



Original Article

Effects of treadmill exercise on skeletal muscle mTOR signaling pathway in high-fat diet-induced obese mice

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Abstract. [Purpose] The aim of this study was to investigate the effects of regular treadmill exercise on skeletal muscle Rictor-Akt and mTOR-Raptor-S6K1 signaling pathway in high-fat diet-induced obese mice. [Subjects and Methods] Four-week-old C57BL/6 mice were adopted and classified into normal diet group (ND, n = 10), normal diet and training group (NDT, n = 10), high-fat diet group (HF, n = 10), and high-fat diet and training group (HFT, n = 10). The exercise program consisted of a treadmill exercise provided at low intensity for 1–4 weeks, and moderate intensity for 5–8 weeks. [Results] The Western blot method was used to measure the expression of mTOR, Raptor, S6K1, Rictor, and Akt proteins in the soleus muscle. mTOR levels were significantly higher in the HF group than in the ND and NDT groups. Raptor/mTORC1 and S6K1 levels were significantly higher in the HF group than in all the other groups. Akt levels were significantly lower in the HF group than in the NDT group. The risk of obesity may be associated with the overactivation of the mTOR-Raptor-S6K1 signaling pathway and a decrease in Akt levels. [Conclusion] This study also indicates that performing aerobic exercise may be associated with the downregulation of the mTOR-Raptor-S6K1 pathway.

Key words: Exercise, High-fat diet, MTOR

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INTRODUCTION

Obesity increases the risk of metabolic disorders, such as high blood pressure, type 2 diabetes, and fatty liver^{1–3}. It can also reduce skeletal muscle mass and exert a negative effect on normal muscle function^{4,5}.

Mammalian target of rapamycin (mTOR), a serine/threonine protein kinase regulating the functions and activities of various cells in the skeletal muscle, consists of 2 distinct protein complexes: mTORC1 and mTORC2⁶. mTORC1, a master regulator of protein synthesis and cell growth, contains the scaffold protein Raptor, whereas glucose mTORC2, a regulator of Akt activity, contains Rictor. These 2 complexes are involved in signaling pathways^{7,8}. Akt, which transduces important signals for regulating cell metabolism and cell growth, is activated by Rictor. The activated Akt, in turn, induces protein synthesis and muscle fiber hypertrophy via the S6 kinase 1 (S6K1) pathway^{9–11}. However, the overactivation of the Raptor-S6K1 signaling pathway causes obesity by promoting fat deposition in the muscle, liver, and white adipose tissue¹². In spite of the Raptor-S6K1 activity, an over-nutrition condition such as obesity leads to muscle wasting and functional damage because of the low efficacy of protein anabolism¹³. The reduction in Rictor-Akt activity causes a reduction in muscle mass by promoting protein catabolism⁷; and further, it causes metabolic imbalance leading to increase in oxidative stress, inflammatory responses, and the risk of cardiovascular diseases and obesity¹².

Many studies have reported that both acute and regular resistance exercises promoted protein synthesis and muscle hypertrophy by activating the mTOR pathway^{14–16}. Yet, there is very limited research that investigated the change in the mTOR

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signaling pathway according to regular aerobic exercise, though aerobic exercise is the most effective type of exercise for treating obesity. Moreover, it is notable that aerobic exercise can inhibit mTORC1 by inducing adenosine monophosphate-activated protein kinase (AMPK) activity that promotes oxidative metabolism^{17, 18}. This suggests that aerobic exercise can reduce muscle hypertrophy, and therefore, induce a different pattern of change in the mTOR signaling pathway compared to resistance exercise.

Thus, although exercise is an important factor that induces muscle protein synthesis and muscle hypertrophy by enhancing the positive effect of the mTOR signaling pathway, most of the preceding researches have been limited to resistance exercise. In particular, there has been insufficient research that involves obesity models to investigate the effect of regular aerobic exercise training on the Rictor-Akt and mTOR-Raptor-S6K1 signaling pathways.

Therefore, the present research studied the effect of 8 weeks of regular treadmill exercise on the skeletal muscle Rictor-Akt and mTOR-Raptor-S6K1 signaling pathways in high-fat diet-induced obese mice.

SUBJECTS AND METHODS

Forty male C57BL/6 mice (4 weeks old) were housed in cages and fed freely with standard rat chow and water (Samtako, Inc., Korea). Four mice were housed in each cage and maintained under standardized conditions in an animal facility (Laboratory of animals, College of Medicine, Dong-A University), at a room temperature of 22 ± 2 °C, $60 \pm 5\%$ relative humidity, and a 12 h light/dark cycle. Animal experiments were approved by Dong-A University Medical School Institutional Animal Care and Use Committee (DIACUC-13-21), and all procedures were conducted in accordance with committee guidelines.

After 1 week of adaptation maintenance, mice were randomly divided into 2 groups to induce obesity by high-fat diet for 8 weeks: a normal diet (ND) group (6% fat; Samtako, Inc., Korea, $n = 20$), and a high fat diet (HF) group (45% fat; Research Diets Inc., USA, $n = 20$). Dietary and body weight were measured every week at the same time (11:00) during the entire experimental period.

After inducing obesity, mice were randomly subdivided into the ND, ND, and training (NDT), HF, HF, and training (HFT) groups (10 mice per group). Exercise protocol was adapted for 5 days in order to perform the treadmill exercise. Exercise for mice in the training groups was conducted on a treadmill for 40 min once a day, 5 times a week, for 8 weeks. Exercise intensity consisted of 5 m/min (5 min), 10 m/min (30 min), and 5 m/min (5 min) at 0% slope for weeks 1 to 4 (low intensity). During weeks 5 to 8, exercise intensity was increased to 5 m/min (5 min), 14 m/min (30 min), and 5 m/min (5 min) at the same slope (moderate intensity).

Tissues were collected at 48 h after the completion of training in order to rule out temporary exercise effects. The feed supply was discontinued 12 h prior to sampling with water continuously supplied. Animals were anesthetized with ethyl ether. Muscles were removed to collect the soleus muscle (10 g) and stored in a deep freezer (NIHOW freezer, Japan) at -80 °C until analysis.

To extract protein from the soleus muscle, tissues were crushed after adding a solution containing 150 mM NaCl, 5 mM EDTA, 50 mM Tris-HCl (pH 8.0), 1%-NP 40, 1 mM aprotinin, 0.1 mM leupeptin, and 1 mM pepstatin. The solution was centrifuged for 30 min at $16,600 \times g$. Supernatants were collected and assayed for protein content prior to storage at -80 °C. Protein samples were mixed with Laemmli sample buffer (LSB) and placed in a boiling water bath for 5 min. Proteins were resolved by 10, 12, or 15% SDS-polyacrylamide gel electrophoresis (SDS-PAGE; each loaded with same amount in μg of total protein per lane), and transferred to nitrocellulose membranes. Thereafter, protein membranes were incubated with the following primary antibodies: Akt (1:1000; cell signaling), mTOR (1:1000; cell signaling), Raptor (1:1000; cell signaling), Rictor (1:1000; cell signaling) and S6K1 (1:1000; cell signaling) for 1 hour, and washed 3 times (15 min each) in a PBS solution containing 0.1% tween 20. The washed membrane was then treated with secondary antibody (goat-anti-mouse [or rabbit] IgG) conjugated with horseradish peroxidase (HRP). Immune-reactive bands were developed on Fuji film. The relative strengths of bands were quantitated by ImageQuant™ LAS-4000 (GE Healthcare, SWE).

All data were analyzed using SPSS Software Version 21.0 for Windows (SPSS Inc., Chicago, IL, USA). Data were expressed as mean \pm standard error (SE). Body weight and mTOR signaling pathway (mTOR, Raptor, S6K1, Rictor, Akt) were analyzed by one-way ANOVA. Tukey post-hoc analysis was performed for any intergroup difference observed. All data were tested for normal distribution using the Shapiro-Wilk test. Statistical significance was defined as a p value less than 0.05.

RESULTS

The body weight of mice at 8 weeks of exercise training was significantly higher in the HF (49.1 ± 0.8 g) group than that in the ND (38.1 ± 1.5 g) and NDT (31.7 ± 1.3 g) groups ($p < 0.05$). In addition, body weight in the HFT (42.4 ± 1.1 g) group was significantly lower than that in the HF group ($p < 0.05$); body weight in the NDT group was also significantly different from that in the ND and HFT groups ($p < 0.05$).

The mTOR, Raptor/mTORC1, S6K1, Rictor/mTORC2, and Akt levels for the 4 groups after 8 weeks of exercise training are shown in Table 1. mTOR levels were significantly higher in the HF group than that in the ND and NDT groups ($p < 0.05$). Raptor/mTORC1 and S6K1 levels were significantly higher in the HF group than in all the other groups ($p < 0.05$). Akt levels were significantly lower in the HF group than that in the NDT group ($p < 0.05$). In contrast, Rictor/mTORC2 levels were not significantly different among all the groups.

Table 1. Changes in mTOR signaling pathway factors in skeletal muscle after 8 weeks of exercise training

Variables	ND (n = 10)	NDT (n = 10)	HF (n = 10)	HFT (n = 10)
mTOR (Arbitrary Unit)	0.55 ± 0.22	0.51 ± 0.24	0.80 ± 0.16 ^{+,#}	0.68 ± 0.16
Raptor (Arbitrary Unit)	0.65 ± 0.14	0.64 ± 0.20	0.93 ± 0.15 ^{+,#J}	0.62 ± 0.21
S6K1 (Arbitrary Unit)	0.55 ± 0.18	0.53 ± 0.14	0.80 ± 0.13 ^{+,#J}	0.58 ± 0.11
Rictor (Arbitrary Unit)	0.69 ± 0.38	0.73 ± 0.21	0.57 ± 0.15	0.71 ± 0.23
Akt (Arbitrary Unit)	0.64 ± 0.12	0.69 ± 0.12	0.53 ± 0.11 [#]	0.60 ± 0.11
β-actin				

Data are presented as mean ± SE. ND: normal diet group; NDT: normal diet and exercise training group; HF: high fat diet group; HFT: high fat diet and exercise training group; ⁺p < 0.05 vs. ND; [#]p < 0.05 vs. NDT; ^Jp < 0.05 vs. HFT

DISCUSSION

An obese individual may suffer deterioration of muscle function owing to the reduction of muscle mass caused by imbalance in protein synthesis and degradation¹⁹⁾. It is, therefore, important to maintain or increase skeletal muscle mass through mechanical stimulus such as exercise. Russell²⁰⁾ reported that skeletal muscle mass is regulated by the synergy between the growth and loss of muscle and is closely associated with the mTOR signaling pathway.

mTOR has been reported to be regulated by repetitive muscle contraction and, as a mediator of the adaptation induced by exercise, to promote the growth and hypertrophy of muscle fiber by being involved in protein synthesis²¹⁾. Richard-Bulteau et al.²²⁾ reported that mTOR expression decreased in damaged skeletal muscle but increased during the initial period of exercise, and that mTOR played an important role in muscle growth. In the present study, the HF group showed significantly higher mTOR levels compared to the ND and NDT groups. This result was because of high-fat diet-induced obesity, which suggests that regular aerobic exercise could not induce mTOR activity. Of interest, Li et al.²³⁾ reported the tendency of the mTOR signaling pathway to be overactivated in the skeletal muscle of obese individuals. Our results are also supported by those of Møller et al.²⁴⁾, who reported that resistance exercise could induce mTORC1 activation in skeletal muscle but endurance exercise exerted no significant effect.

It has been reported that Raptor, a component of mTOR complex, plays an important role in regulating protein synthesis, mitochondrial biosynthesis, and oxidative metabolism in muscle⁷⁾, and that repetitive muscle contractions could induce skeletal muscle growth by increasing Raptor activity²⁵⁾. On the contrary, defective Raptor in muscle could reduce oxidative metabolism in mitochondria and cause early death²⁶⁾. In the present study, the HF group showed a significantly higher Raptor levels compared to the other 3 groups (the ND, NDT, and HFT groups). This result is in accordance with those of preceding researches which showed significantly higher level of Raptor in the muscles of high fat-fed obese rats²⁷⁾, which proved that aerobic exercise could decrease Raptor activity¹⁸⁾. It can be interpreted from our results that Raptor activity was abnormally overactivated by high-fat diet-induced obesity rather than by skeletal muscle synthesis, but that aerobic exercise positively

contributed to normalizing the Raptor signaling pathway. This interpretation is supported by the suggestion that chronic Raptor activation or abnormal Raptor signaling might rather weaken the skeletal muscle response to growth signals in obesity models^{28, 29)} and also by the suggestion from Laplante and Sabatini⁷⁾ that no increase of muscle mass, in spite of high level of Raptor activity in obesity models, is because of protein catabolism.

S6K1, which is involved in the mechanism of Raptor downstream signaling, integrates various external signals for controlling cell growth and metabolism to induce protein synthesis and muscle growth³⁰⁾. Pagano et al.³¹⁾ reported an increase in S6K1 during recovery after moderate- or high-intensity endurance exercise. In the present study, the HF group showed a significantly higher S6K1 levels than the other 3 groups, similar to that of Raptor. Considering the high level of Raptor shown by the HF group, it can be interpreted from our results that obesity is the main contributor to S6K1 activity, but that aerobic exercise suppressed the obesity-induced overactivation of S6K1. This interpretation is supported by previous studies, which reported that the activation of the Raptor-S6K1 signaling pathway was associated not only with protein synthesis but also with obesity^{32, 33)}. Liao and Xu³²⁾, who investigated mTOR/S6K1 signaling in high-fat diet-induced obese rats, also demonstrated the remarkable increase of S6K1 in their muscles. In addition, Khamzina et al.²⁷⁾ suggested that high-fat diet-induced increase in mTORC1 activity is because of increased S6K1 phosphorylation, whereas Williamson et al.¹⁸⁾ suggested that the decrease in Raptor activity caused by aerobic exercise is because of the decrease in S6K1.

Rictor plays an important role in maintaining the life and size of cells by being involved in the long-term activation of Akt/protein kinase B^{34, 35)}, and its interaction with Akt is associated with the regulation of carbohydrate metabolism in cells³⁶⁾. The role of exercise-induced Rictor activation is not yet clearly known, though it has been reported that aerobic exercise-induced mTORC2 activity promoted metabolism⁸⁾. In the present study, while there was no significant difference in Rictor, Akt level was found to be significantly lower in the HF group than in the NDT group. Preceding studies have reported that Akt was closely associated with the regulation of mTOR and S6K1 levels, and that increase in Akt expression contributed to improving high-fat diet-induced obesity^{37, 38)} and increased anabolism in muscles³⁹⁾. It can be interpreted from our results that Akt expression was suppressed by obesity in the HF group, but that Akt expression increased in the NDT group as an efficacy of regular aerobic exercise.

Altogether, the results suggest that risk of obesity may be associated with the overactivation of the mTOR-Raptor-S6K1 signaling pathway and a decrease in Akt levels. We also found that performing aerobic exercise may be associated with the downregulation of the mTOR-Raptor-S6K1 pathway and an increase in Akt levels. Whereas a reduction in the concentration of proteins related to mTOR signaling according to aerobic exercise may not reflect its negative effects on metabolism and protein synthesis in the skeletal muscle, it may rather indicate the normalization of obesity-induced abnormal signaling.

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