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Blood cell respirometry is associated with skeletal and cardiac muscle bioenergetics: Implications for a minimally invasive biomarker of mitochondrial health

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ABSTRACT

Blood based bioenergetic profiling strategies are emerging as potential reporters of systemic mitochondrial function; however, the extent to which these measures reflect the bioenergetic capacity of other tissues is not known. The premise of this work is that highly metabolically active tissues, such as skeletal and cardiac muscle, are susceptible to differences in systemic bioenergetic capacity. Therefore, we tested whether the respiratory capacity of blood cells, monocytes and platelets, are related to contemporaneous respirometric assessments of skeletal and cardiac muscle mitochondria. 18 female vervet/African green monkeys (*Chlorocebus aethiops sabaeus*) of varying age and metabolic status were examined for this study. Monocyte and platelet maximal capacity correlated with maximal oxidative phosphorylation capacity of permeabilized skeletal muscle (R=0.75, 95% confidence interval [CI]: 0.38–0.97; R=0.51, 95%CI: 0.05–0.81; respectively), isolated skeletal muscle mitochondrial respiratory control ratio (RCR; R=0.70, 95%CI: 0.35–0.89; R=0.64, 95%CI: 0.23–0.98; respectively), and isolated cardiac muscle mitochondrial RCR (R=0.55, 95%CI: 0.22–0.86; R=0.58, 95%CI: 0.22–0.85; respectively). These results suggest that blood based bioenergetic profiling may be used to report on the bioenergetic capacity of muscle tissues. Blood cell respirometry represents an attractive alternative to tissue based assessments of mitochondrial function in human studies based on ease of access and the minimal participant burden required by these measures.

1. Introduction

Mitochondrial dysfunction is well recognized to play a key role in a wide variety of diseases, particularly those associated with aging. For this reason, assessments of mitochondrial function have long been proposed to have significant diagnostic and prognostic applications. There is mounting evidence that blood cells can report on systemic mitochondrial function. Diabetes, atherosclerosis, and neurodegeneration are all related to the deterioration of various mitochondrial parameters in multiple cell types, including leukocytes and platelets [1–4]. Changes in mitochondrial DNA (mtDNA), mitochondrial enzyme activity, and electron transport chain (ETC) activity measured in peripheral blood mononuclear cells, monocytes, lymphocytes, and platelets have been associated with mortality, diabetes, HIV, cardiovascular disease, Parkinson's disease, Alzheimer's disease, cancer, inflammation, cognition, Huntington's disease, sepsis, and fibromyalgia [1,5–18]. More recently, blood based bioenergetic profiling strategies utilizing cellular respirometry have been associated with key features of aging such as gait speed, physical function decline, inflammation, and depression [19–21].

Blood based measures of mitochondrial function may provide a minimally invasive test that is particularly well suited for diagnostic use. In addition, these measures are highly amenable for use in large scale clinical trials, including those with serial longitudinal assessments, because they require minimal patient burden and

Abbreviations: ACD, acid citrate dextrose; BMI, body mass index; BSA, bovine serum albumin; CP, Chappell-Perry buffer; CII, complex 2; EGTA, triethylene glycol diammine tetraacetic acid; ELISA, enzyme-linked immunosorbent assay; ETC, electron transport chain; FCCP, Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone; HOMA-IR, homeostatic model assessment of insulin resistance; MAS, mannitol and sucrose buffer; MAX, maximal FCCP-linked bioenergetic capacity; MES, 2-(N-morpholino)ethanesulfonic acid; O2k, Oroboros Oxygraph 2K; OXPHOS, oxidative phosphorylation; PBS, phosphate buffered saline; PGE_1, prostaglandin E_1; PmFBs, permeabilized fiber bundles; RCR, respiratory control ratio; SD, standard deviation; XF, extracellular flux

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cost compared to biopsy based measures of mitochondrial function. Blood based bioenergetic profiling strategies are also well suited for use in older adults who often present with multiple contraindications to biopsy. However, little is known about whether these blood based measurements are able to recapitulate measures of mitochondrial function performed in other tissues.

In this study, we examined the relationships of monocyte and platelet mitochondrial respiration with assessments of mitochondrial function performed in muscle tissues. We focused on skeletal and cardiac muscle because age-related bioenergetic decline is thought to be most significant in highly metabolically active tissues and based on our previous findings that blood based respirometry is positively correlated with measures of physical function and strength in human subjects [20]. For example, skeletal muscle from older adults is reported to have reduced ATP production, maximal bioenergetic capacity, and mitochondrial content compared to younger counterparts [22]. In addition, cardiac tissue oxidative capacity and phosphocreatine/ATP ratio are reduced in the earliest stages of heart failure in humans [23], which is further impaired by diabetes and obesity [24,25]. These are similar to findings from rodent models [26,27]. The use of a non-human primate model provided us with reliable access to heart and skeletal muscle tissue and sufficient volumes of blood to permit the analyses of multiple blood cell populations. Vervet macaques are susceptible to naturally occurring changes in body composition [28], physical function [29], and chronic diseases ranging from obesity [30], diabetes [31], and heart disease [32] as they age in a manner similar to humans [33]. The present study utilized a group of female vervet macaques specifically selected to represent a wide range of metabolic health status and insulin resistance as well as body mass indices from lean to obese across young and old age groups. This design was utilized in order to maximize the potential bioenergetic differences between animals.

Blood cell respiration was compared to skeletal muscle mitochondrial function in two ways. *Vastus lateralis* muscle fibers were permeabilized and analyzed by high resolution respirometry [34] to examine bioenergetic capacity in a manner that maintains potential differences in mitochondrial content and architecture [35]. In addition, we examined respiratory control in isolated *vastus lateralis* mitochondria [36] to determine whether blood based measures might be related to differences in intrinsic electron transport chain function. Similar methods using isolated organelles were carried out for analysis of cardiac muscle mitochondrial function. We hypothesized that because blood cells are continuously exposed to circulating factors such as inflammatory cytokines, redox stress [37], and recently described mitokines [38]; which are known to affect mitochondrial function across tissues; respirometric analyses of monocytes and platelets will recapitulate differences in systemic bioenergetic capacity.

2. Materials and methods

2.1. Animal participants

This study included 18 female vervet/African green monkeys (*Chlorocebus aethiops sabaeus*) ranging in age from 8.2 to 23.4 yrs. The monkeys originally lived in stable social groups of 11–49 in indoor-outdoor housing units with approximately 28 m^2 indoors and 111 m^2 outdoors which contained perches, platforms, elevated climbing structures and a base composed of smooth stones. Seven of the 18 animals were moved to indoor housing (pair- or individually-housed) prior to study initiation. All animals were fed a standard monkey chow diet (LabDiet 1538), supplemented with fruits and vegetables 5 times per week. Water was available ad libitum. Blood samples were obtained from anesthetized animals immediately prior to necropsy, and the harvest of skeletal and cardiac muscle tissues. Euthanasia was carried out with IM ketamine (10–15 mg/kg) followed by IV sodium pentobarbital (60–100 mg/kg) to attain deep surgical anesthesia and exsanguination in accordance with guidelines established by the Panel on Euthanasia of the American Veterinary Medical Association. All procedures were approved and performed according to the guidelines of state and federal laws, the US Department of Health and Human Services, and the Animal Care and Use Committee of Wake Forest School of Medicine.

2.2. Body mass measurements

BMI was estimated as the ratio of body mass to the square of trunk length measured from the suprasternal notch to the pubic symphysis using an electronic caliper (in kg/m^2) and was calculated 4 months prior to necropsy while weight was measured at the time of necropsy [39].

2.3. Insulin, glucose, and insulin sensitivity measurements

Fasting glucose was determined using reagents and instrumentation (ACE ALERA autoanalyzer) from Alfa Wasserman Diagnostic Technologies (West Caldwell, NJ). Insulin was determined by an enzyme-linked immunosorbent assay (ELISA; Mercodia, Uppsala, Sweden). All analyses were performed in the Wake Forest Comparative Medicine Clinical Chemistry and Endocrinology Laboratory 4 months prior to necropsy. Homeostatic model of insulin resistance (HOMA-IR)=([mg/dL fasting glucose X mIU/L fasting insulin]/405) was used as an estimate of insulin resistance [40].

2.4. Isolation of blood cells

Blood (8 mL) was collected from fasted monkeys into acid citrate dextrose (ACD) tubes (Vacutainer; Becton Dickinson, Franklin Lakes, NJ) and processed immediately to obtain platelet and CD14+ monocyte preparations. Platelets and CD14+ monocytes were isolated using methods similar to those described by Chacko et al. [41]. Briefly, whole blood in ACD tubes was centrifuged at $500 \times g$ for 15 min at room temperature with the brake off. Platelet rich plasma was removed and platelets were isolated by centrifugation at $1500 \times g$ for 10 min, washed in phosphate-buffered saline (PBS) with prostaglandin E_1 (PGE1; Cayman Chemical, Ann Arbor, MI), and resuspended in extracellular flux (XF) assay buffer (Seahorse Biosciences, North Billerica, MA) containing 1 mM Na^+-pyruvate, 1 mM GlutaMAX (Gibco, Grand Island, NY), 11 mM D-glucose, and PGE1 (pH 7.4) for respirometry experiments. The buffy coat layer was extracted, diluted 4× in RPMI 1640 (Gibco) and layered onto 3 mL of polysucrose solution at a density of 1.077 g/mL (Sigma Histopaque®-1077, St. Louis, MO) in 15 mL centrifuge tubes and centrifuged at $700 \times g$ for 30 min with no brake. The buffy coat layer was obtained, washed in PBS, and divided into 2 tubes. CD14+ monocytes were isolated from 1 tube using CD14-labeled magnetic microbeads (Miltenyi Biotec, San Diego, CA) according to manufacturer instructions using modified RPMI 1640+fatty-acid free bovine serum albumin (BSA) media. Monocytes were washed in modified RPMI 1640 media and resuspended in XF assay buffer without PGE1 for respirometry experiments.

2.5. Respirometry of blood cells

A total of 250,000 monocytes and 25,000,000 platelets per well were plated in quadruplicate in the Seahorse microplate. Bioenergetic profiling using selected inhibitors and uncoupler have