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Combined Effects of High Fat Diet and Exercise on Autophagy in White Adipose Tissue of Mice

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Running title: Exercise Training and High-Fat Diet on Autophagy

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Abstract

Aim: The effects of nutrition and exercise on autophagy are not well studied. This study aimed to investigate the combined effects of high-fat diets (HFD) and exercise training (ET) on autophagy in white adipose tissue of mice.

Materials and methods: Male C57BL/6 mice were assigned into four groups of 7 mice per group: (1) Control, (2) High-Fat Diet-induced Obesity (HFD-Ob), (3) Exercise Training (ET), and (4) High-Fat Diet with Exercise Training (HFD-ET). The HFD-Ob group was fed a high-fat diet for 14 weeks, while the ET group continuously ran on a treadmill for five sessions per week for seven weeks, and the HFD-ET group had both HFD and exercise training. qReal-time-PCR and western blot were used to measure the mRNA and protein levels of autophagy markers in white adipose tissue.

Results. Mice from the HFD group showed higher levels in autophagy-related gene5 (ATG5, $p=0.04$), ATG7 ($p=0.002$), cathepsin B (CTSB, $p=0.0004$), LC3-II ($p=0.03$) than control. Mice in the ET group displayed higher levels of genes for ATG7 ($p=0.0003$), microtubule-associated protein1-light chain 3 (LC3, ($p=0.05$), lysosome-associated membrane protein 2 (LAMP2, $p=0.04$) and cathepsin L (CTSL, $p=0.03$) than control. Mice from the HFD-ET group had higher levels of genes for ATG7 ($p=0.05$) and CTSL ($p=0.043$) and lower levels of genes for CTSB ($p=0.045$) compared to the HFD group and lower levels of LAMP2 ($p=0.02$) compared to the ET group.

Conclusion. There were increases in autophagosome formation in the white adipose tissue from Mice in the HFD and ET groups. A combination of HFD and ET enhances autophagosome formation and modulates lysosomal degradation in white adipose tissue.

Keywords: Obesity, High-fat diet, Exercise training, Autophagy, Autophagy, Adipose tissue

1. Introduction

Obesity increases the risk of developing metabolic syndrome, type 2 diabetes, hyperlipidemia, and heart diseases [1], and is associated with inactivity and high-calorie diets [2]. Lifestyle changes, such as a healthy diet and exercise can prevent and treat obesity and its complications [3]. Energy-dense foods cause fat to accumulate in adipose depots, leading to abnormal expansion of white adipose tissue (WAT) at the onset of obesity, dysregulation of adipose properties and the development of obesity-related complications [4, 5].

Autophagy is a lysosomal self-degrading process whereby some damaged cytoplasmic molecules and organelles are hydrolyzed so as to maintain cellular integrity [6-8]. Autophagy also has an important role in adipose tissue growth to regulate lipid-depositing capacity [9]. Impairment of autophagy can cause abnormal adipose-tissue growth linked to metabolic disorders [5, 10].

Increased intake of foods rich in fat leads to the remodeling of adipocytes and adipose tissue, resulting in the adipose hyper expansion [11]. The role of high-fat diets (HFD) in autophagy is unclear. For example, studies by Budi et al (2022) and Zhang et al (2020) reported that HFD impairs autophagy in white adipose tissue [12, 13], while findings by Deng et al (2014) and Mizunoe et al (2017) indicate that HFD promotes autophagy [14, 15].

Exercise training (ET) alleviates obesity and obesity-related complications [16, 17] and enhances autophagy in cardiac and hepatic tissue [18, 19] and skeletal muscle [20]. However, there is conflicting information on the effects of exercise on autophagy in adipose tissue. For example, Tanaka et al. (2015) reported that exercise training increased autophagy in the epididymal adipose tissue, while Rocha-Rodrigues et al. (2017) could not identify changes in autophagy in visceral adipose tissue after endurance training [21, 22].

Our study tests the hypothesis that combined ET and HFD induce autophagy in white adipose tissue. To this end, we examined the effects of a combination of HFD and ET on factors involving autophagy in white adipose tissue. We measured mRNA or protein levels related to autophagy in epididymal fat depots in exercise-trained / untrained mice that were fed either low-fat or high-fat diets.

2. Materials and Methods

2.1. Experimental animals

Twenty-eight male C57BL/6 mice aged five weeks and weighing 12 ± 2 grams were purchased from Pasteur Institute (Iran) and housed under the following circumstances: temperature of $22\pm 2^{\circ}\text{C}$,

moisture of $55\pm 10\%$, and a 12:12 h light/dark cycle. Following a week of acclimatization, the mice were divided into four groups at random: (1) Control (C, n=7), (2) Exercise training (ET, n=7), (3) High fat diet-induced obese (HFD-Ob, n=7), and (4) High-fat diet with exercise training (HFD-ET, n=7) (**Fig.1**). The experiment was conducted based on ARRIVE guidelines and certified by the Medicine Research Ethics Committees at Tehran University. (*IR.TUM.MEDICINE.REC.98.671*).

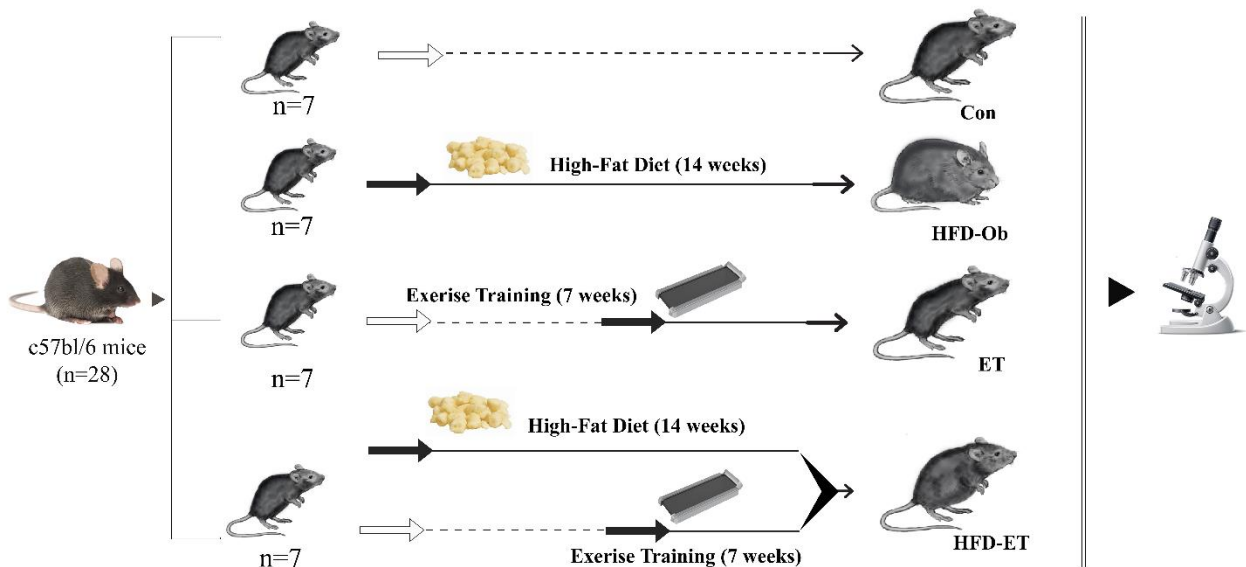


Fig. 1. Graphical representation of the experimental protocol

Con, Control group; **ET**, Exercise Training; **HFD-Ob**, High fat diet-induced obese group; **HFD-ET**, High fat diet with exercise training group

2.2. HFD-induced obesity

Mice in the HFD-Ob group were fed a high-fat diet for fourteen weeks. The total calories of the diet were 4.6 kcal/g, with 45% of those calories coming from fat, 20% from protein and 40% from carbohydrates [23]. The control mice were given a standard diet consisting of 3.5 kcal/g of total calories, with 60% kcal from carbohydrates, 25% kcal from protein and 15% kcal from fat [24]. The diets were provided by Animal Food Company (Behparvar, Iran).

2.3. Exercise-training program

Mice in the ET and HFD-ET groups were familiarized with running on a treadmill before initiating the exercise training protocol, where they ran for 40 min on a rodent treadmill (at 0% slope) for seven weeks (five sessions per week). The exercise sessions started with 5 min warm-up running at 40-50% vVO_2 peak, followed by 30 min ceaseless running at 60-70% vVO_2 peak, and concluded with

5 min cool-down jogging at 40-50% vVO_2 peak [25, 26]. The details of training such as speed of training and distance run are presented in **Table 1 (supplementary)**.

vVO_{2peak} was calculated by an increasing intensity test [27], which consisted of a few two-minute running stages. The speed of the first stage was 2m/min and the speeds of the next stages were increased by 2m/min. Running speeds were increased until the mice were unable to keep running for 10 s, and the stage that the mice were unable to continue running was considered as vVO_{2peak} [27].

2.4. Blood collection and tissue extraction

Mice were euthanized 48 h after the last training session by injecting a combination of ketamine (90 mg/kg) and xylazine (10 mg/kg) [28]. The blood sample was taken from the heart, and fat was dissected from its attachment to the epididymis and testis and stored at -78°C .

2.5 Plasma biochemical analysis

Plasma glucose was measured by a glucose oxidase method using an Auto-Analyzer (*Hitachi 902, Boehringer Mannheim, Germany*). Lipid profiles including triglycerides (TG), non-esterified fatty acids (NEFA), total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were determined by Auto Analyzer using enzymatic methods using *ParsAzmon* kits (*Iran*) using the manufacturer's instructions. Insulin levels were measured by an ELISA method (kit: *10-1247-01; Mercodia AB, Sweden*).

2.5. Gene expression (mRNA) by Real-Time PCR

1 ml of TRIzol reagent (*Thermo Fisher Scientific, USA*) was mixed with 50 mg of fat sample. Total RNA was extracted by TRIzol based on the manufacturer's instructions (*Cat. No. 15596026*). RNA concentration and purity were determined by a spectrophotometer (*OD: 260/280; Ultraspec 3000, Pharmacia Biotech, Sweden*). RNA was transcriptionally reversed to DNA by SuperScript II Reverse Transcriptase (*Thermo Fisher Scientific, USA; Cat. No. 18-064-014*). The content of mRNA was assessed by real-time PCR carried out using Rotor-Gene 6000 (*Corbett, Australia*) with SYBR Green RT-qPCR Kit (*QR0100-Sigma-Aldrich- Germany*). The thermal cycling profile was 95°C for 10 min, followed by 40 cycles at 95°C for 10 s and 55°C to 58°C for 30 s to 40 s as the annealing temperature. The primers, with specifications shown in **Table 2 (supplementary)**, were synthesized by SinaClon (*Iran*). The relative mRNA expression was determined by the Pffaf1 method using Genex software (7.0).

2.6. Western blotting

The fat sample was pulverized using a glass mortar, and the resulting powder was homogenized in RIPA lysis buffer (*Sigma-Aldrich; German*). The homogenate was centrifuged at 4°C for 15 min at 13000 × g and the supernatant was separated. The total protein of the supernatant liquid was assessed with a Bradford-protein assay kit (*ZellBio; German*) using bovine serum albumin as a standard. Protein concentrations were determined by spectrophotometers (absorbance at 595 nm) (*Ultrospec 3000, Pharmacia Biotech, Sweden*). After providing an SDS-polyacrylamide gel (4% and 12%), 30 µg of the supernatant liquid was boiled for 5 minutes and loaded onto the gel. Using electrophoresis at 110 V for 60 min, proteins were segregated, and the separated protein on the gel was transferred to nitrocellulose polyvinylidene difluoride (PVDF) membranes (*Sigma-Aldrich*) by electroblotting at 80 V for 180 min. The PVDF membranes were blocked using 5% non-fat milk, in Tris buffer saline comprising 0.1% Tween 20, at room temperature for 60 min. The membranes were then incubated with primary antibodies (1 µg/ml) against ATG7, ATG5, SQSTM, LC3A/B, CTSB, LAMP2, CTSL, and GAPDH, at 4°C for an overnight time. The membranes were then incubated with secondary antibodies conjugated with horseradish peroxidase (1:1000) at room temperature for two hours. The details of the antibodies are presented in **Table 3 (supplementary)**. Bands were observed using Enhanced Chemiluminescence (ECL) reagent (*Sigma- Aldrich; Germany*) in a Gel Doc system (*Uvidoc, England*). The band densities were analyzed by ImageJ software (*15.1, NIH, USA*).

2.7. Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). A two-way analysis of variance (ANOVA) was used to analyze the effects of HFD and ET, while a one-way analysis followed by a Tukey post hoc test was used for intergroup comparisons of variables. SPSS 21.0 (SPSS Inc., Chicago, IL, USA) and Prism 8 (GraphPad, USA) were utilized for data analysis and graph design, respectively.

3. Results

3.1 General characteristics

The general characteristics of mice at the end of the experiments in the different study groups are shown in **Table 1**. Consumption of HFD lead to obesity, increased plasma levels of glucose level and impaired lipid profile, and exercise training opposingly was able to alleviate it.

Table 1. General characteristics of mice in the four experimental groups (n=7 per group)

	Con	HFD-Ob	ET	HFD-ET
Body weight (g)	25.0±0.7	33.2±1.5*	22.1±0.8	29.5±1.2* [#]
Body weight gain (g)	12.3±0.4	19.6±0.9*	10.4±0.4	16.6±0.7* [#]
Epididymal pad (g)	0.41 ± 0.07	1.08 ± 0.1	0.31 ± 0.1	0.81 ± 0.1

Food intake (kcal/week)	78.2±10.3	106±14.7*	88.5±11.6	101.8±12.9*
Glucose (mg/dl)	95.6 ± 5.4	130.1 ± 10.8*	89.6 ± 4.4	111.6±11.1*. [#]
Insulin (ng/ml)	2.17 ±0.51	2.87 ± 0.94	2.07 ±0.51	2.51 ± 0.86
Triglyceride (TG)(mg/dl)	70.45 ± 9.3	90.4 ± 14.8*	61.25 ± 8.3*	80±13.6*. [#]
NEFA (mmol/l)	0.14±0.01	0.31±0.05*	0.09±0.01	0.23±0.07
Total cholesterol (TC) (mg/dl)	180 ± 11.01	200 ± 20.02*	170 ± 10.03*	190 ± 18*. [#]
HDL cholesterol (mg/dl)	125.63±7.6	140.1±9.3*	134.24±8.3*	145.63±8.5*
LDL+VLDL (mg/dl)	85.11± 11.04	143± 18.06*	55±7.04*	121±10*. [#]

Data are expressed as mean±SEM and were analyzed by one-way ANOVA followed by the Tukey test. * p<0.05 compared control; [#] p<0.05 compared HFD-Ob. **Con**, Control; **ET**, Exercise Training; **HFD-Ob**, High fat diet-induced obese; **HFD-ET**, High fat diet with exercise training

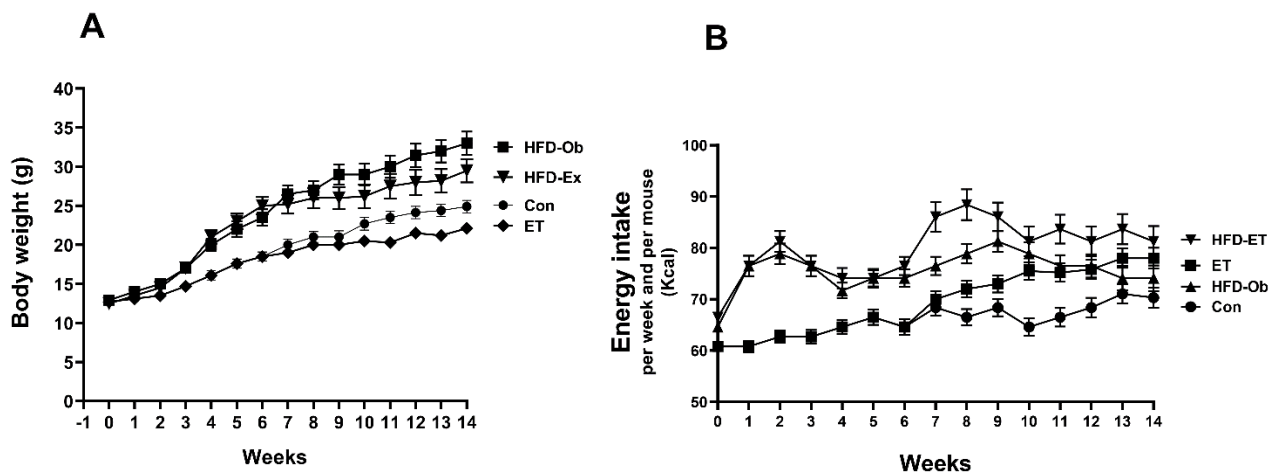


Fig 2. Changes in body weight (A) and energy intake (B) in animal groups (n= 7 per group)

Data are expressed as the mean±SEM.

Con, Control; **ET**, Exercise Training; **HFD-Ob**, High fat diet-induced obese; **HFD-ET**, High fat diet with exercise training

3.2. Autophagy-related gene5 (ATG5) and autophagy-related gene7 (ATG7)

A two-way ANOVA indicated that HFD and ET significantly affected the expressions of ATG5 (p=0.002) and ATG7 (p=0.001) genes, but with no interaction between HFD and ET detected (p>0.05).

Intergroup comparisons determined that mRNA levels of ATG5 (p=0.04) and ATG7 (p=0.002) were higher in HFD-induced obese mice than in controls (**Fig.3**). Further, mice in the HFD-ET group had higher levels of these genes compared with the control group (ATG5: p=0.007 and ATG7: p=0.0001). In addition, we also observed higher ATG7 mRNA levels in the ET group than in the control (p=0.0003), and in the HFD-ET group compared to the HFD-Ob group (p=0.05) (**Fig.3b**).

These findings indicate that HFD and ET increased the expression of markers of the early stages of autophagy, such as ATG5 and ATG7, and that there was a greater effect when HFD and ET were combined.

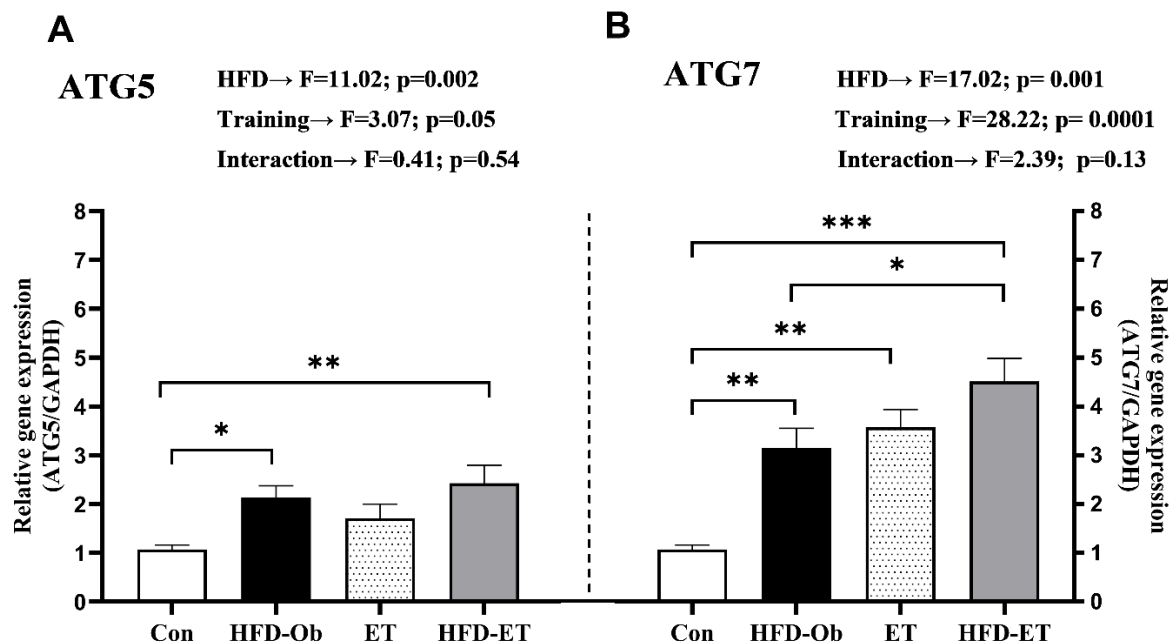


Fig 3. mRNA levels of ATG5 (A) and ATG7 (B) in experimental groups

Data are expressed as the mean±SEM. Data were analyzed by two-way and one-way ANOVA followed by the Tukey test. * p<0.05; ** p<0.01; *** p<0.001

ATG5, autophagy-related genes5; **ATG7**, autophagy-related genes7; **GAPDH**, Glyceraldehyde 3-phosphate dehydrogenase; **Con**, Control; **ET**, Exercise Training; **HFD-Ob**, High fat diet-induced obese ; **HFD-ET**, High fat diet with exercise training

3.3. Microtubule-associated protein1- light chain 3 (LC3)

Findings from a two-way ANOVA indicate that HFD and ET increased LC3-I and LC3-II protein contents ($p < 0.05$), but with no interaction between HFD and ET ($p > 0.05$). Intergroup comparisons indicate that mice from the HFD-Ob, ET and HFD-ET groups had higher LC3-I and LC3-II levels than the control group ($p < 0.05$) (**Fig.4**). These findings suggested that HFD and ET induce autophagosome formation.

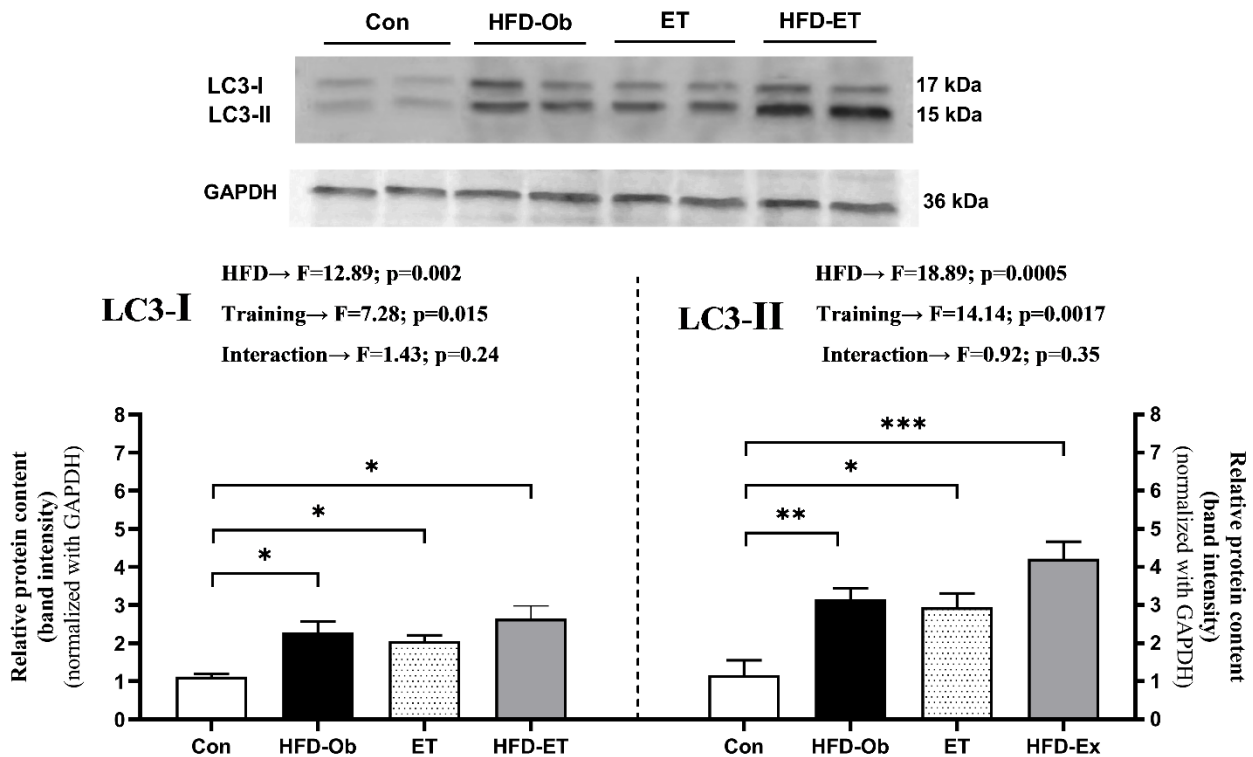


Fig 4. Protein levels of LC3 in experimental groups

Data were analyzed by two-way and one-way ANOVA followed by the Tukey test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

LC3, microtubule-associated protein 1-light chain 3, **GAPDH**, Glyceraldehyde 3-phosphate dehydrogenase; **Con**, Control; **ET**, Exercise Training; **HFD-Ob**, High fat diet-induced obese; **HFD-ET**, High fat diet with exercise training

3.4. Sequestosome 1 (SQSTM1/P62) and Lysosome-associated membrane protein 2 (LAMP2)

Our findings indicate that HFD reduced protein levels of LAMP2 ($p < 0.05$), while ET increased protein levels of LAMP2 protein levels ($p < 0.05$) (**Fig.5a**). Further, LAMP2 protein levels were lower in the HFD-ET group compared to the ET group ($p = 0.02$), indicating that HFD negated ET-induced upregulation of LAMP2 (**Fig.5a**). There were no changes in P62 protein levels in the various study groups ($p > 0.05$) (**Fig.5b**).

These findings indicate the upregulation of autophagolysosome by ET and downregulation by HFD. However, levels of P62, a substrate of autophagy, were not changed by either HFD or ET. The

unchanged levels of P62 along with increases in LC3 suggest that ET and HFD lead to an accumulation of autophagosomes in white adipose tissue. We explored this further by measuring levels of markers of lysosomal function such as CTSL and CTSB mRNA.

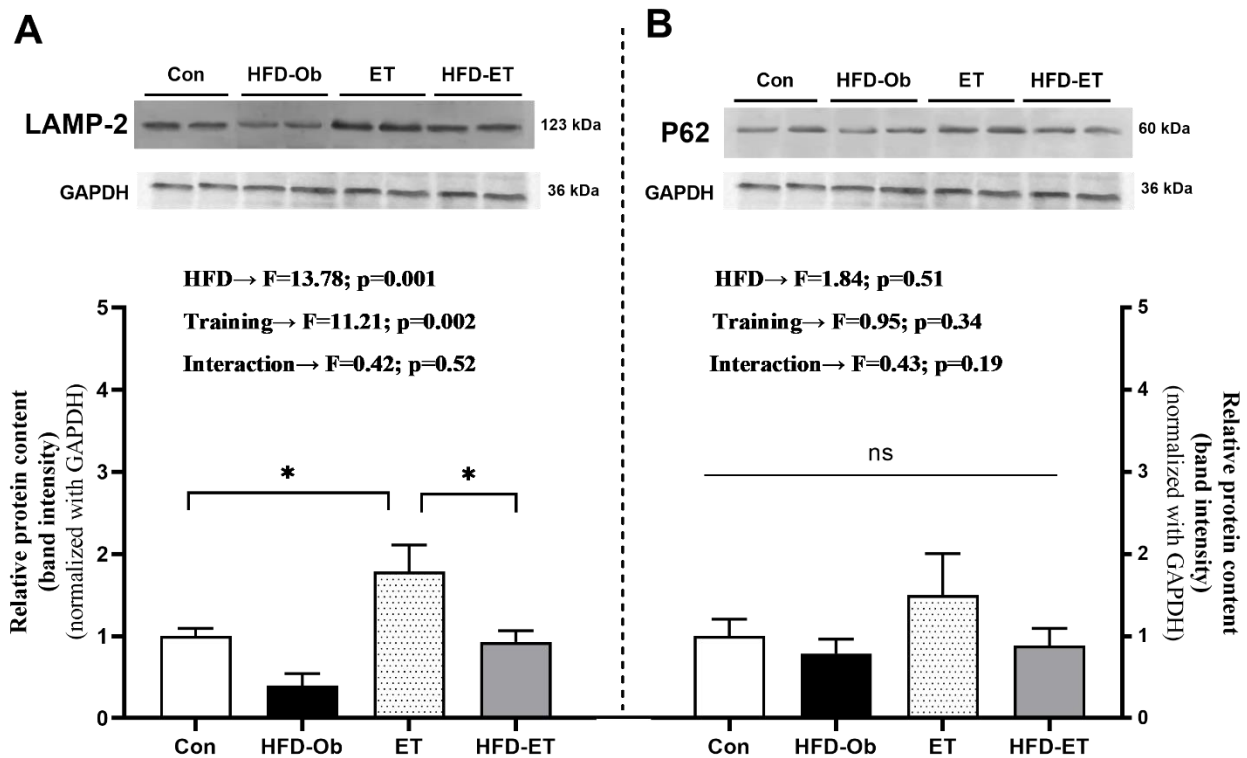


Fig 5. Protein levels of LAMP2 (A) and P62 (B) in the experimental groups
Data were analyzed by two-way and one-way ANOVA followed by the Tukey test.
* p<0.05 **P62**, sequestosome 1; **LAMP2**, lysosome-associated membrane protein 2;
GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; **Con**, Control group; **ET**,
Exercise Training; **HFD-Ob**, High fat diet-induced obese group; **HFD-ET**, High fat
diet with exercise training group

3.5. Cathepsin B (CTSB) and Cathepsin L (CTSL)

A two-way ANOVA analysis indicated that HFD significantly increased CTSL (p=0.005) and CTSB (p=0.0001) gene expressions, and that ET caused increases in CTSL (p=0.005) but not CTSB (p=0.14) (**Fig.6**). Intergroup comparisons confirmed that CTSB levels were significantly higher in the HFD group (p=0.0004) compared to the control group, and that CTSL levels were higher in the ET group (p=0.03) compared to the control group. Further, the HFD-ET group had significantly higher levels of CTSL (p=0.043) and lower levels of CTSB (p=0.045) compared to the HFD group (**Fig.6**). These data indicated that ET augmented the HFD-induced upregulation of CTSL and modified the HFD-induced upregulation of CTSB.

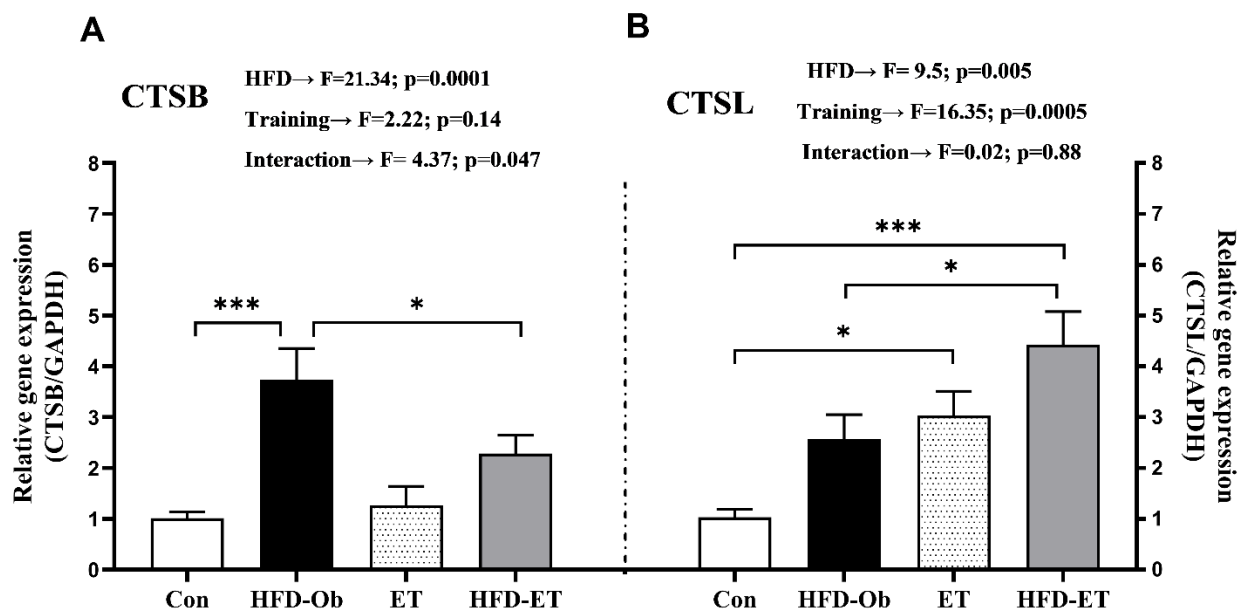


Fig 6. mRNA levels of CTSB (A) and CTSL (B) in the experiment groups
 Data were analyzed by two-way and one-way ANOVA followed by the Tukey test. * p<0.05; *** p<0.001
CTSB, cathepsin B; **CTSL**, cathepsin L; **GAPDH**, Glyceraldehyde 3-phosphate dehydrogenase. **Con**, Control group; **ET**, Exercise Training; **HFD-Ob**, High fat diet-induced obese group; **HFD-ET**, High fat diet with exercise training group

4. Discussion

Consuming high-fat diets cause lipid accumulation in fat pads, leading to the hyper-expansion of white adipose tissue. Exercise increases fat lipolysis and oxidation, reducing the size and activity of white adipose tissue. Autophagy mediates the remodeling of adipose tissue through three main phases: (I) autophagosome formation, (II) autolysosome formation, and (III) degradation of autolysosome contents [29]. We investigated the effect of an HFD and ET on markers of the three phases of autophagy in the white adipose tissue of mice.

We report that mRNA levels of ATG5 and ATG7 and protein levels of LC3II were upregulated in white adipose tissue of HFD-induced obese mice, indicating that HFD-induced obesity upregulates the early phases of the autophagic process i.e., autophagosome formation. Our results are supported by previous findings that HFD-induced obesity stimulates autophagosome formation [11, 14, 15, 30, 31]. However, a study by Zhang et al (2020) reported that the ratio of LC3-II/LC3-I (representing autophagosome formation) was reduced in HFD-induced obesity [13], and Sakane et al (2021) reported no changes in markers of autophagosome formation (LC3, ATG5, ATG7, Beclin1) in HFD-fed mice [32]. The reasons for these discrepant findings are unclear, however, the difference in high-fat diet composition and measurement procedure may have contributed to these discrepancies.

We observed that ET, similar to HFD, increased ATG5 and ATG7 mRNA and LC3II reported that nine weeks of endurance training increased ATG7 and LC3II proteins in epididymal fat depots [21, 33]. However, a study by Li et al (2022) described reductions in LC3-II in abdominal fat following

eight weeks of exercise training [34]. These inconsistent findings may be related to the different fat pads studied.

Our study suggests that markers of autophagosome formation such as ATG7 increased when exercise (ET) was combined with HFD compared to the effect of either in isolation. A related report by Rocha-Rodrigues et al (2017) indicated that the expression of Beclin-1 was increased in the adipose tissue of HFD-fed rats compared to the HFD-fed group following eight-week endurance training [22]. These findings suggest that the HFD and ET can upregulate autophagosome formation, and that their effects may be additive. We speculate that HFD facilitates the early phases of the autophagic process as an adaptive mechanism to promote adipogenesis and fat storage and to preclude ectopic fat accumulation. ET also enhances the early phases of the autophagic process, possibly as a mechanism to repair/remove cell organelles that may be damaged by ET.


Pro-inflammatory cytokines such as TNF- α mediate HFD-induced upregulation of agents involved in the early phase of the autophagy process [35]. FGF21/AMPK and AMPK/SIRT1 are also involved in ET-induced upregulation of pro-inflammatory cytokines [36, 37]. One mechanism for the combined effects of HFD and ET on autophagosome formation could result from adipocyte loading and unloading lipids in succession, where HFD can induce lipid synthesis and its accumulation in adipocytes, while ET induces lipolysis and lipophagy in fat cells [9].

The accumulation of LC3-II augments the formation of the autophagosome [30, 38], and paradoxically can also represent either a development or a deficit in the late step of the process of autophagy (i.e., autolysosome formation or lysosomal degradation) [15, 19, 30, 39]. We thus also measured LC3-II as an indicator of the late step of the process of autophagy, i.e., LAMP2a, P62 and cathepsin levels.

LAMP2 is a common lysosomal protein needed for the combination of autophagosomes and lysosomes in the production of autolysosomes. [40]. We observed that HFD reduced LAMP2 protein levels in white adipose tissue, raising the possibility of an impaired fusion of lysosomes with autophagosomes. Similarly, Li et al (2022) and Zhang et (2020) also reported that LAMP2 protein was reduced in HFD-induced obese mice [13, 34]. It is likely that sirtuin 3 (SIRT3) mediates the downregulation of LAMP expression by activating AMPK [13].

Our study indicates that LAMP2 levels were higher in exercise-trained mice compared to the control, suggesting the increased formation of autolysosomes. This finding is inconsistent with a previous study by Tanaka et al (2015) that did not find changes in LAMP2 protein in the adipose tissue of rats following nine weeks of exercise training [21]. The discrepancy may be related to differences in the endurance training protocols related to the speed and duration of the exercise sessions in the two studies. Further, our results indicate that ET combined with HFD negated the upregulation of ET on LAMP2, suggesting that the HFD may impair the ET-induced formation of autolysosomes. Taken together, we speculate that autolysosome formation, as the intermediate phase of the autophagy process, is enhanced by ET, but impaired by HFD feeding.

Lysosomes degrade P62, and reductions in P62 levels reflect increased lysosome activity. We did not observe changes in p62 protein levels in mice from HFD-obese, ET, and ET-HFD groups. These findings suggest that increased autophagosome formation induced by HFD and ET was not related to



P62 degradation. However, for greater exploration of lysosome degradation, we also measured levels of CTSL and CTSB which contributed to the lysosome step of the autophagic clearing.

Autophagy is mediated by the lysosome cysteine proteases CTSL and CTSB. Our findings that levels of CTSB and CTSL mRNA were increased by the effect of HFD are in agreement with previous studies [15, 31, 41-43]. These findings indicated that unlike the changes in P62, lysosome degradation is promoted during HFD-induced obesity, likely because of the upregulation of transcription factor EB (TFEB) in HFD obesity [44].

Our study also indicates that exercise did not affect CTSB expression, although it increased CTSL expression. ET inhibited the upregulation of CTSB by HFD, but increased the upregulation of CTSL by HFD, indicating a modulating effect of ET in lysosome proteases. This also suggested a protective role of ET against insulin resistance, as supported by findings that CTSB promotes inflammation [41] and lipolysis by degrading perilipin-1 to induce insulin resistance [31, 45]. However, the upregulation of CTSL has not been associated with unfavorable outcomes.

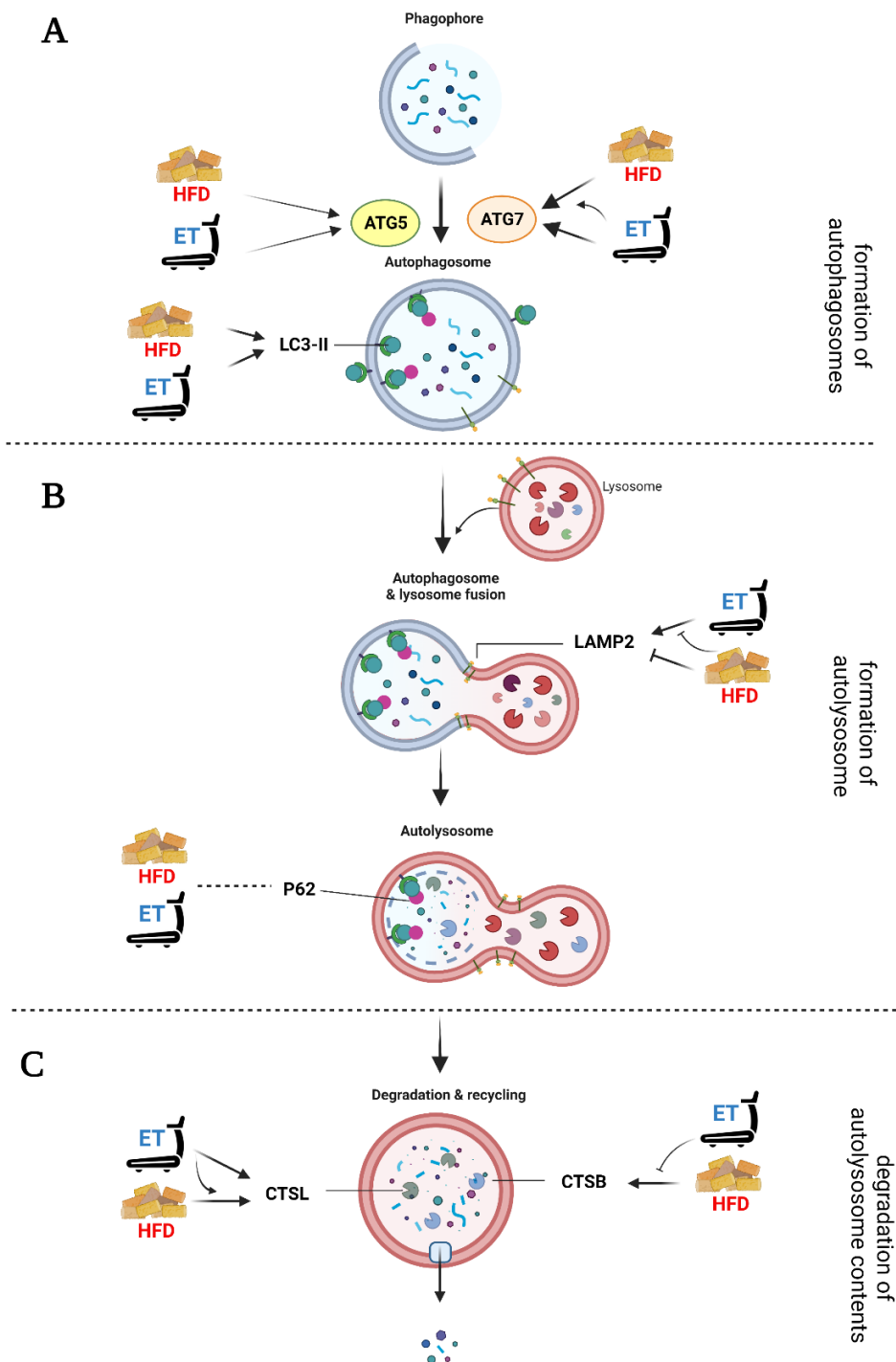


Fig 7. Schematic diagram of the effect of High-fat diet (HFD) and Exercise training (ET), subjected in the experiment, on Autophagy-process factors. **A.** The initial stage of autophagy (autophagosome formation). In this stage, HFD and ET could induce the expression of ATG5 and ATG7, and also cause the increase of LC3-II protein content. **B.** The intermediate stage of autophagy (autolysosome formation). In this stage, HFD decreased and ET increased LAMP2 protein content. **C.** The final stage of autophagy (degradation of the autolysosome content). In this stage, HFD and ET did not affect the P62 level, however, they affect CTSL and CTSB expressions. **ATG5.** Autophagy-related genes5; **ATG7.** Autophagy-related genes7; **LC3-II.** lipidated form of Microtubule-associated protein1- light chain 3; **LAMP2.** Lysosome-associated membrane protein 2; **P62.** Sequestosome 1 (SQSTM1); **CTSL.** Cathepsin L; **CTSB.** Cathepsin B.

Study Limitations

Our study has some limitations including: (1) it was not possible to measure both mRNA levels and protein levels of factors regulating autophagy, (2) it was not feasible to assess autophagy flux *in vitro*, e.g. by adding a lysosome inhibitor, and (3) there are no methods available to reliably count the numbers of autophagosomes.

Conclusion. HFD and ET upregulate autophagosome formation (markers of the early phase of the autophagy process), and also lysosome degradation last phase of the autophagy process) in white adipose tissue. However, the combination of HFD and ET enhances HFD-induced autophagosome formation and improves autolysosome formation impaired by HFD. Further, ET modulates lysosome degradation by upregulating CTSL and down-regulating CTSB in white adipose tissue.

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Conflicts of Interests

The authors report no conflict of interest.

Authors' Contributions

SD, GT, MD, AS, and HZ participated in the conception and design of the research; SD, MB, and SD and SB performed the experiment protocol and were responsible for collecting data. SD, MD, AS, IL and HZ were responsible for the writing and finalization of the manuscript. All authors approved the final version of the manuscript.

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