Scholar Publishing Group
Zoology and Animal Physiology
https://doi.org/10.38007/ZAP.2021.020203
ISSN 2789-8369 Vol. 2, Issue 2: 27-40

Transcription and Translation Levels of PGC-1α in Skeletal Muscle of C57BL6 Mice

Kazuyoshier Yoshii*

University of Sydney, Australia

**corresponding author*

Keywords: PGC-1α Transcription, Skeletal Muscle, C57BL6 Mice, Aerobic Exercise, Protein Levels

Abstract: Skeletal muscle plays a very important role in the growth and development of the human body, but with the increase of age, the activity of various substances in skeletal muscle will also be limited. This study mainly explored the transcription and translation levels of PGC-1α in skeletal muscle of C57BL6 mice under aerobic exercise. Select 60 C57BL6 mice (N=4), the training time of the mice is fixed at 10:00, use zH-PT to control the animal's running path, and monitor the entire exercise process to confirm the exercise intensity of the mice. After the 9th week of training, the mouse was hunger striked for 6 hours, and his neck was broken and killed. The thigh bones, calf bones and thigh muscles closest to the surface of the thigh bones were obtained. The OD value was measured with a spectrophotometer, and a reverse transcription system was prepared based on an efficient cDNA inversion system. The reverse recording reaction was performed in the MyCyclePCR amplification device. After high-fat diet, the discovery of PGC-1α in skeletal muscle of mice was significantly higher than that of normal group, that is, the discovery of PGC-1α in HC group was 36.5% higher than that of NC group ($P<0.05$). The level of PGC-1α in skeletal muscle of HE group was 13% lower than that of HC group ($P<0.05$). The results show that high-fat diet can greatly increase the performance level of skeletal muscle PGC-1α, while aerobic exercise can greatly reduce the performance level of skeletal muscle PGC-1α. Appropriate aerobic exercise can reduce PGC-1α transcription and translation levels in skeletal muscle of C57BL6 mice.

1. Introduction

The function of skeletal muscle often involves physical training. As the main part of human sports, skeletal muscles participate in various sports. The body is constantly exchanging skeletal muscle energy, and exercise stimulation changes the structure and function of the skeletal muscle of

the body. The generation of various human movements mainly depends on the contraction and relaxation of skeletal muscle, and the contraction ability of skeletal muscle is related to the activity of substances in the muscle.

PGC-1α is present in mitochondria and is very important for mitochondrial activity. Normally, PGC-1α will be maintained at a normal level, but proper aerobic exercise can regulate the transcription and translation levels of PGC-1α, which is very important for improving the body's endurance. Therefore, it is necessary to study the expression level of PGC-1α in muscle.

Muscle wasting associated with chronic diseases is associated with decreased expression of PGC-1α, while overexpression of PGC-1α is associated with muscle damage. Rahnert believes that the combination of CREB and CREB-regulated transcriptional coactivator (CRTC2) is a direct factor affecting PGC-1α transcription. Despite the high level of CREB phosphorylation (that is, activation), the expression of PGC-1α in skeletal muscle of diabetic mice decreased, indicating that the CRTC2-CREB signal may be unregulated. He studied the relationship between CREB/CRTC signal transduction and PGC-1α expression in L6 myotubes treated with dexamethasone (Dex, 48h) to induce muscle atrophy. Dex reduced the levels of PGC-1α mRNA and protein and CRTC1 and CRTC2 in the nucleus. Dex also changed the level of two known CRTC2 positioning regulators. In order to evaluate the transcription of PGC-1α, PGC-1α luciferase was used to explore the transfection of plasmid (PGC-1α-Luc) into muscle cells [1]. His research did not control related variables, and the experimental process was not rigorous. The population of hepatitis B combined with many metabolic disorders is increasing significantly. Shi believes that resveratrol (RSV) has been used as a preclinical drug to treat metabolic disorders. However, the effect of RSV on HBV replication is still unknown. He used HBV-expressing hepatocellular carcinoma cell lines and hydrodynamic injection of viral DNA to create a mouse model. It was found that RSV activates Sirt1, thereby deacetylating PGC-1α, and subsequently increasing the transcriptional activity of PPARα, resulting in enhanced HBV transcription and replication in vivo and in vitro. In addition, he found that this pathway is also necessary for fasting-induced HBV transcription. By combining the two, his research determined that RSV enhances HBV transcription and replication, especially on the core promoter, which depends on the transcriptional alpha pathway of PGC-1α. Therefore, RSV may exacerbate the development of hepatitis B, and patients with hepatitis B infection should take RSV as a dietary supplement with caution [2]. His research did not conduct group experiments on mice, and lacked experimental controls. Exercise and exposure to low temperature increased PGC-1αmRNA. However, transcription control has not been checked during the cold exercise. Shute believes that the need for cold exposure to the environment after exercise may not be the actual way to recover. In order to determine the expression of mitochondrial-related genes and the transcriptional regulation of PGC-1α after exercise in cold environment compared to cold environment. 11 trained male animals completed two 1-hour exercises in a cold (7℃) or room temperature (20℃) environment, and then stood and recovered for 3 hours under standard indoor conditions, respectively . Biopsies were taken from the lateral femoral muscle before exercise, after exercise and after 3 hours of recovery. The relationship between gene expression and transcription factors and PGC-1α promoter was analyzed. PGC-1αmRNA recovered from 3 hours before exercise, and there was no difference between the tests. The activity of ERRα, MEF2 and NRF2 mRNA at low temperature is lower than room temperature [3]. The number of specimens he studied is too small and has no practical value. During the development of goat follicles, the abnormal expression of peroxisome proliferator-activated receptor γcoactivator-1 alpha (PGC-1α) in granulosa cells (GC) may lead to follicle atresia, and its regulatory mechanism is not yet clear. Zhang investigated the effect of ectopic expression or PGC-1α interference on apoptosis of goat granulosa cells (FGCs) in vitro. The RNA (shRNA) in goat FGC silences PGC-1α and thus reduces the mitochondrial DNA (mtDNA) copy number ($P < 0.05$), changes the mitochondrial ultrastructure, and induces apoptosis

(P <0.05). The transcription and translation levels of the protein X of the apoptosis-related gene BCL-2 (BAX), caspase 3 and caspase 9 were significantly up-regulated (P<0.05, respectively). In addition, the ratio of BAX/B cell lymphoma 2 (BCL-2) decreased (P <0.05), and the release of cytochrome c (cyt c) and lactate dehydrogenase (LDH) was significantly enhanced (P <0.05) [4] . His research did not discuss the process of cell apoptosis, and the experimental process is not detailed.

In this study, a C57BL6 mouse model was constructed, and the transcription and translation of PGC-1α in mouse skeletal muscle was thoroughly explored by extracting the mouse skeletal muscle.

2. Skeletal Muscle PGC-1α

2.1. Physiological Function of Skeletal Muscle PGC-1α

PGC-1α can sense the changes of nutrient content, energy substance levels and growth factor signals outside the cell, and regulate cell growth, differentiation and protein synthesis by activating downstream proteins. There are two complex forms of PGC-1, PGC-1-raptor and PGC-1-ricTOR, in cells. The former is blocked by rapamycin and plays an important role in cells. PGC-1α affects protein translation and phosphate synthesis and other metabolic processes by regulating the activity of a series of downstream target proteins, nutrient levels and growth factors in the cell, thereby changing the growth and metabolic state of the cell. PGC-1-ricTOR is not sensitive to rapamycin. The PGC-1-ricTOR special complex activates PKB by phosphorylating a series of amino acid residues of the PKB molecule and participates in the construction of cell skeleton. Usually the amount of transport RNA in the cell indirectly reflects the activity of the mitochondria P .

$$P = 1 - \left\{ \left[\left(F^2 + 8F \right)^{1/2} - F \right] / 2 \right\}^{1/r} + 2N_{xv} / \left(N_x + N_y \right) \tag{1}$$

Among them, F is shared fragment information of a single fragment RNA, and N_x and N_y are shared fragment values.

PGC-1α is a general inducer of mitochondrial respiration. Mitochondrial ATP production requires translation products derived from cytoplasmic ribosomes. Here, PGC-1α of some cells is located in the nucleolus, the site of RNA polymerase I transcribed by the ribosome. After activation, PGC-1α associates with ribosomal DNA and promotes the expression of RNA polymerase I and RNA promoter. This induces RNA polymerase I transcription under different stress conditions in cell culture and mouse models and healthy humans. This novel molecular link between ribosomes and mitochondria helps explain sarcopenia in neurodegenerative diseases. The genetic rules between cells can best represent the relevant characteristics between organisms [5-6].

$$\psi_A = \sum A_{Aj} P_{ij} + A_i B_j P_{ij} - \frac{\pi_A + \pi_B}{2} \tag{2}$$

Among them, ψ_A represents the correlation coefficient between populations, A_i and B_i represent the cell types of different populations.

PGC-1 regulatory factors include PGC-1α, PGC-1β and PRC, which play a leading role in controlling the synthesis of mitochondria and the regulation of respiratory function. NRF1 and NRF2 are related to the functions of various mitochondria. Nrf-1 can be identified by combining translational sequences of transcribed genes, which is related to the function of the respiratory chain and the appearance of certain genes. Similarly, NRF-2 is a multiple subunit activation factor, and in the expression of phosphatase, animal NRF2 is transcribed in the same way as humans. These two

factors well reflect the specific function of mitochondria and work together on the expression of most nuclear genes. Other nuclear factors are involved in the control of mitochondrial respiration. Creb-camp response element binding protein can participate in the formation and regulation of cytochrome C together with NRF1. Mononuclear receptors (ERRa) (estrogen-related receptors) are highly expressed in muscle tissue and promote oxidation through controlled intermediate chain subnuclear dehydrogenase (MCAD) [7-8].

2.2. Harm of Fatty Acids in Skeletal Muscle Tissue

Skeletal muscle accounts for a large part of the body mass and is an indispensable part of exercise and metabolic health. The increase in age is related to the decline in muscle mass and function (eg strength-related performance, strength), and the decline in muscle function exceeds the decline in muscle in number. The mechanism behind these declines is multifaceted, involving internal age-related metabolic disorders and environmental impacts such as nutrition and physical activity. Aging is related to a certain degree of "anabolic resistance" of these key environmental inputs, which may accelerate the internal process that drives aging. Both resistant exercise and aerobic exercise may bring functional and health benefits to the elderly. Usually, the mathematical expectation $D(X)$ is used when studying the relevant characteristics of muscles.

$$D(X)=E\left[(X-E[X])^2\right]=E\left[X^2-2XE(X)+[E(X)]^2\right] \tag{3}$$

$$D\xi=\sum_{i=1}^{n}\left[x_i^2-2x_iE\xi+(E\xi)^2\right]\cdot P_i=E\xi^2-(E\xi)^2 \tag{4}$$

Among them, $D\xi$ is often used as the distribution of variance, and ξ is the average value.

A large number of experimental data indicate that different duration, intensity and pattern of aerobic exercise will affect the molecular mechanism regulating skeletal muscle mitochondria. Studies on the effects of acute exercise and exercise training have shown that increasing the aerobic exercise time from 30 minutes to 90 minutes does not provide additional stimulation to activate the regulation of peroxidase-activated receptor gamma and the co-activator PGC-1α post-translational modification Signal pathway and the expression of PGC-1α gene (PPARGC1A). On the contrary, due to the increase of type II muscle fibers, exercise intensity substantially affects the biological commonality of mitochondria, and is accompanied by obvious metabolic disorders, which leads to the activation of signaling cascades and the regulation of mitochondrial gene expression. Therefore, the increase in type II muscle fibers more effectively activates mitochondrial activity [9].

Reducing the concentration of circulating high fatty acids can reduce the accumulation of fatty acids in skeletal tissues, thereby reducing the oxidation of skeletal muscle fatty acids during ischemia and movement disorders. However, excessive reduction or disappearance of FFA in plasma during dyskinesias may also lead to psychological disorders. Moreover, FFA is often widely used as a lipid regulator. This mechanism is selective anodization of PPARa, which increases fatty acid oxidation by increasing the content of fatty acid oxidase and reduces excessive FFA circulation. As we all know, fatty acid metabolism consists of two parts. The first step is to mobilize fat. The second stage is the oxidation of fatty acids. The second stage is the supply source of fatty acids. The third step is the path of fatty acids. Based on this, it is scientifically speculated that when the skeletal muscle energy metabolism disorder leads to skeletal muscle ischemia and dyskinesia, the energy supply is cut off, and the body's energy shortage is compensated by the compensation reaction. At this time, catechol stimulates fat mobilization and increases the metabolic matrix under stress. However, due to ischemia of skeletal muscle and hypoxia, it is difficult to supply energy by fatty acid metabolism of high oxygen consumption, so switching to glucose metabolism is also

suitable for the circulation theory. In this way, the increase of free fatty acids in plasma and the reduction of pathways will cause damage to skeletal muscle tissue and lipid toxicity and further decrease of cardiac function. However, if the fatty acid pathway is strengthened, not only will the free fatty acids in the plasma decrease, but the energy supply to the skeletal muscle will also increase. In addition, skeletal muscle tissue is divided into ischemic areas and non-ischemic areas. There is no hypoxia in non-ischemic areas. In non-ischemic areas, the body can provide enough oxygen to promote the energy metabolism of bone tissue. Combined with the above speculation, the inhibition of fatty acid metabolism and muscle fatty acid metabolism can indicate that it has a protective effect on cardiac function in clinical diagnosis and treatment [10].

2.3. Aerobic Exercise on Mitochondrial Autophagy in C57BL6 Mice

Autophagy is the process of using cells to carry out normal physiological activities after cell breakdown. Although cell autophagy has been studied for nearly seventy years, it is regarded as a normal physiological process and has not received much attention from scientists. In the past, researchers have become increasingly interested in this process. With the progress of human research, cell autophagy disorder and excessive autophagy are very harmful to cells. Therefore, studying the mechanism of cell autophagy and its physiological effects is very important for the development of biology and medicine [11-12].

Studies have shown that changes in the membrane permeability of damaged mitochondria can induce the occurrence of autophagy, which breaks down mitochondria through the autophagy decomposition pathway. This process is a type of selective autophagy. Mitochondrial autophagy is triggered when oxygen is taken away from the cell or when it is hungry. For example, yeast bacteria trigger mitochondrial autophagy when starved. This process includes the phosphorylation of the autophagy-related protein ATG32 in yeast and the interaction of ATG8. In addition, the interaction between ATGI1 and ATG32 is also promoted, causing the occurrence of mitochondrial autophagy.

2.4. Exercise on Skeletal Muscle

There are few reports on the metabolism of skeletal muscles and the improvement of molecular structures during resistance exercise. In clinical diagnosis and treatment, by regulating different metabolic pathways of skeletal muscle cells, various metabolic diseases can be prevented and treated, and aerobic exercise can delay the aging and death of skeletal tendon cells caused by metabolic disorders. Recent studies have shown that in many cases accompanied by insulin-resistant skeletal muscle cells, long-term aerobic exercise will enhance the sensitivity of the Akt/mTOR signal transmission path, increase the speed of skeletal muscle cell protein synthesis, and play a role in preventing skeletal muscle atrophy. Aerobic exercise may be related to protein synthesis, which can reduce aging skeletal muscle cells, at the same time increase insulin sensitivity, improve sugar metabolism, and also promote the metabolism of aging skeletal muscle cells. Although there is no significant increase in muscle function, it is also an effective method for interventional improvement. During the pathogenesis of senile critical illness, the mitochondrial activity of skeletal muscles in rats is reduced, the ability to converge is reduced and it is easier to fall. This may be related to the appearance of the aging process. Long-term aerobic exercise effectively improves the aging-related The performance of skeletal muscle particles to activate proteins improves the activity of mitochondria.

Most changes in muscle cells during exercise are related to alienation. In other words, exercise will greatly change the molecular and biochemical mechanisms in the cell, promote the oxidation of carbohydrates and fatty acids, and release energy [13-14]. The flexibility of healthy people in the conversion of glucose and fatty acids during acute exercise depends largely on the intensity and

time of exercise. High-intensity exercise mainly depends on the anaerobic effect of energy. With the increase of exercise time, the energy supply more and more depends on the phosphorylation of glucose. Because the circulating level of insulin is usually lower during exercise, this process has nothing to do with insulin. The oxidation of fatty acids did not change significantly with the increase of exercise intensity. However, the supply of fatty acids to the overall energy increases with the increase during exercise. From the perspective of energy release, skeletal muscle can promote the supply of oxidative energy for fatty acids and glucose during exercise. In addition, skeletal muscle can also produce changes corresponding to acute movements, including cell gene transcription, promotion of cell metabolism and cell remodeling and other processes that promote overall energy metabolism. During exercise, the skeletal muscle changes in extensional curvature, transgenic efficiency, and metabolic pathways all change, providing more energy and metabolic flexibility for subsequent skeletal muscle physiological activities.

3. Aerobic Exercise Intervention Experiment

3.1. Research Object

60 mice were selected, weighing 20-40g. Feed indoors and keep the feeding environment clean. Air circulation, at the same time, the indoor temperature is set to 25 degrees Celsius, sufficient light time.

3.2. Experimental Grouping

(1) Quiet group (C): No treatment, free diet.

(2) Endurance exercise group (E): After two weeks of training, another week of adaptation training begins, and after 8 weeks of endurance training, mice are trained every 6 days. After that, adaptive feeding is performed.

(3) Quiet medicine group (CM): taking medicine for 5 weeks in natural environment.

(4) Sports Medicine Group (EM): After two weeks of physical training, another week of drug training and fitness training begins. After the end of the 8-week endurance training, drug feeding is performed.

The training was maintained every half an hour, and during the 5th week of forced oral administration, one mouse died in CM and one mouse died within 6 weeks. This may be due to inadequate medication and excessive exercise load.

3.3. Experimental Materials

After the 9th week of training, the mouse was hunger striked for 6 hours, and his neck was broken and killed. The thigh bones, calf bones and thigh muscles closest to the surface of the thigh bones were obtained. The left thigh bone was used for extraction experiments, the right thigh bone and the middle thigh muscle were used for protein extraction experiments, and the right thigh bone and calf fibula were used for RNA transcription studies. All operations are performed on ice cubes in order to keep the tissue free from infection. Then save it in a low-temperature refrigerator at -80 ℃.

3.4. Total RNA Extraction and RNA Reverse Transcription

Put 40 mg of thigh muscle tissue measured on ice into 4 crushed tubes with steel balls, and add 1 mol of Trizol. Use a grinding machine, 60 seconds each time, divided into 6 times, and destroy the

tissue at the same time interval. After that, it was allowed to stand at room temperature for 10 minutes. Centrifuge for 10 minutes in a high-speed refrigerated centrifuge (number of rotations: 126675pm, temperature: 5 ℃). Place the supernatant in a centrifuge tube without transcriptase and add 300ul of isopropanol. The supernatant was shaken and left at room temperature for 8 minutes. Centrifuge again for 8 minutes using a high-speed refrigerated centrifuge (speed: 15000 rpm, temperature: 5 ℃). Discard the supernatant, precipitate, and add 1 ML 77% ethanol. After removing the suspended sediment, centrifuge it with a high-speed refrigerated centrifuge (rotating 9000 rpm, cooling to a temperature of 7 ℃) for 9 minutes and repeat 2 times. After that, he discarded the supernatant, leaving only a white transparent precipitate. Dry for 6 minutes at room temperature, add 20ULDEPC water, and soak for 8 minutes (40-56 ℃). Finally, the OD value was measured with a spectrophotometer, and a reverse transcription system was prepared based on an efficient cDNA inversion system. The reverse recording reaction was performed in the MyCyclePCR amplification device.

3.5. RNA Purity Analysis

Agarose electrophoresis solution 0.5XTAE: 0.037g EDTA, 0.54g Tris, 0.275g boric acid, add 10ml of distilled water to dissolve, adjust the pH to 8.0, and then dissolve to 100ml.

Sample buffer solution: 0.56% brominated phenol, 60% sucrose aqueous solution, stored at 5 ℃ environment. Put 0.4g of sugar in a test tube, add 40ML of XTAE, dissolve in boiling water, wait for the temperature to drop to about 50 degrees Celsius, add 2ulB dye, mix thoroughly, and pour into the gel tank (the gel tank is treated with DEPC water in advance Done), put it in a test tube and leave it for 30 minutes. Slowly remove the pipette from one side and inject the electrophoresis solution. Put a safety film on the surface of the ice pack, absorb 4 ulRNA sample and 1 ul sample buffer, and mix thoroughly to add the sample. Note that the sample flows from the negative electrode to the positive electrode. When the bromide reaches 5 cm from the bottom of the rubber, electrophoresis ends. The RNA band showed 4 bands, namely 15s, 6s, 9s, the 18s band was twice the brightness of 9s, and the 6S band was the brightest, showing better RNA quality.

3.6. Quality Control

(1) The training time of the mouse is fixed at 10:00, use zH-PT to control the animal's running path, and monitor the entire exercise process to confirm the exercise intensity of the mouse.

(2) All types of containers are immersed in 1% DEPC water overnight, then placed in a sterilization container, and continue to be sterilized at high temperature for 12 hours.

(3) In order to avoid the failure of the experiment due to the expired reagents, the reagents used in the experiment were prepared and stored according to the experimental requirements and instructions.

(4) Preparatory experiments are conducted before the formal experiment, and the formal experiment begins after the experimental technique is mastered.

3.7. Data Processing and Analysis

All data are shown as average standard deviation. Statistical analysis of the data was performed using SPSS 20.0 software package. Under the condition of dispersion uniformity determination, the difference of the same factor was compared using the method of one-component configuration dispersion analysis[15]. $P<0.05$ is regarded as the effective difference.

4. Analysis of Skeletal Muscle PGC-1α Expression

4.1. PGC-1α and FGF-2 Proteins in the Femoral Intermediate Muscles of Each Group

The expression of PGC-1α in each group is shown in Figure 1. Compared with group C, after 8 weeks of exercise intervention, the IGF-1 protein expression in the middle femoral muscles of the NC group and the NE group was significantly increased (P<0.05); similarly, the middle femur of the NC group and the NE group The expression of FGF-2 protein in muscle was also higher than that in group C, and there was a significant difference (P<0.05); in addition, compared with group D, the FGF-2 protein in the middle femoral muscle of mice in NC group and NE group The expression level was significantly increased (P<0.05). Although the expression of IGF-1 and FGF-2 protein in the femoral middle muscle of group D was slightly higher than that of group C, there was no statistical difference between the two groups (P>0.05).

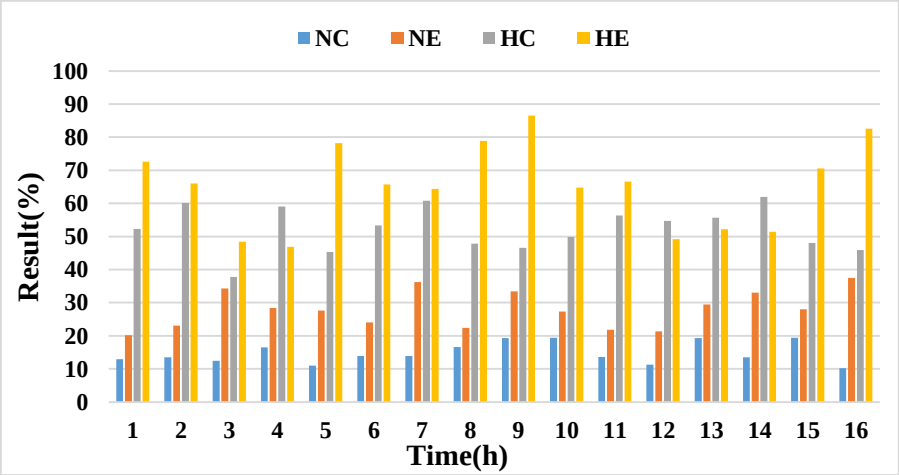

Figure 1. Expression of PGC-1α in each group

4.2. Analysis of PGC-1α mRNA and Protein Expression in Skeletal Muscle

The statistical results of PGC-1αmRNA expression in skeletal muscle are shown in Table 1. In the main effect, diet and exercise can significantly affect its mRNA expression, and the interaction effect of exercise and diet is not statistically significant. The expression of PGC-1αmRNA in skeletal muscle of NE group was 31.16% higher than that of NC group (P<0.001); the expression of PGC-1αmRNA in skeletal muscle of HE group was 128.30% higher than that of HC group (P<0.01). Diet also had a significant effect on its expression, that is, compared with the NC group, the PGC-1αmRNA expression in the HC group was 72.01% lower (P<0.001); compared with the NE group, the PGC-1αmRNA expression in the HE group skeletal muscle 51.28% lower (P<0.001). This shows that high-fat diet can significantly reduce the expression level of PGC-1αmRNA in skeletal muscle, and exercise can significantly increase the expression level of PGC-1αmRNA in skeletal muscle.

Table 1. Statistics of PGC-1αmRNA expression in skeletal muscle

Source	F	Significance
Medicine	S	0.000
Exercise	94.170	0.000
Medicine + Exercise	60.199	0.088

The statistical results of PGC-1α protein expression in skeletal muscle are shown in Table 2. In

the main effect, diet and exercise significantly affect its protein expression, while the interactive effect of exercise and diet has no effect on its protein expression. Compared with NC, PGC-1α protein expression in NE group was 23.95% higher (P<0.05), PGC-1o protein expression in HE group was 76.80% higher than HC group (P<0.001). Compared with the NC group, the PGC-1α protein expression in the skeletal muscle of the HC group was 47.98% lower (P<0.001); compared with the NE group, the PGC-1α mRNA expression in the HE group was 25.80% lower (P<0.05). This shows that high-fat diet can significantly reduce the expression level of PGC-1α protein in skeletal muscle, while exercise can significantly increase the expression level of PGC-1α protein in skeletal muscle.

The expression of PGC-1α protein in skeletal muscle is consistent with its mRNA expression. The statistical results show that the main effects of diet and exercise have significant effects on protein expression, but the interaction between diet and exercise has no statistical significance. After high-fat diet, the discovery of PGC-1 in skeletal muscle of mice was significantly higher than that of normal group, that is, the discovery of PGC-1 in HC group was 36.5% higher than that of NC group (P<0.05). The level of PGC-1 in skeletal muscle of HE group was 13% lower than that of HC group (P<0.05). Therefore, a high-fat diet can greatly increase the performance level of skeletal muscle PGC-1, while aerobic exercise can greatly reduce the performance level of skeletal muscle PGC-1. In particular, the protein level of PGC-1 in the HE group was much lower than that in the HC group.

Table 2. Statistical results of PGC-1α protein expression in skeletal muscle

Groups(N=4)	PGC-1α mRNA (% control)	PGC-1α protein (% control)
NC	100.00±3.07	100.00±6.30
NE	90.74±4.55	93.87±9.61
HC	105.1 7±4.10	111.00±733
HE	125.61±10.43	137. 41±7.86

4.3. Correlation Analysis of PGC-1α, NT-PGC-1α and Mouse ECG in Mouse Model

The expression level of PGC-1α after lack of exercise is shown in Figure 2. The expression level of PGC-1α decreased in mice after lack of exercise. We randomly selected 5 mice each in the Sham group and the surgery group for research. The study found that after 4 weeks of modeling, the expression level of PGC-la mRNA was positively correlated with the left ventricular ejection fraction in wild-type mice (correlation coefficient r=0.515, n=10, p<0.05); NT-PGC-1α mRNA expression level is positively correlated with ejection fraction (correlation coefficient 2=0.549, n=10, p<0.05); PGC-lα and NT-PGC-1α mRNA expression trends are consistent, and correlation analysis has statistics Academic differences (correlation coefficient 2=0.725, n=l0, p<0.01). Because PGC-1α and NT-PGC-1α are transcribed from the same gene, the actual NT-PGC-1α mRNA level cannot be detected.The mRNA level of NT-PGC-la represents the actual NT-PGC-1α and full-length PGC-1α The overall level of PGC-1α represents the full-length PGC-1α level. In order to further understand the role of CRM1 inhibitor Selinexor in cardiomyocytes, we found in vitro experiments that Selinexor can inhibit PE and angiotensin-induced myocardial hypertrophy, Selinexor can inhibit PE-induced increase in MHC overexpression. These results indicate that CRM1 inhibitors can not only increase the distribution in the nucleus of NT-PGC-la cells, but also play an anti-cardiac hypertrophy effect. In addition, the regulation of NT-PGC-1α into the nucleus can increase the transcription level of metabolism-related mRNA, suggesting that we may increase the energy metabolism level after giving CRM1 inhibitor. In our results, the simple administration

of CRM1 inhibitors partially increased the level of NT-PGC-1α in the nucleus, which means that NT-PGC-1α did not enter the nucleus in a large amount. The use of CRM1 inhibitor alone may also avoid NT-Excessive activation of PGC-1α-related genes in the nucleus. Previous studies have found that overexpression of PGC-1α causes a large number of non-functioning mitochondria in myocardial tissue, mitochondrial disorder, and mice with cardiomyopathy. No benefit was seen in the TAC mouse model overexpressing PGC-1α. It shows that the over-expression of PGC-la is harmful to the transitional activation of mitochondria, and the enhancement of metabolic level is not as high as possible. Only by maintaining it in a moderate range can it benefit. Therefore, the use of CRMI inhibitor Selinexor can not only play a role in inhibiting myocardial hypertrophy, but also moderately activate the expression of NT-PGC-1α energy metabolism related genes, which has potential therapeutic value.

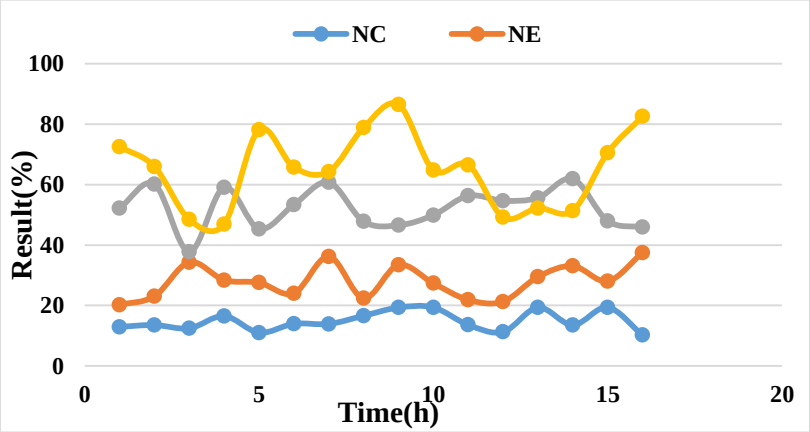

Figure 2. PGC-1α expression level after lack of exercise

4.4. Transcription and Translation Levels of PGC-1α, NT-PGC-1α and Related Proteins in Mouse Models

The translation level of PGC-1α in mouse skeletal muscle is shown in Figure 3. After 4 weeks of modeling, the total RNA and total protein of the myocardium tissues of the mice lacking the exercise group and the sham operation group were extracted, and related gene and protein expression levels were detected by qPCR and WestermBloting technology. The study found that over-exercise mice after four weeks had lower mRNA expressions of PGC-lα and NT-PGC-1α compared to mice in the sham-operated group: at the protein level, PGC-1α and NT-PGC-1α also decreased: meanwhile PGC- 1α downstream related protein and SOD2 expression levels also decreased. Immunohistochemistry also showed a decrease in PGC-la expression after myocardial infarction. In myocardial infarction-induced heart failure mice, NT-PGC-la function decreased, fatty acid metabolism was inhibited, and energy metabolism was impaired. Based on this, we speculate that regulating the entry of NT-PGC-1α into the nucleus can increase its downstream gene expression. NT-PGC-1α can be regulated into the nucleus through fusion expression of NLS sequence. In addition, NT-PGC-1α can also be regulated into the nucleus by inhibiting CRM1. By simply adjusting the subcellular distribution of NT-PGC-1α, it may play a role similar to that of overexpressing NT-PGC-1α. In other words, NT-PGC-1α in the nucleus can exert biological effects, and increasing NT-PGC-la entering the nucleus can increase its biological effects.

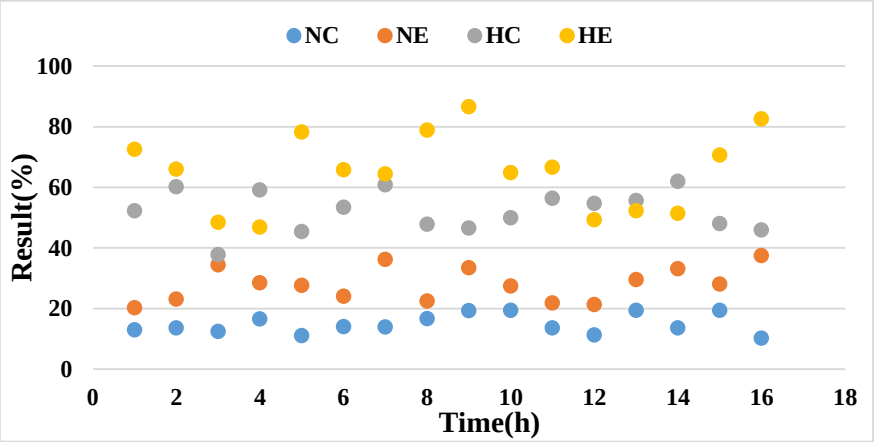

Figure 3. Translational levels of PGC-1α in mouse skeletal muscle

Compared with group C, the expression of PGC-1 α mRNA in Group E, cm and EM was significantly decreased ($P < 0.01$); the expression of PGC-1 α mRNA in group cm was lower than that in Group E, but there was no difference between group EM and group E; compared with group EM and group cm, the expression of PGC-1 α mRNA was significantly increased ($P < 0.01$). There are two non parallel lines in the interaction between endurance and drug on PGC-1 α mRNA in the sural of SD mice, which indicates that there is interaction between endurance and drugs on PGC-1 α mRNA. There are two non parallel lines in the interaction between endurance and drug on PGC-1 α mRNA in the sural of SD mice, which indicates that there is interaction between endurance and drugs on PGC-1 α mRNA. By comparing the expression of PGC-1 α mRNA in gastrocnemius muscle of four groups, it was found that the content of PGC-1 α mRNA in cm group and EM group was significantly higher than that in C group ($P < 0.05$) and very significant ($P < 0.01$); that in cm group was significantly higher than that in e group ($P < 0.05$); that in EM group was significantly higher than that in cm group, but there was no statistical significance between E group and C group. The expression of PGC-1 α in gastrocnemius muscle of aged quiet group was significantly lower than that of young group, while the expression of PGC-1 α in skeletal muscle of senile exercise group was significantly higher than that of elderly quiet group, indicating that long-term aerobic exercise can effectively maintain mitochondrial biosynthesis of aging skeletal muscle. The expression of mitochondrial respiratory chain key protease cox4 in the aged quiet group was significantly lower than that in the young group, while the content of Cox 4 in the skeletal muscle of the elderly exercise group was significantly increased, indicating that aerobic exercise can promote the expression of mitochondrial oxidative respiration related proteins in aging skeletal muscle and improve their aerobic exercise ability. In the elderly quiet group, the expression of PINK1 in skeletal muscle decreased significantly, indicating that the mitochondrial autophagy ability of aging skeletal muscle decreased, while the content of p62 was significantly increased, indicating that the overall autophagy flow of aging skeletal muscle cells was inhibited. On the one hand, long-term aerobic exercise could increase PINK1 expression and mitochondrial specific autophagy. On the other hand, it can reduce p62 accumulation and improve the overall autophagy level of aging skeletal muscle cells.

4.5. Aerobic Exercise on the Content of PGC-1α

The changes of PGC-1 α content in skeletal muscle were shown in Figure 4. The expression of PGC-1 α in 6-month-old app / PS1 mice was significantly higher than that in the normal control group ($P < 0.05$). Although there was an increasing trend in the Podocarpus, there was no statistical

significance. 8 weeks aerobic exercise can significantly reduce the abnormal high expression of PGC-1 α ($P < 0.01$). At the same time, under the electron microscope, the cell structure of frontal lobe and Po lobe in normal control group was normal, microfilaments and microtubules in axoplasm were arranged orderly, and the membrane structure of organelles such as mitochondria was clear. However, 6-month-old app / PS1 mice showed disordered arrangement of microfilaments and microtubules in frontal lobe and Po lobe, which tended to form rod-shaped structure and showed obvious early vacuolar degeneration. The prognosis of exercise F can improve the early structural disorder of its cells. The total protein expression of PGC-1α in frontal cortex of APP / PS1 mice at 6 months old was significantly up-regulated ($P < 0.01$), and the expression level was significantly decreased after 8 weeks of exercise ($P < 0.01$); although the expression of PGC-1 α in temporal lobe of 6-month-old app / PS1 mice showed an upward trend, there was no statistical significance, and exercise significantly decreased RhoA expression in the title lobe ($P < 0.05$). According to the ratio of PGC-1 α to total protein, there was no significant difference in PGC-1 α activity among groups. When NLS was fused with nt-pgc-1 α, the distribution of nls-nt-pgc-1 α increased slightly, but the change was not obvious. However, the distribution of nls-nt-pgc-1 α in nucleus was significantly increased after stimulation with selinexor, a CRM1 inhibitor, suggesting that CRMI inhibitor has a more obvious effect on the distribution of NLS expressing protein subcellular. Further studies showed that CRMI protein interacted with PGC-1 α and NT-PGC-1 α. In conclusion, the effect of NT-PGC-1 α in and out of nucleus was regulated by CRM1. PGC-1 α is a kind of nuclear receptor coactivator, which plays a transcriptional role by binding with nuclear nuclear transcription factors. We examined the level of gene transcription. Further studies showed that the mRNA expression level of NLS-NT-PGC-1 α overexpression cells under the stimulation of CRM1 inhibitor selinexor was higher than that of NLS-NT-PGC-1 α only. PPAR-a, TFAM, err-y, cpt1b, PDK4 and Nrf2 were statistically significant, although NT-PGC-1 α was mainly present in the cytoplasm, However, it can play a role in nuclear transcription, which also indicates that CRM1 inhibitor can play a role by increasing the distribution of NT-PGC-1 α nucleus. There are quite a lot of PGC-1 α protein in the cytoplasm of skeletal muscle cells under resting state. After exercise stress, PGC-1 α is activated and enters the nucleus to play its physiological function. Aerobic exercise can delay or improve the occurrence of aging skeletal muscle atrophy, reduce aging, promote mitochondrial production, improve mitochondrial function, improve mitochondrial autophagy, and reduce the production of abnormal mitochondria[16].

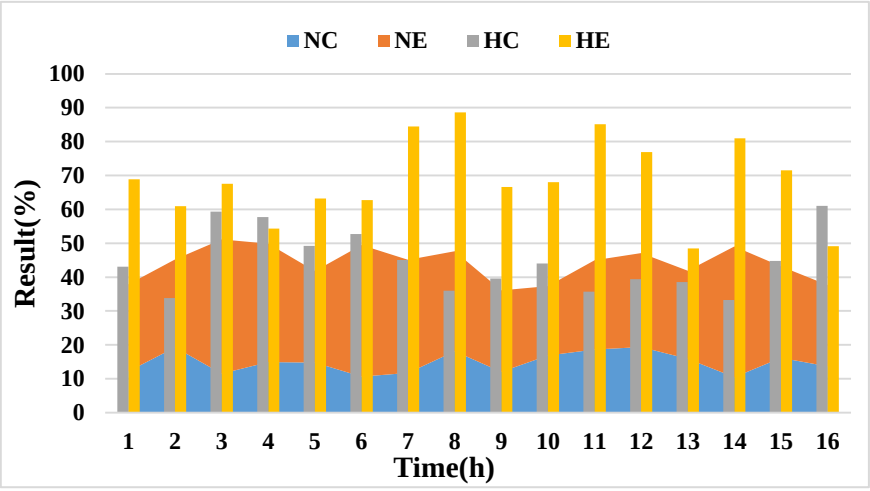

Figure 4. Changes in PGC-1α content in skeletal muscle

5. Conclusion

Skeletal muscle is involved in various sports. The body is constantly exchanging skeletal muscle energy. Today, changes in skeletal muscle fibers under exercise pressure are the focus of academic research. It is very important for the general athletes' fitness guidance to study the differences in skeletal muscle fiber types of different age groups and the effect of sports training on muscle fiber types.

As a related substance carrier of skeletal muscle, PGC-1α has great potential value for the growth process of skeletal muscle. Since PGC-1 is the core substance of the receptor in the cell nucleus, it performs its function by binding to the nucleus and related factors of the nucleus. Further research shows that compared with cells that only overexpress NRM-NT-PGC-1, if the cells are stimulated with CRM1 inhibitors, the mRNA levels of related genes in cells that overexpress NL-NT-PGC-1 will further increase.

Aerobic exercise can promote the appearance of oxidative respiration-related proteins during the aging of skeletal muscles and improve aerobic exercise capacity. In addition, in the skeletal muscle of old mice, the frequency of occurrence of the PGC-1 protein Pink1, which is related to mitochondrial autophagy, has been greatly reduced, showing a decrease in the ability of mitochondrial autophagy in skeletal muscle of old mice.

Funding

This article is not supported by any foundation.

Data Availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Conflict of Interest

The author states that this article has no conflict of interest.

References

[1]Rahnert, J. A. , Bin, Z. , Hudson, M. B. , Woodworth-Hobbs, M. E. , Russ, P. S. , & Ashok, K. . (2016). "Glucocorticoids Alter Crtc-Creb Signaling in Muscle Cells: Impact on Pgc-1α Expression and Atrophy Markers",Plos One, 11(7),pp.e0159181.https://doi.org/10.1371/journal.pone.0159181

[2]Shi, Y. , Li, Y. , Huang, C. , Ying, L. , Xue, J. , & Wu, H. , et al. (2016). "Resveratrol Enhances Hbv Replication through Activating Sirt1-Pgc-1α-Pparα Pathway", Entific Reports, 6(1),pp. 24744. https://doi.org/10.1038/srep24744

[3]Shute, R. J. , Heesch, M. W. , Zak, R. B. , Kreiling, J. L. , & Slivka, D. R. . (2018). "Effects of Exercise in a Cold Environment on Transcriptional Control of Pgc-1α", AJP Regulatory Integrative and Comparative Physiology, 314(6),pp.R850-R857. https://doi.org/10.1152/ajpregu.00425.2017

[4]Zhang, G. M. , Deng, M. T. , Zhang, Y. L. , Fan, Y. X. , Wan, Y. J. , & Nie, H. T. , et al. (2016). "Effect of Pgc-1α Overexpression or Silencing on Mitochondrial Apoptosis of Goat Luteinized Granulosa Cells",Journal of Bioenergetics & Biomembranes, 48(5), pp.493-507. https://doi.org/10.1007/s10863-016-9684-6

[5] *Brook, M. S. , Wilkinson, D. J. , Phillips, B. E. , Perez-Schindler, J. , Philp, A. , & Smith, K. , et al. (2016). "Skeletal Muscle Homeostasis and Plasticity in Youth and Ageing: Impact of Nutrition and Exercise", Acta Physiologica,216(1), pp.15-41. https://doi.org/10.1111/apha.12532*

[6] *Ye, Q. , Huang, W. , Li, D. , Si, E. , Wang, J. , & Wang, Y. , et al. (2016). "Overexpression of Pgc-1α Influences Mitochondrial Signal Transduction of Dopaminergic Neurons",Molecular Neurobiology, 53(6), pp.3756. https://doi.org/10.1007/s12035-015-9299-7*

[7] *Li, X. , Pan, E. , Zhu, J. , Xu, L. , Chen, X. , & Li, J. , et al. (2018). "Cisplatin Enhances Hepatitis b Virus Replication and Pgc-1α Expression through Endoplasmic Reticulum Stress", Entific Reports, 8(1), pp.3496. https://doi.org/10.1038/s41598-018-21847-3*

[8] *Koh, J. H. , & Kim, K. J. . (2017). "the Effects of Tfam Expression by Endurance Exercise on Ampk, Pparβ/δ and Pgc-1α in Mouse Skeletal Muscle",Korean Journal of Physical Education, 56(2), pp.517-526. https://doi.org/10.23949/kjpe.2017.03.56.2.37*

[9] *Fang, Z. , Li, P. , Jia, W. , Jiang, T. , & Xiang, Y. . (2016). "Mir-696 Plays a Role in Hepatic Gluconeogenesis in Ob/Ob Mice by Targeting Pgc-1α",International Journal of Molecular Medicine, 38(3), pp.845. https://doi.org/10.3892/ijmm.2016.2659*

[10] *Kristensen, T. , Pedersen, P. , Larsen, J. , Feldthusen, A. D. , Jelstrup, Søren, & Ellervik, C. . (2019). "Reduced Gene Expression of Peroxisome Proliferator-Activated Receptor Gamma Coactivator-1α in Whole Blood in Euthyroid Patients One Year after Hemithyroidectomy for Benign Euthyroid Goiter", Hormone & Metabolic Research, 51(02), pp.127-133. https://doi.org/10.1055/a-0822-3066*

[11] *Nishida, T. , & Yamada, Y. . (2016). "Sumoylation of the Krab Zinc-Finger Transcription Factor Paris/Znf746 Regulates its Transcriptional Activity",Biochem Biophys Res Commun,473(4), pp.1261-1267. https://doi.org/10.1016/j.bbrc.2016.04.051*

[12] *Wu, H. , Deng, X. , Shi, Y. , Su, Y. , Wei, J. , & Duan, H. . (2016). "Pgc-1 Alpha, Glucose Metabolism and Type 2 Diabetes Mellitus", Journal of Endocrinology, 229(3), pp.R99-R115. https://doi.org/10.1530/JOE-16-0021*

[13] *Giorgio Nordo , Arif Mehmood , Said Broumi, Single Valued Neutrosophic Filters, International Journal of Neutrosophic Science, 2020, Vol. 6, No. 1, pp: 08-21.* https://doi.org/10.54216/IJNS.060101

[14] *Rabeb Touati , Dr. Imen Ferchichi , Dr. Imen Messaoudi , Dr.Afef Elloumi Oueslati , Dr. Zied Lachiri, Pre-Cursor microRNAs from Different Species classification based on features extracted from the image, Journal of Cybersecurity and Information Management, 2020, Vol. 3, No. 1, pp: 05-13. https://doi.org/10.54216/JCIM.030101*

[15] *Mahmoud Miari , Mohamad Taher Anan , Mohamed Bisher Zeina, Neutrosophic Two Way ANOVA, International Journal of Neutrosophic Science, 2022, Vol. 18, No. 3, pp: 73-83. https://doi.org/10.54216/IJNS.180306*

[16] *Choi, G. H. , Ko, H. , Pedrycz, W. , Singh, A. K. , & Pan, S. B. . (2020). Recognition system using fusion normalization based on morphological features of post-exercise ecg for intelligent biometrics. Sensors, 20(24), 7130. https://doi.org/10.3390/s20247130*