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Lipogenic regulators are elevated with age and chronic overload in rat skeletal muscle

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Abstract

Aim—Both muscle mass and strength decline with ageing, but the loss of strength far surpasses what is projected based on the decline in mass. Interestingly, the accumulation of fat mass has been shown to be a strong predictor of functional loss and disability. Furthermore, there is a known attenuated hypertrophic response to skeletal muscle overload with ageing. The purpose of this study was to determine the effect of 28 days of overload on the storage of intramuscular triglycerides (IMTG) and metabolic regulators of lipid synthesis in young and old skeletal muscle.

Methods—The phosphorylation and expression of essential lipogenic regulators were determined in the plantaris of young (YNG; 6-month-old) and aged (OLD; 30-month-old) rats subjected to bilateral synergist ablation (SA) of two-thirds of the gastrocnemius muscle or sham surgery.

Results—We demonstrate that age-induced increases in IMTG are associated with enhancements in the expression of lipogenic regulators in muscle. We also show that the phosphorylation and concentration of the 5′AMP-activated protein kinase (AMPK) isoforms are altered in OLD. We observed increases in the expression of lipogenic regulators and AMPK signalling after SA in YNG, despite no increase in IMTG. Markers of oxidative capacity were increased in YNG after SA. These overload-induced effects were blunted in OLD.

Conclusion—These data suggest that lipid metabolism may be altered in ageing skeletal muscle and is unaffected by mechanical overload via SA. By determining the role of increased lipid storage on skeletal muscle mass during ageing, possible gene targets for the treatment of sarcopenia may be identified.

Keywords

lipid metabolism; mechanical overload; sarcopenia; skeletal muscle

The loss of muscle mass and function with age, or sarcopenia, is associated with substantial social and economic costs as a result of impairments in strength and function, which ultimately lead to physical disability and institutionalization (Frontera *et al*. 1991, Baumgartner *et al*. 1998). Despite these observations, the idea that a decline in physical

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functioning is fundamentally related to a decrease in muscle mass remains controversial. Both muscle mass and strength decline with age, but the loss of strength far surpasses what is projected based on the decline in mass (Hughes *et al*. 2001, Lauretani *et al*. 2003, Goodpaster *et al*. 2006, Stenholm *et al*. 2008). This may be explained by deterioration in muscle quality including decreased fibre size and number, reduced contractility of fibres, motor neurone loss and intramyocellular lipid infiltration (Lexell *et al*. 1988, Sipila & Suominen 1994, Larsson *et al*. 1997, Visser *et al*. 2000, Goodpaster *et al*. 2001, Delbono 2003, Cree *et al*. 2004). Interestingly, in several epidemiological studies the accumulation of fat mass has been shown to be a strong independent predictor of subsequent functional loss and disability (Sipila & Suominen 1994, Visser *et al*. 2000, Goodpaster *et al*. 2001).

The master transcription factor, sterol regulatory element-binding protein (SREBP) 1, mediates transcriptional effects on lipogenic genes including fatty-acid synthase (FAS), acetyl-CoA carboxylase (ACC) and stearoyl-CoA desaturase (SCD) (Osborne 2000, Dobrzyn & Dobrzyn 2006, Sampath *et al*. 2007, Wang *et al*. 2009). These enzymes catalyse the synthesis of fatty acids that are the key substrates for the formation of complex lipids such as triglycerides, diglycerides and ceramides, which have been shown to be upregulated in obesity and insulin resistance (Hyde *et al*. 2005, Summers & Nelson 2005, Brownsey *et al*. 2006, Dobrzyn & Dobrzyn 2006, Holland *et al*. 2007, Lessard *et al*. 2007, Sampath *et al*. 2007, Erion & Shulman 2010). However, their function in age-associated skeletal muscle loss and anabolic resistance are currently unknown.

The 5′AMP-activated protein kinase (AMPK) is a sensor of cellular energy homeostasis and has well-established roles in the regulation of glucose and lipid metabolism (Hawley & Lessard 2008, Osler & Zierath 2008, Witczak *et al*. 2008, Steinberg & Kemp 2009). Recently, it has been recognized that AMPK activation is increased in both aged skeletal muscle (Thomson & Gordon 2005, Thomson *et al*. 2008, 2009) and in the presence of increased lipids or a high-fat diet (Watt *et al*. 2006, Lessard *et al*. 2007) and may have a function in the inhibition of the response of aged skeletal muscle to contraction-induced anabolic stimulation (Thomson *et al*. 2009). In support of this contention, the AMPKα1 catalytic subunit is thought to have a role in the prevention of excess muscle growth by the inhibition of the mammalian target of rapamycin (mTOR) anabolic pathway (Mounier *et al*. 2009, McGee *et al*. 2008, Rivas *et al*. 2009a).

We (Parkington *et al*. 2004, Funai *et al*. 2006, Chale-Rush *et al*. 2009) and others (Cutlip *et al*. 2006, Thomson & Gordon 2006, Baker *et al*. 2008, Dennis *et al*. 2008, Drummond *et al*. 2008, Kumar *et al*. 2009) have previously demonstrated that the ability of aged humans (Dennis *et al*. 2008, Drummond *et al*. 2008, Kumar *et al*. 2009) and rodents (Parkington *et al*. 2004, Thomson & Gordon 2005, 2006, Cutlip *et al*. 2006, Funai *et al*. 2006, Baker *et al*. 2008, Chale-Rush *et al*. 2009) to adapt (i.e. hypertrophy, increased anabolic signalling, etc.) following overload is blunted compared with young. Therefore, the aim of the present study was to determine what role age-related lipid accumulation in skeletal muscle has on muscle growth in response to 28 days of muscle overload. For this purpose, we determined the storage of triglycerides in skeletal muscle, the concentration of SREBP1 and its transcriptional target genes, the activation of AMPK and the concentration of the isoforms of AMPKα. We hypothesized that the storage of lipids would be increased in aged skeletal

muscle and this would be associated with an increase in lipogenic capacity leading to the activation of AMPK even in animals that have undergone chronic skeletal muscle overload.

Methods

Experimental animals

Young adult (YNG; 6-month-old, *n* = 16) and aged (OLD; 30-month-old, *n* = 16) male Fischer 344 × Brown Norway rats were purchased from the National Institute on Aging. The Fischer 344 × Brown Norway rat strain is less susceptible to disease (Lipman *et al*. 1996) and shows muscle atrophy that is similar to aged skeletal muscle in humans (Blough & Linderman 2000). Rats were housed in a temperature-controlled animal room (21 °C) maintained on a 12-h light–dark cycle. Animals were provided with standard chow diets and water *ad libitum*. Rats were acclimatized for 14 days and fasted overnight before initiation of experimental protocol. All animal experimentation procedures were carried out with the approval of Institutional Animal Use and Care Committee of the Jean Mayer USDA Human Nutrition Research Center at Tufts University.

Experimental design and synergist ablation

Following the 14-day acclimatization period, YNG and OLD rats were randomly assigned to one of the four groups (*n* = 8/group): YNG control (YNG CON), YNG surgically ablated (YNG SA), OLD control (OLD CON) and OLD surgically ablated (OLD SA). Surgical procedures were performed under aseptic conditions after rats were anaesthetized with 2–3% isofluorane gas supplemented with oxygen. The animals underwent either bilateral SA (YNG, *n* = 8; OLD, *n* = 8) of two-thirds of the gastrocnemius muscle or sham operation (YNG CON, *n* = 8; OLD CON, *n* = 8). SA was performed to induce overload for 28 days and promote compensatory hypertrophy in the plantaris (PLAN) as previously described (Chale-Rush *et al*. 2009).

Analysis of intramuscular lipid storage and citrate synthase activity

Portions of PLAN muscle were freeze–dried, powdered and analysed for the content of glycerol and maximal citrate synthase (CS) activity as previously described (Lessard *et al*. 2011, Rivas *et al*. 2011). Briefly, freeze–dried muscle was powdered and cleaned of all visible connective tissue and blood under magnification. Portions (4–5 mg) of the sample (8–10/group) were used to fluorometrically determine skeletal muscle triacylglycerol (total glycerol) content, following Folch lipid extraction and saponification. Portions (3–4 mg) of the sample (8–10/group) were homogenized in 100 mM potassium phosphate buffer (pH 7.3, 1 : 400 dilution), and CS was assayed spectrophotometrically at 25 °C by the reduction of DTNB as previously described (Srere 1969, Chi *et al*. 1983).

Western blotting analysis

The phosphorylation and concentration of signalling proteins were quantified with Western blot analyses as previously described (Rivas *et al*. 2009b). Muscle samples were cut and weighed, frozen and homogenized in an ice-cold homogenization buffer (1 : 10 wt/vol) containing 50 mM Tris–HCl (pH 7.5), 5 mM Na-pyrophosphate, 50 mM NaF, 1 mM EDTA, 1 mM EGTA, 10% glycerol (v/v), 1% Triton-X, 1 mM DTT, 1 mM benz-amidine, 1 mM PMSF,

10 μg mL^{-1} trypsin inhibitor and 2 μg mL^{-1} aprotinin. Following centrifugation (21 000 ***g***, 4 °C) for 15 min, the supernatant was collected and assayed for protein content. PLAN (30 μg) were solubilized in Laemmli buffer, separated by SDS-PAGE, and transferred to nitrocellulose membranes. The membranes were then blocked (5% NFDM), and incubated overnight at 4 °C with primary antibodies specific for SREBP1 (ab3259; Abcam, Cambridge, MA, USA), SCD1 (ab19862; Abcam), FAS1 (ab22759; Abcam), Phospho-ACC Ser79 (3661; Cell Signaling Technology, Danvers, MA, USA), ACC (3676; Cell Signaling), AMPKα1 (2795, Cell Signaling), AMPKα2 (2757; Cell Signaling), Phospho-AMPKα Thr172 (3562; Cell Signaling), Cytochrome *c* (4280; Cell Signaling), Cytochrome *c* Oxidase IV (COX IV; 4850; Cell Signaling). Membranes were probed with α/β-tubulin (T6074; Sigma, St Louis, MO, USA) antibody to monitor protein loading. The immunoreactive proteins were detected with Supersignal Chemiluminescent Substrate (Thermo Scientific, Rockford, IL, USA). Images were scanned and band intensities quantified by optical density using standardized bandwidths (Alpha Innotech Corporation, San Leandro, CA, USA).

Quantitative mRNA analysis

The quantification of mRNA was determined on SCD1, FAS1, ACC1 and ACC2 as previously described (Lessard *et al*. 2009). RNA was extracted from muscle using RNeasy Fibrous Tissue Mini Kit (74704; Qiagen, Germantown, MD, USA). cDNA levels of SCD1 (QT02285493), FAS1 (QT00371210), ACC1 (QT00190946) and ACC2 (QT01082655) were measured using commercially available primer mixtures (QuantiTect Primer Assays; Qiagen). All reactions were run using a commercially available reaction mixture (QuantiFast SYBR Green RT-PCR Kit; Qiagen) on a Stratagene MX3000P (Agilent, Santa Clara, CA, USA). Changes in gene expression were normalized to the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (QT00199633). Efficiencies of each primer set were assessed using a standard curve, analysed using 0.001–10 ng of control cDNA.

Statistical analysis

Differences between groups were identified using a two-way analysis of variance (ANOVA) with Bonferroni posttest performed with GRAPHPAD PRISM version 5.00 for Windows (GraphPad Software, CA, USA, http://www.graphpad.com). Results are expressed as mean ± SEM and statistical significance was accepted at $P < 0.05$.

Results

Animal body weights, muscle wet weights and total protein contents

OLD animals had a significant decrease in body weight from day 0 to day 28 compared with YNG ($P < 0.0001$ vs. YNG; Table 1) and SA were significantly different after 28 days of overload compared with CON ($P = 0.01$ vs. CON; Table 1). Older animals had significantly smaller PLAN muscle wet weights ($P < 0.0001$ vs. YNG; Table 1) and 28 days of overload significantly increased PLAN wet weight in both YNG and OLD ($P = 0.0002$ vs. CON; Table 1). However, we have previously reported in these animals that the PLAN muscle weights when normalized to body weight were significantly higher in YNG animals with SA and this effect was attenuated in OLD (Chale-Rush *et al*. 2009). The total protein content of the PLAN muscles was also lower in OLD ($P = 0.0036$ vs. YNG; Table 1) while overload

caused a significantly higher total protein content of 80% in SA and 64% in YNG and OLD respectively ($P = 0.0004$ vs. CON; Table 1).

Intramuscular triglycerides

Ageing led to a 52% higher storage of intramuscular triglycerides (IMTG) ($P = 0.0002$ vs. YNG; Fig. 1).

Concentration of active SREBP1 and lipid synthesis genes

There was a significantly lower concentration of the inactive uncleaved form of SREBP1 in OLD ($P = 0.037$ vs. YNG; Fig. 2a) while there was no change in SA in either age group (Fig. 2a). The active cleaved form of SREBP1 was higher with ageing ($P = 0.024$ vs. YNG; Fig. 2b) and after muscle overload ($P = 0.004$ vs. CON; Fig. 2b). In addition, there was a trend for an interaction between age and overload ($P = 0.073$, CON vs. SA; Fig. 2b) with a 90% increase in YNG and a 17% increase in OLD.

We determined the gene expression of selected transcriptional targets of SREBP1. The gene expression of SCD1 was fourfold higher in OLD ($P < 0.05$ vs. YNG; Fig. 2c), although there was no effect in either age group by SA. However, there was no change in the expression of ACC1, ACC2 and FAS1 with ageing or muscle overload (Fig. 2c).

Protein expression of ACC1/2, FAS1 and SCD1

We next determined the protein concentration of ACC1/2, FAS1 and SCD1 by Western blotting. There was a 32% higher concentration of ACC1/2 in OLD ($P < 0.0001$ vs. YNG; Fig. 3a), which was not affected by SA (Fig. 3a). FAS1 was also higher in OLD ($P = 0.003$ vs. YNG; Fig. 3b) and was augmented by SA ($P = 0.0008$ vs. CON; Fig. 3b). The protein expression of SCD1 mirrored the gene expression results in the older animals ($P < 0.0001$ vs. YNG; Fig. 3c) and was further increased in SA ($P = 0.002$ vs. CON; Fig. 3c) in both groups.

Phosphorylation of AMPKα and concentration of the AMPKα isoforms

We determined the phosphorylation of the AMPKα activation site, Thr172, and the total protein concentration of AMPKα1 and AMPKα2. The phosphorylation of AMPKα on Thr172 was 40% higher in OLD ($P = 0.001$ vs. YNG; Fig. 4a) and with overload ($P = 0.0008$ vs. CON; Fig. 4a). The total protein expression of the AMPKα1 isoform was higher in OLD ($P = 0.01$ vs. YNG; Fig. 4b). The concentration of AMPKα1 was further increased by SA ($P = 0.006$ vs. CON; Fig. 4b). In addition, there was a significant interaction between age and overload ($P = 0.032$, CON vs. SA; Fig. 4b) with a 50% increase in YNG and a ~20% increase in OLD. The protein concentration of AMPKα2 was 20% lower in OLD ($P = 0.001$ vs. YNG; Fig. 4c), but was unaffected in either group by SA.

Markers of skeletal muscle oxidative capacity

Citrate synthase activity in PLAN muscle was lower in OLD ($P = 0.036$ vs. YNG) while SA increased CS activity in both groups ($P = 0.017$ vs. CON; Fig. 5a). There was a significant increase in ACC Ser79 phosphorylation after SA in YNG ($P = 0.04$; Fig. 5b) and a

significant interaction between age and SA ($P = 0.005$; Fig. 5b). However, there was no difference in the phosphorylation of ACC on Ser79 between age groups (Fig. 5b). The levels of cytochrome *c* were unaffected with ageing (Fig. 5c). Conversely, there was a significant increase after overload in both groups ($P = 0.009$; Fig. 5c) and a further significant interaction between age and SA ($P < 0.05$; Fig. 5c). In contrast to cytochrome *c*, there were no difference in the protein expression of COX IV with ageing and SA (Fig. 5d).

Discussion

Here, we demonstrate for the first time that age-induced increases in intramuscular lipids are associated with increased lipid synthetic regulators including SREBP1, ACC1/2, FAS1 and SCD1 (Figs 1–3). We also demonstrate that AMPK activation (Fig. 4c) and concentration of the AMPKα1 isoform are higher in aged skeletal muscle (Fig. 4a,b). Furthermore, we observed increases in the protein expression of these same lipogenic regulators in YNG after SA, despite no increase in IMTG. Markers of oxidative capacity (CS activity and cytochrome *c*) were also increased after 28 days of muscle overload in YNG, but the response in OLD was blunted (Fig. 5).

We have recently reported that after 28 days of muscle overload the hypertrophic response in the skeletal muscle of aged animals was attenuated compared with young adult animals (Chale-Rush *et al*. 2009). We now report a similar blunted response to the levels of total protein content of PLAN in OLD (Table 1). This is in agreement with previous studies employing overload that have revealed an attenuation of muscle hypertrophy and the activation of the mTOR anabolic pathway in older animals (Blough & Linderman 2000, Thomson & Gordon 2006, Hwee & Bodine 2009).

In addition to decreases in muscle mass, fibre cross-sectional area and changes in fibre type composition, ageing is also associated with an increased accumulation of IMTG that is likely a result of increased lipid uptake and decreased lipid oxidation (Blaak 2000, Tucker & Turcotte 2003, Cree *et al*. 2004, Goodpaster *et al*. 2006, Nakagawa *et al*. 2007). Furthermore, there is an age-related decline in the capacity of skeletal muscle to oxidize fatty acids in the fasting state and during exercise (Coggan *et al*. 1992, Sial *et al.* 1996, Blaak 2000, Tucker & Turcotte 2002). This is in agreement with the current study where we report higher levels of IMTG stored in aged muscle even after 28 days of mechanical overload (Fig. 1). We further report a 44% higher maximal CS activity in YNG, while only 13% higher in OLD after SA (Fig. 1). The cytochrome *c* concentration mirrors CS activity; we report a twofold increase in protein expression of cytochrome *c* in YNG and only a 10% increase in OLD after SA (Fig. 5c). However, the expression of COXIV was unchanged with age or SA (Fig. 5d). Taken together, these data demonstrate there is a reduced ability of aged skeletal muscle to increase oxidative capacity after chronic skeletal muscle overload.

As we observed increased levels of IMTG in aged skeletal muscle, we next determined the role of lipogenic regulators such as SREBP1. This is a membrane-bound transcription factor that regulates the expression of genes involved in the production and uptake of fatty acids, triglycerides and phospholipids (Osborne 2000, Hagen *et al*. 2010). In response to various stimuli (e.g. sterol-repletion, insulin, ER-stress, etc.), SREBP1 is escorted from the ER to

the Golgi where it is then cleaved releasing the amino terminus of SREBP1 (68 kDa), which is able to enter the nucleus and transcriptionally upregulate SREBP1 target genes (Sampath *et al*. 2007, Hagen *et al*. 2010). It has been previously reported that cleaved active SREBP1 is upregulated in high-fat fed diabetic rats and its inhibition was associated with reduced levels of IMTG in this model (Bi *et al*. 2009). We now show an increased expression of mature active SREBP1 (Fig. 2b) in aged skeletal muscle that mirrors the increases in IMTG levels in these animals (Fig. 1). We also noted increases in the protein expression of SREBP1 transcription targets ACC1/2, FAS1 and SCD1 in OLD (Fig. 3a,b,c). Contrary to the protein results, there was a fourfold higher mRNA expression of SCD1 in OLD with no change of any other SREBP1 target gene (Fig. 2c). SCD1 is highly associated with increased lipid accumulation (Dobrzyn & Dobrzyn 2006, Jiang *et al*. 2008) and its inhibition is protective against high-fat diet induced obesity and insulin resistance (Dobrzyn & Dobrzyn 2006, Sampath *et al*. 2007, Miyazaki *et al*. 2009). Although mRNA expressions for the other gene targets are somewhat unexpected it is not unprecedented. Changes in the gene expression may be transient and differences are dependent on a variety of physiological events.

The response of SREBP1 to exercise training is equivocal, with some studies showing an increased expression (Boonsong *et al*. 2007) and others reporting a decreased expression (Nadeau *et al*. 2006). Our study is in agreement with Nadeau *et al*. (2006) who found an exercise training adaptive response of increased SREBP1 in skeletal muscle. We now report a 95% increase in cleaved SREBP1 in YNG and only a 20% increase in OLD after 28 days of SA (Fig. 2b). We observed a 150% and 90% increase in FAS1 protein expression following SA (Fig. 3b) in YNG and OLD animals, respectively. These results were also found in SCD1 protein expression, which increased 150% and 50% after SA in YNG and OLD, respectively. However, there were no changes in the mRNA expression of any measured SREBP1 targets after SA (Fig. 2c).

In addition, we examined the role of the isoforms of the energy regulator, AMPK, in ageing skeletal muscle. Recent studies provide evidence for the existence of distinct regulatory functions for AMPKα1 and AMPKα2 catalytic subunits (McGee *et al*. 2008, Mounier *et al*. 2009). Furthermore, there is support for the idea that AMPK ‘hyperphosphorylation’ has a function in the loss of skeletal muscle mass with ageing (Thomson & Gordon 2005). However, these studies have failed to show a mechanism for these increases of AMPK activation. In agreement with Thomson & Gordon (2005, 2006) and Thomson *et al*. (2008, 2009), we now show significant increases in the Thr172 phosphorylation of AMPKα (Fig. 4a) in the skeletal muscle of aged animals. We (Lessard *et al*. 2007, Yeo *et al*. 2008) and others (Fediuc *et al*. 2006, Watt *et al*. 2006) have previously reported increases in the activation of AMPK in response to a high-fat diet or in the presence of increased lipids. Of interest, previous studies have found that rodent obesity is characterized by a decrease in muscle mass and an impaired response to overload (Almond & Enser 1984, Sitnick *et al*. 2009). In the current study, the increases in AMPK phosphorylation (Fig. 4a) in OLD are associated with the increases of IMTG (Fig. 1) and decreases in muscle mass (Table 1) in this group.

In agreement with Thomson *et al*. (2009), we observed a significant increase in AMPKα1 concentration (Fig. 4b) in OLD animals. This is of interest because, Mounier *et al*. (2009) using mice deleted for AMPKα1 observed a more pronounced hypertrophy after overload in this model. The authors hypothesized it was likely from increases to the activation of components of mTOR pathway, which regulate protein synthesis (Mounier *et al*. 2009). We have previously reported in these animals a decrease of mTOR activity in OLD after SA (Chale-Rush *et al*. 2009). AMPK, because of its role in suppressing energy consuming processes, is a known physiological inhibitor of components of the energy consuming mTOR pathway (Rivas *et al*. 2009b). Our results provide evidence to show an association of increases in AMPKα1 concentration to the attenuation in muscle mass growth observed in OLD even after SA.

AMPK phosphorylation and activation is highly regulated by exercise and contraction (Hawley & Lessard 2008, Witczak *et al*. 2008, Rivas *et al*. 2009b). In contrast to AMPKα1, the AMPKα2 catalytic subunit is hypothesized to regulate metabolic processes (lipid oxidation and glucose uptake) (Hardie & Sakamoto 2006). We now show a 50 and 20% increase in the phosphorylation of Thr172 site of AMPKα (Fig. 4c) after SA in YNG and OLD, respectively. We believe that the increases of AMPKα phosphorylation in OLD are a result of an increase of the AMPKα1 and not the AMPKα2. This is likely the result of lowered total protein concentration of AMPKα2 levels (Fig. 4c) and higher levels of AMPKα1 (Fig. 4b) in the OLD. The AMPKα phosphorylation levels closely mirrored the level of ACC phosphorylation (Fig. 5b) in the young animals after SA. Increases of Ser79 phosphorylation and the subsequent inhibition of ACC are highly associated with the increased lipid oxidation in skeletal muscle (Witczak *et al*. 2008).

In conclusion, we demonstrate that age-induced increases in intramuscular lipids are associated with the increased lipid synthetic regulators including SREBP1, ACC1/2, FAS1 and SCD1. We also demonstrate that the AMPK activation and concentration of the AMPKα1 isoform are higher in aged skeletal muscle. Furthermore, we observed increases in the protein expression of these same lipogenic regulators in YNG after SA, despite no increase in IMTG. Markers of oxidative capacity and lipid metabolism (CS activity, cytochrome *c*, and Phospho-ACC) were also increased after 28 days of muscle overload in YNG, but this increase was attenuated in OLD. This indicates that our results of lower IMTG levels in YNG SA, despite higher concentrations of lipogenic regulators is possibly a result of a significant adaptive increase in skeletal muscle oxidative capacity. Future studies should examine the management of IMTG levels in aged skeletal muscle by inhibition of lipogenic regulators such as, SREBP1 and/or SCD1 via pharmacological treatments or gene manipulation. By determining the role of increased lipid storage on skeletal muscle mass during ageing, possible gene targets for the treatment of sarcopenia maybe identified.

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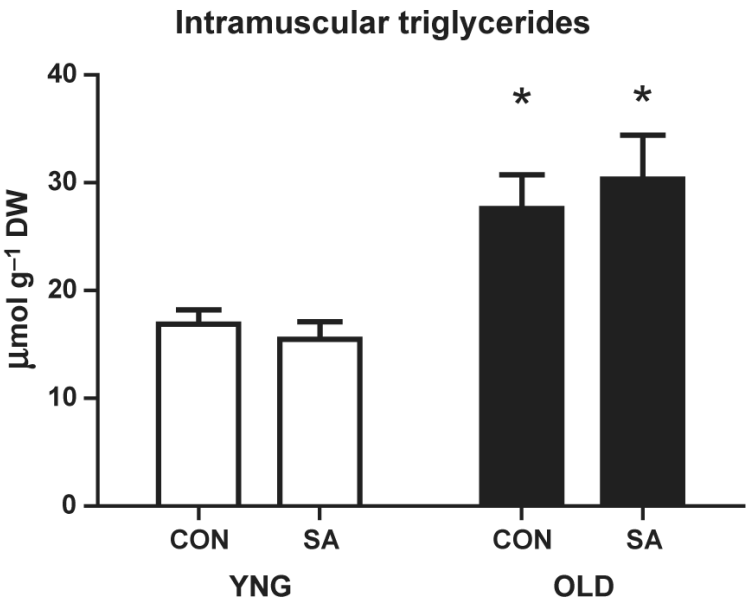

Figure 1.

Intramuscular lipid storage and mitochondrial enzyme activity in the skeletal muscle of young (YNG), old (OLD) and surgically ablated (SA) Fischer 344 × Brown Norway rats. Intramuscular triglyceride content was determined in plantaris on aliquots of freeze–dried/powdered muscle and expressed per mg of dry weight (DW). *$P < 0.05$.

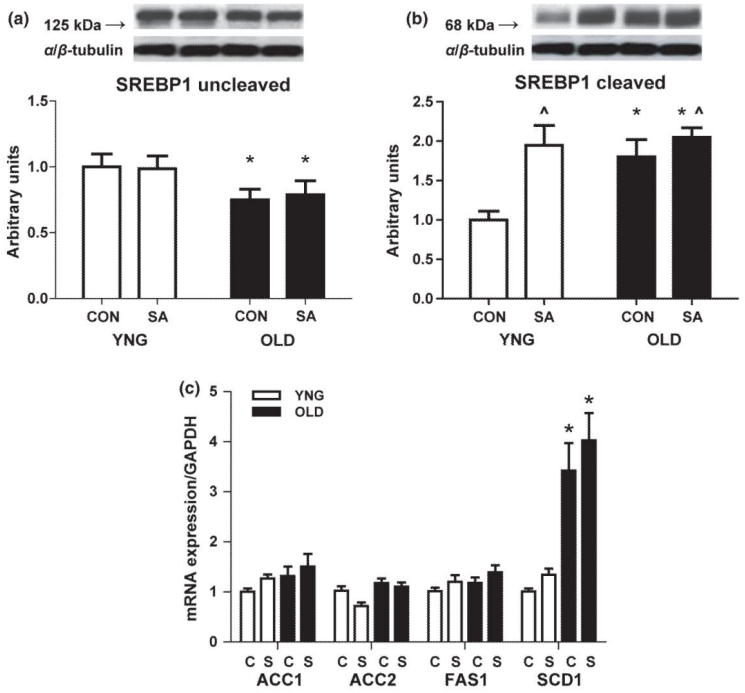

Figure 2.

Total protein content of the lipogenic transcription factor SREBP1 in the plantaris muscle of YNG, OLD and SA rats. Relative protein levels of the inactive uncleaved SREBP1 (a) and the active cleaved SBREP1 (b) were quantified using Western blot analysis and densitometry in plantaris muscle. mRNA expression (c) of the SREBP1 target genes ACC1/2, FAS1 and SCD1 differences between groups (*$P < 0.05$ vs. YNG, ^$P < 0.05$ vs. CON; $n = 8$/group).

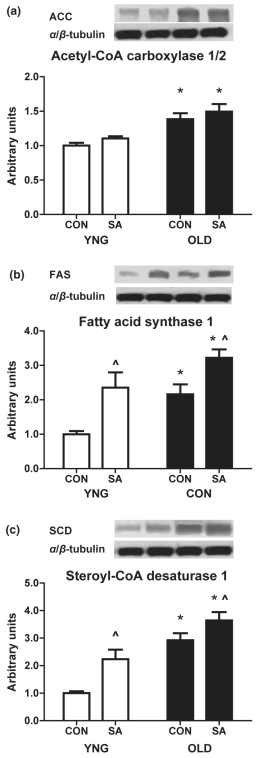

Figure 3.

Total protein content of lipid synthesis regulators in the skeletal muscle of YNG, OLD and SA Fischer 344 × Brown Norway rats. Relative protein levels of ACC1/2 (a), FAS1 (b) and SCD1 (c) were quantified using Western blot analysis in plantaris muscle. Significant differences between groups (*$P < 0.05$ vs. YNG; ^$P < 0.05$ vs CON; $n = 8$/group).

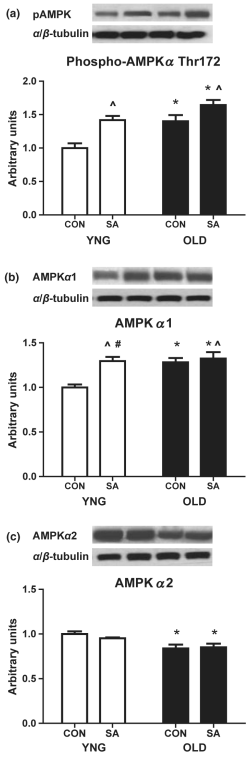

Figure 4.

Phosphorylation and total protein content of the metabolic regulator 5′ AMP-activated protein kinase (AMPK) in the skeletal muscle of YNG, OLD and SA Fischer 344 × Brown Norway rats. Relative protein levels of Phospho-AMPKα (a), the AMPK α1 isoform (b) and the AMPK α2 isoform (c) were quantified using Western blot analysis in plantaris muscle. Significant differences between groups (*$P < 0.05$ vs. YNG, ^$P < 0.05$ vs. CON, $^{\#}P < 0.05$. vs. YNG CON; $n = 8$/group).

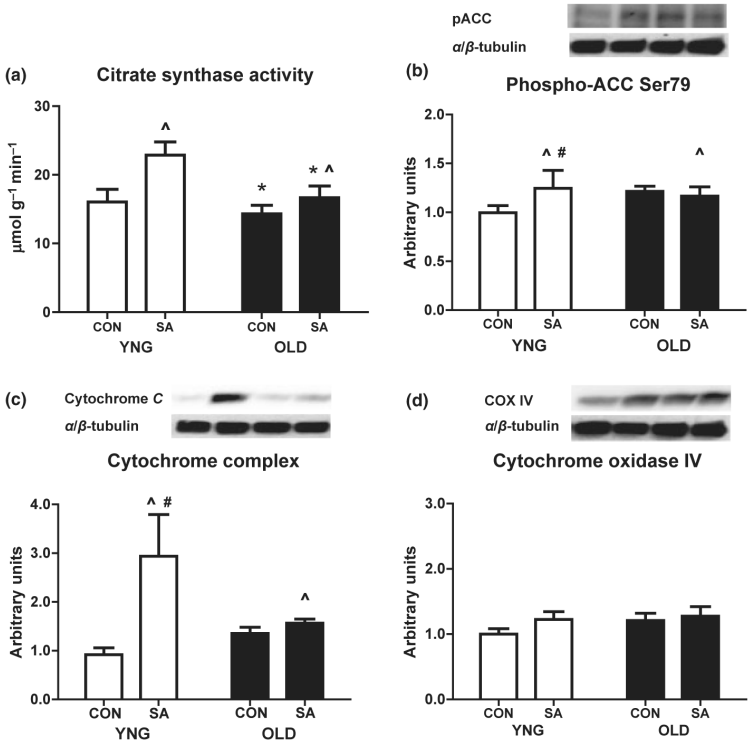

Figure 5.

Marker of mitochondrial enzyme activity and markers of mitochondrial protein expression in the skeletal muscle of young, old and surgically ablated Fischer 344 × Brown Norway rats. Citrate synthase activity (a) as determined by enzymatic assay. Relative protein levels of Phospho-ACC (b), Cytochrome *c* (c) and COX IV (d) were quantified using Western blot analysis in plantaris muscle. Significant differences between groups (*$P < 0.05$ vs. YNG, ^$P < 0.05$ vs. CON, $^{\#}P < 0.05$. vs. YNG CON; $n = 8$/group).

Table 1

Change in whole-body weights (g) from day 0 to day 28, plantaris muscle wet weights and plantaris total protein content of young (YNG), old (OLD) and surgically ablated (SA) Fischer 344 × Brown Norway rats

	YNG		OLD	
	CON	SA	CON	SA
Δ Body weight (g)	27.9 ± 4.0	−0.04 ± 3.7†	−46.3 ± 3.7*	−76.4 ± 4.2*†
Plantaris wet weight (mg)	434.0 ± 13.8	506.5 ± 19.7†	352.8 ± 6.4*	402.1 ± 14.4*†
Total protein content (mg)	25.13 ± 1.8	45.16 ± 5.7†	17.30 ± 1.9*	28.52 ± 4.0*†

*Significant differences between groups $P < 0.05$ vs. YNG

†Significant differences between groups $P < 0.05$ vs. CON; $n = 8$/group.