Intramyocellular lipid content and lipogenic gene expression responses following a single bout of resistance type exercise differ between young and older men

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Short title: (less than 40 letters and spaces) Resistance type exercise and ageing

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Abstract

The aim of this study was to examine the temporal relationship between intramyocellular lipid (IMCL) content and the expression of genes associated with IMCL turnover, fat metabolism, and inflammation during recovery from an acute bout of resistance type exercise in old versus young men. Seven healthy young (23 ± 2 years, 77.2 ± 2.9 kg) and seven healthy older (72 ± 1 years, 79.3 ± 4.9 kg) males performed a single bout of resistance exercise involving 6 sets of 10 repetitions of leg press and 6 sets of 10 repetitions of leg extension at 75% one-repetition maximum (1-RM). Muscle biopsy samples were obtained before and 12, 24 and 48h after the completion of exercise and analysed for IMCL content and the expression of 48 genes. The subjects refrained from further heavy physical exercise and consumed a standardized diet for the entire experimental period. The IMCL content was ~2-fold higher at baseline and 12h post-exercise in old compared with young individuals. However, no differences between groups were apparent after 48h of recovery. There was higher expression of genes involved in fatty acid synthesis (FASN and PPARγ) during the first 24h of recovery. Differential responses to exercise were observed between groups for a number of genes indicating increased inflammatory response (IL6, IkBalpha, CREB1) and impaired fat metabolism and TCA cycle (LPL, ACAT1, SUCLG1) in older compared with younger individuals. A singe bout of resistance type exercise leads to molecular changes in skeletal muscle favouring reduced lipid oxidation, increased lipogenesis, and exaggerated inflammation during post-exercise recovery in the older compared with younger individuals, which may be indicative of a blunted response of IMCL turnover with ageing.

Key words

ageing; genes; IMCL; resistance exercise; skeletal muscle.

Abbreviations

IMCL=Intramyocellular lipid. DAG=diacylglycerol. CPT1= Carnitine palmitoyl transferase1. ACAT1=Acetyl-CoA acetyltransferase1. FASN=Fatty Acid Synthase. SUCLG1=Succinyl-CoA ligase (synthetase). NF-kB=Nuclear Factor kappaB. TNFa=Tumour Necrosis Factor alpha. CREB1=Cyclic AMP responsive element binding protein1. IL6=Interleukin6. COX2=Cyclooxygenase2. PI3KR1=Phosphatidylinositol 3-kinase, regulatory1 (p85 alpha). LPL=Lipoprotein lipase. ATGL=Adipose Triglyceride Lipase. PPARα=Peroxisome Proliferator Activated Receptor alpha. PPARγ=Peroxisome Proliferator Activated Receptor gamma. IkBalpha=Inhibitor of kappaB kinase alpha. Akt2=Protein kinase B/Akt, isoform2. IRS1=Insulin receptor substrate1. PKCα=Protein kinase alpha. GLUT4=Glucose transporter isoform4. PDK2=Pyruvate dehydrogenase kinase 2. HK2=Hexokinase2. LDH=Lactate dehydrogenase. ChREBP=Carbohydrate response element binding protein.

1.0 Introduction

A common consequence of human ageing is increased visceral adiposity and intramyocellular lipid (IMCL) accumulation especially in the subsarcolemmal region of skeletal muscle cells (Chee et al., 2016, Crane et al., 2010, Cree et al., 2004). Interestingly, this ectopic lipid accumulation in elderly individuals is likely due to lifestyle factors (e.g. diet, periods of physical inactivity/disuse and/or sedentariness) rather than inherent ageing of skeletal muscle (Chee et al., 2016).

Regardless of its aetiology, IMCL accumulation is a major factor for the induction of peripheral insulin resistance of skeletal muscle glucose metabolism (Kim et al., 2007, Stefan et al., 2008) and also implicated in ageing-related anabolic resistance to dietary protein (Masgrau et al., 2012, Murton et al., 2015, Rivas et al., 2016, Wall et al., 2015, Stephens et al., 2015). Perturbed protein metabolism underpins the frequently observed loss (wasting) of skeletal muscle with advancing age (sarcopenia). Ectopic lipid species accumulation is also associated with a proinflammatory response which may explain, at least in part, the increased levels of the NF-κB (Rivas et al., 2012) and TNFα (Greiwe et al., 2001) in ageing skeletal muscle.

The contribution of IMCL utilisation to total fat oxidation during endurance exercise is reduced in older when compared with young individuals (Chee et al., 2016), which, over the long term, may contribute to higher IMCL content, inflammation, and insulin resistance commonly observed in older adults. Although a single bout of resistance type exercise leads to a significant utilisation of IMCL and its subsequent resynthesis within the first few hours of recovery in healthy young individuals (Koopman et al., 2006), the impact of resistance type exercise on IMCL depots in older individuals has not been elucidated. This is surprising given

that resistance type exercise leads to substantial increases in muscle mass and strength and is frequently prescribed as part of lifestyle interventions designed to ameliorate the age-related loss of muscle mass (Kosek et al., 2006, Roth et al., 2001).

Although changes in expression of genes related to cell stress and inflammation during recovery from a single bout of resistance type exercise are greater in older when compared with young individuals (Thalacker-Mercer et al., 2010), there is some evidence from gene array studies that molecular changes may be attenuated in the older compared to the young during the first few hours after exercise (Raue et al., 2012). This raises the interesting possibility that the time-course dependent changes in gene expression during recovery from resistance type exercise may differ between young and older individuals.

In the present study we examined the temporal relationship between IMCL content and the expression of genes associated with IMCL turnover, fat metabolism, insulin signalling, and inflammation during recovery from an acute bout of resistance type exercise in old versus younger men. We hypothesised that in older individuals those molecular changes would favour a blunted turnover of IMCL content. Elucidating the molecular responses in skeletal muscle from young and older individuals to a single bout of intense resistance exercise is an important step in understanding the causes of abnormal lipid accumulation in skeletal muscle with ageing, which may predispose to the development of muscle insulin resistance of glucose metabolism.

2.0 Materials and Methods

2.1 Subjects. Seven healthy young [age 23 ± 2 years, body mass 77.2 ± 2.9 kg, Body Mass Index (BMI) 23.5 ± 0.6 kg/m^2, % body fat on DEXA 16.7 ± 1.9] and 7 healthy elderly [age 72 ± 1 years ($P<0.001$ from Young), body mass 79.3 ± 4.9 kg, BMI 25.9 ± 1.2 kg/m^2, % body fat 22.0 ± 1.9) men were recruited to participate in this study. The % type I and type II fibres in *vastus lateralis m.* was 51 ± 8 and 49 ± 8 in the young, respectively, and 62 ± 8 and 38 ± 8 in the older subjects, respectively. There was no significant difference in either fibre type between groups. All subjects were recreationally active, defined as participating in non-competitive sporting activities being performed less than 3 times per week. No participants engaged in any structured resistance exercise program in the past two years. Subjects were informed about the nature and risks of the experimental procedures before their written consent were obtained. The study was approved by the Medical Ethical Committee of the Maastricht University Medical Centre, and complied with the guidelines set out in the declaration of Helsinki. Medical history of all participants was evaluated and a resting electrocardiogram was performed before selection. Individuals with a recent history or current state of cardiovascular disease, COPD, Parkinson, rheumatoid arthritis, musculoskeletal/orthopedic disorders, renal disorder and cognitive impairment were excluded from participation. All eligible subjects participated in a pre-trial session to become familiarized with the resistance type exercise protocol and the equipment used in the main study. Proper lifting technique was demonstrated and then practiced by the subjects for each of the 2 lower-limb exercises (leg press and leg extension). Subsequently, maximal strength (one-repetition maximum, 1RM) was estimated by using the multiple repetitions testing procedure. Furthermore, each subject underwent a DEXA scan to evaluate whole body and leg fat free and fat mass. This study was part of a larger project investigating the muscle

adaptive response to a single bout of resistance type exercise in young and older men (Snijders et al., 2014).

2.2 Experimental design and protocol. Both groups of subjects (young and old) performed a single bout of resistance type exercise involving 12 repeated sets of bilateral leg muscle exercises at 75% 1-RM. Blood and muscle biopsy samples were obtained before and 12, 24 and 48h after the completion of exercise. All volunteers were instructed to refrain from heavy physical exercise for 3 days before the experimental test day and the subsequent 48h recovery period. Furthermore, a controlled diet (based on habitual daily energy requirements and food preferences) was provided to each subject for 24h before and 48h after the single session of resistance type exercise. Subjects' energy requirements were calculated using the Harris and Benedict equations (Harris and Benedict, 1918) with a physical activity index of 1.4. On average, the young subjects consumed 148 ± 9 kJ·kg bm^{-1}·d^{-1}, consisting of 71 ± 2 En% carbohydrate, 13 ± 0.4 En% protein and 20 ± 1 En% fat. Elderly subjects consumed 124 ± 6 kJ·kg bm^{-1}·d^{-1}, consisting of 64 ± 1 En% carbohydrate, 15 ± 0.3 En% protein (equal to 1.1 ± 0.1 g protein kg bw^{-1}·d^{-1}) and 22 ± 1 En% fat.

On the morning of the experimental test, following 24h of the controlled diet, subjects reported to the lab after an overnight fast. Following 30 min of supine rest, a venous blood sample was obtained after which a muscle biopsy was taken from the *vastus lateralis m*. Subjects were then provided with a standardized breakfast and performed a single bout of resistance type exercise, consisting of 6 sets of 10 repetitions at 75% 1-RM on the horizontal leg press machine (Technogym BV, Rotterdam, The Netherlands) and 6 sets of 10 repetitions at 75% 1-RM on the leg extension machine (Technogym BV). A resting period of exactly 2 minutes between sets was allowed. The entire protocol lasted approximately 40 min. There

was no difference in the time it took to complete the exercise session between young and old subjects. All subjects were verbally encouraged during the test to complete the whole protocol. Before and after the exercise session, a 5-10 min warm up/cooling down at low intensity was performed on a cycle ergometer. At the end of the exercise protocol the subjects rested for 3h in a supine position, immediately after which they received a standardized lunch, and were then provided with a standardized dinner and instructed to consume it 8h after the completion of exercise. Twelve hours after the completion of the single bout of resistance type exercise subjects reported back to the laboratory for collection of a second muscle biopsy and blood sample. Fasted muscle biopsies and blood samples were obtained exactly 24 and 48h after the completion of exercise.

2.3 Blood analysis. Blood samples (10 ml) were collected in EDTA and clot-activator gel containing tubes for plasma and serum separation, respectively. EDTA tubes were immediately centrifuged at 1000 × g for 10 min at 4°C to obtain plasma, whereas serum was obtained after allowing the blood to clot for 90 min at 21°C followed by centrifugation at 1000 × g for 15 min. Aliquots of plasma and serum were immediately frozen in liquid nitrogen and stored at −80°C until further analysis. Plasma glucose concentrations were analyzed with a COBAS FARA analyzer (Uni Kit III; Roche). Plasma FFA concentrations were analyzed with the NEFA C test kit from Wako Chemicals. Plasma insulin concentrations were analysed using a radio immunoassay (Linco, Human Insulin RIA kit). Serum IL6 levels were determined using an Enzyme-Linked ImmunoSorbant Assay (ELISA) kit according to manufactures instructions (R&D systems, Inc., USA).

2.4 Skeletal muscle biopsy and analysis. Muscle biopsies were obtained from the middle region of the *vastus lateralis* muscle 15 cm above the patella by means of the percutaneous

needle biopsy technique described by Bergstrom & Hultman (Bergstrom and Hultman, 1966). All muscle biopsies were taken from the same leg spaced by at least 2 cm. Lidocaine with 1% epinephrine was used to anaesthetize the local skin and the muscle fascia. Care was taken to prevent any anesthetic penetrating into the muscle. Upon collection, muscle samples were carefully freed from any visible fat and blood and rapidly frozen in liquid nitrogen cooled isopentane before stored at –80 ºC for subsequent analysis.

At a later date, muscle samples were used to cut 5 µm cross-sections at -20°C using a cryostat. Sections were then mounted on uncoated glass slides and stored at -20°C for subsequent determination of IMCL content, lipid droplet size and number (Stephens et al., 2014). After fixation in 4% paraformaldehyde phosphate buffered saline (pH 7.4). Samples were incubated at room temperature for 1h in 3 µM LD540/dimethyl sulfoxide (DMSO) and, following 3 washes in PBS (pH 7.4), embedded in Antifade gold for subsequent confocal microscopy analysis of LD540 stained lipid droplets (SP2, Leica). Briefly, 1µm z-stacks using a 561nm laser were captured at x40 magnification in order to control for sample depth and background noise, and the area of the fibre, lipid droplet size, and total number of lipid droplets per square micrometer of area covered by fluorescence was calculated using Volocity software (Volocity 6.3, PerkinElmer, Cambridge, UK). LD540 is a lipophilic dye similar to Bodipy, and was manufactured by the University of Nottingham School of Chemistry according to the method of Spandl et al (Spandl et al., 2009).

Total RNA was extracted from 15-20 mg of frozen muscle tissue by the method of Chomczynski and Sacchi (Chomczynski and Sacchi, 1987) using TRIzol reagent (Invitrogen, Paisley, UK). Quantification of RNA and its reverse transcription was carried out as described previously (Tsintzas et al., 2006). Taqman Low density Custom Array using Micro Fluidic

cards (ABI Applied Biosystems, UK) was used for the relative quantification of expression of 48 metabolic genes as previously described (Tsintzas et al., 2013). Each card allowed for 8 samples to be run in parallel against 48 Taqman gene expression assay targets that were pre-loaded into each of the wells on the card (Supplementary online Table S1). A control sample was loaded in each card to assess reproducibility of data between cards and normalise for variations in the expression of the target genes between cards. Briefly, 50 μl of Taqman Universal PCR master mix (2x) (ABI Applied Biosystems, UK) was added to 200 ng RNA equivalent of cDNA into an eppendorf RNAse-free tube. RNAse-free water was added to make the total volume of the reaction mixture up to 100 μl. The reaction mixture was mixed, centrifuged and loaded into one of the fill reservoir of the Micro Fluidic card. The cards were centrifuged (MULTIFUGE 3 S-R, Heraeus) and ran on a 7900HT Fast Real-Time PCR System (ABI Applied Biosystems, UK). Relative quantification of the genes of interest was performed using the comparative CT method. The geometric mean of 3 housekeeping genes [α-actin, 18S ribosomal RNA (18S) and hydroxymethylbilane synthase (HMBS)] was used to normalise the data to minimize variations in the expression of individual housekeeping genes.

2.5 Statistical analysis. All blood and muscle data were analysed using a two-way [age group (young vs. old) x time (pre- and 12, 24, 48h post exercise)] analysis of variance (ANOVA)]. When a significant difference was obtained with the two-way ANOVA, data were further analysed with Student's paired t-tests (when comparing within each age group) or unpaired t-tests (for comparisons between age groups) using the Bonferroni correction. Statistical significance was accepted at a 5% level ($P<0.05$). Statistical tendency was defined as $0.05<P\leq0.10$. Results are presented as means ± SEM.

3.0 Results

3.1 Resistance type exercise. The absolute exercise load at 75% 1-RM was higher in young compared with old subjects for both the horizontal leg press machine (149 ± 4 vs. 133 ± 7 kg, respectively; P=0.05) and the leg extension machine (95 ± 5 vs. 63 ± 3 kg, respectively; P<0.001). The young individuals had higher (P<0.05) fat free mass (FFM) for both legs combined when compared with the old (21.2 ± 0.9 vs. 18.5 ± 0.7 kg, respectively). As a result, the exercise load per kg leg FFM was similar between the young and old groups for leg press (7.1 ± 0.2 vs. 7.2 ± 0.4 kg, respectively) but was lower for leg extension in the old when compared with the young group (3.4 ± 0.1 vs. 4.5 ± 0.2 kg, respectively; P<0.001). Interestingly, there was a strong negative correlation between the exercise load per kg leg FFM and total adiposity (r = -0.82 and r = - 0.71 in the old and young, respectively) for leg extension but not leg press.

3.2 Blood metabolites. Plasma glucose concentrations were higher (P<0.05) at baseline and all time points during the 48h recovery period in old compared with young individuals (Table 1). However, baseline levels of HbA1C were similar between the two groups (5.6 ± 0.1 vs. 5.4 ± 0.1%, respectively). Plasma insulin concentrations were similar at baseline and throughout the recovery period between groups (Table 1). The log HOMA-IR (log of fasting glucose x fasting insulin / 22.5), a surrogate index of insulin resistance, was also similar at baseline between the old and young subjects (0.54 ± 0.08 vs. 0.53 ± 0.04, respectively). There were no differences in plasma FFA concentrations at any time point between groups but a similar decrease (~50-60%) was observed at 12h when compared with pre-exercise levels in both groups (Table 1). Serum IL6 levels were higher at 12h (P<0.05), 24h (P<0.05) and 48h (P<0.01) in old compared with young individuals (Table 1).

Table 1. Blood metabolite concentrations before (pre-exercise) and at 12, 24 and 48h after a single bout of resistance type exercise in young and older men.

		Pre-exercise	Post 12h	Post 24h	Post 48h
Plasma Glucose (mmol/L)	**Young** **Old**	5.1 ± 0.1 5.8 ± 0.2*	5.1 ± 0.3 6.2 ± 0.4*	5.1 ± 0.1 5.6 ± 0.1*	5.1 ± 0.1 5.5 ± 0.1*
Plasma Insulin (mU/L)	**Young** **Old**	15.3 ± 1.3 13.3 ± 3.5	23.8 ± 3.6 35.7 ± 6.9	14.4 ± 2.2 13.8 ± 3.5	14.9 ± 3.4 13.8 ± 3.0
Plasma IL6 (pg/mL)	**Young** **Old**	4.2 ± 1.9 11.4 ± 3.9	4.3 ± 1.0 12.4 ± 3.2*	4.0 ± 1.0 13.6 ± 3.1*	2.6 ± 0.3 14.0 ± 3.0#
Serum FFA (mmol/L)	**Young** **Old**	0.38 ± 0.05 0.42 ± 0.04	0.20 ± 0.03[b] 0.14 ± 0.03[a]	0.37 ± 0.03 0.41 ± 0.05	0.35 ± 0.04 0.38 ± 0.05

Data are expressed as means ± SEM, n=7. * P<0.05 from Young; # P<0.01 from Young; [a] P<0.01 from Pre-exercise; [b] P<0.05 from Pre-exercise.

3.3 IMCL content. The IMCL content (% area of muscle fibre analysed) was higher (P<0.05) at baseline in old compared with young individuals and this difference was maintained at 12h into the recovery period (P<0.01; Fig. 1A). However, the IMCL content increased at 48h in the young subjects (P<0.05) whereas it remained unchanged in the old such that there was no difference at that time point between groups (Fig. 1A). Furthermore, there was a trend that failed to reach statistical significance (P=0.095) for higher lipid droplet number per μm^2 of muscle fibre cross-section area before and 12h after exercise in the old when compared with the young group. Similar to IMCL % area of muscle fibre, the lipid droplet number was higher (P<0.05) at 48h than preexercise in the young subjects only (Fig. 1B). On the other hand, 2-way ANOVA revealed higher (P<0.05) lipid droplet size in the old when compared with the young subjects with no effect of exercise in either group (Fig. 1C).

Figure 1

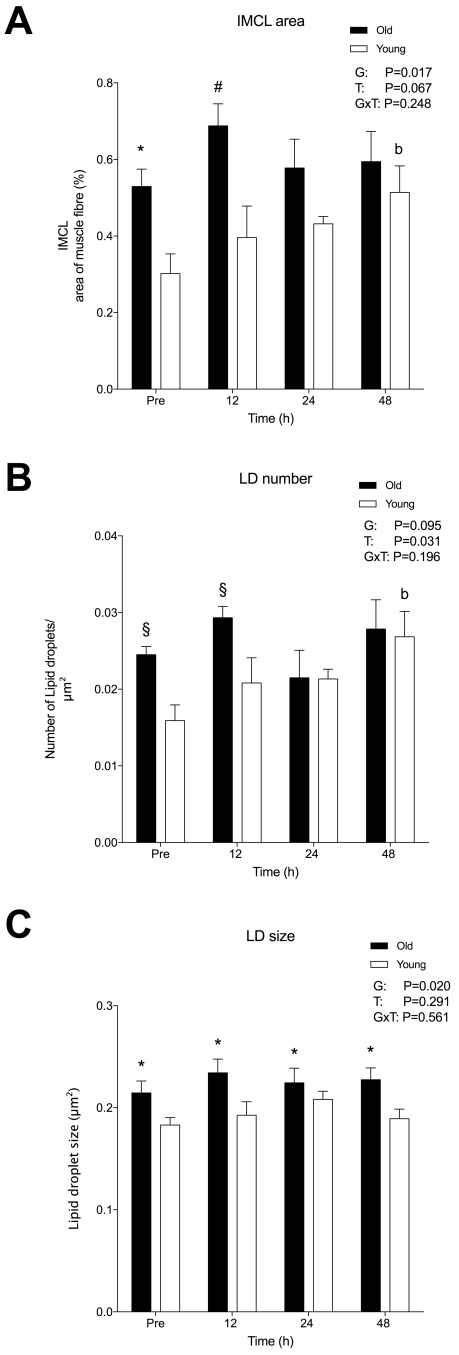

Figure 1. Human skeletal muscle IMCL content (% of muscle fibre area) (A), lipid droplet number per µm^2 of muscle fibre cross-section area (B) and lipid droplet size (µm^2) (C) before (Pre) and at 12, 24 and 48h after a single bout of resistance exercise in young and old individuals. Data are expressed as means ± SEM, n=7. * P<0.05 from Young; # P<0.01 from Young; § P<0.10 from Young**;** ^{b} P<0.05 from corresponding Pre. G=two-way ANOVA group effect; T=two-way ANOVA time effect; GxT=two-way ANOVA interaction effect.

3.4 Skeletal muscle gene expression differences between groups. Two-way ANOVA revealed a significant effect between the young and old groups (with no time or interaction effects) for PPARγ, with higher expression (P<0.05) observed in the old (Fig. 2A). There was also a strong trend (P=0.058) for lower expression of ATGL in old compared with young individuals (Fig. 2B).

Figure 2

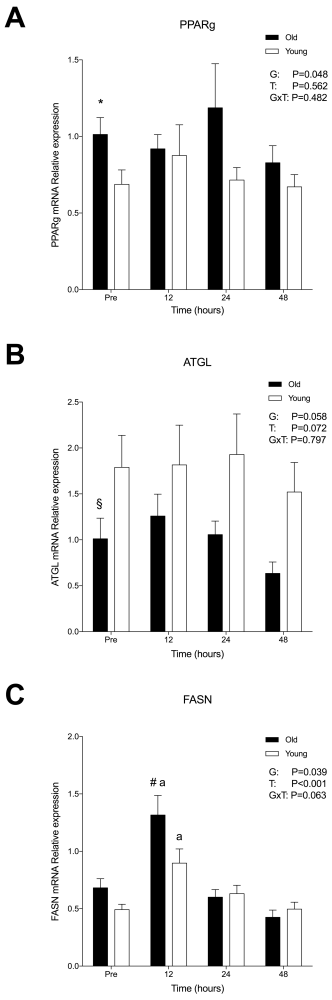

Figure 2. Human skeletal muscle mRNA relative expression for PPARγ (A), ATGL (B) and FASN (C) before (Pre) and at 12, 24 and 48h after a single bout of resistance type exercise in young and old individuals. Data are expressed as means ± SEM, n=7. * P<0.05 from Young; # P<0.01 from Young; [a] P <0.01 from corresponding Pre; [§] P=0.058 from Young. G=two-way ANOVA group effect; T=two-way ANOVA time effect; GxT=two-way ANOVA interaction effect.

Furthermore, there were significant differences between groups ($P<0.05$) and time ($P<0.01$) effects for the FASN (Fig. 2C) and COX2 (Fig. 3A) genes, with higher content observed in the old group when compared with the young. Specifically, the mRNA content of FASN increased during the early phase of recovery from exercise in both groups ($P<0.01$ from baseline) but this response was higher in the older group resulting in higher values at 12h post-exercise when compared with the young ($P<0.01$). Thereafter a decrease in its content was observed with no differences between groups at 24 and 48h.

3.5 Age differences in skeletal muscle gene expression response to exercise. Two-way ANOVA also revealed significant interaction effects between groups (Old vs. Young) and time (pre-exercise vs. post-exercise recovery sampling times) for a number of genes and transcription factors involved in inflammatory (IL6, IkBalpha, CREB1), insulin signalling (PI3KR1, Akt2), CHO metabolism (HK2, LDH, ChREBP), and fat metabolism and TCA cycle (LPL, ACAT1, SUCLG1) pathways.

Expression of IL6 (Fig. 3B) was similar between old and young at baseline but showed a 34-fold increase ($P<0.01$) at 12h into recovery in the old group only resulting in higher levels at 12h post-exercise when compared with the young ($P<0.01$). Thereafter, a rapid decrease in IL6 expression was observed in the old such that no significant differences were observed between groups at 24h and 48h of recovery. In contrast, IkBalpha and CREB1 were higher before exercise in old when compared with younger individuals ($P<0.01$ and $P<0.05$, respectively) but this difference was no longer apparent after 48h of post-exercise recovery (Fig. 3C and 3D, respectively).

Figure 3

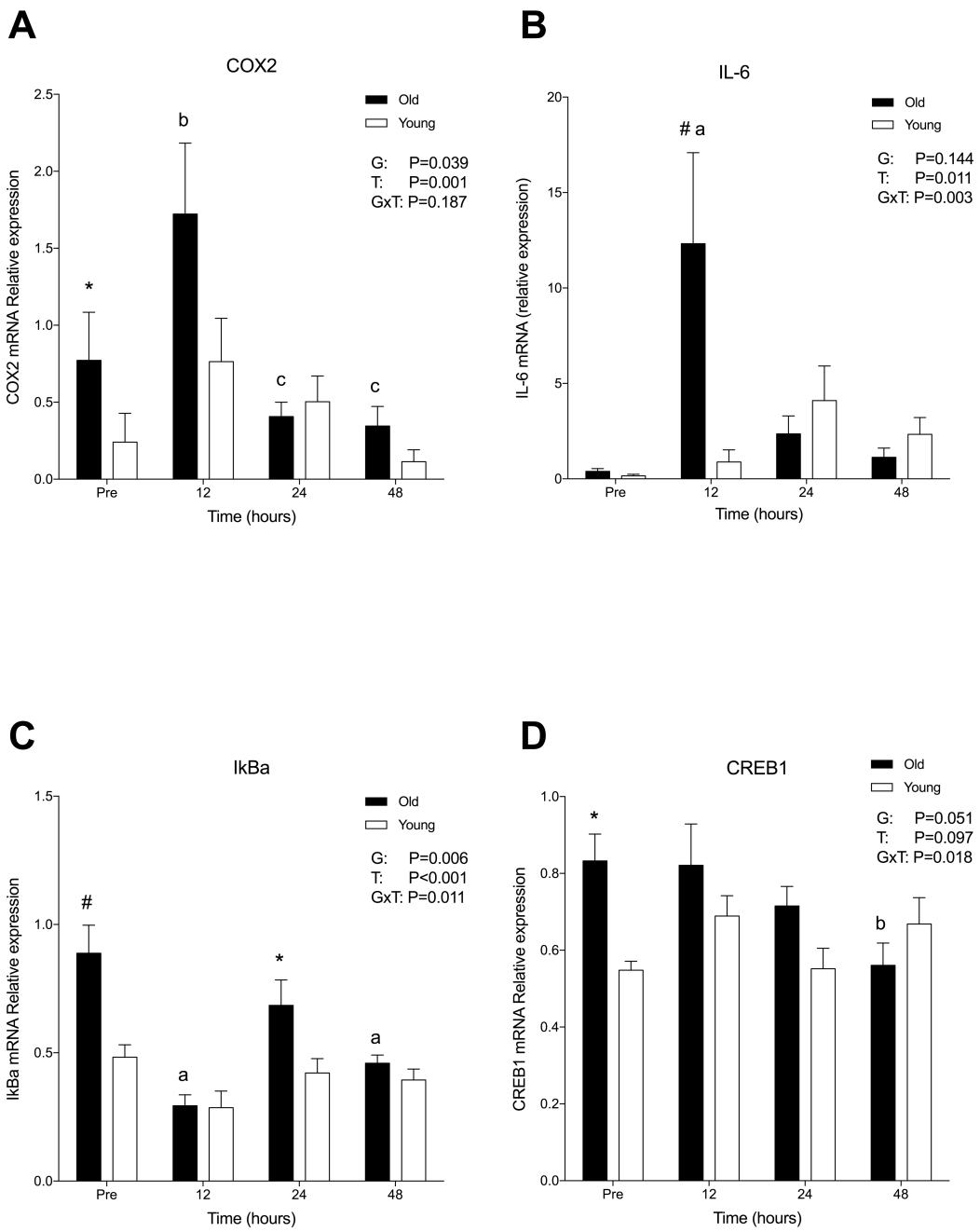

Figure 3. Human skeletal muscle mRNA relative expression for COX2 (A), IL6 (B), IkBa (C) and CREB1 (D) before (Pre) and at 12, 24 and 48h after a single bout of resistance type exercise in young and old individuals. Data are expressed as means ± SEM, n=7. * P<0.05 from Young; # P<0.01 from Young; [a] P <0.01 from corresponding Pre; [b] P <0.05 from corresponding Pre. [c] P <0.05 from corresponding 12h. G=two-way ANOVA group effect; T=two-way ANOVA time effect; GxT=two-way ANOVA interaction effect.

The mRNA content of PI3KR1 (Fig. 4A) was higher before exercise in old when compared with young individuals (between groups effect $P<0.01$) but responded differently to exercise in the young compared with the old (interaction effect $P<0.01$). Its expression remained higher at 12h ($P<0.01$) and 24h ($P<0.05$) of recovery in the old when compared with the young, but then declined such that no significant difference was observed between groups at 48h (Fig. 4A). The mRNA content of Akt2 (Fig. 4B) also declined 48h after exercise in the old subjects ($P<0.05$) whereas it remained unchanged in the young.

The mRNA content of HK2 (Fig. 4C) was similar between old and young at baseline but showed a 12-fold increase at 12h into recovery in the old group only resulting in higher levels at 12h post-exercise when compared with the young ($P<0.01$). This was followed by a decrease in HK2 expression after 24 and 48h in the old, whereas it remained unchanged in the young. On the other hand, a gradual decrease in LDH and ChREBP was observed in response to exercise in the old group ($P<0.01$) whereas they remained unchanged in the young (Fig. 4D and 4E, respectively).

Figure 4

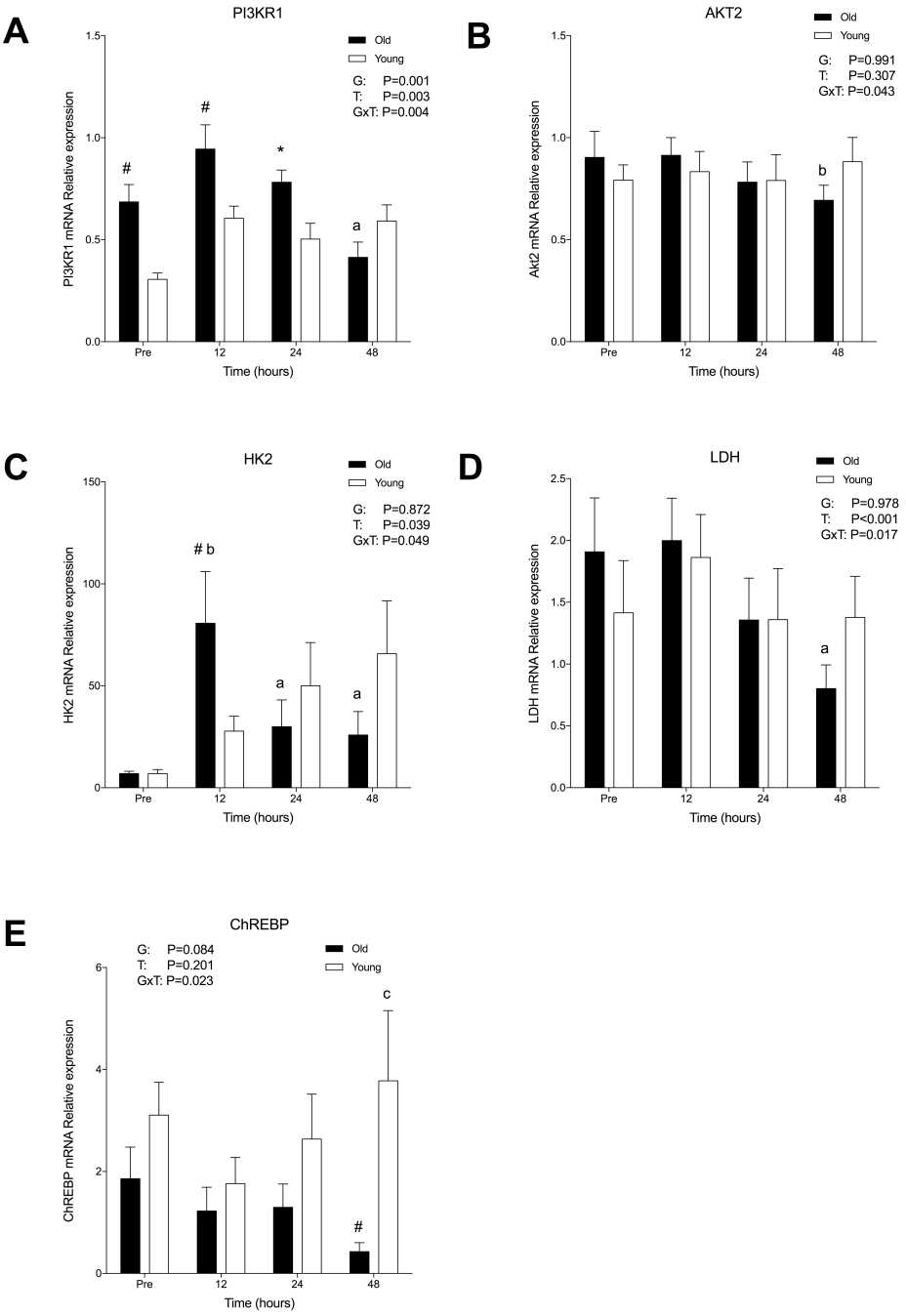

Figure 4. Human skeletal muscle mRNA relative expression for PI3KR1 (A), Akt2 (B), HK2 (C), LDH (D) and ChREBP (E) before (Pre) and at 12, 24 and 48h after a single bout of resistance type exercise in young and old individuals. Data are expressed as means ± SEM, n=7. * P<0.05 from Young; # P<0.01 from Young; [a] P <0.01 from corresponding Pre; [b] P <0.05 from corresponding Pre; [c] P <0.05 from corresponding 12h. G=two-way ANOVA group effect; T=two-way ANOVA time effect; GxT=two-way ANOVA interaction effect.

The expression of LPL (Fig. 5A) decreased between baseline and 48h after exercise in the old ($P<0.05$), whereas it remained unchanged in the young. Similarly, the expression of ACAT1 (Fig. 5B) gradually decreased after exercise in the old ($P<0.01$) whereas it remained unchanged in the young. The expression of SUCLG1 (Fig. 5C) also decreased during the 48h recovery period in the old ($P<0.01$) but not the young.

3.6 Skeletal muscle gene expression in response to exercise. Two-way ANOVA revealed significant time (changes from pre-exercise to post-exercise recovery expression values) but no group or interaction effects for 6 genes involved in insulin signalling (IRS1, PKCα) and energy metabolism (GLUT4, PDK2, PPARα and CPT1). Specifically, there was downregulation of IRS1, GLUT4, PDK2 and CPT1 after exercise, whereas PKCα was upregulated. On the other hand, PPARα showed an initial increase at 12h followed by a decrease at 48h of recovery (Supplementary online Table S2).

Figure 5

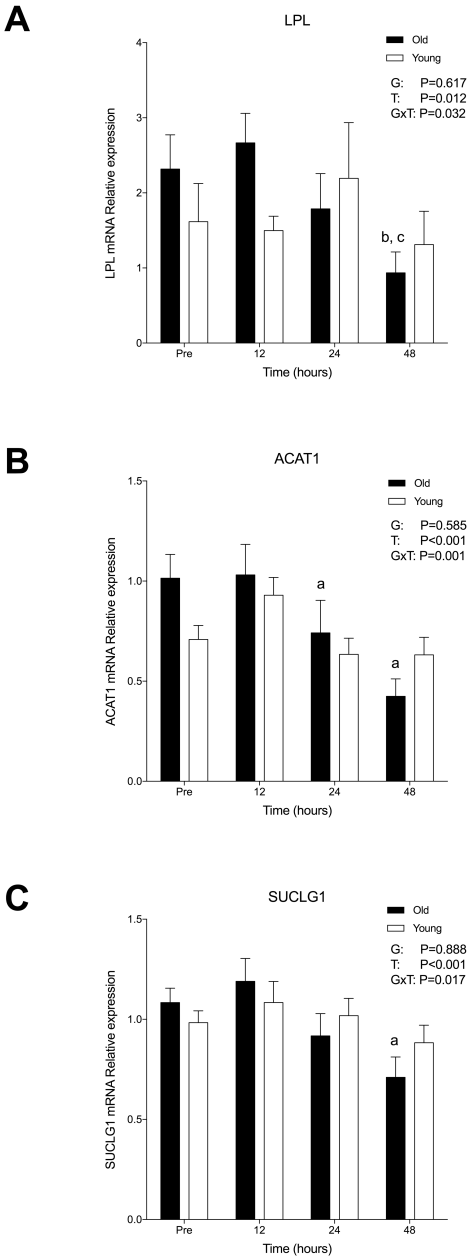

Figure 5. Human skeletal muscle mRNA relative expression for LPL (A), ACAT1 (B) and SUCLG1 (C) before (Pre) and at 12, 24 and 48 h after a single bout of resistance type exercise in young and old individuals. Data are expressed as means ± SEM, n=7. [a] P <0.01 from corresponding Pre; [b] P <0.05 from corresponding Pre; [c] P <0.05 from corresponding 12h. G=two-way ANOVA group effect; T=two-way ANOVA time effect; GxT=two-way ANOVA interaction effect.

4.0 Discussion

The present study showed that acute resistance type exercise performed by healthy older individuals leads to molecular changes in skeletal muscle tissue that are indicative of reduced lipid oxidation, increased lipogenesis of various lipid species, and increased inflammation, which may explain the relative inflexibility (blunted response to exercise) of changes in IMCL content in those individuals.

In accordance with previous studies (Chee et al., 2016, Crane et al., 2010, Cree et al., 2004) the IMCL content was ~2-fold higher at rest (before exercise) but remained unchanged during the recovery period in older when compared with younger individuals, indicating a relative inflexibility in its turnover. In contrast, the IMCL content gradually increased during recovery in the young subjects such that the preexercise differences between groups were no longer present after 48h of postexercise recovery. Interestingly, although the size of lipid droplets was lower in younger compared with older subjects, the increase in IMCL content from pre-exercise to 48h post-exercise in young individuals was due to an increase in lipid droplet number rather than size. This is a novel finding indicating that a single bout of resistance exercise is capable of increasing the number of lipid droplets in human skeletal muscle. This is also consistent with a previous report showing that regular aerobic exercise training in young subjects increases the IMCL area density due to an increase in the number rather than size of lipid droplets (Tarnopolsky et al., 2007). Collectively, these data indicate that exercise in young individuals promotes IMCL turnover and the lipid droplet number.

Previous studies using healthy young individuals have demonstrated a significant utilisation of IMCL and its subsequent resynthesis during recovery from both resistance (Koopman et al., 2006) and endurance (van Loon et al., 2003) type exercise. Although a single bout of

resistance type exercise leads to repletion of the IMCL stores within 2h of recovery in young individuals (Koopman et al., 2006), the impact of resistance exercise on the time course of IMCL resynthesis in older individuals has not been previously investigated. Interestingly, it may take up to 48h for complete repletion of IMCL stores after a single exhaustive bout of endurance type exercise (van Loon et al., 2003), although some studies have observed 30-50% greater IMCL content 24-30h after a single bout of endurance exercise than the preexercise values in healthy young individuals (Decombaz et al., 2001, Schenk and Horowitz, 2007) indicating a dynamic flexibility of IMCL turnover in response to exercise in young subjects. Indeed, chronic endurance training in young males leads to increased resting IMCL content and fractional synthesis rates (FSR) when compared with sedentary individuals (Bergman et al., 2010). In accordance, in young individuals the turnover of IMCL stores seems to correlate with physical training and increased oxidative capacity of skeletal muscle (van Loon and Goodpaster, 2006). Since IMCL accumulation is often (with the exception of exercise training) associated with the development of insulin and anabolic resistance, and subclinical inflammation in skeletal muscle tissue, the capacity (flexibility) to turnover this particular ectopic fat depot in response to exercise may be critical for the maintenance of metabolic health with advancing age. The IMCL insensitivity to acute resistance type exercise with ageing observed in the present study is further supported by the observation that, unlike endurance type exercise training, regular resistance type exercise training does not seem to alter skeletal muscle IMCL contents in older individuals (Ngo et al., 2012).

In the present study, the higher expression of skeletal muscle genes involved in fatty acid synthesis (FASN and PPARγ), and the lower expression of ATGL (a key enzyme involved in lipolysis of IMCL) before and after exercise in old vs. young men indicate a molecular milieu favouring increased lipogenesis of lipid species and reduced reliance on IMCL lipolysis,

respectively, in older men. This is in agreement with a recent study from our laboratory showing that old overweight subjects have greater resting expression of genes involved in ceramide and DAG accumulation in skeletal muscle when compared with young lean subjects (Chee et al., 2016). Furthermore, the abundance of genes involved in fat metabolism and TCA cycle activity (namely LPL, ACAT1 and SUCLG1) was differentially affected by exercise showing a decrease after exercise in the old whereas they remained unchanged in the young. Interestingly, the expression of skeletal muscle ACAT1, a key enzyme in the terminal stage of β-oxidation, was previously shown to be upregulated (along with ATGL as part of the top 6 most differentially expressed genes) in response to chronic carnitine supplementation in humans that led to increased rates of fat oxidation at rest and during low intensity endurance exercise (Stephens et al., 2013). Taken together it would appear that there is a relative inflexibility of IMCL turnover in the older subjects which, combined with increased delivery of FFA to muscle (Chee et al., 2016), ultimately results in a greater IMCL pool size and the associated decline in metabolic health/function. However, whether this is the cause or consequence of the transcriptional changes indicating reduced fat metabolism and increased lipogenesis of metabolically harmful lipid metabolites before and after exercise is not clear.

Since excessive accumulation of lipid metabolites (including DAG, fatty acyl CoAs and ceramides) is strongly linked with the development of muscle insulin resistance (Boden, 2006), it is not surprising that the lipogenic molecular signature observed in the older subjects in the present study was associated with profound changes in the expression of genes implicated in muscle insulin signalling and carbohydrate metabolism. Indeed, the mRNA content of the PI3KR1 gene, which encodes the p85α regulatory subunit of the phosphatidylinositol 3-kinase (PIK3) signal transduction protein in the insulin signalling pathway in human skeletal muscle, was higher before exercise and during the first 24h of

recovery in the old when compared with the young. Free p85α plays a negative role in skeletal muscle PIK3/Akt signalling axis by competing with the p85/p110 dimer for binding to IRS1 and activation of PIK3 (Barbour et al., 2005), which explains why mice lacking the p85a subunit of PIK3 showed increased insulin sensitivity (Terauchi et al., 1999). Interestingly, the mRNA content of Akt2, a positive regulator of muscle insulin signalling, declined 48h after exercise in the old but not young subjects. In accordance, the expression of genes involved in carbohydrate metabolism (such as LDH and ChREBP) showed a gradual decline after exercise in the old but not young subjects. ChREBP appears to be necessary for the induction of glucose-regulated genes (Denechaud et al., 2008) but its downregulation after exercise occurred despite consistently higher blood glucose concentrations before and after exercise in the old when compared with the younger individuals in the present study.

Ectopic lipid accumulation is also frequently associated with a low grade (subclinical) pro-inflammatory response (Glass and Olefsky, 2012) which may explain, at least in part, the increased resting levels of the NF-κB (Rivas et al., 2012) and TNFα (Greiwe et al., 2001) in ageing skeletal muscle. NF-κB, in particular, appears to be a key transcription factor that mediates the postexercise inflammatory response. Indeed, in young, recreationally active subjects acute intense resistance type exercise caused a significant upregulation of the NF-kB signalling in skeletal muscle within the first 2h of recovery, which was associated with elevated mRNA expression of the pro-inflammatory cytokines IL6 and IL8 (Vella et al., 2012). In a separate study, the time course of genes associated with inflammation (such as IL6, IL8 and TNFα) showed peak mRNA expression levels between 2-12h after a single bout of resistance exercise, which were still elevated after 24h in young individuals accustomed to resistance type exercise (Louis et al., 2007). However, changes in expression of genes related to cell stress, inflammation, and regeneration (involved in the activation of the NF-kB and

p38 mitogen-activated protein kinase signalling pathways) are greater in old when compared with young individuals 24h after a single bout of resistance exercise, indicating that senescent muscle is more sensitive to exercise-induced damage and subsequent regeneration (Thalacker-Mercer et al., 2010). Interestingly, gene array studies suggest that post exercise transcriptional changes may be attenuated in old subjects during the first few hours of post-exercise recovery (Raue et al., 2012), which raises the possibility that the time course dependent changes in gene expression during recovery from resistance type exercise may differ between young and old individuals. This contention is supported by the findings from the present study that revealed significant differential responses to exercise between old and young subjects for a number of genes indicating exaggerated inflammatory response (COX2, IL6, IkBalpha, CREB1) in old when compared with young. This response was higher for IL6 and COX2 at 12h into recovery, whereas it was maintained for up to 24h for IkBalpha and CREB1. Interestingly, it appears that changes in the abundance of those pro-inflammatory genes occur early on in the recovery period and precede changes in genes involved in fat metabolism, which supports the notion that ectopic lipid accumulation is closely linked to inflammation in human skeletal muscle (Glass and Olefsky, 2012). Neubauer et al (Neubauer et al., 2014) investigated the time course-dependent changes in global gene expression in human skeletal muscle during recovery from endurance type exercise using microarrays and showed that early upregulation of the CREB1 appears to be critical in the expression of genes involved in cell metabolism and remodelling. In the present study, the mRNA abundance of CREB1 was higher before exercise and early in recovery in old when compared with younger individuals but this difference was no longer apparent after 48h of post-exercise recovery, suggesting that CREB1 may also be important in mediating the early proinflammatory response in skeletal muscle after performing a single bout of resistance type exercise in older individuals.

5.0 Conclusion

A single bout of resistance type exercise induces molecular responses in skeletal muscle of older individuals favouring reduced lipid oxidation, increased lipogenesis, and exaggerated inflammation, which have been associated with abnormal lipid accumulation and consequently the development of insulin resistance and anabolic resistance to feeding. It remains to be investigated whether engaging in regular intense resistance type exercise will attenuate this unfavourable molecular signature in skeletal muscle tissue in older individuals.

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Conflict of interest

There are no conflicts of interest.