Diminished overload-induced hypertrophy in aged fast-twitch skeletal muscle is associated with AMPK hyperphosphorylation

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Thomson, David M., and Scott E. Gordon. Diminished overload-induced hypertrophy in aged fast-twitch skeletal muscle is associated with AMPK hyperphosphorylation. J Appl Physiol 98: 557–564, 2005. First published October 1, 2004; doi:10.1152/japplphysiol.00811.2004.—Skeletal muscle mass declines with age, as does the potential for overload-induced fast-twitch skeletal muscle hypertrophy. Because 5′-AMP-activated protein kinase (AMPK) activity is thought to inhibit skeletal muscle protein synthesis and may therefore modulate muscle mass and hypertrophy, the purpose of this investigation was to examine AMPK phosphorylation status (a marker of AMPK activity) and its potential association with the attenuated overload-induced hypertrophy observed in aged skeletal muscle. One-week overload of fast-twitch plantaris and slow-twitch soleus muscles was achieved in young adult (8 mo; n = 7) and old (30 mo; n = 7) Fischer{	extsubscript{144}} × Brown Norway male rats via unilateral gastrocnemius ablation. Significant (P ≤ 0.05) age-related atrophy (as measured by total protein content) was noted in plantaris and soleus control ( sham-operated) muscles. In fast-twitch plantaris muscles, percent hypertrophy with overload was significantly attenuated with age, whereas AMPK phosphorylation status as determined by Western blotting [phospho-AMPK (Thr172)/total AMPK] was significantly elevated with age (regardless of loading status). There was also a main effect of loading on AMPK phosphorylation status in plantaris muscles (overload > control). Moreover, a strong and significant negative correlation (r = −0.82) was observed between AMPK phosphorylation status and percent hypertrophy in the overloaded plantaris muscles of all animals. In contrast to the plantaris, overload-induced hypertrophy of the slow-twitch soleus muscle was similar between ages, and AMPK phosphorylation in this muscle was also unaffected by age or overload. These data support the possibility that an age-related elevation in AMPK phosphorylation may partly contribute to the attenuated hypertrophic response observed with age in overloaded fast-twitch plantaris muscle.

fibre type; loading; sarcopenia; acetyl CoA carboxylase

SARCOPENIA, OR THE LOSS OF muscle mass with age, is an increasingly serious clinical problem that can lead to a loss of general mobility and independence in the elderly as well as increase the risk of injury from falling because of decreased muscular strength and power (45, 48). Although resistance exercise training is clearly beneficial for older individuals as a therapeutic intervention aimed at increasing muscle mass in humans, it does not appear to be as effective in the old as in the young, especially in fast-twitch fibers, which are particularly prone to age-related atrophy (19, 29, 51). Hypertrophic impairments have also been noted in old rats subjected to chronic skeletal muscle overload (2, 8, 13). The cellular mechanisms underlying this decreased capacity for skeletal muscle hypertrophy in the aged are not yet understood.

One potential regulator of hypertrophy is 5′-AMP-activated protein kinase (AMPK), which mediates the response to decreased cellular energy status. AMPK activation during metabolic stress (such as muscle contraction) is known to stimulate ATP generating processes, such as muscle glucose uptake and lipid catabolism, and inhibit ATP-consuming anabolic processes such as fatty acid synthesis (20). Moreover, AMPK activation has been shown to inhibit skeletal muscle protein synthesis (10), likely because protein synthesis is a bioenergetically expensive process. Suppression of protein synthesis by AMPK suggests that this signaling protein may also regulate skeletal muscle hypertrophy. There is some indication of such a role for AMPK in cardiac muscle, where mutations in the gene encoding the gamma subunit of AMPK are associated with pathological hypertrophy (7), and AMPK activation has been shown to inhibit cultured cardiac myocyte growth (12). However, no studies have examined the possibility of such a function for AMPK in skeletal muscle.

Little is known about the effect of aging on skeletal muscle AMPK. However, it has been demonstrated that AMP concentration is elevated and phosphocreatine concentration is decreased to a greater degree in old vs. young skeletal muscle before and after exercise training (6), conditions that are both known to stimulate AMPK activity (20). We thus hypothesized that AMPK activation would be elevated in skeletal muscle of aging animals. If true, the elevated AMPK activity may also contribute to the impairment of muscle hypertrophy observed in older animals. Therefore, the purpose of this investigation was to examine AMPK phosphorylation status (a marker of AMPK activity) and its potential association with the attenuated overload-induced hypertrophy in aged skeletal muscle. To accomplish this, we examined plantaris and soleus muscles of young adult (8 mo) vs. old (30 mo) rats after 1 wk of compensatory overload-induced hypertrophy (via gastrocnemius ablation) or a sham operation. Here we show that AMPK phosphorylation status is increased with age and is also negatively correlated with overload-induced hypertrophy in fast-twitch plantaris muscles but not in slow-twitch soleus muscles. These results suggest a role for AMPK in the regulation of overload-induced hypertrophy and support the possibility that elevated AMPK phosphorylation may contribute to the

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decreased hypertrophy observed in aged fast-twitch skeletal muscle.

MATERIALS AND METHODS

Animals. Young adult (YA; 8 mo; n = 7) and old (O; 30 mo; n = 7) Fischer344 × Brown Norway F1 hybrid (FBN) male rats were housed in the animal care facility of East Carolina University Brody School of Medicine. They were kept on a 12-h light-dark cycle and had free access to water and chow. The East Carolina University Animal Care and Use Committee approved all procedures before this investigation. We chose the FBN rat for use in this study because it is considered to be the preferred model for the study of age-related skeletal muscle dysfunction (8, 13).

Synergist ablation procedure. In all rats, unilateral 1-wk overload of the plantaris and soleus muscles was achieved through ablation of the synergistic gastrocnemius muscle. By overloading the soleus and plantaris muscles, it was possible to make comparisons between fiber types, because the plantaris muscle is 93% fast-twitch by mass, and the soleus is 89% slow-twitch (3). Rats were weighed and anesthetized with 2–3% isoflurane and supplemental oxygen. Under aseptic conditions, the distal two-thirds of the gastrocnemius muscle were surgically removed from the left hindlimb as previously described (17). A sham (control) operation was performed on the right hindlimb but without disruption of the gastrocnemius muscle. The plantaris and soleus muscles from this limb served as controls. After each procedure, the incision was closed with stainless steel surgical clips, after which the animals were given a one-time subcutaneous injection of an analgesic (Buprenex, 0.03 mg/kg body wt).

We chose unilateral ablation over bilateral ablation because it allows within-subjects comparisons to be made between control and overloaded muscles, thus eliminating bias due to systemic differences between groups of animals and allowing a more precise measurement of muscle hypertrophy in each individual animal. Because of the possibility that some animals may favor one leg after unilateral gastrocnemius ablation, it was necessary to verify that the AMPK phosphorylation was similar regardless of prior overload condition. One week of overload resulted in significant hypertrophy of both the fast-twitch plantaris and slow-twitch soleus muscles (as demonstrated by wet-weight and total protein content measurements) in both YA and O rats. No ablation or sham surgery had been previously performed. These muscles were compared with sham-operated “control” plantaris and soleus muscles of the seven original YA experimental animals (n = 3) in which no ablation or sham surgery had been previously performed. These muscles were compared with sham-operated “control” plantaris and soleus muscles of the seven original YA experimental animals to verify that AMPK phosphorylation was similar regardless of prior surgery.

One week after surgery, animals were weighed and anesthetized with an intraperitoneal injection of ketamine and xylazine (90 and 10 mg/kg body wt, respectively). Animals were then killed by decapitation, after which the plantaris and soleus muscles from both legs were quickly removed, trimmed of excess fat and connective tissue, weighed on an analytical balance, frozen in liquid nitrogen, and stored at −80°C. These samples were later used to assess basal AMPK phosphorylation in fast-twitch muscles of YA vs. O rats not subjected to prior surgery [the mixed gastrocnemius is over 95% fast-twitch by mass (3)]. Secondly, we also removed and examined plantaris and soleus muscles from a separate group of YA animals (n = 3) in which no ablation or sham surgery had been previously performed. These muscles were compared with sham-operated “control” plantaris and soleus muscles of the seven original YA experimental animals to verify that AMPK phosphorylation was similar regardless of prior surgery.

Tissue homogenization and determination of protein concentration. Muscles were homogenized on ice in a ground glass homogenizer as a 3.5% (wt/vol) solution in a homogenization buffer [50 mM HEPES (pH 7.4), 0.1% Triton X-100, 4 mM EGTA, 10 mM EDTA, 15 mM Na3VO4, 10 H2O, 100 mM β-glycerophosphate, 25 mM NaF, 50 μg/ml leupeptin, 50 μg/ml pepstatin, 33 μg/ml aprotinin, and 5 mM Na2VO3]. Sample homogenates were then diluted 1:5 in homogenization buffer before analysis of protein concentration, which was assessed in triplicate using a modified Lowry procedure (DC Protein Assay, Bio-Rad, Hercules, CA). Protein assay results were then used to calculate total protein per whole muscle as an index of muscle hypertrophy.

SDS-PAGE, Western blotting, and immunodetection. Standard Western blotting protocols were used for the detection of phospho-AMPK (Thr172), total AMPK, phospho-acetyl CoA carboxylase (phospho-ACC; at Ser79). Total muscle protein homogenates were solubilized in sample loading buffer (50 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 2% β-mercaptoethanol, 0.1% bromophenol blue) at a concentration of 1 mg/ml and boiled for 5 min. Proteins were then separated by 7.5% SDS-PAGE at 200 mV for ~1 h and Western blotted for 2 h at 4°C onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA) at 100 V in transfer buffer (25 mM Tris-base, 192 mM glycine, 20% methanol). To verify transfer and equal loading among lanes, membranes were then stained with Ponceau S (10 mg/ml in 50 mM Tris-HCl, pH 7.6), serially washed in TBS-T at room temperature, incubated with primary antibody at 4°C in primary antibody buffer (5% BSA in TBS-T, pH 7.6, primary antibody dilution 1:1,000 overnight, serially washed again in TBS-T, incubated with horseradish peroxidase (HRP)-conjugated secondary antibody in blocking buffer for 1 h, and again serially washed in TBS-T. The HRP activity was detected by enhanced chemiluminescence reagent (ECL; Amersham Biosciences, Piscataway, NJ) and exposure to Kodak-XAR5 autoradiographic film.

Antigen concentration was calculated by quantification of the integrated optical density (IOD) of the appropriate band by use of Gel Pro Analyzer software (Media Cybernetics, Silver Spring, MD). Total AMPK-α, phospho-AMPK-α (Thr172), and phospho-ACC (Ser79) antibodies for these procedures were all obtained from Cell Signaling Technology (Beverly, MA). The HRP-conjugated anti-rabbit secondary antibody was from Amersham Pharmacia Biotech (Piscataway, NJ). For Western blots, samples from each of the four experimental conditions were represented as equally as possible on each gel, and the IODs were normalized to the sham-operated condition of the young adult animals. AMPK phosphorylation status (phosphorylation per unit of total AMPK protein) was determined by normalizing the IOD of phospho-AMPK to the IOD of total AMPK.

Statistics. Multivariate analyses of variance were employed for all group comparisons, with repeated measures being used where appropriate. Post hoc comparisons were accomplished via the Fisher’s least significant difference test. All correlations were calculated as Pearson product-moment correlation coefficients. Statistical significance was set at a level of P ≤ 0.05.

RESULTS

Muscle wet weights and total protein contents. Body weights at the time of death were 420.5 ± 10.2 g for the YA and 508.9 ± 10.8 g for the O animals. Because the unilateral ablation model was used, we chose not to normalize muscle weights to body weights because percent hypertrophy is unaffected by normalization. One week of overload resulted in significant hypertrophy of both the fast-twitch plantaris and slow-twitch soleus muscles (as demonstrated by wet-weight and total protein content measurements) in both YA and O animals (Table 1). However, regardless of overload condition, plantaris and soleus muscles from O rats were lighter in terms of wet weight and protein content than their YA counterparts. Furthermore, the fast-twitch plantaris muscle of O rats hypertrophied to a significantly lesser extent (as indicated by percent hypertrophy) with overload than did the plantaris muscle from

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YA rats, whereas no such difference was observed in the slow-twitch soleus muscle. Previous studies in rats have also shown a diminished overload-induced hypertrophy with aging in fast-twitch muscle (2, 8, 13). Our data are very similar to reports of diminished or nonexistent fast-twitch fiber hypertrophy in elderly humans with resistance training, even when slow-twitch fiber hypertrophy is significant (18, 40, 42, 43).

Muscle phospho- and total AMPK concentrations. In the fast-twitch plantaris muscle (Fig. 1), there was a significant main effect of overload on phospho-AMPK (increased with overload) and total AMPK (decreased with overload), regardless of age. Although the plantaris muscles of old animals tended to have higher phospho-AMPK (P = 0.15) and lower total AMPK (P = 0.09) concentrations regardless of overload, these effects were not significant. There were no effects of age or overload on phospho- or total AMPK concentrations in the soleus muscle (Fig. 2). Interestingly, AMPK phosphorylation status [phospho-AMPK (Thr172) IOD/total AMPK IOD] was significantly elevated by both age and overload (main effects) in the plantaris muscle (Fig. 3). AMPK phosphorylation status was also significantly elevated with age (by >80%) in the fast-twitch mixed gastrocnemius muscle that was excised during ablation surgery from the subset of animals (n = 3 per group). In these muscles, phosphorylation status was 1.54 ± 0.24 vs. 2.78 ± 0.30 (means ± SE; arbitrary units) for the YA vs. O animals, respectively (P ≤ 0.05). These data in the fast-twitch gastrocnemius confirm our similar findings in the fast-twitch plantaris and also indicate that the age-related increase in AMPK phosphorylation observed in the plantaris muscle was not likely due to the effects of prior sham surgery or the animals potentially favoring one particular leg during the 1-wk postsurgical period.

To further verify that AMPK phosphorylation was similar between our sham-operated “control” muscles and muscles obtained from rats not subjected to prior surgery, the sham-operated plantaris and soleus muscles of the original YA experimental animals (n = 7) were compared with plantaris and soleus muscles from a separate group of YA animals (n = 3) in which no ablation or sham surgery had been previously performed. Similar AMPK phosphorylation status values (means ± SE; arbitrary units) were obtained for the “prior surgery” group vs. the “no surgery” group in the plantaris muscle (1.15 ± 0.28 vs. 1.30 ± 0.29, respectively) as well as the soleus muscle (1.24 ± 0.48 vs. 0.92 ± 0.56, respectively), suggesting that the unilateral ablation procedure did not significantly affect AMPK signaling in the sham-operated plantaris or soleus muscles.

Plantaris muscle phospho-ACC concentration. Phospho-ACC at Ser79 was significantly elevated by both age and overload (main effects) in the plantaris muscle (Fig. 4), similar to the pattern seen for AMPK phosphorylation status. ACC phosphorylation was also significantly correlated with AMPK phosphorylation status in the plantaris muscle (r = 0.74, P = 0.000006).

Correlation between AMPK phosphorylation and percent hypertrophy in overloaded muscle. A significant negative correlation (r = −0.82; P = 0.00035) was observed between AMPK phosphorylation status and percent increase in muscle protein content in the overloaded fast-twitch plantaris muscle (Fig. 5). A similar significant relationship was also observed when AMPK phosphorylation status was correlated with percent wet weight hypertrophy in these muscles (r = −0.79; P = 0.0008; data not shown). A negative (r = −0.40), but nonsignificant (P = 0.16), relationship was also found between AMPK phosphorylation status and the percent increase in protein content in the overloaded slow-twitch soleus muscles (Fig. 5).

DISCUSSION

Activation of AMPK occurs with low cellular energy status (20). Because cellular energetics in skeletal muscle have been shown to be compromised in old age (6, 15, 33, 34), AMPK activity might therefore be expected to increase with aging in skeletal muscle. Additionally, AMPK activation has also been shown to inhibit skeletal muscle protein synthesis (10), which is important for skeletal muscle hypertrophy (35). Thus we investigated the possibility that AMPK signaling (phosphorylation status) would be elevated in aging skeletal muscle and associated with the attenuated overload-induced hypertrophy known to occur in fast-twitch skeletal muscle of aged animals (2, 8, 13). We found that AMPK phosphorylation status was indeed elevated in O compared with YA fast-twitch plantaris muscles and that these results were highly correlated with ACC phosphorylation, an established marker of AMPK activity (21, 37). Moreover, AMPK phosphorylation status was increased with chronic overload and had a significant negative correlation with percent muscle hypertrophy in fast-twitch plantaris muscles (but not slow-twitch soleus muscles). These results suggest that increased AMPK activity may play a role in the

### Table 1. Muscle wet weights and protein contents after the 1-wk overloading protocol

<table>
<thead>
<tr>
<th></th>
<th>Young Adult (8 mo)</th>
<th>Old (30 mo)</th>
<th>Young Adult (8 mo)</th>
<th>Old (30 mo)</th>
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<tr>
<td><strong>Muscle wet weight, mg</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Sham-operated</td>
<td>383.7±12.8</td>
<td>321.5±6.7*</td>
<td>166.5±3.9</td>
<td>143.7±7.3‡</td>
</tr>
<tr>
<td>Overloaded</td>
<td>497.5±17.7†</td>
<td>352.9±11.2*‡</td>
<td>201.5±4.2§</td>
<td>164.1±7.4‡§</td>
</tr>
<tr>
<td>Percent difference</td>
<td>30.0±4.3</td>
<td>9.7±2.3*</td>
<td>21.4±3.3</td>
<td>15.3±6.1</td>
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<tr>
<td><strong>Muscle protein content, mg</strong></td>
<td></td>
<td></td>
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<tr>
<td>Sham-operated</td>
<td>109.7±6.5</td>
<td>74.0±5.5*</td>
<td>42.2±2.6</td>
<td>34.8±1.9‡</td>
</tr>
<tr>
<td>Overloaded</td>
<td>130.8±8.7†</td>
<td>78.4±6.6*‡</td>
<td>48.8±2.6§</td>
<td>38.3±1.4§</td>
</tr>
<tr>
<td>Percent difference</td>
<td>19.3±2.9</td>
<td>5.6±1.7*</td>
<td>16.7±4.7</td>
<td>11.2±5.1</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 7/group). *Significantly different (P ≤ 0.05) from young adult plantaris muscle; †significantly different from sham-operated plantaris muscle within the specified age group; ‡significant main effect of age in the soleus muscle; §significant main effect of overload in the soleus muscle.
attenuation of overload-induced hypertrophy in aged fast-twitch skeletal muscle.

AMPK phosphorylation status was approximately fivefold and twofold greater in the fast-twitch plantaris muscles of O animals vs. those of YA animals in control (sham-operated) and overloaded muscles, respectively. Further confirmation of these findings is provided by the fact that AMPK phosphorylation status was also elevated with age in resting fast-twitch gastrocnemius muscles obtained from a subset of these animals before sham or overload surgery. To our knowledge, this is the first indication of increased in vivo AMPK activity with age in skeletal muscle. Such a finding is in line with a previous observation that cultured senescent fibroblasts also have an increased AMPK activity, likely because of an elevated AMP-to-ATP ratio (46). Our data are not surprising considering the fact that AMP levels are elevated and phosphocreatine levels decreased with age before and after exercise training in rats (6), both of which are conditions that promote AMPK activation.

![Fig. 1. Fast-twitch plantaris muscle 5'-AMP-activated protein kinase (AMPK) protein phosphorylation (phospho-AMPK) at Thr172 on the α-subunit is increased (A) and total AMPK protein concentration is decreased (B) after 1 wk of functional overload (unilateral gastrocnemius ablation) compared with control (sham-operated) conditions in young adult (YA; 8 mo; n = 7) and old (O; 30 mo; n = 7) rats. Data (means ± SE) are expressed as a percentage of YA, control (sham-operated) values. *Significant main effect (P ≤ 0.05) of overload status, regardless of age.](image1)

![Fig. 2. Slow-twitch soleus muscle AMPK protein phosphorylation at Thr172 on the α-subunit (A) and total AMPK protein concentration (B) is not significantly altered after 1 wk of functional overload (unilateral gastrocnemius ablation) compared with control (sham-operated) conditions in YA (n = 7) and O (n = 7) rats. Data (means ± SE) are expressed as a percentage of YA, control (sham-operated) values.](image2)
In contrast to our findings in the fast-twitch plantaris muscle, we found no age-related differences in AMPK phosphorylation status in slow-twitch soleus muscles, which is interesting because hypertrophy was reduced with age in the fast-twitch plantaris muscle but not in the slow-twitch soleus muscle.

Because the fast-twitch-specific elevation in AMPK phosphorylation status that we observed with age in control muscles was also maintained with overload, it may also explain why the age-related loss in overload-induced muscle hypertrophy was observed primarily in the fast-twitch plantaris muscles of these animals. Although our data do not necessarily establish a direct causative relationship between the two, the evidence that AMPK may negatively regulate the hypertrophic response to overload is strengthened considerably by the significant negative correlation between AMPK phosphorylation status and percent hypertrophy in the overloaded fast-twitch plantaris muscles. This correlation was not significant in the slow-twitch soleus muscle, which is in line with several previous findings of diminished physiological effectiveness of AMPK activation in slow-twitch compared with fast-twitch muscle for reasons that are not yet clear (1, 5, 14, 25). Nevertheless, it is evident from our data that, even in the slow-twitch soleus muscle, there is a definitive threshold for AMPK phosphorylation above which hypertrophy appears to be limited (Fig. 5).

Because activation of AMPK occurs under conditions of increased metabolic stress (20), it is not surprising that AMPK phosphorylation status in the plantaris muscle was elevated by overload in this investigation. Additionally, our finding of a lack of increase in AMPK phosphorylation status in the soleus is consistent with other reports showing no soleus AMPK activation after intense in situ muscle contractions, even despite AMPK activation in other contracting muscles (14, 39). The increased AMPK phosphorylation status that we observed in the overloaded plantaris muscles was driven both by elevated concentrations of phospho-AMPK as well as by decreased concentrations of total AMPK protein. Decreased AMPK protein concentration has been demonstrated in chronically overloaded and hypertrophied cardiac muscle as well (26). It may be that the decrease in total AMPK protein concentration despite its increased phosphorylation status with overload represents an adaptive response to chronic overload that protects against extremely high phospho-AMPK levels and allows hypertrophy to occur despite an increased phosphorylation status. Nevertheless, our calculations showed that total AMPK mass per whole muscle was not different between control and overloaded plantaris muscles of either age group in this investigation (data not shown). Therefore, the decreased

Fig. 3. Phosphorylation status at Thr172 on the α-subunit of AMPK is greater in O (n = 7) than YA (n = 7) rats and is elevated after 1 wk of functional overload in fast-twitch plantaris muscles (A), but not in slow-twitch soleus muscles (B). Phosphorylation status is calculated as the intensity of phospho-AMPK signal relative to total AMPK signal. Data (means ± SE) are expressed as a percentage of YA, control (sham-operated) values. *Significant main effect (P ≤ 0.05) for age, regardless of loading status. #Significant main effect for overload, regardless of age.

Fig. 4. Fast-twitch plantaris muscle phospho-acetyl CoA carboxylase (phospho-ACC) at Ser79 is greater in O (n = 7) than YA (n = 7) rats and is elevated after 1 wk of functional overload. Data (means ± SE) are expressed as a percentage of YA, control (sham-operated) values. *Significant main effect (P ≤ 0.05) for age, regardless of loading status. #Significant main effect for overload, regardless of age.
AMPK concentration may not necessarily represent a downregulation of AMPK synthesis or targeted AMPK degradation. Instead, such an effect may be due to a disproportionately greater upregulation of the synthesis of other muscle proteins (such as myosin, actin, etc.) in the hypertrophying muscle, which would result in a lower AMPK concentration per unit of muscle protein but similar absolute AMPK content per muscle.

The precise mechanisms by which AMPK may regulate hypertrophy are unclear. Because AMPK activation inhibits protein synthesis in skeletal muscle (10) and is inversely related to protein synthesis in cultured cardiac myocytes (22), a likely paradigm is that AMPK limits hypertrophy by impeding protein synthesis at the protein translation level. One pathway that appears to be particularly important for regulating the initiation of translation in hypertrophying skeletal muscle is the Akt/mammalian target of rapamycin (mTOR) pathway (4, 9–11, 38), which also contains multiple potential sites for regulatory integration with AMPK (10, 24). Other points of potential regulation by AMPK are protein elongation (23) or mRNA transcription (30). Elevated protein degradation is also a possibility, but this may be less likely because fast-twitch muscle hypertrophy in response to exercise has been reported to occur because of changes in protein synthesis and not protein degradation (47).

If AMPK is a negative regulator of skeletal muscle hypertrophy, then the increased AMPK phosphorylation status in overloaded fast-twitch muscle presents an interesting paradox. That is, a potential inhibitor of hypertrophy is actually shown to be activated in response to a stimulus that promotes hypertrophy. It should be remembered, however, that increased AMPK phosphorylation status is just one of many responses to the overload stimulus. In this respect, AMPK activation may be important in preventing excessively high energy expenditure on protein synthesis when cellular energy is low by partially counteracting the stimulatory effect of other signaling mechanisms such as the Akt/mTOR pathway (4, 9–11, 38). It therefore follows that the relative degree of AMPK activation could be important in regulating the extent of protein synthesis and hypertrophy that occurs under overload conditions. Accordingly, it appears that AMPK activation beyond a certain threshold must also be considered, because survival curves indicate that 24-mo-old male C57Bl/6 mice are ∼20% younger in biological age than the 30-mo-old male FBN rats used in the present investigation (44). Nevertheless, our present findings may have clinical importance for targeting the underlying mechanisms of age-related fast-twitch muscle atrophy, because in humans the decrease in muscle mass with aging is highly related to functional strength loss (32).

The age-related increase in AMPK signaling in nonoverloaded fast-twitch plantaris muscle (but not slow-twitch soleus muscle) in this investigation is intriguing considering the significantly greater age-related atrophy that we observed in the plantaris muscle (∼33%) compared with the soleus muscle (∼18%). These data are in agreement with the fact that fast-twitch fibers are also atrophied to a greater extent than slow-twitch fibers in aging human skeletal muscle (28, 31, 32, 45). Because skeletal muscle protein synthesis declines with age (49, 50, 52) and AMPK has been shown to negatively regulate protein synthesis (10, 22, 27), a fast-twitch-specific upregulation of AMPK activity may therefore explain in part why atrophy is more pronounced with age in fast-twitch plantaris muscles than in the slow-twitch soleus muscles of these animals. Although our data do not conclusively link elevated AMPK activity with age-related atrophy in such a fashion, the possibility that such a link may exist provides an exciting rationale for future exploration of the role of AMPK in sarcopenia. In this respect, consideration must be given to potential species differences. For instance, others have recently shown that hypoxia-stimulated AMPK-α2 activity in male C57Bl/6 mice may not be significantly elevated by 24 mo of age in the gastrocnemius muscle despite being elevated with age in the heart (16). However, the possibility of an age-related threshold must also be considered, because survival curves indicate that 24-mo-old male C57Bl/6 mice are ∼20% younger in biological age than the 30-mo-old male FBN rats used in the present investigation (44). Nevertheless, our present findings may have clinical importance for targeting the underlying mechanisms of age-related fast-twitch muscle atrophy, because in humans the decrease in muscle mass with aging is highly related to functional strength loss (32).
We did not directly measure AMPK activity in this investigation, but instead measured AMPK phosphorylation at Thr172. Although this is a possible limitation of our findings, it is known that Thr172 phosphorylation is a necessary and sufficient step for AMPK activation (21, 41) and is thus an established marker for AMPK activity (21, 37). Furthermore, we also verified AMPK activity in the plantaris muscles by measuring ACC phosphorylation at Ser79, a downstream site phosphorylated by AMPK in the lipid metabolism pathway (37). It has been suggested that phosphorylation at this site is a better indicator of true in vivo AMPK activity than the in vitro measurement of actual AMPK activity (36, 37). This is because the allosteric effects of AMP and phosphocreatine, which may be important in establishing true in vivo AMPK activity, are lost during sample processing for the in vitro activity assay. We found that ACC phosphorylation at Ser79 was highly correlated with our measures of AMPK phosphorylation status. We therefore believe that the increased AMPK phosphorylation status truly represents increased activity of the enzyme.

To summarize, we found that an age-related increase in AMPK phosphorylation in fast-twitch plantaris muscle, but not slow-twitch soleus muscle, was associated with an impaired hypertrophic capacity in fast-twitch muscle. In fact, a strong negative correlation was found between the degree of AMPK phosphorylation and the degree of hypertrophy in the overloaded plantaris muscles across both age groups. These data indicate that AMPK may play an important role in mediating the regulation of the hypertrophic response to skeletal muscle overload and may also be partly responsible for deficits in fast-twitch muscle hypertrophy with age. It is possible that this regulation by AMPK may occur through interaction with signaling pathways involved in the initiation of protein translation. Further research is needed to establish a causal relationship between AMPK activation and suppression of skeletal muscle hypertrophy, as well as to explore the possibility that increased AMPK activation with age may contribute to age-related atrophy in nonoverloaded fast-twitch skeletal muscle.

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