Angiotensin II Upregulates Protein Phosphatase 2Cα and Inhibits AMP-Activated Protein Kinase Signaling and Energy Balance Leading to Skeletal Muscle Wasting

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Abstract—Congestive heart failure and chronic kidney disease are characterized by chronically elevated angiotensin II (Ang II) and muscle wasting. Ang II causes skeletal muscle wasting by reducing appetite and by enhancing catabolism. The serine/threonine kinase AMP-activated protein kinase (AMPK) functions mainly as a sensor of cellular energy status. It is energy sparing and favors ATP generation. We hypothesized that Ang II induces muscle wasting in part by inhibiting AMPK signaling and altering cellular energy balance. Our results show that Ang II infusion in mice reduced gastrocnemius muscle weight by 26% and depleted ATP by 74%. In addition, Ang II upregulated protein phosphatase 2Cα by 2.6-fold and reduced AMPK phosphorylation and signaling in muscle. Importantly, the pharmacological AMPK activator 5-aminoimidazole-4-carboxamide ribonucleoside restored AMPK activity to levels of pair-fed controls and reversed Ang II–mediated ATP depletion and muscle wasting. Moreover, 5-aminoimidazole-4-carboxamide ribonucleoside activated Akt and inhibited Ang II–induced increases in E3 ubiquitin ligase expression. These novel results demonstrate critical roles for energy depletion and AMPK inhibition in Ang II–induced skeletal muscle wasting and suggest a therapeutic potential for AMPK activators in diseases characterized by muscle wasting. (Hypertension. 2011;58:643-649.) ● Online Data Supplement

Key Words: ATP ▪ AMPK ▪ AICAR ▪ mitochondria ▪ atrogin 1 ▪ muscle RING-finger protein 1 ▪ Akt

Much is known about the actions of angiotensin II (Ang II) on the vasculature, heart, and kidney, but the effects of Ang II on skeletal muscle are much less understood. We originally reported that Ang II infusion promoted loss of body weight by reducing food intake and decreasing skeletal muscle weight, effects that were pressor independent and accompanied by a marked reduction in circulating insulin-like growth factor 1.1-3 These findings were relevant to conditions such as congestive heart failure and chronic kidney disease, in which Ang II levels are elevated, and in which loss of lean body mass correlates strongly with poor prognosis.4,5 Subsequent studies demonstrated that Ang II causes skeletal muscle wasting primarily by increasing rates of protein degradation via activation of forkhead box protein (FoxO) transcription factors, caspase 3, and the ubiquitin proteasome pathway,6,7 while simultaneously decreasing protein synthesis via inhibition of the insulin-like growth factor 1/Akt/mammalian target of rapamycin signaling axis.1-3,7-12 Ang II wasting is mediated via the Ang II type 1 receptor. However, because mature skeletal muscle expresses little or no Ang II receptors (A, B, or Ang II type 2),12 these effects are likely indirect, involving inflammatory cytokines like interleukin 6,12 tumor necrosis factor-α,13,14 serum amyloid A12 glucocorticoids,15 and reactive oxygen species.16,17 Although it has been well established that Ang II induces skeletal muscle atrophy, little is known about potential effects of Ang II on muscle metabolism and energy stores or about the potential link between these effects and Ang II wasting. AMPK is a serine-threonine kinase that plays a pivotal role in cellular and whole-body metabolism. The aims of this study were to determine whether Ang II affects skeletal muscle energy stores, whether energy depletion plays a role in skeletal muscle wasting, and to characterize the effects of Ang II on AMPK signaling in gastrocnemius muscle.

Materials and Methods

A full description of the experimental design, materials, animals, methods used, and statistical analysis can be found in the online Data Supplement. Please see http://hyper.ahajournals.org.

Results

To separate the catabolic and anorexigenic effects of Ang II on skeletal muscle wasting, 2 saline-infused control groups were included, ad libitum and pair-fed mice. Ang II–infused mice lost 12% of total body mass and 26% of gastrocnemius muscle mass compared with ad libitum controls after 4 days (Figure 1A and 1B). Ang II mice ate approximately half of what the saline-infused ad libitum–fed mice consumed.

Received April 25, 2011; first decision May 13, 2011; revision accepted July 19, 2011.

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Hypertension is available at http://hyper.ahajournals.org DOI: 10.1161/HYPERTENSIONAHA.111.174839

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5-Aminoimidazole-4-carboxamide ribonucleoside (AICAR) prevented angiotensin II (Ang II)–induced weight loss and skeletal muscle wasting by day 4 independent of food intake and blood pressure. **A**, Total body mass, **B** gastrocnemius mass, **C** daily food intake, and **D** systolic blood pressure. *P*<0.05 vs ad libitum. ***P*<0.001 vs ad libitum. +++*P*<0.001 vs pair fed. ***P*<0.001 vs Ang II.

Throughout the experiment (Figure 1C). Although food restriction alone (pair-fed controls) resulted in significant 13% reduction in gastrocnemius muscle weight compared with ad libitum fed controls, Ang II elicited an additional highly significant 13% decrease in skeletal muscle mass, independent of food intake (Figure 1B). Of note, this catabolic effect of Ang II was not apparent from measurement of total body mass (Figure 1A) because of fluid retention, as we have reported previously.18 To ascertain the role that AMPK signaling plays in Ang II–induced wasting, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), which reliably activates AMPK, was administered via daily intraperitoneal injection. AICAR markedly blunted Ang II–induced loss in total body mass and completely reversed the 26% loss of skeletal muscle mass induced by Ang II (Figure 1A and 1B). The effects of AICAR were independent of both food intake and blood pressure (Figure 1C and 1D).

Ang II infusion or pair feeding did not alter mitochondrial content as measured by real-time PCR analysis of mitochondrial copy number relative to myofiber nuclei (Figure 2A). However, Ang II significantly reduced mitochondrial activity, specifically Ang II decreased cytochrome C oxidase activity (complex IV of the electron transport chain), by 47% (Figure 2B). Ang II also increased mitochondrial-derived superoxide, consistent with Ang II–induced mitochondrial dysfunction, although mitochondrial-derived reactive oxygen species did not directly contribute to Ang II wasting (Figure S1, available in the online Data Supplement). Ang II also reduced skeletal muscle ATP content by 74% in a food intake–independent manner (Figure 2C), and this effect was completely reversed by AICAR (Figure 2C). Of note, AICAR itself reduced ATP levels in control animals (Figure 2C).

Food restriction (pair-fed controls) increased AMPK activity in gastrocnemius muscle by 75% (assessed by Thr172 phosphorylation; Figure 3), and Ang II completely prevented this increase at 4 days (Figure 3). The inhibitory effect of Ang II on AMPK activation in response to reduced food intake was completely blocked by AICAR (Figure 3). Although caloric restriction-induced AMPK activation was not yet apparent, Ang II also inhibited AMPK phosphorylation at day 1 (Figure S2). To determine potential mechanisms by which Ang II prevented AMPK activation, we analyzed activities of the upstream AMPK kinases LKB1 and TAK1. Caloric restriction, Ang II, or AICAR had no effect on TAK1 activity (Figure S3). Conversely, there was a significant food intake–independent 56% increase in LKB1 activation with Ang II, and LKB1 activity was restored to basal levels in AICAR-treated animals (Figure S3). These findings suggest that LKB1 activation by Ang II was a compensatory response to Ang II–mediated reduction in AMPK activity. In the absence of Ang II inhibition of AMPK activating kinases, we examined the expression levels of protein phosphatase 2Cα (PP2Cα), a serine/threonine protein phosphatase known to dephosphorylate and inactivate AMPK.19,20 We detected a robust food intake–independent 2.6-fold induction of PP2Cα protein after Ang II infusion (Figure 4). These data suggest that the inhibitory effects of Ang II on AMPK activity are
mediated by the upregulation of PP2Cα. Interestingly, AICAR completely prevented this increase in Ang II–induced PP2Cα expression (Figure 4). Neither Ang II nor AICAR altered expression of the β-isofrom of PP2C (data not shown). Although Ang II increased PP2Cα expression, we detected no change in total PP2C activity (Figure S4).

We next examined several downstream targets of AMPK signaling, including peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) and acetyl-coenzyme A carboxylase (ACC). Correlating with caloric restriction–induced AMPK activation, we observed a significant 60% increase in total PGC-1α expression in the gastrocnemius muscle of pair-fed mice, and Ang II prevented this induction of PGC-1α (Figure 5A). Correlating with AMPK inactivation, ACC phosphorylation at Ser79 was also decreased by Ang II, and the ratio of phospho-ACC/total-ACC was reduced by 41%, consistent with activation of ACC by Ang II (because ACC phosphorylation by AMPK is inhibitory). This Ang II–induced activation of ACC was not reversed by AICAR (Figure 5B). Interestingly, there was a significant 65% reduction in total ACC protein in response to Ang II (Figure S5). While having no effect on ACC activity, AICAR restored total ACC to basal levels.

Finally, we examined effects of Ang II and AICAR on the expression of the E3 ubiquitin ligases muscle atrophy F-box protein 1 (atrogin 1) and muscle RING-finger protein 1 in gastrocnemius after 24 hours of treatment. As we have reported previously, Ang II significantly upregulated both atrogin 1 and muscle RING-finger protein 1 mRNA expression (8.2-fold and 6.2-fold, respectively). Intriguingly, AICAR completely prevented their upregulation, without affecting basal expression of these E3 ligases (Figure 6A and 6B). Because FoxO transcription factors regulate E3 ligase expression, and because the principle effects of AMPK on FoxO are believed to be activating,21,22 the ability of AICAR to prevent Ang II–induced increases in E3 ligases was unexpected. To better understand this rescue effect of AICAR, we measured activation of Akt (which is known to inhibit FoxO18,23). Pair feeding increased S473-phospho-Akt levels by 2.8-fold over ad libitum, whereas Ang II blocked this caloric restriction–induced activation. AICAR partially restored S473-phospho-Akt levels in Ang II–treated mice (Figure 6D). Correlating with Akt activation levels by 2.8-fold over ad libitum, whereas Ang II blocked this caloric restriction–induced activation. AICAR partially restored S473-phospho-Akt levels in Ang II–treated mice (Figure 6D). Correlating with Akt activation

**Figure 2.** Angiotensin II (Ang II) impaired gastrocnemius mitochondrial function and reduced skeletal muscle energy stores without altering mitochondrial content, whereas 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) prevented Ang II–induced energy depletion. A, Mitochondrial content, B, cytochrome C oxidase activity, and C, ATP content relative to ad libitum fed controls. \(^*P<0.05\) vs ad libitum, \(+P<0.05\) vs pair fed. \(+++P<0.001\) vs pair fed. \(\alpha \alpha \alpha P<0.01\) vs Ang II.

**Figure 3.** Angiotensin II (Ang II) prevented caloric restriction–induced AMP-activated protein kinase (AMPK) activation in gastrocnemius, whereas 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) rescued from Ang II–induced AMPK inhibition. Representative Western blotting and quantitative data are shown. AL indicates ad libitum–fed controls; PF, pair-fed controls. \(^*P<0.05\) vs Ad libitum. + \(+P<0.01\) vs pair fed. \(\alpha \alpha \alpha P<0.05\) vs Ang II.

**Discussion**

We report for the first time that Ang II caused marked ATP depletion in skeletal muscle independent of food intake and blocked caloric restriction–induced AMPK activation, probably via increased expression of the protein phosphatase

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**Image 2**

- **A**: Mitochondrial content, **B**: cytochrome C oxidase activity, **C**: ATP content relative to ad libitum fed controls.

**Image 3**

- Representative Western blotting and quantitative data are shown. AL indicates ad libitum–fed controls; PF, pair-fed controls. **Image 4**

- Correlating with AMPK inactivation, ACC phosphorylation at Ser79 was also decreased by Ang II, and the ratio of phospho-ACC/total-ACC was reduced by 41%, consistent with activation of ACC by Ang II (because ACC phosphorylation by AMPK is inhibitory). This Ang II–induced activation of ACC was not reversed by AICAR (Figure 5B).

**Image 5**

- Interestingly, there was a significant 65% reduction in total ACC protein in response to Ang II (Figure S5). While having no effect on ACC activity, AICAR restored total ACC to basal levels.

**Image 6**

- Finally, we examined effects of Ang II and AICAR on the expression of the E3 ubiquitin ligases muscle atrophy F-box protein 1 (atrogin 1) and muscle RING-finger protein 1 in gastrocnemius after 24 hours of treatment. As we have reported previously, Ang II significantly upregulated both atrogin 1 and muscle RING-finger protein 1 mRNA expression (8.2-fold and 6.2-fold, respectively). Intriguingly, AICAR completely prevented their upregulation, without affecting basal expression of these E3 ligases (Figure 6A and 6B). Because FoxO transcription factors regulate E3 ligase expression, and because the principle effects of AMPK on FoxO are believed to be activating,21,22 the ability of AICAR to prevent Ang II–induced increases in E3 ligases was unexpected. To better understand this rescue effect of AICAR, we measured activation of Akt (which is known to inhibit FoxO18,23). Pair feeding increased S473-phospho-Akt levels by 2.8-fold over ad libitum, whereas Ang II blocked this caloric restriction–induced activation. AICAR partially restored S473-phospho-Akt levels in Ang II–treated mice (Figure 6D). Correlating with Akt activation levels by 2.8-fold over ad libitum, whereas Ang II blocked this caloric restriction–induced activation. AICAR partially restored S473-phospho-Akt levels in Ang II–treated mice (Figure 6D). Correlating with Akt activation

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**Table**

- Table 1: Mitochondrial content, cytochrome C oxidase activity, and ATP content relative to ad libitum fed controls. \(^*P<0.05\) vs ad libitum, \(+P<0.01\) vs pair fed. \(\alpha \alpha \alpha P<0.05\) vs Ang II.
Importantly, our data also demonstrated that the AMPK activator AICAR restores ATP levels to that of controls and reverses Ang II–induced loss of gastrocnemius muscle mass. In addition, AICAR blocked Ang II–induced increases in E3 ubiquitin ligase expression via Akt activation, thereby contributing to its ant cacabolic effects. These novel findings suggest that AMPK activators may have a therapeutic potential in congestive heart failure and chronic kidney disease, 2 disease states characterized by elevated levels of Ang II and wasting.

AMPK activation by AICAR reversed Ang II–induced skeletal muscle wasting independent of food intake and blood pressure. Of note, AMPK activation can increase appetite24,25; however, our experiment was designed to minimize the orexigenic effect of AICAR to focus on its direct signaling effects in skeletal muscle. To accomplish this, food was provided only during nocturnal hours. Because AICAR was injected in the morning, food was unavailable for 12 hours after administration, and food intake was unaltered in AICAR mice. Also, although AICAR has been reported to reduce blood pressure,26 this occurred at a much higher dose and longer duration of administration (7 weeks) than was used in our study.

Neither caloric restriction nor Ang II altered mitochondrial content or protein expression of electron transport chain components. These results contrast with that reported previously in C2C12 myotubes and C57Bl/6 mice.16 In that study, Ang II, infused for 10 days at a subpressor dose, reduced both mitochondrial content and protein levels of the electron transport chain complexes IV and V. Strain, dosage, and time point differences might have contributed to these differences. In addition, it is possible that meal-interval training27,28 of our mice preconditioned them to states of metabolic stress, a protective effect of AMPK activation observed in a variety of cell types and animal models.29–31 As such, mitochondrial biogenesis could have been activated in the days before the start of the experiment, protecting against Ang II–induced reductions in mitochondrial content. In any case, Ang II

![Figure 4. Angiotensin II (Ang II) increased expression of protein phosphatase 2C\(\alpha\) (PP2C\(\alpha\)) in gastrocnemius independent of food intake, whereas 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) blocked this Ang II–mediated increase.](image)

PP2C\(\alpha\). Importantly, our data also demonstrated that the AMPK activator AICAR restores ATP levels to that of controls and reverses Ang II–induced loss of gastrocnemius muscle mass. In addition, AICAR blocked Ang II–induced increases in E3 ubiquitin ligase expression via Akt activation,
reduced cytochrome C oxidase activity, and this mitochondrial dysfunction led to reduced ATP content in gastrocnemius muscles of Ang II–infused mice.

Despite reduced caloric intake, the reduction in gastrocnemius weight in pair-fed controls was not accompanied by reduced ATP, suggesting that increased AMPK activity was able to maintain normal ATP levels and indicating that there is not a direct correlation between ATP and muscle weight. This is further reflected in the low ATP in gastrocnemius muscles of AICAR-treated control animals (pair fed and ad libitum), in which there was no wasting. Ang II mice were unable to respond to the initial energy imbalance induced by Ang II, leading to prolonged ATP depletion, which potentially contributed to the wasting phenotype. This lack of an appropriate adaptive response in Ang II mice was reflected in gastrocnemius AMPK activity, which was increased by pair feeding but failed to increase with Ang II. Despite severe energy depletion, Ang II inhibited the ability of AMPK to sense the low fuel state and signal accordingly to correct the imbalance. Direct stimulation of AMPK by AICAR was able to override the inhibitory effect of Ang II and to normalize Ang II–induced energy depletion by 4 days. The mechanism by which Ang II enhances AMPK dephosphorylation and inactivation appears to be via upregulation of PP2Cα expression in muscle. A similar mechanism of AMPK inhibition has been described previously in skeletal muscle insulin resistance induced by tumor necrosis factor-α.32 AICAR returned PP2Cα expression to basal levels in Ang II–infused mice, and this reduction in expression of the competing phosphatase might have contributed to the ability of AICAR to prevent Ang II–mediated reduction in AMPK phosphorylation.

Ang II did not activate PP2C enzymatic activity in addition to increasing PP2Cα expression in gastrocnemius. However, our assay could not discriminate between PP2Cα and PP2Cβ activities. In addition, the specific mechanism of Ang II–induced PP2Cα upregulation remains to be elucidated, although we observed no increase in gastrocnemius redox status (Figure S7), suggesting that reactive oxygen species may not be directly involved. However, it remains possible that other types of reactive oxygen species may mediate the effect (ie, NADPH oxidase–derived superoxides, which are important in Ang II wasting33). Although our data suggest

Figure 6. 5-Aminoimidazole-4-carboxamide ribonucleoside (AICAR) blocked angiotensin II (Ang II)–induced increases in E3 ligase expression via Akt activation. A, Atrogin 1, (B) muscle RING-finger protein-1 (MuRF-1) mRNA, (C) Akt phosphorylation at Ser473, and (D) Akt phosphorylation at Thr308. *P<0.05 vs ad libitum. +P<0.05 vs pair fed. +++P<0.001 vs pair fed. oP<0.05 vs Ang II. oooP<0.001 vs Ang II.
that upregulation of PP2Cα by Ang II mediates its inhibitory effects on AMPK phosphorylation and energy balance, further experiments are required to establish a causal link. In particular, it will be necessary to demonstrate that inhibition of PP2Cα in vivo can mimic the rescue effects of AICAR.

ACC phosphorylation status and PGC-1α expression correlated closely with levels of AMPK phosphorylation. As AMPK is activated, PGC-1α transcription is turned on, leading to increased expression of mitochondrial genes and mitochondrial biogenesis in skeletal muscle. Caloric restriction induced AMPK activity with a corresponding increase in PGC-1α levels at 4 days. By blocking AMPK activation, Ang II also likely blocked activation of PGC-1α transcription, and, as such, protein levels of PGC-1α remained unchanged. AICAR maintained AMPK activation in the presence of Ang II and blocked the inhibitory effect of Ang II on PGC-1α expression. Consistent with its inhibitory effect on AMPK activity, Ang II reduced ACC phosphorylation. As AMPK is activated, it phosphorylates and inactivates ACC, in effect turning off fatty acid synthesis. Active ACC catalyzes the synthesis of malonyl-coenzyme A, which, in turn, blocks carnitine palmitoyltransferase-1 function via allosteric inhibition. Phosphorylation and inactivation of ACC relieves this inhibition of carnitine palmitoyltransferase 1 and allows for the transporter to facilitate mobilization of fatty acids to mitochondria, where they can be β-oxidized for acute ATP production in times of metabolic stress. Therefore, Ang II–induced reduction of ACC phosphorylation is consistent with activation of ACC and would be a maladaptive response, because long chain fatty acid synthesis (which is energy consuming) would continue, whereas β-oxidation (which is ATP generating) would be inhibited. Of note, Ang II reduced total ACC at 4 days, which may be a compensatory response. AICAR restored total ACC to basal levels but did not alter ACC activity.

Ang II upregulates the E3 ubiquitin ligases atrogin 1 and muscle RING-finger protein 1 in skeletal muscle, and these are essential to the wasting process. Interestingly, AICAR blocked this upregulation, providing a potential additional mechanism whereby AICAR treatment prevents Ang II–induced wasting. AICAR induced Akt activation and inhibitory phosphorylation of FoxO1, explaining the ability of AICAR to abrogate Ang II–mediated upregulation of E3 ubiquitin ligases. The predominant effects of AMPK-mediated phosphorylation of FoxO are believed to be activating. Therefore, its plausible that Akt-mediated inhibitory phosphorylation of FoxO is dominant over any direct activating effects of AMPK.

Our findings are relevant to wasting conditions in which the renin-angiotensin system is activated. Patients with congestive heart failure have 2- to 4-fold increases in plasma Ang II levels, in many cases even in the presence of angiotensin-converting enzyme inhibitor therapy. There is also evidence for 5-fold increases in circulating Ang II levels in chronic kidney disease patients. Infusion of 1000 ng/kg per minute of Ang II in our model yields a 2.8-fold increase in plasma Ang II, which is well within this range. Furthermore, infusion of low-dose Ang II (80 ng/kg per minute) for 28 days into rats also caused loss of lean body mass with no reduction in food intake, clearly indicating catabolic activation with Ang II. Our finding that AMPK activation reverses Ang II–induced catabolic effects may offer new therapeutic strategies for the treatment of skeletal muscle wasting.

**Perspectives**

Our data show that Ang II–mediated skeletal muscle wasting is characterized by mitochondrial dysfunction-induced skeletal muscle ATP depletion and inhibition of the normal physiological response to energy depletion. Specifically, Ang II prevented AMPK activation, likely via upregulation of the inactivating phosphatase PP2Cα, thereby preventing normalization of muscle energy balance. In addition, Ang II upregulated E3 ubiquitin ligase expression. The AMPK activator AICAR essentially reversed these effects and restored muscle mass. Thus, our studies describe the mechanisms underlying Ang II–induced skeletal muscle wasting and suggest a therapeutic potential for AMPK activators in congestive heart failure and chronic kidney disease, disease states characterized by chronic activation of the renin-angiotensin system and muscle wasting.

**Sources of Funding**

This study was supported by grants from the National Institutes of Health/National Heart, Lung and Blood Institute (R01HL070241, R01HL080682, and HL86787), National Institutes of Health/National Center for Research Resources (P20RR018766). Veterans Affairs Office of Research and Development Service Award (1I01BX00246), and National Institutes of Health (R37 DK37175).

**Disclosures**

None.

**References**

1. Brink M, Price SR, Chrast J, Bailey JL, Anwar A, Mitch WE, Delafontaine P. Angiotensin II induces skeletal muscle wasting through enhanced protein degradation and down-regulates autocrine insulin-like growth factor I. Endocrinology. 2001;142:1489–1496.
2. Brink M, Wellen J, Delafontaine P. Angiotensin II causes weight loss and decreases circulating insulin-like growth factor I in rats through a pressor-independent mechanism. J Clin Invest. 1996;97:2509–2516.
3. Song YH, Li Y, Du J, Mitch WE, Rosenthal N, Delafontaine P. Muscle-specific expression of IGF-1 blocks angiotensin II-induced skeletal muscle wasting. J Clin Invest. 2005;115:451–458.
4. Anker SD, Ponikowski P, Varney S, Chua TP, Clark AL, Webb-Peploe KM, Harrington D, Kox WJ, Poole-Wilson PA, Coats AJ. Wasting as independent risk factor for mortality in chronic heart failure. Lancet. 1997;349:1050–1053.
5. Levenson JW, Skerritt PJ, Gaziano JM. Reducing the global burden of cardiovascular disease: the role of risk factors. Prev Cardiol. 2002;5:188–199.
6. Du J, Hu Z, Mitch WE. Cellular signals activating muscle proteolysis in chronic kidney disease: a two-stage process. Int J Biochem Cell Biol. 2005;37:2147–2155.
7. Rajan V, Mitch WE. Ubiquitin, proteasomes and proteolytic mechanisms activated by kidney disease. Biochim Biophys Acta. 2008;1782:795–799.
8. Foll F, Kahn CR, Hansen H, Bouche JL, Feener EP. Angiotensin II inhibits insulin signaling in aortic smooth muscle cells at multiple levels. A potential role for serine phosphorylation in insulin/angiotensin II crosstalk. J Clin Invest. 1997;100:2158–2169.
9. Foll F, Saad MJ, Velloso L, Hansen H, Carandente O, Feener EP, Kahn CR. Crosstalk between insulin and angiotensin II signalling systems. Exp Clin Endocrinol Diabetes. 1999;107:133–139.
10. Workenhe BT, Mitch WE. Review of muscle wasting associated with chronic kidney disease. Am J Clin Nutr. 2010;91:1128S–1132S.
23. Zhao J, Semprun-Prieto L, Sukhanov S, Delafontaine P. IGF-1 prevents Ang II-induced skeletal muscle atrophy via Akt- and FOXO-dependent inhibition of the ubiquitin ligase atroglin-1 expression. Am J Physiol Heart Circ Physiol. 2010;298:H1565–H1570.

22. Zhang L, Du J, Hu Z, Han G, Delafontaine P, Garcia G, Mitch WE. IGF-6 antisense amylloid a synergy mediates angiotensin II-induced muscle wasting. J Am Soc Nephrol. 2009;20:604–612.

21. Sato H, Watanabe A, Tanaka T, Koitabashi N, Arai M, Kurabayashi M, Yokoyama T. Regulation of the human tumor necrosis factor-α promoter by angiotensin II and lipopolysaccharide in cardiac fibroblasts: different cis-acting promoter sequences and transcriptional factors. J Mol Cell Cardiol. 2003;35:1197–1205.

20. Zera T, Ufnal M, Szczepanska-Sadowska E. Central TNF-α elevation and elevated angiotensin II in the hypothalamus. Trends Mol Med. 2008;14:465–474.

19. Sanders MJ, Grondin PO, Hegarty BD, Snowden MA, Carling D. Investigation of catabolic/anabolic imbalance and cachexia. Int J Cardiol. 2002;85:111–121, discussion 121–114.

18. Yoshida T, Semprun-Prieto L, Sukhanov S. IGF-1 reduces mitochondrial content in skeletal muscle and affects glycolytic control. Diabetes. 2009;58:710–717.

17. Manga D, Olszewski D, Sadoski R, Lecker SH, Goldberg AL. Foxo3 coordinately activates protein degradation by the mammalian foxo3 transcription factor. J Biol Chem. 2007:403:139–148.

16. Yoshida T, Semprun-Prieto L, Sukhanov S, Delafontaine P. IGF-1 protects Ang II-induced skeletal muscle atrophy via Akt- and FoxO-dependent inhibition of the ubiquitin ligase atroglin-1 expression. Am J Physiol Heart Circ Physiol. 2010;298:H1565–H1570.

15. Sanders MJ, Grondin PO, Hegarty BD, Snowden MA, Carling D. Investigating the mechanism for AMP activation of the AMP-activated protein kinase caspase. Biochem J. 2007;403:139–148.

14. Zera T, Ufnal M, Szczepanska-Sadowska E. Central TNF-α elevation and elevated angiotensin II in the hypothalamus. Trends Mol Med. 2008;14:465–474.

13. Sato H, Watanabe A, Tanaka T, Koitabashi N, Arai M, Kurabayashi M, Yokoyama T. Regulation of the human tumor necrosis factor-α promoter by angiotensin II and lipopolysaccharide in cardiac fibroblasts: different cis-acting promoter sequences and transcriptional factors. J Mol Cell Cardiol. 2003;35:1197–1205.

12. Zhang L, Du J, Hu Z, Han G, Delafontaine P, Garcia G, Mitch WE. IGF-6 antisense amylloid a synergy mediates angiotensin II-induced muscle wasting. J Am Soc Nephrol. 2009;20:604–612.

11. Yoshida T, Semprun-Prieto L, Sukhanov S, Delafontaine P. IGF-1 prevents Ang II-induced skeletal muscle atrophy via Akt- and FOXO-dependent inhibition of the ubiquitin ligase atroglin-1 expression. Am J Physiol Heart Circ Physiol. 2010;298:H1565–H1570.