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

Kostas Tsintzas [a,*], Francis B. Stephens [a,c,1], Tim Snijders [b], Benjamin T. Wall [b,c], Scott Cooper [a], Joanne Mallinson [a], Lex B. Verdijk [b], Luc J.C. van Loon [b]

[a] MRC/ARUK Centre for Musculoskeletal Ageing, School of Life Sciences, University of Nottingham, NG7 2UH, UK
[b] Department of Human Biology and Movement Sciences, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre, Maastricht, The Netherlands
[c] School of Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter, Exeter EX1 2LU, UK

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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 48 h 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 12 h post-exercise in old compared with young individuals. However, no differences between groups were apparent after 48 h of recovery. There was higher expression of genes involved in fatty acid synthesis (FASN and PPARγ) during the first 24 h 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.

1. 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

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.

* Corresponding author at: MRC/ARUK Centre for Musculoskeletal Ageing, School of Life Sciences, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH, UK.
E-mail address: kostas.tsintzas@nottingham.ac.uk (K. Tsintzas).
[1] Joint first author.

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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. 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 <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 48 h 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 48 h recovery period. Furthermore, a controlled diet (based on habitual daily energy requirements and food preferences) was provided to each subject for 24 h before and 48 h 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 24 h 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

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

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