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Effect of exercise training on skeletal muscle cytokine expression in the elderly

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ABSTRACT

Aging is associated with increased circulating pro-inflammatory and lower anti-inflammatory cytokines. Exercise training, in addition to improving muscle function, reduces these circulating pro-inflammatory cytokines. Yet, few studies have evaluated changes in the expression of cytokines within skeletal muscle after exercise training. The aim of the current study was to examine the expression of cytokines both at rest and following a bout of isokinetic exercise performed before and after 12 weeks of resistance exercise training in young (n = 8, 20.3 ± 0.8 yr) and elderly men (n = 8, 66.9 ± 1.6 yr). Protein expression of various cytokines was determined in muscle homogenates. The expression of MCP-1, IL-8 and IL-6 (which are traditionally classified as 'pro-inflammatory') increased substantially after acute exercise. By contrast, the expression of the anti-inflammatory cytokines IL-4, IL-10 and IL-13 increased only slightly (or not at all) after acute exercise. These responses were not significantly different between young and elderly men, either before or after 12 weeks of exercise training. However, compared with the young men, the expression of pro-inflammatory cytokines 2 h post exercise tended to be greater in the elderly men prior to training. Training attenuated this difference. These data suggest that the inflammatory response to unaccustomed exercise increases with age. Furthermore, regular exercise training may help to normalize this inflammatory response, which could have important implications for muscle regeneration and adaptation in the elderly.

Introduction

From approximately the fifth decade of life, there is a gradual and inevitable decline in muscle mass (known as 'sarcopenia') in susceptible individuals (Hughes et al., 2001; Janssen and Ross, 2005). This loss of muscle mass is a major contributor to detrimental health outcomes, including increased falls, frailty and mortality risk (Janssen, 2006; Rantanen et al., 2003). The mechanisms underpinning this loss of muscle are complex, but may include age-related changes in immune function (previously described as inflamm-aging (Franceschi et al., 2000)). Inflamm-aging was initially described as an increase in circulating concentrations of classically pro-inflammatory cytokines. However, there are many complex alterations in adaptive and innate immunity that also influence the secretion of anti-inflammatory and pro-resolving cytokines (Franceschi et al., 2007).

In the elderly, elevated inflammatory cytokines, including TNF-α and IL-6, correlate with reduced muscle mass and strength (Pedersen et al., 2003; Visser et al., 2002). Both *in vitro* and rodent models demonstrate a capacity for these predominantly pro-inflammatory cytokines to increase skeletal muscle protein degradation (Frost et al., 2003; Haddad et al., 2005). In addition, protein degradation in response to treatment with pro-inflammatory cytokines is greater in muscle cells isolated from older individuals compared with muscle cells from younger individuals (Lees et al., 2009; Merritt et al., 2013). It is increasingly recognized that skeletal muscle itself is an important source of inflammatory mediators, collectively known as 'myokines'. Expression of these locally generated cytokines (e.g., IL-1β, TNF-α, IL-1ra, IL-10) is elevated in skeletal muscle of elderly individuals, perhaps as part of the inflamm-aging process (Buford et al., 2010; Leger et al., 2008; Przybyla et al., 2006; Thalacker-Mercer et al., 2010).

The concept of the anti-inflammatory effects of exercise training has gained increasing attention in recent years (Gleeson et al., 2011). The cytokine species that exert predominantly anti-inflammatory effects (e.g., IL-4, IL-10 and IL-13) (Prokopchuk et al., 2007) also enhance myogenesis (Deng et al., 2012; Heredia et al., 2013). Thus, it is possible that by enhancing the secretion of anti-inflammatory cytokines in skeletal muscle, exercise may promote muscle hypertrophy—or at least limit muscle atrophy—in the elderly.

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To date, most studies examining the effects of exercise training on the dynamic regulation of cytokines have focused either primarily on circulating cytokine concentrations, or have analyzed cytokine gene expression within skeletal muscle at rest before and after a period of training (Gielen et al., 2003; Greiwe et al., 2001; Lambert et al., 2008; Nader et al., 2010). The aim of the present study was to compare the protein expression of cytokines in skeletal muscle between young and elderly men in response to a single bout of isokinetic exercise performed before and after 12 weeks of regular resistance training. We hypothesized that (1) the cytokine response to isokinetic exercise before training would be smaller in elderly men compared with young men, and that (2) the cytokine response to isokinetic exercise after training would be similar in elderly and young men.

Materials and methods

Ethics statement

Prior to consenting to participate in the study, the nature, purpose and risks of the study were fully explained to all subjects. All experimental procedures involved in this study were formally approved by the Human Research Ethics Committee of Deakin University.

Subjects

Eight healthy young men (aged 18–25 yr) and eight healthy elderly men (aged 60–75 yr) (Table 1), who had not participated in resistance exercise for a minimum of 1 yr prior to commencing the study, were recruited. The elderly men completed a comprehensive medical screening procedure, which included a 12-lead ECG exercise stress test to detect any underlying heart conditions prior to their inclusion in the study.

Experimental design

Single bout of isokinetic exercise

All subjects completed a familiarization session on the Cybex NORM dynamometer (Cybex International Inc., UK) to become familiar with how to perform the isokinetic exercise. This session involved assessing isokinetic maximal voluntary contraction (MVC) strength and peak torque (Nm) during knee extension/flexion over 12 maximal repetitions at a constant speed of $60^\circ\ s^{-1}$ in both concentric and eccentric phases. Exercise was performed using the dominant limb. Subjects were instructed to push maximally, and were verbally encouraged throughout the test. Familiarization was performed at least 7 days prior to the first experimental trial. For this experimental trial, the subjects arrived at the laboratory in the fasted state and, following 30 min of supine resting, a resting muscle sample was collected. Following the resting biopsy, the subjects completed three sets of 12 repetitions of maximal unilateral knee extension exercise on the dynamometer, with 2 min rest between each set, as described for the familiarization session. Two hours after the completion of the exercise session, another muscle sample was collected. This time point was chosen because inflammatory proteins are most abundant in skeletal muscle at 2 h post exercise (Della Gatta et al., unpublished observations).

Table 1
Subject characteristics (mean ± SE).

Characteristics	Young males ($n = 6$)	Elderly males ($n = 8$)
Age (yr)	20.3 ± 0.8	66.9 ± 1.6[a]
Height (m)	1.85 ± 0.02	1.74 ± 0.02[a]
Mass (kg)	76.7 ± 2.2	83.4 ± 4.4
BMI ($kg\ m^{-2}$)	22.4 ± 1.0	27.5 ± 1.0[a]

[a] Significantly different from young male subjects.

12 Week exercise training

Following the first experimental trial, subjects completed 12 weeks of fully supervised progressive resistance exercise training on three days each week, with a minimum of 48 h of rest between exercise sessions. Initially, three training sessions were conducted using light resistance for equipment familiarization and correct execution of the exercises. After the familiarization sessions, strength testing was performed to determine appropriate starting weights for all subjects. One repetition maximum (1-RM) strength was estimated using a 5-RM test for all exercises. A 5-RM test was adopted because this exercise test was the most appropriate for an elderly population with no previous history of strength training. At week 6 and week 12, the subjects' 5-RM was retested, and the training load was adjusted accordingly to ensure that the training was progressive.

Each training session was preceded by a 5 min warm-up on a stationary cycle followed by a full set of exercises with light weights. The exercises consisted of leg press, bench press, seated row, leg extension, dumb-bell shoulder press, and sit-ups. Following the warm-up weights, subjects completed two sets at the required intensity, completing between 8 and 12 repetitions on each exercise. Specified rest periods were allowed between sets. Initially, the exercises were set to 50% of individual estimated 1-RM for 1 week, followed by a progressive increase in the weights lifted each week until the subjects were lifting 80% of estimated 1-RM at week 6. The exercise intensity was set at 80% of estimated 1-RM for the remaining 6 weeks.

At the end of the 12 week training program, subjects again visited the laboratory in a fasted state to complete the exercise trial consisting of a single bout of isokinetic exercise and collection of muscle biopsies. The exercise performed and timing of the muscle biopsies were identical to the exercise trial that the subjects completed before their progressive resistance training.

Muscle biopsy procedure

The subjects were required to rest in a supine position for 30 min prior to muscle sampling. A muscle sample was then collected from the *Vastus lateralis* under local anesthesia (Xylocaine 1%) using the percutaneous needle biopsy technique (Bergström, 1962), including suction (Evans et al., 1982). Excised muscle tissue was immediately frozen and stored in liquid nitrogen for subsequent analysis. To minimize the potential for interference, serial muscle biopsies were collected at least 2 cm from the previous muscle biopsy site. Biopsies were taken at rest and 2 h following the completion of the acute exercise session, before and after 12 weeks of resistance exercise training.

Multiplex analysis

A bio-plex assay (Bio-Rad Laboratories, Hercules, CA) was used to analyze the protein expression of cytokines within skeletal muscle tissue. In the present study, kits were designed for the simultaneous analysis of IL-4, IL-6, IL-8, IL-10, IL-13, MCP-1 and TNF-α. The assay was conducted following the manufacturer's instructions (Bio-Rad Laboratories, Hercules, CA). Tissue samples (10 mg) were homogenized in lysis buffer (20 mM Tris–HCl, 5 mM EDTA, 10 mM Na-pyrophosphate, 100 mM NaF, 2 mM Na_3VO_4, 1% Igepal CA-630, 10 μg/ml Aprotinin, 10 μg/ml Leupeptin, 3 mM Benzamidine, 1 mM phenylmethylsulfonyl fluoride (PMSF) using a hand-held homogenizer. The homogenate was rotated at