

Effects of curcumin and aerobic exercise on apoptosis and the IGF-1/PI3K/AKT/mTOR pathway in the muscle of aged rats with sarcopenia

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Abstract

Background

This study aimed to investigate the effects of curcumin supplementation and aerobic exercise on delaying age-related skeletal muscle atrophy and to further explore their potential synergistic effects. The findings are expected to provide a theoretical basis for alleviating skeletal muscle dysfunction and reducing muscle mass loss in the elderly, thereby offering a feasible strategy for the intervention of age-related sarcopenia.

Methods

In this study, 18-month-old rats were randomly divided into six groups. The rats underwent 8 weeks of aerobic exercise and curcumin intervention. Hematoxylin and eosin staining and a terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay were used to compare the morphological structure and apoptosis of skeletal muscle cells in each group, as well as the skeletal muscle tension. The expression levels of proteins related to the IGF-1/PI3K/AKT/mTOR, AMPK/PGC-1, and CaMK signaling pathways, as well as LC3-1 and ULK1, were determined to investigate the anti-aging effects of curcumin and aerobic exercise on skeletal muscle.

Results

Constant-load aerobic exercise alleviated skeletal muscle loss, reduced the apoptosis index, and upregulated IGF-1/PI3K/AKT/mTOR pathway proteins in aged rats. It also activated AMPK/PGC-1 and CaMK signaling to regulate mitochondrial function, lowered LC3-I and ULK1 expression, and improved muscle tension. Curcumin yielded comparable benefits, albeit with weaker effects than constant-load aerobic exercise.

Conclusion

Curcumin combined with constant-load aerobic exercise increased the cross-sectional area of skeletal muscle, improved muscle tension, attenuated myocyte apoptosis, and upregulated the expression of growth-associated proteins in aged rats. Meanwhile, the expression levels of LC3-1 and ULK1 were markedly downregulated. Among all intervention regimens, constant-load aerobic exercise yielded the most pronounced beneficial outcomes; however, no synergistic effects were detected in the combined treatment with curcumin. Collectively, this study demonstrates that supplementation with the exogenous antioxidant curcumin combined with constant-load aerobic exercise can effectively restrain skeletal muscle apoptosis and mitigate age-related sarcopenia.

Introduction

Sarcopenia is a syndrome characterized by a progressive and systemic decline in skeletal muscle mass, strength, and physical function [1]. Studies have shown that muscle strength begins to decline around the age of 40, decreasing by approximately 12%–15% per decade after the age of 50, with an accelerated reduction occurring beyond 65 years of age [2, 3]. A similar trend is observed in skeletal muscle mass, which decreases by 25%–30% between 50 and 75 years of age [4]. These age-related alterations result in skeletal muscle atrophy, increasing the risk of falls, disability, and mortality among older adults, thereby imposing a substantial burden on both families and public healthcare systems [5]. Consequently, identifying effective strategies to delay the onset and progression of sarcopenia holds considerable practical significance. Although the etiology of aging-related sarcopenia is multifactorial and its precise mechanisms remain unclear, it is widely recognized that sarcopenia is closely associated with hormonal alterations, enhanced apoptosis [6], reduced physical activity and sedentary behavior [7], oxidative stress, mitochondrial dysfunction [6, 8], and chronic low-grade inflammation [9]. For example, aging leads to a widespread decline in circulating and tissue levels of insulin-like growth factor 1 (IGF-1), which regulates processes such as PI3K/AKT/mTOR-mediated protein synthesis, degradation, and apoptosis, thereby influencing skeletal muscle homeostasis and mass [10, 11]. In aged skeletal muscle, decreased calcium/calmodulin-dependent protein kinase II (CaMKII) activity may contribute to the development of muscle aging and sarcopenia [12, 13], while also impairing the regulatory role of AMP-activated protein kinase (AMPK) in mitochondrial and oxidative metabolism through its effect on peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1). Furthermore, the proteins LC3-1 and ULK1 are implicated in mitochondrial dysfunction, impaired autophagy, chronic inflammation, and oxidative stress [9, 14], all of which exacerbate the progression of sarcopenia.

Current research aimed at delaying skeletal muscle aging primarily focuses on two areas: nutritional or pharmacological supplementation and exercise interventions. The former involves administration of exogenous antioxidants to enhance free radical scavenging, regulate expression of related proteins, and activate phase enzymes to restore cellular function and delay aging. The latter seeks to improve physical performance and adaptability through structured exercise programs.

Numerous studies have demonstrated that physical activity exerts a significant beneficial effect in mitigating muscle atrophy, and an increasing body of research has focused on elucidating how physical activity can modify or even reverse functional decline. Evidence suggests that the age-related reduction in aerobic capacity and the decline in neuromuscular endurance can be delayed through appropriately designed training regimens [15]. Curcumin plays an essential role in antioxidant research and has shown substantial clinical application potential. Multiple studies have confirmed its anti-inflammatory and antioxidant activities, as well as its ability to effectively scavenge reactive oxygen species and nitric oxide free radicals. Moreover, curcumin at specific concentrations may exert an anti-oxidative stress effect by activating the mTOR signaling pathway, upregulating the expression of the anti-apoptotic protein BCL-2, and downregulating the pro-apoptotic protein Bax [16].

The optimal approach to stimulating muscle growth likely involves the integration of exercise training and nutritional interventions. However, most existing studies have examined the mechanisms through which either nutrition or exercise independently delays skeletal muscle aging, while relatively few have investigated their combined effects. Based on previous research, this study established a sarcopenia model in aged rats and implemented combined intervention with exogenous curcumin supplementation and aerobic exercise training. During the 8-week experimental period, multiple indicators were measured, including variations in body weight, the wet weight of the soleus muscle, grip strength, morphological and structural alterations to skeletal muscle, and the apoptosis level of skeletal muscle cells. Protein expression levels associated the PI3K/AKT/mTOR signaling pathway, AMPK, and PGC-1 were also determined. This study aimed to explore the therapeutic effects and underlying molecular regulatory mechanisms of curcumin combined with aerobic exercise on delaying skeletal muscle aging. The results provide a theoretical basis and empirical evidence for the prevention and clinical treatment of sarcopenia via pharmaceutical, nutritional, and exercise-based strategies.

Materials and methods

Subjects and groups

Sixty healthy male Sprague-Dawley rats (555.61 ± 57.44 g, 18 months old), classified as specific pathogen-free (SPF), were provided by Changsha Tianqin Biotechnology Co., Ltd. (Animal Permit No: SCXK [Hunan] 2014-0032). The rats were housed under standard conditions, including a room temperature of 20–24°C and relative humidity of 45%–55%. They were fed a nationally standardized Glires diet and allowed free access to boiled, cooled tap water. Water and appropriate feed were replenished daily, and litter and cages were changed every other day. After one week of acclimation, the rats were randomly assigned to six groups: control group (BC, $n = 10$), curcumin group (QC, $n = 10$), constant-load exercise group (CE, $n = 10$), incremental-load exercise group (IE, $n = 10$), curcumin combined with constant-load exercise group (CC, $n = 10$), and curcumin combined with incremental-load exercise group (IC, $n = 10$). Following eight weeks of regular aerobic exercise, the actual age of the rats was 20 months, equivalent to approximately 52 years in humans.

Establishment of exercise experimental model

After one week of acclimatization, 18-month-old rats were weighed and administered a curcumin suspension at a dosage of 100 mg/kg. The BC group received 1% carboxymethyl cellulose sodium by gavage and did not undergo any exercise, thereby maintaining normal living conditions. The QC group was gavaged with curcumin based on body weight and also did not participate in exercise. The CE group followed the Bedford exercise model, incorporating modifications based on the training protocols of Radak et al. [17] and Pietrelli et al. [18]. Rats in this group underwent three days of adaptive training ($10 \text{ m} \cdot \text{min}^{-1}$ for 10 min on day one, 20 min on day two, and 25 min on day three, with a 0° incline). After one day of rest, the formal experiment began. The treadmill speed was set at $15 \text{ m} \cdot \text{min}^{-1}$ for 50 min per

session, five days per week, for eight weeks. The CC group was gavaged with curcumin six hours before exercise, following the same training regimen as the CE group. The IE group was based on the exercise model proposed by Liu et al. [19] and Lei [20], with further modifications. The specific protocol is detailed in Supplementary Table S1, ensuring that the total exercise intensity matches that of the CE group. The total exercise volume was calculated as follows: speed (m/min) × running time (min) × days. The curcumin combined with IC group was gavaged with curcumin six hours before exercise and followed the same training load as the IE group. All of the exercise groups trained five days per week and rested on weekends.

Determination of body weight and soleus wet muscle weight

Prior to the experiment, the rats in each group were weighed. During the 8-week intervention, their body weight was measured every Saturday to monitor any changes in it. At the end of the 8-week training period, the rats in the control and exercise groups were weighed under resting conditions or 20 hours after the last exercise session, respectively. Following this, all rats were intraperitoneally anesthetized with 10% chloral hydrate at a dose of 0.5 mL/100 g of body weight. Successful anesthesia was confirmed by the disappearance of the corneal reflex alongside the exhibition of stable respiration and relaxed muscles. Subsequently, body surface fur was disinfected with 75% ethanol. The soleus muscle was then completely isolated from the hind limbs, rinsed with ice-cold normal saline, and stripped of adipose and connective tissues. After blotting off excess saline with filter paper, the wet weight of the soleus muscle was measured using an electronic balance.

Rope hanging test

Referring to the method described by Jänicke et al. [21], adaptive training was conducted three days before the end of the exercise intervention, followed by administration of the horizontal rope test on two consecutive days. A rope with a diameter of 0.4 cm and a length of 83 cm was fixed at both ends at a height of 43 cm above the tabletop. Each rat was placed so that it could grasp the rope with its forelimbs, and the animals spontaneously performed pulling-up movements, hindlimb grasping, or tail winding around the rope. The hanging duration of each rat was recorded. The test was repeated once after a 5-minute interval for each rat, and the better result of the two trials was selected for subsequent statistical analysis. After the hanging times obtained on the two testing days were averaged, one-way analysis of variance was applied for statistical comparison.

Hematoxylin-eosin (H&E) staining and Masson staining

The soleus and gastrocnemius muscle tissues of Sprague-Dawley rats were collected, sectioned, and subjected to H&E and Masson staining [22, 23]. Microscopic images of the stained samples were captured using a Leica microscope (Wetzlar, Germany). Morphological changes in the skeletal muscle

tissues were analyzed using ImageJ software (Version 5.0), developed by the National Institutes of Health (NIH, Bethesda, Maryland, USA).

Immunohistochemistry staining

Paraffin-embedded tissue sections were sequentially processed through deparaffinization, hydration, antigen retrieval, endogenous enzyme inactivation, and blocking procedures, followed by incubation with primary and secondary antibodies. The sections were subsequently rinsed with phosphate-buffered saline, stained with 3,3'-diaminobenzidine, and subjected to terminal deoxynucleotidyl transferase dUTP nick end labeling. After counterstaining with toluene, the samples were dehydrated using graded alcohol solutions, treated with xylene for tissue clearing, and finally mounted with neutral gum. Images were acquired using a Leica microscope, and the integrated optical density was quantified using Aipaitong software (Saier Biotechnology Co., Ltd., Wuhan, China).

Enzyme-linked immunosorbent assay

The expression levels of IGF-1 in rat serum, soleus, and gastrocnemius muscles were determined using an enzyme-linked immunosorbent assay kit obtained from Elabscience Biotechnology Co., Ltd. (Wuhan, China).

Western blot assay

Total proteins from rat skeletal muscle tissues were extracted using RIPA lysis buffer supplemented with protease and phosphatase inhibitors. The total protein concentration was measured using a BCA kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The expression levels of target proteins were assessed via Western blot analysis [24]. Briefly, 30 µg of protein per sample was separated by 10% or 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by transfer onto 0.45 µm or 0.22 µm polyvinylidene fluoride membranes. After blocking with 5% non-fat milk, the membranes were incubated overnight at 4°C with the respective primary antibodies. Protein bands were visualized and analyzed using a gel imaging system (Tanon, Shanghai, China) and ImageJ software. Detailed information on the antibodies used is provided in Supplementary Table S2.

Statistical analysis

Data were expressed as the mean $\pm$ standard deviation (SD). All statistical analyses were performed using GraphPad Prism 9.0 software (GraphPad Software, Inc., La Jolla, CA, USA). The experimental unit was defined as a single animal. A total of 6 groups were included in this study. Each group contained 10 animals per independent experiment, and a total of three independent experiments were performed, resulting in a final sample size of $n = 30$ per group for all statistical analyses. All 10 animals in each independent experiment were included in every detection, and no animals or samples were excluded

during the experiment or statistical analysis. The three independent experiments represent biological replicates, which were conducted using different batches of animals under identical experimental conditions to ensure the reproducibility of the results. For each sample, three technical replicates were performed during detection to reduce measurement error, and the mean value of the three technical replicates was used for subsequent statistical analysis. The normality of the data distribution was assessed using the Shapiro-Wilk test, and the homogeneity of variance was evaluated with Levene's test. Both tests were implemented in GraphPad Prism 9.0. For comparisons among the 6 groups, one-way analysis of variance followed by the Bonferroni post hoc test for multiple comparisons was used when the data were normally distributed and the variance was homogeneous. If the assumptions of normality or homogeneous variance were violated, the Kruskal–Wallis H test (a nonparametric test) was applied. A two-tailed P value < 0.05 was considered statistically significant.

Results

Effects of curcumin combined with aerobic exercise on changes in body weight and skeletal muscle index (SI) in aged rats before and after intervention

As shown in Table S3, there were no significant differences in body weight among the groups before exercise training ($P > 0.05$). With the initiation of intervention, the body weight of the rats in each group exhibited a decreasing trend; this trend was most pronounced in the CC group, which was significantly different from the BC group ($P < 0.05$). The body weight of rats in the IE group decreased markedly at the 7th and 8th weeks, exhibiting significant differences relative to the BC group ($P < 0.05$). In all the training groups, body weight exhibited an extremely significant difference after intervention versus the baseline level before exercise ($P < 0.01$).

The SI was defined as the ratio of the wet weight of the target skeletal muscle to the body weight of the animal, and it was used to normalize body weight and evaluate the relative skeletal muscle mass. Based on the SI values of the soleus muscle presented in Table S4, intergroup difference analysis yielded the following results. Compared with the BC group, the SI values of the QC, CC, CE, IC, and IE groups were significantly increased, with these differences being extremely significant ($P < 0.01$). A significant difference was observed between the QC group and the CC group ($P < 0.05$). Although the CC group showed a marginally higher SI value than the CE group and the QC group had a higher soleus muscle SI value than the CE group, no significant differences were detected in terms of the above comparisons ($P > 0.05$). In addition, the QC group exhibited a significant difference relative to the IC group ($P < 0.05$). Finally, the IC group had a higher SI value than the IE group, though this difference was not statistically significant ($P > 0.05$).

Effects of curcumin and aerobic exercise on horizontal rope grasping time in aged rats

The first horizontal rope grasping test, the results of which are shown in Table S5, was performed immediately following adaptive training and was therefore excluded from statistical analysis. The second horizontal rope grasping test, the results of which are also shown in Table 5, was conducted 24 hours later. The results revealed that, compared with the BC group, the grasping time was significantly prolonged in all treatment groups ($p < 0.05$), with highly significant differences relative to the baseline observed in the CC and IE groups ($p < 0.01$). In comparison with the QC group, both aerobic exercise groups exhibited significant increases in grasping time ($p < 0.05$). Additionally, the CC group exhibited a significantly longer grasping time than the CE group ($p < 0.05$), and the IE group showed significantly improved grasping capacity relative to the IC group ($p < 0.05$).

Effects of curcumin and aerobic exercise on skeletal muscle cell morphology and cross-sectional area in aged rats

Skeletal muscle tissues were stained with H&E, where muscle fibers appeared pink and cell nuclei were stained purplish blue. Under a light microscope, two fields of view were randomly selected from each section for imaging and storage following H&E staining. In Fig. 1, morphological images of the soleus muscle cells in aged rats from each group are shown. Whole-section scans were conducted to analyze and document tissue abnormalities across groups. Nuclear centralization is indicated by thick arrows, while muscle fiber circularization is shown by thin arrows; fiber miniaturization is also depicted. Histopathological abnormalities were identified when skeletal muscle tissues exhibited any of the following characteristics: muscle fiber circularization, fiber miniaturization, or centralization of muscle cell nuclei. The experimental findings indicated that the soleus and gastrocnemius muscles of middle-aged and aged rats exhibited varying degrees of age-related histopathological alterations. In the comparison of cross-sectional area percentages, the aerobic exercise groups exhibited significant differences compared with the control and curcumin groups in the soleus and gastrocnemius muscle ($p < 0.01$).

Effects of curcumin combined with aerobic exercise on the expression of proteins related to the IGF-1/PI3K/AKT/mTOR signaling pathway in the skeletal muscle tissue of aged rats

As illustrated in Fig. 2, the protein activity of insulin-like growth factor-1 (IGF-1) in the blood, soleus muscle, and gastrocnemius muscle of the rats in each group was quantified via enzyme-linked immunosorbent assay (ELISA). Significant intergroup differences in IGF-1 activity were observed across all tested tissues ($p < 0.05$). Specifically, in the blood and gastrocnemius muscle, extremely significant differences in IGF-1 activity were detected between the BC and CE groups ($p < 0.01$). In the soleus muscle, IGF-1 activity exhibited substantial intergroup variability: compared with the CE group, the BC and QC groups showed significant differences ($p < 0.05$), whereas the CC group exhibited extremely significant differences ($p < 0.01$). Additionally, a significant difference was noted between the CE and IE groups ($p < 0.05$). Western blot analysis demonstrated significant differences in the protein expression levels of PI3K, AKT, and mTOR in both the soleus and gastrocnemius muscles. Relative to the IE group, the BC and QC groups exhibited significantly altered PI3K expression in both skeletal muscle types. In

the soleus muscle, PI3K expression in the CC and IE groups differed extremely significantly from that in the CE group ($p < 0.01$). In contrast, in the gastrocnemius muscle, a significant difference in PI3K expression was observed between the CE and CC groups ($p < 0.05$). Moreover, significant overall intergroup differences were observed in terms of AKT expression in the soleus muscle. Extremely significant differences were identified among the BC, QC, and CE groups ($p < 0.01$), as well as between the CE and CC groups and between the IC and IE groups ($p < 0.01$). A comparable expression trend was observed in the gastrocnemius muscle, where significant differences in mTOR expression were exclusively observed between the BC and QC groups ($p < 0.05$), with no statistical differences detected among the other groups ($p > 0.05$). However, when compared with each aerobic exercise group, both the BC and QC groups displayed significant differences in gastrocnemius mTOR expression ($p < 0.05$).

Effects of curcumin combined with aerobic exercise on skeletal muscle cell apoptosis in aged rats

As shown in Fig. 3, it was found that the BC group exhibited a relatively higher number of apoptotic cells, with chromatin displaying obvious condensation and fragmentation into clumps. In the QC group, fewer apoptotic nuclei were present, and these nuclei appeared smaller than normal nuclei and were clustered together. In the CE group, the brown-stained apoptotic nuclei were relatively fewer and displayed more orderly arrangement. In the IE group, the overall number of nuclei was lower; apoptotic nuclei were smaller than normal ones, and the brown apoptotic nuclei were centrally condensed and more densely arranged. The CC group showed a relatively higher number of brown and yellow apoptotic nuclei, though their distribution was somewhat orderly. In the IC group, apoptotic nuclei were mainly brown to black in color, dispersed irregularly, with darker surrounding nuclei. Consistent trends in changes were observed in both the soleus and gastrocnemius muscles. Statistical analysis of the apoptosis index of soleus and gastrocnemius muscle cells revealed a significant difference between the BC group and the QC group ($p < 0.01$), and extremely significant differences were found between the BC group and both the CE group and the IE group ($p < 0.001$). Specifically, the apoptosis index of the CE group was lower than that of the BC group, whereas the apoptosis index of the IE group was higher than that of the BC group. These results indicate that aerobic exercise with a constant load is conducive to reducing skeletal muscle cell apoptosis, while aerobic exercise with a progressive load exacerbates the apoptotic process of skeletal muscle cells. When curcumin was combined with aerobic exercise, a statistically significant difference between the CC group and the CE group was observed ($p < 0.05$), with a higher apoptosis index in the CC group than in the CE group, suggesting that the combined intervention of curcumin supplementation and constant-load aerobic exercise exerts no synergistic effect on skeletal muscle cell apoptosis. Additionally, an extremely significant difference was detected between the IC group and the IE group ($p < 0.001$), with the apoptosis index of the IE group being markedly higher than that of the IC group, implying that exogenous antioxidant supplementation helped alleviate the pro-apoptotic effects of high-intensity exercise on skeletal muscle cells.

Effects of curcumin combined with aerobic exercise on the AMPK/PGC-1 and CaMK signaling pathways and mitochondrial functional status in the skeletal muscle tissue of aged rats

In the present study, the protein expression levels of AMPK, PGC-1, and CaMK were detected in the soleus and gastrocnemius muscles of the rats in each experimental group. Additionally, the opening status of the mitochondrial permeability transition pore (PTP), ATPase activity, and activities of mitochondrial complexes III and IV were assessed in the quadriceps femoris.

As shown in Fig. 4, in the soleus muscle, PGC-1 protein expression exhibited extremely significant differences among the BC, QC, and CE groups ($p < 0.001$). A significant difference was also noted between the CE and IE groups ($p < 0.01$). In contrast, no statistical differences were observed between BC vs. QC, CC vs. CE, or IC vs. IE ($p > 0.05$). AMPK expression in the soleus muscle exhibited a similar tendency. Considerable intergroup variations in CaMK protein expression within the soleus muscle were found, with remarkable differences detected among the BC, QC, CC, CE, and IE groups ($p < 0.05$).

In the gastrocnemius muscle, the differential expression pattern of PGC-1 across groups was highly analogous to that in the soleus muscle. In terms of AMPK expression, statistical disparity existed only between the BC and CE groups ($p < 0.01$). Likewise, the dynamic changes in CaMK expression in the gastrocnemius muscle shared a high similarity with those of PGC-1.

With regard to PTP opening in the quadriceps femoris, significant differences were observed between the BC and QC, QC and IE, and IC and IE groups ($p < 0.01$). Meanwhile, the activities of ATPase and mitochondrial complexes III and IV differed significantly among the control group, curcumin-treated group, and aerobic exercise group ($p < 0.01$).

Effects of curcumin combined with aerobic exercise on the expression of LC3-1 and ULK1 in the skeletal muscle tissue of aged rats

As illustrated in Fig. 5, the expression levels of LC3-I and ULK1 proteins were detected in the skeletal muscle of rats from each group. In both the soleus and gastrocnemius muscles, LC3-I protein expression was the highest in the BC group and the lowest in the CE group, with extremely significant differences relative to the CE group ($p < 0.01$). Meanwhile, a remarkable difference was also observed between the CE and IE groups ($p < 0.01$). Distinct alterations in ULK1 expression were likewise identified in the soleus and gastrocnemius muscles among different groups. Significant differences in ULK1 expression between the control group and aerobic exercise groups were also confirmed ($p < 0.05$).

Discussion

In this study, 18-month-old Sprague-Dawley rats (equivalent to approximately 50 years of age in humans) were used to establish a sarcopenia model to replicate the complex pathophysiological alterations associated with the natural aging process, including motor unit loss, chronic low-grade inflammation, mitochondrial dysfunction, imbalances in protein synthesis and degradation, and changes in hormone levels [25, 26]. Although previous studies have confirmed that curcumin and aerobic exercise exert extensive protective effects—such as preventing oxidative stress [27], enhancing mitochondrial function, promoting myofiber remodeling, improving muscle fatigue resistance [28], inhibiting skeletal muscle cell

apoptosis, and delaying skeletal muscle senescence [29]. Nevertheless, the therapeutic effects of aerobic exercise combined with exogenous curcumin supplementation on ameliorating sarcopenia remain poorly elucidated. This study revealed that exogenous antioxidant supplementation and different modes of aerobic exercise regulate the expression levels of key proteins involved in multiple signaling pathways, including IGF-1/PI3K/AKT/mTOR, AMPK/PGC-1, CaMK, LC3-1, and ULK1. These interventions further modulate a series of biological processes in skeletal muscle, such as muscle morphological characteristics, cellular apoptosis, muscle mass, muscle tension, and mitochondrial functional homeostasis.

Skeletal muscle atrophy or injury is frequently accompanied by excessive oxidative stress responses. Interventions such as exercise or antioxidant supplementation typically elevate levels of IGF-1 and antioxidant enzymes like superoxide dismutase (SOD) in skeletal muscle, thereby reducing excessive oxidative stress in cells and increasing skeletal muscle SOD levels. IGF-1 plays a critical role in the growth and development of skeletal muscle and initiates rapid repair responses following injury. It mediates the repair of damaged sarcolemma, reactivates multiple developmental pathways, and promotes skeletal muscle regeneration by stimulating the differentiation and fusion of skeletal muscle precursor cells. These processes support skeletal muscle growth and regeneration, ultimately preventing the onset of sarcopenia [30, 31]. IGF-1 can activate the PI3K/AKT/mTOR signaling pathway, which promotes protein synthesis in skeletal muscle cells via P70S6K, thereby facilitating skeletal muscle hypertrophy and reducing muscle tissue fibrosis. By activating the PI3K/AKT/mTOR signaling pathway, inhibits protein ubiquitination, degradation, and apoptosis in skeletal muscle cells, thereby reducing tissue necrosis and mitigating sarcopenia associated with aging and denervation [32, 33]. As shown in Fig. 3, IGF-1 activity and the protein expression levels of AKT, PI3K, and mTOR in the skeletal muscle of aging rats were all higher in the QC group compared with the BC group. For example, curcumin treatment led to increased expression of PI3K and AKT in the soleus muscle, although the magnitude of the increase was modest. However, curcumin exerted an opposing effect on mTOR expression compared to aerobic exercise. This contrasting regulation may be due to curcumin influencing not only upstream components of the mTOR signaling pathway but also pathways involved in cell regeneration. Further, curcumin may regulate skeletal muscle cell apoptosis by activating the PI3K/AKT/mTOR signaling pathway [34]. Similar results were observed in this study. The findings demonstrated that constant-load aerobic exercise effectively upregulated the expression of PI3K and AKT, whereas incremental-load exercise exerted a limited impact on their expression. This observation could be attributed to the differential effects of high exercise intensity during the late stage of incremental-load exercise on skeletal muscle anabolism and cell apoptosis. In skeletal muscle tissues, the expression levels of PI3K and AKT remained relatively stable under different incremental-load exercise conditions. This phenomenon might result from the direct effect of exercise intervention on the synthesis of PI3K and AKT, or from the regulation of upstream molecules that subsequently modulate the expression of downstream regulatory proteins. This study investigated the effects of curcumin and aerobic exercise on the IGF-1/PI3K/AKT/mTOR signaling pathway in the soleus muscle of middle-aged and older rats. The experimental results indicated that both curcumin intervention and constant-load aerobic exercise could

independently stimulate the expression of key regulatory factors in the IGF-1/PI3K/AKT/mTOR pathway, reduce cell apoptosis in skeletal muscle tissue, and enhance skeletal muscle tension. However, when curcumin was combined with aerobic exercise, the observed effects were not consistently superior to those of single intervention measures. For example, as shown in Fig. 2, the combination of curcumin and aerobic exercise did not enhance PI3K expression, but instead showed a downward trend. Similarly, curcumin alone, constant-load exercise, and incremental-load exercise all increased AKT expression, while the combination of curcumin and aerobic exercise decreased AKT expression. These findings suggest that the interaction between curcumin and aerobic exercise in regulating the IGF-1/PI3K/AKT/mTOR signaling pathway, cell apoptosis, and skeletal muscle aging may not represent a simple additive or synergistic effect, but may instead be context-dependent. The interaction between these two intervention may vary according to physiological state or regulatory feedback mechanisms within the protein network. Therefore, further studies are required to clarify the specific roles of curcumin and aerobic exercise in regulating the mTOR signaling pathway, as well as their potential combined effect in delaying skeletal muscle aging.

Empirical research has demonstrated that curcumin and aerobic exercise upregulate AMPK/PGC-1 and CaMK in skeletal muscle, thereby modulating mitochondrial functional status. Compared with curcumin alone or its combination with constant-load aerobic exercise, eight weeks of constant-load aerobic exercise exerted a more pronounced effect on alleviating skeletal muscle mass loss and improving muscle tissue integrity. The present study found that curcumin combined with increasing-load aerobic exercise significantly elevated mitochondrial ATPase activity in the quadriceps femoris of middle-aged and aged rats. This improvement may result from structural enhancement of mitochondria induced by aerobic exercise, the antioxidant and anti-inflammatory effects of curcumin, or the maintenance of mitochondrial homeostasis via their combined intervention.

Additionally, the co-intervention of curcumin and increasing-load aerobic exercise tended to reduce the opening probability of the mitochondrial PTP in the quadriceps femoris, thereby decreasing apoptosis caused by excessive or abnormal pore opening and maintaining normal cellular metabolism, as shown in Fig. 4. This combination also limited the non-selective entry of cytoplasmic macromolecules into the mitochondrial inner membrane, preventing membrane depolarization and uncoupling of oxidative phosphorylation. The combined intervention enhanced mitochondrial complex III (CIII) activity, thereby strengthening mitochondrial respiration and oxidative phosphorylation capacity, while also increasing complex IV (CIV) activity to improve the efficiency of the electron transport chain, H^+ transfer, and oxygen utilization. Mitochondrial ATPase activity was likewise elevated, enhancing ATP synthesis capacity and maintaining normal mitochondrial energy metabolism and calcium homeostasis.

After eight weeks of intervention, curcumin monotherapy, constant-load aerobic exercise, and their combined application all downregulated LC3-1 and ULK1 expression in rat skeletal muscle, with significant reductions observed in both constant-load and increasing-load aerobic exercise groups. Supplementation with exogenous curcumin also decreased LC3-1 and ULK1 expression. However, a mild antagonistic effect emerged when curcumin was combined with exercise, suggesting complex

regulatory interactions. Therefore, curcumin and aerobic exercise jointly regulate the morphology and apoptotic status of skeletal muscle through multiple signaling pathways, thereby alleviating the progression of age-related sarcopenia.

Study findings and limitations

Sarcopenia has a multifactorial etiology. Both autophagy and apoptosis contribute to muscle atrophy, while the metabolism, synthesis, and regeneration of skeletal muscle cells are regulated by multiple signaling pathways and molecular mechanisms. This study focused on signaling pathways associated with apoptosis, autophagy, and mitochondrial function in the skeletal muscle fibers of rats. It examined the effects of exogenous antioxidant (curcumin) supplementation, aerobic exercise training, and curcumin combined with different exercise modalities on skeletal muscle in aging rats. The main limitation of this study lies in its restricted research scope, as only the expression levels of related proteins were analyzed without further mechanistic exploration. To fully elucidate the pathophysiological mechanisms underlying sarcopenia, future research should include more extensive analyses of protein phosphorylation and gene expression.

Conclusion

This study concluded that curcumin combined with constant-load aerobic exercise effectively increases muscle cross-sectional area and muscle tension, reduces the skeletal muscle apoptosis index, upregulates IGF-1 activity and the protein expression of PI3K/AKT/mTOR, AMPK/PGC-1, and CaMK, and downregulates LC3-I and ULK1 expression in skeletal muscle tissues, thereby delaying skeletal muscle aging in aged rats. Among all intervention protocols, constant-load aerobic exercise exerted the most significant beneficial effects, and no synergistic effect was observed when it was combined with curcumin. In contrast, incremental-load aerobic exercise promoted skeletal muscle cell apoptosis in aged rats, indicating that moderate-to-high intensity aerobic exercise could induce oxidative stress and exacerbate skeletal muscle damage. Notably, curcumin supplementation alleviated such exercise-induced damage, as evidenced by lower apoptosis levels and improved overall exercise performance in the curcumin combined with incremental-load exercise group compared with the incremental-load exercise alone group. Collectively, exogenous curcumin supplementation and regular constant-load aerobic exercise effectively reduced the skeletal muscle apoptosis index and enhanced the expression of proteins related to skeletal muscle cell growth. Constant-load aerobic exercise remains the more effective intervention strategy for delaying skeletal muscle aging, while curcumin may serve as a beneficial adjuvant to mitigate exercise-induced muscle damage under moderate-to-high intensity exercise conditions.

Directions for further research

This study preliminarily investigated the effects of exogenous antioxidant curcumin combined with various aerobic exercise protocols on delaying skeletal muscle aging with the aim of exploring whether

the combination exhibited synergistic or antagonistic effects. The findings revealed that curcumin combined with constant-load aerobic exercise did not yield a synergistic effect in delaying sarcopenia, which may be attributable to differences in underlying regulatory mechanisms. However, curcumin supplementation effectively alleviated skeletal muscle injury caused by high-intensity exercise. Further research is warranted to elucidate the specific mechanisms involved, as well as to determine the optimal timing, dosage, and administration strategies for curcumin supplementation in the context of exercise interventions.

Abbreviations

Insulin-like growth factor 1 (IGF-1)

Phosphatidylinositol 3-kinase (PI3K)

Protein kinase B (AKT)

Mechanistic target of rapamycin (mTOR)

Hematoxylin-eosin (HE)

Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL)

Adenosine monophosphate-activated protein kinase (AMPK)

Peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1)

Calmodulin-dependent protein kinase (CaMK)

Microtubule-associated protein 1 light chain 3 isoform I (LC3-1)

Unc-51-like autophagy activating kinase 1 (ULK1)

Declarations

Ethics approval and consent to participate

This study involving animal experiments was approved by the Animal Ethics Committee of Hunan Normal University (Approval No. 2023-016). All procedures were conducted in accordance with relevant guidelines and regulations. All coauthors provided their consent to participate in the research.

Consent for publication

All coauthors have provided their consent for the publication of this research.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests.

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Author contributions

L.T., Z.C., and L.L. performed the experiments. L.T., C.S, and C.T. drafted the manuscript. L.T., C.S, and Z.C. analyzed the data. L.T., Z.C., L.L., and C.T. designed the study. All the authors read and approved the final version of the article. L.T. assumes responsibility for the work in its entirety.

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Supplementary Tables

Table S1
Incremental load training program for rats [speed (m/min) × duration (min), slope 0°]

Fitness training			10×10	10×20	10×30	Rest	Rest
1st W	12×35	12×35	12×35	12×35	12×35	Rest	Rest
2nd W	15×35	15×35	15×35	15×35	15×35	Rest	Rest
3rd W	15×40	15×40	15×40	15×40	15×40	Rest	Rest
4th W	18×40	18×40	18×40	18×40	18×40	Rest	Rest
5th W	18×45	18×45	18×45	18×45	18×45	Rest	Rest
6th W	21×45	21×45	21×45	21×45	21×45	Rest	Rest
7th W	21×45	21×45	21×45	21×45	21×45	Rest	Rest
8th W	23×45	23×45	23×45	23×45	23×45	Materials	
Note: All tissues were collected immediately after completion of the exercise intervention.							

Table S2
Main reagents used in experiments

Reagent	Manufacturer	Catalog Number	Dilution Ratio/Usage Instructions	Validation Reference
PI3K	Proteintech (Chicago, IL, USA)	17846-1-AP	1:1000	Li Y, et al. PI3K/mTOR signaling pathway regulates autophagy in liver injury. J Cell Physiol. 2022; 237(5): 3892–3905.
mTOR	Proteintech (Chicago, IL, USA)	29834-1-AP	1:1000	Zhang W, et al. mTOR activation is associated with autophagy dysfunction in experimental fibrosis. Int J Mol Sci. 2021; 22(11): 5876.
AKT	Cell Signaling Technology (Danvers, MA, USA)	#9272	1:1000	Chen J, et al. AKT signaling mediates cell survival in inflammatory responses. J Biol Chem. 2020; 295(47): 15987–16000.
AMPK	Thermo Fisher Scientific (Waltham, MA, USA)	PA5-108342	1:1200	Wang H, et al. AMPK regulates autophagy and energy metabolism in hepatic cells. Biochem Biophys Res Commun. 2022; 615: 102–108.
PGC-1	Cell Signaling Technology (Danvers, MA, USA)	#5165	1:800	Liu Z, et al. PGC-1 α modulates mitochondrial function in liver fibrosis. Hepatology. 2021; 74(3): 1389–1405.
CaMK	Cell Signaling Technology (Danvers, MA, USA)	#3361	1:1000	Zhao L, et al. CaMK II regulates autophagy via AMPK signaling in hepatocytes. J Hepatol. 2020; 73(4): 923–934.
LC3	Thermo Fisher Scientific (Waltham, MA, USA)	PA1-16935	1:1000	Sun M, et al. LC3-mediated autophagy in liver injury: a review. Int J Biochem Cell Biol. 2021; 136: 105987.
ULK1	Thermo Fisher Scientific (Waltham, MA, USA)	PA5-85897	1:1000	Guo F, et al. ULK1-dependent autophagy in hepatic steatosis. J Lipid Res. 2022; 63(2): 100186.

Reagent	Manufacturer	Catalog Number	Dilution Ratio/Usage Instructions	Validation Reference
Beclin protein reagent	Bioworld Technology (St. Louis Park, MN, USA)	AP1052	1:800	Han J, et al. Beclin 1 regulates autophagy and apoptosis in liver cancer cells. <i>Oncol Rep.</i> 2020; 44(3): 1029–1040.
GAPDH	Cell Signaling Technology (Danvers, MA, USA)	#2118	1:5000	Zhang H, et al. GAPDH as a reliable internal control for Western blot analysis in liver tissues. <i>Biochem Biophys Res Commun.</i> 2018; 499(2): 301–306.
Goat Anti-Rabbit IgG (H&L)-HRP (No. BS13278)	Bioworld Technology (St. Louis Park, MN, USA)	BS13278	1:5000	Bioworld Technology Product Manual, Cat. No. BS13278; validated in our laboratory.
SuperECL Plus	Thermo Fisher Scientific (Waltham, MA, USA)	34096	-	Thermo Fisher Scientific Product Manual, Cat. No. 34096.
Bradford protein quantification kit	Tiagen Biochemical Technology Co., Ltd. (Beijing, China)	PA102	-	Tiagen Biochemical Technology Product Manual, Cat. No. PA102; validated in our laboratory.

Table S3
Changes in the body weight of the rats in each group before and after 8 weeks of training

Group	BC (n = 10)	QC (n = 10)	CC (n = 10)	CE (n = 10)	IC (n = 10)	IE (n = 10)
Pre-experiment	668.2 ± 38.82	559.8 ± 29.64	563.7 ± 51.65	571.3 ± 79.15	531.7 ± 8.622	567.3 ± 69.4
1st W	646.4 ± 45.65	530.3 ± 43.2*	541.3 ± 47.23*	531 ± 2.944*	545.7 ± 80.59	545.7 ± 45.47*
2nd W	629 ± 55.96	519.3 ± 50.6*	521.1 ± 42.3▲	517 ± 11.78*	551 ± 82.46	534.4 ± 43.3*
3rd W	587.2 ± 65.46	487.5 ± 50.19	478.3 ± 37.21*	501.8 ± 27.54	514.3 ± 73.01	504.1 ± 46.21
4th W	572.8 ± 67.36	487.5 ± 58.62	476.9 ± 28.18*	501.8 ± 23.2	498.7 ± 66.53	497.6 ± 47.77
5th W	585.2 ± 68.87	478.3 ± 47.42	494.6 ± 32.32	502 ± 31.55	509.3 ± 77.53	523.3 ± 58.47
6th W	575.2 ± 71.37	489.3 ± 22.68	479.9 ± 31.6*	468.8 ± 51.52	487.3 ± 63.61	492.1 ± 57.41
7th W	573.8 ± 67.43	497.3 ± 7.095	483.9 ± 34.99*	491.7 ± 8.505	498.3 ± 73.45	488 ± 44.91*
8th W	577.2 ± 31.68	489.3 ± 19.14##	467.8 ± 45.71▲##	494.3 ± 12.42##	478.3 ± 52.7*##	479.4 ± 61.25▲##
Note: BC: control group; QC: curcumin group; CC: curcumin combined with constant-load exercise group; CE: constant-load exercise group; IC: curcumin combined with incremental-load exercise group; and IE: incremental-load exercise group. * indicates a significant difference compared with the control group (BC), P < 0.05; ▲ indicates an extremely significant difference compared with the BC group, P < 0.01. # denotes a significant difference in body weight within the same group before and after the intervention (P < 0.01); ## denotes a significant difference in body weight within the same group before and after the intervention (P < 0.05).						

Table S4
Comparison of skeletal muscle index (SI) in rats from each group after intervention

Group	Soleus muscle weight/rat body weight (mg/g)
BC (n = 10)	0.4634 ± 0.0058
QC (n = 10)	0.5215 ± 0.0248**
CC (n = 10)	0.5503 ± 0.0045**▲
CE (n = 10)	0.5139 ± 0.0251**
IC (n = 10)	0.5342 ± 0.135**▲
IE (n = 10)	0.5213 ± 0.079**
Note: BC: control group; QC: curcumin group; CC: curcumin combined with constant-load exercise group; CE: constant-load exercise group; IC: curcumin combined with incremental-load exercise group; and IE: incremental-load exercise group. * indicates p < 0.05 versus the BC group; ** indicates p < 0.01 versus the BC group; ▲ indicates p < 0.05 versus the QC group; ▲▲ indicates p < 0.01 versus the CC group.	

Table S5
Results of the horizontal rope test in rats from each group

Group	First horizontal rope grasping time (s)	Secondary rope hanging duration (s)
BC (n = 10)	3.92 ± 2.16	2.58 ± 0.87
QC (n = 10)	3.28 ± 0.93	3.86 ± 1.73*
CC (n = 10)	5.63 ± 1.24	5.81 ± 1.70**##Δ▲
CE (n = 10)	5.56 ± 1.67	5.17 ± 0.43*#▲
IC (n = 10)	5.05 ± 1.25	5.53 ± 1.24*#
IE (n = 10)	5.23 ± 1.19	6.58 ± 1.35**##▲
Note: BC: control group; QC: curcumin group; CC: curcumin combined with constant-load exercise group; CE: constant-load exercise group; IC: curcumin combined with incremental-load exercise group; and IE: incremental-load exercise group. * indicates a significant difference versus the BC group, P < 0.05; ** indicates an extremely significant difference versus the BC group, P < 0.01; # indicates a significant difference versus the QC group, P < 0.05; ## indicates an extremely significant difference versus the QC group, P < 0.01; Δ represents a significant difference versus the CE group, P < 0.05; ▲ represents a significant difference versus the IC group, P < 0.05.		

Figures

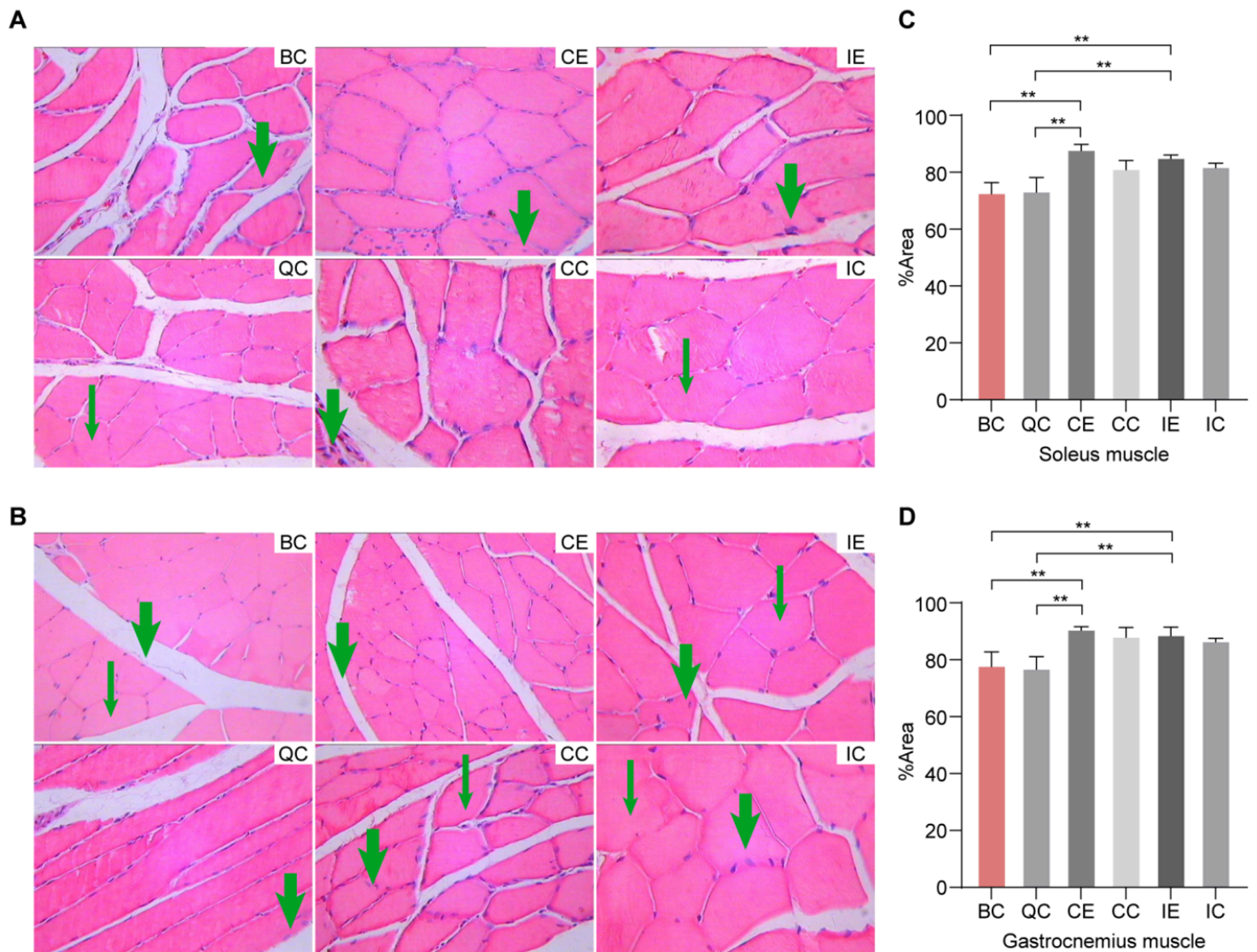


Figure 1

Hematoxylin-eosin (H&E) staining of skeletal muscle cells in rats across different experimental groups magnification: ($\times 200$). **A** Soleus muscle; **B** Gastrocnemius muscle; **C** Cross-sectional area of muscle fibers in the soleus muscles of each group; **D** Cross-sectional area of muscle fibers in the gastrocnemius muscles of each group. Data are shown as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

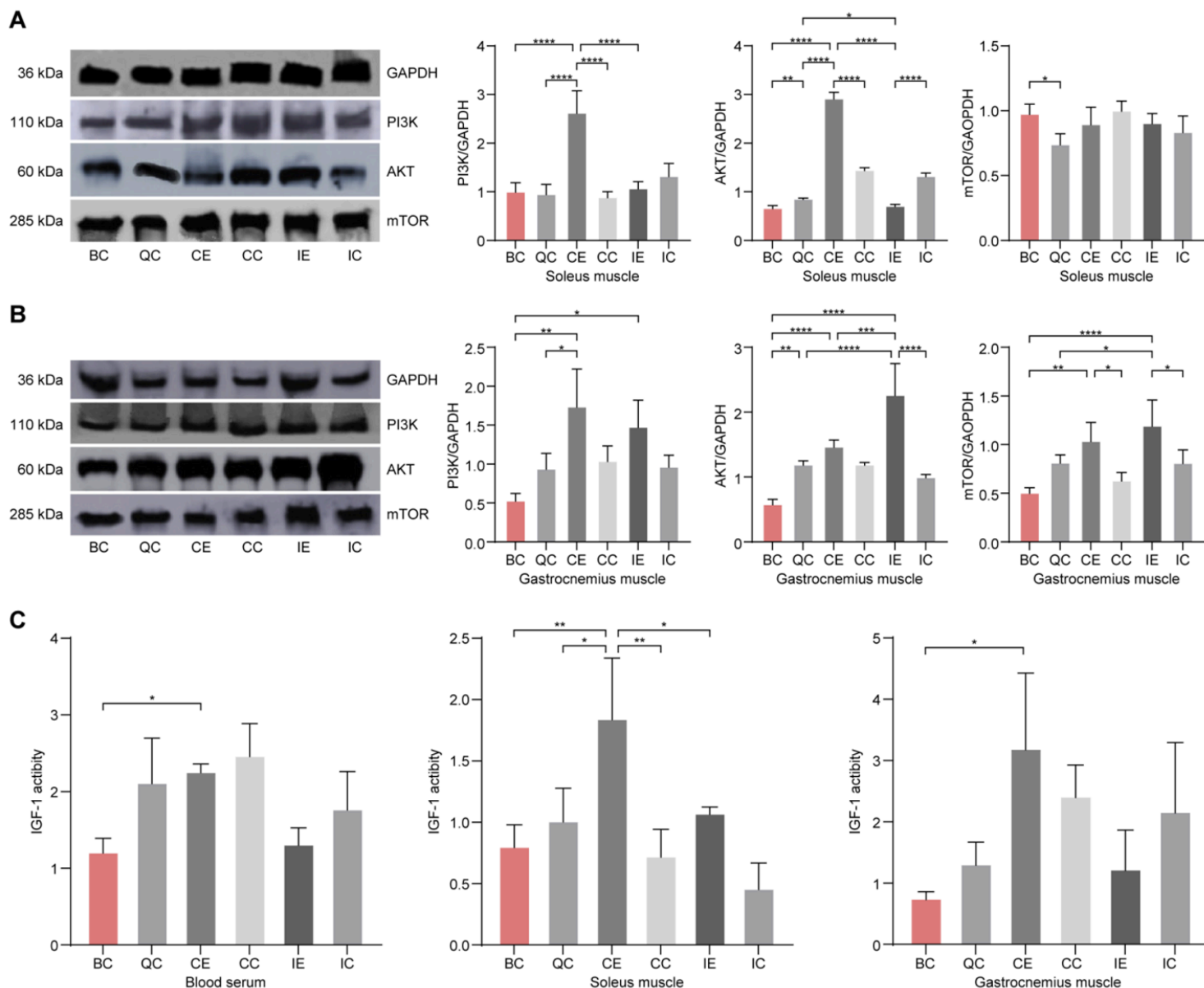


Figure 2

Expression of the IGF-1/PI3K/AKT/mTOR signaling pathway in rats across different experimental groups. **A** Expression of PI3K/AKT/mTOR proteins in the soleus muscle; **B** Expression of PI3K/AKT/mTOR proteins in the gastrocnemius muscle; **C** IGF-1 activity in serum, the soleus muscle, and the gastrocnemius muscle. Data are shown as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

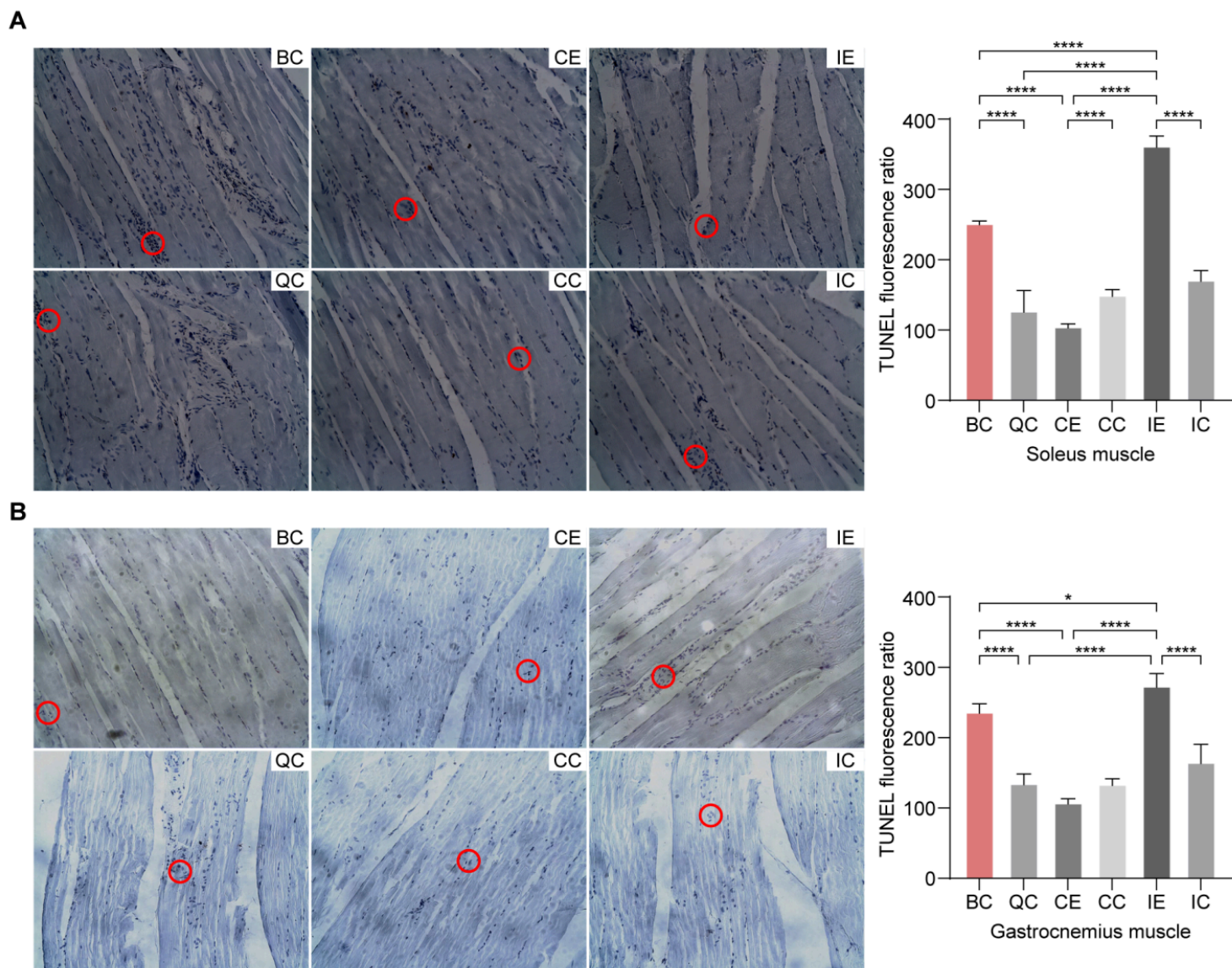


Figure 3

Apoptosis of muscle cells in different groups of rats (apoptotic cells are circled in red). **A** Soleus muscle; **B** Gastrocnemius muscle magnification: ($\times 400$). Data are shown as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

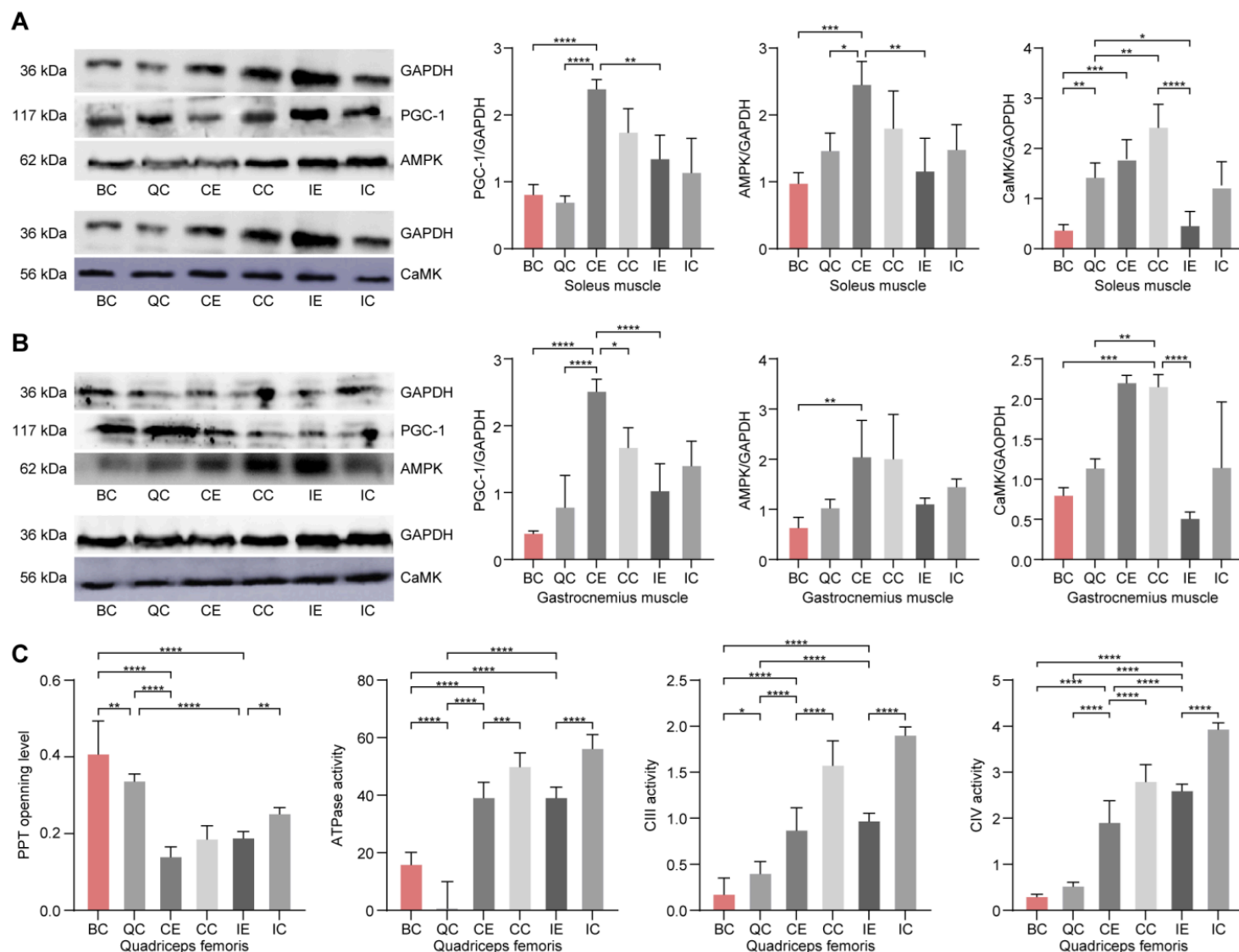


Figure 4

Expression of PGC-1, AMPK, and CaMK in the skeletal muscles of rats from different groups. **A** Soleus muscle; **B** Gastrocnemius muscle; **C** Mitochondrial permeability transition pore (PTP) opening degree, ATPase activity, and mitochondrial complex III and IV activities in the quadriceps femoris. Data are shown as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

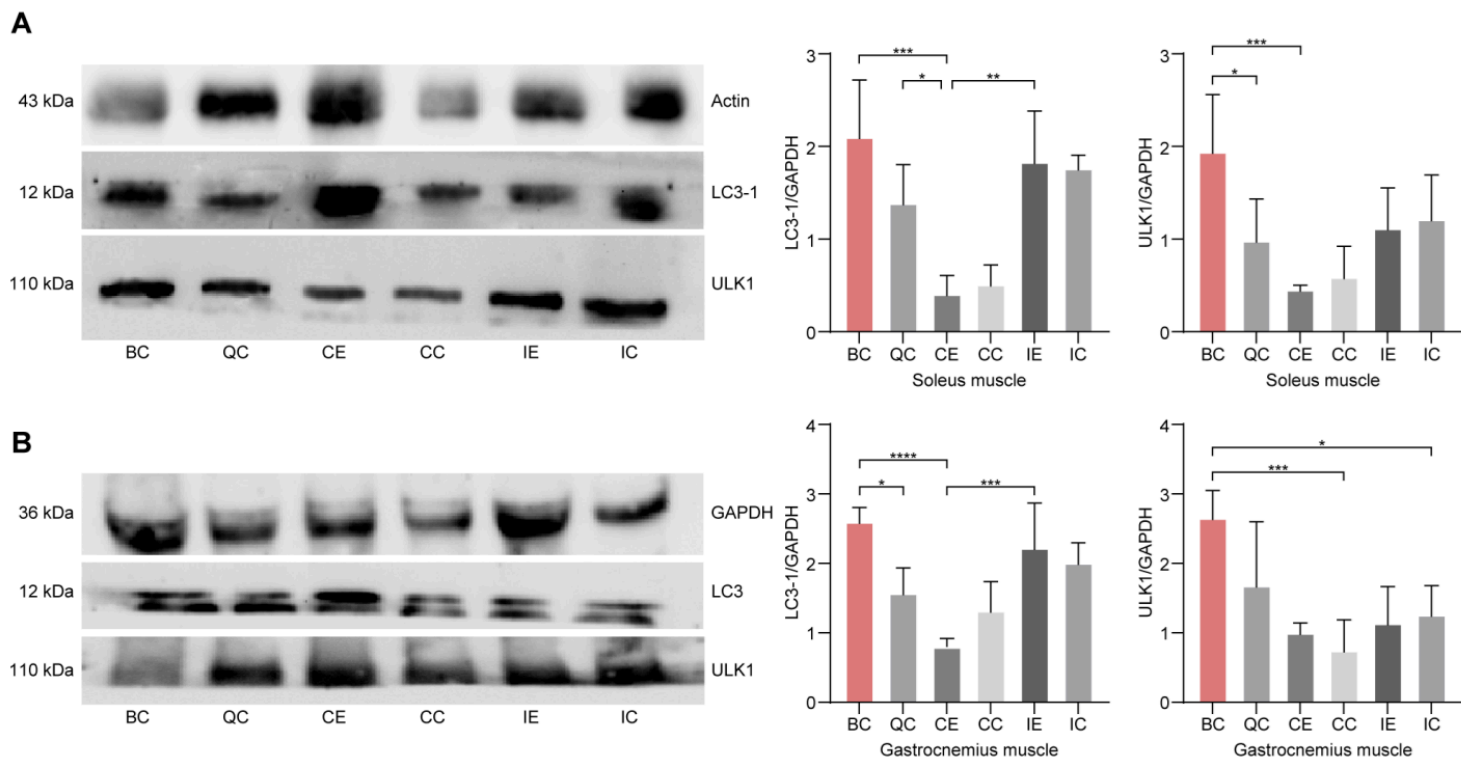


Figure 5

Expression of LC3-1 and ULK1 in the skeletal muscles of rats from different groups. **A** Soleus muscle; **B** Gastrocnemius muscle. Data are shown as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.