

# CHANGES RELATED TO MUSCLE AUTOPHAGY AFTER EXHAUSTIVE EXERCISE AND BLUNT TRAUMA

ALTERAÇÕES RELACIONADAS À AUTOFAGIA MUSCULAR APÓS EXERCÍCIO EXAUSTIVO E TRAUMA CONTUSO

CAMBIOS RELACIONADOS CON LA AUTOFAGIA MUSCULAR TRAS EJERCICIO EXAUSTIVO Y TRAUMATISMO CONTUSO

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## ABSTRACT

**Objective:** To study the temporal changes of autophagy related factors in skeletal muscle of rats after exhaustive exercise and blunt trauma. **Methods:** Forty-two male SD rats were divided into 7 groups with 6 rats in each group: Quiet control group (C), immediately after exhaustive exercise (E0), 24 hours after exhaustive exercise (E24), 48 hours after exhaustive exercise (E48), immediately after blunt trauma (D0), 24 hours after blunt trauma (D24), 48 hours after blunt trauma (D48). All groups of rats were killed and sampled respectively at different time points specified above, and the right gastrocnemius muscle was taken, which was divided into two parts, one for mRNAs of Lamp-2, BNIP3 and NIX by real-time fluorescent quantitative PCR, and the other for p62 protein by Western blotting. **Results:** (1) Compared with group C, mRNA levels of p62, Lamp-2 and NIX in group E48 were significantly increased after exhaustive exercise ( $P < 0.05$ ), suggesting that autophagy increased in 48h after exhaustive exercise. (2) Compared with group C, p62mRNA and Lamp-2 mRNA levels were significantly increased immediately after blunt trauma ( $P < 0.05$ ) and decreased significantly in 48h after blunt trauma ( $P < 0.05$ ), suggesting that autophagy activity was enhanced immediately after blunt trauma and decreased in 48h after injury. **Conclusions:** Generally, there were differences at each recovery phase between blunt trauma and exhausted exercise models, and the basal autophagy factors and mitochondrial autophagy factors were also inconsistent. Basal autophagy factors p62 and Lamp-2 increased significantly 48 hours after eccentric exhaustive exercise and immediately after blunt trauma. Mitochondrial autophagy factor BNIP3 did not increase after exhaustive exercise and blunt trauma, but NIX only increased after exhaustive exercise. Its molecular mechanism needs to be further studied. **Level of Evidence III; Therapeutic Studies Investigating the Results of Treatment.**

**Keywords:** Autophagy-Related Proteins; Muscle, Skeletal; Wounds, Nonpenetrating; Muscle Fatigue.

## RESUMO

**Objetivo:** Estudar as alterações temporais dos fatores relacionados à autofagia no músculo esquelético de ratos após exercício exaustivo e trauma contuso. **Métodos:** Quarenta e dois ratos machos SD foram divididos em 7 grupos com 6 ratos em cada grupo: Grupo de controle silencioso (C), imediatamente após o exercício exaustivo (E0), 24 horas após o exercício exaustivo (E24), 48 horas após o exercício exaustivo (E48), imediatamente após o trauma contuso (D0), 24 horas após o trauma contuso (D24), 48 horas após o trauma contuso (D48). Todos os grupos de ratos foram mortos e rotulados, respectivamente, em diferentes momentos especificados acima, e o músculo gastrocnêmio direito foi retirado, dividido em duas partes, uma para mRNAs de Lamp-2, BNIP3 e NIX por PCR quantitativo fluorescente em tempo real, e a outra para a proteína p62 por imunotransferência. **Resultados:** (1) Em comparação com o grupo C, os níveis de mRNA de p62, Lamp-2 e NIX no grupo E48 aumentaram significativamente após o exercício exaustivo ( $P < 0,05$ ), sugerindo que a autofagia aumentou em 48 horas após o exercício exaustivo. (2) Em comparação com o grupo C, os níveis de mRNA de p62mRNA e Lamp-2 foram significativamente aumentados imediatamente após o trauma contuso ( $P < 0,05$ ) e diminuíram significativamente em 48 horas após o trauma contuso ( $P < 0,05$ ), sugerindo que a atividade de autofagia foi aumentada imediatamente após o trauma contuso e diminuiu em 48 horas após a lesão. **Conclusão:** Houve, via de regra, diferenças em cada fase de recuperação entre os modelos de trauma contuso e de exercício exaustivo, sendo que os fatores de autofagia basal e os fatores de autofagia mitocondrial também foram inconsistentes. Os fatores de autofagia basal p62 e Lamp-2 aumentaram significativamente 48 horas após o exercício excêntrico exaustivo e imediatamente após o trauma contuso. O fator de autofagia mitocondrial BNIP3 não aumentou após o exercício exaustivo e o trauma contuso, mas o NIX aumentou somente após o exercício exaustivo. Seu mecanismo molecular precisa ser investigado com mais detalhes. **Nível de Evidência III; Estudos Terapêuticos que Investigam os Resultados do Tratamento.**

**Descritores:** Proteínas Relacionadas à Autofagia; Músculo Esquelético; Ferimentos não Penetrantes; Fadiga Muscular.

## RESUMEN

**Objetivo:** Estudiar los cambios temporales de los factores relacionados con la autofagia en el músculo esquelético de ratas tras el ejercicio exaustivo y el traumatismo contuso. **Métodos:** Se dividieron 42 ratas SD macho en 7 grupos



con 6 ratas en cada grupo: grupo de control silencioso (C), inmediatamente después del ejercicio exhaustivo (E0), 24 horas después del ejercicio exhaustivo (E24), 48 horas después del ejercicio exhaustivo (E48), inmediatamente después de un traumatismo contuso (D0), 24 horas después de un traumatismo contuso (D24), 48 horas después de un traumatismo contuso (D48). Todos los grupos de ratas fueron sacrificados y rotulados, respectivamente, en diferentes momentos especificados anteriormente, y se extrajo el músculo gastrocnemio derecho, dividido en dos partes, una para los ARNm Lamp-2, BNIP3 y NIX mediante PCR cuantitativa fluorescente en tiempo real, y la otra para la proteína p62 mediante Western blotting. Resultados: (1) En comparación con el grupo C, los niveles de ARNm de p62, Lamp-2 y NIX en el grupo E48 aumentaron significativamente tras el ejercicio exhaustivo ( $P < 0,05$ ), lo que sugiere que la autofagia aumentó en las 48 horas posteriores al ejercicio exhaustivo. (2) En comparación con el grupo C, los niveles de ARNm de p62 ARNm y Lamp-2 aumentaron significativamente inmediatamente después del traumatismo contuso ( $P < 0,05$ ) y disminuyeron significativamente a las 48 horas después del traumatismo contuso ( $P < 0,05$ ), lo que sugiere que la actividad de autofagia aumentó inmediatamente después del traumatismo contuso y disminuyó a las 48 horas después de la lesión. Conclusión: En general, hubo diferencias en cada fase de recuperación entre los modelos de traumatismo contuso y ejercicio exhaustivo, y los factores de autofagia basal y los factores de autofagia mitocondrial también fueron inconsistentes. Los factores de autofagia basal p62 y Lamp-2 aumentaron significativamente 48 horas después del ejercicio excéntrico exhaustivo e inmediatamente después del traumatismo contuso. El factor de autofagia mitocondrial BNIP3 no aumentó tras el ejercicio exhaustivo y el traumatismo contuso, pero NIX sólo aumentó tras el ejercicio exhaustivo. Su mecanismo molecular debe investigarse con más detalle. **Nivel de Evidencia III; Estudios Terapéuticos que Investigan los Resultados del Tratamiento.**

**Descriptor:** Proteínas Relacionadas con la Autofagia; Músculo Esquelético; Heridas no Penetrantes; Fatiga Muscular.

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## INTRODUCTION

After skeletal muscle injury, the body will repair the damaged part. In addition to muscle satellite cells' repair, autophagy is also an important repair procedure of skeletal muscle injury.<sup>1</sup> Mitochondrial autophagy mainly refers to the depolarization damage of intracellular mitochondria under the stimulation of ROS, nutritional deficiency, cellular aging, etc., and the damaged mitochondria are specifically wrapped into the autophagosome and integrated with the lysosome, thus completing the degradation of damaged mitochondria and maintaining the stability of the intracellular environment.<sup>2,3</sup> BNIP3/NIX is an important mitochondrial autophagy pathway under hypoxia conditions.<sup>4</sup> P62 is an autophagy receptor protein, which can bring ubiquitinated autophagy substrates into the autophagosome and play an important role in the degradation of toxic protein aggregates. Since it can be degraded together with substrates in lysosomes, p62 is often used as a marker for autophagy degradation.<sup>5</sup> The autophagy process ends with the degradation of autophagy substrate in lysosome. Lamp-2, as a lysosome membrane protein, mediates the fusion of autophagosome and lysosome to promote the degradation of substrate.<sup>6</sup>

Non-motor injury can also cause changes in skeletal muscle autophagy. Studies have shown that mitochondrial autophagy related proteins Drp1, BNIP3, Pink1 and Parkin are significantly increased after skeletal muscle freezing injury and mitochondrial dysfunction in mice, LC3II is increased and induced mitochondrial autophagy.<sup>7</sup>

In this study, two injury models were established such as eccentric exhaustive exercise and acute blunt trauma, and autophagy related genes Lamp-2, P62, BNIP3/NIX mRNA expression and p62 protein content were detected at immediately, 24h and 48h after exhaustive exercise and blunt trauma, so as to analyze the effects of different phase after skeletal muscle injury on autophagy factors, and explore the related biological significance and possible mechanism.

## METHODS

### Animals and grouping

Male Sprague Dawley rats (aged 8 weeks, weighted  $130 \pm 20$  g,  $n = 6$  per group) were obtained from Comparative Medicine Centre, Jiangsu

University (Jiangsu, China). Rats were breed and raised in Human Movement Science Laboratory in Yangzhou University. All animal experiments fit in the 'Guide for the Care and Use of Laboratory Animals' guidelines (NIH Publications no. 8023, revised 1978) and approved by the Animal Care and Use Committee of Yangzhou University for the treatment of animals (Jiangsu, China. SCXK 2013-0011). All rats were controlled at approximately 22°C with a 12/12 h light-dark cycle and housed in a home cage 30 x 41 cm and 25 cm height in a clean room. Solid diet and water were provided ad libitum.

### Grouping

Forty-two male SD rats were divided into 7 groups with 6 rats in each group: Quiet control group (C), immediately after blunt trauma (D0), 24 hours after blunt trauma (D24), 48 hours after blunt trauma (D48). immediately after exhaustive exercise (E0), 24 hours after exhaustive exercise (E24), 48 hours after exhaustive exercise (E48). All rats were euthanized by sodium pentobarbital overdose (200 mg/kg, intraperitoneal) and killed respectively at different time points according to above groups. The rats were sampled immediately or 24 and 48 hours after exhaustive exercise and blunt trauma respectively. The right gastrocnemius muscle of the hind limbs were taken and stored at  $-80^{\circ}\text{C}$ , then mixed and ground the middle section of the sample for Real-time PCR and Western blotting.

### A single bout of exhaustive treadmill exercise

The graded exhaustive exercise protocol was adapted by a modification of the method of Lin et al.<sup>8</sup> The treadmill installed electric shock grid on the rear obstacle to give the animal power to exercise. In the exhaustive exercise groups, the rats were tested for 15 - 20 minutes of accommodate treadmill exercise at 10 - 30 meters / minute for 6 day. During the exercise test, they were asked to run in six lanes inclined treadmill ( $-10^{\circ}$ ). The treadmill speed were increased gradually to 10, 15, 20, 25m/min for 10min in each gear, and then accelerated to 30m/min until exhaustion of rats. The exhaustion standard is that the rats can no longer keep pace with the treadmill at the last speed 30 meters / minute, and can't stand erect when placed on the back at rest. The average exhaustion time was 102 minutes in our experiment.

## Blunt trauma

The blunt trauma to the rat hind-limb was produced using the mass-drop model injury first described by Kami et al<sup>9</sup> and optimized for our laboratory. This blunt trauma was moderately severe, did not result in bone injury or affect gait in the injured animals. Briefly, the technique entails dropping a 200 g weight of cylinder (Diameter of basal surface is 1 cm with 1.0J of kinetic energy) from the height of 50 cm onto the medial surface of the right gastrocnemius muscle of anaesthetized rats with ethyl ether.

## Real-time reverse transcription-PCR

In the present study, the mRNA expression of BNIP3, NIX, Lamp-2, and p62 was assessed by real-time reverse transcription-polymerase chain reaction (RT-PCR). Using Takara SYBR Premix Ex Taq TMII for mRNA, synthesized cDNA was applied to real-time RT-PCR (ABI 9700 Thermal Cycler Dice, USA) and analyzed with 7500 Real-Time PCR System (ABI, USA). Using RNAiso Plus (Takara Bio, Japan), total RNA was extracted from the proximal portion of gastrocnemius muscle according to the manufacturer's instructions. Using the firststrand cDNA Synthesis kit, Samples (~10 ng of RNA) were reverse-transcribed according to the manufacturer's protocol [PrimeScript RT Master Mix (Perfect Real Time) for mRNA, Takara Bio, Japan].

GAPDH was used as an internal standard, to normalize the amount of total RNA present in each reaction. The real-time cycle conditions were 95°C for 30 sec, followed by 40 cycles at 95°C for 5 sec, and at 60°C for 34 sec for mRNA. Expression levels for each mRNA transcript were determined by normalizing each group to the sedentary group by the 2-ΔΔCT method. Primers used for detection of rat cDNA were as follows, which were designed by Sangon Biotech (Shanghai, China).

P62	FORWARD: CCAGCACAGGCACAGAAGACAAG REVERSE: TGAGCCAGCCGCCTTCATCC
Lamp-2	FORWARD: CCTAGGAGCCGTTCACTCCA REVERSE: GCCCAAGAGACAGCGAATCC
BNIP3	FORWARD: AGCGTTCAGCCTCCGTCTC REVERSE: GAGAGTAGCTGTGCGCTTCGG
NIX	FORWARD: GTTCTTCTCTCGTCTCCATCCAC REVERSE: GGCTCCACTTCTCATGCTTAG
GAPDH	FORWARD: AATGGTGAAGGTCGG TGTGAACG REVERSE: TCGCTCCTGGAAGATGGTGATGG

## Western blot analyses

An appropriate amount of mixed gastrocnemius muscle were taken and shred. RIPA lysate containing PMSF (PMSF: RIPA = 100: 1, Shanghai Biyuntian Co., China) was added to the samples. After electric homogenization, the samples were sonicated and lysed at 4°C overnight, then centrifuged at 12000 rpm and 4°C for 30 min. The supernatant was sub-packed. The above sample was prepared at 20 ug / time, and SDS polyacrylamide gel electrophoresis was performed (electrophoresis instrument 325BR051391, BIO-RAD, USA. Separating gel concentration 12%), then transferred to a PVDF membrane for 1 hour and blocked with 5% skim milk. The primary antibody Anti-p62 (Sangon Biotech Co., China, 1:500) and the internal reference Anti-GAPDH (Sangon Biotech Co., China, 1:10000) were diluted with 5% skim milk according to the ratio of antibody instructions. Then the membrane was soaked, and incubated at 4°C in a three-dimensional shaker overnight. The membrane was washed 4 times and incubated the secondary antibody (Sangon Biotech Co., 1:5000) for 1 h. Then the membrane developed by ECL (ECL Plus Ultra Sensitive Kit, Phygene Life Sci Co., China). The target protein and GAPDH are evaluated in the

same membrane. The final result was expressed as the OD ratio of the target protein p62 to the internal reference GAPDH. Image Lab5.1 software was used to analyze the gray value.

## Statistical analysis

Statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to correct for multiple comparisons (SPSS 21.0, SPSS Inc). Data are expressed as mean ± standard error of mean (SEM). P < 0.05 was considered statistically significant.

## RESULTS

In the eccentric exhaustive exercise groups, compared with the C group, the expression level of p62 mRNA in E48 group was significantly increased (P<0.05), but there was no significant difference between E0 and E24 groups. The mRNA level of Lamp-2 in E48 group was significantly increased (p<0.05), while that in E0 and E24 groups was significantly decreased (p<0.05). The expression level of NIX mRNA in E48 group was significantly increased (P<0.05), while that in E0 group was significantly decreased (P<0.05). The mRNA expression level of BNIP3 was not significantly different in E48 group, but significantly decreased in E0 and E24 groups (P<0.05) (Table 1, Figure 1). On the other hand, compared with C group, p62 protein expression in E0 and E24 groups had no significant difference, but was significantly increased in E48 group (P<0.05). Compared with E0 group, the above factors were significantly increased in E48 group (P<0.05), but there was no significant difference in E24 group (except NIX mRNA in E24). (Table 2, Figure 2)

In blunt trauma groups, compared with group C, the levels of p62 mRNA and Lamp-2 mRNA in group D0 were significantly increased (P<0.05), while those in groups D24 and D48 were significantly decreased (P<0.05); Compared with D0 group, the levels of p62mRNA and Lamp-2 mRNA in D48 group were significantly decreased (P<0.01). On the other hand, compared with group C, the expression levels of BNIP3 mRNA and NIX mRNA were not significantly different in group D0, but significantly decreased in group D24 and group D48 (P<0.05). Compared with D0 group, it was significantly decreased in D24 and D48 groups (P<0.05) (P<0.01) (Table 1, Figure 1). In addition, compared with group C, there was no significant difference in the expression of p62 protein in blunt trauma groups. (Table 2, Figure 2)

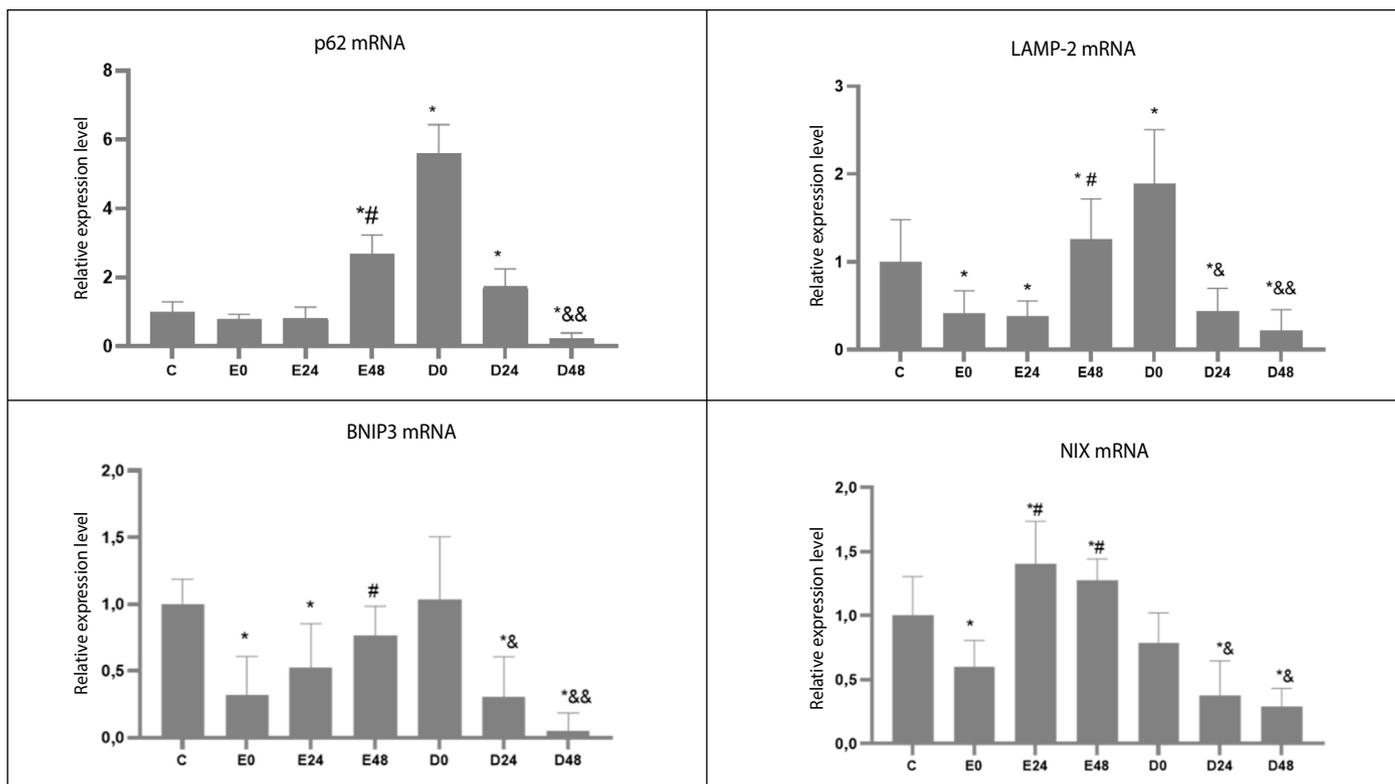
## DISCUSSION

Eccentric exhaustive exercise can cause microdamage and ultrastructural changes in skeletal muscle, which has been demonstrated by previous studies. Ultrastructural changes have also been reported in previous work in our laboratory. Blunt trauma can cause relatively significant swelling of muscle fibers, but the dynamic changes of autophagy under both injury models remain unclear, which motivated this study. The differences and degree of autophagy in the two models will be explored and evaluated by synchronous comparison.

**Table 1.** Genes expression in all groups at different time points after exhaustive exercise (E0, E24, E48) and contusion (D0, D24, D48).

group (n=6)	p62mRNA	Lamp-2 mRNA	BNIP3 mRNA	NIX mRNA
C	1.00±0.30	1.00±0.49	1.00±0.17	1.00±0.39
E0	0.81±0.14	0.43±0.27*	0.33±0.30*	0.59±0.22*
E24	0.83±0.39	0.38±0.19*	0.53±0.49*	1.38±0.32*#
E48	2.71±0.57 *#	1.26±0.46*#	0.77±0.23#	1.29±0.17*#
D0	5.59±0.84 *	1.90±0.62 *	1.04±0.29	0.79±0.25
D24	1.74±0.49 *	0.42±0.25 *&	0.31±0.28*&	0.39±0.26*&
D48	0.22±0.15 *&&	0.21±0.19*&&	0.05±0.34*&&	0.29±0.13*&

[\*] Significant difference compared with control, P < 0.05. [#] Significant difference compared with E0, P < 0.05. [&] Significant difference compared with D0, P < 0.05. [&&] P < 0.01. n = 6/group.



**Figure 1.** Genes expression in all groups at different time points after exhaustive exercise(E0, E24, E48) and blunt trauma(D0, D24, D48). (A) p62, (B) Lamp-2, (C) BNIP3, (D) NIX gene expressions were measured using qRT-PCR. Data are mean  $\pm$  SEM, n = 6/group.[\*]Significant difference compared with control, P < 0.05. [#] Significant difference compared with E0, P < 0.05. [&] Significant difference compared with D0, P < 0.05. [&&] P < 0.01. n = 6/group.

**Table 2.** Protein expression of p62 at different time points after exhaustive exercise(E0,E24,E48) and blunt trauma(D0,D24,D48).

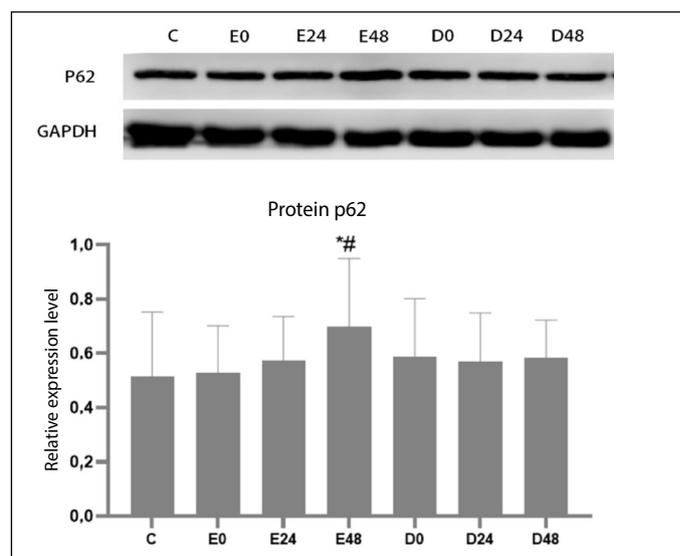
group (n=6)	Protein expression of p62
C	0.5098 $\pm$ 0.2286
E0	0.5301 $\pm$ 0.1697
E24	0.5689 $\pm$ 0.1588
E48	0.7012 $\pm$ 0.2409*#
D0	0.5902 $\pm$ 0.2089
D24	0.5697 $\pm$ 0.1803
D48	0.5778 $\pm$ 0.1279

[\*]Significant difference compared with control, P < 0.05. [#] Significant difference compared with E0, P < 0.05.

### Influence of exercise training on autophagy related factors

There are few reports on autophagy related factors in exhaustive exercise and acute blunt trauma models. Previous reports have shown that high-intensity interval training (HIIT) improves athletic performance more effectively than continuous moderate intensity training (CMT), and that this improvement is associated with basal autophagy adaptation and mitochondrial function of cardiac and skeletal muscles. The results showed that Beclin-1, BNIP3, P62, ATG-3, LC3-II and LC3III/LC3-1 expressions were significantly increased in soleus muscle and myocardium after HIIT alone.<sup>10,11</sup> Other reports have shown that autophagy regulation is weakened in old skeletal muscle. But levels of autophagy can be upped with some exercise training.<sup>12,13</sup>

Regarding BNIP3/NIX mediated mitochondrial autophagy, studies have shown that four weeks treadmill exercise of moderate intensity promoted mitochondrial autophagy BNIP3 mediated, while high intensity exercise blocked the BNIP3 mitochondrial autophagy pathway. After four weeks of high intensity exercise, the expression of p62 and BNIP3 protein decreased. In addition, Bnip3 and Nix have different responses to exercise. Exercise training plus dietary restriction can effectively promote the significant expression of Nix protein in gastrocnemius of mice, while BNIP3 has no significant expression.<sup>14-16</sup>



**Figure 2.** Protein expression of p62 were measured by Western Blot at different time points in all groups after blunt trauma(D0, D24, D48) and exhaustive exercise(E0, E24, E48). Date are mean  $\pm$  SEM, n = 6/group.[\*]Significant difference compared with control, P < 0.05. [#] Significant difference compared with E0, P < 0.05.

Wohlgemuth et al. found that the expression of LC3 and LAMP-2 mRNA and protein in skeletal muscle of male rats showed age-related decline, and autonomous exercise combined with caloric restriction could up-regulate the expression of LC3 and LAMP2 to maintain certain autophagy activity in skeletal muscle and promote cell survival.<sup>17</sup>

The results of this experiment showed that p62 mRNA decreased significantly immediately and 24h after exhaustive exercise, and increased significantly at 48h. After exhaustive exercise, misfolded proteins and oxidative damage of proteins gradually increased, which activated the transcription of p62 gene and promoted the isolation of toxic proteins

by autophagosomes. The mRNA of Lamp-2 decreased significantly immediately and 24h after exhaustive exercise, and increased significantly at 48h. The results indicated that the intracellular autophagosomes were gradually increased, and the rise of Lamp-2 promoted the fusion of autophagosome and lysosomes and the degradation of substrates in autophagy. BNIP3 after exhaustive exercise was lower than that in the control group, and NIX was significantly decreased immediately after exercise and significantly increased at 24h and 48h. These results suggest that Nix-mediated mitochondrial autophagy may be activated more easily after exhaustive exercise. P62 protein did not decrease significantly in each group after exhaustive exercise and blunt trauma, which may indicate that the effects of injury on autophagy genes and proteins are not synchronized, and autophagy proteins can play a role only when the substrate to be degraded accumulates to a certain extent.

### Effects of muscle injury on autophagy related factors

There are also few reports on autophagy related factors in the acute blunt trauma model. Recent studies have shown that markers of mitochondrial fission are elevated and fusion proteins are decreased after sciatic nerve transection.<sup>18</sup> The total expressions of mitochondrial markers Beclin1, PINK1, LC3-II, p62 and phosphorylated ULK1 (S555) were increased. As a result, gastrocnemius paralysis leads to a progressive increase in mitochondrial protein expression and mitochondrial fission. Repair or drug intervention to limit excessive mitochondrial phagocytosis may be effective therapies to protect the mass and function of paralyzed muscle. In addition, aerobic exercise also had effective effects on several autophagy related factors in the skeletal muscle of hypertensive and normal blood pressure rats.<sup>19</sup>

Regarding the repair of acute skeletal muscle blunt trauma,<sup>20</sup> studies have shown that the expressions of P62 and LC3-II are first high and then low. LC3-II and P62 expressions were significantly increased in the 3, 5, and 7 day groups after acute blunt trauma compared with the control group and the 14th-day group. The results suggest that the rate of damage repair may be related to the level of autophagy.

The results of this experiment showed that compared with group C, the levels of p62 mRNA and Lamp-2 mRNA in group D0 were significantly increased in blunt trauma group ( $P < 0.05$ ), while those in groups D24 and D48 were significantly decreased ( $P < 0.05$ ). On the other hand, compared with group C, the expression levels of BNIP3 mRNA and NIX mRNA were not significantly different in group D0, but significantly decreased in group D24 and group D48 ( $P < 0.05$ ). The basal autophagy factors and mitochondrial autophagy factors were inconsistent, and its molecular mechanism needs to be investigated in the future.

### BNIP3/NIX and mitochondrial autophagy

Six weeks of endurance exercise training combined with dietary improvement can alleviate the decline of autophagy level in nutritionally obese mice. During the process of improving the autophagy of skeletal muscle mitochondria in nutritionally obese mice, the transcription levels of NIX/BNIP3 and PINK1/Parkin signal are increased adaptively, so as to regulate the number and function of skeletal muscle mitochondria. BNIP3 and NIX are related multifunctional mitochondrial outer membrane proteins. BNIP3 regulates mitochondria during hypoxia, and mitochondria require NIX during erythroid lineage development. Ney discussed recent advances in the field of mitochondrial phagocytosis mediated by BNIP3 and NIX. In addition, impaired function of BNIP3 or NIX may lead to defective mitochondrial quality control, which may lead to mitochondrial dysfunction and cell death.<sup>21</sup>

Some studies have reported that Bnip3 induces transposition of DRP1 in cells mitochondrial, and DRP1-mediated mitochondrial division is associated with increased autophagy.<sup>22</sup> Inhibition of DRP1 can reduce BNIP3-mediated autophagy, and inhibit mitochondrial division or increased death of cardiomyocytes that autophagy lead to overexpressing BNIP3. Other studies have shown that BNIP3 gene knockout can reduce mitochondrial fragmentation and lead to the restoration of mitochondrial morphology and integrity. Markers of endoplasmic reticulum stress and mitochondrial apoptosis were also significantly reduced during BNIP3 gene knockout. In contrast, increased BNIP3 expression decreased myocardial diastolic and systolic function.<sup>23</sup>

In conclusion, the low expression of BNIP3/NIX during the severe injury period in 24 and 48 hours after blunt trauma may reduce the excessive autophagy of mitochondria and maintain a certain amount of mitochondrial function, thus reducing cell death.

### CONCLUSIONS

Generally, there were differences at each recovery phase between blunt trauma and exhausted exercise models, and the basal autophagy factors and mitochondrial autophagy factors were also inconsistent. Basal autophagy factors p62 and Lamp-2 increased significantly 48 hours after eccentric exhaustive exercise and immediately after blunt trauma. Mitochondrial autophagy factor BNIP3 did not increase after exhaustive exercise and blunt trauma, but NIX only increased after exhaustive exercise. Its molecular mechanism needs to be further studied.

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All authors declare no potential conflict of interest related to this article

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**AUTHORS' CONTRIBUTIONS:** Each author made significant individual contributions to this manuscript. TP, JJ conceived and designed the experiments. TP, JJ performed the experiments. LY, JJ analysed the data. QW, XT, MJ, LY contributed reagents/materials/analysis tools. Tongbin Pan wrote the manuscript. All authors read and approved the manuscript

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