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Exercise Stimulates *PINK-1*, *PARKIN*, *MFN-1*, and *ATG-3* Genes Expression Despite High-fat Diet: Tissue-specific Responses

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Abstract

Background/Aim: Adipose tissue is pivotal in regulating metabolism, functioning not only as an energy store but also as an endocrine organe. Our investigation focused on the gene expression levels of *PARKIN*, *ATG-3*, *PINK-1* and *MFN-1* in C57BL/6J mice subjected to a high-fat diet and exercise regimen. The C57BL/6J mouse model was chosen due to its well-documented genetic background to diet-induced obesity for studying obesity.

Materials and Methods: The study consisted of three groups: control (C), high-fat diet (HFD), and exercise-high fat diet (E-HFD) groups. While HFD and E-HFD mice received a 60% fat diet, controls received a 10% fat diet. Real-time polymerase chain reaction system was applied to evaluate gene expression levels in hepatic, adipose, and heart tissues. Pro-inflammatory cytokine levels in the serum were quantified using ELISA kits according to the protocols.

Results: In contrast to the control and HFD groups, the E-HFD group exhibited notably elevated gene expressions of *ATG-3* and *PINK-1* ($p<0.05$). In the hepatic tissue of E-HFD group, gene expression of *MFN-1* and *PARKIN* were significantly upregulated ($p<0.05$). However, there was a significant reduction in *PARKIN* and *MFN-1* gene expression in the hepatic tissues of the HFD group ($p<0.05$).

Conclusion: Exercise and high-fat diet intervention significantly alter expression levels of mitophagy-related genes *PINK-1*, *PARKIN*, *MFN-1*, and *ATG3* and suggest that the examined genes may serve as potential molecular candidates for further research on obesity-related mitochondrial dysfunction and exercise-mediated metabolic improvement.

Keywords: Obesity, *ATG-3*, *PINK-1*, *PARKIN*, *MFN-1.*

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Introduction

Obesity is defined as an abnormal accumulation of fat that disrupts the balance of metabolism and energy homeostasis. It is a chronic disease of multifactorial origin, developing as a result of the interaction of environmental, metabolic, and molecular factors (1, 2). Adipose tissue is composed of adipocytes, a matrix containing collagen, blood and lymphatic vessels, endothelial cells, epithelial cells, muscle cells, fibroblasts, immune system cells, preadipocytes, and mesenchymal stem cells (3). This tissue is highly plastic and primarily functions in energy homeostasis by storing and releasing free fatty acids (FFAs). However, recent studies have demonstrated that functions as a very dynamic endocrine organ, influencing the functions of various organs, including skeletal and cardiac muscles (4, 5).

The mitochondrion plays a crucial role in various pathways of adipocyte, hepatocyte and cardiac cell metabolism. The synchronized initiation of adipogenesis and mitochondrial biogenesis indicates that mitochondria are vital for adipocyte differentiation and maturation (6). Furthermore, mitochondria are a vital organelles in cellular metabolism in keeping adipocyte pathophysiology in check, including lipid and fat homeostasis, hormonal sensitivity, antioxidant and oxidative capacity, adaptive thermogenesis, and WAT differentiation (7). In hepatocytes, mitochondria are important components, especially in nitrogen metabolism, through ammonia detoxification within the ornithine cycle. (8). One of the most important and unique features of liver cell mitochondrial fraction is their involvement in hepatic process of ketogenesis, where acetyl coenzyme A (acetyl-CoA) is degraded into ketone bodies (acetoacetate, β-D-hydroxybutyrate, and acetone) to supply energy to peripheral tissues during fasting when fatty acid supply exceeds cellular energy requirement (8). Cardiomyocytes have a high number and density of mitochondria to provide energy for cardiac contraction and relaxation. Approximately 90% of the cell's ATP is used to maintain the process of contraction-relaxation in the myocardium, and 70% of cardiac ATP is derived from fatty acid oxidation, thereby leading to increased reactive oxygen species (ROS) production (9). Mitochondria are the primary organelles responsible for generating ROS via lipid oxidation and oxidative phosphorylation reactions (10, 11). A surplus of ROS beyond the cell's antioxidant capacity can lead to an increase in ROS that eventually results in mtDNA and protein damage. This eventually leads to a reduction in the efficiency of the electron transport chain (ETC) and beta-oxidation capacity, resulting in mitochondrial dysfunction (12-14).

Mitophagy, or autophagy of mitochondria, is a protective process for selective elimination of defective or structurally impaired mitochondria by autophagic vesicles and subsequent degradation by lysosomes. Mitochondrial autophagy is involved in the regulation of mitochondrial number and quality in cells. Mitochondrial autophagy is typically initiated by two major pathways: the ubiquitin-mediated oxidative stress-controlled *PTEN-induced kinase 1(PINK-1)/PARKIN* pathway and the ubiquitin-independent hypoxia-controlled receptor-mediated pathway (15-17). Physical exercise is a central component in the management of adult obesity and plays a major role in obesity complication risk reduction, weight reduction, and weight maintenance. Exercise can change the expression of genes involved in mitochondrial dynamics, oxidative phosphorylation, antioxidant defense pathways, and cell growth (18, 19). These actions assist in reducing mortality risk, increasing insulin sensitivity, and exerting non-pharmacologic effects on several obesity-linked chronic conditions such as type 2 diabetes (T2DM), nonalcoholic fatty liver disease (NAFLD), high blood pressure, and cardiovascular ailments (20-22).

In our study, we aimed to investigate the expression levels of the genes including *MFN-1*, *PINK-1*, *ATG-3*, and E3 *PARKIN*, which play significant roles in the mitochondrial and cellular autophagy pathways in the adipose, heart and liver tissues of experimental C57BL/6J mice subjected to a 60% high-fed diet and running exercise regimen. Additionally, we examined whether these genes can serve as molecular markers for obesity. Understanding the relationship between exercise and these genes associated with mitophagy (*PINK-1*, *PARKIN*, *MFN-1*, *ATG-3*) will

contribute to the literature by unveiling new pathways in mitochondrial dynamics associated with obesity.

Materials and Methods

The experimental groups. The research was approved by the Local Ethics Committee for Animal Experiments at Istanbul University Rectorate (Ethics Committee Approval Number: 2021/18). The research investigation was conducted at the Department of Laboratory Animal Science and the Department of Molecular Medicine of the Aziz Sancar Institute of Experimental Medicine in conjunction with the Institute of Graduate Studies in Health Sciences, Istanbul, Türkiye. The animals were observed every day for the presence of signs of suffering such as impaired mobility, lack of grooming, and marked weight loss (>15%). The humane endpoints were used on the animals, which showed persistent suffering and anorexia/cachexia. On the completion of the experiment, the mice were sacrificed via cervical dislocation after approval by the Department for the Protection of Animal Welfare & Experimentation at the Institute (HADYEK), Turkish guidelines for animal welfare (Law No. 5199).

The research was carried out on a total of 30 female C57BL/6J mice; all were 8 weeks old and had a mean body weight of 20 grams and included three groups: control group (n=10), high-fat diet group (HFD, n=10), and exercise-high-fat diet group (E-HFD, n=10) (23). Female animals were selected as they have a higher susceptibility to develop adiposity. Both E-HFD and HFD groups were provided a diet containing 60% fat kcal, the control group was given a diet consisting of 10% kcal of fat (Catalog No: #58Y2, 58Y1; Purina and TestDiet, St. Louis, MO, USA). Beginning in the sixth week of the study and continuing until the end, the E-HFD group engaged in daily exercise using a running wheel. Exercise training was initiated at the 6th week of the study and continued on a treadmill until sacrifice, 5 days per week for 1 hour per day. Based on widely accepted mice endurance exercise protocols, the intensity was adjusted to correspond approximately to 60-70% of VO_2max, which represents moderate-intensity endurance training in mice (24-26). Conversely, the control and HFD groups remained sedentary within their experimental cages, where they were allowed standard movement without additional exercise interventions. For welfare reasons, mice were group-housed in cages of five; however, for biological and statistical purposes, each mouse was treated as an independent experimental unit. A total of 30 mice were used, and all analyses were performed at the level of the individual animal. The mice were allocated to the Control, HFD, and E-HFD groups using simple randomization. Blinding was not adopted since knowledge of group allocation was required to conduct dietary and exercise procedures. We ensured that the study groups were placed in a noise-controlled environment, provided ad libitum food and water, maintained at a controlled laboratory temperature of 21˚C with approximately 50% relative humidity, subjected to 12-hour photoperiod (light/dark cycle), and exposed to a light intensity of 40 lux. Mice were euthanized by cervical dislocation at the completion of the experiment. Blood was drawn by cardiac puncture, and the liver, adipose, and heart tissues were harvested and stored at -80°C.

RNA isolation and real-time polymerase chain reaction. RNA isolation from adipose, liver, and heart tissue samples was carried out using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The isolated RNA was then stored at -80°C, following established protocols (27). RNA purity was checked by the A260/280 spectrophotometric ratios, and only samples possessing acceptable values of purity were used. cDNA synthesis from 1 µg RNA was performed using the iScript™ cDNA Synthesis Supermix (Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocol. Gene expression was quantified by real-time quantitative PCR (qRT-PCR) using the Bio-Rad C1000™ Thermal Cycler (Bio-Rad) with each reaction being performed three times in a final volume of 25 µl. The reaction mixture included 6.8 µl of nuclease-free water, 0.6 µmol/l of forward and reverse primers, 10 µl of PCR Master Mix with SYBR green, and 2 µl of cDNA, resulting

in a total volume of 20 μl. The qRT-PCR parameters were optimized to initial denaturation of 5 min at 95°C and then 45 cycles of 10 s at 95°C for denaturation, 30 s at 60°C for primer-specific annealing temperature, and 20 s at 72°C for extension. To ensure accurate mRNA expression levels, mouse-specific primers were employed and *β-actin* used as the reference gene for normalization. The primer sequences were designed by using the Primer3 Program.

ATG-3: Forward primer: 5' ATAGTTTTTGACTCCCCTGTG 3', Reverse primer : 5' CAATGTAGTGGAGAGGCTATA 3'; *MFN-1*: Forward primer: 5' ATGACCTGGTGTTAGTAGACAGT 3', Reverse primer: 5' AGACATCAGCATCTAGGCAAAAC 3', *PINK-1*: Forward primer: 5' AGTGATTGACTACAGCAAGGCTGA T 3', Reverse primer: 5' ATCTTGTCTAACTTCAGATTCTTCAGG 3', *PARKIN*: Forward primer: 5' GTGTTTGTCAGGTTCAACTCCA 3', Reverse primer: 5' GAAAATCACACGCAACTGGTC 3'.

Enzyme-linked immunosorbent assay (ELISA). At the end of the experimental period, mice were sacrificed and blood samples were collected by cardiac puncture. The samples were stored under appropriate conditions until ELISA analysis. The blood samples were centrifuged to obtain serum, which was aliquoted into 15 μl aliquots and stored at −80 °C until ELISA analysis. Serum levels of TNF-α, IL-6, and IL-1B were quantified and calculated using ELISA kits, according to the manufacturer's instructions (cat. no: ab222503, ab208348, ab205577; Abcam, Cambridge, MA, USA).

Statistical analysis. RT-PCR Cycle threshold (CT) values were normalized to the reference housekeeping gene *β-actin* to compensate for potential variations in RNA quantity and quality. Relative gene expression fold change differences (FC, $2^{-\Delta\Delta CT}$) and log2 and fold change differences (log2FC) were computed based on the Livak method. GraphPad Prism 6.2 software (GraphPad Software, San Diego, CA, USA) was applied for analysis of all statistical data (28). Gene expression among groups and tissues was compared performing the Mann-Whitney *U* test for *ATG3*, *PINK-1*, *PARKIN* and *MFN-1* gene expression values and their correlation was assessed using Spearman's correlation test, data being presented as mean±standard error of the mean (SEM). The discriminatory power of the gene expression between the three groups and tissues was evaluated through receiver operating characteristic (ROC) curve analysis, performed using MedCalc Statistical Software (Version 12.7, MedCalc Software Ltd., Ostend, Belgium). The *p*-value <0.05 was taken as indicating statistical significance.

Results

Analysis of animal body and organ weights. The study evaluated the influence of HFD and exercise (E-HFD) on gene expressions in the heart, liver, and adipose tissues of mice, as well as their body and tissue weights (grams). The findings revealed that total body weight and individual tissue weights in the HFD group were significantly elevated compared to the E-HFD group and the control (respectively, control: 26.89±0.94, HFD: 39.36±4.28, E-HFD: 18.93±2.66) (Figure 1).

mRNA expression levels of related genes. Substantial increases in *ATG-3* gene expression were observed in the heart tissue of E-HFD group (2.87-fold, p=0.0164), liver (12.96-fold, p<0.0001), and adipose (9-fold, p=0.002) tissues compared to the control. In the HFD group, a notable increase was noted only in the adipose tissue (4.26-fold, p=0.0001), with no important changes in the heart and liver tissues (p>0.05) (Table I). The relative log2FC of *ATG-3* in the E-HFD group were 1.75 for the heart and liver, 3.83 for the heart and adipose, and 2.08 for the liver and adipose tissues. In the HFD group, the log2FC for *ATG-3* were 8.95 (p<0.0001) for the heart and liver, 7.34 (p<0.0001) for the heart and adipose, and -0.29 for liver and adipose tissues (Figure 2).

For the *PINK-1* gene, expression markedly increased in the E-HFD group across all tissues: heart (p=0.0079), liver (p=0.001), and adipose (p<0.0001) tissues compared with the control. In the HFD group, significant increases were found in the liver (p=0.0158) and adipose (p=0.0013) tissues, but not in the heart tissue (p>0.05) (Table I). The

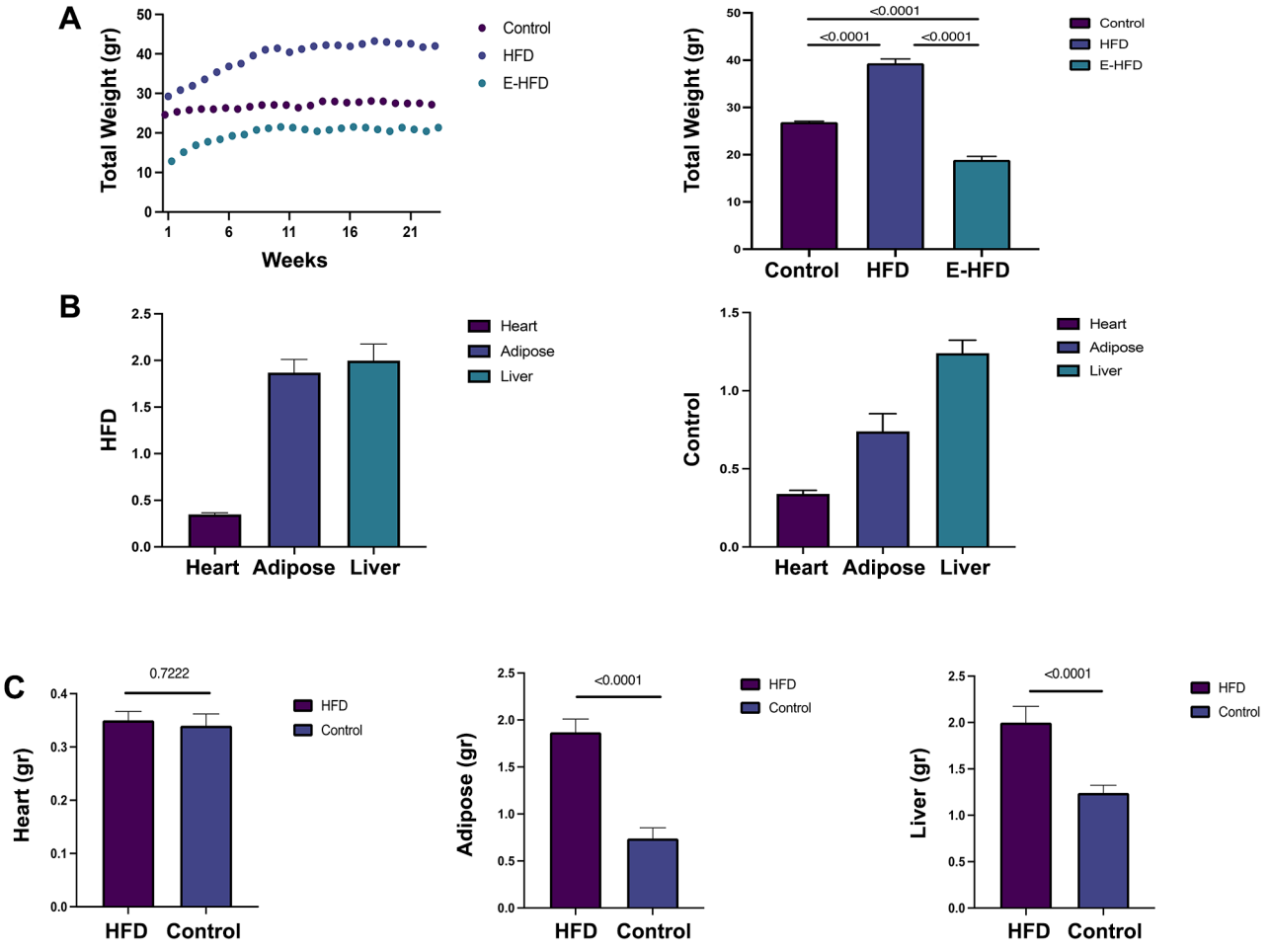


Figure 1. *The body and tissue weight of mice in the study groups. After 24 weeks of feeding and exercise, the body and tissue weight of the mice in the high-fat diet (HFD) group are dramatically higher than that of the control and exercise and high-fat diet group (E-HFD) group. A) the total body weight (g) of mice in each experimental group. B) Individual organ and tissue weights (g), including the heart, adipose tissue, and liver for each mouse. C) Compares organ weights (g) across the experimental groups. All values represent individual animals, and units are reported in grams (g). C: Control group.*

relative log2FC of *PINK-1* in the E-HFD group were 1.75 for the heart and liver, 3.83 for the heart and adipose, and 2.08 for the liver and adipose tissues. In the HFD group, the log2FC for *PINK-1* were 3.59 (p=0.0065) for the heart and liver, 19.19 (p<0.0001) for the heart and adipose, and 5.34 for the liver and adipose tissues (Figure 2).

The *PARKIN* gene showed significant increases in expression in the E-HFD group's heart (16.66-fold, p<0.0001) and liver (3.11-fold, p<0.0001) tissues in comparison with control group, with no significant change in adipose tissue (p>0.05). *PARKIN* gene expression levels significantly increased in the heart (6.37-fold, p<0.0001) and significantly decreased in the liver (2.7-fold, p<0.0001) tissues relative to the control, with no significant change in adipose tissues (p>0.05) in the HFD group (Table I). The relative log2FC of *PARKIN* in the E-HFD group were 0.44 for the heart and liver, 5.94 for the heart and adipose and 5.50 for the liver and adipose tissues. In the HFD group, the log2FC for *PARKIN* were 1.84 for the heart and liver, 4.26 for the heart and adipose, and 2.42 for the liver and adipose tissues (Figure 2).

Table I. *Fold-change in expression levels of ATG3, PINK-1, MFN-1, and PARKIN in the study groups.*

Tissues and Genes		Control (Mean±SE)	E-HFD (Mean±SE)	HFD (Mean±SE)	p1 *(Control & E-HFD)*	p2 *(Control & HFD)*	HFD (Mean±SE)	E-HFD (Mean±SE)	p3 *(E-HFD & HFD)*
Liver	*ATG3*	1.00±0.13	12.96±3.01	1.187±0.39	**<0.0001**	0.4813	1.00±0.33	10.92±2.53	**<0.0001**
	PINK1	1.00±0.33	32.10±12.22	6.10±2.638	**<0.0001**	**0.036**	1.00±0.43	5.26±2.00	**0.0068**
	MFN1	1.00±0.086	2.61±0.88	0.22±0.11	**0.05**	**0.0002**	1.00±1.53	12.02±4.07	**0.0001**
	PARKIN	1.00±0.086	3.11±0.47	0.37±0.11	**<0.0001**	**<0.0001**	1.00±0.51	6.92±1.26	**0.0002**
Adipose	*ATG3*	1.00±0.26	9.00±1.71	4.26±0.97	**0.0002**	**0.0001**	1.00±0.23	2.114±0.40	**0.0338**
	PINK1	1.00±0.39	180.7±80.63	29.49±23.58	**<0.0001**	**0.013**	1.00±0.80	6.13±2.73	**0.0021**
	MFN1	1.00±0.27	1.58±1.12	0.63±0.28	0.3054	0.1835	1.00±0.45	2.52±1.78	0.7959
	PARKIN	1.00±0.23	2.13±0.87	2.60±1.16	0.469	0.2101	1.00±0.45	0.82±0.34	0.9705
Heart	*ATG3*	1.00±0.18	2.87±0.57	0.70±0.03	**0.0164**	0.0829	1.00±0.05	4.09±0.82	**0.0048**
	PINK1	1.00±0.15	4.47±0.78	0.82±0.19	**0.0079**	0.3784	1.00±0.22	5.44±0.95	**0.001**
	MFN1	1.00±0.12	4.81±0.60	6.81±0.99	**0.0009**	**<0.0001**	1.00±0.15	0.71±0.09	**0.159**
	PARKIN	1.00±0.09	16.66±1.60	6.37±0.91	**<0.0001**	**<0.0001**	1.00±0.14	2.61±0.25	**<0.0001**

The significance between groups was analyzed using the non-parametric Mann-Whitney *U* test, and the fold changes are presented as Mean±Standard Error of the Mean (SEM). Statistically significant values are shown in bold ($p<0.05$). p1: mRNA expression fold change of the E-HFD group compared to the control group; p2: mRNA expression fold change of the HFD group compared to the control group; p3: mRNA expression fold change of the E-HFD group compared to the HFD group. C: control group; HFD: high-fat diet group; E-HFD: exercise and high-fat diet group; PINK-1: PTEN-induced kinase 1; PARKIN: E3 ubiquitin-protein ligase Parkin; MFN-1: mitofusin-1; ATG-3: autophagy-related gene 3.

For the *MFN-1* gene, significant increases were detected in the E-HFD group in the heart (4.81-fold, $p=0.0009$) and liver (2.61-fold, $p=0.05$) tissues in comparison to the control, with no significant change in adipose tissue ($p>0.05$). In the HFD group, significant increases were seen in the heart tissue (6.81-fold, $p<0.0001$) and significant decreases in the liver tissues (4.55-fold, $p=0.0002$) in comparison to the control, with no significant change in adipose tissue ($p>0.05$) (Table I). The relative fold changes (log2FC) of *MFN-1* in the E-HFD group were 1.95 ($p=0.023$) for the heart and liver, 37.63 ($p<0.0001$) for the heart and adipose, and 19.32 ($p<0.0001$) for the liver and adipose tissues. In the HFD group, the log2FC for *MFN-1* were 33.15 ($p<0.0001$) for the heart and liver, 134.2 ($p<0.0001$) for the heart and adipose, and 4.05 for the liver and adipose tissues (Figure 2).

ELISA results. We also determined the levels of the serum pro-inflammatory cytokines interleukin-6 (IL-6), IL-1β, and tumor necrosis factor alpha (TNF-α) in the HFD and control groups. The results indicated that the serum levels of IL-6, IL-1β, and TNF-α in the HFD group were considerably greater than those in the controls, indicating a higher systemic inflammatory response in the setting of HFD consumption (IL-6: 47.39→94.7, $p=0.0234$; IL-1B: 26.1→73.3, $p=0.0052$; TNF-α: 39.65→110.7, $p=0.0173$). Furthermore, TNF-α correlated significantly with *MFN-1*, *PINK-1*, and *PARKIN* gene expression in the liver tissue of the HFD group (*PINK-1 vs*. TNF-α: r=0.8146, $p=0.006$; *PARKIN vs*. TNF-α: r=0.8268, $p=0.0048$; *MFN-1 vs*. TNF-α: r=0.8511, $p=0.0029$). In addition, IL-6 correlated with *ATG-3* gene expression in adipose and heart tissues of the HFD group (Adipose: *ATG-3* vs. IL-6, r=-0.8085, $p=0.0065$; Heart: *ATG-3 vs*. IL-6, r=-0.7477, $p=0.0161$) (Figure 3).

Discussion

Obesity, defined as deranged or abnormal lipid accumulation that adversely affects health, occurs when calorie intake exceeds calorie expenditure, resulting in the storage of excess calories as triacylglycerol in adipose tissue (29). Adipose tissue not only regulates energy metabolism but is also considered an endocrine organ because it secretes adipokines, which are key players in

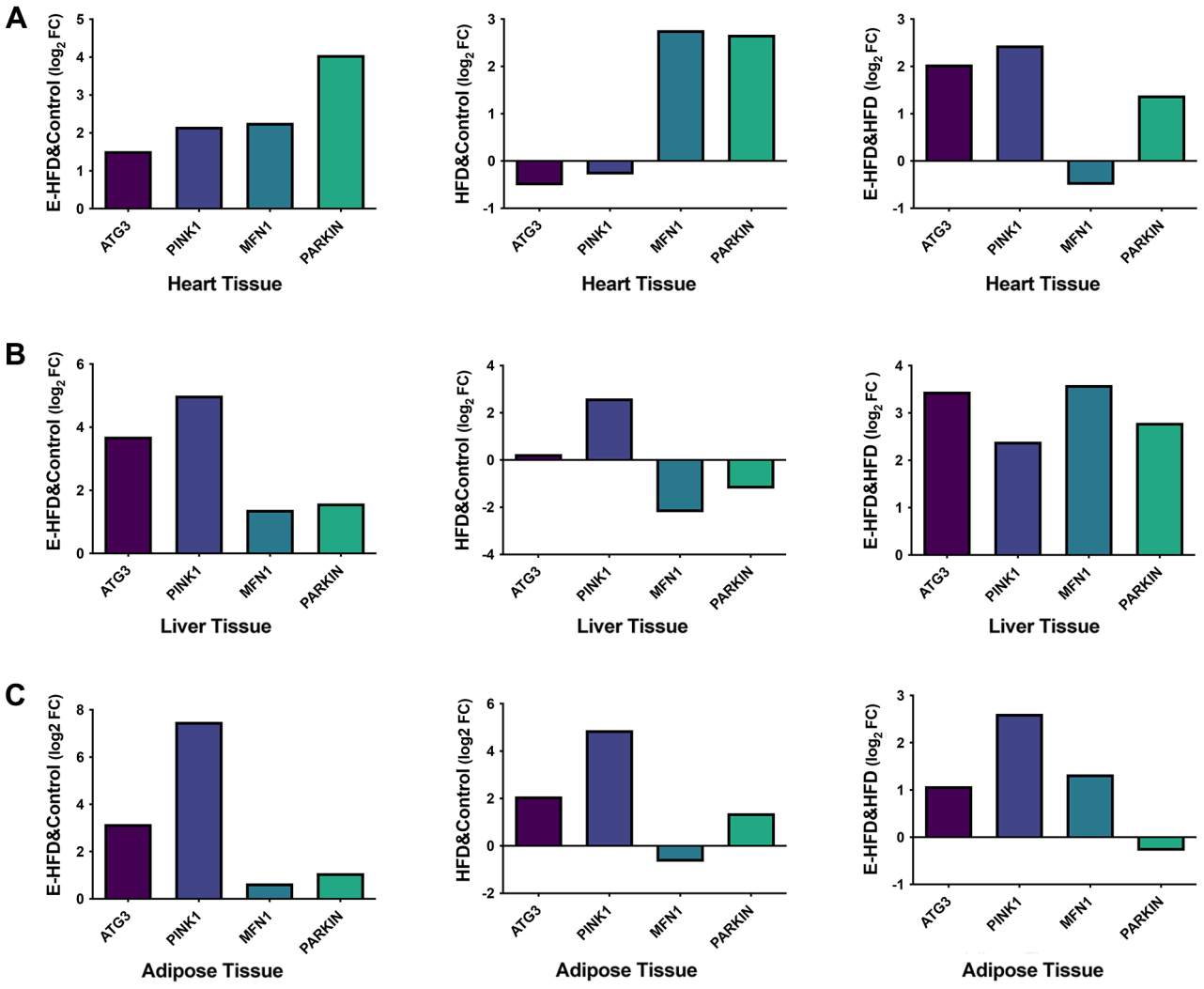


Figure 2. *The log2FC of ATG3, PINK1, PARKIN and MFN1 expression in the liver, adipose and heart tissue of mice in the study groups. Exercise and high-fat diet group (E-HFD) and high-fat diet group (HFD) groups are compared to the control group, while the comparison between the exercise and HFD groups is conducted relative to the HFD group. Data are shown as log_2 fold change (log_2FC). C: Control group.*

carbohydrate and lipid metabolism (30). Mitochondria are the cell powerhouses and are engaged in cellularr homeostasis, cellular energy metabolism, and cell signaling pathways (31). Obesity is associated with increased generation of ROS, which are primarily generated in mitochondria as byproducts of imperfect oxidative phosphorylation. The overproduction of ROS results in oxidative stress, thereby leading to damage of cellular components like lipids, proteins, and nucleic acids (32). Consequently, elevated mutation frequencies are observed in nuclear and mtDNA, resulting in genomic instability and mitochondrial dysfunction. Exercise plays a crucial role in promoting weight loss, maintaining current weight, and decreasing the risk of developing obesity-associated complications (Type 2 diabetes, heart diseases, stroke, sleep apnea and fatty liver disease) (33). In the present research, we examined the expression of mitophagy genes (*ATG-3*, *PINK-1*, *PARKIN*, *MFN-1*) in

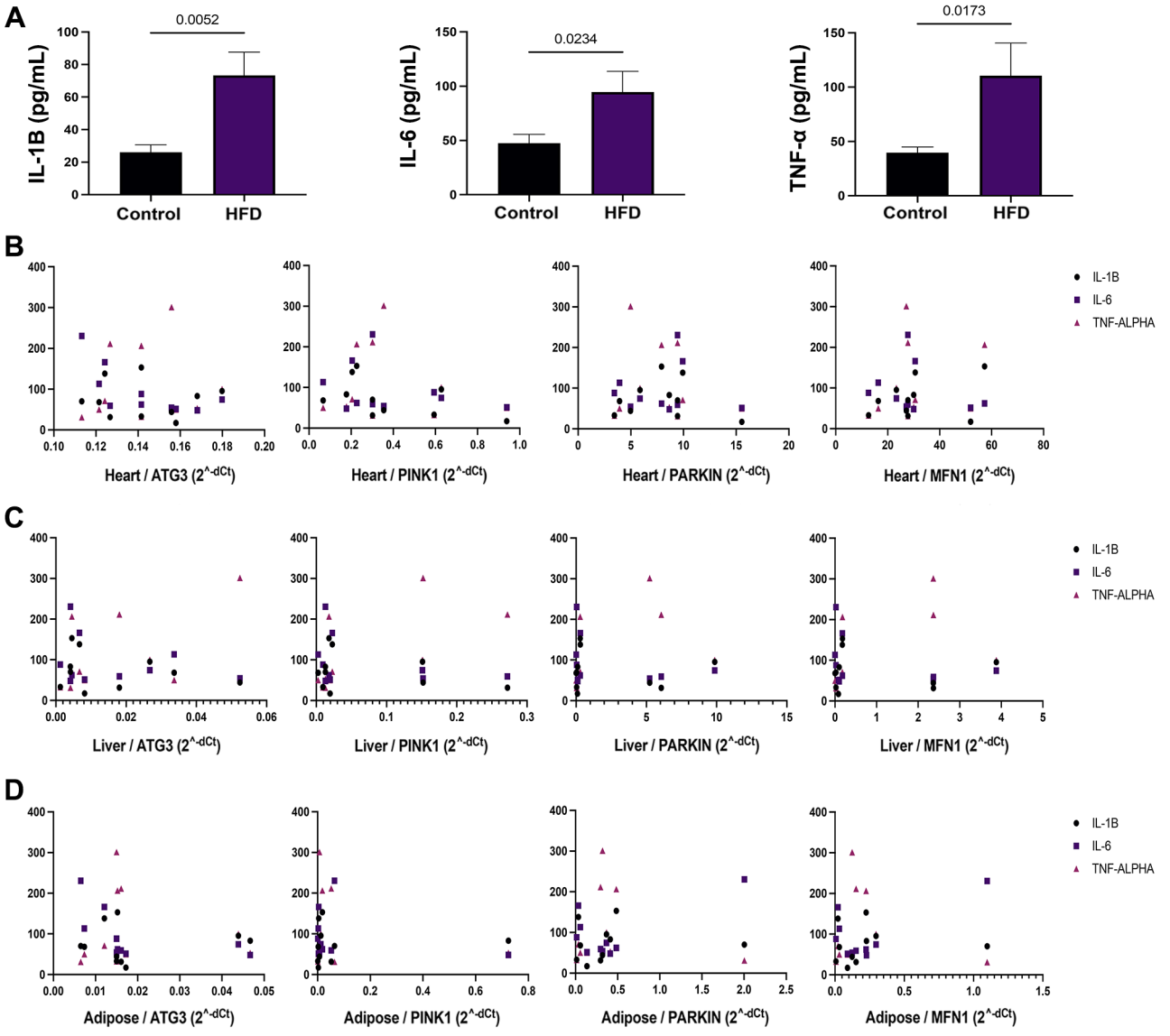


Figure 3. *Proinflammatory cytokine serum levels and correlation analyses of their relationship with mitophagy-related genes. A) The serum levels of TNF-α, IL-6 and IL-1B in the high-fat diet (HFD) and control groups. B) TNF-α, IL-6 and IL-1B correlation with ATG3, PINK1, PARKIN and MFN1 in the heart tissue of the HFD group. C) TNF-α, IL-6 and IL-1B correlation with ATG3, PINK1, PARKIN and MFN1 in the liver tissue of the HFD group. D) TNF-α, IL-6 and IL-1B correlation with ATG3, PINK1, PARKIN and MFN1 in the adipose tissue of the HFD group. C: Control group.*

adipose, liver and cardiac tissues of C57BL/6J mice subjected to HFD and running exercise regimen (32, 34). The *PINK-1-PARKIN* pathway represents one of the key processes regulating mitophagy to remove damaged mitochondria for the purpose of maintaining cellular homeostasis. When the mitochiondrial membrane potential is compromised, the buildup of *PINK-1* on the outer membrane leads to recruitment and subsequent ubiquitination of the outer membrane proteins, thus triggering the initiation of the mitophagic process, especially during oxidative stress conditions. Zhao *et al.* reported the expression levels of both *PINK-1* and *PARKIN*

within the heart tissue of mice undergoing swimming exercise and a low-calorie diet. The results showed an induction in the gene and protein expression levels of *PINK-1* and an induction in the gene level of *PARKIN*, although the protein level remained unchanged. Notably, swimming exercise alone significantly elevated *PINK-1* expression, whereas a low-calorie diet did not induce a significant increase (35). As expected, the levels of *PINK-1* gene expression in the E-HFD group showed a marked increment compared to the control and HFD groups. Unlike the result obtained by the study of Zhao *et al.,* the levels of gene expression for the *PARKIN* gene showed marked increment in the E-HFD group than in the control and HFD groups. In the same context, Rosa-Caldwell *et al*. examined the protein expression levels of *PINK-1* and *PARKIN* in the liver under the high-fat and regular diets, with the inclusion of exercise. Their results showed that the protein levels of *PINK-1* among mice fed the low-fat diet without exercise increased by approximately 40% compared to those fed the low-fat diet with exercise. Furthermore, the protein levels of *PARKIN* among mice fed the low-fat diet and having exercise decreased by approximately 40% compared to those fed the low-fat diet only (36, 37). Cui *et al*. found enhanced mRNA levels of both *PINK-1* and *PARKIN* in the adipose tissue of HFD-treated mice, consistent with the results obtained in the current study showing enhanced level of *PINK-1* mRNA expression in the HFD and E-HFD groups. Furthermore, it has been shown that palmitic acid regulates the levels of mitophagy markers in pre-adipocytes in a dose-dependent manner, where low concentrations enhance the levels of both *PINK-1* and *PARKIN* mRNAs, whereas high concentrations inhibit their levels (38). HFD-induced circulating FFAs have been proposed to chronically suppress PARKIN activation and contribute to impaired mitochondrial function. In contrast, the results unequivocally indicate the capability of exercise to abrogate these inhibitory effects. Specifically, the level of *PARKIN* mRNA was increased in the E-HFD group.

MFN-1 is a crucial regulator for the process of mitochondrial fusion. Through the maintenance of the mitochondrial structure and function, MFN-1 facilitates the metabolic functions of the cell and has also previously been associated with the dysfunction of the mitochondria during conditions of metabolic stress (39). Rosa-Caldwell *et al*. found an insignificant difference in the gene expression of MFN-1 in the liver of mice fed a Western diet and undergoing exercise compared to the control group. Although the protein levels of MFN-1 showed a considerably high difference between the exercise and non-exercise group (36). Conversely, Gonçalves *et al.* found a significant upregulation of the protein levels of MFN-1 in the liver, indicating an increased activity of mitochondrial fusion under conditions of metabolic stress and exercise (40). In agreement with the literature, we found that the MFN-1 gene expression was significantly decreased in the liver tissue of the HFD group compared to the control group, while a significant upregulation was observed in the exercise group compared to controls (41). Furthermore, the present experiment found the MFN-1 gene to be significantly decreased in the liver of HFD-fed mice, yet significantly induced by exercise. Conversely, in the adipose tissue, the MFN-1 gene showed non-significant differences among the groups, which correlates well with the variable effects of exercise on the MFN-1 gene in the literature. This increased expression of hepatic MFN-1 after exercise may indicate the restoration of mitochondrial fusion, which is important for improving fatty acid oxidation capacity to counteract lipotoxicity (42).

ATG-3 plays an essential role in lipidation during autophagosome formation and macroautophagy. Fang *et al.* found that *ATG-3* expression was significantly increased in the heart tissues of exercised animals compared to controls (43). Our study confirmed this, showing a significant increase in *ATG-3* gene expression in the heart tissue of the E-HFD group. Pires et al. reported a 40% reduction in *ATG-3* protein in obese mice hearts, which we also observed as a non-significant decrease in *ATG-3* mRNA expression in the obese group (44). Han *et al.* noted increased *ATG-3* expression in maternal obese mice livers, though not statistically significant, consistent with our findings of non-significant increases in the HFD

group (45). Interestingly, the HFD group displayed a reduction in *MFN-1* gene expression in adipose tissue, hinting at unresolved mitochondrial dysfunction through the fusion process. Conversely, the heightened *ATG-3* gene expression-suggests that adipocytes may be involved in the autophagic process.

A comprehensive review of the literature combined with our study's findings suggests that inactive mice on a HFD demonstrate increased gene expressions of *PINK-1* and *ATG-3*, which are involved in mitochondrial quality control and macroautophagy, respectively, in adipose tissue. This observation is consistent with the recognized roles of *PINK-1* in marking damaged mitochondria for degradation and *ATG-3's* crucial role in macroautophagy (37, 46, 47). The protective effects of exercise, as evidenced through mitochondrial biogenesis, suggest a potential regulation of genes involved in the mitophagy pathway, such as *PINK-1*, *PARKIN*, *MFN-1*, and *ATG-3*, during physical activity. Exercise seems to facilitate the clearance of damaged mitochondria, thus maintaining cellular homeostasis. Additionally, the significant increase in *ATG-3* and *PINK-1* gene expression levels in the liver, heart, and adipose tissues of the E-HFD group raises questions regarding the possibility biological agents (adipokines?) to promote gene interactions throughout various tissues. Exercise produced more significant increases in ATG-3 and PINK-1 expression levels in the E-HFD group versus the two other groups, both in adipose and liver tissues. This pattern of expression suggests that these genes are major markers of the exercise-mediated enhancement of mitochondrial quality control, biogenesis, and autophagic activity. The gene expression profile further validates that exercise supports not just mitochondrial biogenesis but also enhances the autophagic apparatus, providing an potentially protective effect against metabolic disturbances in high-fat diets. Future research should explore various diet and exercise protocols with larger sample sizes to enhance the accuracy and generalizability of the findings. Such studies will contribute to the development of more effective strategies for the treatment of obesity and related metabolic disorders.

Conflicts of Interest

The Authors declare that there are no non-financial or financial conflicts of interest in relation to this study.

Authors' Contributions

All Authors contributed to the study project conception and design; collection of data: O.K., I.Y., Y.Y.; examination and evaluation of data: U.Z., S.D., O.K.; writing of the article: S.D. and S.B.A.S.; critical revision of the manuscript: U.Z. and F.C.; managerial and technical support: S.D. and A.C..; and project and article supervision: U.Z. and S.D.

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Artificial Intelligence (AI) Disclosure

No artificial intelligence (AI) tools, including large language models or machine learning software, were used in the preparation, analysis, or presentation of this manuscript.

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