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Research article

Time course change of IGF1/Akt/mTOR/p70s6k pathway activation in rat gastrocnemius muscle during repeated bouts of eccentric exercise

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Abstract
The purpose of this study was to examine whether insulin-like growth factor (IGF-1) and Akt/mTOR/p70S6K pathway activity is altered by chronic eccentric exercise in rat medial gastrocnemius muscle. Male Wistar rats (n = 24) were randomly assigned to 1 of the 2 groups: eccentric exercise (ECC) group or sham-operated control (CON) group. Rats in the ECC group were trained every second day for 10 days (5 sessions in total) or 20 days (10 sessions in total). After either 5 or 10 exercise sessions, muscle specimens were dissected and weighed. The mRNA expression of IGF-1 and its variant, mechano growth factor (MGF), was evaluated using real time reverse transcriptase-polymerase chain reaction (RT-PCR). Tissue concentrations of Akt (P), mTOR (P), and p70S6K (P) were measured by using western blot analysis. The medial gastrocnemius muscle mass of the ECC group did not show any significant difference after 5 exercise sessions, whereas the muscle mass increased significantly after 10 exercise sessions with a concomitant increase in the cross-sectional area of muscle fibers ($p < 0.05$). The expression of IGF-1 mRNA and the tissue concentrations of Akt (P) and p70S6K (P) after 10 exercise sessions was significantly higher than those of the age-matched controls and the rats that received 5 exercise sessions. The expression of MGF mRNA in both ECC5S and ECC10S were significantly higher than that in each period-matched control ($p < 0.01$). The tissue concentration of mTOR (P) after 10 sessions showed a significant increase when compared with period-matched controls ($p < 0.01$). These results suggest that activation of the IGF-1/Akt/mTOR/p70S6K signaling pathway becomes dominant in the later phase of chronic exercise, when significant muscular hypertrophy is observed.

Key words: Signal pathway, skeletal muscle, eccentric contractions, hypertrophy.

Introduction

Previous studies have shown that exercise increases muscle mass and strength (Armstrong et al., 1983; Wirth et al., 2003). Skeletal muscle hypertrophy is due to increase in both the cross-sectional area (hypertrophy) and the number of muscle fibers (hyperplasia). Hyperplasia and hypertrophy are closely related to both the proliferation/differentiation of satellite cells and the enhancement of protein synthesis (Appell et al., 1988; Giddings and Gonyea. 1992; Perry and Rudnick. 2000; Vierck et al., 2000).

Protein synthesis in skeletal muscle is promoted by insulin-like growth factor (IGF-1) (Barton-Davis et al., 1999). The activation of Akt/mTOR/p70S6K signal transduction pathway is partly induced by the autocrine/ paracrine response mediated by IGF-1, thereby, leads to muscular hypertrophy (Bodine et al., 2001; Glass 2003; Rommel et al., 2001). Mechanical stimuli, such as exercise, increase IGF-1 expression and activate the Akt/mTOR/p70S6K signal transduction pathway extensively (Burry et al., 2007; Sakamoto et al., 2002, Parkington et al., 2003; Spangenburg and McBride, 2006). Sakamoto et al. reported that P-Akt increased at 1 h after 1 bout of treadmill exercise; IGF-1 increased immediately after exercise in an electrical stimulation model [Sakamoto et al., 2002]. In addition, the phosphorylation of mTOR and p70S6K, downstream of Akt, occurs after muscular contractions during a single bout of exercise (Burry et al., 2007; Parkington et al., 2003; Spangenburg and McBride. 2006). Above all, a single bout of exercise induces protein synthesis in exercised muscle.

The effects of chronic functional overload and exercise on changes in IGF-1 and Akt/mTOR/p70S6K signal transduction pathway have also been examined (Adams and Haddad, 1996; Bodine et al., 2001; DeVol et al., 1990; Thomson et al., 2006). With regard to functional overload, IGF-1 concentration increased up to 7days, but then decreased thereafter (Adams and Haddad, 1996; DeVol et al., 1990). On the other hand, activation of the Akt/mTOR/p70S6K pathway gradually increased after more than 1 week, and the activation of these proteins, especially p70S6K, correlated with the amount of muscle hypertrophy (Bodine et al., 2001; Thomson et al., 2006). Taken together, elevated IGF-1 levels and activation of Akt/mTOR/p70S6K seemed to correlate within 1 week after tenonectomy. But after one week, activation of Akt/mTOR/p70S6K was independent from IGF-1 in functional overload model.

In chronic exercise, Hameed et al. showed that during chronic exercise the concentrations of IGF-1 and its variants were elevated after 5 and 12 weeks of resistance training, respectively, in aged humans (Hameed et al., 2004. Leger et al. (2006) reported that phosphorylated Akt and mTOR levels were elevated after 8 weeks of anabolic muscular exercise in human subjects. In an animal exercise model, 20 days of exercise induced significant hypertrophy independent of contraction modes such as eccentric, isometric, and concentric (Adams et al., 2004). On the contrary, isometric and concentric, but not eccentric, modes of exercise increased muscle IGF-1 and its variant, mechano growth factor (MGF) (Adams et al.,

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2004). Although these results suggest that chronic exercise induces significant hypertrophy, IGF-1 elevation, and protein synthesis activation, direct comparison of IGF-1, Akt, mTOR, and p70S6K has not been examined in the same chronic exercise model.

We hypothesized that the increase in muscle mass is associated with elevation of IGF-1 mRNA content and activation of the Akt/mTOR/p70S6K pathway. We previously reported significant increases in skeletal muscle mass and muscle strength using an eccentric exercise model in the rat gastrocnemius muscle (Ochi et al., 2007). Therefore, in this study, we used this model to induce 10- and 20-day eccentric exercise in rat medial gastrocnemius. After chronic exercise, we measured the muscle wet mass, muscle fiber cross-sectional area (CSA), IGF-1 mRNA expression, and activation of the Akt/mTOR/p70S6K signal transduction pathway.

Methods

Animals

Male Wistar rats (age, 12 weeks; body mass, 303–365 g; n = 24) were randomly assigned into 4 groups: 5 sessions of eccentric exercise group (ECC5S, n = 6); 5 sessions of sham-operated control group (CON5S, n = 6); 10 sessions of eccentric exercise group (ECC10S, n = 6); 10 sessions of sham-operated control group (CON10S, n = 6). They were housed individually in a steel case maintained at 22–24°C and 12-h light/dark cycle. The right hindlimb of each animal was shaved, and the animals were anesthetized with isoflurance during the exercise. Water and food were available *ad libitum*. No significant differences in age or mass were found between the groups (as shown in Table 1). The study was approved by the Ethical Committee for Animal Experiments at the University of Tokyo.

CSA of medial gastrocnemius muscle fibers

A part of each frozen sample was subjected to hematoxylin-eosin (H&E) staining (Sakura Finetek Japan Co. Japan). Before staining, the muscle sample was sectioned at 10 μm thickness at -20°C. Sections from each group were placed on the same glass slide to eliminate variability in staining conditions. After staining, the fibers were observed under a light microscope. CSA of muscle fibers was determined using NIH image (version 1.61; National Institutes of Health, USA). We measured 200 fibers from each animal.

Eccentric exercise for medial gastrocnemius muscle

The eccentric exercise system of the rat ankle joint was similar to the system described in our previous study (Ochi et al., 2007). Rats were anesthetized and firmly fixed on the platform of an isokinetic dynamometer in a prone position, and the triceps surae muscle of the right hindlimb was electrically stimulated. Rats in the eccentric exercise group were trained every second day for 10 days (5 sessions in total; ECC5S) or 20 days (10 sessions in total; ECC10S). The stimulus voltage was adjusted to produce submaximal isometric twitch torque through an adhesive surface electrode (7.5 mm × 7.5 mm) connected to an electric stimulator and isolator (Nihon Koden, Japan). The muscle was then stimulated for 3 s to cause submaximal tetanic contraction. One second after the onset of stimulation, forced isokinetic dorsi-flexion was given to the ankle joint to cause an eccentric contraction of the triceps surae muscle. The speed and the range of forced lengthening were 30°/s and from 0° (defined as the angle at which the sole of the foot and tibial bone are orthogonally positioned) to 45°, respectively. Each exercise session consisted of 4 sets of 5 contractions, with a 5-min interval between each set. The sham-operated groups (CON5S and CON10S) were only anesthetized, not exercised. Within 12 h after the last exercise session, the muscle specimens were dissected, weighed, and immediately frozen in liquid N_2 and stored at -80°C until analysis.

Analysis of mRNA by real-time reverse transcriptase-polymerase chain reaction (RT-PCR)

The expressions of IGF-1 and MGF mRNAs in medial gastrocnemius muscle was measured using real-time (RT-PCR) as described by Nakazato et al. (2007). The RNA isolation and RT-PCR methods were performed according to the manufacturer's instructions (RNeasy MiniKit, QIAGEN, USA, High-capacity cDNA Archive kit, Applied Biosystems, USA); RT-PCR was performed with a master mix in a final volume of 12.5 μl (SYBR Green PCR Master Mix, Applied Biosystems, USA). Amplification was carried out on an ABI Prism system (Applied Biosystems, USA) using the following program: 2 min at 50°C and 10 min at 95°C, followed by 15 s at 95°C and 1 min at 60°C for 40 cycles. External standard curves were used for quantitative analysis, and the mRNA levels of each target gene were normalized to those of 18S, which served as the control. The primers used were 5′-GCATTGTGGATGAGTGTTGC-3′ (forward primer) and 3′-GGCTCCTCCTACATTCTGTA-5′ (reverse primer) for IGF-1 (GenBank: X06043) and 5′-GCATTGT GGATGAGTGTTGC-3′ (forward primer) and 3′-CTTTTCTTGTGTGTCGATAGG-5′ (reverse primer) for MGF-1 (GenBank: X06108) based on Adams et al.(2004). Since the amplicon ranges in the sharing region of IGF variants, mixture of all IGF variants mRNAs were evaluated by this primer set. Data were analyzed using a comparative critical threshold (Ct) method where the amount of target gene was normalized to that of the endogenous control gene.

Analysis of signal transduction markers by western blotting

The medial gastrocnemius muscle samples were macerated in liquid N_2 and homogenized in 50 mM Tris-Cl (pH

Table 1. Whole body mass, muscle wet mass, and muscle wet relative to body mass. Data are means (±SD).

	CON5S (n =6)	ECC5S (n =6)	CON10S (n =6)	ECC10S (n =6)
Whole body mass (g)	342.9 (12.0)	347.2 (12.0)	337.6 (7.2)	336.5 (9.9)
Medial gastrocnemius (mg)	720.6 (39.7)	740.1 (48.9) ††	753.5 (16.7) *	820.3 (48.4)
Medial gastrocnemius per body mass (mg·g^{-1})	2.105 (.1687)	2.134 (.1627) ††	2.233 (.0496) *	2.440 (.1612)

* $p < 0.05$ CON10S vs. ECC10S, †† $p < 0.01$ ECC5S vs. ECC10S

7.4), 150 mM NaCl, 5 mM EDTA, 0.5% sodium dodecyl sulfate (SDS), 1% deoxycholate, 0.1% Triton X-100, 1% Nonidet P-40 (NP-40), 0.05% mercaptoethanol, 10 mg/ml PMSF, 0.5 mg/ml leupeptin, 0.2 mg/ml aprotinin, and 1mM Na_3VO_4. The homogenate was centrifuged at 15,000 × *g* for 25 min at 4°C. Protein concentrations were determined using a protein concentration determination kit (Protein Assay II, Bio-Rad, Richmond, CA, USA). An equivalent 25 µg of total protein extract from each sample was mixed with the sample buffer, boiled, and loaded onto SDS polyacrylamide gels. Proteins were separated by discontinuous PAGE through 12.5% acrylamide gels. The samples were electrophoretically separated at 180mA for 90min and then transferred onto PVDF membranes (ATTO, Japan). The membranes were blocked with PBS containing 5% skim milk and incubated for 1 h. All primary antibodies were incubated overnight at 4°C: a monoclonal anti-Akt (P) (Cell Signaling Technology, USA), a polyclonal anti-mTOR (P) (Cell Signaling Technology, USA), and a monoclonal anti-p70S6K (Cell Signaling Technology, USA), each diluted 1/1,000. Membranes were washed and incubated for 10 min 2 times at room temperature. The secondary HRP-conjugated goat anti-mouse or anti-rabbit IgG was used at a dilution of 1:10,000 (Santa-Cruz, Biotechnology, USA). HRP activity was detected by chemiluminescence (Supersignal West Dura; Pierce, USA) using a chemiluminescence detector (AE6961, ATTO, Japan) and quantified using CS Analyzer (ATTO). To minimize the effect of reaction conditions, samples from each gel contained each experimental group.

Statistical analyses

All values are expressed as means and standard deviations. One-way analysis of variance (ANOVA) was used to compare the CSA of muscle fibers in 10 sessions. Two-way ANOVA followed by Bonferroni test was used for the whole body mass, muscle wet mass, muscle wet mass per body mass, expression of IGF-1 mRNA, and tissue concentrations of Akt (P), mTOR (P), and p70S6K (P). The significance level was set at $p < 0.05$.

Results

The wet mass and relative wet mass of muscles and CSA of muscle fibers

Both medial gastrocnemius muscle mass and the muscle mass per body mass in ECC5S did not increase significantly, whereas those in ECC10S were significantly greater than those in CON10S, with a concomitant increase in the CSA of muscle fibers ($p < 0.05$; Table 1 and Figure 1). In addition, those in the ECC10S samples were also significantly larger than those in the ECC5S group ($p < 0.01$).

Expression of IGF-1 mRNA and MGF mRNA

Expression of IGF-1 mRNA in the gastrocnemius was determined by RT-PCR, and it was found to be significantly greater in ECC10S than that in CON10S and ECC5S ($p < 0.05$); Figure2A). On the other hand, the MGF mRNA expressions in both ECC5S and ECC10S were significantly higher than that in each period-matched control ($p < 0.01$; Figure 2B).

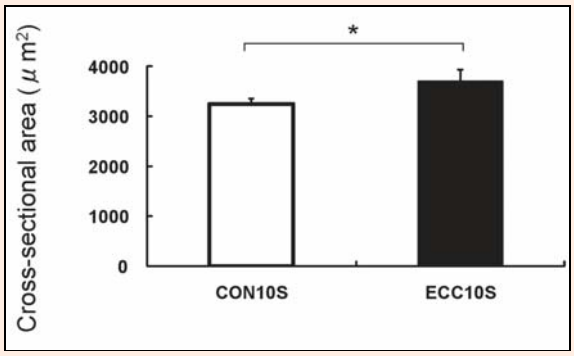

Figure 1. Cross-sectional area (CSA) of muscle fibers in the medial gastrocnemius muscle by H&E staining. Values are means ± S.D. Total fiber number = 1200 for each group. * $p < 0.05$.

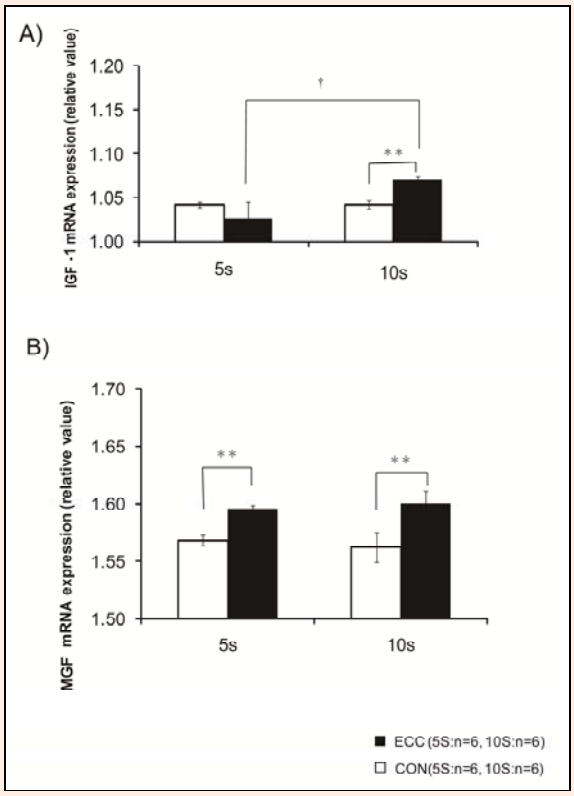

Figure 2. Muscle mRNA expression for IGF-1 A) or MGF B) determined by using real time RT-PCR. The mRNA expression of IGF-1 in ECC10S was significantly higher than that in CON10S and ECC5S. The mRNA expression of MGF in ECC5S and ECC10S were significantly higher than that in each period-matched control (CON5S or CON10S, respectively). Values are means ± S.D. * $p < 0.05$, ** $p < 0.01$, eccentric training groups vs. their sham-operated groups. † $p < 0.05$, ECC10S vs. ECC5S.

Phosphorylation of signal transduction proteins

The tissue concentrations of Akt (P), mTOR (P), and p70S6K (P) were determined by using western blot analysis. The tissue concentration of Akt (P) in ECC10S was significantly higher than those in both CON10S and

ECC5S ($p < 0.01$; Figure 3). The tissue concentration of mTOR (P) in ECC10S showed a significant increase when compared with their age-matched controls ($p < 0.01$; Figure 4). The tissue concentration of p70S6K in ECC10S was significantly higher than in CON10S and ECC5S ($p < 0.001$; Figure 5).

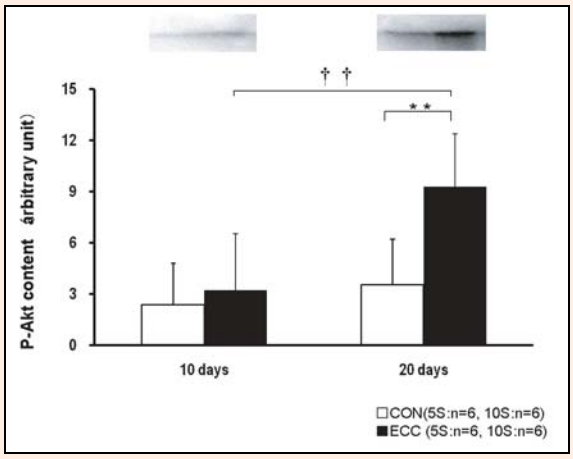

Figure 3. Muscle Akt(P) content determined by using western blotting. Values are means ± S.D. ** $p < 0.01$, ECC10S vs. CON10S. †† $p < 0.01$, ECC10S vs. ECC5S.

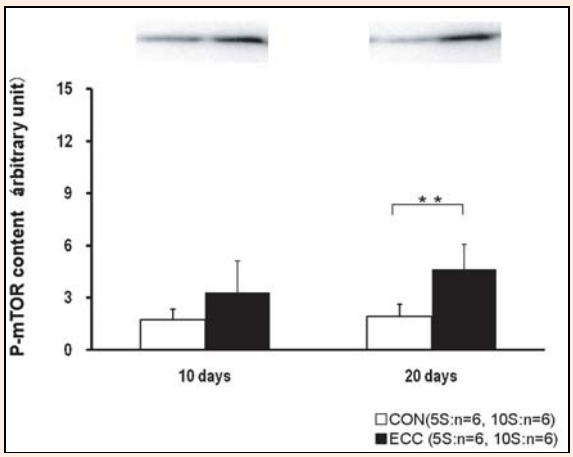

Figure 4. Muscle mTOR(P) content determined by using western blotting. Values are means ± S.D. ** $p < 0.01$, ECC10S vs. CON10S.

Discussion

By using our exercise model, we showed that 20 days, but not 10 days, of eccentric exercise causes an increase in muscle mass (Table 1). Since no changes were observed in the medial gastrocnemius muscle mass or the muscle per body mass ratio after 5 exercise sessions, we conclude that 5 exercise sessions are inadequate to cause muscular hypertrophy in this model system. On the other hand, significant hypertrophy was observed after 10 exercise sessions, and it was accompanied by a significant increase in the expression of IGF-1 mRNA and activation of the Akt/mTOR/p70S6K pathway. Taken together, these results suggest that the IGF-1/Akt/mTOR signaling pathway is associated with significant muscle hypertrophy in chronic exercise. Future work using this model should be performed with more attention on the timing of dissection. Dissections in this study were performed within 12 h after the last exercise session. The present results suggest that the observed increases in IGF-1 mRNA, mTOR (P), Akt (P), and p70S6k (P) after 10 exercise sessions may be maintained for up to 12 h.

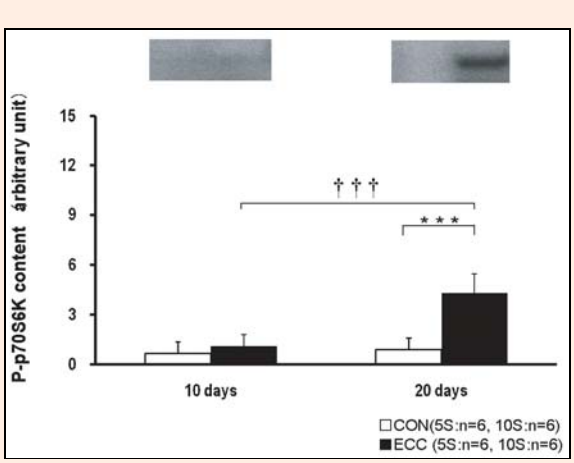

Figure 5. Muscle p70S6K(P) content determined by using western blotting. Values are means ± S.D. *** $p < 0.001$, ECC10S vs. CON10S. ††† $p < 0.001$, ECC10S vs. ECC5S.

In this study, the expression of IGF-1 mRNA was quantified. Although no changes were observed after 5 exercise sessions, a significant increase was observed after 10 exercise sessions ($p < 0.05$; Figure 2A). These results are consistent with IGF-1 responses after anabolic exercise in humans (Bamman et al., 2001; Hameed et al., 2004). Repeated bouts of eccentric contractions n a rat gastrocnemius model also induced higher expression of IGF-1 mRNA after 10 exercise sessions than in controls, but the observed difference was not statistically significant (Adams et al., 2004). In the present study, significant expression of IGF-1 mRNA was observed after 10 sessions of eccentric exercise. Since the range of forced lengthening in our model and that in Adams et al. (2004) were different (45° and 20°, respectively), the expression of MGF after both 5 and 10 sessions showed significant increases (Figure 2B). That was not in the case of Adams et al. (2004). Since MGF is defined as the loading sensitive IGF-1 isoform, the MGF increase might be the cause of greater mechanical loading in comparison to Adams et al. (2004).

The time course change of IGF-1 mRNA is another point to be considered. In a functional overload model, IGF-1 was only observed within 1 week (Adams and Haddad 1996; Ishido et al., 2008). Since the timing of increase in IGF-1 mRNA was associated with significant muscular hypertrophy, we hypothesize that the activation of IGF-1 plays a role in the onset of muscle hypertrophy during chronic strength exercise.

Previous studies have shown that a single session of muscular exercise causes a transient increase in activation of the Akt/mTOR/p70S6K signal pathway within working muscles (Burry et al., 2007; Parkington et al.,

2003; Sakamoto et al., 2002; Spangenburg and McBride, 2006). However, the relevance between IGF-1 and Akt/mTOR/p70S6K to muscle hypertrophy is controversial. Previous studies have examined the relationship between IGF-1 and Akt/mTOR/p70S6K by using functional loading and exercise models (Adams 2002; Hornberger et al., 2006; Ishido et al., 2008; O'Neil et al., 2009; Spangenburg et al., 2008). Spangenburg et al. reported that activation of the IGF-1 receptor was not necessary for muscular hypertrophy and had no influence on the activation of Akt and p70S6k (Spangenburg et al., 2008). In addition, it has been shown that phospholipase D (PLD) and phosphatidic acid (PA) activate mTOR and p70S6K, independent of IGF-1 (Hornberger et al., 2006). Conversely, it showed that IGF-1 activates the Akt/mTOR/p70S6K pathway in chronic models (Adams 2002; Ishido et al., 2008). Although we believe that IGF-1 and the Akt/mTOR/p70S6K pathway play a role in muscle hypertrophy, we also hypothesize the presence of redundant pathways such as PLD and PA pathways.

Conclusion

We showed that the muscular exercise model in this study increased the wet mass of the medial gastrocnemius muscle and CSA of muscle fibers. These increases could only be achieved after 10 exercise sessions (20 days). Increased IGF-1 mRNA expression was accompanied by phosphorylation of Akt, mTOR, and p70S6K, and such a molecular event was only seen after 10 exercise sessions. These results, along with those of previous studies, suggest that the activation of IGF/Akt/mTOR/p70S6K plays a role in the later phase of chronic exercise, when significant muscle hypertrophy is observed.

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References

Adams, G.R. (2002) Invited review: Autocrine/paracrine IGF-I and skeletal muscle adaptation. *Journal of Applied Physiology* **93,** 1159-1167.

Adams, G.R., Cheng, D.C., Haddad, F. and Baldwin, K.M. (2004) Skeletal muscle hypertrophy in response to isometric, lengthening, and shortening training bouts of equivalent duration. *Journal of Applied Physiology* **96,** 1613-1618.

Adams, G.R. and Haddad, F. (1996) The relationships among IGF-1, DNA content, and protein accumulation during skeletal muscle hypertrophy. *Journal of Applied Physiology* **81,** 2509-2516.

Appell, H.J., Forsberg, S. and Hollmann, W. (1988) Satellite cell activation in human skeletal muscle after training: Evidence for muscle fiber neoformation. *International Journal of Sports Medicine* **9,** 297-299.

Armstrong, R.B., Ogilvie, R.W. and Schwane, J.A. (1983) Eccentric exercise-induced injury to rat skeletal muscle. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology* **54,** 80-93.

Bamman, M.M., Shipp, J.R., Jiang, J., Gower, B.A., Hunter, G.R., Goodman, A., McLafferty, J.L.Jr. and Urban, R.J. (2001) Mechanical load increases muscle IGF-I and androgen receptor mRNA concentrations in humans. *American Journal of Physiology, Endocrinology and Metabolism* **280,** E383-390.

Barton-Davis, E.R., Shoturma, D.I. and Sweeney, H.L. (1999) Contribution of satellite cells to IGF-I induced hypertrophy of skeletal muscle. *Acta Physiologica Scandinavica* **167,** 301-305.

Bodine, S.C., Stitt, T.N., Gonzalez, M., Kline, W.O., Stover, G.L., Bauerlein, R., Zlotchenko, E., Scrimgeour, A., Lawrence, J.C., Glass, D.J. and Yancopoulos, G.D. (2001) Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nature Cell Biology* **3,** 1014-1019.

Burry, M., Hawkins, D. and Spangenburg, E.E. (2007) Lengthening contractions differentially affect p70s6k phosphorylation compared to isometric contractions in rat skeletal muscle. *European Journal of Applied Physiology* **100,** 409-415.

DeVol, D.L., Rotwein, P., Sadow, J.L., Novakofski, J. and Bechtel, P.J. (1990) Activation of insulin-like growth factor gene expression during work-induced skeletal muscle growth. *The American Journal of Physiology* **259,** E89-95.

Giddings, C.J. and Gonyea, W.J. (1992) Morphological observations supporting muscle fiber hyperplasia following weight-lifting exercise in cats. *The Anatomical Record* **233,** 178-195.

Glass, D.J. (2003) Molecular mechanisms modulating muscle mass. *Trends in Molecular Medicine* **9,** 344-350.

Hameed, M., Lange, K.H., Andersen, J.L., Schjerling, P., Kjaer, M., Harridge, S.D. and Goldspink, G. (2004) The effect of recombinant human growth hormone and resistance training on IGF-I mRNA expression in the muscles of elderly men. *The Journal of Physiology* **555,** 231-240.

Hornberger, T.A., Chu, W.K., Mak, Y.W., Hsiung, J.W., Huang, S.A. and Chien, S. (2006) The role of phospholipase D and phosphatidic acid in the mechanical activation of mTOR signaling in skeletal muscle. *Proceedings of the National Academy of Sciences of the United States of America* **103,** 4741-4746.

Ishido, M., Kasuga, N. and Masuhara, M. (2008) Time course changes of the expression of IGF-I, phosphorylated akt and phosphorylated mTOR in myofibers of the early stage of functionally overloaded skeletal muscle. *Advances in Exercise and Sports Physiology* **14,** 25-29.

Leger, B., Cartoni, R., Praz, M., Lamon, S., Deriaz, O., Crettenand, A., Gobelet, C., Rohmer, P., Konzelmann, M., Luthi, F. and Russell, A.P. (2006) Akt signalling through GSK-3beta, mTOR and Foxo1 is involved in human skeletal muscle hypertrophy and atrophy. *The Journal of Physiology* **576,** 923-933.

Nakazato, K., Song, H., Waga, T. (2007) Dietary apple polyphenols enhance gastrocnemius function in wistar rats. *Medicine and Science in Sports and Exercise* **39,** 934-940.

Ochi, E., Nakazato, K. and Ishii, N. (2007) Effects of eccentric exercise on joint stiffness and muscle connectin (titin) isoform in the rat hindlimb. *The Journal of Physiological Sciences* **57,** 1-6.

O'Neil, T.K., Duffy, L.R., Frey, J.W. and Hornberger, T.A. (2009) The role of phosphoinositide 3-kinase and phosphatidic acid in the regulation of mammalian target of rapamycin following eccentric contractions. *The Journal of Physiology* **587,** 3691-3701.

Parkington, J.D., Siebert, A.P., LeBrasseur, N.K. and Fielding, R.A. (2003) Differential activation of mTOR signaling by contractile activity in skeletal muscle. *American Journal of Physiology.Regulatory, Integrative and Comparative Physiology* **285,** R1086-1090.

Perry, R.L. and Rudnick, M.A. (2000) Molecular mechanisms regulating myogenic determination and differentiation. *Frontiers in Bioscience: A Journal and Virtual Library* **5,** D750-767.

Rommel, C., Bodine, S.C., Clarke, B.A., Rossman, R., Nunez, L., Stitt, T.N., Yancopoulos, G.D. and Glass, D.J. (2001) Mediation of IGF-1-induced skeletal myotube hypertrophy by PI(3)K/Akt/mTOR and PI(3)K/Akt/GSK3 pathways. *Nature Cell Biology* **3,** 1009-1013.

Sakamoto, K., Hirshman, M.F., Aschenbach, W.G. and Goodyear, L.J. (2002) Contraction regulation of akt in rat skeletal muscle. *The Journal of Biological Chemistry* **277,** 11910-11917.

Spangenburg, E.E., Le Roith, D., Ward, C.W. and Bodine, S.C. (2008) A functional insulin-like growth factor receptor is not necessary for load-induced skeletal muscle hypertrophy. *The Journal of Physiology* **586,** 283-291.

Spangenburg, E.E. and McBride, T.A. (2006) Inhibition of stretch-activated channels during eccentric muscle contraction attenuates p70S6K activation. *Journal of Applied Physiology* **100,** 129-135.

Thomson, D.M. and Gordon, S.E. (2006) Impaired overload-induced muscle growth is associated with diminished translational signalling in aged rat fast-twitch skeletal muscle. *The Journal of Physiology* **574,** 291-305.

Vierck, J., O'Reilly, B., Hossner, K., Antonio, J., Byrne, K., Bucci, L. and Dodson, M. (2000) Satellite cell regulation following myotrauma caused by resistance exercise. *Cell Biology International* **24,** 263-272.

Wirth, O., Gregory, E.W., Cutlip, R.G. and Miller, G.R. (2003) Control and quantitation of voluntary weight-lifting performance of rats. *Journal of Applied Physiology* **95,** 402-412.

Key points

- We confirmed that the rat muscular exercise model using originally-developed equipment increased the wet mass of the medial gastrocnemius muscle and cross-sectional areas of muscle fibres in 10 sessions (20 days) but not in 5 sessions (10days).
- We clarified that the increases of muscle mass and CSA of muscle fibers were accompanied by IGF-1 mRNA expression, the phosphorylated Akt, mTOR, and p70S6K.
- These results suggest that muscular hypertrophy in our model was achieved after 10 sessions of exercise and associated with the activation of IGF-1/Akt/mTOR/p70S6K signal pathway.

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