Published in final edited form as:

Mech Ageing Dev. 2012 ; 133(0): 655–664. doi:10.1016/j.mad.2012.09.001.

Reduced AMPK-ACC and mTOR signaling in muscle from older men, and effect of resistance exercise

Mengyao Li^{a,b,c}, Lex B. Verdijk^d, Kei Sakamoto^e, Brian Ely^{a,b,c}, Luc J.C. van Loon^d, and Nicolas Musi^{a,b,c,*}

^aGeriatric Research, Education and Clinical Center, Audie L. Murphy Veterans Hospital, 7400 Merton Minter Blvd, San Antonio, TX 78229, United States ^bCenter for Healthy Aging, University of Texas Health Science Center at San Antonio, 8403 Floyd Curl Dr., San Antonio, TX 78229, United States ^cTexas Diabetes Institute, 701 S. Zarzamora St., San Antonio, TX 78207, United States ^dDepartment of Human Movement Sciences, NUTRIM School for Nutrition, Toxicology and Metabolism, Maastricht University Medical Centre+, P.O. Box 616, 6200 MD Maastricht, The Netherlands ^eNestlé Institute of Health Sciences SA, Campus EPFL, Quartier de l'innovation, Bâtiment G, 1015 Lausanne, Switzerland

Abstract

AMP-activated protein kinase (AMPK) is a key energy-sensitive enzyme that controls numerous metabolic and cellular processes. Mammalian target of rapamycin (mTOR) is another energy/nutrient-sensitive kinase that controls protein synthesis and cell growth. In this study we determined whether older versus younger men have alterations in the AMPK and mTOR pathways in skeletal muscle, and examined the effect of a long term resistance type exercise training program on these signaling intermediaries. Older men had decreased AMPK α 2 activity and lower phosphorylation of AMPK and its downstream signaling substrate acetyl-CoA carboxylase (ACC). mTOR phosphorylation also was reduced in muscle from older men. Exercise training increased AMPK α 1 activity in older men, however, AMPK α 2 activity, and the phosphorylation of AMPK, ACC and mTOR, were not affected. In conclusion, older men have alterations in the AMPK-ACC and mTOR pathways in muscle. In addition, prolonged resistance type exercise training induces an isoform-selective up regulation of AMPK activity.

Keywords

Aging; Skeletal muscle; AMPK; mTOR; Resistance exercise

1. Introduction

AMP-activated protein kinase (AMPK) is an enzyme that functions as a metabolic master switch. AMPK is a heterotrimeric protein complex consisting of three subunits, α , β and γ . There are two α subunit isoforms, two β subunit isoforms and three γ subunit isoforms (Hardie and Carling, 1997; Stapleton et al., 1997; Steinberg and Kemp, 2009). The α subunit possesses the catalytic activity of the enzyme. AMPK complexes containing the α 2 isoform are predominant in skeletal muscle, heart, and liver, while AMPK α 1 containing complexes are ubiquitously expressed. Upon increases in the AMP/ATP ratio, the activity of AMPK increases through coordinated regulation of allosteric modification and α subunit

*Corresponding author at: Geriatric Research, Education and Clinical Center, 7400 Merton Minter Blvd, San Antonio, TX 78229, United States. Tel.: +1 210 6175197; fax: +1 210 6175312. musu@uthscsa.edu (N. Musi).

phosphorylation at site Thr172 by upstream kinases such as LKB1 and calcium-calmodulin dependent protein kinase kinase (CaMKK), and decreased dephosphorylation by phosphatases (Hardie and Carling, 1997; Kemp et al., 1999). In human muscle AMPK is phosphorylated and activated robustly by energy-consuming stimuli such as muscle contraction and hypoxia (Musi et al., 2001a; Wadley et al., 2006).

AMPK works to sustain cellular ATP, and it does so by modifying diverse metabolic and cellular pathways. For example, the increases in AMPK activity caused by contraction are thought to mediate, at least partially, exercise-induced increases in skeletal muscle glucose transport (Hayashi et al., 2000; Mu et al., 2001). Activation of AMPK also induces some of the adaptations to prolonged endurance type exercise training and increased activity of key mitochondrial enzymes (Frosig et al., 2004; Winder et al., 2000). Another important role of AMPK is to induce muscle fat oxidation through phosphorylation of acetyl CoA carboxylase (ACC) (Hardie and Carling, 1997). Considering the increasing evidence indicating that several energy-regulating pathways (anaerobic glycolysis, oxidative phosphorylation) may be altered in aging skeletal muscle (Russ and Lanza, 2011), and the key role that the AMPK-ACC pathway plays on metabolic regulation, it is important to establish whether human aging is associated with alterations in the AMPK-ACC axis. Indeed, animal studies have revealed decreased flux through the AMP-ACC pathway in muscle from old rodents (Qiang et al., 2007; Reznick et al., 2007), although there are limited data about this pathway in aging human muscle. Thus, one goal of this study was to determine whether older subjects have alterations in the AMPK-ACC axis in skeletal muscle.

Physical activity plays a fundamental role in promoting good health in older individuals. Resistance type exercise, in particular, stimulates protein metabolism (Yarasheski et al., 1993), which helps to increase muscle mass/strength and improve functional status. Yet, the molecular basis underlying the beneficial effects conveyed by resistance exercise in the older is not fully understood. The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that integrates environmental stimuli such as nutrients (i.e. amino acids) and growth factors (i.e. insulin, insulin-like growth factor 1) to control protein synthesis (Raught et al., 2001). Regulation of protein synthesis by mTOR occurs through the phosphorylation of the downstream targets including initiation factor 4E binding protein 1 (4E-BP1) and S6 kinase (also known as p70S6K) (Dufner and Thomas, 1999; Jefferies et al., 1997; Raught and Gingras, 1999; Sonenberg and Gingras, 1998). Similar to AMPK, mTOR also responds to mechanical stimuli (i.e. muscle contraction). While some studies have reported that resistance type exercise acutely stimulates AMPK and mTOR (Dreyer et al., 2010; Koopman et al., 2006), it is not clear whether chronic resistance training leads to sustained activation of these signaling intermediaries. Therefore, another goal of this study was to investigate whether a long term resistance type exercise program stimulates the AMPK-ACC and mTOR pathways.

2. Materials and methods

2.1. Subjects

32 Younger (aged 19–41) and 32 older (aged 64–86) male subjects were recruited through advertisements in local newspapers and with flyers at the Maastricht University. Each subject underwent a medical history, physical examination, screening laboratory tests, and a 75-g oral glucose tolerance test (OGTT). Subjects with cardiac or peripheral vascular disease, orthopedic limitations and/or type 2 diabetes were excluded. All subjects were living independently, sedentary (not more than one session of exercise per week), and had no history of participating in any structured exercise training program for at least 5 years. Subjects did not report problems in activities of daily living (walking, climbing stairs, rising from a chair), and did not need any assistive equipment (e.g. using a cane) while walking.

Eleven older subjects were taking anti-hypertensive medications, seven older individuals were taking lipid-lowering medications, and six older subjects were taking medications for benign prostate hypertrophy. The study was approved by the Medical Ethics Committee of the Maastricht University Medical Centre and all subjects gave written consent.

2.2. Exercise program and biopsy sampling

Twenty five older subjects were engaged in a supervised resistance type exercise training, which was performed 3 times a week (Mon–Wed–Fri) for a 12 week period. Exercise sessions were always performed in the morning, at the same time of day. Training consisted of a 5 min warm-up on a cycle ergometer, followed by 4 sets on both the leg press and leg extension machines (Technogym, Rotterdam), followed by a 5 min cooling-down period on the cycle ergometer. Workload was increased from 60% of 1RM (1-repetition maximum) in week 1 (10–15 repetitions per set) to 75% of 1RM in week 4 and after (8–10 repetitions per set). Resting periods of 90 s and 3 min were allowed between sets and between exercises, respectively. Workload intensity was adjusted based on 4-weekly 1RM testing (Verdijk et al., 2009b). In addition, workload was increased when more than 8 repetitions could be performed in 3 out of 4 sets. Post-training 1RM strength assessment was performed 2 days after the final training session.

After local anesthesia, percutaneous needle biopsies (50–80 mg) were taken from the *vastus lateralis* muscle, ~15 cm above the patella and 2–3 cm below entry through the fascia (Bergstrom, 1975). Any visible non-muscle tissue was removed immediately, and biopsy samples were frozen in liquid nitrogen and stored at –80 °C until further analyses. All biopsy samples were taken from the right leg in the morning, following an overnight fast. Subjects refrained from any heavy physical exercise/labor in the three days prior to muscle biopsy sampling. To assess the chronic effect of the training intervention in the older subjects post-training biopsies were taken 4 days after the post-intervention strength assessment (Verdijk et al., 2009a).

2.3. Western blotting

Muscle tissue was homogenized in cold lysis buffer containing 20 mmol/l Tris–HCl (pH 7.4), 1% Triton-X 100, 50 mmol/l NaCl, 250 mmol/l sucrose, 50 mmol/l NaF, 5 mmol/l sodium pyrophosphate, 2 mmol/l dithiothreitol (DTT), 4 mg/l leupeptin, 50 mg/l trypsin inhibitor, 0.1 mmol/l benzamidine, and 0.5 mmol/l PMSF, centrifuged at 14,000 g for 20 min at 4 °C, and soluble materials were collected. Proteins (40 µg) from muscle lysates were separated by 8% SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked in Tris-buffered saline with 0.05% Tween 20 (TBST) and 5% nonfat milk or BSA for 1 h at room temperature. Immunoblotting was performed using the following primary antibodies: phospho-AMPK–Thr172, phospho-ACC–Ser221, phospho-mTOR–Ser2448, mTOR, phospho-4E-BP1–Thr37/46, 4E-BP1, phospho-S6K–Thr389, and S6K, from Cell Signaling (Beverly, MA), AMPK Pan α from Upstate Biotechnology (Lake Placid, NY), and LKB1 from Abcam (Cambridge, England). Anti-AMPK α 1 and AMPK α 2 antibodies were kindly provided by Dr. Laurie Goodyear. Bound primary antibodies were detected with anti-rabbit immunoglobulin–horseradish-peroxidase–linked antibodies. The membranes were washed with TBST then incubated with enhanced chemiluminescence reagents (NEN Life Science Products, Boston, MA) and exposed to film. Because ACC is a biotinylated protein, its content in muscle was measured using HRP-conjugated streptavidin (Pierce, Rockford, IL), which has high affinity for biotin. Bands were quantitated with ImageQuant software (Sunnyvale, CA). Two internal control samples were loaded on all the gels and the mean density from these samples was utilized to normalize the values from all the gels.

2.4. AMPK activity assay

The activities of AMPK α 1 and AMPK α 2 were measured after immunoprecipitating from muscle extracts (150 μ g total protein) using antibodies against AMPK α 1 (Upstate, Lake Placid, NY) and AMPK α 2 (amino acid sequences 352–366), as previously described (Musi et al., 2001b).

2.5. Clinical laboratory assays

Plasma glucose concentrations were analyzed with a COBAS FARA analyzer (Uni Kit III; Roche, Basel, Switzerland), insulin was measured by radioimmunoassay (Insulin RIA Kit; LINCO Research Inc., St Charles, MO), and hemoglobin A1c content was analyzed by HPLC (Variant II; BioRad, Munich, Germany). The homeostatic model assessment index of insulin resistance (HOMA-IR) was calculated as described previously (Matthews et al., 1985).

2.6. Computer tomography (CT) scanning

To determine the effect of resistance training on *vastus lateralis* muscle mass in older subjects, CT scanning of the thigh was performed before and after completion of the exercise training program (3 days after the post-training strength assessment) using a Philips Medical System (IDT 8000; Philips, Best, The Netherlands) as described (Verdijk et al., 2009b).

2.7. Statistical analysis

All data are expressed as mean $\pm$ SE. Baseline data between younger and older groups were analyzed with an unpaired *t*-test. The effect of exercise within the older group (pre- and post-training) was analyzed with a paired *t*-test. Analyses were performed using SigmaStat software.

3. Results

3.1. Clinical characteristics

The baseline clinical characteristics of the subjects are shown in Table 1. There were no differences in body weight between younger and older men, however, because the older subject had a lower height, the body mass index (BMI) was higher in this group. Fasting plasma glucose, insulin, and hemoglobin A1c concentrations were elevated by 13% ($P < 0.05$), 27% ($P < 0.05$), and 9% ($P < 0.05$) in older versus younger, respectively. The HOMA index of insulin resistance also was significantly elevated in the older group ($P < 0.05$).

3.2. Clinical effects of resistance type exercise training

The older subjects performed resistance exercise training 3 times a week for 12 weeks. The resistance exercise training program led to significant increases in quadriceps cross-sectional area (9%) and 1RM muscle strength for leg extension (23%) and leg press (31%) (Table 2). Whole body lean mass increased, whereas whole body fat mass decreased after the resistance exercise training program. Glucose, insulin, and hemoglobin A1c concentrations did not change with exercise. The HOMA index also did not change significantly after resistance training. CT scanning did not reveal changes in lean tissue density after resistance training (not shown), suggesting that the exercise program did not affect intramyocellular lipid content.

3.3. AMPK phosphorylation and enzymatic activity, and ACC phosphorylation

At baseline, AMPK Thr172 phosphorylation was significantly lower in elderly (68% of younger, $P < 0.05$) (Fig. 1A). Resistance exercise training did not affect AMPK

phosphorylation (Fig. 1B). Consistent with the decrease in AMPK phosphorylation, the activity of AMPK α 2-complexes also was lower in muscle from older subjects by 21% at baseline ($P < 0.05$ versus younger) (Fig. 2B), whereas AMPK α 1 was not significantly different between groups (Fig. 2A). Resistance type exercise training significantly increased the activity of AMPK α 1 (Fig. 2C) by 1.4-fold ($P < 0.05$), whereas the activity of AMPK α 2 did not change with exercise training (Fig. 2D). The protein content of AMPK α 1 and AMPK α 2 was not different between older and younger (Fig. 3A and B) and resistance exercise training did not affect the content of these proteins (Fig. 3C and D). LKB1 protein content also was not different between groups and did not change with resistance training (not shown). In line with the decreases in AMPK phosphorylation and AMPK α 2 activity, older subjects had a significant decrease in ACC2 Ser221 phosphorylation (Fig. 4A). Prolonged resistance training did not affect the phosphorylation of this enzyme (Fig. 4B).

3.4. mTOR signaling

As shown in Fig. 5A, mTOR phosphorylation was significantly lower in muscle from older subjects by 41% ($P < 0.05$), but mTOR phosphorylation was not affected by exercise training (Fig. 5B). Consistent with the lower mTOR phosphorylation observed in older subjects, the phosphorylation of 4E-BP1 also was lower in this group by 30% (Fig. 6A). Resistance exercise training did not affect 4E-BP1 phosphorylation (Fig. 6B). In contrast to 4E-BP1, S6K phosphorylation was not lower in older subjects (Fig. 7A). Resistance exercise did not affect the phosphorylation of S6K (Fig. 7B).

4. Discussion

Reznick et al. reported that old rats have decreased AMPK activity in muscle upon acute stimulation with the AMPK-activating compound 5'-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside (AICAR) and treadmill exercise (Reznick et al., 2007). AMPK activity also was blunted in old rats after chronic stimulation with β -guanidinopropionic acid (β -GPA) (Reznick et al., 2007). Qiang et al. found that muscles from old rats have decreased basal AMPK and ACC phosphorylation, in association with a marked impairment in insulin-stimulated muscle glucose transport (Qiang et al., 2007). This is also consistent with a study performed in older mono- and dizygotic twins which showed that baseline AMPK γ 3-associated activity is decreased in aging (Mortensen et al., 2009). Collectively, the results from these studies and the present findings indicate that aging leads to a down regulation of the AMPK-ACC axis in the basal state (Qiang et al., 2007; Mortensen et al., 2009) and in response to acute (Reznick et al., 2007) and chronic (Reznick et al., 2007) stimuli. Nonetheless, other studies have not observed a down regulation of the AMPK pathway with aging. Thompson et al. reported enhanced activation of AMPK after acute, electrically-stimulated contraction of *extensor digitorum longus* muscles in old versus young rats (Thomson et al., 2009) and Drummond et al. did not observe differences in baseline of AMPK phosphorylation between young and older subjects (Drummond et al., 2008). Drummond et al. reported that AMPK phosphorylation was accentuated in muscle from older subjects after ingestion of essential amino acids and a 1 h bout of resistance exercise, compared with younger individuals (Drummond et al., 2008). The contrasting results between studies regarding basal and stimulated AMPK activity could be explained by differences in experimental conditions, species, and populations studied (in case of the human studies).

In this study insulin sensitivity was lower in older versus younger men, based on the higher fasting plasma insulin concentrations and the higher HOMA index of insulin resistance. Of note, the older men had a higher BMI than the younger men, which likely contributed to the insulin resistant state in these individuals. We previously reported that obesity and type 2 diabetes actually up regulate AMPK activity in humans (Sriwi-jitkamol et al., 2007).

Therefore, it is unlikely that the increased BMI in the older subjects would explain the down regulation in AMPK and ACC observed in older subjects in the present study.

The effect that prolonged resistance type exercise training has on AMPK and ACC is unclear. In previous studies, acute resistance exercise either stimulated (Dreyer et al., 2006) or did not affect AMPK (Harber et al., 2008). Wojtaszewski et al. evaluated the effect of strength training for 6 weeks in healthy volunteers and type 2 diabetic subjects, and did not observe changes in AMPK and ACC phosphorylation, although resistance exercise did cause a small increase in AMPK α 1 protein (Wojtaszewski et al., 2005). In this study, we found that resistance training significantly increased the activity of α 1 subunit-containing AMPK complexes, without affecting AMPK α 2. This finding is consistent with the increasing evidence suggesting that α 1- and α 2-containing AMPK complexes have distinct regulatory properties and roles in the regulation of skeletal muscle adaptations. The AMPK α 2 isoform is more sensitive to aerobic exercise (Fujii et al., 2000) and is thought to play a more predominant role in regulating adaptations to aerobic exercise training (Jorgensen et al., 2007). In contrast, muscle overload causes a marked activation of AMPK α 1 (McGee et al., 2008) and this isoform plays an important role in limiting skeletal muscle overgrowth during overload-induced hypertrophy (Mounier et al., 2009).

AMPK α 2-containing complexes are more abundant than AMPK α 1 in human muscle (Musi et al., 2001a; Wojtaszewski et al., 2005), which is possibly why the increase in AMPK α 1 activity after resistance training observed in this study was not reflected at the level of pan- α AMPK phosphorylation. Moreover, there is some evidence to suggest that sensitivity of the various AMPK complexes to different stimuli may depend on the specificity of upstream kinases for α subunit phosphorylation/activation. For example, in LKB1null mice, AMPK α 2 activation is severely blunted after electrically-induced muscle contraction, whereas AMPK α 1 activity increases significantly (Koh et al., 2006), suggesting that AMPK α 2, but not α 1 stimulation, depends on LKB1. On the other hand, chronic muscle overload causes a marked increase in the expression and the activity of CaMKK α and CaMKK β (McGee et al., 2008), upstream AMPK kinases which also can activate AMPK α 1- and α 2-containing complexes (Witczak et al., 2007).

Some (Balagopal et al., 1997; Welle et al., 1993), albeit not all studies (Volpi et al., 2001), have shown a reduction in protein synthesis in muscle from older subjects. In this study we found a significant decrease in mTOR phosphorylation in the older subjects. In line with this, the phosphorylation of 4E-BP1 also was lower in the older group. 4E-BP1 inhibits protein translation by binding to eukaryotic initiation factor 4E (eIF4E). 4E-BP1 phosphorylation leads to the release of eIF4E which functions to induce translation of capped cellular mRNAs (Raught and Gingras, 1999). Taken together, the reductions in mTOR and 4E-BP1 phosphorylation in the older group observed in this study suggest that down regulation of this signaling cascade might be implicated in age-related changes in protein metabolism reported by some groups (Balagopal et al., 1997; Welle et al., 1993). Furthermore, Cuthbertson et al. found that older subjects have significant decreases in mTOR, 4E-BP1 and S6K content, which should lead to a net decrease in the activity of these proteins (Cuthbertson et al., 2005) and Wu et al. showed that mTOR phosphorylation is reduced in muscle from very old (33 month old) rats (Wu et al., 2009). Notably, unlike the present findings, others have reported no age differences in mTOR (Drummond et al., 2008) and 4E-BP1 (Kumar et al., 2009; Mayhew et al., 2009) phosphorylation in muscle. More research in this area is needed to better clarify how age affects the mTOR axis in the basal and exercise-stimulated states and to define the physiological consequences of these changes.

Several studies have examined the effect of acute resistance exercise on mTOR signaling in human subjects. While these studies have reported variable responses, in general, mTOR signaling has been found to be enhanced when analyzed 0.5–2 h after a single session of acute resistance exercise (Dreyer et al., 2006; Fujita et al., 2009; Rommel et al., 2001; Terzis et al., 2010). Interestingly, acute resistance exercise had opposite effects on mTOR substrates; 4E-BP1 phosphorylation decreased, whereas phospho-S6K increased with exercise (Dreyer). In contrast, we did not observe differences on mTOR, 4E-BP1 or S6K after the 12 week resistance training program. Our goal was to evaluate the chronic effect of resistance training. Therefore, post-training muscle samples were obtained 4 days after the last bout of exercise, once the acute exercise effect had dissipated. We conclude that, under the experimental conditions tested in this study resistance exercise does not have long-lasting (i.e. chronic) effects on this signaling pathway, suggesting that during resistance exercise training the mTOR pathway induces muscle protein synthesis and enhances muscle mass through its repetitive activation, triggered by each successive exercise session.

In this study both AMPK and mTOR signaling were down regulated in muscle from older subjects. This finding contradicts the notion that AMPK has an inhibitory effect on the mTOR pathway. In corneal epithelial cells AICAR decreases mTOR complex (TORC)1 activity (Kimura et al., 2003), and this agent cannot suppress TORC1 in AMPK α 1/ α 2 double-knockout fibro-blasts (Kalender et al., 2010). Moreover, in HEK293 cells AMPK physically associates with and phosphorylates tuberous sclerosis complex 2, reducing TORC1 activity (Inoki et al., 2003). While the findings from cell culture studies suggest that AMPK decreases mTOR signaling, there is limited evidence demonstrating that this phenomenon occurs in human subjects in vivo. AICAR administration to rats inhibits mTOR signaling (Bolster et al., 2002). Nonetheless, these experiments are limited by the non-specificity of AICAR. This compound has other non-AMPK mediated effects, including stimulation of adenosine receptors (Oei et al., 1991) and inhibition of gluconeogenic enzymes (Vincent et al., 1991). In addition, in vivo AICAR administration causes a whole host of systemic metabolic and hormonal alterations that indirectly could affect mTOR signaling, including lowering of plasma glucose and insulin concentration and increasing lactic and uric acid levels (Aschenbach et al., 2002). The data from the present study, along with other in vivo human investigations that have shown that acute resistance (Vissing et al., 2011) and aerobic (Supplementary Fig. 1) exercise simultaneously increase AMPK and mTOR phosphorylation in muscle, indicate that AMPK activity is not always inversely related to the function of mTOR. Clearly, more research is required to better understand the interaction between the AMPK and mTOR pathways in vivo, and to clarify what are the physiological consequences of such interactions.

One limitation of this study is that the cell signaling assays were carried out in muscle samples conformed of a mixture of muscle fiber types (glycolytic versus oxidative). Although care was taken to standardize the muscle biopsy procedure with respect to location and depth at which the biopsy was collected, each muscle biopsy procedure can yield samples with different mixtures of fiber types. In future studies it will be important to determine whether age-related differences in AMPK-mTOR signaling, and the effect of exercise, varies depending upon the fiber type, and whether these differences/changes are present across all fibers.

In summary, in this study we found that older men have a down regulation of both the AMPK and mTOR signaling pathways. Future studies will help to determine the physiologic relevance of these age-related differences. Also, we found that long-term resistance type exercise training increases the activity of AMPK α 1-, but not AMPK α 2-containing complexes, and does not lead to activation of the mTOR pathway beyond 96 h after the last exercise bout. This suggests that mTOR-regulated protein synthesis and hypertrophy

resulting from resistance training is due to episodic, transient activation, rather than from sustained stimulation of this pathway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grants from the National Institutes of Health (RO1-DK80157 and RO1-DK089229 to N.M.). N.M. was the recipient of a Paul B. Beeson Career Development Award (K23-AG030979) from the American Federation for Aging Research and the National Institute on Aging. L.V. received support from the Anna Foundation (Leiden, the Netherlands) and K.S. from U.K. Medical Research Council.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.mad.2012.09.001>.

Fig. 1.

AMPK phosphorylation. AMPK phosphorylation and AMPK α subunit muscle protein content were measured in older versus younger subjects at baseline (A) and in older subjects pre- versus post-training (B). Data are means $\pm$ SE. $n = 32/\text{group}$ in (A) and $n = 25/\text{group}$ in (B). * $P < 0.05$ older versus younger group. Representative blots are shown for 4 younger and 4 older subjects. IC, internal control.

Fig. 2.

AMPK activity. AMPK $\alpha 1$ (A) and AMPK $\alpha 2$ (B) activities were measured in 32 older versus 32 younger subjects at baseline. AMPK $\alpha 1$ (C) and AMPK $\alpha 2$ (D) activities were also measured in 25 older subjects pre- versus post-training. Data are means $\pm$ SE. * $P < 0.05$ pre- versus post-exercise (B), older versus younger group (C). IC, internal control.

Fig. 3.

AMPK protein content. AMPK α1 (A) and AMPK α2 (B) protein content were measured in the skeletal muscle of 32 older versus 32 younger subjects at baseline. AMPK α1 (C) and AMPK α2 (D) protein content was measured in 25 older subjects pre- versus post-training. Data are means $\pm$ SE. Representative blots are shown for 4 younger and 4 older subjects for AMPK α1, and 3 younger and 4 older subjects for AMPK α2. IC, internal control.

Fig. 4. ACC2 phosphorylation. ACC2 phosphorylation and protein content were measured in older versus younger subjects at baseline (A) and in older pre- versus post-training (B). Data are means $\pm$ SE. $n = 32/\text{group}$ in (A) and $n = 25/\text{group}$ in (B). * $P < 0.05$ older versus younger group. Representative blots are shown for 4 younger and 4 older subjects. IC, internal control.

Fig. 5. mTOR phosphorylation. mTOR phosphorylation and protein content were measured in older versus younger subjects at baseline (A) and in older pre- versus post-training (B). Data are means $\pm$ SE. $n = 32$ /group in (A) and $n = 25$ /group in (B). * $P < 0.05$ older versus younger group. Representative blots are shown for 4 younger and 4 older subjects. IC, internal control.

Fig. 6. 4E-BP1 phosphorylation. 4E-BP1 phosphorylation and protein content were measured in older versus younger subjects at baseline (A) and in older pre- versus post-training (B). Data are means $\pm$ SE. $n = 32$ /group in (A) and $n = 25$ /group in (B). * $P < 0.05$ older versus younger group. Representative blots are shown for 3 younger and 4 older subjects. IC, internal control.

Fig. 7. S6K phosphorylation. S6K phosphorylation and protein content were measured in older versus younger subjects at baseline (A) and in older pre- versus post-training (B). Data are means $\pm$ SE. $n = 32$ /group in (A) and $n = 25$ /group in (B). Representative blots are shown for 3 younger and 4 older subjects. IC, internal control.

Table 1

Baseline clinical characteristics.

	Younger	Older
<i>n</i>	32	32
Age, yrs	24 ± 1	73 ± 1 *
Body weight, kg	75.5 ± 1.6	77.7 ± 1.9
Height, m	1.81 ± 0.11	1.72 ± 0.10 *
BMI, kg/m ²	22.9 ± 0.5	26.2 ± 0.6 *
FPG, mg/dl	92 ± 1	104 ± 2 *
HbA1c, %	5.4 ± 0.1	5.9 ± 0.1 *
FPI, mU/ml	9.1 ± 0.5	11.6 ± 0.7 *
HOMA-IR	2.1 ± 0.1	3.0 ± 0.2 *

Data are means ± SE. BMI, body mass index; FPG, fasting plasma glucose; FPI, fasting plasma insulin.

* $P < 0.05$.

Table 2

Effects of resistance type exercise.

	Older	
	Pre-training	Post-training
<i>n</i>	25	25
Body weight, kg	78.9 ± 2.1	78.7 ± 2.1
BMI, kg/m ²	26.7 ± 0.7	26.7 ± 0.7
FPG, mg/dl	104 ± 2	102 ± 2
Hb A1c, %	5.9 ± 0.1	5.8 ± 0.1
FPI, mU/ml	12.3 ± 0.7	12.4 ± 0.8
HOMA-IR	3.2 ± 0.2	3.2 ± 0.2
Quadriceps CSA, cm ²	74.4 ± 2.5	81 ± 2.5 *
Whole body lean mass, kg	56.6 ± 1.1	57.5 ± 1.1 *
Whole body fat mass, kg	19.3 ± 1.4	18.4 ± 1.4 *
1RM leg extension, kg	85.6 ± 2.6	112.9 ± 3.4 *
1RM leg press, kg	171.8 ± 5.5	213.9 ± 7.6 *

Data are means ± SE. BMI, body mass index; FPG, fasting plasma glucose; FPI, fasting plasma insulin; CSA, cross-sectional area; 1RM, one-repetition maximum strength.

* $P < 0.05$.