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Blood-cell bioenergetics are associated with physical function and inflammation in overweight/obese older adults☆

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ABSTRACT

Background: Physical function and strength decline with age and lead to limited mobility and independence in older adults. Alterations in mitochondrial function are thought to underlie numerous age-related changes, including declining physical ability. Recent studies suggest that systemic changes in bioenergetic capacity may be reported by analyzing mitochondrial function in circulating cells. The objective of this study was to determine whether the bioenergetic capacity of peripheral blood mononuclear cells (PBMCs) is related to differences in physical function among older, overweight/obese, adults. To address this, we tested the hypothesis that greater PBMC respirometric capacity would be associated with better physical function, muscular strength, leg lean mass, and muscle quality. Furthermore, we tested whether the respirometric capacity of PBMCs is related to cellular composition and inflammatory status reported by interleukin-6 (IL-6).

Methods: Fasted PBMC respiration (pmol/min/500,000 cells), expanded short physical performance battery (Ex-SPPB), peak knee extensor (KE) strength (Nm), grip strength (kg), leg lean mass (kg, via dual energy X-ray absorptiometry [DXA]), muscle quality (Nm/kg), and plasma IL-6 (pg/mL) were analyzed in 15 well-functioning, community-dwelling, sedentary overweight/obese older men ($n = 9$) and women ($n = 6$) aged 65 to 78 (mean 68.3 ± 3.5 years). Pearson and partial correlations were calculated to determine associations between PBMC respiration and these variables.

Results: Higher maximal respiration of PBMCs was associated with better Ex-SPPB ($r = 0.58$, $p = 0.02$), greater KE strength ($r = 0.60$, $p = 0.02$), greater grip strength ($r = 0.52$, $p = 0.05$) and lower IL-6 ($r = -0.58$, $p = 0.04$). Higher spare respiratory capacity was associated with better Ex-SPPB ($r = 0.59$, $p = 0.02$), greater KE strength ($r = 0.60$, $p = 0.02$), greater grip strength ($r = 0.54$, $p = 0.04$), greater leg muscle quality ($r = 0.56$, $p = 0.04$), and lower IL-6 ($r = -0.55$, $p = 0.05$). Monocyte and lymphocyte counts were not related to PBMC respiratory capacity.

Conclusions: Our results indicate that respirometric profiles of readily obtainable blood cells are associated with physical function and strength. Future studies should be undertaken in order to determine whether blood-based bioenergetic profiling can provide an objective index of systemic mitochondrial health.

1. Introduction

The maintenance of physical function is a critical factor for mobility and independence in the growing population of adults 65 years and older (Katz et al., 1983; Avila et al., 2012). Measures of physical function, specifically involving the lower extremities, are strong predictors of morbidity and mortality (Guralnik et al., 1995), generalizable to older adults with a wide range of physical abilities, including those that are well-functioning (Simonsick et al., 2001). Physical function tests integrate multiple physiological systems including: nervous, musculoskeletal, and energy production/delivery, which likely underlies their ability to predict morbidity and mortality (Ferrucci et al., 2000). Our group has reported that chronic inflammation, such as elevated interleukin-6 (IL-6) levels in plasma, is associated with poorer physical function in

Abbreviations: ATP, adenosine triphosphate; BMI, body mass index; DXA, dual energy X-ray absorptiometry; ELISA, enzyme-linked immunosorbent assay; ETC, electron transport chain; Ex-SPPB, expanded short physical performance battery; FCCP, carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone; IL-6, interleukin-6; KE, knee extensor; Nm, Newton meters; OCR, oxygen consumption rate; PBMC, peripheral blood mononuclear cell; SRC, spare respiratory capacity; XF, extracellular flux.

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older adults across a variety of diseases/health conditions, supporting the hypothesis that chronic inflammation represents a common mechanism underlying age-related functional decline (Brinkley et al., 2009). Indeed, studies focused on IL-6 have shown that blood levels and monocyte production of IL-6 are higher with advancing age (Ferrucci et al., 2005). In particular, there is a dramatic increase in the number of individuals with elevated IL-6 levels over the age of 70 years (Giuliani et al., 2001). In addition, chronic inflammation, along with other circulating factors, has been shown to detrimentally affect mitochondrial function in muscle tissue (Valerio et al., 2006).

Although measures of physical function are important indicators of health in geriatrics, the biological mechanisms that underlie age-related decline in physical function and associated health consequences are not fully understood. Aging and declining function are associated with bioenergetic decline. For example, lower maximal adenosine triphosphate (ATP) production in *vastus lateralis* skeletal muscle is associated with reduced physical function and aerobic capacity (Joseph et al., 2012; Tyrrell et al., 2014; Coen et al., 2013). Multiple lines of evidence, including these data, suggest that mitochondrial function is vitally important for physical function, particularly in the context of aging.

Bioenergetic profiling using readily obtainable blood-cells has been proposed as a way to assess systemic mitochondrial health (Chacko et al., 2014; Ravi et al., 2014). There is mounting evidence that circulating factors, including inflammatory cytokines, can mediate bioenergetic decline in multiple tissues throughout the body (Salminen et al., 2012). The effects of these factors on mitochondrial bioenergetics may be reflected in the respiratory profiles of circulating cells such as peripheral blood mononuclear cells (PBMCs). In fact, we recently showed that mitochondrial function measured in both skeletal muscle and PBMCs was associated with gait speed among older adults (Tyrrell et al., 2014). To expand on this work, the main objective of the current study was to determine whether physical and muscle function are related to bioenergetic capacity of PBMCs in well-functioning, community-dwelling, overweight/obese older adults. We also determined whether the heterogeneous composition of PBMCs underlies their bioenergetic capacity and whether the proinflammatory cytokine IL-6 is associated with PBMC respiratory capacity.

2. Methods

2.1. Participants

Fifteen participants were included in this study of older (65–79 years), overweight and obese (body mass index [BMI]: 28–35 kg/m^2), sedentary men (n = 9) and women (n = 6) recruited to participate in a clinical trial of resistance training with or without dietary-induced weight loss. The assessments reported here were conducted at baseline, prior to randomization.

Eligible participants were in good health and had normal cognitive function, used no walking aids, and did not have uncontrolled diabetes or hypertension, cardiovascular disease, abnormal liver or kidney function, or cancer requiring treatment in the past 2 years. The study was approved by the Wake Forest School of Medicine Institutional Review Board and all participants provided written, informed consent.

2.2. Physical function

2.2.1. Expanded short physical performance battery

The expanded short physical performance battery (Ex-SPPB) was used as previously reported to prevent ceiling effects when applying the measure to well-functioning, community-dwelling older adults (Simonsick et al., 2001). Briefly, the Ex-SPPB consists of a usual gait speed test, a usual gait speed test using a narrow course (20 cm), 5 repeated chair stands, and 30 second standing balance tests (semi-, full-tandem, and single leg). Scores and times are recorded for each and converted to ratios and summed to get a composite score on a continuous scale ranging from 0 to 4.

2.3. Muscle strength

2.3.1. Peak knee extensor strength

Peak knee extensor (KE) strength (in Newton meters; Nm) was measured on a dynamometer (Biodex Medical Systems, Inc., Shirley, NY) at 60° per second with the participant seated and the hips and knees flexed at 90°. To stabilize the hip joint and the trunk, participants were restrained with straps at the chest, hip and thigh. Seat height and depth, and the position of the lever arm ankle pad were adjusted to accommodate each participant. Participants were asked to extend the knee and push as hard as possible against the ankle pad. Strength of the right leg recorded as peak torque was used for analyses.

2.3.2. Grip strength

Grip strength was measured twice on each hand to the nearest kg using an isometric hydraulic hand dynamometer (Jamar, Bolingbrook, IL) and the maximal value from both hands was used in analyses.

2.4. Leg lean mass and muscle quality

Leg lean mass was measured by dual energy X-ray absorptiometry (DXA, Hologic Delphi QDR, Bedford, MA). Muscle quality was calculated as the ratio of knee extensor peak torque to lean mass of the right leg assessed by DXA (Nm/kg leg lean mass).

2.5. Blood draw

Blood draws were performed in the morning after an overnight fast and were processed immediately after collection. 8 mL of whole blood was collected into cell preparation tubes (Vacutainer; Becton Dickinson, Franklin Lakes, NJ) for PBMC separation.

2.6. Respirometry of peripheral blood mononuclear cells

PBMCs were washed with phosphate-buffered saline and resuspended in extracellular flux (XF) assay buffer containing 1 mM Na^+-pyruvate and 11 mM D-glucose (pH 7.4) for respirometry experiments. Using a defined series of inhibitors and uncoupler, the basal and maximal cellular respiration was quantified and used to calculate spare respiratory capacity (SRC) (Desler et al., 2012; Ferrick et al., 2008). A total of 500,000 cells were plated per well of the Seahorse microplate. Basal oxygen consumption rate (OCR) measures were followed by sequential addition of oligomycin (750 nM), carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP; 1 μM), and antimycin-A/rotenone (both 1 μM). Maximal OCR was calculated after addition of FCCP, a potent mitochondrial uncoupler. The use of FCCP as a chemical uncoupler allows us to estimate maximal respiration. SRC was calculated as the difference between maximal OCR and the basal OCR.

2.7. Inflammatory cytokine quantification

Blood from 12-hour fasted participants was collected, processed, divided into aliquots, stored at −80 °C, and analyzed according to previously published methods (Beavers et al., 2010). Briefly, high-sensitivity IL-6 assays were run using Quantikine enzyme-linked immunosorbent assay (ELISA) kits from R&D systems (Minneapolis, MN). All samples were measured in duplicate, and the average of the two values was used for data analyses.

2.8. Statistical analyses

Normality of distributions was assessed using Shapiro–Wilk tests. All variables were normally distributed except for monocyte cell