composition of the diet consumed plays a role in the regulation of PON1 [12]. Also, postprandial PON1 activity has been shown to increase after the Mediterranean diet [13].

This study aims to examine the effects of exercise and intermittent fasting on weight loss, adipose tissue, oxidative status, and blood parameters in obese rats.

MATERIAL AND METHODS

Ethical aspects

The study was approved by Burdur Mehmet Akif Ersoy University (MAKU) Animal Experiments Local Ethics Committee on 19.07.2018 as a research project with registration number 376, and performed in accordance with the Guide to the Care and Use of Laboratory Animals.

Animals

Sprague Dawley rats (a total of 30 adult male, 90 day-old) were kept under the inverted circadian cycle of 12 hours (lights on at 6 p.m. and lights off at 6 a.m.) with water ad libitum and assigned into 5 different groups. The rats were obtained from and cared for MAKU Experimental Animals Production and Experimental Research Center. All the animals in the groups were kept in the same cage. Rats were randomly selected from healthy offspring of mothers who mated and gave birth on the same day. Brief information on the groups is as follows: Control (C) (n = 6): Sedentary, normal weight and ad libitum. Obese (O) (n = 6): Sedentary, obese and ad libitum. Exercise (E) (n = 5): Obese, swimming, and ad libitum. Intermittent fasting (IF) (n = 6): Sedentary, obese and intermittent fasting. Intermittent fasting + exercise (IFE) (n = 6): Obese, swimming and intermittent fasting.

Cages are numbered, and a distinctive colored marker is used to identify each animal in each cage to avoid confusion. At the beginning of the study, group E consisted of 6 animals. However, at the 12th week of the study, one animal in group E died of unknown cause, so it was excluded from the study and the number of animals in the group decreased to 5. The experiment consists of 2 phases, which are described in detail below. The rats' daily feed and water consumption were measured and recorded during the study.

First phase: formation of diet-induced obesity

A 3-day adaptation period was applied at first. Then except for C, which was fed with standard pellets, the other groups were fed a high-fat diet (Fat: 45%, Protein: 19%, Carbohydrate: 36%; Altromin C 1090 - 45), for 8 weeks to form diet-induced obesity (4.496 cal/g). Obesity formation was determined by calculating the Lee index [14] and body mass index (BMI) [15].

Second phase: intermittent fasting and/or swimming

Intermittent fasting was applied for 18 hours in a 12-hour light and 6-hour dark phase for 8 weeks. Ad-libitum period was applied for 6 hours right after exercise in the dark phase until the light phase started. For 4 days, all rats were kept in shallow water at 31 ºC ± 2 to adapt to exercise and standardize water stress's

adverse effects. Then, swimming was applied 5 days a week, at a depth of 60 cm and for 40 minutes between 12 p.m. and 2 p.m. in the dark phase [9].

During the first and second phases, in which the animals were fed and exercised, the researcher who took care of the animals knew about the groups. After the experiment was terminated and tissue and blood samples were taken, all samples were sorted and numbered. The researchers who did the analysis do not have information about the study groups due to the serial numbering method. The flow chart of the study is shown in Figure 1.

Anthropometric measurements

Animals were weighed with precision scales sensitive to 0.1 grams. The distance between the nose and anus was measured and recorded [13].

Euthanasia - tissue and blood samples

Blood samples were taken from the rats' tails following an overnight (12h) fasting before anesthesia. Anesthesia was applied with intraperitoneal injection of pentobarbital sodium (50 mg / kg) and the toe pinching test showed that contractile reflexes disappeared. Blood was collected from the hearts of rats. Then they were euthanized by cervical dislocation. Fat tissues were taken from the epididymal and retroperitoneal regions. Tissue processing was performed. The blood samples were centrifuged at 3000 rpm for 10 minutes, and serums were stored at -20 °C until the analysis.

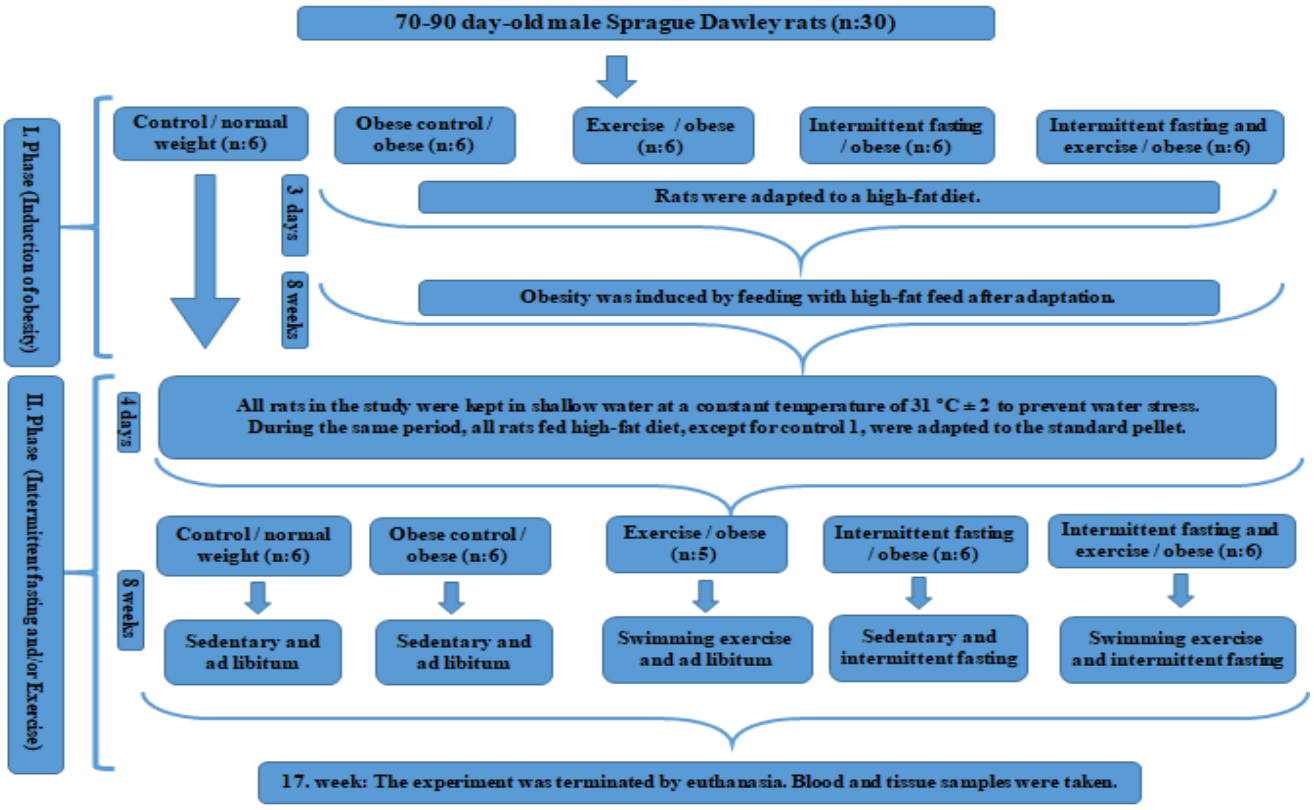

Figure 1. Flow chart of the study

Biochemical analysis

Fasting glucose was measured using a handheld glucometer (Accu-Check glucometer, Roche) [16]. Triglyceride, total cholesterol, HDL-C, and LDL-C analyses were performed in Beckman Coulter AU680 autoanalyzer using enzymatic colorimetric method. Insulin analysis was performed using a sandwich-ELISA kit. Analysis of total antioxidant status (TAS), total oxidant status (TOS), PON1, and ARES activities were performed in a Beckman Coulter AU680 autoanalyzer using commercially available kits developed by Erel (Relassay, Turkey). The oxidative stress index (OSI) is calculated as the ratio percentage of total peroxide to the total antioxidant potential. OSI (arbitrary unit) = TOS (μmol H2O2 equivalent/L) / TAS (μmol Trolox equivalent/L) [17].

Histological assessment of adipose tissue

Small pieces of adipose tissues were fixed with formalin (10% neutral-buffer formalin), and embedded in paraffin. Five-micrometer sections were cut and stained using hematoxylin and eosin (H&E). Digital images of the slides were captured with a camera (DP74 / Olympus) [18].

Assessment of cross-sectional adipocyte size and number of adipocytes

Cross-sectional adipocyte size and number of adipocytes were determined by using previously described method [19].

Statistical analysis

Statistical analyses were performed using SPSS package program. Normality was tested with the Kolmogorov-Smirnov test. The data were assessed for assumptions of normality, linearity, and homogeneity. The data was met the assumption of the statistical method. ANOVA followed by post-hoc Tukey's test were performed. Kruskal-Wallis H test followed by post-hoc Bonferroni correction test was also performed. Pearson correlation coefficient and Spearman correlation coefficient were used. Significance level was considered $p < 0.05$.

RESULTS

Effects of high-fat diet on body weight

The rats' body weights at the study's beginning and end are shown in Figure 2. There was no statistically significant difference in initial mean body weights in all groups. However, at the end of the first phase, a statistically significant difference was found in mean body weights ($F = 4.640$; $p < 0.01$). At the end of this phase, the body weights of C were statistically significantly lower than IFE and O.

Effects of intermittent fasting and/or swimming on body weight

A comparison of the body weight of rats at the end of the study is shown in Table 1. A statistically significant difference was found in body weight at the end of the second phase ($F = 2.950$; $p < 0.05$). The body weights of C were statistically significantly lower than O. No significant difference was found between E, IF, and IFE.

Table.1 Comparison of body weight (g) after the second phase

Groups	$\overline{X} \pm SS$	Median [Min-Max]	
Body weights (g) at the end of second phase (17. week)			
C	389.50±44.05[a]	397.5 [324.5-454.0]	
O	475.92±44.54[b]	465.8 [440.0-561.0]	**F=2.950**
E	436.58±41.85[ab]	446.3 [373.5-487.5]	
IF	431.25±55.76[ab]	449.0 [340.5-494.5]	**p=0.040***
IFE	452.08±38.30[ab]	447.5 [402.5-503.0]	

* $p<0.05$, ANOVA test, The difference between groups was evaluated using the Tukey-HSD Test, a,b,c For groups of different letters $p <0.05$, for groups belonging to the same letters $p> 0.05$, C: Control, O: Obese control, E: Exercise, IF: Intermittent fasting, IFE: Intermittent fasting + Exercise

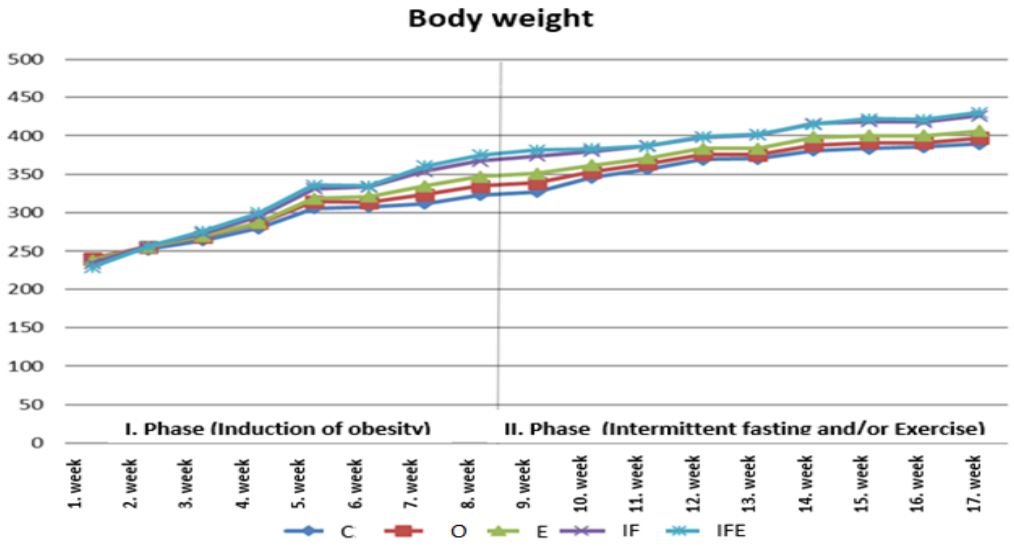

Figure 2. Body weights of rats

Evaluation of water and feed consumption in the second phase

A comparison of water and feed consumption between the groups is shown in Table 2. A statistically significant difference was found in water consumption ($\chi2=157,550$; $p<0.001$). Water consumption is significantly higher in C than in IFE, E, and IF; however, it is significantly lower than O. In addition, the water consumption of IF was significantly higher than IFE and E. Water consumption of O was significantly higher than IFE, E, and IF. A statistically significant difference was found in feed consumption ($\chi2=90,245$; $p<0.001$). The feed consumption of C was significantly higher than E and IF but lower than O. In addition, the feed consumption of IFE was significantly higher than E and IF but lower than O. The feed consumption of O was significantly higher than E and IF. The highest water consumption was observed in O, and the lowest was in E and IFE.

Table 2. Comparison of water (ml/day) and feed (g/day) consumption between the groups in the second phase

Groups	$\bar{X} \pm SS$	Median [Min-Max]	
Water (ml/day)			
C	268.09±24.24	265.0[a] [220.0-350.0]	
O	324.19±33.12	315.0[b] [240.0-450.0]	
E	219.64±42.50	220.0[c] [160.0-370.0]	**χ^2=157.550 p=0.000***
IF	251.00±30.56	250.0[d] [180.0-350.0]	
IFE	225.18±35.29	220.0[c] [160.0-340.0]	
Feed (g/day)			
C	163.78±17.08	160.5[a] [139.0-259.0]	
O	186.76±27.14	191.3[b] [113.0-230.0]	
E	145.85±21.85	148.3[c] [96.0-176.5]	**χ^2=90.245 p=0.000***
IF	145.95±24.48	152.8[c] [70.0-178.0]	
IFE	157.94±27.21	166.0[ad] [92.5-192.0]	

* p<0.05, Kruskal-Wallis Test, The difference between groups was evaluated using the Bonferroni Test, a,b,c For groups of different letters p <0.05, for groups belonging to the same letters p> 0.05, C: Control, O: Obese control, E: Exercise, IF: Intermittent fasting, IFE: Intermittent fasting + Exercise

Table 3. Comparison of biochemical parameters between groups

Biochemical parameters	Groups										
	C		O		E		IF		IFE		
	x̄±SS	Median [Min-Max]	x̄±SS	Median [Min-Max]	x̄±SS	Median [Min-Max]	x̄±SS	Median [Min-Max]	x̄±SS	Median [Min-Max]	
Fasting Blood Glucose (mg/dL)	69.50±11.10	69.0^{a} [55.0-84.0]	78.67±8.57	77.5^{ab} [67.0-92.0]	84.20±12.70	83.0^{abc} [73.0-105.0]	97.00±8.10	94.5^{bc} [90.0-110.0]	114.00±23.51	105.5^{c} [97.0-159.0]	**χ2=19.149 p=0.001**
Plasma Insulin (mg/dL)	0.22±0.03	0.2 [0.2-0.3]	0.21±0.02	0.2 [0.2-0.3]	0.20±0.01	0.2 [0.2-0.2]	0.22±0.03	0.2 [0.2-0.3]	0.20±0.01	0.2 [0.2-0.2]	**χ2=4.198 p=0.380**
HDL-C (mg/dL)	34.50±8.94	35. 0^{a} [24.0-45.0]	55.67±16.02	50.0^{ab} [38.0-75.0]	56.60±17.17	53.0^{b} [42.0-86.0]	44.17±9.99	41.5^{ab} [35.0-63.0]	36.83±8.99	38.0^{a} [26.0-49.0]	**χ2=11.309 p=0.023**
LDL-C (mg/dL)	7.83±2.14	8.0 [5.0-11.0]	10.17±3.13	9.0 [8.0-16.0]	10.00±0.71	10.0 [9.0-11.0]	11.17±4.58	9.5 [8.0-20.0]	7.67±2.07	8.0 [4.0-10.0]	**χ2=7.312 p=0.120**
Triglyceride (mg/dL)	23.17±3.25	23.0^{a} [18.0-27.0]	29.17±5.34	28.5^{ab} [24.0-39.0]	24.40±3.13	25.0^{ab} [21.0-29.0]	31.67±5.43	32.5^{b} [25.0-39.0]	30.50±7.18	32.0^{ab} [17.0-37.0]	**χ2=10.846 p=0.028**
Total Cholesterol (mg/dL)	26.50±6.28	26.0^{ac} [20.0-37.0]	35.00±6.10	33.0^{a} [30.0-47.0]	36.60±2.41	38.0^{b} [34.0-39.0]	39.33±14.56	34.0^{a} [27.0-68.0]	25.50±5.09	24.0^{c} [18.0-32.0]	**χ2=15.253 p=0.004**
TAS (mmol/L)	1.16±0.21	1.1 [0.9-1.5]	1.05±0.13	1.0 [0.9-1.2]	1.05±0.07	1.0 [1.0-1.2]	1.56±0.95	1.1 [1.0-3.5]	1.27±0.18	1.3 [1.1-1.5]	**χ2=8.590 p=0.072**
TOS (μmol/L)	4.10±1.21	3.8 [3.1-6.4]	5.33±0.47	5.5 [4.5-5.7]	4.34±0.75	4.2 [3.5-5.3]	3.82±0.69	3.8 [3.0-4.7]	4.15±1.10	3.8 [3.0-6.1]	**χ2=8.383 p=0.078**
OSI	0.35±0.06	0.3^{a} [0.3-0.4]	0.52±0.09	0.5^{b} [0.4-0.6]	0.41±0.05	0.4^{ab} [0.4-0.5]	0.30±0.12	0.3^{a} [0.1-0.4]	0.33±0.09	0.3^{a} [0.3-0.5]	**χ2=13.632 p=0.009**

*p<0.05, Kruskal-Wallis Test, The difference between groups was evaluated with Bonferroni Test, a,b,c p<0.05 for groups belonging to different letters, p>0.05 for groups belonging to the same letters, HDL-C: High Density Lipoprotein Cholesterol, LDL-C: Low Density Lipoprotein Cholesterol, TAS: Total Antioxidant Status, TOS: Total Oxidant Status, OSI: Oxidative Stress Index, C: Control, O: Obese control, E: Exercise, IF: Intermittent fasting, IFE: Intermittent fasting + Exercise

Evaluation of the relationship between body weight and feed consumption in the second phase

No significant correlation was found between body weight and feed consumption (p>0,05).

Evaluation of biochemical parameters

A comparison of biochemical parameters between groups is shown in Table 3. A statistically significant difference was found in fasting blood glucose ($\chi2=19.149$; $p<0.01$). Fasting blood glucose of C was statistically significantly lower than IFE and IF ($p<0.05$). Fasting blood glucose of O was significantly lower than IFE ($p<0.05$). A statistically significant difference was found in HDL-C ($\chi2=11.309$; $p<0.05$). HDL-C of E were statistically significantly higher than C and IFE ($p<0.05$). A statistically significant difference was found in triglycerides ($\chi2=10.846$; $p<0.05$). The triglycerides of IF were statistically significantly higher than C ($p<0.05$). A statistically significant difference was found in total cholesterol ($\chi2=15.253$; $p<0.005$). Total cholesterol of E was statistically significantly higher than C ($p<0.05$). Total cholesterol of IFE was statistically significantly lower than E, IF, and O ($p<0.05$). A statistically significant difference was found in OSI ($\chi2=13.632$; $p<0.005$). OSI in O was statistically significantly higher than C, IFE, and IF ($p<0.05$). There was no statistically significant difference in plasma insulin, LDL-C, TAS, and TOS ($p>0.05$).

Histometric examination of adipose tissue

A comparison of the fat cell diameter and the number of fat cells between groups is shown in Table 4. A statistically significant difference was found in the fat cell diameter ($\chi2=10.627$; $p<0.01$). The fat cell diameters of C were statistically significantly higher than IFE, but E was statistically significantly lower than IF and O ($p<0.05$). Similarly, the fat cell diameters of IFE were statistically significantly lower than E, IF, and O ($p<0.05$). In addition, the fat cell diameters of O were statistically significantly higher than E and IF ($p<0.05$). The highest fat cell diameter was in O, and the lowest fat cell diameter was in IFE. Lower fat cell diameters were measured in IFE compared to C. The fat cell diameters of IF and E were similar.
A statistically significant difference was in the fat cell counts ($F=6.196$; $p<0.01$). The fat cell counts of IFE and IF were statistically significantly higher than O ($p<0.05$). The lowest number of fat cells was observed in O, and the highest number of fat cells was observed in IF. The number of fat cells was similar in C and E.

Table.4 Comparison of the fat cell diameter (μm) and the number of fat cells between groups

	Value		
Groups	$\bar{X} \pm SS$	**Median [Min-Max]**	
The Fat Cell Diameter (μm)			
C	52.37±12.67	51.2[a] [29.2-98.4]	
O	71.74±10.56	71.4[b] [43.8-110.0]	
E	58.63±14.09	56.0[c] [28.7-97.4]	**χ^2=10.627 p=0.001***
IF	57.30±12.31	58.0[c] [30.7-87.5]	
IFE	47.47±11.87	46.5[d] [26.2-84.2]	
The Fat Cell Count			
C	24.83±4.26[ab]	24.5 [20.0-31.0]	
O	17.50±1.64[a]	17.0 [16.0-20.0]	
E	24.67±3.93[ab]	24.0 [20.0-31.0]	**F=6.196 p=0.001****
IF	32.80±9.26[b]	34.0 [22.0-44.0]	
IFE	30.83±7.39[b]	33.5 [20.0-40.0]	

*p<0.05, Kruskal-Wallis Test, The difference between groups was evaluated with Bonferroni Test, **p<0.05, ANOVA test, Difference between groups was evaluated with Tukey-HSD Test, a,b,c p<0.05 for groups belonging to different letters, p>0.05 for groups belonging to the same letters, C: Control, O: Obese control, E: Exercise, IF: Intermittent fasting, IFE: Intermittent fasting + Exercise.

Compared to C, the rate of change of the fat cell diameters showed a 9.36% reduction in the fat cell diameters of IFE. O showed 36.99% growth in the fat cell diameters compared to C. Compared to O, IFE decreased by 33.83%.

Sample pictures showing the fat cell diameter and fat cell number of the groups are shown in Figure 3. When the microscopic images of the tissues are examined, the increase in the diameters of the fat cells in O can be clearly seen. Compared to O, the cells of C are much more numerous and congested in the measurement area. The fat cells were stuck and shrunk the most in IFE.

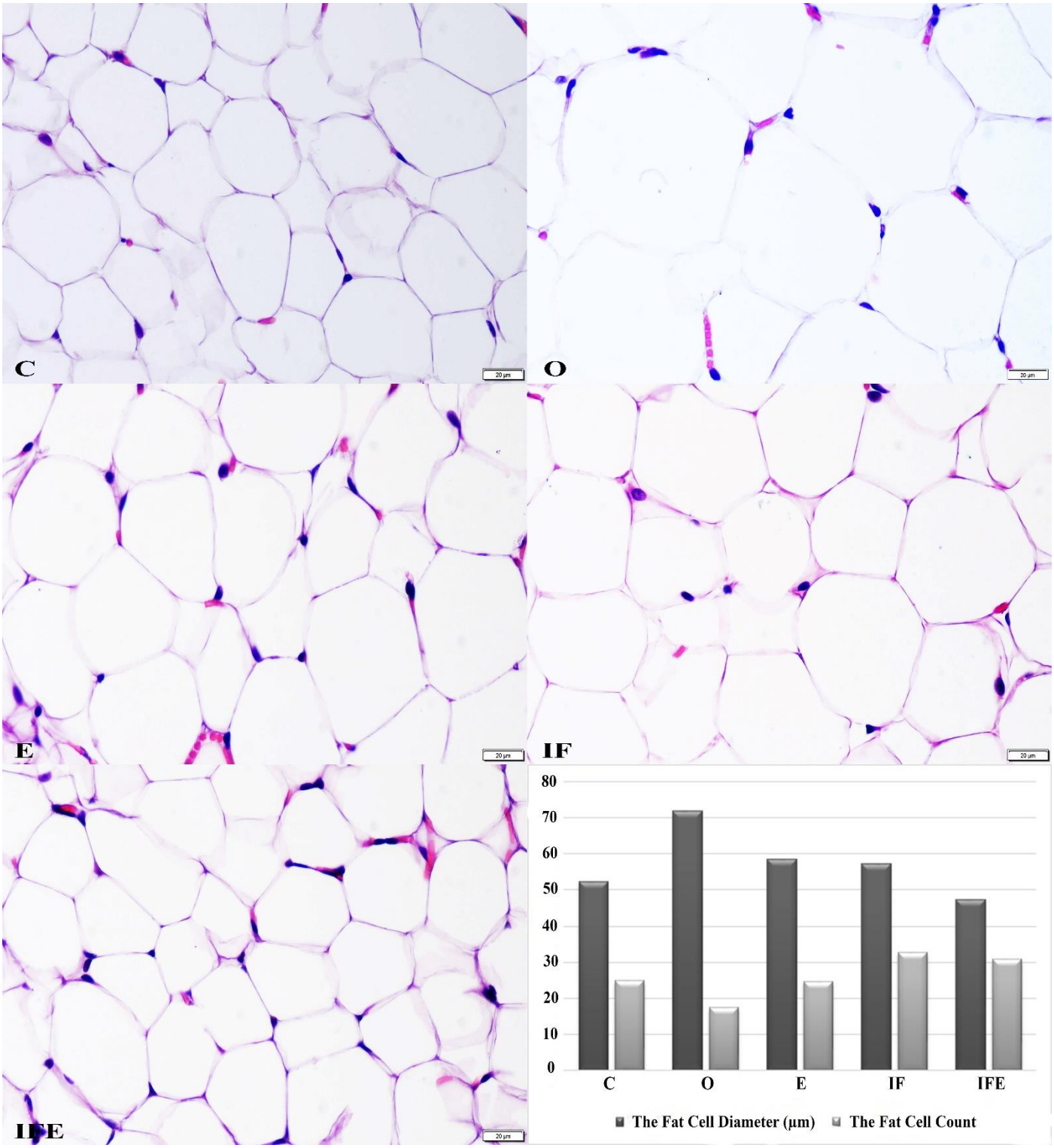

Figure 3. Sample pictures showing the fat cell diameter and fat cell number of the groups

Evaluation of the relationship between paraoxonase and arylesterase enzymes and some other parameters

A comparison of PON1 and ARES between groups is shown in Table 5. A statistically significant difference was found in PON1 ($F=4.201$; $p<0.05$). PON1 of E was statistically significantly higher than C and IFE ($p<0.05$). The highest PON1 was in E. IFE was at a similar level to C, and the lowest PON1 level. When C and O were compared, O was higher than C, although not statistically significant ($p>0.05$). There was no statistically significant difference in ARES ($p>0.05$).

Table 5. Comparison of PON1 (U/L) and ARES (kU/L) between groups

Groups	Values		
	$\overline{X} \pm SS$	Median [Min-Max]	
PON1 (U/L)			
C	532.33±117.37[a]	505.0 [422.0-742.0]	**F=4.201 p=0.010***
O	676.50±125.51[ab]	631.5 [545.0-847.0]	
E	816.80±160.18[b]	807.0 [608.0-987.0]	
IF	658.33±165.76[ab]	638.0 [470.0-887.0]	
IFE	520.50±118.70[a]	501.0 [375.0-707.0]	
ARES (kU/L)			
C	406.50±61.13	410.5 [327.0-476.0]	**F=0.585 p=0.676**
O	379.00±73.86	362.0 [299.0-506.0]	
E	418.40±75.66	406.0 [305.0-491.0]	
IF	371.50±86.95	351.5 [279.0-537.0]	
IFE	361.50±71.90	349.0 [274.0-484.0]	

* p<0.05, ANOVA test, Difference between groups was evaluated with Tukey-HSD Test, a,b,c p<0.05 for groups belonging to different letters, p>0.05 for groups belonging to the same letters, C: Control, O: Obese control, E: Exercise, IF: Intermittent fasting, IFE: Intermittent fasting + Exercise

When examined separately, there was no statistically significant correlation between PON1 and body weight, feed consumption, BMI, Lee index, ARES, and TAS (p>0.05).

In IF, a negative, very high, and statistically significant correlation was found between PON1 and TOS (r=-0.943; p<0.01). Also, a negative, very high, and statistically significant correlation was found between PON1 and OSI (r=-0.943; p<0.01). Accordingly, as OSI increases, PON1 decreases. Also, as TOS increases, PON1 decreases or vice versa. In addition, a positive, very high, and statistically significant correlation was found between PON1 and the number of fat cells in O (r=0.858; p<0.05). As the number of fat cells in O increases, PON1 increases or vice versa.

DISCUSSION

Altering the energy balance through exercise and diet is an effective strategy for improving body composition and managing lifestyle-related metabolic diseases. However, practices involving high energy expenditure, such as intermittent fasting and high-intensity exercise, can cause more significant physiological effects independent of changes in energy balance and positively increase the adaptation of metabolism to the current situation. Interventions combined with exercise and intermittent fasting are thought to further prevent body weight gain by storing fat mass. It is also hypothesized that it causes a decrease in fasting blood glucose, insulin sensitivity, and glucose tolerance and provides a more significant improvement in lipid profile compared to intermittent fasting and exercise intervention alone [20].

This study evaluated the effects of intermittent fasting or exercise alone and whether it would create a synergistic effect when applied together. In addition, the effect of intermittent fasting alone and the combination of intermittent fasting and exercise via PON1 and ARES was investigated for the first time in this study.

Evaluation of body weight, BMI, and lee index in the second phase

In a study, an overall trend of decreased body weight was observed in rats undergoing intermittent fasting. In contrast, a significant decrease in body weight was observed in rats undergoing intermittent fasting and exercising in the third and sixth weeks compared to the control and exercise groups [9]. In another study, ten weeks after application, male rats showed significant reductions in body weight and fat mass in intermittent fasting alone and the combination of intermittent fasting and exercise groups compared to the control and exercise groups. In females, at the end of the 10th week, there was a significant decrease in body weight and body fat mass in the group undergoing intermittent fasting and exercise combination, in the group intermittent fasting alone, and in the group exercising alone, compared to the control groups [20]. In this study, when the groups were compared at the end of the second phase, no statistically significant difference was observed in body weights in any experimental groups (IF, E, IFE) compared to the control groups. During

the first phase, rats in the experimental group who gained weight similarly to O gained less weight due to intermittent fasting and/or exercise than O but could not reach a body weight in C. Since the rats generally continued to gain body weight throughout the study, it is important to gain less body weight than body weight loss during this period. However, when the body weight of the rats was examined individually, some rats lost weight. When the second phase was examined, it was observed that there was a sharp decrease in body weight after the 13th and 16th weeks. Compared to previous studies, the significant decreases in body weight losses in different weeks may have varied depending on the composition of the feed the rats were fed, and the duration and intensity of the exercise applied.

Evaluation of water and feed consumption

The highest water consumption is in O , and the lowest in E. Rats may have consumed less water during swimming since they met the body's water need through the skin, albeit limited. Different results can be obtained when an exercise that causes sweating in the body is applied. The highest water consumption was observed in O , consisting of obese rats. IF group consumed higher water compared to the exercise groups (E and IFE) and lower water compared to both control groups. In this respect, it can be said that intermittent fasting practice may cause low water consumption.

After starting standard pellet feeding from the ninth week, O consisting of obese rats consumed a higher level of feed than C consisting of normal-weight rats. Although obese rats consumed more, their body weight gains were lower. Basal metabolic rate is high in obese people [21]. Therefore, the increase in feed consumption seen in O may be due to the increase in basal metabolic rate as body weight increases. For this reason, the relationship between feed consumption during the second phase and body weight was investigated. However, no statistically significant correlation was found ($p>0.05$).

Evaluation of biochemical parameters

When the fasting blood glucose was examined, it was observed that it increased significantly in the intermittent fasting groups (IF and IFE) compared to C. While doing exercise alone did not cause a significant change in fasting blood glucose, the combination of intermittent fasting and exercise increased the negative effect of intermittent fasting, increasing the fasting blood glucose in comparison to obese controls as well as controls with normal body weight ($p<0.05$). There was no statistically significant difference between the groups regarding plasma insulin ($p>0.05$). In a study, fasting blood glucose was found to be significantly higher in the intermittent fasting group compared to the other three groups after 10 weeks of intermittent fasting and/or exercise in obese rats. This study differs from ours in that the intermittent fasting group showed a higher fasting blood glucose than the combination of intermittent fasting and exercise group. In addition, in this study, similar to our study, no significant difference was observed between the groups in terms of plasma insulin [20]. However, contrary to our results, fasting blood glucose decreased in obese rats who performed alternate-day fasting on 3 non-consecutive days of the week for 8 weeks. In addition, plasma insulin also decreased in this study [22]. In another study, fasting blood glucose was similar in all groups of rats that were applied intermittent fasting on alternate days of fasting for 2 days a week along with swimming exercise for 8 weeks. In addition, plasma insulin decreased in the combination of intermittent fasting and exercise group compared to the control and exercise groups [16]. Different results were obtained in terms of fasting blood glucose and insulin. Different types of intermittent fasting may play a role in the different results obtained. Therefore, it is important to determine whether intermittent fasting will be applied or which type of intermittent fasting will be applied if glucoregulatory factors are not controlled in individuals who are considered to be on an intermittent fasting diet. Changes observed in individuals should be closely monitored. This study observed the highest HDL-C in the exercise group, as expected. However, the combination of intermittent fasting and exercise had a negative effect, causing a significant decrease in HDL-C ($p<0.05$). Intermittent fasting significantly increased triglyceride levels ($p<0.05$). The combination of intermittent fasting and exercise decreased the total cholesterol level, while exercise increased it ($p<0.05$). Results for blood lipids in studies are various and contradictory [20, 23-26]. The fact that the results of blood lipid parameters are quite diverse and inconsistent with each other prevents reaching a definitive decision. Especially in human studies, the fact that the composition of the diet consumed by individuals is not examined in detail, the types of intermittent fasting applied, and the different intervention times lead to different results. In addition, gender, physical activity status, and weight loss also affect lipid parameters. Weight loss reduces LDL-C and triglycerides in humans and animals [27]. Exercise increases HDL-C [28]. By eliminating such factors that affect blood lipid parameters, more consistent results will be obtained on the effect of intermittent fasting on blood lipids with further studies.

This study investigated TOS, TAS, and OSI as indicators of oxidative stress status. There was no statistically significant difference in TAS and TOS ($p>0.05$). It was observed that obesity increased OSI, and OSI decreased significantly in the intermittent fasting groups ($p<0.05$). In the literature review, only 2 studies were found to examine TAS, TOS, and OSI. Both studies were about Ramadan fasting. In the first study, TAS of people with Ramadan fasting was found to be significantly higher. In contrast, TOS and OSI were found to be significantly lower compared to non-fasting people [29]. Conversely, a significant decrease was observed among TOS measured at the beginning of Ramadan and TOS measured at the end of Ramadan, while no change was observed in TAS values [30]. Some studies analyzed different oxidative stress parameters with intermittent fasting. In a 16-week study, IL-6, TNF-α, and CRP levels were decreased in mice undergoing intermittent fasting with 3 hours of ad libitum feeding compared to the control group [31]. In another study, intermittent fasting based on a high-fat diet or a low-fat diet for 4 hours ad libitum was applied. After 18 weeks, IL-6 level remained unchanged, but TNF-α level decreased in both groups compared to controls [23]. In another study, IL-6 and TNF-α plasma levels were decreased in both intermittent fasting groups fed 8 hours ad libitum with high-fat or standard pellet compared to the control groups [32]. TAS, TOS, and OSI analysis were not found in studies where intermittent fasting and exercise were applied together. However, some studies analyzed different oxidative stress parameters. A study observed a significant decrease in malondialdehyde levels in the intermittent fasting and exercise combination group. Statistical analyses show a strong effect of exercise with a small additive effect of intermittent fasting [16]. Contrary to these findings, in a study, liver concentrations of thiobarbituric acid reactive substances (TBARS) and carbonylated proteins, indicators of lipid peroxidation, were found to be higher in animals treated with intermittent fasting after 6 weeks of intermittent fasting and/or exercise. However, there was a decrease in TBARS and carbonyl levels in the intermittent fasting and exercise combination group compared to intermittent fasting group. In addition, the carbonyl content in the liver tissue of exercise group and intermittent fasting and exercise combination group were lower than the control group. When TBARS, carbonyl, and glutathione levels in muscle tissue were examined, similar results were obtained in liver tissue [9]. Apart from one study, it is predicted that intermittent fasting may affect the parameters related to oxidative stress positively in other studies. In two studies examining the combination of intermittent fasting and exercise, it was stated that this combination had a more positive effect compared to intermittent fasting alone. However, in our study, intermittent fasting alone was found to be more effective, although it was not statistically significant. The number of studies on this subject is limited, and further studies are needed.

Histometric evaluation of adipose tissue

In a study examining fat cell size, rats were subjected to modified intermittent fasting for 4 weeks. In the study, 4 groups were formed. The control group was fed ad libitum. In the other 3 groups, 3 different types of intermittent fasting were applied: energy needs were reduced by 25%, 50% on the day of fasting, and fasting throughout the day. At the end of the four-week intervention, inguinal fat cell sizes were found to be 47% smaller in the 50% reduced group compared to the control group and 56% smaller in the all-day fasted group. Epididymal fat cell size was also found to be 34% smaller in the 50% reduced group than in the controls and 36% smaller in the day-long fasted group. No change was observed in the 25% reduced group [33]. Our study observed the most significant retroperitoneal fat cell size reduction in the IFE group, with approximately 34%. IF group showed a decrease of approximately 20%. However, in a study conducted in hypercholesterolemic mice, after 3 months of intermittent fasting, epididymal adipose tissue, carcass adipose tissue, and subcutaneous adipose tissue size increased by 15%, 72%, and 68%, respectively [34]. In another study, an increase in inguinal and epididymal adipocyte sizes was observed in rats who applied time-restricted intermittent fasting for 4 weeks with a western-style diet compared to the control group fed a western-style and ad libitum diet. However, this difference was not reflected in epididymal and retroperitoneal adipose tissue mass or adipocyte size distribution [35]. When these results were examined, the administration of intermittent fasting, including normal fat, resulted in reductions in adipocyte size, and administration of intermittent fasting, including western-style diet/high fat, may cause an increase in adipocyte size. In our study, fat cell size decreased significantly at the end of the second phase in all experimental groups (E, IF, and IFE) rats. The reason for this may be intermittent fasting applied by consuming standard pellets and exercise. In our study, fat cell size decreased significantly at the end of the second phase in all experimental groups (E, IF, and IFE) rats. The reason for this may be intermittent fasting applied by consuming standard pellets and exercise. If the high-fat feed consumed by the rats in the first phase, when they were made obese, is consumed in the second phase, how the fat cell sizes will be affected in the intermittent fasting groups can be evaluated in future studies. In addition, it is seen that many factors, such as the composition of the diet, the type of intermittent fasting, the duration of the intervention, the health status of the animals, and the

application of exercise, can cause a change in the fat size. Studies with more significant reductions in adipocyte size also showed higher body weight losses. However, the decrease in fat cell size and the changes in the number of fat cells were not generally consistent [36-38]. Large and lipid-laden adipocytes are associated with many diseases, such as type 2 diabetes, insulin resistance, metabolic syndrome, and cancer. In the mechanism explaining the relationship between fat cell size and metabolic syndrome, it is stated that ectopic lipidosis increases because the cells that are too enlarged cannot store excess fat. Thus, the accumulation of triglycerides in the liver, skeletal muscle, and pancreas may contribute to the development of insulin resistance and diabetes [39]. In addition, enlarged fat cells can increase carcinogenesis with increased DNA damage by stimulating chronic inflammation [40]. Therefore, reduction in adipocyte size achieved by intermittent fasting and/or exercise may be important in preventing and treating these diseases. In this study, the fat cells were statistically significantly higher in IFE, both in number and smaller in diameter, indicating more fat loss in this group compared to the other groups. However, this is not reflected in body weight loss. At the end of the second phase, no significant difference was found between the experimental groups. An increase in lean body mass may offset the observed loss of fat cells.

Evaluation of PON1 and some other parameters

It has been found that energy restriction decreases PON1 [41], while short-term fasting increases PON1 [42]. However, no study has been conducted on the effect of intermittent fasting applied for a certain period and the combination of intermittent fasting and exercise on PON1. Our study is the first in this regard. In this study, it was determined that PON1 of obese rats undergoing the combination of intermittent fasting and exercise decreased below PON1 of normal body weight and sedentary rats. Exercise provided a significant increase in PON1. Therefore, according to the results of this study, while exercise provides an increase in PON1, intermittent fasting causes a decrease in PON1. On the other hand, the combination of intermittent fasting and exercise caused a higher decrease in PON1 compared to intermittent fasting, despite the positive effect of exercise. Studies have shown that exercise has various effects on PON1. A study observed that PON1 of obese women who exercised for 12 weeks decreased [43]. However, in other studies, it has been shown that regular low/medium intensity aerobic exercise can resist oxidative stress, heavy exercise increases PON1 in both physically active and sedentary people, and exercise done at a level that does not strain health increases PON1 only in physically active people [44-46]. Similar to these studies, the result we obtained in our study showed that PON1 increased. A study examining PON1, during and after exercise showed that PON1, which was at a normal level before exercise, increased during exercise. It was observed that after the exercise, it returned to its pre-exercise level approximately [47]. It is thought that the mechanism responsible for the increase in PON1 due to regular exercise is the adaptation provided by regular exercise to the increase in PON1 that occurs during exercise. When ARES was examined, no statistically significant difference was observed ($p>0.05$). Consistent with the results obtained in this study, no significant difference was observed in ARES in a study of obese children who attended a camp where various exercises and an energy-restricted diet were applied for 2 weeks [44]. In this study, PON1 was observed to be lower in IF than O, although it was not statistically significant. The reason for this situation can be shown as the loss of body weight resulting from intermittent fasting. It is known that body weight loss reduces PON1 [41]. However, it was determined that PON1 increased in IF compared to C, although it was not statistically significant. This result may be due to the difference in body weight between the two groups. This result is supported by the fact that PON1 of O consisting of obese people is higher than C with normal body weight. There are studies stating that PON1 increases [48], decreases [49], or does not change [50] in obese patients. An opinion is that intermittent fasting can increase PON1, and the underlying mechanism can be explained as follows. In fasted rats, energy deprivation in the first hours of fasting increases PON1, with the highest level occurring at the 12th hour. Also, 24 hours later, PON1 drops significantly, reaching levels of non-fasted rats, and stabilizes after 24 hours [42]. Therefore, it can be thought that the underlying mechanism is an adaptation to the increase in PON1 seen with regular short-term fasting in intermittent fasting, similar to the adaptation seen in regular exercise. However, contrary to the situation stated in this mechanism, in our study, it was observed that PON1 decreased due to body weight loss in obese subjects who performed intermittent fasting compared to obese controls, although it was not statistically significant. This mechanism may be activated due to intermittent fasting in studies without body weight loss or in individuals with normal body weight. Further studies are needed to explain this situation. While it was determined in our study that exercise increased PON1, it was observed that the combination of intermittent fasting and exercise decreased PON1 to a lower value than C, contrary to expectations. In a study, a group of overweight and obese people followed an energy-restricted diet and exercised for 4-7 months. At the end of the study, a significant decrease in PON1 was observed, similar to our study [51]. However, an increase in PON1 was observed in a study

conducted on obese children who performed various exercises and followed an energy-restricted diet for 2 weeks [44]. Also, in a study in which diet and exercise were applied for 6 months in obese children, no significant change was observed in PON1 [52]. The differences in the studies' intervention times, diets, and exercises may have led to different results in PON1. There was no correlation between PON1 and body weight, feed consumption, BMI and Lee index, ARES, and TAS in rats ($p>0.05$). A very high correlation was found between PON1 and TOS and also OSI in IF. PON1 expression is downregulated by oxidative stress. Therefore, PON1 decreases under oxidative conditions. Low PON1 is thought to be associated with atherosclerosis. Individuals are prone to the development of atherosclerosis through increased susceptibility to lipid peroxidation. Since the oxidation of LDL-C in arterial walls plays an important role in atherogenesis, the decrease in PON1, one of the mechanisms preventing LDL-C oxidation, increases this tendency. It has been observed that PON1 is decreased in people prone to the development of atherosclerosis, such as diabetes mellitus, hypercholesterolemia, and chronic renal failure [53]. Therefore, when these results are examined, it can be thought that if there is a decrease in PON1 with intermittent fasting in obese individuals, they may become more susceptible to the development of atherosclerosis. In addition, this group's increase in triglyceride and total cholesterol compared to the normal weight and obese control groups supports this idea. There was no correlation between PON1 and body weight, feed consumption, BMI and Lee index, ARES, and TAS in rats ($p>0.05$). A very high correlation was found between PON1 and TOS and also OSI in IF. There was a negative correlation between OSI and PON1 in IF. In addition, a statistically insignificant decrease was observed in IF for PON1, while a statistically significant decrease was observed in IFE. In this case, it is expected that OSI will increase due to the negative correlation between them. However, interestingly, it was observed that OSI decreased in the intermittent fasting groups (IF and IFE). This suggests the existence of another factor that reduces oxidative stress. Further studies are needed to explain this situation.

There was a very high correlation between the fat cell counts and PON1 in O. This result shows that PON1 will decrease as fat size increases, as fat size will increase with the decrease in fat cell count. Increasing the size of the fat cells increases oxidative stress by causing increased free fatty acid release, which can stimulate the release of some proinflammatory peptides such as TNF-α and IL-6 [54]. This mechanism may cause a decrease in PON1. A decrease in PON1 is associated with many diseases [53].

CONCLUSION

In this study, fasting blood glucose increased in those who practiced intermittent fasting alone or combined intermittent fasting and exercise. Therefore, according to the results of this study, the administration of intermittent fasting should not be recommended in diabetics or individuals with impaired glucose tolerance. However, further studies are needed due to the availability of studies showing improvements in glucose tolerance.

The effect of intermittent fasting on blood lipids is complex and challenging to make a firm judgment. Therefore, this should be considered before recommending it to individuals with cardiovascular diseases. Intermittent fasting has been shown to cause a decrease in PON1, which is considered one of the antioxidant defense systems. However, the combination of intermittent fasting and exercise leads to a higher decrease than the decrease induced by intermittent fasting alone, despite the increase in PON1 induced by exercise alone. Further studies are needed to investigate the reasons for this antagonist effect of intermittent fasting and exercise on PON1. In addition, the combination of intermittent fasting and exercise increased the count and size of fat cells compared to intermittent fasting alone or exercise alone. However, no significant reduction in body weight loss was found. The reason for this is thought to be an increase in lean body mass. In light of these data, although the combination of intermittent fasting and exercise in obese individuals causes loss in body weight, reduces fat cell size, and reduces oxidative stress, it raises fasting blood sugar, has mixed effects on blood lipids, and decreases PON1. Considering the positive and negative aspects of intermittent fasting and exercise, it can be recommended to be applied and controlled under strict monitoring. The limitations of the study are as follows. In this study process, one rat in E died in the 3rd week of the second phase due to an unknown reason. Therefore, while there were 6 rats in each of the 5 groups at the beginning of the study, 5 rats remained in E after the 3rd week of the second phase. At the beginning of the second phase, changes in group members could not be evaluated because serum could not be examined due to the risk of animal loss.

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