Am J Physiol Endocrinol Metab 305: E760–E765, 2013.
First published July 30, 2013; doi:10.1152/ajpendo.00302.2013.

Ursolic acid stimulates mTORC1 signaling after resistance exercise in rat skeletal muscle

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Submitted 3 June 2013; accepted in final form 25 July 2013

Ogasawara R, Sato K, Higashida K, Nakazato K, Fujita S. Ursolic acid stimulates mTORC1 signaling after resistance exercise in rat skeletal muscle. *Am J Physiol Endocrinol Metab* 305: E760–E765, 2013. First published July 30, 2013; doi:10.1152/ajpendo.00302.2013.—A recent study identified ursolic acid (UA) as a potent stimulator of muscle protein anabolism via PI3K/Akt signaling, thereby suggesting that UA can increase Akt-independent mTOR complex 1 (mTORC1) activation induced by resistance exercise via Akt signaling. The purpose of the present study was to investigate the effect of UA on resistance exercise-induced mTORC1 activation. The right gastrocnemius muscle of male Sprague-Dawley rats aged 11 wk was isometrically exercised via percutaneous electrical stimulation (stimulating ten 3-s contractions per set for 5 sets), while the left gastrocnemius muscle served as the control. UA or placebo (PLA; corn oil only) was injected intraperitoneally immediately after exercise. The rats were killed 1 or 6 h after the completion of exercise and the target tissues removed immediately. With placebo injection, the phosphorylation of $p70^{S6K}$ at Thr^{389} increased 1 h after resistance exercise but attenuated to the control levels 6 h after the exercise. On the other hand, the augmented phosphorylation of $p70^{S6K}$ was maintained even 6 h after exercise when UA was injected immediately after exercise. A similar trend of prolonged phosphorylation was observed in PRAS40 Thr^{246}, whereas UA alone or resistance exercise alone did not alter its phosphorylation level at 6 h after intervention. These results indicate that UA is able to sustain resistance exercise-induced mTORC1 activity.

exercise; nutrition; supplement; muscle hypertrophy; intracellular signaling

URSOLIC ACID (UA) is a natural pentacyclic triterpenoid carboxylic acid that is widely found in apple (a major compound of apple wax) and other fruits; it is known to exhibit a wide range of biological functions such as antioxidative, antimicrobial, anti-inflammatory, and anticancer activities (6, 10). A recent study based on microarray analysis identified UA as an inhibitor of skeletal muscle atrophy and also, interestingly, revealed that skeletal muscle hypertrophy occurs by daily UA consumption (13). Thus, UA appears to be a potent stimulator of muscle protein anabolism.

On the other hand, muscle contraction such as that occurring in resistance exercise is widely known to accelerate muscle anabolism; repeated application of such stimuli leads to the gradual accumulation of muscle proteins (17, 28). The molecular mechanisms underlying this accumulation are being increasingly clarified by recent studies. The mammalian target of rapamycin complex 1 (mTORC1) is recognized as a key regulator of translation initiation and has been shown to be important in muscle protein synthesis and muscle hypertrophy (4, 8, 9). Recent studies have indicated that resistance exercise activates mTORC1 in contracted skeletal muscle through a mechanism independent of PI3K/Akt signaling (15, 26), although the latter pathway is a well-known upstream signaling pathway involved in the activation of mTORC1 (7, 20, 25). Although little is known about the mechanisms of UA-induced muscle anabolism, a previous study reported that the mRNA levels of IGF-I and Akt phosphorylation are upregulated in the skeletal muscle of mice after daily UA consumption over a long period (13); this implies that UA may acutely activate mTORC1 through IGF-I/Akt signaling. Because of these findings, we hypothesized that UA may additively increase Akt-independent mTORC1 activation induced by resistance exercise through Akt activation.

Therefore, the purpose of the present study was to investigate the effect of UA on mTORC1 activation induced by resistance exercise. To this end, we measured the degree of phosphorylation of the signaling proteins of the Akt/mTORC1 signaling pathway after resistance exercise with and without UA injection. We used a previously developed rat model of resistance exercise (16) leading to muscle hypertrophy as a result of chronic training.

MATERIALS AND METHODS

Animals and Experimental Protocol

Twenty male Sprague-Dawley rats, aged 10 wk (330–360 g), were obtained from CLEA Japan (Tokyo, Japan). All animals were housed for 1 wk in an environment maintained at 22–24°C with a 12:12-h light-dark cycle and were allowed food (CE2; CLEA Japan) and water ad libitum. Subsequently, the rat muscles were exercised after a 12-h overnight fast, and UA (250 mg/kg in corn oil) or placebo (PLA; corn oil only) was injected intraperitoneally immediately after exercise. UA was dissolved in corn oil at a concentration of 25 mg/ml. The rats were killed 1 or 6 h after the completion of exercise, and the target tissues were removed immediately (Fig. 1). After their masses were measured, the tissue samples were rapidly frozen in liquid N_2 and stored at −80°C until use. The study protocol was approved by the Ethics Committee for Animal Experiments at Ritsumeikan University.

Isometric Exercise

Under isoflurane anesthesia, hair was shaved off the right lower leg of each rat, and the shaved leg was cleaned with alcohol wipes. The rats were then positioned with their right foot on the footplate (the ankle joint angle was positioned at 90°) in the prone posture. The triceps surae muscle was stimulated percutaneously with electrodes (Vitrode V, Ag/AgCl; Nihon Kohden, Tokyo, Japan), which were cut to measure 10 × 5 mm, and connected to an electric stimulator and an

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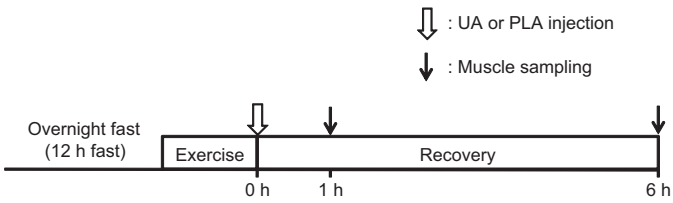

Fig. 1. Schematic diagram of the experimental protocol. UA, ursolic acid; PLA, placebo.

isolator (SS-104J; Nihon Kohden, Tokyo, Japan) (14). The right gastrocnemius muscle was isometrically exercised (stimulating ten 3-s contractions, with a 7-s interval between the contractions, per set for 5 sets, with 3-min rest intervals), while the left gastrocnemius muscle served as the internal control. The voltage (~30 V) and stimulation frequency (100 Hz) were adjusted to produce maximal isometric tension.

Western Blotting Analysis

Western blotting analysis was performed as reported previously (16). Briefly, muscle samples were homogenized with a polytron homogenizer in a homogenization buffer containing 100 mM Tris·HCl of pH 7.8, 1% NP-40, 0.1% SDS, 0.1% sodium deoxycholate, 1 mM EDTA, 150 mM NaCl, and protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific). Homogenates were centrifuged at 10,000 *g* for 10 min at 4°C. The supernatant was removed, and the protein concentration for each sample was determined using a protein concentration determination kit (Protein Assay Rapid kit, WAKO Japan). The samples were diluted in 3× sample buffer [1.0% vol/vol β-mercaptoethanol (β-ME), 4.0% wt/vol SDS, 0.16 M Tris·HCl, pH 6.8, 43% vol/vol glycerol, and 0.2% wt/vol bromophenol blue] and boiled at 95°C for 5 min. Using 5–20% SDS-polyacrylamide gradient gels, 30 μg of protein was separated by electrophoresis and subsequently transferred to polyvinylidene difluoride membranes. After the transfer, the membranes were washed in Tris-buffered saline containing 0.1% Tween 20 (TBST), and membranes were then blocked with 5% powdered milk in TBST for 1 h at room temperature. After blocking, the membranes were washed and incubated overnight at 4°C with primary antibodies, including phospho-Akt (Thr^{308}, cat. no. 9275), phospho-Akt (Ser^{473}, cat. no. 9271), total Akt (cat. no. 9272), phospho-PRAS40 (proline-rich Akt substrate of 40 kDa; Thr^{246}, cat. no. 2640), total PRAS40 (cat. no. 2619), phospho-$p70^{S6K}$ (Thr^{389}, cat. no. 9205), total $p70^{S6K}$ (cat. no. 9202), phospho-S6 ribosomal protein (rpS6; $Ser^{240/244}$, cat. no. 2215), and total S6 ribosomal protein (cat. no. 2217) (Cell Signaling Technology, Danvers, MA). Membranes were then washed again in TBST and incubated for 1 h at room temperature with the appropriate secondary antibodies. Chemiluminescent reagents (ECL Plus, GE Healthcare) were used to facilitate the detection of protein bands. Images were scanned using a chemiluminescence detector (ImageQuant LAS 4000, GE Healthcare). After the scan, the membranes were stained with Coomassie blue to verify equal loading in all lanes. Band intensities were quantified using ImageJ 1.46 (NIH).

Measurement of Serum and Muscle IGF-I Concentrations

IGF-I levels in the serum and skeletal muscle extracts were determined using the mouse/rat IGF-I quantikine ELISA kit (MG100, R&D Systems) according to the manufacturer's instructions.

Statistical Analysis

A two-way analysis of variance (ANOVA) was used to evaluate changes in phosphorylation of signaling proteins with the condition (PLA, PLA + exercise, UA, and UA + exercise) and time (1 h and 6 h) as factors. Post hoc analyses were performed using *t*-tests with the Benjamini and Hochberg false discovery rate correction for multiple comparisons when a significant main effect and/or interaction was observed. All values are expressed as means ± SE. Significance was accepted at $P < 0.05$.

RESULTS

Serum and Muscle IGF-I Concentrations

UA alone did not change the muscle IGF-I concentrations, but resistance exercise increased muscle IGF-I concentrations 1 h and 6 h after resistance exercise (Table 1). Furthermore, UA tended ($P = 0.051$) to further increase the exercise-induced muscle IGF-I concentrations at 6 h after resistance exercise. Serum IGF-I concentrations were not changed with resistance exercise alone, UA alone, or with UA in combination with the resistance exercise (Table 1).

Intracellular Signaling

Representative blots of signaling proteins are shown in Fig. 2.

Akt. UA injection increased the Akt phosphorylation at Thr^{308} both 1 h and 6 h after the injection, whereas resistance exercise alone did not significantly change the phosphorylation of Akt at Thr^{308} (Fig. 3). Akt phosphorylation at Ser^{473} was not affected significantly by UA alone, resistance exercise alone, or the combination of both.

PRAS40. UA alone did not increase PRAS40 phosphorylation at any time point (Fig. 4). In contrast, resistance exercise increased PRAS40 phosphorylation 1 h after resistance exercise. At 6 h after resistance exercise, the increased PRAS40 phosphorylation was maintained when UA was administered immediately after resistance exercise, while the degree of PRAS40 phosphorylation decreased to that in the controls when exercise alone was performed.

$p70^{S6K}$. In the case of resistance exercise alone, the phosphorylation of $p70^{S6K}$ increased at 1 h after resistance exercise (Fig. 5*A*); at 6 h after resistance exercise, the level remained high but lower than that at 1 h after resistance exercise. On the other hand, the increased $p70^{S6K}$ phosphorylation was maintained at 6 h after resistance exercise when UA was adminis-

Table 1. *Serum and muscle IGF-I concentrations*

	1 h				6 h			
UA	−	+	−	+	−	+	−	+
EX	−	−	+	+	−	−	+	+
Serum, ng/ml	1,294 ± 4	NA	1,261 ± 28	1,189 ± 32	NA	NA	1,195 ± 46	1,166 ± 81
Muscle, ng/g	170 ± 9	142 ± 17	448 ± 79*	448 ± 75*	138 ± 13	168 ± 18	378 ± 56*	546 ± 47*#

Values are means ± SE; $n = 5$ per group. UA, ursolic acid; EX, exercise; NA, not analyzed. *$P < 0.05$ vs. control (without UA or EX); #$P = 0.051$ vs. corresponding EX only group.

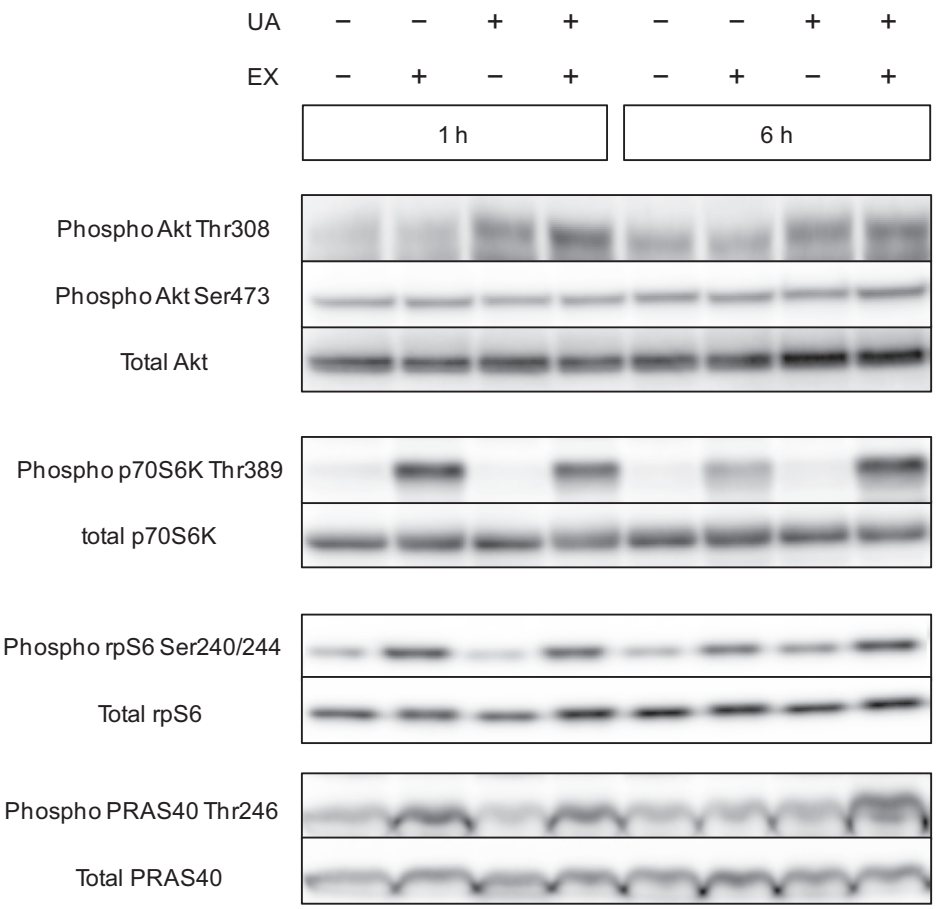

Fig. 2. Representative blots for the proteins. EX, exercise.

tered immediately after resistance exercise. UA alone did not increase the phosphorylation of $p70^{S6K}$ at any time point.

rpS6. Increase in the phosphorylation of rpS6 was observed 1 and 6 h after resistance exercise, but not with UA injection (Fig. 5*B*). There was no additional or synergistic effect of resistance exercise and UA on rpS6 phosphorylation.

DISCUSSION

In this study, we first investigated the acute effects of UA alone and in combination with resistance exercise on the phosphorylation of mTORC1 signaling proteins in in vivo skeletal muscle and observed that UA sustained mTORC1 activity after resistance exercise.

A previous in vivo study using mice reported that chronic daily UA consumption increased Akt phosphorylation at Ser^{473} and mRNA expression of IGF-I (13). Therefore, it was concluded that UA-induced muscle anabolism is mediated by the activation of Akt through the IGF-I/PI3K signaling pathway. However, it is currently unclear whether UA can significantly increase IGF-I protein level. Furthermore, the direct link be-

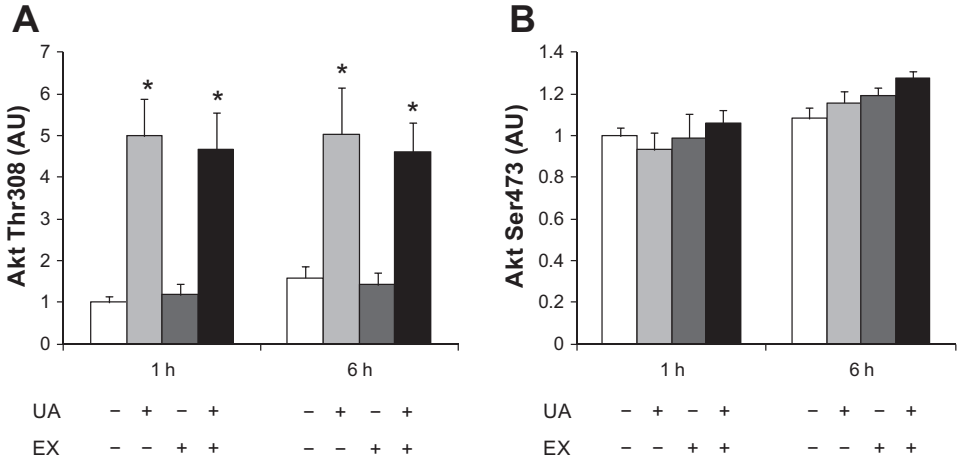

Fig. 3. Phosphorylated Akt Thr^{308} (*A*) and Ser^{473} (*B*) relative to total protein during the protocol ($n = 5$ per group). Values are means ± SE. *$P < 0.05$ vs. control muscle (no exercise and only treated with corn oil).

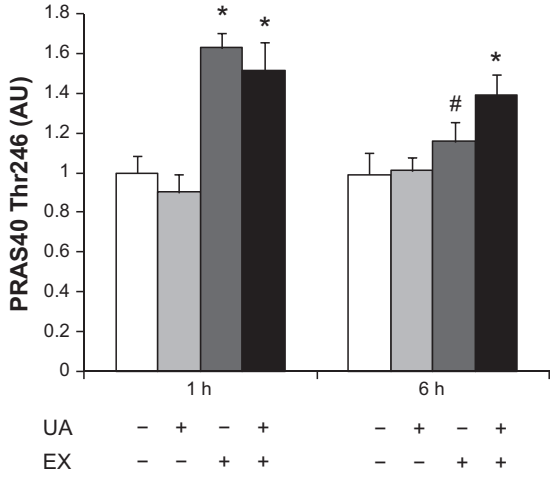

Fig. 4. Phosphorylated PRAS40 Thr^{246} relative to total protein during the protocol ($n = 5$ per group). Values are means ± SE. *$P < 0.05$ vs. control muscle; #$P < 0.05$ vs. 1 h of corresponding treatment.

tween the IGF-I/PI3K signaling pathway and phosphorylation of Akt at Ser^{473} is poorly understood. On the other hand, reports have indicated that the Thr^{308} site of Akt is phosphorylated by the 3-phosphoinositide-dependent protein kinase-1 (PDK-1), whereas the Ser^{473} site of Akt is phosphorylated by mTORC2 (3). Although little is known about the upstream events leading to mTORC2 activation, it is known that PDK-1 is activated through the IGF-I/PI3K signaling pathway (3). Therefore, in the present study, we investigated IGF-I protein expression and phosphorylation of both the Thr^{308} and Ser^{473} sites of Akt to investigate whether UA stimulates the IGF-I/PI3K/Akt signaling pathway. Subsequently, we found that Akt phosphorylation at Thr^{308} increased acutely, whereas IGF-I protein expression and Akt phosphorylation at Ser^{473} did not change at any time point after UA injection alone. Although it is currently unclear why UA did not acutely phosphorylate the Ser^{473} site of Akt in vivo, a finding that is different from previously reported in vitro findings, the results of this and previous studies together suggest that UA acutely stimulates Akt through the PI3K signaling pathway. On the other hand, Akt phosphorylation may not be regulated by change in IGF-I protein expression because UA alone did not alter either serum or muscle IGF-I protein levels.

Although we observed an acute increase in Akt phosphorylation at Thr^{308} by UA alone, these did not contribute to an increase in the phosphorylation of downstream signaling proteins. That is, UA alone did not increase the phosphorylation of $p70^{S6K}$ or rpS6. In contrast, previous in vitro studies observed that acute UA injection increased the phosphorylation of those proteins with the concomitant increase in Akt phosphorylation at Ser^{473} (5, 13). It is known that Thr^{308} phosphorylation is necessary for Akt activation and that Ser^{473} phosphorylation is required for the complete activation of Akt (19). Therefore, increase in the Akt phosphorylation at Ser^{473} may be required to activate mTORC1 and phosphorylate downstream proteins in vivo. The discrepancies between the findings of this and previous studies may be attributable to the differences in the experimental design (i.e., in vivo vs. in vitro studies). Nonetheless, because previous in vivo studies observed that chronic UA injection led to an increase in the phosphorylation of Akt at Ser^{473} (12, 13), we can infer that accumulative UA stimulation may be required to phosphorylate the Ser^{473} site of Akt in vivo. Further studies are required to investigate the effect of chronic UA injection on mTORC1 in vivo.

High-intensity muscle contraction is known to increase the phosphorylation of the mTORC1 downstream targets $p70^{S6K}$ and rpS6 for more than 18 h after muscle contraction (15, 17). In contrast, our preliminary test using the present muscle activation protocol and the findings of previous studies together indicate that, although high-intensity muscle contraction leads to Akt phosphorylation at Ser^{473} transiently (its peak activation state is often observed ~30 min after muscle contraction and return to the baseline level within 1 h of muscle contraction), the phosphorylation of Akt at Thr^{308} is not altered with acute muscle contraction (15, 17, 18). This suggests that muscle contraction-induced mTORC1 activation is independent of the activation of the PI3K/Akt signaling pathway. In accord with the findings of previous studies, our findings indicated that resistance exercise alone increased the phosphorylation of p70S6K at Thr^{389} and rpS6 at $Ser^{240/244}$, but Akt phosphorylation at either residue remained unchanged at 1 h and 6 h after resistance exercise.

PRAS40 is a known component of mTORC1 and acts as a negative regulator of its activity (22, 27, 29). The phosphorylation of PRAS40 weakens its interaction with mTORC1 and results in mTORC1 activation (23, 27). In the present study,

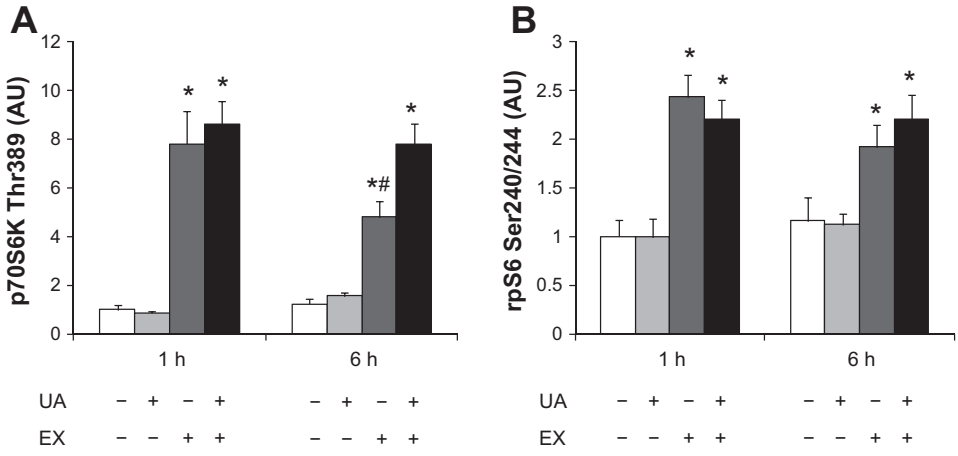

Fig. 5. Phosphorylated p70S6K Thr^{389} (*A*) and rpS6 $Ser^{240/244}$ (*B*) relative to total protein during the protocol ($n = 5$ per group). Values are means ± SE. *$P < 0.05$ vs. control muscle; #$P < 0.05$ vs. 1 h of corresponding treatment.

Akt phosphorylation did not change with resistance exercise but PRAS40 Thr^{246}, a downstream target of Akt, was phosphorylated 1 h after resistance exercise. Although insulin has consistently shown the ability to stimulate Akt and PRAS40 concomitantly (22), the discrepancy between Akt and PRAS40 phosphorylation after resistance exercise has also been reported previously (26). Taken together, PI3K/Akt-independent PRAS40 activation (dissociation from mTORC1) may have contributed to mTORC1 activation induced by resistance exercise.

Interestingly, in the present study, the phosphorylation of p70S6K at Thr^{389} at 6 h after resistance exercise was maintained at the same level as that 1 h after resistance exercise only when UA was injected immediately after the exercise, whereas UA alone did not alter its phosphorylation levels. A similar trend was observed for muscle IGF-I. Although it remains unclear whether IGF-I per se or IGF-I/PI3k/Akt signaling pathway is involved in the muscle hypertrophy induced by mechanical load, the overexpression of IGF-I has been shown to stimulate mTORC1 and induce muscle hypertrophy without increasing Akt phosphorylation (2, 21). Furthermore, a previous in vitro study reported that IGF-I is necessary to stimulate p70S6K phosphorylation by UA, thereby indicating that high muscle IGF-I concentration contributed to the sustained p70S6K phosphorylation. However, the upstream mechanisms are relatively unclear, and further studies are needed to elucidate this. Nonetheless, because the phosphorylation of p70S6K reflects the mTORC1 activity and its associated muscle protein synthesis and subsequent muscle hypertrophy (1, 8, 11, 24), the combination of UA and resistance exercise may afford a greater anabolic outcome than that achieved with resistance exercise alone as a result of training. Future study should be conducted to determine whether resistance exercise and UA have additive or synergistic effects on muscle mass and strength. In conclusion, we suggest that UA has the potential to serve as an anabolic agent for facilitating acute muscle anabolism induced by resistance exercise.

GRANTS

This work was supported by grants-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan #12911148 (S.F.).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: R.O., K.S., K.N., and S.F. conception and design of research; R.O., K.S., and K.H. performed experiments; R.O. analyzed data; R.O., K.H., and S.F. interpreted results of experiments; R.O. prepared figures; R.O. and S.F. drafted manuscript; R.O., K.S., K.H., K.N., and S.F. approved final version of manuscript; K.S., K.H., K.N., and S.F. edited and revised manuscript.

REFERENCES

1. **Baar K, Esser K.** Phosphorylation of $p70^{S6k}$ correlates with increased skeletal muscle mass following resistance exercise. *Am J Physiol Cell Physiol* 276: C120–C127, 1999.
2. **Barton ER, Morris L, Musaro A, Rosenthal N, Sweeney HL.** Muscle-specific expression of insulin-like growth factor I counters muscle decline in mdx mice. *J Cell Biol* 157: 137–148, 2002.
3. **Bhaskar PT, Hay N.** The two TORCs and Akt. *Dev Cell* 12: 487–502, 2007.
4. **Drummond MJ, Dreyer HC, Fry CS, Glynn EL, Rasmussen BB.** Nutritional and contractile regulation of human skeletal muscle protein synthesis and mTORC1 signaling. *J Appl Physiol* 106: 1374–1384, 2009.
5. **Figueiredo VC, Nader GA.** Ursolic acid directly promotes protein accretion in myotubes but does not affect myoblast proliferation. *Cell Biochem Funct* 30: 432–437, 2012.
6. **Frighetto RTS, Welendorf RM, Nigro EN, Frighetto N, Siani AC.** Isolation of ursolic acid from apple peels by high speed counter-current chromatography. *Food Chem* 106: 767–771, 2008.
7. **Fujita S, Dreyer HC, Drummond MJ, Glynn EL, Cadenas JG, Yoshizawa F, Volpi E, Rasmussen BB.** Nutrient signalling in the regulation of human muscle protein synthesis. *J Physiol* 582: 813–823, 2007.
8. **Goodman CA, Frey JW, Mabrey DM, Jacobs BL, Lincoln HC, You JS, Hornberger TA.** The role of skeletal muscle mTOR in the regulation of mechanical load-induced growth. *J Physiol* 589: 5485–5501, 2011.
9. **Iadevaia V, Huo Y, Zhang Z, Foster LJ, Proud CG.** Roles of the mammalian target of rapamycin, mTOR, in controlling ribosome biogenesis and protein synthesis. *Biochem Soc Trans* 40: 168–172, 2012.
10. **Ikeda Y, Murakami A, Ohigashi H.** Ursolic acid: an anti- and pro-inflammatory triterpenoid. *Mol Nutr Food Res* 52: 26–42, 2008.
11. **Kubica N, Bolster DR, Farrell PA, Kimball SR, Jefferson LS.** Resistance exercise increases muscle protein synthesis and translation of eukaryotic initiation factor 2Bepsilon mRNA in a mammalian target of rapamycin-dependent manner. *J Biol Chem* 280: 7570–7580, 2005.
12. **Kunkel SD, Elmore CJ, Bongers KS, Ebert SM, Fox DK, Dyle MC, Bullard SA, Adams CM.** Ursolic acid increases skeletal muscle and brown fat and decreases diet-induced obesity, glucose intolerance and fatty liver disease. *PLoS One* 7: e39332, 2012.
13. **Kunkel SD, Suneja M, Ebert SM, Bongers KS, Fox DK, Malmberg SE, Alipour F, Shields RK, Adams CM.** mRNA expression signatures of human skeletal muscle atrophy identify a natural compound that increases muscle mass. *Cell Metab* 13: 627–638, 2011.
14. **Nakazato K, Ochi E, Waga T.** Dietary apple polyphenols have preventive effects against lengthening contraction-induced muscle injuries. *Mol Nutr Food Res* 54: 364–372, 2010.
15. **O'Neil TK, Duffy LR, Frey JW, Hornberger TA.** The role of phosphoinositide 3-kinase and phosphatidic acid in the regulation of mammalian target of rapamycin following eccentric contractions. *J Physiol* 587: 3691–3701, 2009.
16. **Ogasawara R, Kobayashi K, Tsutaki A, Lee K, Abe T, Fujita S, Nakazato K, Ishii N.** mTOR signaling response to resistance exercise is altered by chronic resistance training and detraining in skeletal muscle. *J Appl Physiol* 114: 934–940, 2013.
17. **Philp A, Hamilton DL, Baar K.** Signals mediating skeletal muscle remodeling by resistance exercise: PI3-kinase independent activation of mTORC1. *J Appl Physiol* 110: 561–568, 2011.
18. **Sakamoto K, Hirshman MF, Aschenbach WG, Goodyear LJ.** Contraction regulation of Akt in rat skeletal muscle. *J Biol Chem* 277: 11910–11917, 2002.
19. **Sarbassov DD, Guertin DA, Ali SM, Sabatini DM.** Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 307: 1098–1101, 2005.
20. **Sengupta S, Peterson TR, Sabatini DM.** Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. *Mol Cell* 40: 310–322, 2010.
21. **Song YH, Godard M, Li Y, Richmond SR, Rosenthal N, Delafontaine P.** Insulin-like growth factor I-mediated skeletal muscle hypertrophy is characterized by increased mTOR-p70S6K signaling without increased Akt phosphorylation. *J Investig Med* 53: 135–142, 2005.
22. **Vander Haar E, Lee SI, Bandhakavi S, Griffin TJ, Kim DH.** Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. *Nat Cell Biol* 9: 316–323, 2007.
23. **Wang H, Zhang Q, Wen Q, Zheng Y, Lazarovici P, Jiang H, Lin J, Zheng W.** Proline-rich Akt substrate of 40 kDa (PRAS40): a novel downstream target of PI3k/Akt signaling pathway. *Cell Signal* 24: 17–24, 2012.
24. **Weigl LG.** Lost in translation: regulation of skeletal muscle protein synthesis. *Curr Opin Pharmacol* 12: 377–382, 2012.
25. **Winter JN, Jefferson LS, Kimball SR.** ERK and Akt signaling pathways function through parallel mechanisms to promote mTORC1 signaling. *Am J Physiol Cell Physiol* 300: C1172–C1180, 2011.
26. **Witkowski S, Lovering RM, Spangenburg EE.** High-frequency electrically stimulated skeletal muscle contractions increase p70s6k phosphory-

lation independent of known IGF-I sensitive signaling pathways. *FEBS Lett* 584: 2891–2895, 2010.
27. **Wiza C, Nascimento EB, Ouwens DM.** Role of PRAS40 in Akt and mTOR signaling in health and disease. *Am J Physiol Endocrinol Metab* 302: E1453–E1460, 2012.
28. **Yarasheski KE.** Exercise, aging, and muscle protein metabolism. *J Gerontol A Biol Sci Med Sci* 58: M918–M922, 2003.
29. **Zoncu R, Efeyan A, Sabatini DM.** mTOR: from growth signal integration to cancer, diabetes and ageing. *Nat Rev Mol Cell Biol* 12: 21–35, 2011.