



Endurance exercise training under normal diet conditions activates skeletal muscle protein synthesis and inhibits protein degradation signaling except MuRF1

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Abstract

Purpose Loss of skeletal muscle mass, which depends on a balance between protein synthesis and degradation, is common in sarcopenia, cachexia, and some diseases. The purpose of this study was to investigate the alterations and interactions of protein synthesis and degradation signaling components induced by 8-week endurance exercise training with a normal diet.

Methods Two exercise ($n=8$) and control ($n=7$) groups of Wistar rats were kept under standard conditions. The exercise group performed 8-week endurance running at 65–70% VO₂max, 30–60 min, on a treadmill with 0° slope, and the rats of the control group were maintained under identical conditions except exercise training. Forty-eight hours after the last exercise session, the dissected soleus muscles were stored at –80 °C for gene and protein expression analyses.

Results Although there was a non-significant increase in mTOR gene expression, Akt1 and S6K1 increased significantly compared with the control group, which was confirmed by Western blot analysis. In addition, given that the FoxO3a did not increase, 4E-BP1 and LC3a were suppressed significantly and were confirmed by Western blot analysis. Contrary to our hypothesis, the MuRF1 gene expression was significantly increased compared with the control group.

Conclusion The results showed that the moderate-intensity endurance exercise training protocol with no calories restrictions, normal diet, not only does not lead to protein degradation but also sufficiently activates protein synthesis signaling. Further investigations including exercise training with different intensities and nutritional status are needed to reveal the cause of the unusual MuRF1 response.

Keywords Muscle protein synthesis and degradation · Signaling pathway · Endurance exercise training · MuRF1 · Wistar rat

Introduction

The important role of skeletal muscles in generating force for locomotion to meet everyday needs, whole-body metabolism, and overall health is well documented. Skeletal muscle mass is dependent on a balance between the rates of protein synthesis and protein degradation. Mechanical loading or unloading (Immobilization), growth factors or inflammatory cytokines, nutrient availability or metabolic stress, and

hormones, can trigger muscle hypertrophy or atrophy signaling pathways leading to skeletal muscle gain or loss, respectively [1–3]. IGF1–PI3K–Akt pathway as major signaling positively regulates protein synthesis and muscle growth. Indeed, Akt is activated by IGF1 through PI3K which is followed by activation of mTOR and its downstream targets to stimulate protein synthesis and consequently muscle growth [4, 5]. S6K1 and 4E-BP1 are two well-known targets of mTORC1 as a major regulator of translation initiation and cell growth [6]. The activated S6K1 by mTORC1 phosphorylates ribosomal protein S6 to induce protein synthesis. In addition, cap-binding protein eIF4E is the rate-limiting member of eIF4F complex, eIF4E–eIF4A–eIF4G. The phosphorylated form of 4E-BP1 is dissociated from the eIF4E–4E-BP1 complex which allows the eIF4F complex is formed to promote the translation initiation, muscle growth, and hypertrophy, otherwise, 4E-BP1 binds to eIF4E which

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results in the reduction of protein synthesis and increasing muscle atrophy [7–9]. Under conditions such as bed rest, aging, cachexia, and food deprivation, muscle atrophy occurs as the result of changes in the balance between anabolic and catabolic processes in favor of protein degradation leading to loss of muscle mass [10, 11]. FoxOs (Forkhead family of transcription factors) play a role in the regulation of cellular homeostasis including oxidative stress resistance, cellular metabolism, cell-cycle arrest, cell survival, and muscle atrophy through ubiquitin–proteasome and autophagy–lysosome pathways [12–14]. It has been shown that under atrophy conditions the expression of FoxOs increases in skeletal muscle to promote the transcriptional activity of two E3 ubiquitin ligases, MuRF1 and MAFbx/atrogen-1 [15, 16]. Moreover, autophagy, known as a cellular degradation pathway, is characterized by the formation of the autophagosome, which is regulated by several proteins encoded by autophagy-related genes (Atg), to degrade cytosolic components. Atg8/LC3, which is essential for autophagosome biogenesis, is activated by FoxO3 leading to muscle atrophy [17, 18]. Resistance training is a well-known method to improve protein synthesis and skeletal muscle hypertrophy. In contrast, the current pieces of evidence suggest that endurance training does not stimulate muscle hypertrophy due to the shift in signaling from Akt-mTOR to AMPK-PGC1 α [19]. AMPK (AMP-activated protein kinase) is a major cellular energy sensor that mediates other related signals to maintain the energy balance in cells. In addition to many factors, such as metabolic stress and some hormonal influences, the activation of AMPK due to changes in ADP/ATP and AMP/ATP ratios induced by for example exercise or food deprivation leads to inhibition of Akt. Conversely, glucose-dependent increased plasma insulin levels induce the phosphorylation and activation of Akt [20]. Recently, a human study reported that 7-week concurrent exercise training, endurance exercise followed by resistance exercise, resulted in larger hypertrophy, compared with resistance training alone [21]; however, those findings are not attributed solely to the impact of endurance exercise training. When we discuss alterations of protein synthesis and degradation signaling components induced by exercise training, as a therapeutic modality for rehabilitation and prevention, some of the following issues should be considered: (1) given the interaction between signaling pathways, as it occurs in the body, most of the studies have been conducted genetically; (2) mostly the effects of a bout of exercise and acute alterations have been investigated; (3) because the level of energy expenditure during exercise has a great impact on cellular metabolism and consequently activation of the signaling, the exercise intensity and duration should be considered. A few studies have examined the endurance exercise training-induced alteration of both protein synthesis and degradation signaling components simultaneously, especially under normal diet conditions. Recently, the alterations

of both signaling components induced by High-Intensity Interval Training have been examined by a study [22]. There is a lack of information regarding the alterations of both skeletal muscle protein synthesis and degradation signaling components induced by endurance exercise training with no nutritional intervention. Accordingly, the effect of 8-week moderate-intensity endurance exercise training on mRNA alterations of protein synthesis signaling components in rat soleus muscle, Akt1, mTOR, and S6K1, concomitant with the atrophy-related genes, 4E-BP1, FoxO3a, MuRF1, and MAP1LC3a (hereafter referred to as LC3a) under normal diet conditions was considered as the purpose of the present study. In addition, for further confirmation, western blot analysis was used to evaluate the S6K1, LC3a, and MuRF1 protein expression.

Methods

Study design

The study procedures were approved by the Regional Research Ethics Committee and performed in accordance with the principles outlined in the Declaration of Helsinki. Sixteen male Wistar rats, aged 8 months, were randomly assigned into two groups; exercise (215.38 ± 18.02 g) and control (219.86 ± 13.79 g). All rats were maintained, 4 rats per cage, on a 12:12 h reverse light–dark cycle and were provided standard rat chow and water ad libitum throughout the study.

Exercise training intervention

Two-week, 5 days/week, familiarization and adaptation to the main exercise training protocol was conducted, including a gradual increase in time and speed of running on a motorized treadmill with an electrical grid installed at the end of the lines to encourage rats to run. Before starting the main exercise protocol, the exercise group underwent the VO₂max (maximal oxygen uptake) test to determine exercise intensity as previously described [23, 24], and the test was repeated every other week throughout the study. Briefly, after 20 min warm-up at 50–60% of VO₂max, the velocity of the treadmill was increased by 0.03 m/s every 2 min until the rat was unable to run further. The exercise group performed an 8-week endurance training protocol, 5 days/week, including 30 min treadmill running at 0° slope in the 1st week, 40 min in the 2nd week, and 50 min from the 3rd to 8th week at 65–70% VO₂max. Each exercise session started with 5 min warm-up and ended with 5 min cool-down at 50–60% VO₂max [25, 26]. The treadmill speed was 17.50 ± 0.71 m/min in the 1st week and progressively increased to 26 ± 1.41 m/min by the 8th week. Accordingly,

the VO₂max was 57.87 ± 2.16 ml/kg/min in the baseline and increased to 80.06 ± 3.45 ml/kg/min at the end of the study, indicating the effectiveness of the exercise training protocol. The control group was maintained under identical conditions except the exercise training. Due to the death, seven rats in the control group were used for later analysis. Given the fact that the slow-twitch soleus muscle responds well to exercise training, including fiber size changes and fiber-type conversion [27], and the gene expression in soleus muscle is more influenced by moderate endurance treadmill running compared to gastrocnemius muscle [28], 48 h after the last exercise session, the dissected soleus muscle of each anesthetized rat was stored at -80 °C for qRT-PCR and Western blot analyses.

Gene expression (qRT-PCR)

The gene expression was evaluated using Quantitative Real-time PCR. Forty to fifty milligram of soleus muscle was homogenized to isolate total RNA using TRizol reagent (Qiazol, cat. no. 79306, USA) according to the instruction of the manufacturer (QIAGEN, Germany). Purity and concentration of RNA were determined spectrophotometrically by NanoDrop 2000 (Thermo Scientific, Rockford, IL, USA), and 1% agarose gel stained with Nancy-520 (Sigma-Aldrich, Sao Paulo, SP, Brazil) was used to check the integrity of RNA electrophoretically. cDNA was synthesized from 2 mg of total RNA using Revertaid™ First Strand cDNA synthesis kit (Fermentas, Glen Burnie, MD, USA). After cDNA synthesis, qRT-PCR was run to assess the messenger RNA (mRNA) levels of the target genes and endogenous reference gene (GAPDH) separately. Amplifications were performed with an ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using SYBR Green/High ROX qPCR Master Mix (Ampliqon, Denmark). Melting point dissociation curves were used to confirm the purity of the amplification products. Results were expressed using the comparative cycle threshold (Ct)

method according to the manufacturer's instruction. The primer sequences are shown in Table 1.

Western blot analysis

The soleus muscles were homogenized in RIPA buffer (Cytomatingene) with a protease inhibitor cocktail (Sigma) and were centrifuged at 15,000 rpm for 10 min at 4 °C. The related supernatant was collected, and protein content was assessed by the Lowry method. Proteins were then separated by polyacrylamide gel electrophoresis (Bio-Rad) via 4–20% gradient polyacrylamide gels containing 0.1% sodium dodecyl sulfate for ~2 h at 95 V. After electrophoresis, the proteins were transferred to PVDF membranes (roth) for 80 min at 80 V (Bio-Rad). Nonspecific sites were blocked overnight at 4 °C in PBS containing Tween and 5% nonfat milk (sigma). Membranes were then incubated for 2 h at room temperature with primary antibodies directed against the proteins of interest. The protein abundance of S6K1, 4E-BP1, LC3a, and GAPDH (served as a loading control to normalize protein loading and transfer) were determined in muscle samples. Following incubation with primary antibodies, membranes were washed extensively with PBS-Tween and then incubated with secondary antibodies 1 h at room temperature. After washing, membranes were developed using DAB (3, 3'-diaminobenzidine) substrate, and images of the membrane were captured and analyzed using the Image J software.

Statistical analysis

Results are expressed as mean \pm standard error of mean (SEM). Considering the lack of normal distribution of data, between-group differences were examined by Mann–Whitney *U* test, with the statistical significance level of $P < 0.05$ using SPSS version 24.

Table 1 Primer sequences of genes

Gene name	Gene ID	Forward sequence	Reverse sequence
Akt1	24185	5'-TGTGGGAAGATGTGTATGAGAA-3'	5'-TTGATGAGGCGGTGTGATGGTGA-3'
mTOR	56718	5'-TGATTTTGGGAGAACAGAAGATGA-3'	5'-GAGGTAACAGGATGGTGGAGTG-3'
S6K1	83840	5'-GTGTTGTGGATTGGTGGAGT-3'	5'-TTGTTGTGTGAGGTAGGGAGG-3'
4E-BP1	116636	5'-GGACACAGTGATGCTTCT-3'	5'-GGAGGTATGTGCTGGTAT-3'
FoxO3a	294515	5'-GCCTCATCTCAAAGCTGGGT-3'	5'-TGCTCTGGAGTAGGGATGCT-3'
MuRF1	140939	5'-GGTGTGACGAAGGTGGAAGAGA-3	5'-CAAGGCAGATGGGGCAGATGAG-3
LC3a	362245	5'-GGGTGGATTAGGCAGAGATGTGA-3	5'-GATGAGGGGCAAGATGGGTAGA-3
GAPDH	108351137	5'-AAGTTCAACGGCACAGTCAAGG-3'	5'-CATACTCAGCACCAGCATCACC-3'

Results

The present study examined the impact of 8-week moderate-intensity endurance exercise training on the alteration of protein synthesis and degradation pathways in Wistar rat soleus muscle under normal fed conditions.

Protein synthesis signaling

The results showed that the exercise training caused a significant increase in gene expression of Akt1 ($P < 0.001$) in comparison with the control group (Fig. 1a), indicating the positive effect of the exercise protocol. Although, mTOR gene expression considerably increased (Fig. 1b), this elevation was not significant ($P = 0.054$). According to the Akt-mTOR-S6K1 signaling, the gene expression of S6K1 (Fig. 1c) significantly increased ($P < 0.001$) as a result of the exercise training.

Moreover, the protein expression level of S6K1 was evaluated using Western blotting to confirm the activation of protein synthesis signaling (Fig. 2a). The results revealed that the expression of S6K1 protein is higher in the exercise group than the control group (Fig. 2b) and the statistical analysis determined this elevation was significant ($P = 0.002$). Altogether, the 8-week moderate-intensity endurance exercise training, with a normal diet, was sufficient to activate the protein synthesis signaling in rat soleus muscle.

Protein degradation signaling

Concerning proteolytic-related genes, although the expression of 4E-BP1 (Fig. 3a) was significantly inhibited by the exercise training ($P < 0.001$), the suppression of the FoxO3a gene expression was not significant ($P = 0.336$); however, the exercise training prevented its expression to be increased when compared with the control group (Fig. 3b).

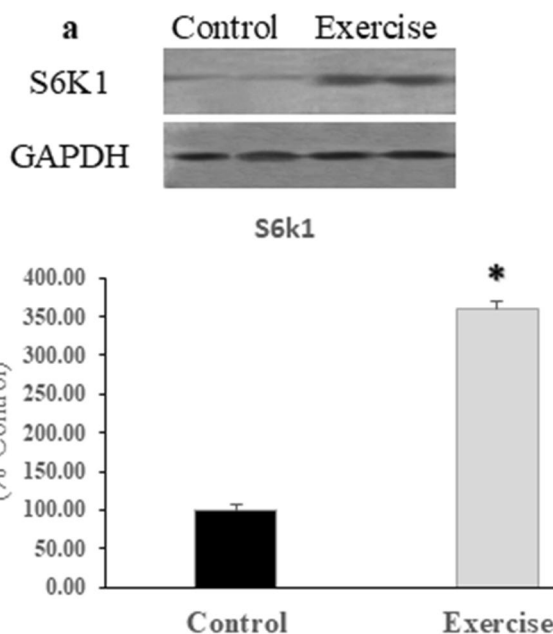


Fig. 2 Data expressed as mean \pm SEM. **a** Protein expression of S6K1 in rat soleus muscle was analyzed by Western blotting. **b** 8-week moderate-intensity endurance exercise training caused a significant increase of the S6K1 protein levels in the exercise group ($n = 8$). *Indicates significant difference ($P = 0.002$) from the control group ($n = 7$)

Unexpectedly, the gene expression of the MuRF1, the downstream target of FoxO, not only was not suppressed (Fig. 3c) but also significantly increased in the exercise group in comparison with the control group ($P < 0.001$). On the other hand, the expression of LC3a, another downstream target of FoxO3, was significantly inhibited ($P = 0.002$) by the exercise training (Fig. 3d).

Western blot analysis was conducted to confirm the results of the qRT-PCR. The results showed a reduction in protein levels of 4E-BP1 (Fig. 4a) and LC3a (Fig. 4c). The statistical analyses revealed that the protein expression of

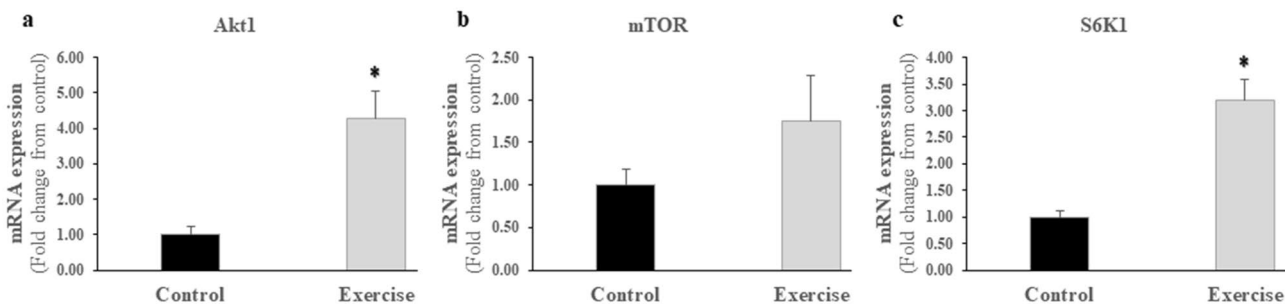


Fig. 1 Data expressed as mean \pm SEM. The alteration of mRNA expression involved in protein synthesis pathway in rat soleus muscle after the 8-week moderate-intensity endurance exercise training. Accordingly, compared with the control group ($n = 7$), the gene

expressions of Akt1 (a) and S6K1 (c) was significantly increased in the exercise group ($n = 8$), except the mTOR (b); however, the increase in its gene expression was considerable ($P = 0.054$). *Indicates significant difference from the control group ($P < 0.001$)

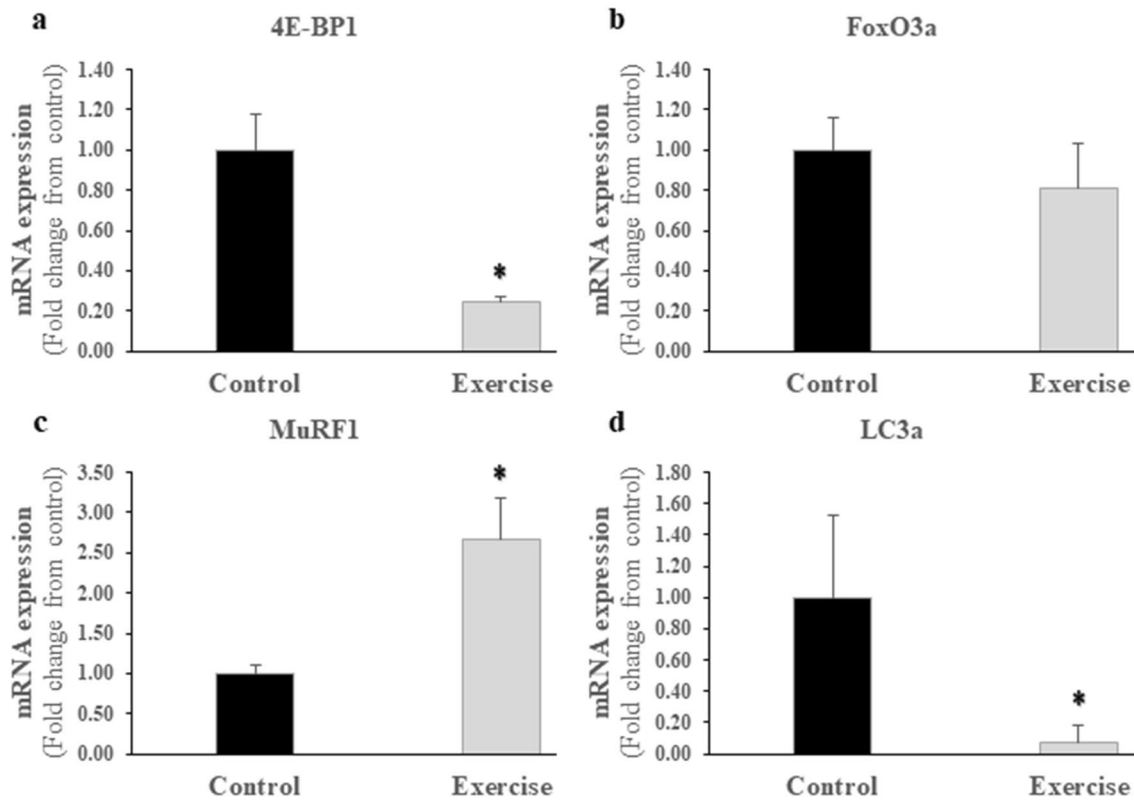


Fig. 3 Data expressed as mean ± SEM. The alterations of atrophy-related mRNA expression in rat soleus muscle after the 8-week moderate-intensity endurance exercise training. The expression of 4E-BP1 (a) was inhibited in the exercise group ($n=8$) compared with the control group ($n=7$). According to the lack of increase in FoxO3a

gene expression (b), the autophagy-related LC3a gene expression (d) was significantly repressed in the exercise group. Contrary to our hypothesis, ubiquitin ligase MuRF1 (c) increased in comparison with the control group. *Indicates significant difference from the control group ($P < 0.001$)

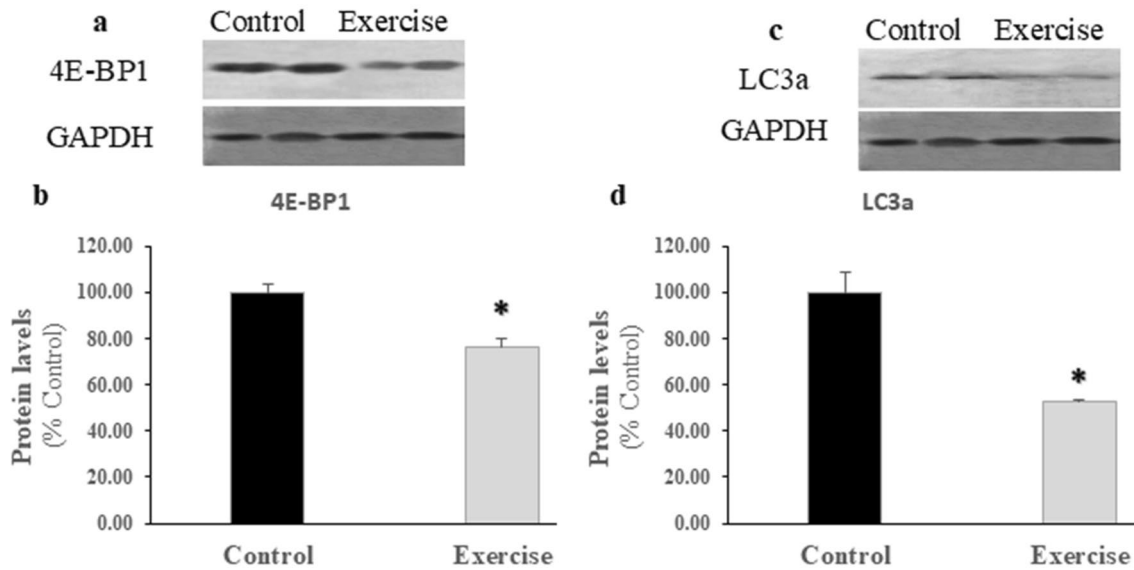


Fig. 4 Data expressed as mean ± SEM. The protein levels of 4E-BP1 (a) and LC3a (c) in rat soleus muscle were analyzed by Western blotting after 8-week moderate-intensity endurance exercise training. The

protein levels of 4E-BP1 (b) and autophagy-related LC3a (d) were significantly reduced in the exercise group ($n=8$). *Indicates significant difference ($P \leq 0.042$) from the control group ($n=7$)

4E-BP1 (Fig. 4b) and LC3a (Fig. 4d) were significantly suppressed by the exercise training compared with the control group ($P=0.044$ and $P=0.031$, respectively).

Discussion

To the best of our knowledge, this is the first study that examines the effect of moderate-intensity endurance exercise training with a normal diet on protein synthesis and degradation signaling.

Protein synthesis signaling

Akt-mTOR-S6K1 is well-known signaling in the regulation of protein synthesis, which enhances muscle hypertrophy. Along with other mechanisms, such as insulin-mediated signaling, it has been accepted that mechanical loading, for example during resistance exercise, induces IGF-1 expression which in turn stimulates PI3K/Akt pathway. Activated Akt then activates mTORC1 to phosphorylate downstream targets S6k and 4E-BP1 to promote translation initiation [29]. In the present study, the moderate-intensity endurance exercise training caused a significant increase in the gene expression involved in the Akt–mTOR–S6K1 pathway, except mTOR (Fig. 1a–c). Given the fact that the mTOR acts as a key regulator in controlling protein synthesis and skeletal muscle mass, the lack of significant increase in its gene expression is a marked question and needs to be investigated; however, the increased level of mTOR expression in the present study was considerable (Fig. 1b). In this regard, Akt indirectly activates mTORC1. In other words, Akt phosphorylates TSC1/2 to prevent the GAP (GTPase-activating protein) inhibitory activity of the TSC1/2 on small G protein Rheb (Ras homolog enriched in brain), which in turn mTORC1 is activated [30]. Nevertheless, the reason for the lack of TSC1/2 inhibition and/or stimulation of Rheb activity is unclear. In addition, mTORC1 is directly activated, independently of PI3K/Akt pathway, by amino acids signaling mediated by Rag family GTPases [31, 32]. In the present study, the amino acid supplement was not included and may be considered as a reason for the non-significantly increase of the mTOR gene expression; however, the effect of amino acid diet combined with long-term moderate-intensity endurance exercise training on mTOR expression need to be elucidated by further investigation. In addition, the activation of S6K1 by mTOR is inconsistent with our findings, because the increase in mTOR gene expression level was not significant. These contradictory events can be explained by the fact that S6K1 is also phosphorylated independently of mTOR. It has been shown that activated SGK1 (serum- and glucocorticoid-responsive kinase 1) by IGF-1 through PI3K and PDK1 (phosphatidylinositol 3,4,5P₃ dependent kinase

1) can phosphorylate S6K1 at T²²⁹ in mTOR-independent signaling [33, 34]. Moreover, the increased level of S6K1 protein compared with the control group was confirmed by the result of the Western blot analysis (Fig. 2a, b), indicating that the exercise training protocol, with a normal diet, was sufficient for activation of protein synthesis signaling which may promote skeletal muscle hypertrophy.

Protein degradation signaling

4E-BP1 is another downstream target that is phosphorylated and inhibited by mTORC1, leading to its dissociation from eIF4E to promote protein synthesis and muscle hypertrophy. Previous studies have reported that PI3K/Akt/mTOR pathway is a crucial regulator of protein synthesis and muscle growth through the downstream targets S6K1 and 4E-BP1. The reduced mTORC1 expression leads to overexpression of 4E-BP1, in which protein translation is attenuated [35, 36]. Inconsistent with this notion, the expression of the 4E-BP1 in the present study was suppressed (Fig. 3a), whereas the increase in mTOR gene expression was not significant. This finding indicates that other mechanism/mechanisms may be involved in the suppression of 4E-BP1 induced by the 8-week moderate-intensity endurance exercise training. It has been shown that ERK (extracellular signal-regulated kinase) phosphorylates 4E-BP1 at Ser65 [37]. Moreover, 4E-BP1 is upregulated by FoxO [38, 39], and accordingly, the lack of increased FoxO3a gene expression in the present study (Fig. 3b) corresponds with the suppression of 4E-BP1. On the other hand, the FoxO transcription factors play a critical role in muscle atrophy and consistent with the result of the present study are regulated by Akt [40]. Activated Akt phosphorylates and inactivates FoxO to promote its translocation from the nucleus to cytoplasm, which results in muscle protein synthesis. Otherwise, under catabolic conditions and among other regulators, the reduced level of Akt expression facilitates the nuclear localization and activation of FoxO to upregulate the atrophy-related LC3 and MuRF1 gene expression. It has been shown that activation of FoxO3 is sufficient to induce protein degradation and muscle atrophy [41, 42, 15]. There are three primary types of autophagy, including macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). According to macroautophagy, LC3, as a marker of autophagy, is localized with autophagosomes to deliver damaged organelles and superfluous proteins to the lysosome to be degraded, the role of macroautophagy in cell survival and maintenance. This process helps cells respond to stresses, including nutrient starvation. Depending on nutrient availability, AMPK and mTORC1 regulate macroautophagy. Under nutrient-rich conditions, especially glucose and amino acids, activated mTORC1 by PAK (cAMP-dependent protein kinase A) inhibits macroautophagy by phosphorylating ULK1

(Unc-51-like autophagy activating kinase 1) to prevent its interaction with AMPK. Under food deprivation conditions or low-energy levels (i.e., increased AMP/ATP ratio which occurs during exercise session) AMPK directly and indirectly inhibits mTORC1 by phosphorylating and activating TSC1/2 [43, 44]. Accordingly, LC3a gene expression was inhibited in the present study due to the lack of increase in FoxO3a gene expression (Fig. 3d) which was confirmed by Western blot analysis (Fig. 4c, d). These findings indicate that the 8-week moderate-intensity endurance exercise training protocol is sufficient and suitable for the suppression of autophagy-related LC3a. Moreover, contrary to our hypothesis, not only the gene expression of MuRF1 was not suppressed but also significantly increased compared with the control group (Fig. 3c). In this regard, recently a human study reported that 7-week concurrent exercise training, endurance exercise followed by resistance exercise, resulted in larger muscle hypertrophy compared with resistance exercise alone, where MuRF1 was not repressed [21]. MuRF1 plays a major role in skeletal muscle degradation and is upregulated by dexamethasone, a synthetic glucocorticoid [45]. Moreover, plasma concentrations of catabolic hormones are increased after an acute high-intensity endurance treadmill exercise, a progressive continuous cardiopulmonary exercise test [46]. In other words, endurance exercise with high intensity, especially long-distance, leads to the secretion of catabolic hormones, such as cortisol, increase in expression of atrophy-related genes, and consequently skeletal muscle degradation. One study has recently reported that a prolonged low-intensity endurance exercise, 4-day 45 min of one-arm cranking at 15% of maximal intensity followed by 8 h of walking, attenuated the FoxO3a with no significant changes in MuRF1. The authors concluded that the exercise protocol preserved the muscle mass; however, no significant changes in protein synthesis signaling were observed [47]. MuRF1 is upregulated during muscle degradation induced by starvation, disuse, and stress [3, 16]. In the present study, the effects of disuse have been eliminated and exercise-induced stress could not be effective, due to the moderate-intensity of the exercise. Regarding starvation, nutrition status has a crucial effect on AMPK, Akt, and their downstream targets related to protein synthesis and degradation pathways. Insulin plasma concentrations depend on glucose availability. Hyperglycemia-induced insulin secretion in fed status, and conversely, reduction of blood insulin level in food deprivation conditions, and consequently, its effects on AMPK and Akt are temporary. Recently, it has been shown that 12 h starvation induces minimal changes in the phosphorylation of Akt; however, the levels of phosphorylated form decreased during 24–36 h starvation [48]. In the present study, dissection of the soleus muscle 48 h after the last exercise session and most importantly, free access to food and water throughout the study inhibit the AMPK

mRNA expression. Thus, the effects of the AMPK on the Akt–mTOR pathway are eliminated. These findings indicate that endurance exercise training does not cause skeletal muscle loss per se, but the intensity and duration of the activity along with the nutrition status and energy balance play an important role. Moreover, it has been proved that continuous overloading, such as progressive resistance exercise, is necessary for muscle hypertrophy and strength gains [49]. Endurance exercise has been known as a low-resistance loading method, so it may be interpreted that endurance exercise training with low to moderate intensity improves hypertrophy in involved muscles as long as body weight plays a role as an overload [50], which this issue needs to be fully elucidated. This study has limitations, including lack of muscle mass measurement and evaluation of the phosphorylated form of the expressed proteins.

Conclusion

Taken together, our findings revealed that the 8-week moderate-intensity endurance exercise training with normal diet conditions sufficiently improves the expression of genes involved in protein synthesis signaling pathway and suppresses the gene expression of protein degradation signaling components in rat soleus muscle, except MuRF1. According to these findings, applying this exercise training protocol may be considered for preventing and/or treatment of the conditions, such as aging, cachexia, and chronic diseases. Moreover, regarding unusual MuRF1 response, further investigation is needed to compare the influence of different exercise training intensities and diet on both signaling components.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All experiments and procedures used in this study were in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. In addition, approval was obtained from the ethics committee of the Sport Sciences Research Institute of Iran.

Informed consent For this type of study formal consent is not required.

Human and animal rights All procedures of this study were approved by the Payame Noor University Research Ethics Committee.

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