Experimental Gerontology 107 (2018) 37–49

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Aging-related effects of bed rest followed by eccentric exercise rehabilitation on skeletal muscle macrophages and insulin sensitivity

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ARTICLE INFO

Article history:
Received 29 March 2017
Received in revised form 29 June 2017
Accepted 1 July 2017
Available online 10 July 2017

Section Editor: Chennai Guest Editor

Keywords:
Aging
Atrophy
Immune cells
M1
M2
Muscle-resident
Rehabilitation
Regrowth
Recovery
Eccentric exercise
Training

ABSTRACT

The pro- and anti-inflammatory macrophages are associated with insulin sensitivity and skeletal muscle regeneration. Infiltrating macrophages in skeletal muscle during a period of physical inactivity and subsequent reloading/rehabilitation in older adults is unknown, but may provide insight into mechanisms related to the development of metabolic disease and changes in muscle cell size. The purpose of this study was to determine if skeletal muscle macrophage infiltration is modulated differently between young and older adults after bed rest and exercise rehabilitation and if these responses are related to muscle and insulin sensitivity changes. 14 young and 9 older adults underwent 5-days of bed rest followed by 8-weeks of lower limb eccentric exercise rehabilitation (REHAB). Dual-energy X-ray absorptiometry, magnetic resonance imaging and myofiber analysis were used to identify muscle morphology and CLIX-IR and CLIX-β were used to assess insulin sensitivity. Skeletal muscle macrophages, CD68 (pan), CD11b (M1), CD163 (M2), CD206 (M2), were characterized using immunohistochemistry and gene expression. Insulin sensitivity, independent of age, decreased ~38% following bed rest and was restored following REHAB. We found robust age-related differences in muscle atrophy during bed rest, yet older and younger adults equally hypertrophied during REHAB. Interestingly, there were age-related differences in macrophage content ($CD68^+CD11b^+$ and $CD68^+CD11b^-$ cells) but both young and old similarly increased macrophages with REHAB. Satellite cell changes during rehab corresponded to macrophage content changes. Muscle tissue resident macrophages and gene expression, were not associated with changes in insulin sensitivity following bed rest and REHAB. These data suggest that muscle macrophages are modulated as a result of exercise rehabilitation following bed rest and may more associated with muscle regrowth/hypertrophy rather than insulin sensitivity in young or older adults.
This trial was registered at clinicaltrials.gov as NCT01669590.

1. Introduction

Bed rest during hospitalization contributes to severe functional decline in older adults and is associated with adverse outcomes such as increased rates of repeated hospitalization and death (Brown et al., 2004; Gill et al., 2004). We have shown that after 5-days of bed rest, older adults experience a significantly increased muscle loss compared to young subjects. Following 2-months of rehabilitation consisting of

Abbreviations: AFM, absolute fat mass; ALM, arm lean mass; AUC, area under the curve; β2M, beta-2-microglobulin; BCAA, branch chained amino acids; CCTS, Center for Clinical and Translational Science; CD11b, cluster of differentiation 11b; CD68, cluster of differentiation 68; CD163, cluster of differentiation 163; CD206, cluster of differentiation 206; CLIX-β, clamp-like index-beta; CLIX-IR, clamp-like index-insulin resistance; COX-2, cyclooxygenase 2; CSA, cross sectional area; DAPI, 4′,6-diamidino-2-phenylindole; DXA, dual-energy X-ray absorptiometry; IL-1 β, interleukin 1 beta; IL4Rα, interleukin 4 receptor alpha; IL-10, interleukin 10; LLM, leg lean mass; M1, pro-inflammatory macrophages; M2, anti-inflammatory macrophages; MHC I, myosin heavy chain 1; MHC II, myosin heavy chain 2; MRI, magnetic resonance imaging; NHS, normal horse serum; NOS3, nitric oxide synthase 3; OCT, optimal cutting temperature; OGTT, oral glucose tolerance test; Pax7, paired box protein 7; PBS, phosphate buffered saline; RFM, relative fat mass; RT, room temperature; TGFβ1, transforming growth factor beta 1; TLM, total lean mass; TNFα, tumor necrosis factor alpha; WBLM, whole body lean mass; WGA, wheat germ agglutinin.

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http://dx.doi.org/10.1016/j.exger.2017.07.001
0531-5565/

eccentric exercise, these older adults were able to reverse muscle mass loss and strength deficits due to short-term bed rest (Tanner et al., 2015).

The process of aging involves physiological and functional decline that can be described by various cellular changes including the accumulation of inflammatory cells within skeletal muscle. Macrophages in skeletal muscle modulate the process of regeneration and repair following exercise and muscle disuse in rodents, which is dependent upon their polarization state (Tidball and Wehling-Henricks, 2007). Following injury, neutrophil invasion peaks in skeletal muscle within 24 h. Neutrophils recruit M1 macrophages to infiltrate the muscle within 2 days and this subpopulation of macrophages produces pro-inflammatory cytokines that promote the clearance of necrotic debris. M2 macrophages peak around 4 days post injury and produce anti-inflammatory cytokines that enhance regeneration of muscle fibers and fibrosis (Tidball and Wehling-Henricks, 2007). However, there is a lack of evidence in human skeletal muscle suggesting that macrophages can be altered after disuse and following recovery and if there are any age-related differences.

Macrophages have also been shown to modulate insulin sensitivity in vitro. CD11c expression was increased in skeletal muscle of mice fed a high fat diet and conditioned media from FFA-treated bone-marrow cells induced insulin resistance in L6 myotubes (Nguyen et al., 2007). In an in vitro model using a human cell line, pro-inflammatory M1 macrophages inhibit insulin-stimulated glucose disposal, whereas M2 macrophages enhance this response (Groshong, 2013). It is well known that bed rest results in insulin resistance (Hamburg et al., 2007), which is primarily located in skeletal muscle (Stuart et al., 1988). Therefore, the insulin resistance associated with bed rest may be modulated by the inflammatory infiltrate and in turn contribute to the impaired muscle regrowth post bed rest.

Our goal was to elucidate the effects of bed rest and exercise rehabilitation on macrophage polarization within skeletal muscle of younger and older healthy adults while also determining if a relationship exists between macrophage infiltration and insulin resistance.

2. Methods

2.1. Subjects

We recruited 23 healthy community dwelling subjects from the Salt Lake City area in two age groups of 18–35 and 60–75 years and with a BMI <30 kg/m^2 for this study: 14 young (7 male, 7 female) and 9 older adults (2 male, 7 female). Most of these subjects were used in a prior study assessing the role of muscle protein synthesis after bed rest and following rehabilitation (Tanner et al., 2015). Subjects were recruited through campus and community flyers, radio announcements, health fairs, ClinicalTrials.org and word-of-mouth. Subjects were screened through personal interview, physician evaluation and a blood panel to meet an extensive set of inclusion/exclusion criteria. The exclusion list contained various physical, mental, muscle or metabolic dysfunctions that may impact on protein metabolism or contraindicated for bed rest. These included uncontrolled hypertension, diabetes, HIV, hepatitis B and C, hyper/hypothyroidism, cardiovascular, kidney, respiratory, vascular and liver disease, history of DVT, neurological disorders, recent cancer treatment (within 1 year of enrolling), osteopenia, depression, and alcohol/substance abuse. Physical activity status, medication usage of the subjects and menstrual status of young female subjects are previously described (Tanner et al., 2015). Of the 14 young subjects that underwent bed rest, 7 young subjects were randomly assigned (restricted randomization in blocks of 4–1:1 ratio) to continue onto the rehabilitation phase (REHAB) with a protein supplement whereas the remaining 7 conducted the REHAB but did not have supplementation. We did not find any differences during the REHAB phase in the young subjects according to protein supplementation so we collapsed the young subject groups for the majority of the outcomes. However, we present a few of the outcomes in young subjects according to protein supplement for use as supplemental data (Supplemental Fig. 1). All subjects read and signed the informed consent form. The study was approved by the University of Utah Institutional Review Board (no. 00050933) and conformed to the Declaration of Helsinki and Title 45, US Code of Federal Regulations, Part 46, "Protection of Human Subjects".

2.2. Experimental design: general

The experimental design can be summarized as follows: body composition and lower extremity power was assessed before bed rest (PRE), after bed rest (BED REST) and after 8 weeks of exercise rehabilitation (REHAB). Bed rest consisted of a 5-day, 4-night (Monday–Friday) physical inactivity experiment within the University of Utah's clinical research unit. REHAB encompassed 8 weeks of high intensity eccentric resistance exercise (3× per week) of both legs coupled with or without (for young only) post-exercise branch chained amino acid-enriched protein supplementation.

2.3. Tissue composition and power testing

Two weeks before bed rest, and after an overnight fast, lean tissue was determined using a dual-energy X-ray absorptiometry (DXA) scan by a trained technician at the University of Utah's Centre for Clinical and Translational Sciences, Clinical Research Unit. Subjects also underwent MRI testing for their lower legs before and after bed rest and after rehabilitation. Finally, subjects underwent knee extension power testing using the Leg Extension (Nottingham) Power Rig (Queen's Medical Centre, Nottingham, UK) as described previously (Marcus et al., 2011). Following five practice trials, each leg was tested individually and data were reported as peak power (Watts) from the average of maximal efforts from both legs. Power testing was monitored by a research assistant. Tissue composition and power testing were repeated after BED REST and after REHAB.

A habitual dietary assessment was measured approximately 2-wk. prior to bed rest and was recorded over a 5–7 day period. Caloric intake was later calculated using the Food Processor Nutrition Analysis software (Salem, OR, USA). There were no caloric and macronutrient differences between young and older adults at baseline (Tanner et al., 2015).

2.4. Biopsy study

After an overnight fast, application of 1% lidocaine and using aseptic technique, a muscle biopsy was sampled from the *vastus lateralis* using a modified Bergstrom needle approach with manual suction (Bergstrom, 1962). Muscle tissue was immediately washed with cold saline and dissected of visible non-muscle tissue, flash-frozen in liquid nitrogen, or mounted in isopentane-cooled OCT and stored at −80 °C for later analysis. Biopsy samples used for these analyses were taken before bed rest (PRE), after bed rest (BED REST) and following exercise rehabilitation (REHAB).

2.5. 5-Day bed rest

Prior to bed rest and after an overnight fast, subjects arrived at the University of Utah Center for Clinical and Translational Sciences (CCTS) Clinical Research Unit and underwent a DXA scan for assessment of body composition and an oral glucose tolerance test (OGTT). Approximately 1–2 wk. later, subjects participated in a 5-day/4-night bed rest protocol at the CCTS. After the completion of the first biopsy study, subjects adhered to five continuous days of bed rest as previously described in detail (Tanner et al., 2015). Adherence to bed rest was monitored by nursing staff 24 h a day who also performed standard of care (Tanner et al., 2015) for hospitalized/bed-ridden patients while monitoring subject safety. The night before entering the Clinical Research Unit, subjects