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Research Article

Study on the Antifatigue Effect of Compound Amino Acid Capsules

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Supplementing amino acids was proven to relieve fatigue caused by exercise. This study explored the antifatigue effects of compound amino acid capsules (CAAC) on rats undergoing the forced swimming test (FST). CAAC augmented the endurance of FST in rats and alleviated the damage of skeletal muscle tissue and reduced the content of biochemical indicators in the serum. Furthermore, CAAC prevented skeletal muscle dysfunction in FST rats by modulating inflammation and oxidation reactions. After the treatment with CAAC, apoptosis and apoptosis-related protein and p-p65 were weakened, while the levels of SIRT1 and SIRT1/PGC-1α/Nrf2 pathway-related proteins were enhanced. The antifatigue properties of CAAC were associated with its antioxidant and anti-inflammatory capabilities, which were realized by activating the SIRT1/PGC-1α/Nrf2 pathway.

1. Introduction

Exercise can not only prevent obesity and hypertension but also reduce the risk of cancer, type 2 diabetes, and cardiovascular diseases [1, 2]. However, it should not be ignored that when the body continues to exercise under a certain intensity, its physical strength and exercise endurance decline, resulting in a state of fatigue [3]. Fatigue is a complex physiological and psychological phenomenon, which is caused by excessive mental and physical activities, excessive mental stimulation, and repeated monotonous activities [4]. Skeletal muscle is the most important target organ in the occurrence, development, and damage of exercise-induced fatigue, and research has confirmed that the outcome of the fatigue is closely related to changes in the structure and enzymology of skeletal muscle cells [5]. The mechanism of fatigue is very complex, and at present, there are many reasons that may account for, such as the depletion of energy, the accumulation of metabolites, the disorders of both ion metabolism and endocrine regulation disorder, and oxidative stress-induced damage [6, 7]. Among these potential factors, oxidative stress inflammatory response plays the most

important role in the etiology of fatigue [8–10]. Therefore, the body needs to take necessary measures to prevent possible fatigue symptoms or shorten the recovery time when fatigue symptoms appear.

The main physiological and biochemical manifestations of fatigue are the increase of destructed tissues and cells, the hypermetabolism of muscle protein and red blood cells, and the regulation of hormone and nerve during stress. Given that the recovery process is particularly important, the main contributors, nutrients, are increasingly needed, especially protein and amino acids [11, 12]. Amino acids and vitamins are essential organic substances to maintain the activities of daily living and also important active substances to maintain human health. Amino acids provide energy during exercise and are closely related to the ability of exercise; however, the lack of amino acids and vitamins is one of the main reasons that lead to and aggravate fatigue and also one of the important factors that cause retarded recovery from fatigue [13]. Scientific research has clarified that the supplementation of glutamine obviously increases the synthesis of glycogen and reduces the accumulation of ammonia, thereby exerting an antifatigue effect [14]. Also,

compound amino acid capsules (CAAC) are rich in essential amino acids and vitamins, which comprise 8 kinds of essential amino acids and 11 kinds of vitamins (http://www.wanhe-phar.com). Our pre-experiments showed that CAAC effectively attenuated the damage in the rat skeletal muscle caused by exhaustion and regulated muscle injury-related enzymes to improve the body's antifatigue ability. However, the alleviative effects of CAAC on fatigue and its exact mechanism have not been reported yet.

Considering the abovementioned previous reports, this study hypothesized that CAAC could exert its antifatigue effect by improving the oxidative and inflammatory systems in skeletal muscle. The forced swimming test (FST) has become an effective animal model widely used to evaluate the antifatigue properties of various bioactive compounds [15]. In FST, the mouse's inactive behavior reflects the animal's despair, simulates the human's depression, and causes mental stimulation and mental fatigue. In addition, FST can also cause muscle fatigue through long-term weight-bearing exercise on mice [16]. Ascorbic acid can enhance the body's immunity and promote the synthesis of collagen and the absorption of minerals. At the same time, it is also an efficient antioxidant, which has a certain antifatigue effect. The study mainly observed and determined the effect of CAAC on the fatigue-related metabolic factors of rats subjected to weight-loaded FST and unveils and revealed the possible mechanism of CAAC on alleviating sports-caused fatigue by comparing with ascorbic acid,

2. Materials and Methods

2.1. Ethics Statement.
This retrospective study was authorized by the ethics committee of Nanfang Hospital. The protocol on the animal was approved by the committee of experimental animals of Nanfang Hospital (no. YF2019030026).

2.2. Animals.
A total of 40 specific pathogen-free (SPF) male Sprague-Dawley (SD) rats (weighing 200-220 g) were obtained from Chengdu Dashuo Experimental Animal Co. (http://www.cd-dossy.cn/). The animal license number is SCXK (Chengdu) 2019-0401. SD rats were raised in the SPF laboratory animal center of Nanfang Hospital with the temperature and humidity set at $25 \pm 2°C$ and 45%–55%, respectively, with free access to food and water.

2.3. Animal Grouping and Drug Administration.
SD rats were randomly divided into 4 groups ($n = 10$ for each group), namely, the control group, model group, ascorbic acid group (AA) (PHR1008, Supelco, USA), and CAAC group. The AA (100 mg/kg) and CAAC (25 mL/kg) were each dissolved in 500 mL of drinking water to prepare each group of solutions. The doses of CAAC and AA were determined based on a previous study [5]. CAAC and AA were dissolved in distilled water and taken orally once a day 1 hour before the FST for 3 weeks. The rats in the control group and the model group took the same amount of distilled water orally in the same way.

2.4. Weight-Loaded FST.
FST was slightly modified based on the previous report [17]. All animals were not disturbed by any stimuli before the experiment, and they were allowed to adapt to the environment for 5 days prior to the experiment. Except for those in the control group, all SD rats were subjected to weight-loaded FST once every two days. The FST experiment lasted for three weeks, and SD rats were subjected to a constant load (fixed on the tail) equivalent to 5% of their body weight. Before each experiment, they were placed in a water tank ($50 \times 50 \times 40$ cm) with a water depth of 30 cm and a water temperature of 25 2°C for 1 hour. Then, the SD rats were allowed to return to the breeding cage and the exhausted swimming time was recorded. The end standard of the exhausted swimming time was that the rats could not return to the water surface within 5 s after submerging. After the last exhaustive swimming, SD rats were sacrificed by overanesthesia (intraperitoneal injection of sodium pentobarbital (P0500000, Merck, Germany) at a dose of 200 mg/kg) [18]. Blood sample was collected from the abdominal aorta, liver, and skeletal muscle. Meanwhile, the separated skeletal muscle tissues and blood samples were stored in a −80°C refrigerator (TSE600V, Thermo Scientific, USA).

2.5. Preparation of Serum Samples and Skeletal Muscle Tissue Samples.
Blood samples were centrifuged (3000 rpm) at 4°C for 15 minutes to obtain the supernatant of serum, which was stored in a −80°C refrigerator. Part of the skeletal muscle tissues was taken out from the −80°C refrigerator and placed in a high-throughput tissue grinder for 20 minutes at a frequency of 9.5 Hz. Then, the ground skeletal muscle tissues were centrifuged (12000 rpm) at 4°C for 10 minutes to collect the supernatant and preserved for the analyses of ELISA and Western blot. The remaining skeletal muscle tissues were frozen in a −80°C refrigerator and used for hematoxylin-eosin (H&E) staining, TUNEL staining, and immunohistochemical (IHC) analysis.

2.6. H&E Staining.
We used 4% paraformaldehyde (158127, Sigma-Aldrich, USA) to fix the skeletal muscle tissues, which were subsequently dehydrated with gradient ethanol (E111991, Aladdin, China) and embedded in paraffin. Afterwards, the paraffin-embedded tissues were cut into $3 \mu m$ thick slices and were dewaxed with xylene (X139941, Aladdin, China). Then, we used hematoxylin solution (C0107, Beyotime, China) to stain the slices for 5 minutes. Thereafter, the slices were differentiated with 0.7% hydrochloric acid ethanol for a few seconds and washed immediately. After the color of slices turned blue, we used 1% eosin solution (C0109, Beyotime, China) to stain the slices for 1 minute. Following the dehydration with gradient ethanol, xylene was used to transparentize the slices and neutral balsam (D054-1-1, Jiancheng, China) was applied for sealing. Finally, the slices were observed and the photos were captured under an optical microscope (Z735515, Sigma, USA) under a magnification of 100x.

2.7. Enzyme-Linked Immunosorbent Assay (ELISA).
The human BUN kit (C013-2-1) and human LA kit (A019-2-1) were obtained from Jiancheng (Nanjing, China). For the detection of BUN, the prepared serum samples were well

mixed with the buffer enzyme solution and then were soaked in the water at 37°C for 10 minutes. Subsequently, the enzyme chromogenic agent and alkaline sodium hypochlorite were added to the abovementioned mixture, followed by being soaked in water at 37°C for 10 minutes. Finally, the absorbance of each tube was detected by a microplate reader (PLUS 384, Molecular Devices, USA) at a wavelength of 640 nm and an optical path of 1 cm. For the quantification of LA, the prepared serum samples were also well mixed with enzyme solution and chromogenic agent and then soaked in water at 37°C for 10 minutes, after which the termination solution was added into the mixture above and the absorbance of each tube at 530 nm and 1 cm light path was read in a microplate reader.

Those commercial kits for BUN (BPE30566), lactate dehydrogenase (LDH) (BPE30024), LA (lactic acid) (BPE30821), liver glycogen (LG) (BPE30180), alanine aminotransferase (ALT) (BPE30255), reactive oxygen species (ROS) (BPE30810), malondialdehyde (MDA) (BPE30266), superoxide dismutase (SOD) (BPE30267), glutathione peroxidase (GSH-Px) (BPE30800), and tumor necrosis factor-alpha (TNF-α) (BPE30635) of rats were purchased from Lengton (Shanghai, China). The prepared supernatants of serum or skeletal muscle tissue were diluted as needed and added to the 96-well plates along with the antibodies against BUN/LDH/LA/LG/ALT/ROS/MDA/SOD/GSH-Px/TNF-α, where streptavidin-HRP was added as well. Then, the plates were incubated at 37°C for 60 minutes. After that, the color developing agents A and B were added to each well and incubated at 37°C for 10 minutes in the dark. Finally, the stop solution was added to each well and the absorbance (450 nm) was measured by a microplate reader.

Additionally, the commercial kits for muscle glycogen (MG) (A043-1-1), aspartate transaminase (AST) (C010-2-1), interleukin-1β (IL-1β) (H002), and IL-6 (H007) were bought from Jiancheng (Nanjing, China). For detecting the level of MG, the serum sample was made into a glycogen detection solution according to the instructions. Subsequently, the double-distilled water and color-developing solution were added to the glycogen detection solution, well mixed, and then boiled for 5 minutes in boiling water. After cooling at room temperature, the absorbance of each well was measured at a wavelength of 620 nm and an optical path of 1 cm. In order to determine the levels of AST, IL-1β, and IL-6, the matrix solutions for AST/IL-1β/IL-6 and the prepared supernatants of serum or skeletal muscle tissue were added into 96-well plates and well mixed and then placed in a water bath of 37°C for 30 minutes. Afterwards, the color-developing solution was added into a 96-well plate, followed by being well mixed, and placed in a water bath of 37°C for 20 minutes. Finally, the applied termination solution was added to the 96-well plate, which was gently and horizontally shaken, and let stand for 15 minutes at room temperature. The absorbance (510 nm) of each well was measured by a microplate reader.

2.8. TUNEL Staining. We used the TUNEL kit (C1098, Beyotime, China) to evaluate the apoptosis of skeletal muscle. Paraffin-embedded tissues were sectioned (3 μm thick) and dewaxed with xylene. Next, the sections were incubated firstly with DNase-free proteinase K (ST532, Beyotime, China) at 37°C for 20 minutes and then with 3% H_2O_2 solution (P0100B, Beyotime, China) at 37°C for another 20 minutes. Subsequently, the sections were reacted firstly with the prepared biotin labeling solution at 37°C for 1 hour in the dark and then with the labeling reaction stop solution at 37°C for 10 minutes. Then, the sections were incubated with the prepared Streptavidin-HRP working solution at 37°C for 30 minutes, following which the DAB color developing solution was added and incubated at 37°C for 5–30 minutes. Finally, the sections were dehydrated with gradient ethanol, transparentized with xylene, and mounted. We used an optical microscope (100x) to observe the TUNEL-positive signal.

2.9. Western Blot. The treated skeletal muscle tissues were lysed by a RIPA buffer (abs9229, absin, China) [19]. Afterwards, we used the BCA kit (P0009, Beyotime, China) to detect the concentration of protein. The extracts of protein were then separated by electrophoresis and transferred onto the nitrocellulose membranes (abs932, absin, China). Following being blocked with 5% nonfat milk at 37°C for 2 hours, the membranes were incubated with the antibodies against Bcl-2 (1 : 2000, 26 kDa, ab194583, Abcam, UK), cleaved caspase-3 (1 : 500, 17 kDa, ab49822, Abcam, UK), cleaved poly ADP-ribose polymerase 1 (PARP1) (1 : 8000, 25 kDa, ab32064, Abcam, UK), silent information regulator 2 homolog 1 (SIRT1) (1 : 1000, 81 kDa, ab189494, Abcam, UK), peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1α) (1 : 1000, 92 kDa, ab188102, Abcam, UK), nuclear factor erythroid 2-related factor 2 (Nrf2) (1 : 8000, 54 kDa, ab175932, Abcam, UK), heme oxygenase-1 (HO-1) (1 : 40000, 33 kDa, ab68477, Abcam, UK), phospho (p)-p65 (1 : 1000, 60 kDa, ab76302, Abcam, UK), p65 (1 : 1000, 60 kDa, ab16502, Abcam, UK), and GAPDH (1 : 8000, 36 kDa, ab181602, Abcam, UK) at 4°C overnight, followed by the incubation with goat anti-rabbit secondary antibody (1 : 5000, ab6721 Abcam, UK) or anti-mouse secondary antibody (1 : 5000, ab190475, Abcam, UK) at 37°C for 1 hour. An ECL reagent (PE0010, Solarbio, China) and a gel imaging system (A44114, Invitrogen, USA) were employed for the visualization process. GAPDH was used as a housekeeping gene.

2.10. IHC Assay. First, the fixed skeletal muscle tissues were dehydrated and embedded in paraffin and then cut into pieces with 3 μm thickness [20]. The sections were then dewaxed with xylene and hydrated with gradient ethanol. Subsequently, the sections were put into 0.01 M citrate buffer (pH 6.0, I012-1-1, Jiancheng, China), boiled (95°C, 15–20 minutes), and then naturally cooled at room temperature. The sections were then soaked in 3% H_2O_2 solution for 20 minutes to remove the endogenous peroxidase. Thereafter, the sections were reacted with anti-SIRT1 antibody (diluted at 1 : 500) at 4°C overnight. After another 1 hour of incubation with the secondary antibodies (1 : 1000, ab6721, Abcam, UK) at 37°C, the sections were reacted with the DAB kit (DA1015, Solarbio, China) and counterstained by haematoxylin, followed by being dehydrated and mounted by
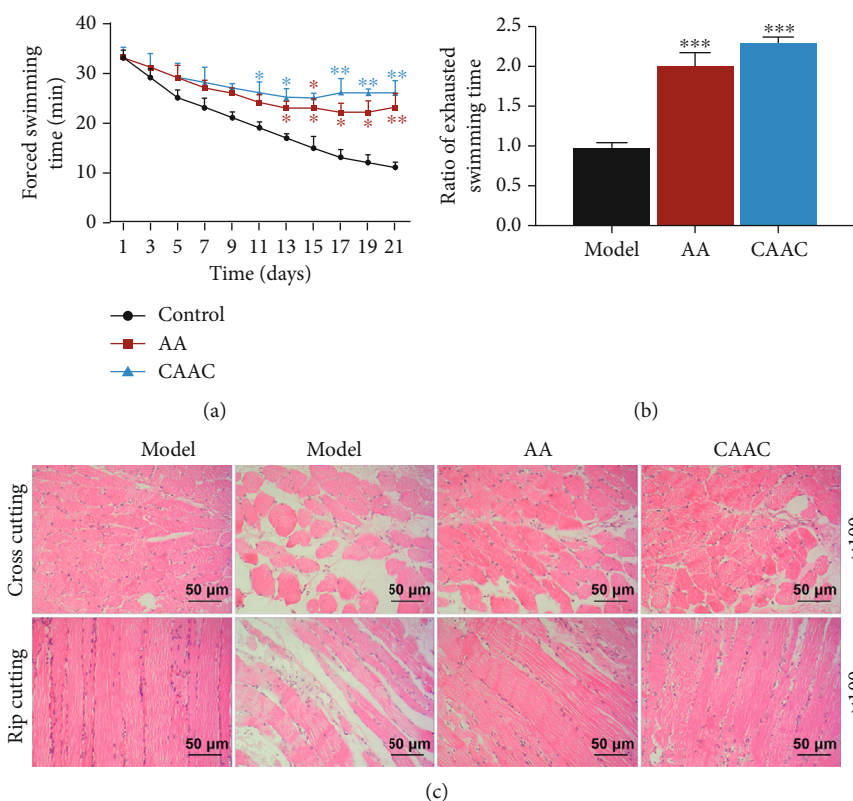
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FIGURE 1: The effects of CAAC on the swimming ability and skeletal muscle pathology of FST model rats. (a) Compound amino acid capsules (CAAC) prolonged the forced swimming time of forced swimming test (FST) model rats (the oral dose of CAAC was 25 mL/kg). (b) CAAC prolonged the ratio of exhausted swimming time. (c) The effect of CAAC on the histological appearance of the skeletal muscle was detected by hematoxylin-eosin staining (magnification: 100x, scale bar = 50 μm). All experiments have been performed in triplicate, and data were expressed as mean ± standard deviation (SD). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs. the model group.

neutral balsam. Finally, an optical microscope (100x) was employed to observe and capture the positive expression of SIRT1.

2.11. Statistical Analysis. Data were analyzed by SPSS 20.0 (IBM, NY, USA) and represented as mean ± standard deviation. Differences among multiple groups were done using one-way analysis of variance followed by Tukey's post hoc test. $P < 0.05$ was deemed as statistically significant.

3. Results

3.1. The Effect of CAAC on the Swimming Ability and Skeletal Muscle Pathology of FST Model Rats. Then, we performed the animal experiments and we observed that the CAAC intervention prolonged the forced swimming time and the ratio of exhausted swimming time in rats and the effect of AA treatment was the same as that of CAAC (Figures 1(a) and 1(b), $P < 0.05$). It was manifested in the results of H&E staining that compared with that in the control group, the structure of skeletal muscle fiber in the model group was irregular and the size of fibers was decreased, while the intervention of CAAC and AA greatly improved the growth and development of muscle and augmented the size of fibers in the muscle (Figure 1(c)).

3.2. CAAC Improved the Level of Biochemical Indicators in the Serum of FST Model Rats. As shown in Figures 2(a)–2(c), the levels of BUN, LA, and LDH in the model group were extremely enhanced than those in the control group, while CAAC and AA largely repressed the increased levels of BUN, LA, and LDH in FST model rats ($P < 0.01$). Meanwhile, we also confirmed that the contents of LG and MG were decreased in FST model rats, while the treatments with AA and CAAC obviously increased the contents of LG and MG (Figures 2(d) and 2(e), $P < 0.01$). Besides, we detected the levels of ALT and AST and found that the levels of ALT and AST in the model group were augmented in comparison with those in the control group, whereas the elevated effects were repressed by the treatment with AA and CAAC (Figures 2(f) and 2(g), $P < 0.01$).

3.3. CAAC Decreased the Levels of ROS and MDA Levels and Increased those of SOD and GSH-Px in the Skeletal Muscle Tissue of FST Model Rats. We detected the oxidative stress-related indicators in the skeletal muscle by ELISA, the results of which revealed that the levels of ROS and MDA were largely enhanced, whereas those of SOD and GSH-Px in the skeletal muscle tissue of the model group were greatly attenuated (Figures 3(a)–3(d), $P < 0.01$). In contrast, the supplementation of CAAC and AA strongly inhibited the FST-induced higher levels of ROS and MDA and lower
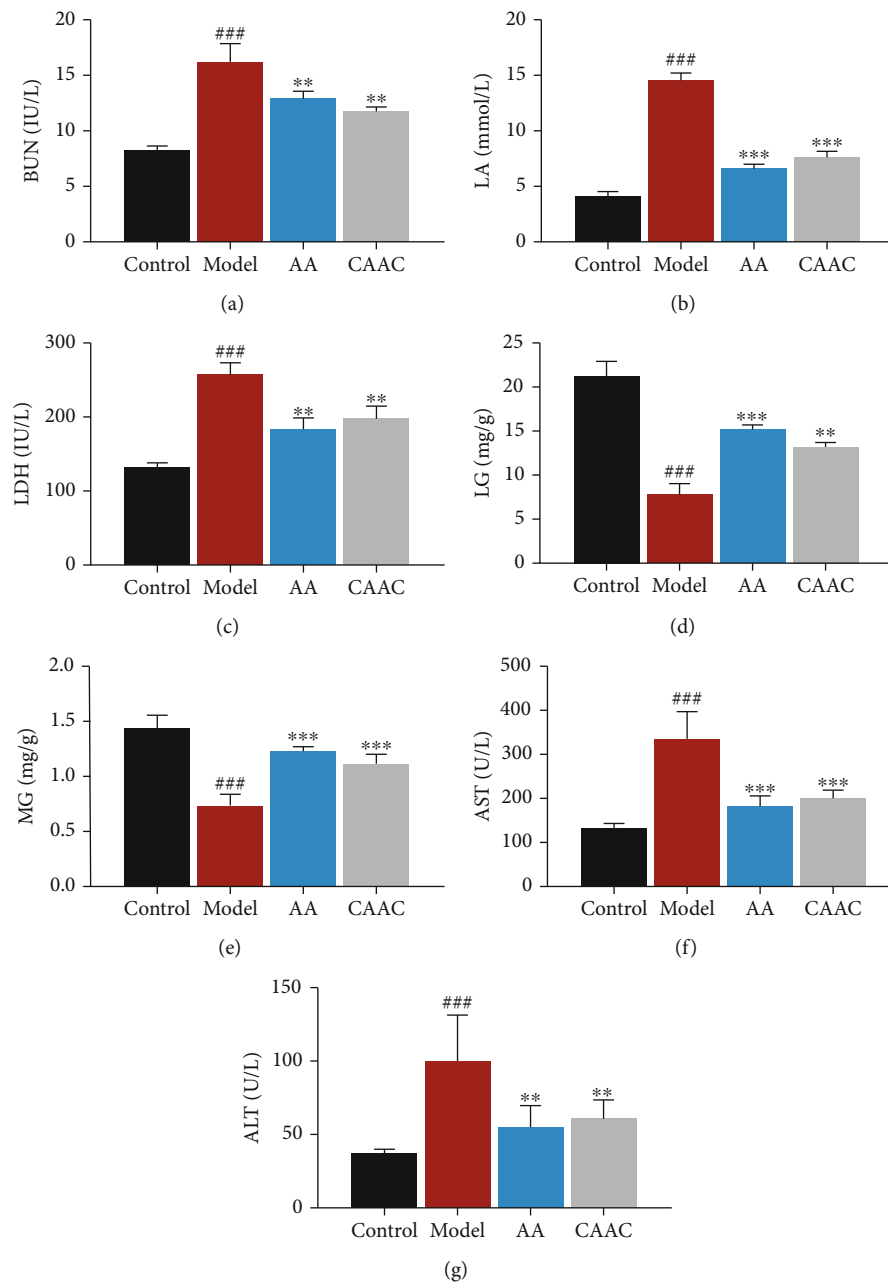
FIGURE 2: CAAC improved the level of serum biochemical indicators in FST model rats. (a–g). The effect of CAAC on the biochemical parameters of serum and glycogen storage in the rats after exhaustive swimming was determined by enzyme-linked immunosorbent assay (ELISA). All experiments have been performed in triplicate and data were expressed as mean ± standard deviation (SD). $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the model group; $^{###}P < 0.001$ vs. the control group.

levels of SOD and GSH-Px in the skeletal muscle tissue (Figures 3(a)–3(d), $P < 0.05$).

3.4. CAAC Weakened Apoptosis and Diminished the Levels of Inflammatory Cytokines and Apoptosis-Related Proteins in the Skeletal Muscle Tissue of FST Model Rats. In Figures 4(a)–4(c), the results indicated that the contents of TNF-α, IL-1β, and IL-6 in the rats of the model group were augmented relative to those of the control group ($P < 0.001$). After CAAC treatment, the contents of TNF-α, IL-1β,

and IL-6 were attenuated and the treatment of AA had similar effects on inflammatory cytokine (Figures 4(a)–4(c), $P < 0.001$). Additionally, the results of TUNEL staining clarified that the number of TUNEL-positive cells in the skeletal muscle tissue of FST model rats enhanced significantly, while the intervention of CAAC and AA strongly reduced the number of TUNEL-positive cells (Figure 5(a), $P < 0.001$). The Western blot assay clarified that the level of Bcl-2 was decreased, whereas those of cleaved caspase-3 and cleaved PARP-1 in the rats of the model group were increased than
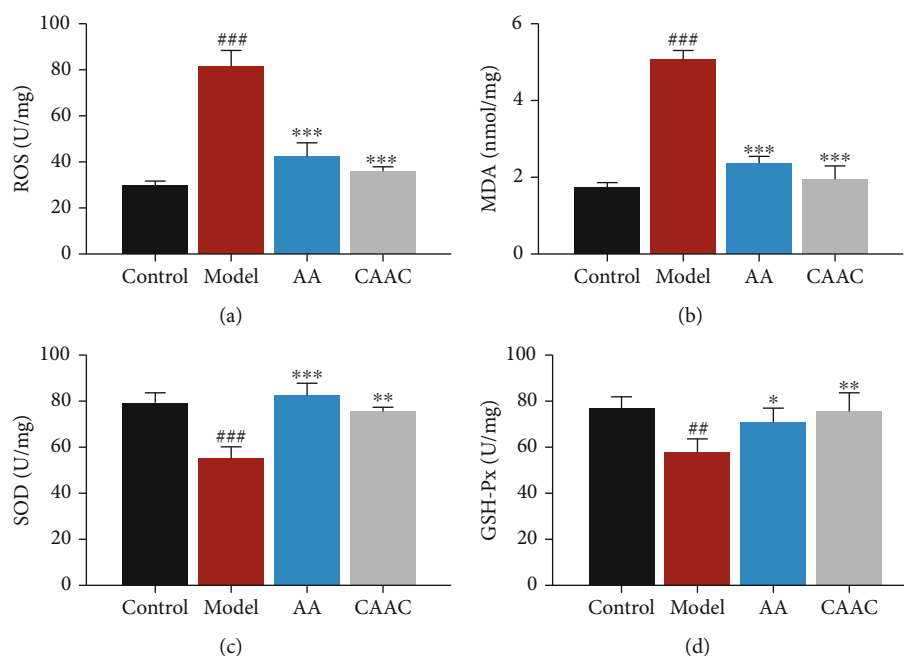
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(d)

FIGURE 3: CAAC blunted the levels of ROS and MDA and increased those of SOD and GSH-Px in the skeletal muscle tissue of FST model rats. (a–d) The effects of CAAC on the levels of reactive oxygen species (ROS), malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) in the skeletal muscle tissue of FST model rats were detected by ELISA. All experiments have been performed in triplicate, and data were expressed as mean ± standard deviation (SD). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the model group; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. the control group.
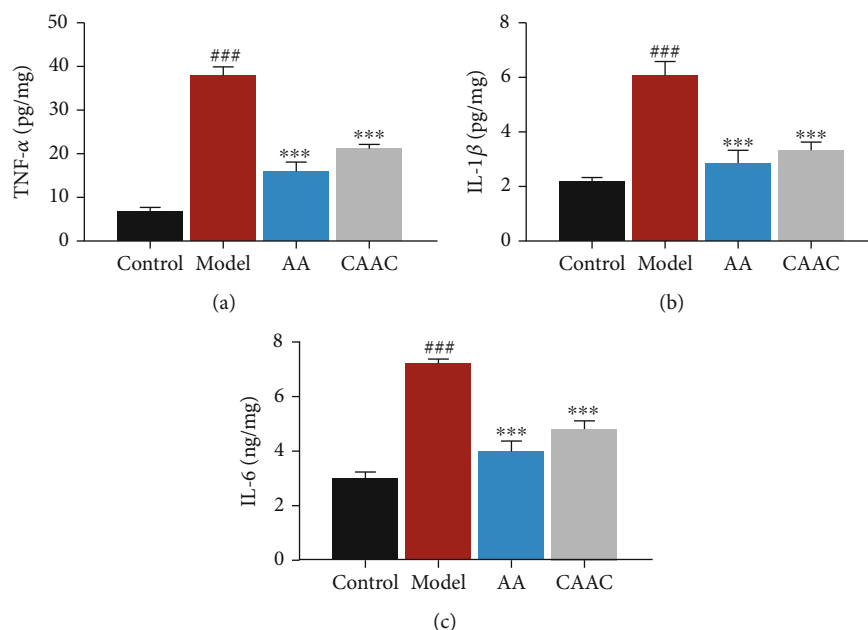

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FIGURE 4: CAAC alleviated the levels of inflammatory cytokines in the skeletal muscle tissue of FST model rats. (a–c) The effects of CAAC on the levels of interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-alpha (TNF-α) in the skeletal muscle tissue of FST model rats were detected by ELISA. All experiments have been performed in triplicate and data were expressed as mean ± standard deviation (SD). $^{***}P < 0.001$ vs. the model group; $^{\#\#\#}P < 0.001$ vs. the control group.

those in the control group, while CAAC and AA elevated the level of Bcl-2 and blunted those of cleaved caspase-3 and cleaved PARP-1 (Figure 5(b), $P < 0.001$).

3.5. CAAC Enhanced the Expression of SIRT1 and SIRT1/ PGC-1α/Nrf2 Pathway-Related Proteins Yet Attenuated That of p-p65 in FST Model Rats. The IHC assay revealed
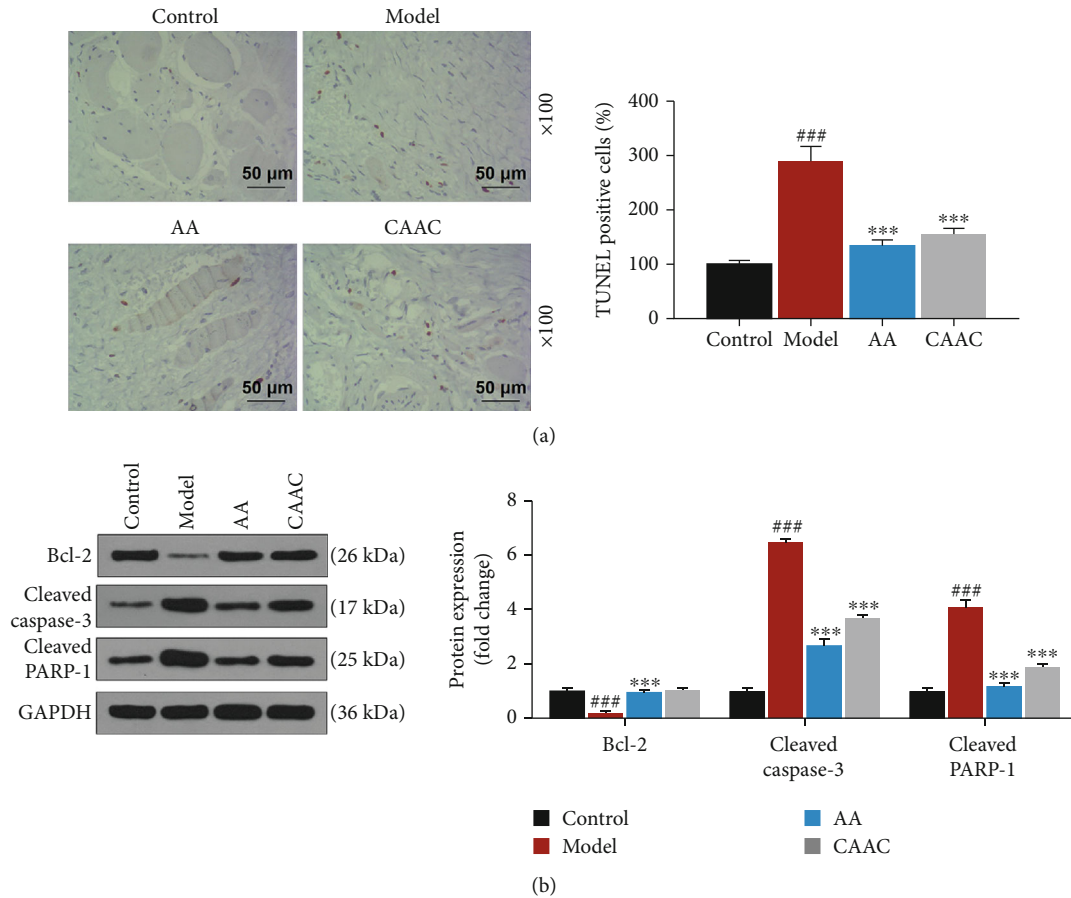
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FIGURE 5: CAAC diminished the apoptosis and the levels of apoptosis-related proteins in the skeletal muscle tissue of FST model rats. (a) The effect of CAAC on the apoptosis in the skeletal muscle tissue of FST model rats was unveiled using terminal-deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) staining (magnification: 100x, scale bar = $50\,\mu$m). (b) The effect of CAAC on the levels of apoptosis-related proteins in the skeletal muscle tissue of FST model rats was illustrated based on the results of Western blot. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. All experiments have been performed in triplicate, and data were expressed as mean ± standard deviation (SD). ***$P < 0.001$ vs. the model group; ###$P < 0.001$ vs. the control group.

that the content of SIRT1 protein in the skeletal muscle tissue of the model group was extremely reduced, while AA and CAAC treatment obviously elevated the content of SIRT1 protein in the skeletal muscle tissue of FST model rats (Figure 6(a), $P < 0.001$). Furthermore, the data from Western blot demonstrated that the expression of SIRT1, PGC-1α, Nrf2, and HO-1 in the skeletal muscle tissue of the model group was largely blunted yet the expression of p-p65 and the ratio of p-p65/p65 were elevated (Figure 6(b), $P < 0.001$). After AA and CAAC treatment, the expression of SIRT1, PGC-1α, Nrf2, and HO-1 was enhanced, whereas the expression of p-p65 and the ratio of p-p65/p65 were repressed (Figure 6(b), $P < 0.001$). However, no evident change on the expression of p65 was evidenced in each group of rats.

4. Discussion

It is generally believed that fatigue is a complex phenomenon and a physiological change where efficiency decreases after continuous work or exercise, which is mainly manifested by the decrease of muscle tension and exercise endurance resulted from the accumulation of metabolites [21].

Here, we evaluate the antifatigue properties of CAAC by evaluating the functional activity of skeletal muscle. This is the first time to analyze the effect of CAAC on exhaustive swimming rats in the FST model. The extension of exercise time is the visual appearance of antiexercise fatigue, so the duration of the exhausted swimming time is used as an indicator of this experiment [22]. Based on our results, it was suggested that CAAC intervention prolonged the forced swimming time and the ratio of exhausted swimming time in rats and ameliorated the damage in the skeletal muscle tissue of FST rats, revealing that CAAC might have antifatigue properties. A research on exercise physiology clarified that the reservation of MG and LG and the maintenance of blood glucose levels directly affect the body's ability to resist exercise-induced fatigue [23]. It was found for the first time that CAAC treatment obviously enhanced the contents of LG and MG in FST model rats. LA is the end product of the anaerobic metabolism of sugar in the body. In the process of strenuous exercise, glycolysis of the body is accelerated and MG is consumed in large quantities and a large amount of LA is produced, causing postexercise fatigue [24]. Meanwhile, similar changes are also displayed when it
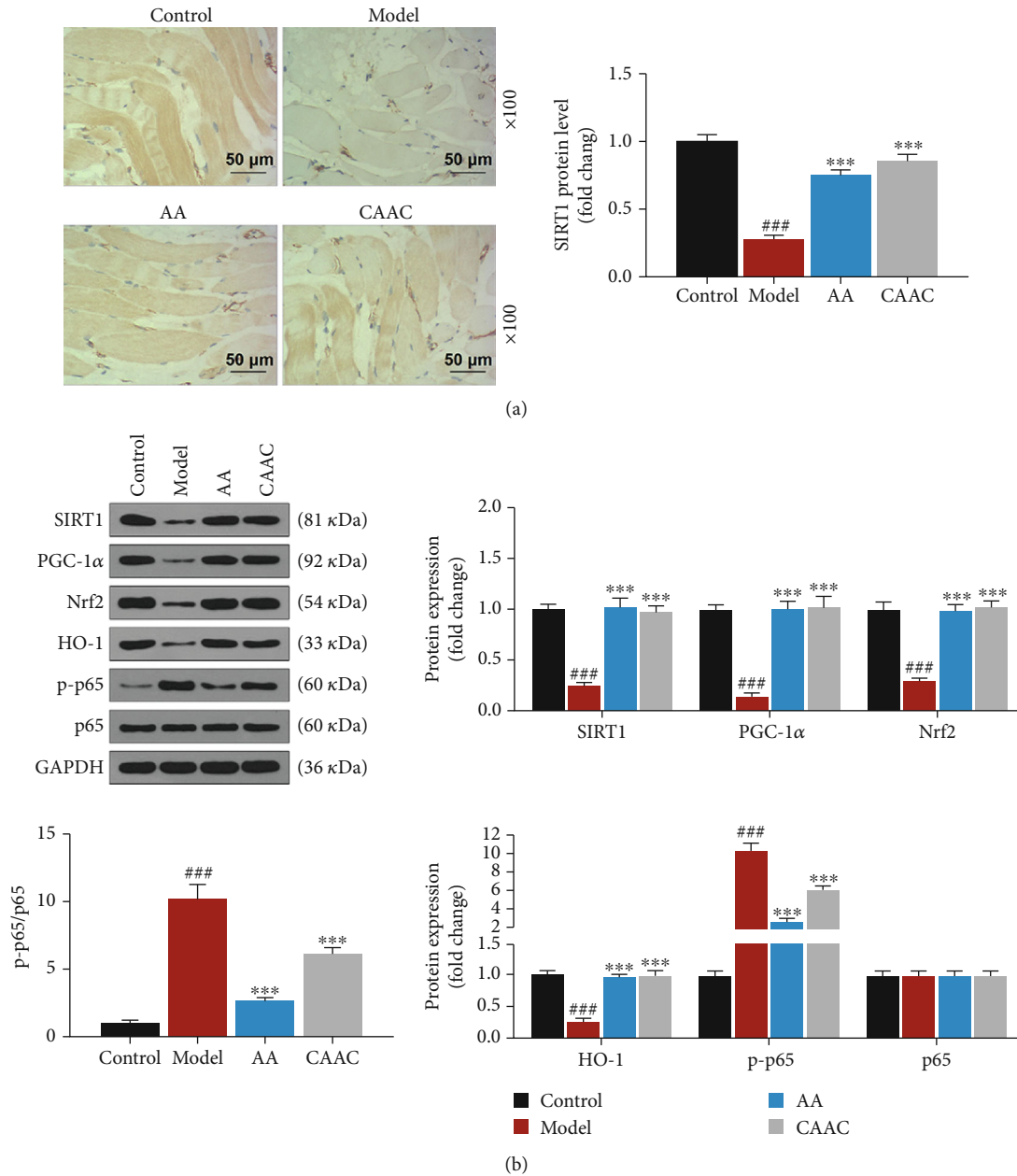
(a)

(b)

FIGURE 6: CAAC enhanced the expression of SIRT1 and SIRT1/PGC-1α/Nrf2 pathway-related protein in FST model rats and attenuated the p-p65 level. (a) The effect of CAAC on the expression of silent information regulator 2 homolog 1 (SIRT1) in the skeletal muscle tissue of FST model rats was uncovered using immunohistochemical assay (magnification: 100x, scale bar = 50 μm). (b) The results of Western blot showed that CAAC enhanced the expression of SIRT1/peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1α)/nuclear factor erythroid 2-related factor 2 (Nrf2) pathway-related factors in FST model rats and attenuated the p-p65 level. All experiments have been performed in triplicate, and data were expressed as mean ± standard deviation (SD). ***$P < 0.001$ vs. the model group; ###$P < 0.001$ vs. the control group.

comes to LDH, the key enzyme for the production of LA [25]. Many factors (fatigue and stress) can also affect the level of BUN, an important indicator of the body's exercise tolerance [26]. As expected, it was demonstrated in the results that the levels of BUN, LA, LDH, ALT, and AST were extremely enhanced in FST model rats, while all of the enhanced levels in FST model rats were greatly repressed by the administration of CAAC.

The study has manifested that long-term exhaustive exercise can easily promote the level of free radicals, which can increase the levels of MDA and ROS in skeletal muscles and other tissues and organs, while decreasing the activities of SOD and GSH-Px [27]. As the primary substance for scavenging free radicals in the body, SOD is both an important antioxidant enzyme in the body's defense system and a sensitive indicator to measure the metabolism of free

radicals [28]. GSH-Px is an important antioxidant and free radical scavenger *in vivo*, and it can also specifically catalyze the reduction of glutathione to hydrogen peroxide and protect cell membrane [29]. Therefore, increasing the activities of SOD and GSH-Px and decreasing the contents of MDA and ROS can alleviate oxidative stress and improve exercise-induced fatigue. Consistent with the results of previous studies, the levels of ROS and MDA were largely enhanced yet those of SOD and GSH-Px were greatly attenuated in rats following the construction of the FST model and it was further confirmed in our research that CAAC effectively elevated the activities of SOD and GSH-Px, whilst blunting the contents of MDA and ROS in skeletal muscle [30].

Inflammation is a condition that appears in response to the exercise-mediated oxidative stress. TNF-α, IL-1β, and IL-6 are important proinflammatory factors. The damage caused by exercise-induced fatigue can increase the levels of TNF-α, IL-1β, and IL-6 in tissues [30]. We therefore used the ELISA to further determine the effects of CAAC on the proinflammatory factors, the results of which indicated that the contents of TNF-α, IL-1β, and IL-6 in FST model rats were augmented, while CAAC treatment inhibited the levels of TNF-α, IL-1β, and IL-6 in skeletal muscle. In addition to these discoveries, we found that CAAC elevated the level of Bcl-2 and blunted those of cleaved caspase-3 and cleaved PARP-1 in the skeletal muscle of FST model rats.

To further elucidate the molecular mechanism by which CAAC alleviates fatigue in FST model rats, we examined the expression of oxidative stress-related signal genes in skeletal muscle tissue of rats. SIRT1 and PGC-1α-mediated inhibition of the antioxidant signaling pathway are the main causes of fatigue. SIRT1 is a nicotinamide adenosine dinucleotide- (NAD^+-) dependent protein deacetylase, which has a significant antagonistic effect on the damage caused by oxidative stress [31]. PGC-1α is a coactivator and pleiotropic regulator of many transcription factors in the energy metabolism pathway, and it participates in the regulation of oxidative metabolism within the body [32]. A scientific study clarified that rutin greatly enhanced the levels of SIRT1 and PGC-1α in the skeletal muscle and brain of FST mice. Meanwhile, it has been reported that Nrf2 was not only the main regulator of the antioxidant response but also the promoter of many antioxidant and detoxification genes (such as HO-1, NQO1, and GCLC) that bind and activate to maintain redox homeostasis [33, 34]. Our study showed that the expression levels of SIRT1, PGC-1α, Nrf2, and HO-1 were significantly decreased in the skeletal muscle of FST model rats and standardized treatment with CAAC could restore these indicators to normal. In addition, we also found that CAAC could reduce the phosphorylation of p-p65/p65. These findings indicated that CAAC improved the body's exercise ability by regulating the expression of exercise-related signal genes so as to exert antifatigue effects.

5. Conclusion

Our results suggest that CAAC plays an antifatigue role by enhancing the activity of the antioxidant enzyme system, modulating the inflammatory response induced by fatigue-caused damage, and regulating the protein expression of the SIRT1/PGC-1α/Nrf2 pathway and p-p65.

Data Availability

The data used and/or analyzed during the current study are available from the corresponding author.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Wen Huang and Huaqiang Hui contributed equally to this work.

Acknowledgments

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