Muscle Type-Specific Modulation of Autophagy Signaling in Obesity: Effects of Caloric Restriction and Exercise

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Background:

Obesity is a global health issue that contributes to the development of various diseases through metabolic dysregulation. Recent findings indicate that obesity leads to autophagy dysregulation, a cellular degradation process. Caloric restriction (CR) and CR combined with exercise (CR+Ex) are effective strategies for managing obesity and modulating autophagy. However, the regulation of autophagy and its signaling pathways in skeletal muscle under conditions of obesity, CR, and CR+Ex remains poorly understood.

Method:

Mice were divided into six groups: normal diet, normal diet CR, normal die CR+Ex, high-fat diet, high-fat diet CR, and high-fat diet CR+Ex. All mice were fed ad libitum with either a normal or high-fat diet for the first four months, followed by the respective interventions for the subsequent four months. We examined body composition, skeletal muscle functions, and expression of autophagy signaling pathway in these mice.

Result:

Obesity resulted in increased body weight, lean mass, fat mass, and fat mass in tissue; decreased grip strength and endurance ($P < 0.05$). CR+Ex decreased body weight, lean mass, and fat mass in obese mice ($P < 0.05$). In red muscle, P62, LC3B-I and LC3B-II levels were elevated ($P < 0.05$), regardless of dietary conditions. High-fat diet induced Cathepsin L was reduced in red muscle ($P < 0.05$).

Conclusion:

Obesity leads to altered body composition and impaired skeletal muscle function, which are partially improved by CR+Ex. The modulation of the autophagy pathway was more pronounced in red muscle compared to white muscle, irrespective of the intervention. Autophagic activity was higher in red muscle compared to white muscle.

Keywords: Obesity, Autophagy, Calorie Restriction, Exercise

1. Introduction

Obesity, characterized by excessive fat accumulation, poses a significant global health challenge, with prevalence rates continuing to rise [1]. It is a major risk factor for numerous diseases, including cardiovascular diseases, type 2 diabetes, certain cancers, and musculoskeletal disorders [2]. The economic and health burdens associated with obesity necessitate the exploration of effective management strategies. Obesity-induced diseases are often caused by metabolic dysregulation, with autophagy playing a key role [3, 4]. Therefore, understanding autophagy can provide new insights into the management and treatment of obesity.

Autophagy involves three sub-mechanisms: chaperone-mediated autophagy, microautophagy, and macroautophagy, which work coordinately to clear damaged cellular matter and maintain homeostasis [5]. Macroautophagy (hereafter referred to as autophagy) utilizes a phagophore to engulf damaged material, then associates with a lysosome to form an autophagolysosome, leading to the hydrolysis of the selected components [6]. Autophagy, a cellular degradation process, is complex and influenced by various internal and external factors, including obesity, calorie restriction (CR), and exercise (Ex) [7]. Obesity induced-abnormal autophagy can contribute to the development of various diseases, such as non-alcoholic fatty liver disease and chronic kidney disease [3, 4].

CR and CR combined with Ex (CR+Ex) have garnered significant attention as non-pharmacological interventions for obesity management [8, 9]. CR, defined as a reduction in calorie intake without malnutrition, has been shown to extend lifespan, decrease body fat ratio, and improve metabolic health across various species [10]. Compared to CR alone, CR+Ex yields greater metabolic benefits for obesity, including improved insulin sensitivity [11], reduced inflammation [12], and enhanced lipid metabolism [13].

CR and CR+Ex are also known to modulate autophagic activity [14]. Both CR and Ex can regulate autophagy synergistically/individually through multiple pathways, including AMP-activated protein kinase (AMPK), mammalian target of rapamycin (mTOR) and forkhead box O3 (FoxO3) [15-17]. For example, CR and CR+Ex have been demonstrated to regulate the expression of autophagy signaling pathways in adipose tissue [18], liver [19], and heart [19], presenting a dual mechanism of action in combating obesity. Despite the promising potential of CR and CR+Ex in managing obesity, the specific regulatory mechanisms of autophagy in skeletal muscle under these conditions remain poorly understood.

Skeletal muscle plays a pivotal role in whole-body health [20]. It is composed of different fiber types, primarily red and white muscle fibers, each with distinct metabolic and functional properties [21]. The differential response of these muscle fiber types to autophagy regulation under conditions of obesity, CR, and CR+Ex is of significant interest. However, muscle type-specific modulation of autophagy under these conditions is currently understudied.

This study seeks to refine our understanding of the effects of obesity on autophagy and the mechanisms by which CR and CR+Ex modulate these effects. Additionally, it will explore how the type specificity of skeletal muscle conditions the regulation of autophagy in response to obesity, CR, and CR+Ex. The results of this study will provide scientific support for future treatment and management strategies for obesity and offer a scientific basis for prescribing exercise interventions.

2. Methods

2.1 Animals and Experimental Design

Male C57BL/6N mice (n=42, 12-months-old) were random divided into six experimental groups: normal diet control (NDC), normal diet control 20% calorie restriction (NDR), normal diet control 20% calorie restriction + voluntary wheel exercise (NDRE), high-fat diet control (HFC), high-fat diet control 20% calorie restriction (HFR), high-fat diet control 20% calorie restriction + voluntary wheel exercise (HFRE). For the first 4 months, all mice were fed either a standard chow diet (ND; Teklad Global 2018, Envigo Inc.) or a high-fat diet (HF; 45% kcal from fat, D12451, Research Diets Inc.) (Table 1). In the subsequent 4 months, 20% calorie restriction (CR) and 20% calorie restriction + voluntary wheel exercise (CR+Ex) interventions were applied to the respective groups (Figure 1). The mice were housed in a controlled environment, maintained at a temperature of 22 ± 2 °C with 50–60% humidity, and subjected to a 12-hour light/dark cycle. All animals had ad libitum access to water and were allowed physical activity. The experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Hanyang University (HYU 2021-0239A).

Table 1. Diet Composition

Group	NDC, NDR, and NDRE		HFC, HFR, and HFRE	
Diet	Teklad Global 2018		D12451	
	gm%	kcal%	gm%	kcal%
Protein	18.4	30	24	20
Carbohydrate	44.2	50	41	35
Fat	6	20	24	45
Total		100		100
Kacl/gm	3.1		4.73	

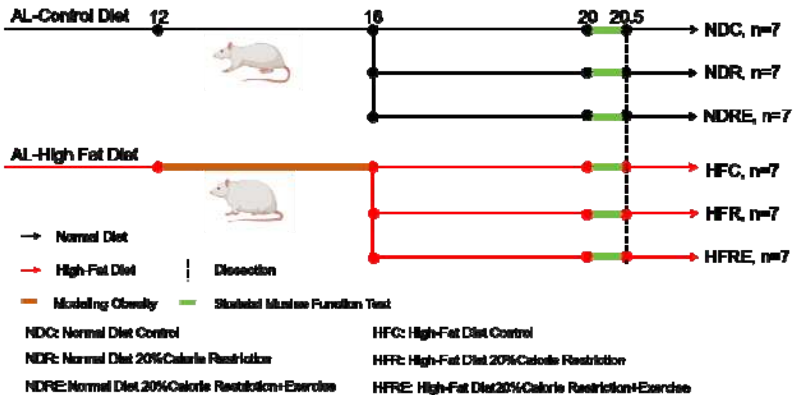


Figure 1. Schematic overview experimental study design.

2.2 Skeletal Muscle Function Tests

Skeletal muscle function was examined in mice two weeks prior to euthanasia. The function was evaluated through tests measuring walking speed, endurance, physical activity, and grip strength across six groups of mice.

Walking Speed: Walking speed was evaluated using the Rota-Rod test. An adaptation period was conducted over the first week, involving a pre-test exercise at a constant speed of 5 rpm for 1 minute, once per day. For the official test, the Rota-Rod was set to acceleration mode, increasing the speed from 5 to 50 rpm over a 5-minute period (Model 76-0770, Harvard Apparatus Inc., Holliston, MA, USA). The latency to fall from the device was recorded for each mouse. Each mouse underwent three trials with a ten-minute interval between tests, and the best performance among the three trials was used as the final outcome measure.

Endurance Capacity: Endurance capacity was assessed using a treadmill test. One week prior to the official test, an adaptation phase was conducted where the mice ran at 3 cm/s for 10 minutes daily on a 0-degree slope. During the official test, the treadmill speed increased by 1 cm/s every 20 seconds, starting from 5 cm/s, on a 0-degree slope. The trial concluded when the mouse touched the shock pad (set at 0.5 mA) three times.

Physical Activity: Physical activity was measured using the voluntary wheel running test. Running distance was recorded with a voluntary wheel (MAN86130, Lafayette Instrument Company, Lafayette, IN, USA), where each wheel rotation equaled a distance of 0.4 meters. The average running distance over a 5-day period was documented for each mouse.

Grip Strength: Grip strength was evaluated using the inverted-cling grip test. An adaptation phase was conducted once daily during the first week. During the official test, each mouse was placed in the center of a wire mesh screen, and a stopwatch was started. The screen was then rotated to an inverted position over the course of 2 seconds, with the mouse's head descending first, and held 40-50 cm above a padded surface. The time until the mouse fell was recorded. This procedure was repeated three times with ten-minute intervals between tests, and the longest time recorded was used as the final measurement.

2.3 Body Composition

Body composition was examined after skeletal muscle function test. The mice were anesthetized with 40 mg/kg ketamine and 0.8 mg/kg medetomidine, and their body composition was analyzed using an InAlyzer Dual-energy X-ray Absorptiometry (DEXA) system from Micro Photonics Inc. (PA, USA). The measured parameters included total mass (g), fat mass (g), lean mass (g), and fat mass in tissue (%).

2.4 Western-Immunoblot (WB)

Then euthanize the anesthetized mouse by cervical dislocation. Following euthanasia, the red and white gastrocnemius were dissected separately [22]. The muscles were lysed using an EzRIPA Lysis Kit (WSE-7420, ATTO). Protein concentration was determined using the Pierce™ Bicinchoninic Acid Protein Assay Kit (Thermo Scientific, Waltham, MA, USA). Equal amounts of protein samples (35 µg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electro-transferred to nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA, USA). The membranes were blocked with 5% non-fat milk dissolved in Tris-buffered saline with Tween-20 (TBST; 10 mM Tris, 150 mM NaCl, and 0.1% Tween-20; pH 7.6) for 1.5 hours at room temperature and then incubated with primary antibody overnight at 4°C. Subsequently, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody for 1.5 hours at room temperature. The following primary and secondary antibodies were used: Beclin-1 (Cell signaling, #3495, 1:1000), ATG-7 (Cell signaling, #8558, 1:1000), P62 (Cell signaling, #23214, 1:1000), LC3B (Sigma, L7543, 1:500), LAMP-2 (Invitrogen, #PA1-665, 1:1000), Cathepsin-L (Abcam, Ab133641, 1:1000), Goat anti Rabbit (Invitrogen, #G-21234, 1:5000), m-IgG Fc (SANTA, sc-525409, 1:5000). Ponceau S staining was used to normalize quantitative protein. Targeted bands were quantified via densitometry using Image J software (National Institutes of Health, Bethesda, MD, USA).

2.5 Statistical Analysis

Statistical analyses were performed using GraphPad Prism software (version 9). Two-way analysis of variance with Bonferroni' s post hoc test was applied. All results are expressed as the mean ± SD. Statistical significance was set at $p < 0.05$, with asterisks indicating the following levels of significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

3. Results

3.1 *CR+Ex Improved Some of the Body Composition Changes Resulting From Obesity*

The data showed that a normal diet did not alter mice total mass, lean mass, fat mass, or fat mass in tissue ($P > 0.05$). However, obesity induced by a high-fat diet resulted in a significant increase in all these indices ($P < 0.05$) (Figure 2C). Additionally, neither CR nor CR+Ex altered body composition in normal diet mice ($P > 0.05$). In contrast, CR+Ex resulted in a significant decrease in total mass, lean mass, and fat mass in obese mice ($P < 0.05$), but not in fat mass in tissue ($P > 0.05$) (Figure 2C). These results indicate that high-fat diet-induced obesity leads to significant changes in body composition, and that only CR+Ex improves some aspects of body composition in high-fat diet-induced obese mice.

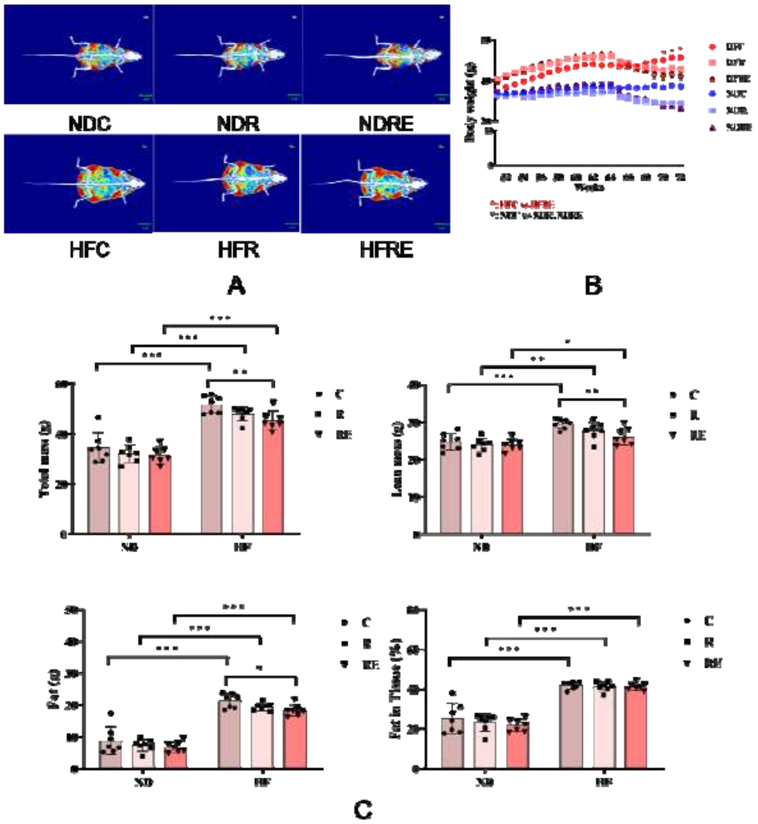


Figure 2. Only CR+Ex Improved Some of the Body Composition Changes Resulting From Obesity (A) Representative DEXA-scanned images of NDC, NDR, NDRE, HFC, HFR, and HFRE mice; skeletal muscle, fat tissue, and bone are shown in blue, red, and white, respectively. (B) Weekly body weight changes (group × weeks). (C) Quantitative data of final total mass, lean mass, fat mass, and fat mass in tissue, respectively, were acquired by DEXA scanning. Significant differences are denoted by asterisks: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****). All values are presented as mean ± SD. Normal diet control (NDC, 20months, n=7), normal diet control 20% calorie restriction (NDR, 20months, n=7), normal diet control 20% calorie restriction + voluntary wheel exercise (NDRE, 20months, n=7), high-fat diet control (HFC, 20months, n=7), high-fat diet control 20% calorie restriction (HFR, 20months, n=7), high-fat diet control 20% calorie restriction + voluntary wheel exercise (HFRE, 20months, n=7).

3.2 Obesity Induced Certain Skeletal Muscle Dysfunction Not Reversed by CR and CR+Ex

The results showed that high-fat diet-induced obesity resulted in reduced grip strength and endurance in mice ($P < 0.05$) (Figure 3A and B). However, physical activity and walking speed were not altered ($P > 0.05$) (Figure 3C and D). Additionally, it was unexpectedly found that neither CR nor CR+Ex significantly improved skeletal muscle function, regardless of dietary condition. These data suggest that high-fat diet-induced obesity reduces some aspects of skeletal muscle function, CR and CR+Ex do not have a significant effect on reversing these impairments.

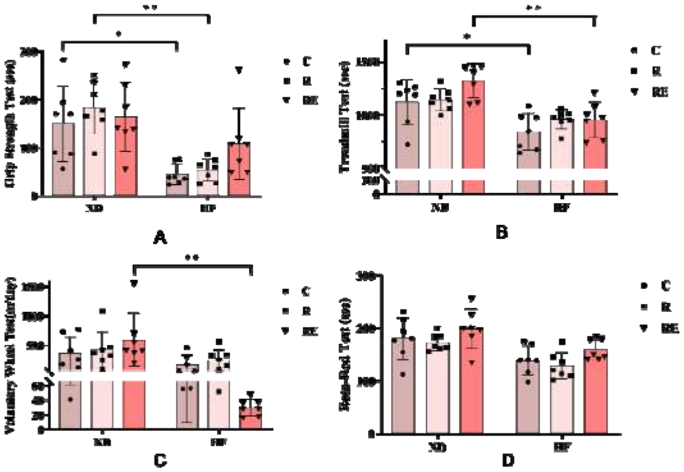


Figure 3. Part of Skeletal Muscle Function Reduced by Obesity and Not Reversed by CR and CR+Ex. (A) Strength test. (B) Endurance test. (C) Physical activity test. (D) Walking speed test. Significant differences are denoted by asterisks: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****). All values are presented as mean ± SD. Normal diet control (NDC, 20months, n=7), normal diet control 20% calorie restriction (NDR, 20months, n=7), normal diet control 20% calorie restriction + voluntary wheel exercise (NDRE, 20months, n=7), high-fat diet control (HFC, 20months, n=7), high-fat diet control 20% calorie restriction (HFR, 20months, n=7), high-fat diet control 20% calorie restriction + voluntary wheel exercise (HFRE, 20months, n=7).

3.3 Increased Autophagic Activity via P62, LC3B-I and LC3B-II in Red Muscle Compared to White Muscle.

First, we examined the role of same skeletal muscle types in modulating autophagy pathway signaling expression under various dietary (normal diet and high-fat diet) and intervention conditions (CR and CR+Ex) (Figure 4). The results showed that neither CR nor CR+Ex significantly modulated the expression of autophagy pathway signaling under different dietary conditions, regardless of skeletal muscle type ($P > 0.05$).

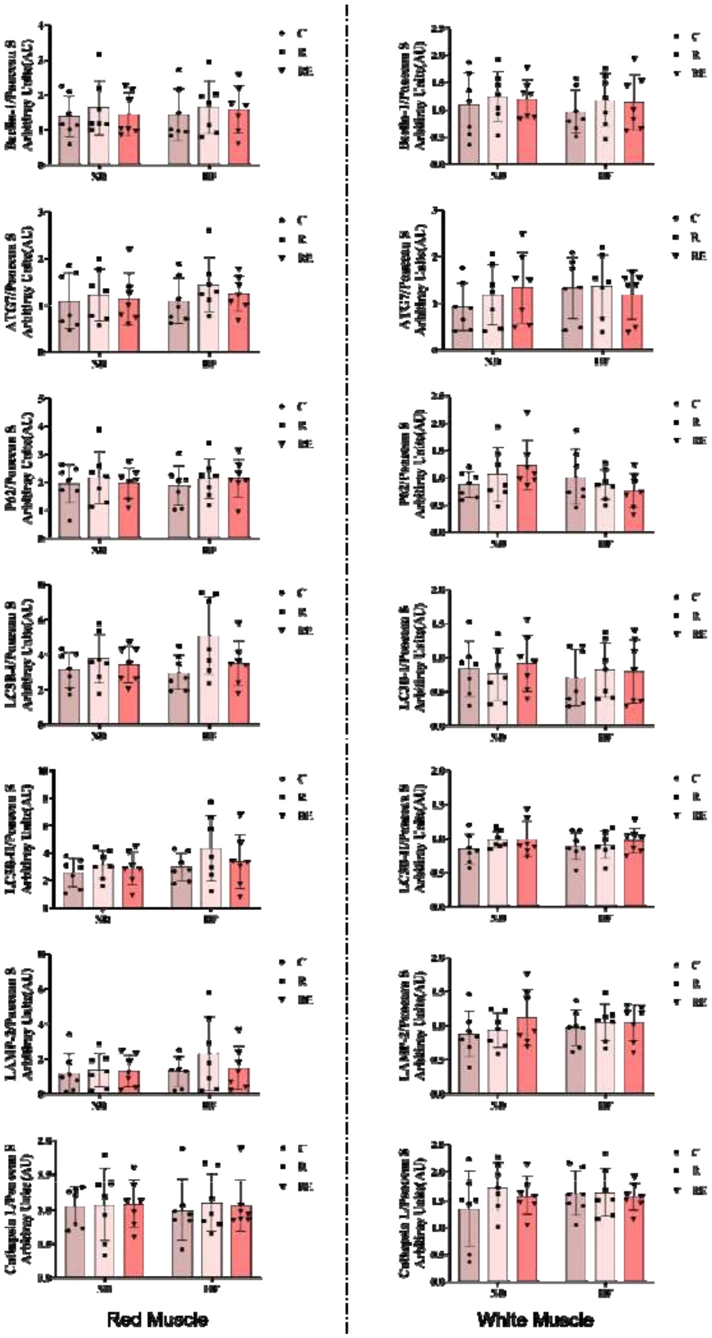


Figure 4. No significant differences in autophagy pathway signaling between the same skeletal muscle types. All values are presented as mean ± SD. Normal diet control (NDC, 20months, n=7), normal diet control 20% calorie restriction (NDR, 20months, n=7), normal diet control 20% calorie restriction + voluntary wheel exercise (NDRE, 20months, n=7), high-fat diet control (HFC, 20months, n=7), high-fat diet control 20% calorie restriction (HFR, 20months, n=7), high-fat diet control 20% calorie restriction + voluntary wheel exercise (HFRE, 20months, n=7).

Next, we examined the role of the same dietary conditions in modulating autophagy pathway signaling expression under different skeletal muscle types (white and red) and intervention conditions (CR and CR+Ex) (Figure 5). The results showed increased expression of P62, LC3B-I, and LC3B-II in red muscle compared to white muscle (P < 0.05), regardless of diet conditions (Figure 5). Changes similar to those observed with a normal diet, high-fat diet-induced obesity also causes specific alterations in autophagy signaling in mice skeletal muscle. Compared to white muscle, a high-fat diet results in decreased expression of Cathepsin L in red muscle (P < 0.05). Furthermore, compared to a normal high-fat diet, CR leads to a significant increase in the expression of LC3B-I in red muscle. These results indicate that skeletal muscle types regulate autophagy signaling more significantly compared to diet, CR, and CR+Ex.

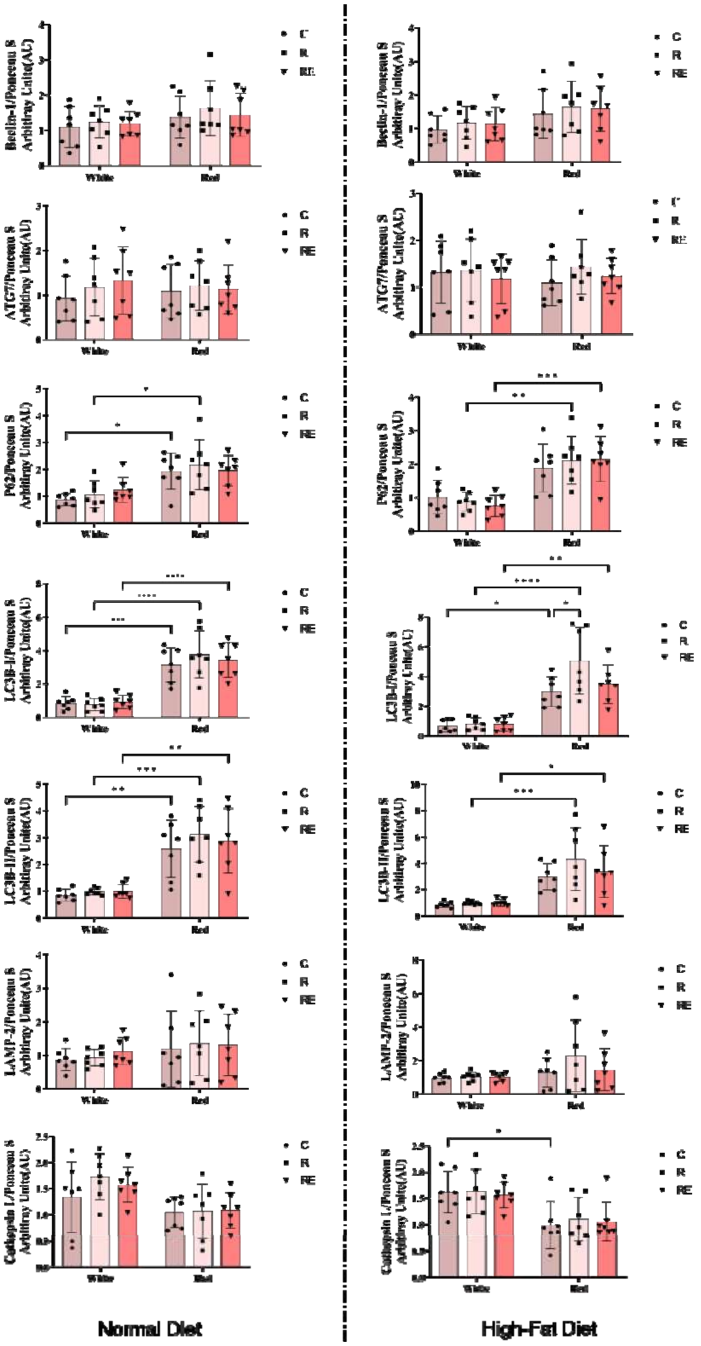


Figure 5. Increased Autophagic Activity via P62, LC3B-I and LC3B-II in Red Muscle Compared to White Muscle. Significant differences are denoted by asterisks: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****). All values are presented as mean ±SD. Normal diet control (NDC, 20months, n=7), normal diet control 20% calorie restriction (NDR, 20months, n=7), normal diet control 20% calorie restriction + voluntary wheel exercise (NDRE, 20months, n=7), high-fat diet control (HFC, 20months, n=7), high-fat diet control 20% calorie restriction (HFR, 20months, n=7), high-fat diet control 20% calorie restriction + voluntary wheel exercise (HFRE, 20months, n=7).

4. Discussion

Obesity has consistently been a prominent topic due to its significant threat to human health [1, 2]. Although Obesity induced-abnormal autophagy can lead to the development of various diseases, there is a lack of consensus on the mechanisms through which obesity regulates autophagy and the potential intervention strategies [3, 4]. Our results show that diet (normal and high-fat) and intervention (CR and CR+Ex) do not significantly modulate autophagy within the same skeletal muscle type. However, skeletal muscle type (white and red) and intervention (CR and CR+Ex) do modulate autophagy under the same dietary conditions. This suggests that skeletal muscle type specificity exerts a stronger influence on autophagy regulation. These findings enhance our understanding of the mechanisms underlying obesity and autophagy, providing a scientific basis for further in-depth studies on obesity and cellular degradation mechanisms.

4.1 Appropriateness of Obesity Mouse Model

In this experiment, we utilized 12-months-old mice, which correspond to humans aged 30-40 years [23]. It is well-documented that the prevalence of obesity in humans increases with age, especially after 35 years [24]. To model the onset of obesity around age 35 in humans, the mice were subjected to a high-fat diet at 12-months-old. We employed a high-fat diet (D12451, Research Diets Inc.) for 4 months to induce obese mouse model. This particular high-fat diet (D12451, Research Diets Inc.) is widely used for developing obese mouse models [25-27]. Moreover, while there is no universal definition for high-fat diet-induced obesity in mice, most studies use a minimum of 8 weeks of ad libitum high-fat diet (D12451, Research Diets Inc.) to establish such models [25, 28, 29]. Therefore, a four-month high-fat diet is sufficient to successfully establish an obese mouse model.

Upon analyzing the mice body composition using DEXA, significant increases were observed in body weight, lean mass, fat mass, and fat mass in tissue after the high-fat diet. These changes in body composition are consistent with those reported in previous studies on obese mice [30-32]. Additionally, obesity was found to impair skeletal muscle functions, supporting prior research that identifies skeletal muscle dysfunction as a characteristic of obesity [33, 34]. Therefore, our results indicate that a four-month high-fat diet effectively induces obesity in mice, providing a robust experimental model for further study.

4.2 Body Composition and Skeletal Muscle Function

Our results differ from those of earlier studies [35, 36]. Under normal dietary conditions, CR and CR+Ex did not alter body composition in mice. Additionally, in high-fat diet-induced obese mice, CR failed to alter body composition, while only CR+Ex significantly reduced total mass, lean mass, and fat mass. The difference in results may be related to the intensity of CR and the age of the experimental mice. In our study, CR intensity was lower (20%) and the mice were older (12 to 20-months-old) compared to the prior studies. Increasing age leads to slower metabolism and reduced responsiveness and adaptability to external stimuli [37]. High-intensity CR has a negative effect on older mice [38], so we chose low CR to protect the survival of late middle-aged mice and successfully complete the experiment.

CR+Ex altered only part of the body composition in the high-fat diet group, but not in the normal diet group, likely due to the higher initial body weight [39] and higher fat oxidation efficiency [40], making these mice more sensitive to the effects of CR and Ex. Overall, obesity caused by a high-fat diet altered the body composition of mice, and CR+Ex improved some of these effects.

Our data show that high-fat-induced obesity leads to reduced grip strength and endurance, consistent with prior studies [41, 42]. However, physical activity and walking speed were not affected. Contrary to predictions, CR and CR+Ex did not significantly increase skeletal muscle function in late middle-aged mice, regardless of dietary condition. This may be due to the combination of low-intensity CR and exercise, as well as the reduced responsiveness and adaptability to external stimuli at advanced age [37].

4.3 Regulation of Skeletal Muscle Autophagy by CR and CR+Ex

To investigate autophagy and regulatory mechanisms in the skeletal muscle of obese late middle-aged mice, we first examined the expression of autophagy signaling pathways in the same muscle type under different dietary conditions (high-fat diet and normal diet) and intervention conditions (CR and CR+Ex).

We did not observe any changes in the expression of autophagy pathway signals due to high-fat diet-induced obesity. Although such results are uncommon, there have been reports of no significant changes in autophagy signaling in the heart [43], skeletal muscle [44], and liver [45] following high-fat diet-induced obesity. Obesity induced by a high-fat diet may interfere with autophagy signaling pathways through various mechanisms. Obesity caused by a high-fat diet can affect cellular nutrient sensing mechanisms, leading to the dysregulation of autophagy signaling pathways. For instance, a high-fat diet can result in insulin resistance and metabolic syndrome, thereby disrupting the regulatory mechanisms of autophagy [46, 47]. Studies have shown that despite the appropriate upregulation of autophagy-related genes at the mRNA level, there is a failure to correspondingly upregulate at the protein level, indicating an inconsistency in cellular autophagy regulation under obese conditions [43]. Additionally, oxidative stress and inflammatory responses triggered by a high-fat diet induced-obesity may disrupt the normal function of autophagy signaling pathways. The accumulation of fatty acids and the increase of inflammatory factors such as TNF-α and IL-1β can inhibit the autophagy process [48].

We also did not observe changes in the autophagy pathway in the same skeletal muscle type due to CR and CR+Ex, regardless of diet conditions. Previous studies support our findings, as some research has indicated that long-term CR and/or CR+Ex do not induce significant changes in autophagy signaling proteins [14, 19, 43]. The impact of these interventions on autophagy signaling pathways may not always be consistent. For example, long-term CR and/or CR+Ex were found to upregulate autophagy-related protein mRNA expression, but this upregulation did not translate into significant changes at the protein level [14, 43]. Additionally, while these interventions can activate autophagy to some extent, the response of autophagy signaling in tissues such as the heart and skeletal muscle may not be significant [19, 43]. This suggests tissue-specific expression of autophagy.

Both CR and Ex promote autophagy by inhibiting mTOR signaling pathway and activating AMPK. The overlap of these pathways might lead to a lack of significant enhancement in autophagy activity when combined, as they operate through similar mechanisms [49]. Research indicates that CR and Ex can independently activate AMPK and inhibit mTOR, thereby promoting autophagy. However, when CR and Ex are combined, these signaling pathways might already be maximally activated, preventing further enhancement of autophagy activity [50, 51]. Additionally, both CR and Ex can independently regulate autophagy through the PI3K/AKT/mTOR and ERK1/2-MAPK signaling pathways. When used together, these pathways might already be fully activated, resulting in no additional significant effects from their combination [52].

4.4 Regulation of Autophagy by Skeletal Muscle Type-Specific

Subsequently, we examined the differences in autophagy expression between different types of skeletal muscle. The expression of certain autophagy-related proteins was higher in red muscle compared to white muscle. Additionally, dietary conditions and intervention methods did not alter the muscle type-specific regulation of autophagy.

The expression of P62 is higher in red muscle than in white muscle, primarily due to differences in metabolic demands and functions. Red muscle has higher oxidative metabolic activity, producing more reactive oxygen species (ROS). P62 helps mitigate oxidative stress by activating the transcription factor NFE2-related factor 2 (Nrf2) signaling pathway, which enhances the expression of antioxidant enzymes [53]. Secondly, red muscle contains more mitochondria and has higher oxidative metabolism, necessitating elevated autophagic activity to maintain mitochondrial quality and function. P62 plays a crucial role in autophagy [54]. Additionally, red muscle has a greater capacity for fatty acid oxidation, requiring high levels of P62 to regulate fatty acid uptake and metabolism [55]. P62 also acts as a bridge in autophagy, promoting the degradation of damaged proteins. Red muscle needs higher levels of P62 to maintain proper protein degradation and recycling [56]. Furthermore, there are significant differences in gene expression between red and white muscle, with many autophagy and metabolism-related genes being more highly expressed in red muscle, including P62 [57].

Studies have shown that the expression of LC3B-I and LC3B-II is significantly higher in red muscle than in white muscle. Red muscle has higher oxidative metabolic activity and more mitochondria, which requires higher autophagic activity to maintain mitochondrial quality and function. LC3B plays a key role in this process [54, 58]. Additionally, gene chip analysis and protein expression studies have found that the expression levels of various genes and proteins are significantly higher in red muscle than in white muscle, including those related to autophagy [57, 59]. These findings indicate that the high expression of LC3B in red muscle is primarily to meet its higher oxidative metabolic demands and to respond to oxidative stress, maintaining cellular homeostasis and function.

In an obesity state induced by a high-fat diet, the expression of Cathepsin L is higher in white muscle than in red muscle, likely due to differences in metabolic demands, inflammatory responses, and autophagic needs. Firstly, white muscle fibers primarily undergo glycolytic metabolism, and a high-fat diet increases their metabolic burden, leading to a higher demand for protein degradation, thereby promoting Cathepsin L expression [58]Secondly, obesity induced by a high-fat diet is accompanied by chronic inflammation and oxidative stress. White muscle is more susceptible to these stress factors, resulting in increased Cathepsin L expression [60]. Finally, Cathepsin L plays a crucial role in autophagy. A high-fat diet increases the protein degradation demand in white muscle, leading to its high expression to meet autophagic needs [61]. These mechanisms collectively contribute to the high expression of Cathepsin L in white muscle to cope with metabolic stress and protein degradation demands.

Overall, the expression of autophagy-related proteins was higher in red muscle than in white muscle, which is closely related to different metabolic demands and functions. Red muscle have a higher oxidative capacity, making them more reliant on the autophagy process to cope with cellular stress and maintain intracellular homeostasis [62]. Furthermore, autophagy activity in Red muscle helps remove damaged mitochondria and proteins, maintaining muscle function and metabolic stability [63]. These characteristics enable Red muscle to better adapt to prolonged low-intensity activities and more effectively carry out autophagy to maintain cellular health.

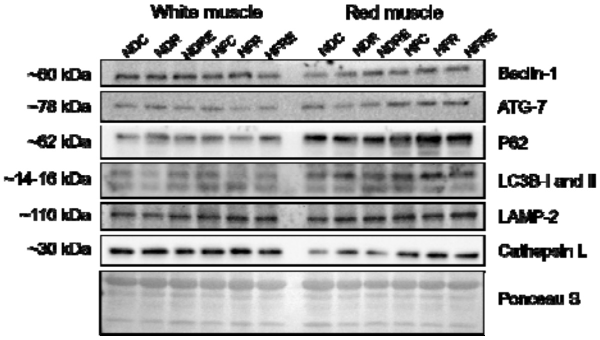
Conclusion

Among the various interventions studied, only the combination of CR+Ex was effective in partially reversing the high-fat diet-induced increases in body weight, lean mass, and fat mass. Moreover, muscle type specificity plays a crucial role in the regulation of autophagy, which is not easily modified by dietary or exercise interventions. This study provides valuable insights into the muscle type-specific mechanisms of autophagy in the context of obesity and highlights the potential for tailored exercise and dietary strategies in managing obesity-related health issues.

Limitation

This study exclusively used male mice, which may not fully represent the responses of other breeds or female mice. Additionally, more advanced research methods, such as spatial proteomics, should be employed to more accurately analyze the muscle type-specific regulation of autophagy pathways.

Representative Picture of Western-Immunoblot



Ethical approval and consent to participate

All the procedures followed in this experiment were approved by the Institutional Animal Care and Use Committee (IACUC) of Hanyang University (HYU 2021-0066A).

Consent for publication

Not applicable.

Availability of data and materials

The data used to support the findings of this study are presented here. Any further data requirements are available from the corresponding author upon request.

Competing interests

No conflicts of interest, financial or otherwise, have been declared by the author(s).

Funding

This research was supported by the National Research Foundation of Korea (NRF-2020R1F1A1061726).

Author' s contributions

F.J.J. and J.H.K. conceived and designed the study. F.J.J. and Y.J.P. performed the experiments. F.J.J. analyzed the data and prepared the figures. F.J.J. and J.H.K. interpreted the results, drafted, edited, and revised the manuscript. J.H.K. acquired funding. All authors approved the final version of the manuscript.

Acknowledgments

We thank Haesung Lee (Hanyang University, Korea) for providing technical support with the statistical analysis of the experimental data.

We thank Jian Guo (Hanyang University, Korea) for his generous help in dissecting the mice.

We are grateful to Prof. Dr. Gwang-woong Go (Hanyang University, Korea) for generously providing the DEXA machine for body composition analysis of the mice.

We thank the support of Skill Learning from Kaixin Doctor and MASCU (Medical Association with Science, Creativity, and Unity), Inc, Shenzhen, China (mascu_forever@163.com).

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