Inflammatory markers in skeletal muscle of older-adults

Marissa K Caldow ¹, David Cameron-Smith ^{1,2}, Pazit Levinger ³, Michael J. McKenna ⁴,
Itamar Levinger ^{4,5}

¹ School of Exercise and Nutrition Sciences, Deakin University, Melbourne Australia

² Liggins Institute, University of Auckland, Auckland New Zealand.

³ Musculoskeletal Research Centre, La Trobe University, Melbourne Australia

⁴ Institute of Sport, Exercise and Active Living (ISEAL), Victoria University, Melbourne Australia.

⁵ School of Sport and Exercise Science, Victoria University, Melbourne Australia.

Address for correspondence:

Dr Itamar Levinger

Institute of Sport, Exercise and Active Living (ISEAL), Victoria University,

School of Sport and Exercise Science,

Victoria University

PO Box 14428, Melbourne, VIC 8001, Australia.

Tel: (61-3) 9919 5343, Fax: (61-3) 9919 5532, E-mail: itamar.levinger@vu.edu.au

Abstract

Background: Older-adults have an increase in circulating markers of inflammation. The current study examined whether there is an increase in the expression of inflammatory markers within the *vastus lateralis*, a major locomotive muscle, of older-adults, and if so, whether the reduction in muscle strength and aerobic capacity in older-adults is related to increased muscle inflammation.

Methods: Skeletal muscle biopsies were taken from older adults (n=17, 67 ± 1.6 years) and young individuals (n=16, 24 ± 0.6 years) under resting and fasting conditions. Muscle was analyzed for mRNA levels of intracellular inflammatory molecules (MCP1, $\text{TNF}\alpha$ and IL- 1β), and total cellular protein abundance of cytokines, chemokines and kinases (IL-6, IL-8, MCP1, $\text{TNF}\alpha$, p65 (NF- κ B), JNK1/2 and STAT3).

Results: MCP1 expression was significantly higher ($P < 0.05$; 50%, mRNA and 40%, protein) in elderly than younger participants, as was IL-8 (4%). No detectable difference in kinase protein expression was observed for STAT3, JNK or p65 (NF- κ B), $\text{TNF}\alpha$ or IL-6. Muscle strength was lower in the elderly compared to the young group (1.55 ± 0.17 versus 2.56 ± 0.13 Nm/kg, $p < 0.001$). The elderly group also had a significant lower $\text{VO}_{2\text{peak}}$ compared to the young group (24.9 ± 1.9 versus 39.3 ± 1.9 , $p < 0.001$), but muscle strength and $\text{VO}_{2\text{peak}}$ were not correlated with the examined inflammatory markers.

Conclusion: Older-adults have increased MCP1 (mRNA and protein abundance) and IL-8 (protein abundance) and also reduced muscle strength and $\text{VO}_{2\text{peak}}$. However the reduction in muscle strength and $\text{VO}_{2\text{peak}}$ was not related to the increase in muscle inflammatory markers in this cohort.

Keywords: inflammation, older-adults, cytokines, skeletal muscle

Background

Inflammation is an essential adaptive response needed for muscular adaptation and repair. The maintenance of muscle mass and strength confer significant survival advantage, but also improves the quality of life, with greater mobility and independence. For this, there must be a balance of protein synthesis and degradation, as well as pro- and anti-inflammatory molecules. One hallmark feature of aging is the loss of muscle mass, muscle function and muscle strength (sarcopenia) that commences from as early as the fifth decade of life, with loss of muscle accelerating with advancing age and in the presence of additional chronic diseases (Fielding et al. 2011; Hughes et al. 2002). As we age, reduced protein synthesis is apparent, and older individuals present with chronic low grade inflammation, characterized by increases in circulating pro-inflammatory mediators; a phenomena known as “inflammaging” (Franceschi et al. 2000; Franceschi et al. 2007; Toth et al. 2005). Chronically elevated circulating inflammation has been suggested as a contributing feature in the pathogenesis of many age-related diseases, such as dementia, atherosclerosis, type 2 diabetes and Alzheimer’s disease (Bruunsgaard et al. 2003; Bruunsgaard & Pedersen 2003; Ferrucci et al. 2005; Krabbe, Pedersen & Bruunsgaard 2004).

The cellular mechanisms for the loss of regenerative capacity in old age are not well understood. Firstly, age-related loss in the number of myonuclei and satellite cell number per fibre may be one possible limiting factor for muscle regeneration (Fulda & Debatin 2002). However aging satellite cells and skeletal muscle retain their functionality (Conboy & Rando 2005) and plasticity (Fiatarone et al. 1994; Kosek et al. 2006), therefore there may be systemic factors present to modulate the signaling necessary for the activation of satellite cells for regeneration (Conboy & Rando 2005). Secondly, there is a complex interplay between tightly regulated signaling pathways which are sensitive to damaging external stimuli, including; the mitogen activated protein kinases (MAPK), nuclear factor kappa-B

(NF- κ B), c-Jun N-terminal kinases (JNK) and the signal transducer and activator of transcription (STAT) pathway (Peake, Della Gatta & Cameron-Smith 2010; Simon et al. 1998). These pathways intersect to transcriptionally regulate the expression of pro-inflammatory mediators, such as tumor necrosis factor (TNF α), interleukin (IL)-8, IL-1 β and monocyte chemoattractant protein-1 (MCP1). However, to date, it is not clear whether older-adults exhibit increases in these pro-inflammatory mediators, particularly in skeletal muscle.

MCP1 and IL-8 are pro-inflammatory chemokines involved in the recruitment of monocytes, macrophages and neutrophils, and other inflammatory cells (Baggiolini, Dewald & Moser 1997). Through the modulation of these cells, these chemokines are important for tissue degradation, particularly after trauma or injury, yet are important in facilitating a faster rate of regeneration and recovery. Conversely, MCP1 and IL-8 are also potent activators of NF- κ B, thus potentially eliciting and maintaining a chronic cycle of maladaptation, impairing muscle regeneration and repair (Handel, McMorro & Gravalles 1995; Peake, Della Gatta & Cameron-Smith 2010). Monocyte and macrophage activation stimulates the release of the majority of circulating TNF α , following damage or inflammation (Figarella-Branger et al. 2003). As a mediator of muscle wasting, TNF α inhibits myoblast differentiation (Langen et al. 2002), resulting in a reduced regenerative capacity following injury. As such, it appears that circulating MCP1, IL-8 and TNF α may contribute to muscle wasting associated with injury (and potentially inactivity); however, it is not clear whether those inflammatory markers are increased in older-adults, particularly in skeletal muscle.

Despite the importance of redox-sensitive pathways as regulators of pro-inflammatory cytokine and chemokine production, very few studies have examined these pathways in the skeletal muscle of older individuals (for review see (Peake, Della Gatta & Cameron-Smith 2010). To understand the molecular and signaling events present within skeletal muscle prior

to the onset of accelerated muscle wasting in the very old, it is important to address whether these intra-muscular regulators are altered during the course of aging, similarly to what has already been identified in circulation (plasma/serum) (Bruunsgaard & Pedersen 2003). Furthermore, it is not clear whether the reduction in muscle strength seen in elderly individuals is related to increased muscle inflammation, which was demonstrated in elderly people with severe musculoskeletal disease, such as osteoarthritis (Levinger et al. 2011). Therefore, the main aim of this study was to compare inflammatory gene and protein expression in the *vastus lateralis* of older adults versus young individuals. A secondary aim was to examine whether the reduction in muscle strength and aerobic power in older-adults is related to muscle inflammatory markers. We hypothesized that older participants would have higher levels of inflammatory molecules compared to their younger counterparts, and this would be related to their reduction in muscle strength and peak aerobic capacity.

Methods

Participants

Seventeen elderly and 16 young people from the general population were recruited for a skeletal muscle biopsy under resting and fasting conditions. As aging is strongly associated with many chronic diseases we included individuals who suffer from asymptomatic cardiovascular (such as hypertension) or metabolic (obesity and type 2 diabetes) conditions. The two groups reported a similar weekly duration of physical activity, although the Young were typically engaged in much higher intensity of exercise (eg running rather than walking). Some elderly individuals were on prescribed medications (Table 1). None of the participants were on anti-inflammatory medications. All participants were informed of the nature and risks of the study before their written consent was obtained. The study was approved by the Victoria University Human Research Ethics Committee.

Muscle strength

Muscle strength was measured in a smaller cohort of the elderly (n=7) and the young (n=9) participants in the leg from which the muscle biopsy was taken. Strength was measured using a portable purposely built non-extendable strain gauge (load cell) as described previously (Levinger et al. 2011). In brief, the strain gauge was attached to the participant's leg using a webbing strap with a Velcro fastener. The participant sat in a tall chair with a strap around the lower leg approximately 10 cm above the ankle joint, and the hip and knee joint angles positioned at 90 degrees. The distance from the knee joint to the strap around the ankle was measured with a tape measure and was used for the calculation of torque [force (N) x distance (m)]. The participant extracted maximal force against the strap assembly for 3 s. Three trials

were recorded and the highest score was used for the analysis. Data was normalized for body mass (Nm/kg).

Aerobic fitness:

Aerobic power ($\text{VO}_{2\text{peak}}$) was assessed during a symptom-limited graded exercise test on Cybex MET 100 cycle (Cybex Metabolic Systems, Ronkonkoma, NY, USA) as we described previously (Levinger et al. 2007)

Muscle biopsy

The *vastus lateralis* of either leg was sampled by percutaneous needle biopsy technique as described previously (Trenerry et al. 2007).

RNA extraction and RT-PCR

Samples (10 mg) from each participant were subjected to RNA analysis. First-strand cDNA was generated from 1.0 μg total RNA (Applied Biosystems, Foster City, CA) and RT-PCR was performed in duplicate using Power SYBR Green (Applied Biosystems, Foster City, CA), forward and reverse primers and cDNA template (1.25 ng/ μl) (Trenerry et al. 2007). The efficacy of TATA box binding protein (TBP) as an endogenous control was examined using the equation $2^{-\Delta\text{Ct}}$. Primer sequences were designed using Primer Express (Applied Biosystems, Foster City, CA) and validated by BLAST sequence homology (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and melt-curve analysis, as previously described (Trenerry et al. 2007) (see Table 2 for primer details).

Protein extraction and Western blot analysis

Tissue samples from each individual were extracted using cell lysis buffer (Bio-Rad Laboratories, Hercules, CA). Approximately 10 mg of sample was homogenized using a FastPrep tissue disruptor (Thermo-Fisher Scientific) (20 sec, setting 5.5) using 15 μl lysis

buffer per mg tissue. The homogenate was rotated at 4°C for 1 h, and then centrifuged at 13,000 rpm at 4°C for 10 min, and the supernatant was collected. Protein concentration was determined using the BCA protein assay kit (Thermo-Fisher Scientific) as per manufacturer's instructions. Total protein (50 µg) was separated by 10% SDS-PAGE, transferred onto a nitrocellulose membrane and blocked in either 5% (w/v) bovine serum albumin, 5% (w/v) cold fish gelatin or 5% skim milk powder (w/v) in PBS with 0.1% Tween 20 (PBST). Primary antibodies, diluted in blocking buffer were applied and incubated overnight at 4°C; STAT3, p65 (NF-κB), JNK (Cell Signaling Technology Inc., Danvers, MA) and actin (Sigma-Aldrich, Sydney, Australia). Secondary anti-rabbit HRP-conjugated antibodies were used and visualized by enhanced chemiluminescence (Perkin-Elmer, Boston, MA) with densitometry (Kodak Imaging software, Kodak ID 3.5, Perkin-Elmer, Boston, MA).

Multiplex suspension array system

A Bio-Plex suspension array for IL-6, IL-8, MCP1 and TNFα was conducted in triplicate following the manufacturer's instructions (Bio-Rad Laboratories, Hercules, CA) and as previously described (Trenerry et al. 2010). Protein samples were prepared as described above, diluted to 1000 µg/ml, using reagents from the Cytokine reagent kit (Bio-Rad Laboratories). Protein abundances were calculated based on standard curve data with a weighted five-parameter logistic fit analysis using Bio-Plex Manager Software v 5.0 (Bio-Rad Laboratories, Hercules, CA). Standards were between 20 – 10364 pg/ml for each protein of interest.

Statistical analysis

Unpaired t tests were used to compare BMI, strength, VO_{2peak} , mRNA expression and protein abundance data between groups (elderly and young). Spearman Rho correlation was conducted to assess the correlation between inflammatory markers, age, muscle strength and

VO_{2peak}. All data are reported as mean $\pm$ standard error of the mean (SEM) and statistical analyses were conducted at the 95% level of significance.

Results

Aging elevates basal levels of inflammatory molecules in muscle

Skeletal muscle MCP1 and IL-1 β mRNA were elevated (20-55%) in the elderly compared to the younger participants (Fig 1), although only MCP1 was significant ($p < 0.01$). Protein abundance of MCP1 was significantly elevated in the elderly participants (40%, $p < 0.01$), along with IL-8 (4%, $p < 0.05$) (Fig 2). TNF α mRNA did not reach statistical significance ($p > 0.05$) (Fig 1). Both muscle MCP1 mRNA and protein abundance were significantly correlated with age ($r = 0.54$, $p < 0.01$; $r = 0.42$, $p < 0.05$ respectively). Age was also associated with IL-8 protein abundance ($r = 0.37$, $p < 0.05$). There was no difference observed between groups for IL-6 protein abundance ($p > 0.05$, Fig 2). In the majority of subjects IL-6 mRNA was below the detection threshold. In the remaining subjects the expression was low and highly variable. Gene expression was not statistically different within groups (i.e. young and older adults) between subjects for which strength measurement and VO_{2peak} were obtained and those from whom those data were not collected.

Inflammatory-associated kinases are not altered with age

The total protein abundance of JNK1/2, p65 (NF- κ B) and STAT3 were measured to evaluate possible downstream signaling events of the aforementioned inflammatory cytokines. There was no difference in the protein expression of these molecules between young and elderly participants (Fig 3).

Muscle strength and aerobic power

Muscle strength was lower in the elderly compared to the young group (1.55 ± 0.17 versus 2.56 ± 0.13 Nm/kg, $p < 0.001$). The elderly group had a significant lower VO_{2peak} compared to the young group (24.9 ± 1.9 versus 39.3 ± 1.9 , $p < 0.001$). Both muscle strength and VO_{2peak} were negatively correlated with age ($r = -0.78$, $p < 0.001$ and $r = -0.72$, $p < 0.01$, respectively).

Muscle strength and $VO_{2\text{peak}}$ did not correlate with any of the examined inflammatory markers. $VO_{2\text{peak}}$ however, tended to be negatively correlated with MCP1 mRNA ($r=-0.45$, $p>0.05$).

Discussion

Skeletal muscle mass, strength and quality are impaired in older individuals compared to that of younger adults (Degens 2007; Janssen et al. 2000) and this may be related to increased inflammation in aging (Peake, Della Gatta & Cameron-Smith 2010). The current study examined several inflammatory markers associated with muscle wasting and atrophy within skeletal muscle. We have found that in older-adults there was increased muscular MCP1 (mRNA and protein abundance) and IL-8 (protein abundance). However, the reduction in muscle strength in these adults was not associated with the expression of inflammatory markers in muscle.

Under normal conditions, MCP1 is involved in the recruitment of inflammatory cells and is important for muscle repair and regeneration via the regulation of macrophages (Baggiolini, Dewald & Moser 1997; Shireman et al. 2007). Macrophage release is necessary for the stimulation of satellite cell activation for skeletal muscle repair (Tidball 2005). It has been suggested that during aging there is an increase in the susceptibility of satellite cells to cytokines such as MCP1, which may alter regeneration capacity of skeletal muscle that in turn may lead to accumulation of damaged cellular components leading to cellular dysfunction (Shireman et al. 2007). In healthy adults, circulating levels of MCP1 in the blood are elevated in an age-dependent manner (Deo et al. 2004; Gerli et al. 2000; Inadera et al. 1999; Kanda et al. 2006; Mariani et al. 2006; Miles et al. 2008; Seidler et al. 2010). We show here that MCP1 is also increased with age in skeletal muscle. A chronic increase in the levels of circulating MCP1 in the blood has previously been suggested to be associated with developing weight gain and health problems (Inadera et al. 1999; Kanda et al. 2006; Miles et al. 2008; Seidler et al. 2010). However, we saw no such associations in the current study, which is similar to other studies who showed elevated MCP-1 without evidence of atherosclerosis (Deo et al. 2004; Mariani et al. 2006).

Similarly to MCP1, IL-8 is also involved in the recruitment of inflammatory cells important for muscle adaptation (Baggiolini, Dewald & Moser 1997). High circulating levels of IL-8 have been observed in healthy elderly (Wieczorowska-Tobis et al. 2006), and it was suggested that the levels of this chemokine may be indicative of healthy aging (Wieczorowska-Tobis et al. 2006), contrary to that described for MCP1. An age-related shift in the immunological network has been proposed to occur in serum/plasma, that has not yet been described in skeletal muscle; with an apparent elevation and preferential activation of type 2 cytokines, as well as CC and CXC chemokines, in healthy aging individuals (Antonelli et al. 2006; Deo et al. 2004; Gerli et al. 2000; Inadera et al. 1999; Mariani et al. 2006; Seidler et al. 2010; Wieczorowska-Tobis et al. 2006). Based on the results of the present study, it appears that the same could be said to be occurring for CC (MCP-1) and CXC (IL-8) chemokines within skeletal muscle. To further elucidate an association between circulating and intramuscular cytokine and chemokine levels, future studies should sample both serum and muscle biopsies for comparisons between the two, with the inclusion of investigating expression profiles of their receptors.

Inflammatory molecules activate a myriad of signaling pathways to mediate cellular repair. Within skeletal muscle, there is complex crosstalk between several signal transduction cascades, due to the commonality of the ligands which stimulate the activation of these pathways. With age, there may be a dysregulation of this process (Trenerry et al. 2008); therefore we sought to investigate differences that may be present in the basal levels of important kinases involved in inflammation and skeletal muscle repair. The expression of two common STAT3 activators, IL-6 and TNF α (protein abundance), did not differ between the age groups in the present study, consistent with the lack of difference with age in basal STAT3 levels. However, this is contrary to previously reported age-related differences in total STAT3 protein expression in both humans and rodents muscle (Haddad & Adams 2006;

Thalacker-Mercer et al. 2010), where it was suggested that STAT3 increases in older adults were indicative of greater inflammatory signaling at rest (Thalacker-Mercer et al. 2010). As skeletal muscle biopsies contain a mixture of cell types, age-related differences in cell types could be an important factor contributing to the differences observed in inflammatory status between studies. The contrasting findings may also be related to different levels of engagement in physical activity (i.e sedentary lifestyle versus recreational active). Some of the elderly individuals in the current study were involved in recreational physical activity which may have a positive (lower) effect on muscle inflammation. A somewhat support for this hypothesis can be found in the trend for negative correlation found between VO_{2peak} and MCP1 mRNA. It is possible that in older adults who live sedentary lifestyle inflammatory markers will be higher than reported in the current study.

As expected, the elderly group had a significant reduction in muscle strength compared to the young group. Although muscle strength was negatively correlated with age, an important finding was that muscle strength was not associated with any of the measured inflammatory markers in muscle in this older-adult cohort. This is in contrast to the negative correlation observed between muscle strength and inflammatory markers in the very old (Greiwe et al. 2001) and in clinical populations, such as patients with knee osteoarthritis (Levinger et al. 2011). Taking together previous results and those from the current study, it is possible that a correlation exists between muscle strength and inflammatory markers in people aged 75 or older when muscle wasting is evident and there is a substantial reduction in the capacity to perform activity of daily living that accompanies chronic clinical conditions. It is possible that in a different cohort of elderly people with a more sedentary lifestyle, that these inflammatory markers may be further elevated, leading to detection of a correlation between reduced muscle strength and increase inflammatory markers. This hypothesis should be explored in future research. In addition, it is possible that a correlation between muscle

strength and inflammatory markers may exist in the presence of musculoskeletal disease, where further muscle impairment may be present as was reported previously (Levinger et al. 2011).

JNK and NF- κ B are two prominent signaling molecules associated with inflammation, cellular stress and aging. Both kinases are activated by the pro-inflammatory molecule TNF α (Liu 2003). Very few studies have been conducted in older-adults measuring the levels of NF- κ B proteins or JNK1/2 in skeletal muscle, at rest. We observed no age-related differences in the protein abundance of p65 (NF- κ B) and JNK1/2, consistent with unchanged TNF α protein abundance. However, the results for NF- κ B contrast with previous research, which have reported increases in phosphorylated I κ B (an NF- κ B regulator) and nuclear p65 expression in the older group, but not p50 (Buford et al. 2010); or elevations of p50 in the older adults but no difference in I κ B (Thalacker-Mercer et al. 2010). Differences in the ages of the sample populations (67 years in current study versus 73 years) and physical activity levels (recreationally active versus sedentary) of the participants may account for some of these discrepancies (Thalacker-Mercer et al. 2010). It was also proposed that in rodents the abundance of NF- κ B isoforms might vary between muscle type (eg type I versus type II) in the hindlimb (Phillips & Leeuwenburgh 2005). Additionally, the studies investigating NF- κ B within skeletal muscle obtained from aged animals failed to compare the resting levels of p65 prior to immobilization, precluding firm conclusions regarding the effect of age on basal NF- κ B levels (Bar-Shai et al. 2005; Bar-Shai, Carmeli & Reznick 2005). Similarly to NF- κ B, there appears to be a difference in JNK expression between muscle types. Age-related changes in JNK protein levels occur in the *extensor digitorum longus* (EDL) of rats, but not in the soleus (Mylabathula et al. 2006). While, in humans, JNK protein expression in the *vastus lateralis* was not different in a population of young and older men similar to the

current study (Williamson et al. 2003). It is possible the different protein expression in skeletal muscle may occur due to the physical properties of the fibres, however this requires further investigation.

A potential limitation of the current study is the relative small sample size. Thus in one sense, this new data should be seen as hypothesis generating. It is possible that with a larger sample size other correlations, especially between muscle strength and inflammatory markers, will become significant. Another potential limitation is the clinical profile of some elderly participants. We cannot exclude that some of the differences reported are partly related to differences in clinical profile, **obesity level**, dietary habits (including the possibility of suppressed caloric intake in the elderly) and pharmaceutical use (including NSAIDS), rather than aging per se; however, in current Western societies where 60% or more of the adult population may be overweight/obese, these clinical profiles are part of aging.

Conclusions

Older adults have increased skeletal muscle MCP1 (mRNA and protein abundance) and IL-8 (protein abundance) and also reduced muscle strength and VO_{2peak} . However the reduction in muscle strength and VO_{2peak} was not related to the increase in muscle inflammatory markers in this cohort.

Acknowledgements

This study was partly funded by the L.E.W Carty Charitable Fund. The authors wish to thank Dr Andrew Garnham (Deakin University) and Dr Mitchell Anderson for performing the muscle biopsies; Dr Paul Della Gatta and Dr Jessica Mathers (Deakin University) for helping with participant recruitment, and all participants for volunteering for this study. Dr Itamar Levinger is a Heart Foundation Postdoctoral Research Fellow (PR 11M 6086).

Competing interest

The authors declare that they have no competing interests

References

- Antonelli, A, Rotondi, M, Fallahi, P, Ferrari, SM, Paolicchi, A, Romagnani, P, Serio, M & Ferrannini, E 2006, 'Increase of CXC chemokine CXCL10 and CC chemokine CCL2 serum levels in normal ageing', *Cytokine*, vol. 34, no. 1-2, pp. 32-8.
- Baggiolini, M, Dewald, B & Moser, B 1997, 'Human chemokines: an update', *Ann Rev Imm* vol. 15, pp. 675-705.
- Bar-Shai, M, Carmeli, E, Coleman, R, Rozen, N, Perek, S, Fuchs, D & Reznick, AZ 2005, 'The effect of hindlimb immobilization on acid phosphatase, metalloproteinases and nuclear factor-kappaB in muscles of young and old rats', *Mech Ageing Develop*, vol. 126, no. 2, pp. 289-97.
- Bar-Shai, M, Carmeli, E & Reznick, AZ 2005, 'The role of NF-kappaB in protein breakdown in immobilization, aging, and exercise: from basic processes to promotion of health', *Ann N Y Acad Sci*, vol. 1057, pp. 431-47.
- Bruunsgaard, H, Andersen-Ranberg, K, Hjelmberg, JB, Pedersen, BK & Jeune, B 2003, 'Elevated levels of tumor necrosis factor alpha and mortality in centenarians', *Am J Med*, vol. 115, no. 4, pp. 278-83.
- Bruunsgaard, H & Pedersen, BK 2003, 'Age-related inflammatory cytokines and disease', *Immunol Allergy Clin North Am*, vol. 23, no. 1, pp. 15-39.
- Buford, TW, Cooke, MB, Manini, TM, Leeuwenburgh, C & Willoughby, DS 2010, 'Effects of age and sedentary lifestyle on skeletal muscle NF-kappaB signaling in men', *J Gerontol Series A Bio Med Sci*, vol. 65, no. 5, pp. 532-7.
- Conboy, IM & Rando, TA 2005, 'Aging, stem cells and tissue regeneration: lessons from muscle', *Cell Cycle*, vol. 4, no. 3, pp. 407-10.
- Degens, H 2007, 'Age-related skeletal muscle dysfunction: causes and mechanisms', *J Musculoskelet Neuronal Interact*, vol. 7, no. 3, pp. 246-52.
- Deo, R, Khera, A, McGuire, DK, Murphy, SA, Meo Neto Jde, P, Morrow, DA & de Lemos, JA 2004, 'Association among plasma levels of monocyte chemoattractant protein-1, traditional cardiovascular risk factors, and subclinical atherosclerosis', *J Am Coll Cardiol*, vol. 44, no. 9, pp. 1812-8.
- Ferrucci, L, Corsi, A, Lauretani, F, Bandinelli, S, Bartali, B, Taub, DD, Guralnik, JM & Longo, DL 2005, 'The origins of age-related proinflammatory state', *Blood*, vol. 105, no. 6, pp. 2294-9.

Fiatarone, MA, O'Neill, EF, Ryan, ND, Clements, KM, Solares, GR, Nelson, ME, Roberts, SB, Kehayias, JJ, Lipsitz, LA & Evans, WJ 1994, 'Exercise training and nutritional supplementation for physical frailty in very elderly people', *New Engl J Med*, vol. 330, no. 25, pp. 1769-75.

Fielding, RA, Vellas, B, Evans, WJ, Bhasin, S, Morley, JE, Newman, AB, Abellan van Kan, G, Andrieu, S, Bauer, J, Breuille, D, Cederholm, T, Chandler, J, De Meynard, C, Donini, L, Harris, T, Kannt, A, Keime Guibert, F, Onder, G, Papanicolaou, D, Rolland, Y, Rooks, D, Sieber, C, Souhami, E, Verlaan, S & Zamboni, M 2011, 'Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia', *J Am Med Dir Assoc*, vol. 12, no. 4, pp. 249-56.

Figarella-Branger, D, Civatte, M, Bartoli, C & Pellissier, JF 2003, 'Cytokines, chemokines, and cell adhesion molecules in inflammatory myopathies', *Muscle Nerve*, vol. 28, no. 6, pp. 659-82.

Franceschi, C, Bonafe, M, Valensin, S, Olivieri, F, De Luca, M, Ottaviani, E & De Benedictis, G 2000, 'Inflamm-aging. An evolutionary perspective on immunosenescence', *Ann N Y Acad Sci*, vol. 908, pp. 244-54.

Franceschi, C, Capri, M, Monti, D, Giunta, S, Olivieri, F, Sevini, F, Panourgia, MP, Invidia, L, Celani, L, Scurti, M, Cevenini, E, Castellani, GC & Salvioli, S 2007, 'Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans', *Mech Ageing Develop*, vol. 128, no. 1, pp. 92-105.

Fulda, S & Debatin, KM 2002, 'IFN γ sensitizes for apoptosis by upregulating caspase-8 expression through the Stat1 pathway', *Oncogene*, vol. 21, no. 15, pp. 2295-308.

Gerli, R, Monti, D, Bistoni, O, Mazzone, AM, Peri, G, Cossarizza, A, Di Gioacchino, M, Cesarotti, ME, Doni, A, Mantovani, A, Franceschi, C & Paganelli, R 2000, 'Chemokines, sTNF-Rs and sCD30 serum levels in healthy aged people and centenarians', *Mech Ageing Develop*, vol. 121, no. 1-3, pp. 37-46.

Greiwe, JS, Cheng, B, Rubin, DC, Yarasheski, KE & Semenkovich, CF 2001, 'Resistance exercise decreases skeletal muscle tumor necrosis factor alpha in frail elderly humans', *FASEB J*, vol. 15, no. 2, pp. 475-82.

Haddad, F & Adams, GR 2006, 'Aging-sensitive cellular and molecular mechanisms associated with skeletal muscle hypertrophy', *J Appl Physiol* vol. 100, no. 4, pp. 1188-203.

Handel, ML, McMorrow, LB & Gravalles, EM 1995, 'Nuclear factor-kappa B in rheumatoid synovium. Localization of p50 and p65', *Arth Rheum*, vol. 38, no. 12, pp. 1762-70.

- Hughes, VA, Frontera, WR, Roubenoff, R, Evans, WJ & Singh, MA 2002, 'Longitudinal changes in body composition in older men and women: role of body weight change and physical activity', *Am J Clin Nut*, vol. 76, no. 2, pp. 473-81.
- Inadera, H, Egashira, K, Takemoto, M, Ouchi, Y & Matsushima, K 1999, 'Increase in circulating levels of monocyte chemoattractant protein-1 with aging', *J Interf Cytokine Res*, vol. 19, no. 10, pp. 1179-82.
- Janssen, I, Heymsfield, SB, Wang, ZM & Ross, R 2000, 'Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr', *J Appl Physiol*, vol. 89, no. 1, pp. 81-8.
- Kanda, H, Tateya, S, Tamori, Y, Kotani, K, Hiasa, K, Kitazawa, R, Kitazawa, S, Miyachi, H, Maeda, S, Egashira, K & Kasuga, M 2006, 'MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity', *J Clin Inv*, vol. 116, no. 6, pp. 1494-505.
- Kosek, DJ, Kim, JS, Petrella, JK, Cross, JM & Bamman, MM 2006, 'Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. older adults', *J Appl Physiol*, vol. 101, no. 2, pp. 531-44.
- Krabbe, KS, Pedersen, M & Bruunsgaard, H 2004, 'Inflammatory mediators in the elderly', *Exp Gerontol*, vol. 39, no. 5, pp. 687-99.
- Langen, RC, Schols, AM, Kelders, MC, Van Der Velden, JL, Wouters, EF & Janssen-Heininger, YM 2002, 'Tumor necrosis factor-alpha inhibits myogenesis through redox-dependent and -independent pathways', *Am J Physiol Cell Physiol*, vol. 283, no. 3, pp. C714-21.
- Levinger, I, Goodman, C, Hare, DL, Jerums, G & Selig, S 2007, 'The effect of resistance training on functional capacity and quality of life in individuals with high and low numbers of metabolic risk factors', *Diabetes Care*, vol. 30, no. 9, pp. 2205-10.
- Levinger, I, Levinger, P, Trenerry, MK, Feller, JA, Bartlett, JR, Bergman, N, McKenna, MJ & Cameron-Smith, D 2011, 'Increased inflammatory cytokine expression in the vastus lateralis of patients with knee osteoarthritis', *Arthr Rheum*, vol. 63, no. 5, pp. 1343-8.
- Liu, ZG 2003, 'Adding facets to TNF signaling. The JNK angle', *Molecular Cell*, vol. 12, no. 4, pp. 795-6.
- Mariani, E, Cattini, L, Neri, S, Malavolta, M, Mocchegiani, E, Ravaglia, G & Facchini, A 2006, 'Simultaneous evaluation of circulating chemokine and cytokine profiles in elderly subjects by multiplex technology: relationship with zinc status', *Biogerontol*, vol. 7, no. 5-6, pp. 449-59.

- Miles, EA, Rees, D, Banerjee, T, Cazzola, R, Lewis, S, Wood, R, Oates, R, Tallant, A, Cestaro, B, Yaqoob, P, Wahle, KW & Calder, PC 2008, 'Age-related increases in circulating inflammatory markers in men are independent of BMI, blood pressure and blood lipid concentrations', *Atheroscl*, vol. 196, no. 1, pp. 298-305.
- Mylabathula, DB, Rice, KM, Wang, Z, Uddemarri, S, Kinnard, RS & Blough, ER 2006, 'Age-associated changes in MAPK activation in fast- and slow-twitch skeletal muscle of the F344/NNiaHSD X Brown Norway/BiNia rat model', *Exp Gerontol*, vol. 41, no. 2, pp. 205-14.
- Peake, J, Della Gatta, P & Cameron-Smith, D 2010, 'Aging and its effects on inflammation in skeletal muscle at rest and following exercise-induced muscle injury', *Am J Physiol. Reg Int Comp Physiol*, vol. 298, no. 6, pp. R1485-95.
- Phillips, T & Leeuwenburgh, C 2005, 'Muscle fiber specific apoptosis and TNF-alpha signaling in sarcopenia are attenuated by life-long calorie restriction', *FASEB J*, vol. 19, no. 6, pp. 668-70.
- Seidler, S, Zimmermann, HW, Bartneck, M, Trautwein, C & Tacke, F 2010, 'Age-dependent alterations of monocyte subsets and monocyte-related chemokine pathways in healthy adults', *BMC Immunol*, vol. 11, p. 30.
- Shireman, PK, Contreras-Shannon, V, Ochoa, O, Karia, BP, Michalek, JE & McManus, LM 2007, 'MCP-1 deficiency causes altered inflammation with impaired skeletal muscle regeneration', *J Leukocyte Biol*, vol. 81, no. 3, pp. 775-85.
- Simon, AR, Rai, U, Fanburg, BL & Cochran, BH 1998, 'Activation of the JAK-STAT pathway by reactive oxygen species', *Am J Physiol*, vol. 275, no. 6 Pt 1, pp. C1640-52.
- Thalacker-Mercer, AE, Dell'Italia, LJ, Cui, X, Cross, JM & Bamman, MM 2010, 'Differential genomic responses in old vs. young humans despite similar levels of modest muscle damage after resistance loading', *Physiol Genomics*, vol. 40, no. 3, pp. 141-9.
- Tidball, JG 2005, 'Inflammatory processes in muscle injury and repair', *Am J Physiology Reg Int Comp Physiol*, vol. 288, no. 2, pp. R345-53.
- Toth, MJ, Matthews, DE, Tracy, RP & Previs, MJ 2005, 'Age-related differences in skeletal muscle protein synthesis: relation to markers of immune activation', *Am J Physiol Endocrinol Metabol*, vol. 288, no. 5, pp. E883-91.
- Trenerry, MK, Carey, KA, Ward, AC & Cameron-Smith, D 2007, 'STAT3 signaling is activated in human skeletal muscle following acute resistance exercise', *J Appl Physiol*, vol. 102, no. 4, pp. 1483-9.

Trenerry, MK, Carey, KA, Ward, AC, Farnfield, MM & Cameron-Smith, D 2008, 'Exercise-induced activation of STAT3 signaling is increased with age', *Rejuvenation Res*, vol. 11, no. 4, pp. 717-24.

Trenerry, MK, Della Gatta, PA, Larsen, AE, Garnham, AP & Cameron-Smith, D 2010, 'Impact of resistance exercise training on interleukin-6 and JAK/STAT in young men', *Muscle Nerve*, vol. 43, no. 3, pp. 385-92.

Wieczorowska-Tobis, K, Niemir, ZI, Podkowka, R, Korybalska, K, Mossakowska, M & Breborowicz, A 2006, 'Can an increased level of circulating IL-8 be a predictor of human longevity?', *Med Sci Monit*, vol. 12, no. 3, pp. CR118-21.

Williamson, D, Gallagher, P, Harber, M, Hollon, C & Trappe, S 2003, 'Mitogen-activated protein kinase (MAPK) pathway activation: effects of age and acute exercise on human skeletal muscle', *J Physiol*, vol. 547, no. Pt 3, pp. 977-87.

List of abbreviations

BMI, body mass index

IL, interleukin

JNK, c-Jun N-terminal kinases

MAPK, mitogen activated protein kinases

MCP1, monocyte chemotactic protein-1

NF- κ B, nuclear factor kappa-B

STAT, signal transducer and activator of transcription

TNF α , tumor necrosis factor

VO_{2peak}, peak aerobic power

Figure legends

Fig 1 Elevations of inflammatory molecules are evident with aging

mRNA expression of MCP1, TNF α and IL-1 β between young and elderly individuals was examined under resting conditions using RT-PCR. Values are arbitrary units (AU) normalized to the expression of the reference gene TBP. Data reported as mean $\pm$ SEM. Significantly different from young participants: * indicates $p < 0.05$, ** indicates $p < 0.01$.

Fig 2 Expression of total protein abundance of inflammatory markers in skeletal muscle

Total protein expression of MCP1, TNF α , IL-6 and IL-8 between young and elderly individuals was examined under resting conditions using a multiplex system. Values are AU. Data reported as mean $\pm$ SEM. Significantly different from young participants: * indicates $p < 0.05$, ** indicates $p < 0.01$.

Fig 3 There is no age-associated difference in the expression of inflammatory-associated kinases total protein levels in skeletal muscle

Total protein expression of JNK1/2, p65 (NF- κ B) and STAT3 between young and elderly individuals was examined under resting conditions using Western blotting. Values are AU, normalized to the expression of actin. Data reported as mean $\pm$ SEM.

Fig 1 Elevations of inflammatory molecules are evident with aging

mRNA expression of MCP1, TNF α and IL-1 β between young and elderly individuals was examined under resting conditions using RT-PCR. Values are arbitrary units (AU) normalized to the expression of the reference gene TBP. Data reported as mean $\pm$ SEM. Significantly different from young participants: * indicates $p < 0.05$, ** indicates $p < 0.01$.

Fig 3 There is no age-associated difference in the expression of inflammatory-associated kinases total protein levels in skeletal muscle

Total protein expression of JNK1/2, p65 (NF-κB) and STAT3 between young and elderly individuals was examined under resting conditions using Western blotting. Values are AU, normalized to the expression of actin. Data reported as mean ± SEM.

Fig 2 Expression of total protein abundance of inflammatory markers in skeletal muscle

Total protein expression of MCP1, TNFα, IL-6 and IL-8 between young and elderly individuals was examined under resting conditions using a multiplex system. Values are AU. Data reported as mean $\pm$ SEM. Significantly different from young participants: * indicates $p < 0.05$, ** indicates $p < 0.01$.

Table 2. Details of primers used for RT-PCR

Gene	Accession Number	Primer Sequence	
IL-1 β	NM_000576	F -	GCACGATGCACCTGTACGAT
		R -	AGACATCACCAAGCTTTTTTGCT
MCP1	NM_002982	F -	TCCCAAAGAAGCTGTGATCTTCA
		R -	CAGATTCTTGGGTGGAGTGA
TBP	NM_003194	F -	CGAATATAATCCCAAGCGGTTT
		R -	CCGTGGTTCGTGGCTCTCT
TNF- α	NM_000594	F -	GGAGAAGGGTGACCGACTCA

Primer sequences were designed using Primer Express version 3.0 software (Applied Biosystems) using sequences accessed through GenBank and checked for specificity using Nucleotide-Nucleotide Blast search. MCP1, monocyte chemoattractant protein; TBP, TATA box binding protein.

Table 1: **Groups characteristics (Mean±SEM)**

	Elderly	Young	p-value
Age (yrs)	67 ± 1.6	24 ± 0.6	<0.001
Sex (M : F)	9 : 8	8 : 8	0.871
Height (cm)	168.6 ± 1.9	171.7 ± 2.3	0.295
Weight (kg)	82.7 ± 4.1	71.2 ± 3.7	0.046
BMI (kg/m ²)	29.0 ± 1.1	23.9 ± 0.8	0.001
Physical activity levels (h.wk ⁻¹)	11.6±10.2	7.4±8.2	0.333
Chronic diseases			
– Hypertension	3	0	
– Type II diabetes	3	0	
– Obesity (BMI≥30 kg.m ⁻²)	6	1	
Medications			
– metformin	2	0	
– anti-cholesterol	2	0	
– platelet inhibitor	1	0	
– ACE Inhibitor	3	0	
– beta-blockers	3	0	
– Ang II receptor antagonist	2	0	
– aspirin	2	0	

BMI (body mass index), Ang (angiotensin).