Research Paper

Anti-fatigue and anti-oxidant effects of curcumin supplementation in exhaustive swimming mice via Nrf2/Keap1 signal pathway

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ABSTRACT

Demands for dietary supplements with anti-fatigue effects are growing fast due to increasing societal demands. Moreover, in highly physically active individuals, there are also significant needs for supplements to improve exercise performance. The present study evaluated the potential anti-fatigue and anti-oxidant effects of curcumin in mice using exhaustive swimming test. Male C57BL/6J mice were randomized into six groups: blank control (Rest), swimming control (Con), Vitamin C (Vc), low-dose curcumin (C50), middle-dose curcumin (C100), and high-dose curcumin (C200). After a 4-week intervention, the mice in all groups except the Rest group were subject to an exhaustive swimming test. Then, mice were sacrificed to examine serum biochemical markers and fatigue-related enzymes. Moreover, the gene and protein expressions of signal transduction factors involved in the Nrf2/Keap1 signaling pathway were measured. The results indicated that curcumin significantly enhanced the exercise tolerance of mice in the exhaustive swimming test. Particularly, the swimming time of mice in the C200 group was increased by 273.5% when compared to that of mice in the Con group. The levels of blood urea nitrogen, blood ammonia, lactic acid, creatine kinase and lactate dehydrogenase in the C200 group were decreased by 13.3%, 21.0%, 18.6%, 16.7% and 21.9%, respectively, when compared to those of mice in the Con group. Curcumin alleviated exercise-induced oxidative stress and significantly enhanced the activities of superoxide dismutase, catalase and glutathione peroxidase by activating the Nrf2 signaling. These findings indicated that curcumin supplementation exerted remarkable anti-oxidant and anti-fatigue effects in mice, providing additional evidence supporting the use of curcumin as functional food, especially by those engaged in sports-related activities.

1. Introduction

Nowadays, chronic diseases such as cardiovascular disease, cancer, obesity and diabetes have become major factors for global morbidity and mortality (Alageel et al., 2017; Marunaka et al., 2020; Stoner, 2020). Physical inactivity contributes 12%–19% to the risks associated with the 5 major NCDs in China (Zhang and Chaaban, 2013). Regular physical activity helps to improve physical and mental functions as well as reverse some effects of chronic diseases. The health benefits of physical activity for improving NCDs has drawn more attentions. With increasing people’s awareness of health, regular sports training is becoming more and more popular. As a result, improving exercise performance and reducing exercise-induced muscle damage in physically active are frequent reasons for seeking sport nutrition and dietary supplements (Chen et al., 2020b). Functional foods could no only provide health-promoting effects beyond basic nutrition, but also offer the prevention or supplementary treatment of chronic diseases (Peng et al., 2020; Xie et al., 2019).

Many phytonutrients have been shown to activate nuclear factor E2-related factor 2 (Nrf2) and this process could occur by modifications of cysteine residues which are different from those targeted through exercise (Chen et al., 2022; Jia et al., 2018; Pala et al., 2016). The Nrf2 signaling pathway is involved in many key genes, and the interaction
between Nrf2 and Keap1 is highly conserved across species, indicating its important regulatory role (Kobayashi et al., 2002). Nrf2 usually is present in the cytoplasm and binds to Keap1 as a redox-sensitive master regulatory transcriptional factor. Nrf2 dissociates from Keap1 and then binds to ARE in the promoters of phase II anti-oxidant enzyme genes. HO-1 and NQO1 are the most important downstream targets of the Nrf2/Keap1 signal pathway. In addition, NAD(P)H: quinone oxidoreductase 1 (NQO1) and heme oxygenase-1 (HO-1), also known as glutamate-cysteine ligase, play important roles in Nrf2 signaling. NQO1 is a flavonoid that catalyzes reductive metabolic detoxification of reductase quinones (Surh, 2021). HO-1 catalyzes the first and rate-limiting step in heme catabolism. Therefore, the above Nrf2 signaling-related genes are primary candidates to study the effects of phytonutrients on exercise performance.

Turmeric (Curcuma longa L.) is a yellow-colored rhizomatous herbaceous perennial plant of the Zingiberaceae family, containing turmeric, essential oils, and curcuminoids. Curcumin with the molecular structure composition of (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is the principal curcuminoid of turmeric and a primary bioactive phytochemical that has been used widely as a dietary supplement (Wang et al., 2021; Sun et al., 2018). Epidemiological studies have consistently confirmed the beneficial effects of dietary curcumin on certain aspects of physiological functions, such as preventing fatigue and muscle damage by its anti-oxidant and anti-inflammatory activities (Boz et al., 2015; Huang et al., 2015; Sahin et al., 2016). Moreover, increasing evidence supports that the anti-oxidant activity of curcumin is highly associated with its regulating ability in the Nrf2 signaling pathway (Peng et al., 2018; Soetikno et al., 2018). Numerous studies suggested that curcumin exerts anti-oxidant activity, but few studies have reported that curcumin could improve exercise-induced fatigue by activating the Nrf2 pathway (Huang et al., 2015; Wafi et al., 2019). Even though the anti-oxidant properties of curcumin might significantly contribute to its anti-fatigue effect, the regulation of curcumin on the fatigue-related enzymes involved in the glycolytic pathway is not well understood. Therefore, this study aimed to investigate the anti-oxidant and anti-fatigue activities of curcumin in mice challenged using exhaustive swimming test and to examine its potential regulation of the glycolytic and Nrf2/Keap1 signaling pathways.

2. Materials and methods

2.1. Materials and reagents

Curcumin with purity over 99% was obtained from Shanghai Winherb Medical Science Co., Ltd (Shanghai, China). Carboxymethylcellulose (CMC) was purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). The following kits, hepatic glycogen, muscle glycogen, pyruvate kinase (PK), succinate dehydrogenase (SDH), malate dehydrogenase (MDH), Na+K+ATPase, Ca2+-Mg2+-ATPase, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were all purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All other reagents were of analytical grade and stored according to the instructions.

2.2. Animals and experimental protocols

A total of 48 adult male C57BL/6J mice (6 weeks old, weight 18–20 g; SLAC Laboratory Animal Co., Ltd., Shanghai) were housed in the Animal Experiments Center of Zhejiang University under the controlled conditions (temperature of 22 °C ± 1 °C, a 12-h light/12-h dark cycle and humidity of 55% ± 5%). All the animals were treated strictly according to the recommendations of the Guide for the Care and Use of Laboratory Animals of the Science and Technology Commission of P.R. C. (STCC Publication No. 2, revised 1988). The protocol with the ethical approval code of 12628 was approved by the Committee on the Ethics of Animal Experiments Center of Zhejiang University.

After acclimatization for one week, mice were randomized into six groups (n = 8): blank control (Rest), swimming control (Con), Vitamin C (Vc), low-dose curcumin (C0.5), middle-dose curcumin (C100), and high-dose curcumin (C200). Vc group was administered Vc orally with 40 mg kg⁻¹ BW/day for four weeks. The three curcumin groups were fed daily by gavage, 50, 100, and 200 mg curcumin/kg BW/day for 4 weeks, respectively. Vc and curcumin were weighed and dissolved to the appropriate concentration with 0.3% CMC solution to ensure that the gavage volume was about 0.1 mL. Also, the mice in the Rest and Con group were given the same volume of CMC solution by gavage every day. During the 4-week experimental period, the weights of mice were recorded every week and the weight changes were calculated according to the initial weight and final weight.

2.3. Weight-loaded swimming test

The weight-loaded swimming test (WST) was carried out according to the previous studies with mild modifications (Hu et al., 2020; Huang et al., 2015). Briefly, 30 min after the last oral administration, a lead sheath, weighing 5% of their corresponding BW, was fixed to the root of the mouse tail. Then, the mice in all groups except the Rest group were subjected to swim individually in a plastic pool (50 × 50 × 40 cm) filled with water (25 ± 1 °C) to a depth of 30 cm. The weight-loaded swimming time was recorded when the mice sank into the water and failed to rise to the surface for breath within a period of 10 s. During the

### Abbreviations

- ALT: Alanine aminotransferase
- ARE: Antioxidant response element
- AST: Aspartate aminotransferase
- BSA: Bovine serum albumin
- BUN: Blood urea nitrogen
- GLU: Blood glucose
- CAT: Catalase
- CK: Creatine kinase
- CMC: Carboxymethylcellulose
- EST: Exhaustive swimming test
- GSH-Px: Glutathione peroxidase
- HG: Hepatic glycogen
- HO: Heme oxygenase
- LD: Lactic acid
- LDH: Lactate dehydrogenase
- MDA: Malondialdehyde
- MDH: Malate dehydrogenase
- NCDs: Noncommunicable diseases
- Nrf2: Nuclear factor E2-related factor 2
- NQO1: NAD(P)H: quinone oxidoreductase 1
- PK: Pyruvate kinase
- qPCR: Quantitative real-time PCR
- ROS: Reactive oxygen species
- SDH: Succinate dehydrogenase
- SOD: Superoxide dismutase
- TP: Total protein
- WST: Weight-loaded swimming test

### References

- Kobayashi et al., 2002
- Wafi et al., 2019
- Peng et al., 2018
- Soetikno et al., 2016
- Surh, 2021
- Boz et al., 2015
- Huang et al., 2015
- Sahin et al., 2016
- Peng et al., 2018
- Soetikno et al., 2018
- Huang et al., 2015
- Wafi et al., 2019
- Hu et al., 2020
- Huang et al., 2015

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swimming test, a glass rod was used for stirring gently to make the water
in the pool moving continuously and to keep the mice swimming to
exhaustion. Then, the mice were removed from the water and dried
immediately. After resting for 60 min, the mice were fully anesthetized
by intraperitoneal injection of 2% pentobarbital sodium (40 mg kg
-1) and sacrificed by cervical dislocation to collect the whole blood and
tissues for further analyses. All these experimental and care procedures
used for animal euthanasia were performed in accordance with the
relevant guidelines and regulations, and approved by the Committee on
the Ethics of Animal Experiments Center of Zhejiang University. After
dissection, the tissues were weighed to calculate the organ indexes ac-
cording to the formula as follows:

\[
\text{Organ index} = \frac{\text{Organ weight}}{\text{Final body weight}} \times 100
\]

2.4. Histological analysis

The skeletal muscles and kidney tissues were harvested for histo-
pathologic evaluation. Histological examination was done according to
the previous studies (Chen et al., 2020b; Soetikno et al., 2013). Briefly,
formalin (10%) fixed the tissues were processed for dehydration. After
dehydration, the tissues were embedded in paraffin and 4 μm thick
sections were cut. Then, the sections were rehydrated through a graded
series of ethanol solutions after removing wax by xylene. Dyed sections
were stained with hematoxylin & eosin and examined using light
microscopy. Histological scoring for tissues according to an injury
grading score system (Peng et al., 2018) with some modifications (Grade
0–4: Grade 0, no pathological change; Grade 1, presence of rare foci of
necrosis; Grade 2, small area of mild necrosis; Grade 3, area of mild
necrosis severer than Grade 2; and Grade 4, the necrosis severer than
Grade 3), was performed by pathologists from Hangzhou Huashu
Biotechnology Co., Ltd.

2.5. Determination of serum biochemical parameters

Serum was separated from whole blood after centrifugation at 3000
rpm for 10 min at 4 °C. The serum levels of alanine aminotransferase
(ALT), aspartate aminotransferase (AST), total protein (TP), blood
glucose (GLU), blood urea nitrogen (BUN), blood ammonia (BA), lactic
acid (LD), lactate dehydrogenase (LDH) and creatine kinase (CK) were
measured by using a 3100 automatic biochemistry analyzer (Hitachi
Lite (Biodrop, UK). The ViiA™ 1 Real-Time PCR system with fast 96-well block and SYBR Premix Ex Taq
(TliRNaseH Plus) (TaKaRa) were used in the qPCR analysis. Cycling
conditions were 95 °C for 5 min followed by 40 repeated cycles of 95 °C
for 10 s and 60 °C for 30 s. The mRNA expression was determined by
comparison with those of the control sample after normalization to
GAPDH levels and calculated by using the 2 -ΔΔCT method. All the anal-
yses were repeated six times.

2.9. Western blotting

Western blot analyses were performed as described previously (Chen
et al., 2020b). Primary antibodies for Nr2f2, Keap1, HO-1 and NQO1
(1:1000) were incubated overnight, followed by incubation with sec-
ondary antibodies for 1 h (goat anti-rabbit 1:5000 dilution for both Nr2f2,
Keap1, HO-1 and NQO1). Antibodies of Nr2f2, Keap1, HO-1, NQO1 and
GAPDH were purchased from Abcam (Abcam, Cambridge, MA, USA).
Bands were detected by the ECL Western blot detection reagents
(Thermo Fisher Scientific, Waltham, MA, USA) and exposed to a
Mini-Protean 3 System (Bio-Rad, Atlanta, GA, USA). Protein band
intensities were normalized to GAPDH band intensities using the Image J
software. All experiments were performed in triplicate.

3. Results

Table 1

| Gene      | Primer sequences                                      |
|-----------|-------------------------------------------------------|
| GAPDH     | Forward (5′-3′) CTTGCGGCTGGAGAAACCTCAGGCAAGGCTGGC   |
|           | Reverse (5′-3′) TGGAAAGCTGGAGATCTGCTTGAGTGG           |
| Nr2f2     | Forward (5′-3′) TCTTCTCGCTGGAGAAAAAGAAGAAGTTA         |
|           | Reverse (5′-3′) AATGTGGTTCTGCTGGGTTGAAGAAG            |
| HO-1      | Forward (5′-3′) CCTCCAGTCGAGGAAATCTACCTTGAG          |
|           | Reverse (5′-3′) CTTGTGGAGACCCGTCCTCCATCA             |
| Keap1     | Forward (5′-3′) AAGGCCATTCGTGAGGGACCA                 |
| NQO1      | Reverse (5′-3′) CCTGTACCCAGTGTTAGAT                  |
|           | Reverse (5′-3′) TGTCCATGAGCATCTCAGAGTTAGCA           |

Western blot analyses were performed as described previously (Chen
et al., 2020b). Primary antibodies for Nr2f2, Keap1, HO-1 and NQO1
(1:1000) were incubated overnight, followed by incubation with sec-
ondary antibodies for 1 h (goat anti-rabbit 1:5000 dilution for both Nr2f2,
Keap1, HO-1 and NQO1). Antibodies of Nr2f2, Keap1, HO-1, NQO1 and
GAPDH were purchased from Abcam (Abcam, Cambridge, MA, USA).
Bands were detected by the ECL Western blot detection reagents
(Thermo Fisher Scientific, Waltham, MA, USA) and exposed to a
Mini-Protean 3 System (Bio-Rad, Atlanta, GA, USA). Protein band
intensities were normalized to GAPDH band intensities using the Image J
software. All experiments were performed in triplicate.
3.2. Effect of curcumin supplementation on the weight-loaded swimming capacity

As shown in Fig. 1-A, the weight-loaded swimming time of mice in Vc and curcumin supplementation groups was significantly higher than that of mice in the Con group (P < 0.0001), which indicated that curcumin supplementation could markedly reinforce the exercise tolerance and ease the degree of physical fatigue of mice. Particularly, the weight-loaded swimming time of mice in the C100 group was increased by 273.5%, compared to that of mice in the Con group, and was 1.76 times that of mice in the Vc group.

3.3. Effect of curcumin supplementation on the serum biochemical parameters of mice

In order to determine the effect of curcumin supplementation on the metabolism of mice after exhaustive swimming, a total of ten serum biochemical parameters of mice in each group were measured. The results were shown in Table 3. Firstly, there were no significant differences in the total protein (TP) and AST/ALT ratio of all groups. Secondly, compared to that of mice in Rest group, the GLU of mice in Con group was significantly decreased, but no significant differences were found in Vc and curcumin supplementation groups. On the other hand, the ALT, AST, BUN, BA, LA, CK and LDH levels of mice in the Con group were remarkably increased after exhaustive swimming compared to those of mice in the Rest group. Interestingly, these levels were decreased in mice treated with curcumin when compared to those of mice in the Con group. Among all these levels, the levels of BUN, BA, LA, CK and LDH were analyzed to determine the metabolic changes in the anti-fatigue activity of curcumin supplementation. Taking the results of mice in the C100 group that showed the best performance in weight-loaded swimming time as an example, we found that the levels of BUN, BA, LA, CK and LDH of mice in the C100 group were significantly decreased by 13.3%, 21.0%, 18.6%, 16.7% and 21.9%, compared to those of mice in the Con group, respectively. Thus, the results of serum biochemical parameters indicated that curcumin supplementation could improve the exercise tolerance and remit the degree of physical fatigue of mice by modulating physiological and metabolic changes in fatigue-related factors.

3.4. Effect of curcumin supplementation on hepatic and muscular glycogen levels

The glycogen levels in the liver and skeletal muscle were shown in Fig. 1-C, D. Regarding the hepatic glycogen (HG) level (Fig. 1-C), there were no significant differences between those of mice in the Rest group and Con group. However, compared to that of mice in the Con group, the HG levels of mice after 4-week of Vc or curcumin supplementation were significantly increased (P < 0.0001), and the HG levels of mice in the C100 group were 1.29 times of that in the Con group. On the other hand, the muscular glycogen levels of mice in the Con, Vc and C50 groups were remarkably reduced after exhaustive swimming compared with that of
mice in the Rest group (Fig. 1-D). However, compared to that of mice in the Con group, the muscular glycogen levels of mice in all the intervention groups were significantly improved by Vc or curcumin supplementation. Consequently, the results illustrated that curcumin supplementation enhanced the glycogen content of mice during prolonged or high-intensity exercise.

3.5. Effect of curcumin supplementation on fatigue-related enzyme activities in liver

To understand the anti-fatigue mechanisms of curcumin supplementation, we examined the anti-oxidation activities in mouse liver in relation to curcumin supplementation. In the present study, the levels of MDA, SOD, CAT and GSH-Px of mouse liver were measured and summarized in Fig. 3. Compared to those of mice in the Rest group, the MDA (Fig. 3-A) level of mice in the Con group was extensively boosted by 119.4%. However, the SOD (Fig. 3-B), CAT (Fig. 3-C) and GSH-Px (Fig. 3-D) levels of mice in the Con group were significantly reduced by 36.7%, 35.6% and 41.2%, respectively. In contrast, compared to those of Con mice, the MDA levels in the intervention groups were significantly decreased and the SOD, CAT and GSH-Px levels were increased. Particularly, there were no significant differences in all the four indexes between the Rest group and C200 group, suggesting that curcumin supplementation showed regulation capabilities in anti-oxidation-related enzyme activities of mice during prolonged or high-intensity exercise. These findings provided important clues for clarifying the mechanism of the anti-fatigue activity of curcumin supplementation on exhaustive swimming mice.

3.6. Effect of curcumin supplementation on the morphology of skeletal muscle and kidney in mice

Table 3

| Biochemical indexes | Rest (mmol/L) | Con (mmol/L) | Vc (mmol/L) | C50 (mmol/L) | C100 (mmol/L) | C200 (mmol/L) |
|---------------------|---------------|--------------|-------------|--------------|---------------|---------------|
| ALT (U/L)           | 56.50 ± 6.63a | 137.50 ± 15.19b | 79.75 ± 5.09b | 77.25 ± 8.95b | 85.38 ± 6.61b | 87.5 ± 8.23b  |
| AST (U/L)           | 154.75 ± 15.96c | 418.75 ± 32.52a | 245.13 ± 17.82c | 241.25 ± 22.1b | 277.5 ± 25.16c | 233.75 ± 24.85c |
| TP (g/dL)           | 6.59 ± 0.19    | 6.58 ± 0.13   | 6.58 ± 0.08   | 7.11 ± 0.23   | 7.11 ± 0.24   | 7.05 ± 0.24   |
| GLU (mg/dL)         | 5.74 ± 0.29    | 4.75 ± 0.26   | 5.85 ± 0.16   | 6.45 ± 0.28   | 6.29 ± 0.35   | 6.58 ± 0.47   |
| BUN (mmol/L)        | 4.47 ± 0.29    | 8.69 ± 0.49   | 6.03 ± 0.32   | 6.83 ± 0.35   | 7.54 ± 0.26   | 6.65 ± 0.18   |
| BA (mmol/L)         | 77.27 ± 6.13   | 141.09 ± 9.09 | 112.06 ± 5.52 | 100.90 ± 8.73c | 111.42 ± 7.47c | 98.95 ± 6.52c |
| LA (mmol/L)         | 5.43 ± 0.18    | 11.77 ± 0.69  | 8.31 ± 0.34c | 7.46 ± 0.28   | 9.58 ± 0.29   | 8.79 ± 0.39   |
| CK (U/L)            | 455.31 ± 40.99c | 858.78 ± 36.82 | 624.49 ± 27.50 | 630.75 ± 24.50 | 715.31 ± 32.41b | 638.65 ± 61.08b |
| LDH (U/L)           | 456.38 ± 38.43c | 2237.13 ± 138.10 | 1274.8 ± 106.96 | 1051.02 ± 88.09 | 1816.13 ± 59.42 | 1462.88 ± 134.16c |
| AST/ALT              | 2.99 ± 0.48   | 3.30 ± 0.47   | 3.14 ± 0.27   | 3.31 ± 0.41   | 3.49 ± 0.52   | 2.76 ± 0.25   |

Note: Differences were analyzed with one-way ANOVA and Duncan’s new multiple range test. Different letters mean significant difference (P < 0.05). Data are expressed as mean ± S.E.M. (n = 6). The abbreviation of groups used in the figure were: blank control (Rest), swimming control (Con), Vitamin C (Vc), low-dose curcumin (C50), middle-dose curcumin (C100), and high-dose curcumin (C200).

The results of the biochemical analysis indicated that curcumin supplementation showed significant protective effects compared to the Vc group.

3.7. Effect of curcumin supplementation on anti-oxidation related enzyme activities in liver

To understand the anti-fatigue mechanisms of curcumin supplementation, we examined the anti-oxidation activities in mouse liver in relation to curcumin supplementation. In the present study, the levels of MDA, SOD, CAT and GSH-Px of mouse liver were measured and summarized in Fig. 3. Compared to those of mice in the Rest group, the MDA (Fig. 3-A) level in mouse liver was extensively boosted by 119.4%. However, the SOD (Fig. 3-B), CAT (Fig. 3-C) and GSH-Px (Fig. 3-D) levels of mice in the Con group were significantly reduced by 36.7%, 35.6% and 41.2%, respectively. In contrast, compared to those of Con mice, the MDA levels in the intervention groups were significantly decreased and the SOD, CAT and GSH-Px levels were increased. Particularly, there were no significant differences in all the four indexes between the Rest group and C200 group, suggesting that curcumin supplementation showed regulation capabilities in anti-oxidation-related enzyme activities of mice during prolonged or high-intensity exercise. These findings provided important clues for clarifying the mechanism of the anti-fatigue activity of curcumin supplementation on exhaustive swimming mice.

3.8. Regulation of curcumin supplementation on mRNA and protein expressions of Nrf2/Keap1 signal pathway

According to the results of anti-oxidation-related enzyme activities in the liver, the protein expression of the Nrf2/Keap1 signal pathway were chosen for further study of the regulation mechanism of curcumin in enhancing the anti-oxidant function on mice. The mRNA expressions of Nrf2 (Fig. 4-A), Keap1 (Fig. 4-B), HO-1 (Fig. 4-C) and NQO1 (Fig. 4-D) were examined and summarized. Among these genes, only the mRNA expression of HO-1 of mice in the Con group was significantly increased and no significant differences were found in the other three gene expressions compared to the Rest group. However, there were significant differences in all these gene expressions of mice in intervention groups (supplemented by Vc or curcumin) compared to those of mice in the Rest group. Therefore, the results indicated that curcumin supplementation exerted the anti-oxidative and anti-fatigue effects, possibly by regulating related gene mRNA expressions in the Nrf2/Keap1 signal pathway.

To further verify the mechanisms of curcumin supplementation in improving the exercise tolerance of mice, the related protein expressions of the Nrf2/Keap1 signal pathway were examined by Western blotting. As presented in Fig. 5, after the weight-loaded swimming test, the protein levels of Nrf2 (Fig. 5-A) and Keap1 (Fig. 5-B) of mice in the Con
group were significantly decreased and no significant differences were found in protein levels of HO-1 (Fig. 5-C) and NQO1 (Fig. 5-D) when compared to that of mice in the Rest group. However, compared to protein levels in the Con group mice, remarkable increases were found in mice after Vc or curcumin supplementation, especially for the mice in C100 group, which had the best performance in the weight-loaded swimming test. Consequently, combining the results of mRNA expressions and protein levels related to the Nrf2/Keap1 signal pathway, it was of great certainty that curcumin supplementation exerted anti-fatigue activities in mice subjected to excessive exercise.

4. Discussion

Curcumin, as one of the best known phenolic compounds with numerous medicinal properties and health benefits. However, less information exists about exercise-related effects of curcumin supplementation (Suhett et al., 2021). Moreover, the underlying mechanisms for the improvements of curcumin in physical function remain to be elucidated. Our study demonstrates that curcumin supplementation significantly increases the swimming time of mice probably due to a combination of anti-fatigue and anti-oxidant effects providing preliminary support for its use in the general population and by those engaged in sports-related activities.

Previous studies have shown that LA, BA, CK and LDH levels were closely related to exercise. The high levels of LA formed during high-intensity physical exercise can reduce the pH of muscles and blood, harm certain organs and develop fatigue (Westerblad et al., 2010). In this study, curcumin supplemented groups had lower LA levels,
suggesting anti-fatigue activity by preventing LA accumulation and accelerating the LA clearance. On the other hand, ammonia, a fatigue factor was significantly reduced in the curcumin groups. According to previous reports, this decrease of ammonia levels suggests lower muscle catabolism and muscle damage (Chen et al., 2018c). Similarly, LDH is an accurate indicator of muscle activity, whereby elevated serum levels of LDH indicate muscle damage (Huang et al., 2011). Therefore, LDH was identified as an indicator of fatigue in the body and the results demonstrated the anti-fatigue properties of curcumin supplementations by decreasing the LDH levels (Suhett et al., 2021). CK is also a valuable biomarker for skeletal muscle, since it is released during tissue damage, particularly skeletal muscle damage (Westerblad et al., 2010). Prior evidence shows that curcumin supplementation decreased CK activity and muscle damage of rats after eccentric exercise (Boz et al., 2015).
this study, the pathological examination of muscle tissue showed that curcumin supplementation had significant protective effects on the skeletal muscle of mice after exhaustive swimming. Consequently, our data provide evidence that curcumin supplementation regulates fatigue-related biochemical indicators and modulate muscle damage.

Glycogen is a vital energy material that can replenish energy when the blood sugar level is low. Therefore, glycogen plays an essential role in improving exercise tolerance (Xie et al., 2020). During strenuous exercise, hepatic and muscular glycogen are the key sources to satisfy the large amounts of energy consumption. Furthermore, energy metabolism is regulated by various biological enzymes involved in anabolism and catabolism (Greenberg et al., 2006). An exhaustive exercise that may lead to energy metabolism and fatigue imbalance is definitively a very energy-consuming process. The increasing activities of fatigue-related enzymes, such as PK, SDH, MDH, Na\(^+\)-K\(^+\)-ATPase and Ca\(^{2+}\)-Mg\(^{2+}\)-ATPase are primary evidence for early fatigue and exercise intolerance. SDH is a key enzyme associated with the regulation of the tricarboxylic acid cycle, catalyzing the synthesis of ATP. In addition, Na\(^+\)-K\(^+\)-ATPase and Ca\(^{2+}\)-Mg\(^{2+}\)-ATPase, which play an essential role in the physiological process of material transport, energy conversion, and information transmission, are crucial enzymes to degrade ATP. In the present study, curcumin supplementation remarkably improved the activity of these enzymes to maintain the balance between anabolism and catabolism once the extreme fatigue situation presents through improving the gluconeogenesis of liver energy metabolism in mice subjected to exhausting swimming.

Exhaustive exercise induces a significant increase of reactive oxygen species (ROS) and impairs anti-oxidant defense systems (Banerjee et al., 2003; Ma et al., 2021). Previous evidence has shown that dietary curcumin could enhance anti-oxidant defense systems by activating the anti-oxidant enzymes (Wafi et al., 2019). In our study, the gene and protein expressions of key factors of Nrf2, Keap1, HO-1 and NQO1, participating in Nrf2/Keap1 signal pathway were examined and confirmed the anti-oxidant modulation mechanism of curcumin on exhaustive exercise mice. As a result, curcumin effectively attenuated oxidative stress and prolonged the exercise tolerance, suggesting that curcumin holds promising potential anti-fatigue function.

To date, several theories, including the “clogging theory”, “exhaustion theory”, “radical theory”, “homeostasis disturbance theory”, “mutation theory” and “protective inhibition theory” have been advanced to interpret the mechanisms of fatigue (Zhong et al., 2017). Among these mechanisms, “exhaustion theory”, “clogging theory” and “radical theory” have attracted more attention (You et al., 2011). The “clogging theory” is concentrated on fatigue-related factors such as LA, BA, CK and LDH levels. The “exhaustion theory” is focused on energy sources like

**Fig. 5.** Regulation of curcumin supplementation on the protein expressions of NRF2/Keap1 signal pathway. Protein band intensities were normalized to GAPDH band intensities using the Image J software. All experiments were performed in triplicate. Differences were analyzed with one-way ANOVA and Duncan’s new multiple range test. *Different letters indicate a significant difference (P < 0.05). Data are expressed as mean ± S.E.M. (n = 6). The abbreviation of groups used in the figure were: blank control (Rest), swimming control (Con), Vitamin C (Vc), low-dose curcumin (C\(_{50}\)), middle-dose curcumin (C\(_{100}\)), and high-dose curcumin (C\(_{200}\)).
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5. Conclusions

In summary, the present study demonstrated the anti-fatigue and anti-oxidative effects of curcumin in a fatigue mouse model induced by exhaustive swimming stress. Curcumin showed protective effects on exercise-induced fatigue by inhibiting the production of reactive oxygen species. Furthermore, the significant anti-fatigue activity of curcumin worked by efficiently regulating the energy metabolic biomarkers in the glycolytic pathway and activating anti-oxidant response via Nrf2/Keap1 signaling pathway. These results suggest that curcumin might be an anti-fatigue promising candidate applied in exercise performance improvement in the future.

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CRediT authorship contribution statement

Yong Chen: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. Jiajun Wang: Validation, Formal analysis, Investigation, Writing – review & editing. Ziheng Jing: Investigation. Jose M. Orduvos: Writing – original draft, Writing – review & editing. Jing Wang: Supervision, Writing – original draft, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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