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AMP-activated protein kinase response to contractions and treatment with the AMPK activator AICAR in young adult and old skeletal muscle

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One characteristic of ageing skeletal muscle is a decline in mitochondrial function. Activation of AMP-activated protein kinase (AMPK) occurs in response to an increased AMP/ATP ratio, which is one potential result of mitochondrial dysfunction. We have previously observed higher AMPK activity in old (O; 30 months) *vs* young adult (YA; 8 months) fast-twitch muscle in response to chronic overload. Here we tested the hypothesis that AMPK would also be hyperactivated in O *vs* YA fast-twitch extensor digitorum longus muscles from Fischer$_{344}$ × Brown Norway (FBN) rats ($n = 8$ per group) in response to high-frequency electrical stimulation of the sciatic nerve (HFES) or injection of AICAR, an activator of AMPK. Muscles were harvested immediately after HFES (10 sets of six 3-s contractions, 10 s rest between contractions, 1 min rest between sets) or 1 h after AICAR injection (1 mg (g body weight)$^{-1}$ subcutaneously). The phosphorylations of AMPKα and acetyl-CoA carboxylase (ACC2; a downstream AMPK target) were both greatly increased ($P \leq 0.05$) in response to HFES in O muscles, but were either unresponsive (AMPK α) or much less responsive (ACC) in YA muscles. AMPK α2 activity was also greatly elevated in response to HFES in O muscles (but not YA muscles) despite a lower total AMPK α2 protein content in O *vs* YA muscles. In contrast, AMPK α2 activity was equally responsive to AICAR treatment in both age groups. Since mitochondrial content and/or efficiency could potentially underlie AMPK hyperactivation, we measured levels of mitochondrial proteins as well as citrate synthase (CS) activity. While CS activity was increased by 25% in O *vs* YA muscles, uncoupling protein-3 (UCP-3) protein level was upregulated with age by 353%. Thus, AMPK hyperactivation in response to contractile activity in aged fast-twitch muscle may be the result of compromised cellular energetics and not necessarily due to an inherent defect in responsiveness of the AMPK molecule per se.

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One of the major regulators of cellular energy metabolism is the enzyme AMP-activated protein kinase (AMPK). AMPK is a heterotrimeric enzyme that has been described as an intracellular 'low-fuel warning system' owing to the primary mechanism by which it is activated (Hardie & Carling, 1997). As energy-consuming processes within the cell break down ATP, the concentration of ADP begins to rise. Some of this ADP is used to regenerate ATP through the adenylate kinase reaction, which converts two ADP into one ATP and one AMP molecule. The resultant increase in AMP concentration leads to the activation of AMPK. This activation results both from phosphorylation of Thr^{172} on AMPK's α subunit (without which AMPK is essentially inactive), and also by way of allosteric activation of phosphorylated AMPK (Winder & Thomson, 2007).

Phosphorylation of AMPK is accomplished by several upstream kinases. In skeletal muscle the primary AMPK kinase is LKB1, which requires two binding partners, mouse protein 25 (MO25) and Ste-related adapter protein (STRAD), for full activity. Ablation of LKB1 in skeletal muscle essentially eliminates AMPK phosphorylation (Sakamoto *et al.* 2005; Koh *et al.* 2006; Thomson *et al.* 2007*a*,*b*, 2008), although, calcium–calmodulin-dependent protein kinase kinase

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(CaMKK) also appears to play a role in the phosphorylation of AMPK in skeletal muscle under some circumstances (Jensen *et al.* 2007; Witczak *et al.* 2007). While phosphorylation at Thr^{172} is a requisite for its activity, AMPK's phosphorylation state appears to be regulated primarily at the level of its suitability as a target for protein phosphatase 2C (PP2C), rather than as a function of its interaction with upstream kinases (Davies *et al.* 1995; Suter *et al.* 2006). Additionally, AMPK activity may be regulated by hormonal signals, and thus may be controlled via cross-talk between signalling pathways (Winder & Thomson, 2007).

Once activated, the general function of AMPK is to restore the energy charge of the cell. This is accomplished through signalling to many targets. For instance, AMPK acutely stimulates glucose uptake (Merrill *et al.* 1997; Hayashi *et al.* 1998) and fat oxidation (Merrill *et al.* 1997), while inhibiting protein synthesis (Bolster *et al.* 2002). When chronically activated, AMPK promotes mitochondrial biogenesis by increasing the concentrations of peroxisome proliferator activated receptor γ (PPARγ) coactivator-1 (PGC-1) and associated mitochondrial proteins (Winder *et al.* 2000; Bergeron *et al.* 2001; Suwa *et al.* 2003).

ATP content and rate of ATP production under low-energy conditions are lower in old (O) compared to young muscles (Chen Scarabelli *et al.* 2008). This is probably due to a decline in mitochondrial function that occurs with old age in human (Tonkonogi *et al.* 2003; Short *et al.* 2005) and rodent (Mansouri *et al.* 2006; Figueiredo *et al.* 2008) skeletal muscle. The impairment in ATP generating capacity may be due to a decline in mitochondrial content in aged skeletal muscle (Corsetti *et al.* 2008), or increased mitochondrial uncoupling (efficiency or amount of ATP produced per unit of oxygen consumed) observed in aged muscles, particularly type II fibres (Conley *et al.* 2007*a*,*c*). Given the mechanism by which AMPK is activated, one might postulate that AMPK activity should therefore be elevated in old compared to young muscles, particularly under conditions of energy stress. In support of this hypothesis, we previously observed that AMPK phosphorylation was greater in fast-twitch plantaris muscles from O *vs* YA rats after a week of synergist ablation-induced overload (Thomson & Gordon, 2005). Our findings are essentially in agreement with human data reported by Drummond *et al.* (2008), which showed that vastus lateralis AMPK phosphorylation was elevated after resistance exercise in old, but not young individuals after resistance exercise. However, in contrast to these findings, Reznick *et al.* (2007) reported that, unlike that in young muscles, AMPK activity in old muscles was responsive to neither exercise training nor the AMPK activators 5′-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) and β-guanidinopropionic acid (β-GPA). The authors suggested that reduced AMPK activity might contribute to age-related impairment of mitochondrial function. Further, Qiang *et al.* (2007) reported that AMPK phosphorylation was lower in old *vs* young muscles basally, but that old muscles were nonetheless responsive to AICAR treatment. Thus, our understanding of the effect of age on skeletal muscle AMPK activity is still unclear. In an effort to further clarify, we studied the effect of AICAR or muscle contraction upon AMPK activity in YA and O extensor digitorum longus (EDL; fast-twitch) muscles from FBN rats. Given our previous findings, we hypothesized that AMPK would be hyperactivated in response to both stimuli in O compared to YA muscles.

Methods

Animals and ethical approval

YA (8 months; $n = 8$) and O (30 months; $n = 8$) male $Fischer_{344}$ × Brown Norway (FBN) hybrid rats were used in this study. They were housed in the East Carolina University animal care facility at the Brody School of Medicine on a 12 h light–dark cycle with free access to water and standard chow. All procedures were approved by the East Carolina University Animal Care and Use Committee.

AICAR injection

Rats (YA and O) were injected subcutaneously with AICAR dissolved in saline (1 mg (g body wt)$^{-1}$; 75 mg AICAR (ml saline)$^{-1}$) or with an equivalent volume of saline without AICAR. After injection, rats were anaesthetized by isoflurane inhalation (2–4% with nitrous oxide in supplemental oxygen) sufficient to achieve surgical anaesthetic depth. Fifty minutes after AICAR or saline injection, ketamine (11.25 mg (100 g body wt)$^{-1}$) and xylazine (1.25 mg (100 g body wt)$^{-1}$) were injected intraperitoneally. This was done to facilitate the removal of tissues by eliminating the need for administering vapourized anaesthetic through a nose-cone. Ten minutes after this injection, tibialis anterior (TA) and EDL muscles were removed from the hindlimbs, frozen between metal tongs cooled to the temperature of liquid nitrogen, and frozen at −80°C until later analysis. Thus, the rats were anaesthetized for 1 h prior to the removal of tissues, allowing substantial time for AMPK activity to equilibrate to true resting levels.

Electrical stimulation of sciatic nerve

After anaesthesia with isoflurane, the sciatic nerve of the left hindlimb of saline-injected rats was isolated just proximal to the point of trifurcation. Stainless steel electrodes were attached, and a HFES electrical

stimulation bout was elicited using a Grass Stimulator (100 Hz; Grass Model S48 Stimulator, Quincy, MA, USA). The bout consisted of 10 sets of six maximal contractions (3 s duration with 10 s rest between contractions) of the hindlimb musculature, with 1 min rest periods between sets, for a total contraction bout of about 22 min. The contralateral (right) hindlimb was not subjected to the operation or electrical stimulation. Timing of the contraction bout was such that it ended 1 h after saline injection. Ketamine–xylazine was administered 10 min prior to the end of stimulation, after which TA and EDL muscles were removed immediately (within 1–3 min) after cessation of contractions (60 min after saline injection) and harvested as described above.

Homogenization

Muscles were ground-glass homogenized in 20 volumes of homogenization buffer (50 mM tris-HCl, pH 7.4; 250 mM mannitol; 50 mM NaF; 5 mM sodium pyrophosphate; 1 mM EDTA; 1 mM EGTA; 1% Triton X-100; 50 mM β-glycerophosphate; 1 mM sodium orthovanadate; 1 mM DTT; 1 mM benzamidine; 0.1 mM phenylmethane sulfonyl fluoride; 5 μg ml^{-1} soybean trypsin inhibitor). A portion of the raw EDL homogenate was separated for citrate synthase activity determination. The remaining raw homogenate was centrifuged at 800 *g*. The clarified supernatant was separated into a fresh tube, and the pellet was then centrifuged at 10 000 *g*, and this supernatant was combined with the previous one. Homogenates were then analysed for protein content using the DC Protein Assay (Biorad Laboratories, Hercules, CA, USA).

Citrate synthase activity assay

Citrate synthase activity was measured from diluted whole EDL muscle homogenates as described by Srere (1969).

Glycogen concentration assay

Glycogen concentration was determined in TA muscles ($n = 6$ per group) as previously described (Passonneau & Lowry, 1993).

AMPK activity assay

AMPK activity of α1 and α2 subunits immunoprecipitated from EDL homogenates towards SAMS peptide was assessed and expressed as picomoles phosphate incorporated into HHMRSAMSGLHLVKRR-OH (SAMS) peptide per gram tissue per minute, as described previously (Park *et al.* 2002). Due to a lack of adequate tissue for all muscles, this assay was only performed on six samples per condition.

Western blotting

Clarified muscle homogenates were diluted in 2× loading buffer at a concentration of 1 μg μl^{-1}. Equal amounts of protein were separated via SDS-PAGE then transferred to PVDF membranes, which were then stained with Ponceau S, and examined for equal protein loading and transfer. Membranes were then washed with tris-buffered saline + 0.1% tween (TBST), and blocked using 5% non-fat dry milk (NFDM) in TBST. Membranes were washed briefly with TBST and then incubated overnight on a shaker at 4°C with primary antibody diluted in 1% BSA, pH 7.6. Primary antibody dilutions were as follows: phospho-AMPK α (Cell Signalling Technology no. 2531, Beverly, MA, USA), 1 : 4000; total AMPK α (Cell Signalling no. 2532), 1 : 5000; AMPK α1 and α2 (custom made through Affinity Bioreagents, Golden, CO, USA), 1 : 50 000; phospho-ACC (Cell Signalling no. 3661), 1 : 4000; total ACC (streptavidin-HRP, GE Life Sciences, RPN1231), 1 : 20 000; LKB1 (Upstate no. 07-694, Lake Placid, NY, USA), 1 : 5000; PGC-1 (Calbiochem no. 516557, La Jolla, CA, USA), 1 : 2000; cytochrome c oxidase subunit 1 (COX-1; Invitrogen no. A6403, Carlsbad, CA, USA), 1 : 4000; cytochrome C (Santa Cruz no. 13156), 1 : 10 000; UCP-2 (Millipore no. AB3226, Temecula, CA, USA), 1 : 1000; UCP-3 (Affinity Bioreagents no. PA1-055, Golden, CO, USA), 1 : 2000.

Statistics

Statistical comparisons were made by either a 1-way or 2-way ANOVA, using NCSS statistical analysis software (NCSS, Kaysville, UT, USA). Data are presented as means ± standard error of the mean (S.E.M.). Statistical significance was set at $P \leq 0.05$.

Results

AMPK activity

In vitro activity of the AMPK α1 subunit was higher in O *vs* YA muscles regardless of treatment, and increased with AICAR, but not HFES, in YA but not O muscles (Fig. 1*A*). In control muscles, *in vitro* activity of the AMPK α2 subunit was not different between ages, and increased in both YA and O muscles after AICAR treatment (Fig. 1*B*). HFES, on the other hand, failed to activate AMPK α2 in YA muscles, but significantly increased activity by 220% in O muscles (Fig. 1*B*). AMPK phosphorylation at Thr^{172} closely corresponded to the *in vitro* activity data for AMPK α2 (Fig. 1*C*). Phosphorylation of ACC2, representing *in vivo* activity of AMPK (Park *et al.* 2002), was also similar to AMPK α2 *in vitro* activity, except that it was significantly increased by HFES in YA muscles. Nevertheless, this increase was significantly less than in

O muscles (Fig. 1*D*). Also, the relative increase in ACC2 phosphorylation was similar between ages after AICAR treatment, suggesting that *in vivo* AICAR-stimulated AMPK activity toward ACC is not different with age.

Protein concentrations for AMPK and LKB1

AMPK α1 protein concentration was 120% greater in O *vs* YA muscles (Fig. 2*A*). Conversely, AMPK α2 protein concentration in O muscles was only 75% of that in YA muscles (Fig. 2*B*). Protein levels of LKB1, the major upstream kinase for AMPK in skeletal muscle, were not different between ages (Fig. 2*C*).

Mitochondrial protein concentrations

Protein concentrations for PGC1α (a key factor in the regulation of mitochondrial biogenesis) as well as cytochrome C and UCP-2 (a mitochondrial protein) were not significantly different between O and YA muscles (Fig. 3*A*, *B* and *D*). On the other hand, COX-1 and UCP-3 concentrations, as well as citrate synthase activity, were significantly greater in O *vs* YA muscles (181%, 353% and 25% higher *vs* YA, respectively; Fig. 3*C*, *E* and *F*).

Glycogen concentration

The ANOVA analysis indicated that glycogen concentration declined significantly (main effect) with HFES in TA muscles (31.8 ± 1.6 *vs* 28.3 ± 2.0 μmol (g muscle)$^{-1}$ in YA and 36.1 ± 3.1 *vs* 25.6 ± 2.8 μmol (g muscle)$^{-1}$ in O). This main effect of HFES in the two-way ANOVA was obviously due in large part to the decline in glycogen concentration in O muscle; subsequent one-way ANOVAs within each age group indicated a significant glycogen decrease in O ($P = 0.03$)

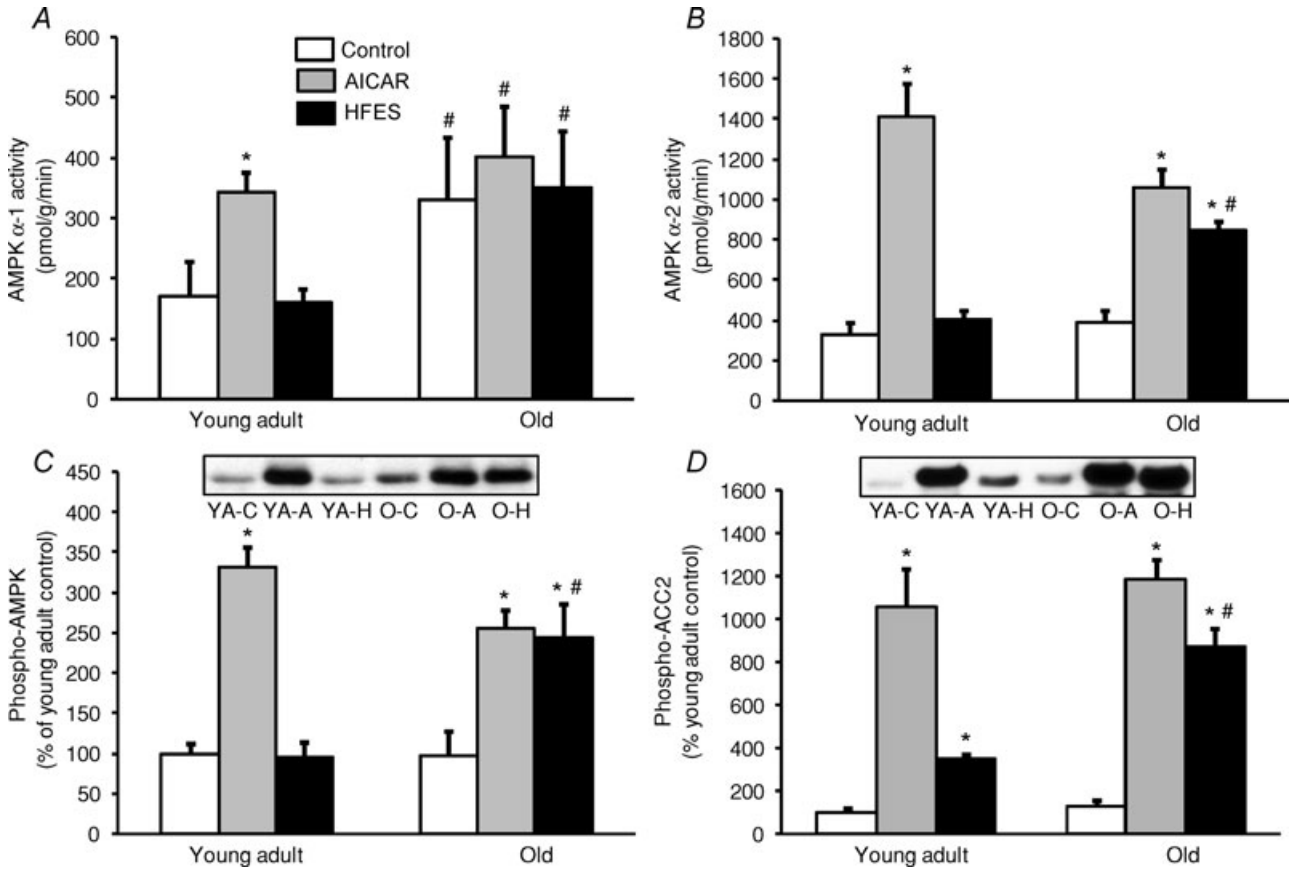

Figure 1. AMPK activation in extensor digitorum longus muscles from young adult (8 months) and old (30 months) rats after AICAR treatment and high-frequency electrical stimulation (HFES)
Rats were injected with 1 mg (g body wt)$^{-1}$ AICAR or an equivalent volume of saline (control and HFES). Contractions were induced by HFES of the sciatic nerve. Muscles were harvested 1 h after saline or AICAR treatment, and immediately post-HFES. *A*, incorporation of radiolabelled phosphate into SAMS peptide by immunoprecipitated AMPK α1 ($n = 6$). *B*, incorporation of radiolabelled phosphate into SAMS peptide by immunoprecipitated AMPK α2 ($n = 6$). *C*, AMPK (α1 and α2) phosphorylation ($n = 8$). *D*, phosphorylation of the AMPK substrate acetyl-CoA. carboxylase (ACC)-2 ($n = 8$). YA, young adult; O, old; C, control (saline treated); A, AICAR treated; H, HFES treated. *Significant difference ($P < 0.05$) *vs* corresponding saline-treated control muscles within age group. #Significant difference *vs* corresponding young adult muscles.

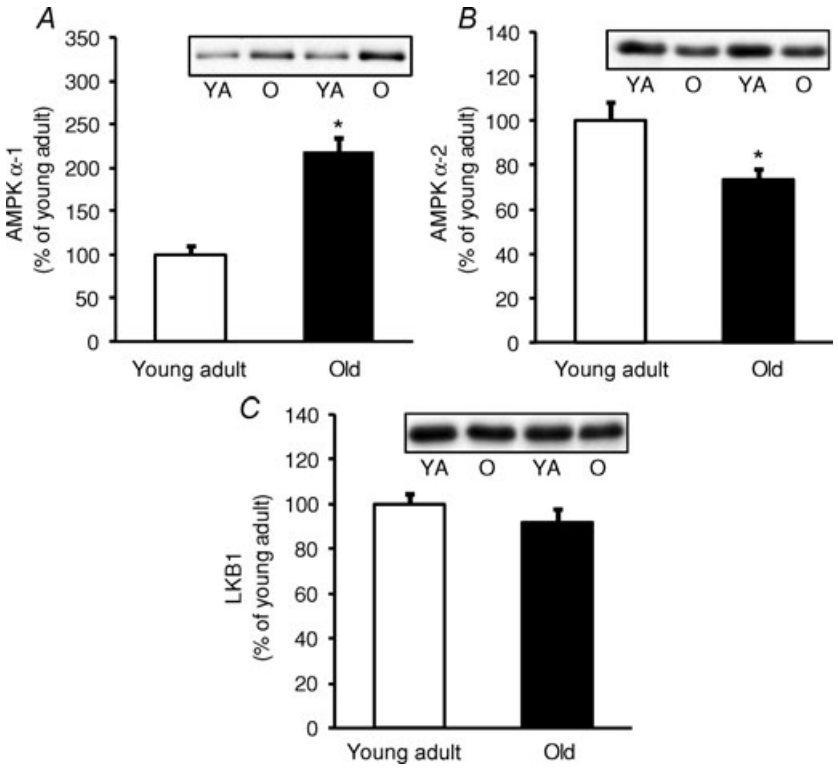

Figure 2. AMPK and LKB1 protein concentrations in extensor digitorum longus muscles from young adult (8 months) and old (30 months) rats
A, AMPK α1 protein concentration ($n = 8$). *B*, AMPK α-2 concentration ($n = 8$). *C*, LKB1 protein concentration ($n = 8$). YA, young adult (8 months); O, old (30 months). *Significant difference *vs* young adult ($P < 0.05$).

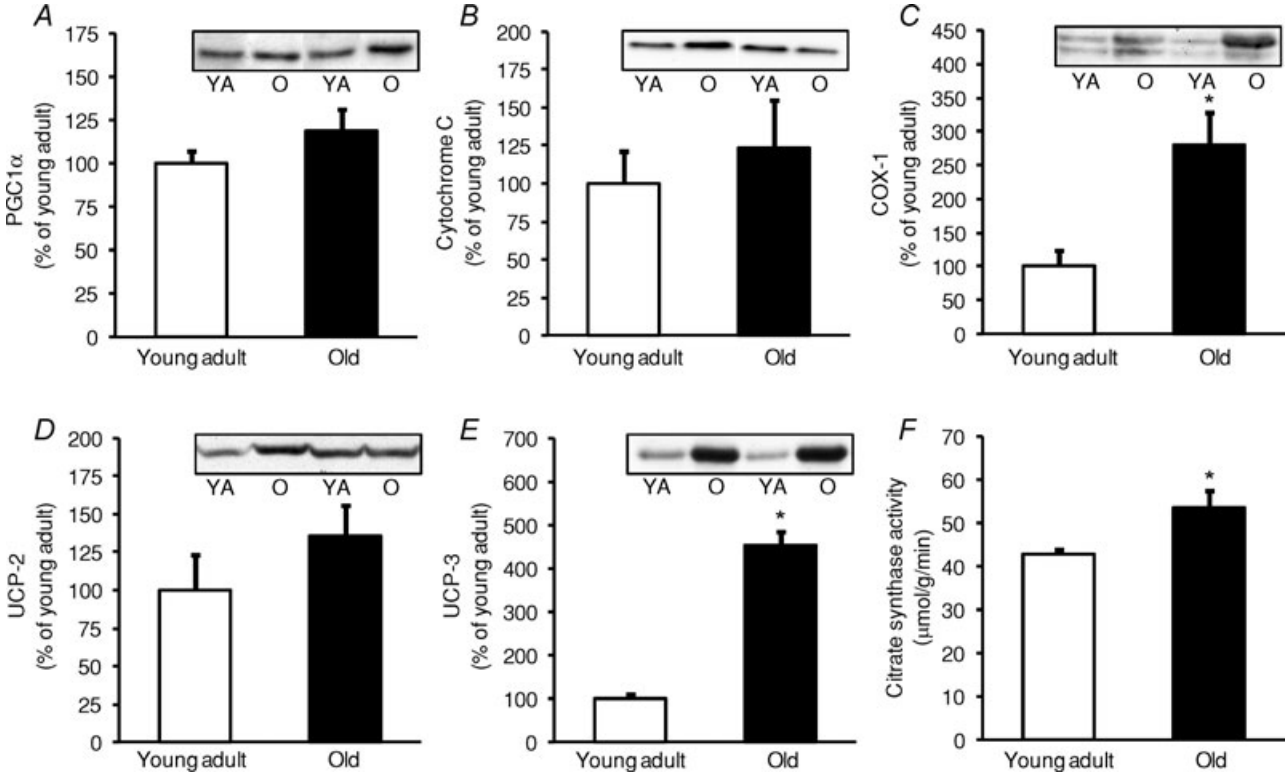

Figure 3. PGC-1 and mitochondrial protein concentrations in extensor digitorum longus muscles from young adult (8 months) and old (30 months) rats
A, PGC-1α protein concentration ($n = 8$). *B*, cytochrome C concentration ($n = 8$). *C*, COX-1 protein concentration ($n = 8$). *D*, UCP-2 protein concentration ($n = 8$). *E*, UCP-3 protein concentration ($n = 8$). *F*, citrate synthase activity ($n = 8$). YA, young adult (8 months); O, old (30 months). *Significant difference *vs* young adult ($P < 0.05$).

but not YA ($P = 0.20$) muscles with HFES. Furthermore, when expressed as percentage decline in glycogen in HFES *vs* contralateral control muscles from the same animal, glycogen depletion was 3-fold greater after contraction in O ($28.7 \pm 5.5\%$ decline) *vs* YA ($9.6 \pm 9.2\%$ decline) muscles.

Discussion

The primary finding of this study is that HFES-induced muscle contractions activate AMPK to a much greater extent in O than in YA skeletal muscle from FBN hybrid rats, and that this AMPK hyperactivation in O muscles is specific to the $\alpha 2$ subunit. In contrast, there were no differences with age in the AICAR-induced AMPK response. The HFES contraction bout employed here was designed to simulate vigorous resistance exercise. As such, when it is repeated chronically it promotes activation of signalling pathways that promote hypertrophy within the muscle (Baar & Esser, 1999). However, it is ineffective at activating AMPK, at least covalently, in YA muscle, probably due to the relatively abundant rest periods included as compared with other AMPK-activating contraction protocols (Hutber *et al.* 1997; Vavvas *et al.* 1997; Hayashi *et al.* 1998). When applied *in vitro*, a similar intermittent HFES contraction protocol also failed to activate AMPK in muscles from YA rats whereas continuous low-frequency stimulation strongly activated AMPK (Atherton *et al.* 2005). The intermittent rest periods during HFES probably prevent an appreciable accumulation of AMP during the bout in YA muscles, thereby preventing activation of AMPK.

In contrast to YA, O muscles exhibited an accentuated and robust AMPK $\alpha 2$ response to HFES. This result could not have been due to elevated $\alpha 2$ protein content, as this was lower in the O compared with YA muscles. Furthermore, the enhanced activation of AMPK in O muscle cannot be attributed to an increased concentration of LKB1 (the major upstream AMPK kinase (AMPKK) in skeletal muscle (Sakamoto *et al.* 2005; Koh *et al.* 2006; Thomson *et al.* 2007b)), because it was equal between ages. Also, previous reports (Suter *et al.* 2006; Sanders *et al.* 2007) indicate that its activity towards AMPK is constitutive and not regulated directly by phosphorylation or other known means. Thus, assuming that the AMPK $\alpha 2$ hyperactivation in O muscles after contractions is due to the classic activating mechanism of an increased AMP/ATP ratio, one possibility is that it occurred because of an inability to sufficiently replenish ATP during the rest periods between contractions, which could suggest a mitochondrial insufficiency.

Although cytochrome C content was not different between ages, we did observe 25% greater activity for citrate synthase in EDL muscles from O *vs* YA rats, which agrees with some previously reported findings (Sanchez *et al.* 1983; Masuda *et al.* 2008). Likewise, COX-1 protein content was elevated by 180% in O *vs* YA EDL. Moreover, by far the most pronounced difference in mitochondrial protein content that we observed was the 353% higher skeletal EDL muscle UCP-3 content in O compared to YA EDL. While the functional role of UCP-3 in skeletal muscle is controversial (Bezaire *et al.* 2007), if present in sufficient quantity above normal levels, it can act as an uncoupling protein, thereby decreasing mitochondrial efficiency (ATP produced/O_2 consumed). For example, overexpression of UCP-3 in cells and transgenic mice leads to mitochondrial uncoupling (Clapham *et al.* 2000; Cadenas *et al.* 2002). Likewise, decreased proton leak and increased mitochondrial coupling (Gong *et al.* 2000; Vidal-Puig *et al.* 2000), as well as increased rate of fasting ATP synthesis (Cline *et al.* 2001) have been reported in UCP-3 knockout mice, although this is not a consistent finding (Cadenas *et al.* 2002). Induction of mild UCP-3-mediated uncoupling via superoxide has also been demonstrated in skeletal muscle mitochondria (Echtay *et al.* 2002), which is important in regards to ageing because reactive oxygen species production is elevated in aged skeletal muscle (Chabi *et al.* 2008). Whether or not the increased UCP-3 protein concentration in aged muscle that we have reported here is sufficient to cause significant uncoupling is not known; however, mild uncoupling has been observed in aged human (Amara *et al.* 2007) and rodent (Marcinek *et al.* 2005) skeletal muscle, which, along with generalized mitochondrial dysfunction, are indicative of a potential mechanism that may drive the hyperactivation of AMPK under conditions of energy stress. That is, as the energy demand of the muscle fibre is increased, such mitochondrial dysfunction and/or uncoupling could certainly limit the ATP-generating capacity of the fibre in aged muscle, thus leading to activation of AMPK under circumstances that normally would not strain the energy-generating machinery. In the case of the present study, uncoupling of oxidative phosphorylation may result in a mitochondrial inefficiency that would negatively impact on the ability of the cell to recover from the energy stress of the contraction bout. It must be emphasized, however, that this hypothesized relationship between UCP-3 levels and AMPK hyperactivation is based only upon the correlational data presented above, and will therefore need to be confirmed through further experimentation.

Another potential factor that may have contributed to the contraction-induced AMPK hyperactivation that we observed in O muscles is glycogen concentration. High glycogen has been shown to inhibit AMPK activation in skeletal muscle (Derave *et al.* 2000; Wojtaszewski *et al.* 2002). Although we were unable to assess glycogen concentration in the EDL muscles from our rats due to a lack of tissue, we did measure it in a limited number of

tibialis anterior (TA) muscles from the same animals. Like the EDL, the TA contracts in opposition to the gastrocnemius muscle when the sciatic nerve is stimulated. Accordingly, ACC phosphorylation in the TA responded similarly to that in the EDL after contraction, and UCP-3 levels were similarly elevated with age (data not shown). Although glycogen concentration significantly declined in both YA and O TA muscles after contraction (main effect), this statistical effect was probably driven primarily by the glycogen decline in O muscles which was 3 times greater than the decline in YA muscles. Although this age-related difference was not statistically significant in our study ($P = 0.09$; probably due to the limited number of TA muscles available for analysis) previous reports have also shown greater glycogen depletion in O muscle during contraction compared to YA (Cartee, 1994; Hopp, 1996). If glycogen levels did indeed decline in the EDL as they appear to have done in the TA muscle, this could explain, at least in part, the enhanced activation of AMPK that we observed in the O EDL muscles.

Reznick *et al.* have reported that AMPK activation was completely impaired in old Fischer$_{344}$ rats in response to aerobic exercise, AICAR and β-GPA feeding, and that β-GPA failed to stimulate mitochondrial biogenesis in their old rats (Reznick *et al.* 2007). This would theoretically indicate that AMPK is somehow unresponsive to stimuli in aged muscle; however, the present investigation presents evidence in strong opposition to the notion of impaired contraction-induced AMPK activation with age, at least in response to HFES. Moreover, we have also previously shown a similar AMPK hyperactivation in aged muscle in response to the increased activity of chronic overload (Thomson & Gordon, 2005). The fact that our present data show no age-related difference in AICAR-stimulated AMPK activity (i.e. in response to an AMP analogue that does not disturb cellular energetics (Corton *et al.* 1995)) indicates that there may be no age-related dysfunction in the AMPK molecule *per se*. Similarly, Qiang and co-workers also showed that AMPK phosphorylation in old muscle increases in response to AICAR (Qiang *et al.* 2007). Since AICAR-stimulated AMPK activity is independent of the effect of cellular bioenergetics, our data collectively indicate that AMPK hyperactivation in response to contractile activity in aged fast-twitch muscle probably results from upstream bioenergetic impairments such as mitochondrial dysfunction.

The findings of Reznick *et al.* (2007) also contrast with findings in human muscle in which AMPK hyperphosphorylation was observed after a strength training bout (Drummond *et al.* 2008). Furthermore, exercise-induced improvements in mitochondrial function and content similar to those in young individuals have been observed with exercise in the elderly (Conley *et al.* 2007*b*). These studies suggest that the exercise training-stimulated signalling mechanisms promoting mitochondrial biogenesis are intact in old muscles, and argue against a cross-species generalized defect in AMPK signalling with age. Our findings are likewise in opposition to the notion of impaired contraction-induced AMPK activation with age.

The conflicting findings mentioned above may be due to a number of factors, but prominent amongst them is the difference in strain or species studied. Reznick *et al.* (2007) used the Fischer$_{344}$ strain in their study, which is commonly used in ageing studies, but may not be the rodent model that is most representative of ageing human muscle. In our study, which agreed more closely with the human study of Drummond *et al.* (2008), Fischer$_{344}$ $\times$ Brown Norway hybrid (FBN) rats were used. This strain of rat is considered by some to be a preferable ageing model compared to the Fischer$_{344}$ strain for muscle studies because of the relatively low incidence of diseases with age (Lipman *et al.* 1996). Such pathological disorders could result in observed differences between young and old animals being erroneously attributed to the ageing process that in truth result from the presence of disease. Furthermore, FBN rats exhibit an ageing process that is more similar to that in human muscle in terms of loss of muscle function and mass (Rice *et al.* 2005, 2008). In regards to the lack of exercise-induced AMPK activation observed by Reznick *et al.* (2007), several methodological factors may also have contributed to the conflicting results. In that study, treadmill running was used, and running speed was normalized to 85% of maximum capacity for both old and young rats. Since maximal running capacity is lower for old than for young rats, the old rats ran at nearly half the speed of the young rats. Since exercise capacity could be affected by cardiovascular or neurological factors extrinsic to skeletal muscle, the metabolic stimulus for AMPK activation may have been different between ages for that treadmill running bout. Additionally, the rats were trained for 4 days after the determination of maximum running capacity and prior to the experimental exercise bout. It is known that exercise training diminishes the AMPK response to a subsequent exercise bout (Durante *et al.* 2002), but whether this phenomenon begins within four training bouts or is affected by ageing is unknown. Therefore, the training prior to the exercise bout may have been another confounding factor. Finally, the possibility that activation of AMPK by aerobic-type muscle activity such as treadmill running is affected by ageing differently than intermittent resistance-type exercise such as that used in this study and that by Drummond *et al.* (2008) cannot be discounted.

AMPK α1 activity was elevated in O *vs* YA muscles across treatments. AICAR treatment in YA muscles activated AMPK α1 to a similar level seen in saline-treated O muscles, while AMPK α1 activity in O muscles did not increase. We cannot say definitively whether the lack of

AICAR-stimulated activation in the O muscles is due to a defect in $\alpha 1$ signalling, or simply because basal levels of $\alpha 1$ activity are already elevated to a near-maximal level. Regardless, the importance and role of $\alpha 1$ AMPK complexes in skeletal muscle is not currently well understood, and AMPK $\alpha 2$ activity and responsiveness to exercise is much greater than that of AMPK $\alpha 1$ (Durante *et al.* 2002).

In conclusion, we observed that AMPK was activated in O, but not YA fast-twitch skeletal muscle in response to a bout of electrically stimulated muscle contractions designed to mimic resistance exercise. We also observed similar AICAR-stimulated activation of AMPK in O compared to YA muscles. Our findings demonstrate that AMPK activation in response to HFES in the EDL muscle is not defective with age in FBN rats. The disproportionately elevated content of UCP-3 protein relative to other mitochondrial proteins in O EDL muscles suggests that mitochondrial inefficiency may potentially lie upstream of AMPK hyperactivation in response to HFES contractions in aged muscle. Our findings are in contrast to data previously collected from other rat strains, which illustrates the importance of taking species and potential strain differences into account when interpreting age-related data.

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