



Astaxanthin ameliorates dexamethasone-induced skeletal muscle atrophy and disorders of glucolipid metabolism

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ARTICLE INFO

Keywords:

Astaxanthin
Dexamethasone
Glucolipid metabolism
Skeletal muscle atrophy

ABSTRACT

Long-term use of glucocorticoids, such as dexamethasone, can lead to skeletal muscle atrophy and disturbances in glucolipid metabolism. Astaxanthin, a ketocarotenoid, has a variety of physiological activities. In this study, we investigated the effects of astaxanthin on dexamethasone-induced skeletal muscle atrophy and disorders of glycolipid metabolism. Male C57Bl/6J mice were administered dexamethasone alone or supplemented with different doses of astaxanthin (30 mg/kg/d, 60 mg/kg/d, 120 mg/kg/d) for 4 weeks. The results showed that astaxanthin improved locomotor performance and attenuated dexamethasone-induced reductions in skeletal muscle mass and cross-sectional area in mice. Further exploration of the mechanism revealed that astaxanthin was able to balance the catabolism and synthesis of skeletal muscle proteins and protect mitochondria by reducing the expression of the mitochondrial autophagy-associated proteins Lc3B and BNIP3. In addition, astaxanthin reduced the accumulation of fatty acid metabolites and visceral fat and improved the ability of skeletal muscle to utilize sugars, suggesting that astaxanthin may alleviate dexamethasone-induced disturbances in glycolipid metabolism. This study illustrates that astaxanthin can alleviate skeletal muscle atrophy and metabolic disturbances induced by long-term dexamethasone treatment. This suggests that astaxanthin may be a functional dietary supplement to reduce the side effects of glucocorticoid therapy.

1. Introduction

Glucocorticoids such as dexamethasone are known for their potent anti-inflammatory and immunosuppressive properties. It is widely used in medicine for the treatment of various acute and chronic inflammatory and autoimmune diseases [1]. However, prolonged use of glucocorticoids may result in the development of skeletal muscle atrophy, lipid accumulation, and hyperglycemic syndrome.

The cross-regulation of glucocorticoid receptor and mTOR in skeletal muscle promotes the expression of E3 ubiquitin ligase, leading to an imbalance between anabolism and catabolism in skeletal muscle [2]. In addition, dexamethasone accelerates skeletal muscle degradation through mitochondrial autophagy, causing skeletal muscle atrophy [3, 4]. In addition, metabolic dysregulation is one of the side effects of dexamethasone. Prolonged use of glucocorticoids causes decreased insulin sensitivity and dysregulation of glucose metabolism in skeletal muscle, liver, and adipose tissue, and it accelerates lipolysis, leading to

increased fatty acid levels and accumulation of visceral fat[5–7].

For this reason, it is necessary to take certain measures to mitigate the side effects of glucocorticoid therapy. For example, Huang-Gui solid dispersion (HGSD), a highly bioavailable preparation of chaparral, can alleviate glucocorticoid-induced insulin resistance by reducing glucocorticoid receptor binding to PI3K [8]. However, there are no effective drugs or targets for skeletal muscle atrophy. Nutritional supplementation with active ingredients is another effective strategy. For example, *Luffa cylindrica Roemer* (LCR) inhibits dexamethasone-induced up-regulation of E3 ubiquitin ligases (Atrogin-1 and MuRF1) expression and promotes myotube development [9]; and the combination of essential amino acids and impedance exercise accelerates skeletal muscle protein synthesis and inhibits autophagy, which alleviates muscle damage and insulin resistance in dexamethasone mice [10]. Therefore, it is necessary to find a safe natural ingredient for dietary supplementation to simultaneously alleviate skeletal muscle atrophy and dysregulated glycolipid metabolism.

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<https://doi.org/10.1016/j.bbrc.2024.151138>

Received 22 September 2024; Received in revised form 3 December 2024; Accepted 4 December 2024

Available online 4 December 2024

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Astaxanthin is a ketocarotenoid with high antioxidant capacity that is widely distributed in foods of marine origin seafood such as shrimp and crab, and possesses a variety of physiological activities such as anti-inflammatory, anti-aging, immune-enhancing, and anti-tumor. In a previous study by our team, astaxanthin was found to alleviate skeletal muscle atrophy caused by cachexia by inhibiting the expression of E3 ligases MAFbx and MURF-1 in skeletal muscle and improving mitochondrial function [11]. It has been found that astaxanthin inhibits oxidative stress and protein hydrolysis to attenuate immobilization-induced skeletal muscle atrophy [12]. Sun, L et al. found that astaxanthin prevented unloading-induced skeletal muscle atrophy by attenuating mitochondrial oxidative stress and mitochondria-mediated apoptosis [13]. These studies suggest that astaxanthin has enormous potential in treating skeletal muscle atrophy. In addition, astaxanthin can stimulate mitochondrial genesis through the AMPK pathway to improve skeletal muscle metabolism in obese mice [14,15]. Astaxanthin can reduce the accumulation of abdominal lipids, control blood glucose levels, increase insulin sensitivity, and also have an ameliorative effect on metabolic diseases such as obesity and diabetes [16–19].

Thus, this study has examined the impact of astaxanthin on skeletal muscle atrophy induced by dexamethasone, as well as its effects on glucose and lipid metabolism. Additionally, it has explored potential mechanisms related to metabolism and mitochondrial damage in skeletal muscle. This study offers a solid foundation for the creation of functional foods centered around astaxanthin as the primary component.

2. Material and methods

2.1. Material

The astaxanthin used in this study was derived from *Haematococcus pluvialis* (*H. pluvialis*). The astaxanthin was obtained from Yunnan Alphy Biotech. Co. Ltd. (Yunnan, China), in which the astaxanthin concentration was 10 % (w/w). The astaxanthin consisted of 71 % astaxanthin monoesters, 28 % astaxanthin diesters and <1 % free astaxanthin, as previously determined by the group [20].

2.2. Animal experiments

Healthy 8-week-old male C57BL/6J mice (20 ± 2 g) were purchased from Beijing VitalLiver Laboratory Animal Technology Co Ltd (Beijing, China). These animals were housed in a constant environment (22 ± 0.5 °C, 50 ± 5 % relative humidity, 12 h light 12 h dark cycle) in a specific pathogen free animal center. The animals had free access to water and food. The maintenance diets for the experimental animals were purchased from Xie Tong Biologicals (Jiangsu, China), and the composition and the energy provided are shown in Tables S1–3. This experiment was approved by the Laboratory Animal Ethics Committee of the Ocean University of China (Permission No. SPXY2023083110). After 7 days of acclimation feeding, the mice were randomly divided into the following groups: (1) control group (Con, $n = 8$), intraperitoneal injection of saline and olive oil by gavage; (2) dexamethasone group (Dex, $n = 8$), intraperitoneal injection of dexamethasone (5 mg/kg/d) and olive oil by gavage; (3) dexamethasone + low-dose astaxanthin (Dex + LA, $n = 8$), intraperitoneal injection of dexamethasone (5 mg/kg/d), and low-dose astaxanthin (30 mg/kg/d) by gavage; (4) dexamethasone + medium-dose astaxanthin (Dex + MA, $n = 8$), intraperitoneal injection of dexamethasone (5 mg/kg/d), and medium-dose astaxanthin (60 mg/kg/d) by gavage; (5) dexamethasone + high-dose astaxanthin (Dex + HA, $n = 8$), intraperitoneal injection of dexamethasone (5 mg/kg/d), and high-dose astaxanthin (120 mg/kg/d) by gavage. The gavage volume was 10 mL/kg mouse body weight. The treatment was administered for a period of 4 weeks, during which the body weight of the mice was continuously monitored every other day. In

addition, the mice's food consumption was recorded every third day. At the end of the experiment, the mice were anaesthetized and euthanized. The blood samples were collected by puncturing the retro-orbital sinus, centrifuged at 7500 rpm for 15 min, and serum specimens were collected and stored at -80 °C until analysis. The spleen, liver, and skeletal muscle (including gastrocnemius, soleus, and tibialis anterior) were weighed, and skeletal muscle was stored at -80 °C until further analysis.

2.3. Behavioral tests

2.3.1. Grip strength test

At the end of the experiment, the grip strength of the mice's forelimbs was measured using a precise electronic dynamometer (Handpi HP-5N, CHINA). Briefly, a slender crossbar is securely attached to the front of the horizontally positioned dynamometer, allowing the mice's forelimbs to firmly grip the crossbar. Gently pull back on the mouse's tail to release the mouse's forelimbs from the crossbar. Simultaneously, measure and record the magnitude of the traction force exerted as the mouse's forelimbs separate from the crossbar. The experiment was repeated three times and the highest force was recorded.

2.3.2. Hanging experiment

At the end of the experiment, the mice were tested for hanging time. A metal mesh (30 cm long, 20 cm wide, wire diameter 1.4 mm) was placed on the foam box 40 cm from the bottom. The mice were placed on the mesh, and after grasping, the mesh was inverted and placed on the foam box, causing the mice to hang from the metal mesh. The time taken for the mice to become fatigued and fall was documented.

2.3.3. Exercise fatigue experiment

Mice were evaluated for fatigue and athletic ability using the Mouse Rotary Wheel Fatigue Meter (YSL-10B, Yiyan Technology Development Co., Ltd, Jinan, China). The mice underwent three acclimatization sessions before the official test, and the official experiment took place on the final day of the study. The mice were placed in the tunnel and given the opportunity to run on the wheel. When the mice became fatigued, they stopped running, positioned themselves near the bottom of the wheel, and received electrical stimulation. Three breaks within 15 min were considered as exhaustion and the experiment was stopped. The rotational speed of the wheel was 30 rpm, the tolerance time for electric shock was 2 s, and the rest period was 45 s.

2.4. Histopathological evaluation

The gastrocnemius muscle tissue was immersed in 4 % paraformaldehyde, paraffin-embedded, and made into sections, which were stained with hematoxylin and eosin (HE) (Servicebio, Wuhan, China). The histomorphology of the gastrocnemius muscle was observed under a light microscope. The cross-sectional area of skeletal muscle was analyzed using ImageJ software.

2.5. Western blotting experiment

The gastrocnemius muscle was immersed in RIPA lysate containing phosphatase inhibitor, and total skeletal muscle protein was obtained after grinding and centrifugation for subsequent experiments. Skeletal muscle nuclear proteins were extracted using a kit (G-CLONE Biotechnology Co., Ltd., Beijing, China) according to the reagent manufacturer's instructions. Twenty-five micrograms of protein from each group was subjected to SDS-PAGE electrophoresis, followed by transfer to polyvinylidene tetrafluoride (PVDF) membranes and blocked with 5 % nonfat dry milk diluted in TBST for 2 h at room temperature. Primary antibodies including Myod1 (ABclonal, 1:1000), MuRF1 (ABclonal, 1:1000), BNIP3 (ABclonal, 1:1000), Lc3B (ABclonal, 1:500), Foxo3 (ABclonal, 1:1000), PGC-1 α (ZEN BIO, 1:1000) and GAPDH (Servicebio 1:2000) were added and sealed overnight at 4 °C. PVDF membranes

Table 1
Primer sequences.

Gene	Forward (5'-3')	Reverse (5'-3')
MuRF1	ACCACAGAGGGTAAAGAAGAACA	GCAGAGAGAAGACACACTTCCC
P70S6K	TGTCAGCCCGAGTCAAATTCTCTCC	ACATCCATCTGCTCTATCCCACTTG
BNIP3	TTCTCACTGTGACAGCCAC	TCTTCCTCAGACAGAGTGCT
Lc3B	TTATAGAGCGATACAAGGGGGAG	CGCCGTCTGATTATCTTGATGAG

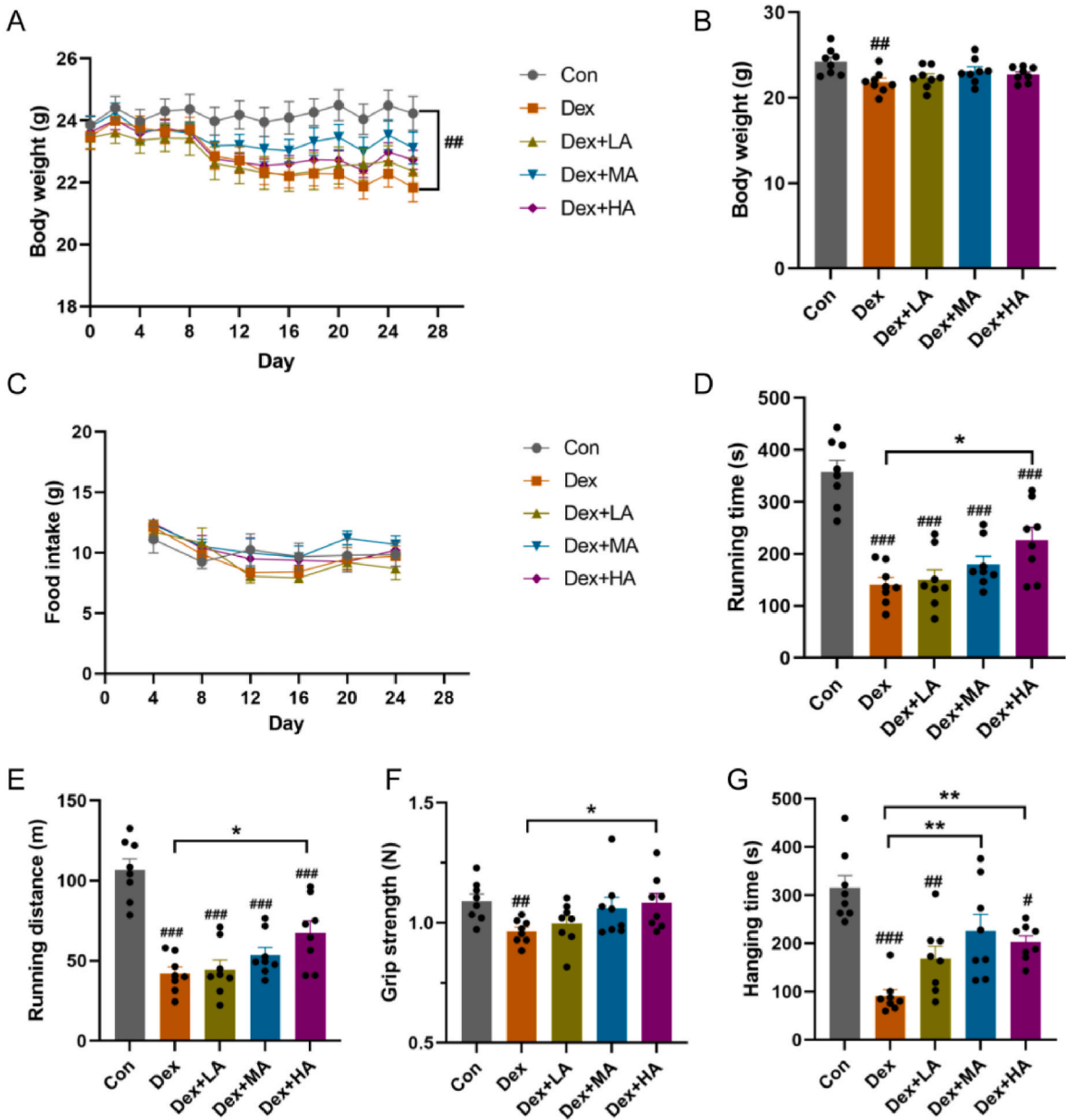


Fig. 1. Effect of astaxanthin on body weight and skeletal muscle function in dexamethasone treated mice. A: Changes trends in body weight, B: Final body weight, C: Food intake, D: Running time, E: Running distances, F: Grip strength, G: hanging time. Data are expressed as mean $\pm$ SEM, $n = 8$ for each group. $\#P < 0.05$, $\#\#\#P < 0.01$, compared with the Con group. $*P < 0.05$, $**P < 0.01$, compared with the Dex group.

were washed with TBST followed by the addition of HRP-labelled goat anti-rabbit IgG secondary antibody and incubated for 2 h at room temperature. After washing the membrane with TBST, the membrane was imaged using a chemiluminescence imaging device (Tanon-5200, China). The bands were analyzed using ImageJ software.

2.6. Biochemical assays

The levels of glucose, pyruvate, lactate, glycogen, cholesterol, tri-glyceride, LDL, and HDL were determined with the appropriate commercial kits according to the manufacturer's protocol using a microplate reader (Nanjing Jiancheng Bioengineering Institute, China). Serum insulin levels were measured using an ELISA kit (Alesia Biotechnology Co.

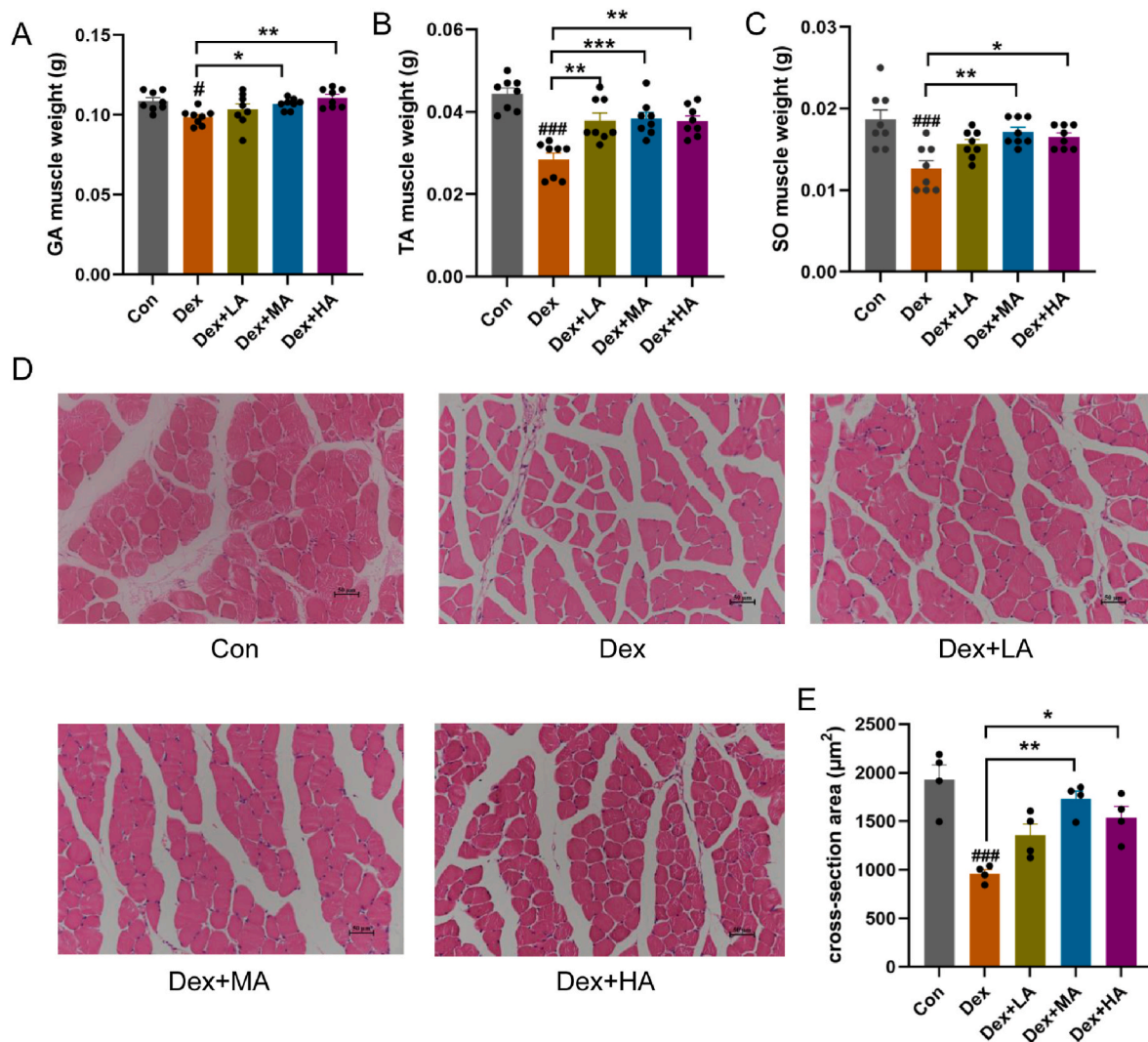


Fig. 2. Astaxanthin improves dexamethasone-induced skeletal muscle atrophy. A: Gastrocnemius muscle weight. B: Tibialis anterior muscle weight. C: Soleus muscle weight. D: H&E staining of myofiber cross-section of Gastrocnemius muscle, scale bar = 50 μm. E: Mean cross-sectional area of muscle fibers. Data are expressed as mean ± SEM, n = 8 for each group. ## $P < 0.05$, ### $P < 0.01$, compared with the Con group. * $P < 0.05$, ** $P < 0.01$, compared with the Dex group.

Ltd. Shanghai, China).

2.7. RNA extraction and RT-qPCR

The total RNA was extracted from gastrocnemius muscle using Trizol reagent according to the manufacturer's instructions. The cDNA was reverse transcribed using All-In-One 5X RT MasterMix (Abmgood, Shanghai, China) according to the instruction manual. cDNA was diluted 10-fold and analyzed by RT-qPCR using BlasTaq™ 2X qPCR MasterMix kit (Abmgood, Shanghai, China). Primers were synthesized by Shanghai Sangon Biotechnology Co Ltd (Shanghai, China). The primer sequences of the target genes are listed in Table 1. The relative expression of each gene was calculated by $2^{-\Delta\Delta CT}$ using GAPDH as an internal reference.

2.8. Statistical analysis

This experiment was performed using GraphPad Prism 9.5.0 (San Diego, CA, USA) software for data analysis and plotting, and the measured data were expressed as mean ± SEM. Normality was tested using the Shapiro-Wilk test. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnet's post hoc test, with $P < 0.05$ indicating a statistically significant difference.

3. Results

3.1. Astaxanthin ameliorates weight loss and impaired motor function induced by dexamethasone

Dexamethasone induced weight loss in mice (Fig. 1A and B). Astaxanthin effectively attenuated the weight loss induced by dexamethasone in mice ($P < 0.05$, Fig. 1A and B), and its effect was not influenced by their dietary food intake ($P > 0.05$, Fig. 1C). Behavioral experiments are a valuable tool for assessing the motor function of organisms. Dexamethasone resulted in a significant decrease in the movement time and distance of mice. On the other hand, astaxanthin led to a increase in movement time and distance in mice, with a dose-dependent effect observed ($P < 0.05$, Fig. 1D and E). Dexamethasone had a negative impact on forelimb grip strength in mice, while astaxanthin had a positive effect and showed a dose-dependent response ($P < 0.05$, Fig. 1F). Additionally, dexamethasone shortened the hanging time in mice, whereas astaxanthin increased it, with the most significant effect observed at a dose of 60 mg/kg ($P < 0.05$, Fig. 1G). These results indicate that astaxanthin effectively counteracted the weight loss and motor function impairment induced by dexamethasone in mice.

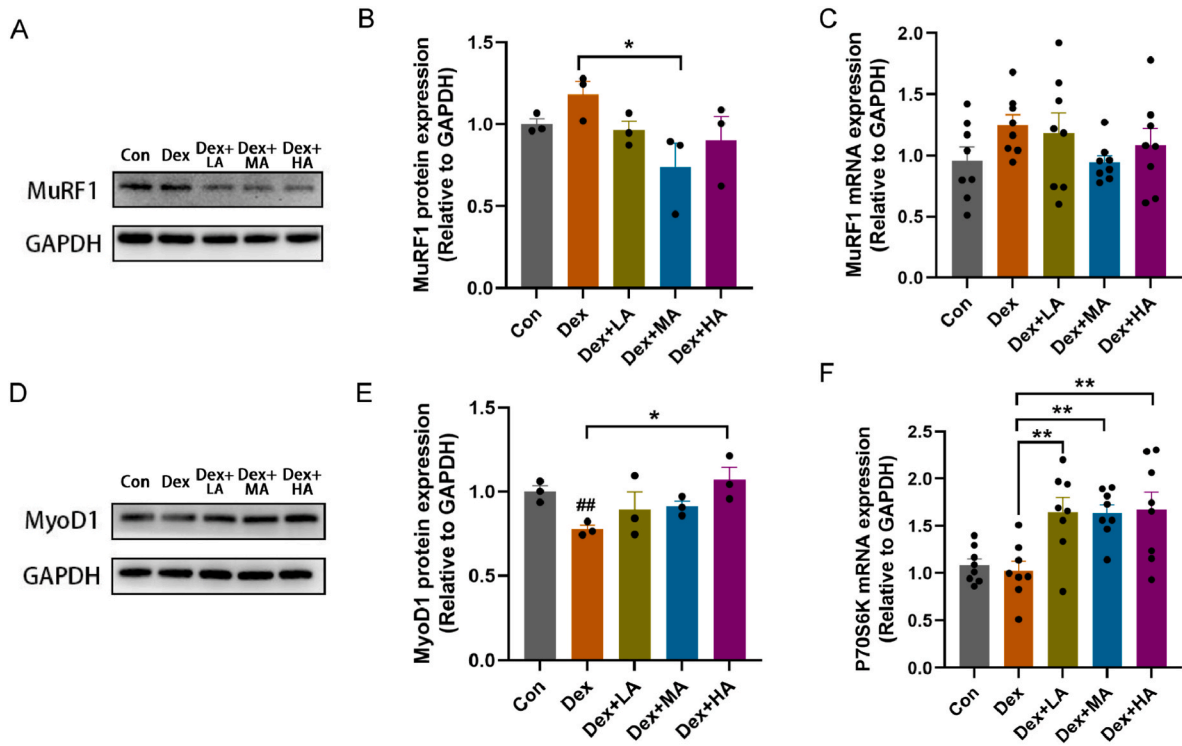


Fig. 3. Astaxanthin modulates the synthesis and breakdown of skeletal muscle in dexamethasone-treated mice. A: Western blot analysis of MuRF1, B: Protein expression level of MuRF1, C: mRNA expression level of MuRF1, D: Western blot analysis of MyoD1, E: Protein expression level of MyoD1, F: mRNA expression level of P70S6K. Data are expressed as mean $\pm$ SEM, $n = 8$ for each group. ## $P < 0.05$, ### $P < 0.01$, compared with the Con group. * $P < 0.05$, ** $P < 0.01$, compared with the Dex group.

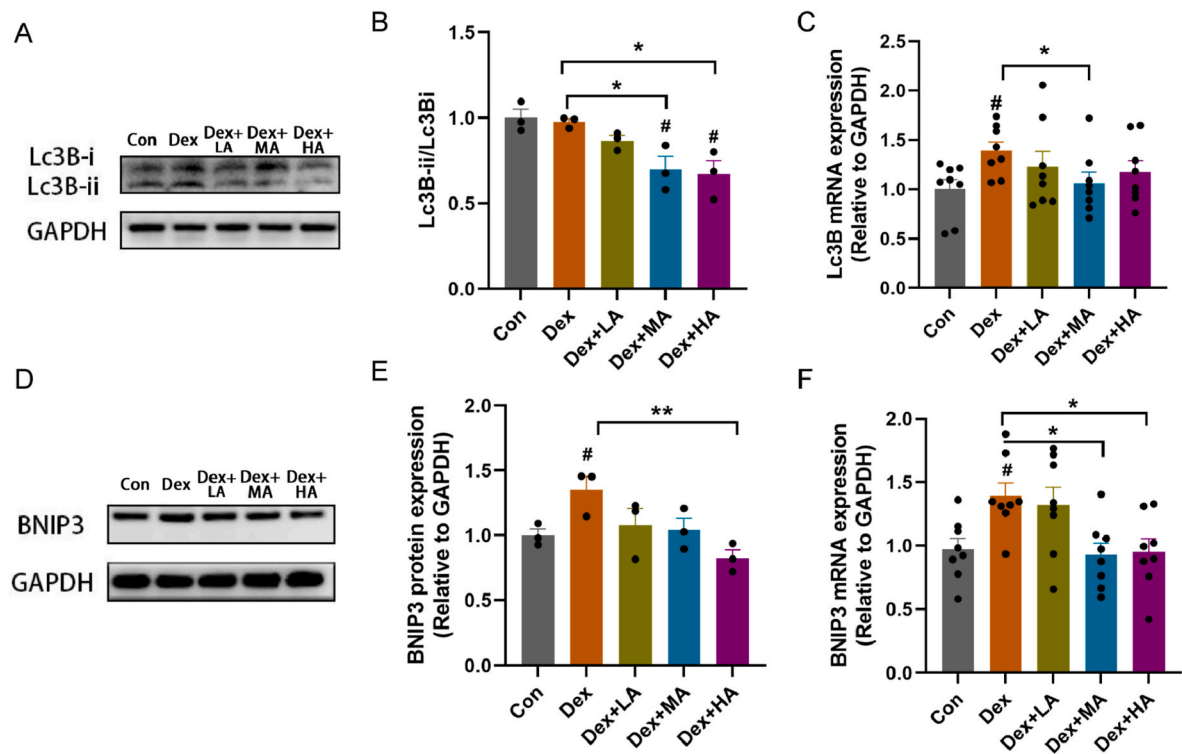


Fig. 4. Astaxanthin inhibits DEX-induced mitochondrial autophagy in skeletal muscle. A: Western blot analysis of Lc3B, B: Ratio of Lc3Bii/Lc3Bi, C: mRNA expression level of Lc3B, D: Western blot analysis of BNIP3, E: Protein expression level of BNIP3, F: mRNA expression level of BNIP3. Data are expressed as mean $\pm$ SEM, $n = 8$ for each group. ## $P < 0.05$, ### $P < 0.01$, compared with the Con group. * $P < 0.05$, ** $P < 0.01$ compared with the Dex group.

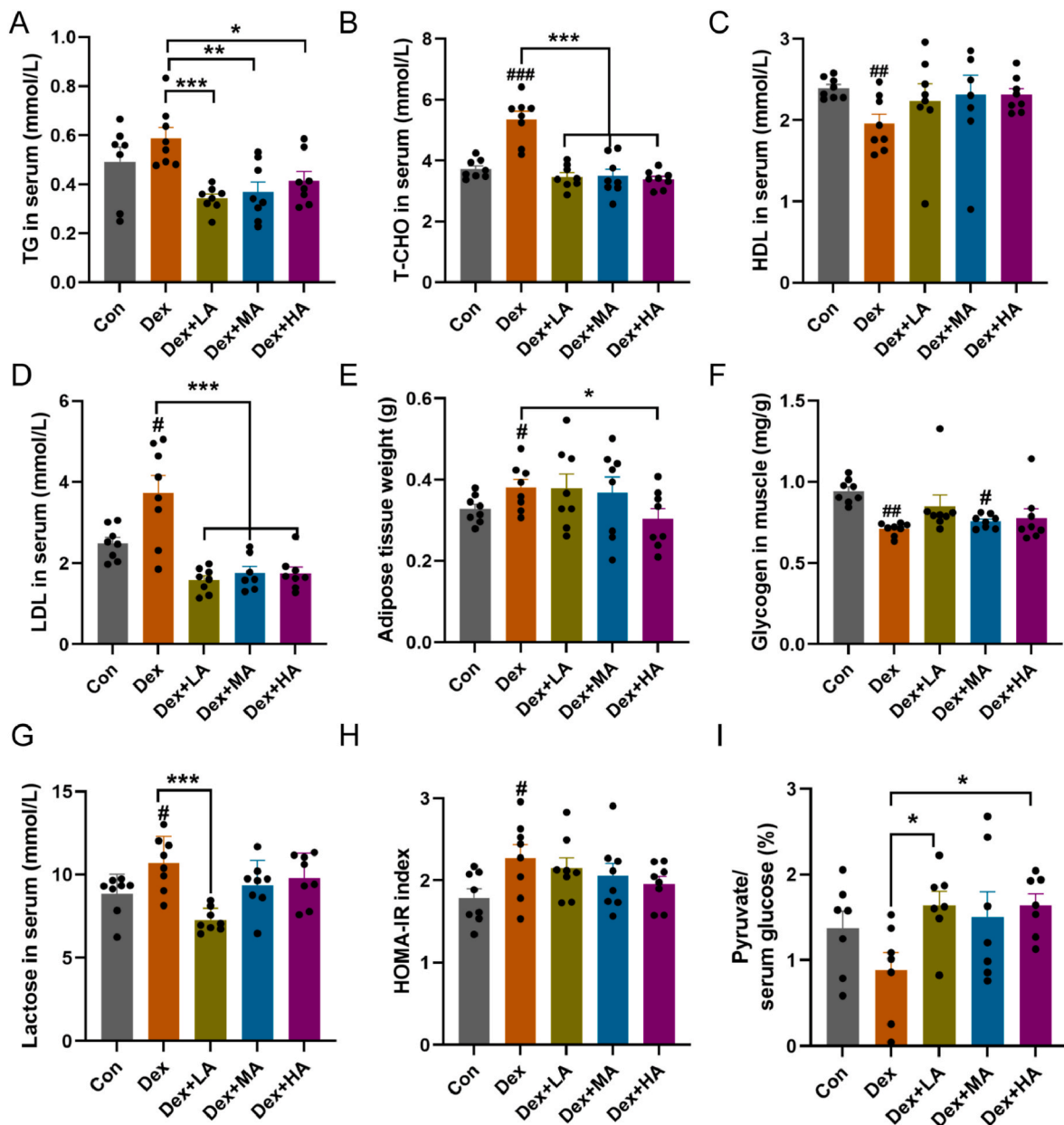


Fig. 5. Astaxanthin ameliorates disorders of glycolipid metabolism in dexamethasone-treated mice. A: Serum TG content, B: Serum T-CHO content, C: Serum HDL content, D: Serum LDL content, E: Adipose tissue weight, F: Glycogen content, G: Serum lactate content, H: HOMA-IR index, I: Ratio of gastrocnemius pyruvate to serum glucose. Data are expressed as mean $\pm$ SEM, n = 8 for each group. ## P < 0.05, ### P < 0.01, compared with the Con group. * P < 0.05, ** P < 0.01 compared with the Dex group.

3.2. Astaxanthin alleviates skeletal muscle atrophy induced by dexamethasone treatment

The skeletal muscle is the primary organ responsible for the body's motor function. Prolonged use of dexamethasone causes the skeletal muscle to atrophy, resulting in a decrease in motor performance [21]. Dexamethasone significantly reduced the muscle weight of the gastrocnemius, tibialis anterior, and soleus muscles in mice. Astaxanthin attenuated the dexamethasone-induced decrease in skeletal muscle mass in the gastrocnemius, tibialis anterior, and soleus muscles (P < 0.05, Fig. 2A–C). The pathological examination of the gastrocnemius muscle revealed that dexamethasone caused a disruption in the arrangement of muscle cells and a decrease in the cross-sectional area of muscle fibers in skeletal muscle (P < 0.05, Fig. 2D and E). Astaxanthin slowed the dexamethasone-induced reduction in muscle fiber cross-sectional area

in skeletal muscle (P < 0.05, Fig. 2D and E). These results indicate that astaxanthin is effective in preventing dexamethasone-induced skeletal muscle atrophy.

3.3. Astaxanthin slows down ubiquitination degradation in skeletal muscle

The imbalance between skeletal muscle degradation and synthesis is an important cause of dexamethasone-induced skeletal muscle atrophy [22]. MuRF1 plays a key role in muscle catabolism and MyoD1 plays a critical role in controlling muscle protein synthesis. Dexamethasone increased both protein expression and transcript levels of MuRF1 in skeletal muscle. Conversely, astaxanthin decreased the protein expression and transcript levels of MuRF1 in skeletal muscle, with the 60 mg/kg dose of astaxanthin showing a significantly better effect (P <

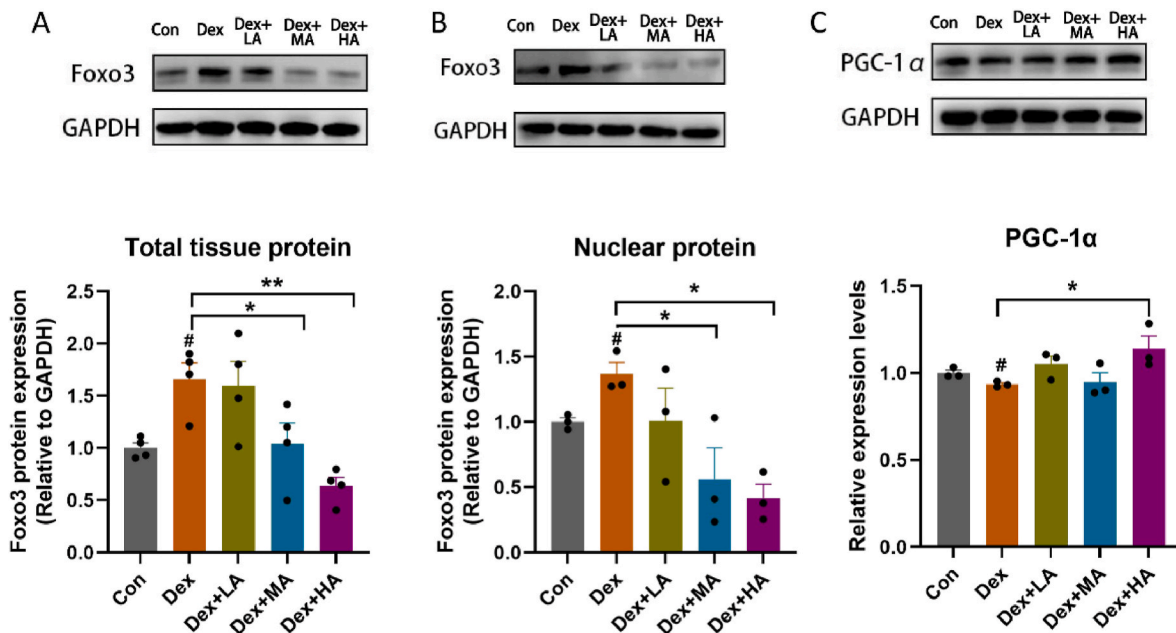


Fig. 6. Changes of metabolism-related proteins in skeletal muscle. A: Western blot analysis and expression level of total Foxo3, B: Western blot analysis and expression level of nuclear Foxo3, C: Western blot analysis and expression level of PGC-1 α . Data are expressed as mean $\pm$ SEM, $n = 8$ for each group. $\#P < 0.05$, $\#\#\#P < 0.01$, compared with the Con group. $*P < 0.05$, $**P < 0.01$, compared with the Dex group.

0.05, Fig. 3A–C). Dexamethasone significantly lowered the protein expression of MyoD1 in skeletal muscle ($P < 0.05$, Fig. 3D and E), while astaxanthin dramatically enhanced the protein expression levels of MyoD1 and the transcription levels of P70S6K in skeletal muscle ($P < 0.05$, Fig. 3D–F). These results suggest that astaxanthin promotes skeletal muscle synthesis and reduces skeletal muscle degradation to alleviate skeletal muscle atrophy.

3.4. Astaxanthin inhibits DEX-induced mitochondrial autophagy in skeletal muscle

Mitochondrial damage is an important pathway for skeletal muscle atrophy [23]. Medium and high doses of astaxanthin significantly reduced the ratio of autophagy markers Lc3B-ii/1 ($P < 0.05$, Fig. 4A and B). Dexamethasone markedly increased the production of BNIP3, a protein that stimulates programmed cell death and mitochondrial degradation, compared to the control group. However, astaxanthin treatment inhibited the increased expression of BNIP3 ($P < 0.05$, Fig. 4D–F). These results suggest that astaxanthin protects mitochondria by reducing mitochondrial autophagy, which in turn attenuates skeletal muscle atrophy.

3.5. Astaxanthin ameliorates disorders of glycolipid metabolism in dexamethasone-treated mice

Dexamethasone treatment causes disorders of glycolipid metabolism and increases the risk of obesity and diabetes [24]. Skeletal muscle, as the largest metabolic organ, plays an important role in regulating glycolipid metabolism [25]. Dexamethasone treatment increased serum triglyceride, cholesterol, and LDL levels and decreased serum HDL levels, which suggests that prolonged dexamethasone treatment dysregulated lipid metabolism in mice. Astaxanthin significantly reduced triglyceride, cholesterol, and LDL levels, and increased HDL levels (Fig. 5A–D). Astaxanthin also reduced the accumulation of visceral fat (Fig. 5E). Compared to the control group, dexamethasone resulted in a significant reduction in skeletal muscle glycogen stores, a change that was ameliorated by astaxanthin (Fig. 5F). Dexamethasone significantly increased serum lactate levels, and astaxanthin reduced lactate

accumulation (Fig. 5G). Dexamethasone significantly increased the HOMA-IR index compared to the normal group, and astaxanthin treatment decreased the HOMA-IR index (Fig. 5H). Compared with the dexamethasone group, astaxanthin significantly increased gastrocnemius pyruvate to serum glucose ratio and enhanced the ability of skeletal muscle to utilize glucose (Fig. 5I). These results suggest that astaxanthin improves lipid metabolism and glucose metabolism disorders induced by dexamethasone treatment in mice.

3.6. Effects of astaxanthin on metabolism-related proteins in skeletal muscle

Numerous studies have shown that dexamethasone increases the expression of Foxo3, and overexpression of Foxo3 decreases the body's glucose uptake and affects glycogen synthesis [26,27]. Dexamethasone markedly increased Foxo3 levels in skeletal muscle, while medium and high doses of astaxanthin effectively suppressed the expression of Foxo3 (Fig. 6A). Foxo3 nuclear protein expression was significantly increased in the Dex group compared to the normal group. Similar to the trend of Foxo3 expression in total protein, medium and high doses of astaxanthin significantly inhibited the expression of Foxo3 nuclear protein. (Fig. 6B). PGC-1 is a transcriptional activator of mitochondrial biogenesis that promotes fatty acid oxidation and metabolism [28,29]. Dexamethasone significantly reduced the expression of PGC-1 α in skeletal muscle (Fig. 6C). Astaxanthin increased the level of PGC-1 α compared to the dexamethasone group, which was significant in the high-dose group (Fig. 6C).

4. Discussion

Dexamethasone, a widely used glucocorticoid, has the side effect of causing skeletal muscle atrophy and metabolic abnormalities [30,31]. Astaxanthin is a natural ingredient of marine origin with a variety of bioactivities [32]. In this study, astaxanthin was found to attenuate dexamethasone-induced skeletal muscle atrophy and regulate glycolipid metabolism by balancing muscle production and degradation and protecting mitochondria.

Skeletal muscle plays an important role in maintaining the body's

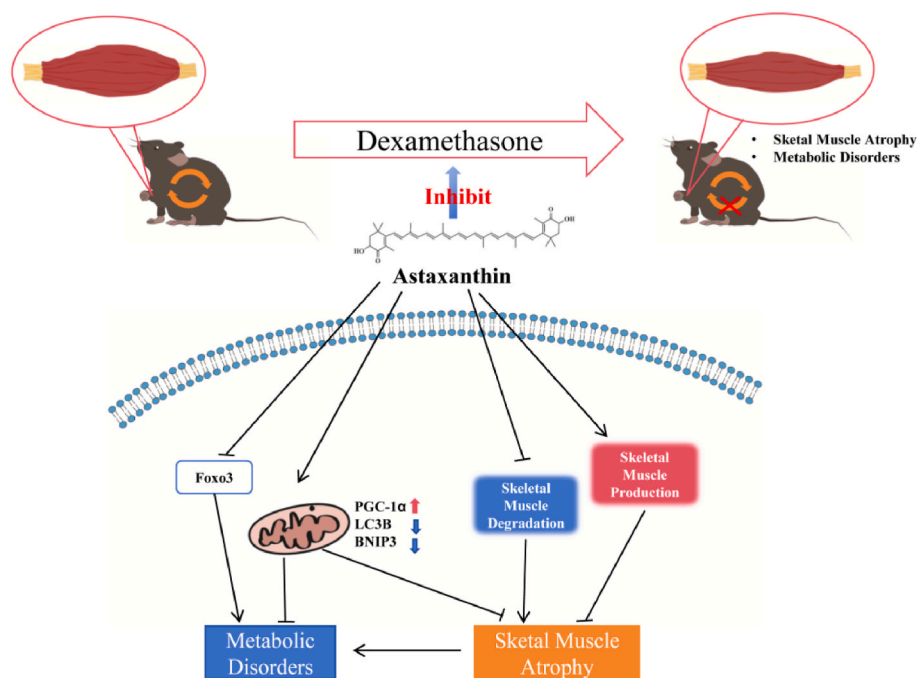


Fig. 7. Astaxanthin improves the mechanism of skeletal muscle atrophy and metabolic disorders caused by dexamethasone.

ability to move. Dexamethasone can lead to skeletal muscle atrophy, impacting the body's motor function. The results of the behavioral experiments revealed that dexamethasone had a significant effect on the exercise time, exercise distance, forelimb grip strength, and hanging time of the mice. These results suggest that dexamethasone had a negative effect on the motor ability of the mice. Astaxanthin significantly improved the motor function of the mice by increasing movement time, movement distance, forelimb grip strength, and mouse hanging time of the mice. This indicates that astaxanthin effectively repaired the impaired motor function caused by dexamethasone treatment. Previous study highlighted the potential benefits of astaxanthin in addressing motor degeneration associated with aging or disease. A study showed that an astaxanthin formulation containing 12 mg astaxanthin, 10 mg tocotrienol and 6 mg zinc enhanced the effects of exercise training in improving endurance and mobility in older people. This was achieved by enhancing muscular strength and size [33]. In this study, astaxanthin was found to reverse the dexamethasone-induced decline in motor function.

Skeletal muscle wasting is the main cause of reduced motor function in the body. Prolonged use of dexamethasone can lead to skeletal muscle weakness, but astaxanthin has shown promise in counteracting this effect. Numerous studies have shown that astaxanthin is effective in improving immobilization-induced skeletal muscle atrophy [13,34,35]. In this study, astaxanthin significantly enhanced the weight and cross-sectional area of skeletal muscle in dexamethasone-treated mice. It is possible that the efficacy of astaxanthin in preventing degradation and promoting muscle production in skeletal muscle is due to its expertise. In this study, dexamethasone was found to have a positive effect on MuRF1 protein expression and transcription levels. This indicates that dexamethasone stimulates the process of skeletal muscle ubiquitination in mice. Astaxanthin effectively decreased the protein expression level of MuRF1 in mice treated with dexamethasone. Shibaguchi, T et al. found that astaxanthin was able to inhibit the increase in the expression of CuZn-superoxide dismutase, cathepsin L, calpain, and ubiquitin in skeletal muscle and alleviate immobilization-induced skeletal muscle atrophy [12]. These findings suggest that astaxanthin may inhibit the degradation process in skeletal muscle. Notably, a dose of 60 mg/kg of astaxanthin demonstrated a significantly more positive impact. It is

worth noting that in a previous study, the administration of astaxanthin at a dose of 60 mg/kg resulted in a notable reduction in MuRF1 expression levels in the skeletal muscle of mice treated with sorafenib [36]. Furthermore, the imbalance between catabolism and myogenesis in skeletal muscle is a significant factor contributing to skeletal muscle atrophy. Astaxanthin not only prevents degradation in skeletal muscle, but also enhances the expression of MyoD1 and P70S6K, indicating its potential to stimulate myogenesis. Myoblast determinant protein 1 (MyoD1) is an important regulator of skeletal muscle growth and development, and prolonged dexamethasone treatment leads to decreased expression of MyoD1, which inhibits skeletal myogenesis [37]. P70S6K is a downstream regulator of the skeletal myogenesis signaling pathway. Phosphorylated mTOR promotes protein synthesis by activating P70S6K [38,39].

Autophagy removes senescent and damaged cellular components and remodels cellular structures. Moderate levels of autophagy are important for maintaining skeletal muscle homeostasis. Excessive activation or excessive lack of autophagy can have detrimental effects on skeletal muscle health [40]. However, Troncoso et al. found that dexamethasone stimulated early activation of autophagy in L6 myotubes and increased the expression of several autophagy genes, including ATG5, LC3, BECN1 and SQSTM1, and triggered AMPK-mediated mitochondrial fragmentation [3]. This suggests that hyperactivation of autophagy is one of the causes of dexamethasone-induced skeletal muscle atrophy. Numerous studies have confirmed that functional components can ameliorate dexamethasone-induced skeletal muscle atrophy by protecting mitochondria [41–43]. Therefore, in this study, we investigated whether astaxanthin can ameliorate excessive autophagy and protect mitochondria. We found that astaxanthin reduced the expression of mitochondrial autophagy-related proteins Lc3B and BNIP3 and promoted the expression of PGC-1α, which is consistent with the results of studies reporting that astaxanthin can protect mitochondria through various signalling pathways such as AMPK/AKT [44]. A study conducted by Nishida et al. revealed that astaxanthin had a positive effect on myogenesis in mice with insulin resistance [14]. The researchers observed that astaxanthin was able to repair mitochondrial dysfunction, leading to improved muscle myogenesis. However, whether the intake of astaxanthin in the normal state will excessively inhibit autophagy in

this study did not set up a relevant control group. Further studies will be conducted in the research group to determine the safety of astaxanthin.

In addition to skeletal muscle loss, metabolic dysregulation is also a common adverse effect of dexamethasone treatment [24,31]. Skeletal muscle is a key organ in the regulation of metabolism and can influence metabolism through its own mitochondrial oxidation or release of cytokines [25,45]. In this study, astaxanthin intake significantly alleviated the dexamethasone-induced elevation of TG, T-CHO, and LDH in serum, and reduced the accumulation of visceral fat. In terms of glucose metabolism, astaxanthin supplementation reduced the accumulation of lactic acid and improved muscle glucose uptake. It is worth noting that astaxanthin can restore abnormal mitochondrial function in skeletal muscle. Mitochondria play a crucial role in the oxidation of fatty acids and the metabolism of amino acids in cells. By restoring mitochondrial function, the disturbances in skeletal muscle energy metabolism were reduced, leading to a slowing of the abnormalities in glucose and lipid metabolism in dexamethasone-treated mice. PGC-1 α is a marker of mitochondrial genesis, it can promote fatty acid metabolism and regulate metabolic homeostasis throughout the body [29]. Liu et al. found that astaxanthin accelerates the utilization of lipids by increasing the expression of PGC-1 α [46]. Foxo3 is thought to be related to skeletal muscle glucose metabolism, and inhibiting its expression improves muscle glucose uptake and increases glycogen reserves [27]. In the present study, astaxanthin inhibited the expression of Foxo3 and promoted the expression of PGC-1 α , suggesting that the regulation of glycolipid metabolism by astaxanthin may be related to its ability to improve skeletal muscle atrophy and protect mitochondrial function. Further research is necessary to uncover the mechanisms by which astaxanthin affects mitochondrial function.

In conclusion, the results of the present study suggest that astaxanthin may alleviate skeletal muscle atrophy and metabolic disorders induced by long-term dexamethasone treatment by inhibiting skeletal muscle catabolism, promoting skeletal muscle production, and improving mitochondrial status (Fig. 7). This suggests that astaxanthin may be a functional dietary supplement to alleviate the side effects of glucocorticoid therapy, especially in improving skeletal muscle atrophy.

CRedit authorship contribution statement

Han Yue: Writing – original draft, Investigation, Data curation, Conceptualization. **Yuchen Huan:** Methodology, Formal analysis, Conceptualization. **Pengfei Ren:** Methodology, Formal analysis, Conceptualization. **Xinyue Yu:** Visualization, Formal analysis. **Qingjuan Tang:** Visualization, Project administration, Investigation, Funding acquisition, Conceptualization. **Changhu Xue:** Resources, Project administration. **Jie Xu:** Project administration, Conceptualization.

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.

Acknowledgement

This work was supported by grants from National Key R&D Program of China , 2023YFF1105305.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbrc.2024.151138>.

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