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BLOOD-BASED BIOENERGETICS: AN EMERGING TRANSLATIONAL AND CLINICAL TOOL

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Abstract

Accumulating studies demonstrate that mitochondrial genetics and function are central to determining the susceptibility to, and prognosis of numerous diseases across all organ systems. Despite this recognition, mitochondrial function remains poorly characterized in humans primarily due to the invasiveness of obtaining viable tissue for mitochondrial studies. Recent studies have begun to test the hypothesis that circulating blood cells, which can be obtained by minimally invasive methodology, can be utilized as a biomarker of systemic bioenergetic function in human populations. Here we present the available methodologies for assessing blood cell bioenergetics and review studies that have applied these techniques to healthy and disease populations. We focus on the validation of this methodology in healthy subjects, as well as studies testing whether blood cell bioenergetics are altered in disease, correlate with clinical parameters, and compare with other methodology for assessing human mitochondrial function. Finally, we present the challenges and goals for the development of this emerging approach into a tool for translational research and personalized medicine.

Keywords

Mitochondria; bioenergetics; platelets; blood cell; biomarker; precision medicine

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Introduction

Bioenergetic alteration is now recognized as a hallmark of many chronic diseases and represents a potential therapeutic avenue. As such, devising strategies for the assessment of human bioenergetics during physiology, and its alteration in pathology is currently an intense area of translational research. Mitochondria are central to bioenergetics due to their “powerhouse” function of adenosine triphosphate (ATP) generation through oxidative phosphorylation (OXPHOS). However, it is now well established that in addition to maintaining energy homeostasis, mitochondria perform a myriad of other functions. Production of reactive oxygen species (ROS) by electron transport chain complexes I and III, as well as calcium buffering, contribute to cell signaling, and release of the electron transport protein cytochrome c from the organelle initiates apoptotic cell death (1–3). Notably, since these functions involve electron transport chain machinery, they are intimately linked to OXPHOS, and thus alterations in any of these functions may directly or indirectly alter bioenergetics.

Bioenergetics has long been studied in organs that have high energy demand and contain large numbers of mitochondria, such as the brain, heart, liver, and skeletal muscle. However, the role of these organelles in circulating cells and their regulation of blood cell function has only relatively recently begun to be uncovered. In adults, the majority of blood cells are derived through hematopoiesis in the bone marrow, a process in which multipotent progenitor hematopoietic stem cells (HSCs) differentiate into precursor cells of the myeloid or lymphoid lineage. Myeloid progenitors serve as the precursors for erythrocytes and megakaryocytes (which give rise to platelets), as well as the myoblast which generates multinucleated basophils, neutrophils, and eosinophils and mononuclear monocytes. Likewise, lymphoid progenitor cells differentiate into T and B-lymphocytes and natural killer cells. (For a comprehensive review on hematopoiesis see (4)). Notably, hematopoietic cellular differentiation is a highly energetic process that relies on OXPHOS and ATP generation (5–8) and is accompanied by functional changes in mitochondrial mass, membrane potential, and mitochondrial reactive oxygen species (mtROS) production (6, 9–14). This ultimately results in the incorporation of different numbers of mitochondria in each terminally differentiated cell type, with the exception of mature erythrocytes which do not contain functional mitochondria (15). Regardless of mitochondrial abundance, accumulating data demonstrate that the organelle plays vital signaling and homeostatic roles in all mature blood cell types (16–18). For example, in addition to generating energy in these newly reprogrammed cells, increases in mtROS production and mitochondria-driven apoptosis have been linked to the activation of platelets (19, 20) and sensitization of dendritic cells (21–23), while an increase in mitochondrial oxygen consumption has been associated with lymphocyte activation (24). Thus, it is now clear that mitochondrial function is integral to both blood cell formation and homeostasis.

Even prior to understanding the role of mitochondria in blood cell homeostasis, researchers displayed a keen interest in mitochondrial measurement of circulating cells. It has long been recognized that circulating cells are unique in that they are relatively easily obtainable, abundant in supply, and come into contact with all other organ systems in the body. As early as 1915, Cowdry and colleagues used Janus green to stain leukocyte mitochondria (25). The

concept that blood cell mitochondria could interact with and influence peripheral tissue mitochondria was also proposed as early as 1924 by Kropp and May, who tested in animal models whether inhalation of gaseous mediators could change leukocyte mitochondrial morphology and transfer these morphologic alterations to other tissues (26). In the 1960's and 1970's, platelets were proposed as a model to study human mitochondria and methodology to isolate human platelet mitochondria became an intense area of focus (27, 28). Building on this idea, numerous labs began to utilize platelets and leukocytes to measure mitochondrial changes in human disease populations in the early 1990's (29–32). However, these types of studies were stymied by traditional technology to assess mitochondrial function, some of which did not provide a comprehensive readout of mitochondrial function and required large volumes of blood. For example, many of these studies utilized spectrophotometric kinetic assays to determine the enzymatic activity of individual complexes of the electron transport chain (ETC) in lysed mitochondria or blood cells (31, 33). However, this methodology only represents isolated enzymatic function and does not provide any information on total OXPHOS or ATP production by the mitochondria. In more recent years, high-resolution respirometry has been adapted for the measurement of intact and permeabilized blood cells. Further, with the advent of the Extracellular Flux Analyzer (Seahorse, Agilent), measurement of oxygen consumption in a high throughput manner, using small numbers of cells, has become possible. Additionally, the ability to concomitantly assess glycolysis in this system has enabled a mechanism to generate comprehensive bioenergetic profiles. This technology has renewed interest in the measurement of bioenergetics in circulating cells. In this review, we provide a survey of the burgeoning field of using circulating blood cells as a bioenergetic biomarker, a tool for understanding mitochondrial mechanisms, and its translational implications for personalized and mitochondrial medicine.

Mitochondrial Dysfunction Plays a Role in the Pathogenesis of Non-metabolic Diseases

Functional mitochondria are the product of the coordinated expression of proteins coded by both nuclear and mitochondrial DNA (mtDNA). Mitochondrial DNA differs from nuclear DNA in that it is a 16.5kb circular molecule and is localized within each mitochondrion. Though mtDNA composes only a small portion of the total DNA in eukaryotic cells, its function is crucial since mtDNA encodes 37 genes, of which 13 are essential subunits of electron transport chain complexes (34, 35). It has long been recognized that sporadic or inherited mutations in mtDNA or in mitochondrial genes encoded by nuclear DNA, which alter the expression of mitochondrial electron transport proteins, can cause a wide range of metabolic diseases. These diseases, such as Kearns-Sayre syndrome (KSS), mitochondrial encephalomyopathy, lactic acidosis, and stroke-like syndrome (MELAS), Leigh syndrome sub-acute necrotizing encephalopathy, and myoneurogenic gastrointestinal encephalopathy (MNGIE), affect multiple organs and have slow and progressive chronic symptoms that vary in onset from birth to late adulthood (36–40). Since mitochondrial function underlies the pathogenesis of these diseases, the systems that are frequently affected are those that require high energy and give rise to symptoms that include vision, hearing and respiratory problems,

cardiovascular abnormalities, neurological and learning problems, and gastrointestinal disorders (37, 41–43).

In addition to inherited metabolic diseases, mitochondrial genetics also influence susceptibility to and pathogenesis of many common diseases across all major organs systems, most of which have traditionally not been considered to be metabolic in nature (44, 45). For example, acquired mtDNA mutations in pancreatic β -cells contribute to abnormal glucose metabolism in diabetes (46, 47), the accumulation of somatic mtDNA deletions is linked to the pathogenesis of Parkinson's Disease (48–50), and mtDNA mutation can modulate tumor cell metastasis in many types of cancer (45, 51, 52). While the concept that pathogenic mtDNA mutations contribute to disease pathogenesis is well accepted, the role of non-pathogenic physiologic variants in the mtDNA sequence is more controversial.

However, accumulating studies demonstrate that mtDNA background can influence disease progression. Human studies examining mtDNA sequence have linked particular mtDNA haplotypes to susceptibility of a number of diseases including asthma (53), Parkinson's Disease (54), Alzheimer's Disease (55), and heart disease (56–58), as well as longevity (59–61). Murine studies utilizing the Mitochondrial-Nuclear eXchange (MNX) mouse model, in which different combinations of mtDNA and nuclear DNA from various mouse strains can be expressed, have more robustly demonstrated the influence of mtDNA. For example, using this model, Ballinger and colleagues inserted mtDNA from the C57/BL6 mouse strain background on the C3H/HeN mouse nuclear background and vice versa and subjected these mice to cardiac volume overload. These studies demonstrated that the C57/BL6 mtDNA background increased mitochondrial ROS production and susceptibility to cardiac stress, independent of nuclear DNA background (62). Similar studies of MNX mice on an atherogenic diet showed that specific mtDNA-nuclear DNA combinations influence inflammatory signaling and pathogenesis of non-alcoholic fatty liver disease (63). Importantly, more recent studies in these mice demonstrated that mtDNA background influences nuclear gene expression (64). These studies suggest that mtDNA potentially impacts disease progression concomitantly through regulation of mitochondrial function as well as through crosstalk with nuclear DNA.

Alterations in mitochondrial function in non-metabolic diseases are not exclusively secondary to mtDNA mutation. Changes in mitochondrial energetics, ROS production and apoptotic signaling have been reported as part of the pathogenesis of the majority of diseases across all organ systems independent of mtDNA alteration (65–68). Notably, while the pathogenic stimuli for mitochondrial functional alterations are multi-factorial and have not yet been completely elucidated in all these diseases, the contribution of mitochondrial dysfunction to disease progression is clearer in some cases. For example, in heart failure, impaired electron transport chain function leading to a lack of ATP production and increased generation of mtROS propagates cardiac bioenergetic deficits in the myocardium (69, 70). Likewise, in pulmonary hypertension (PH), mitochondrial dysfunction resulting in decreased oxidative phosphorylation (with concomitant increase in glycolysis) and suppressed mitochondrial apoptotic signaling is linked to pulmonary artery smooth muscle cell hyperproliferation and resistance to apoptosis, two major molecular hallmarks of the disease that result in vascular remodeling (71–73). In contrast to pathologies with diminished mitochondrial function, airway cells from asthmatic individuals show increased oxidative

phosphorylation and TCA cycle flux (74–76). Recent studies demonstrate that this is due to enhanced mitochondrial arginine metabolism and functions to modulate hypoxia-inducible factor expression and downstream inflammatory signaling (75).

It is interesting to note that while the majority of studies focus on the measurement of mitochondrial function in the solid organ that represents the primary site of pathology, accumulating studies suggest that pathology-induced alterations in bioenergetics may occur systemically. For example, in Parkinson's disease, the mitochondrial complex I inhibition observed in neurons of the substantia nigra has also been measured in circulating platelets (77, 78). Animal and human studies of PH demonstrate that the decrease in oxidative phosphorylation and shift to glycolytic metabolism is not only apparent in pulmonary vascular cells (79, 80), but also in the heart (81, 82), bone marrow-derived immune cells (83, 84), and skeletal muscle (85, 86). Similarly, in asthmatic individuals, increased oxidative phosphorylation has been observed not only in airway cells, but also in circulating platelets (74, 87). Additionally, whole body metabolic studies have shown that mild asthmatics are metabolically more efficient than healthy controls, further suggesting systemic bioenergetic changes as part of the asthma pathogenesis (88).

The types of studies highlighted in this section demonstrate that mitochondrial function can play a pivotal role in disease susceptibility and pathogenesis. Further, bioenergetic changes are potentially not always confined to the primary site of pathology, but rather are systemic in nature. Therefore, there is growing appreciation in the field that systemic assessment of bioenergetic function may, not only provide an understanding of pathogenic mechanisms, but also may potentially be utilized as a personalized medicine tool to diagnose disease, monitor disease progression, and assess therapeutic efficacy.

Blood cells as a systemic biomarker for bioenergetics

While the value of bioenergetic assessment is well recognized, many barriers exist to measuring human mitochondrial function in solid organs. Perhaps the greatest barrier is the lack of accessibility to adequate amounts of intact viable tissue. Functionality of the mitochondrial electron transport chain is dependent on intact mitochondrial membranes. Thus, frozen or damaged tissue will not provide an accurate picture of mitochondrial function. Currently, muscle biopsies remain the gold standard in terms of acquiring mitochondrial rich human tissue. However, these studies are highly invasive and are limited in the amount of tissue that can be obtained (89, 90). Slightly less invasive is the use of skin grafts to acquire and culture dermal fibroblasts. While this methodology yields higher numbers of cells, questions remain about whether mitochondrial function can change over time in these cells with long-term culture (91).

Non-invasive methods of metabolic measurement have gained popularity due to relative ease for the human subject and the ability to perform repeated measures over time. However, these methodologies also have limitations. Near-infrared spectroscopy (NIRS) provides a real-time measure of tissue oxygenation through the measurement of the optical absorption of the oxy- and deoxy-heme groups of hemoglobin, myoglobin and cytochrome c (92). While this method is relatively inexpensive, data interpretation is confounded by difficulty in

differentiating between the multiple heme-containing groups in tissue and measurement can be affected by adiposity and variable blood flow of the subject (93, 94). Phosphorous magnetic resonance spectroscopy (^{31}P -MRS) can be used to measure metabolism of human tissues through the visualization of key phosphate groups, specifically phosphocreatine, the phosphate groups of ATP and inorganic phosphate (95). While this method can be utilized to get a snapshot of the energy status of static organs, it is particularly useful to determine the kinetics of ATP generation in the working heart or recovery after exercise in skeletal muscle (96–100). Non-invasive positron emission tomography (PET) provides a sensitive measure of specific aspects of metabolism based on the detection of injected positron-emitting radionuclide tracers (101, 102). ^{18}F -fluorodeoxyglucose is most routinely used for the imaging of glucose metabolism (103, 104). While PET and MRS can provide robust metabolic data, both methodologies require access to specialized equipment, can be cost prohibitive to perform, and require a high degree of training for the acquisition of reproducible data and accurate analysis. While existing techniques are suitable for the measurement of metabolism in select studies, a minimally invasive and more cost-effective method that requires less expertise to execute would make assessment of human mitochondrial function more practical and increases throughput for use in translational research studies and potentially in the clinic as a personalized medicine tool.

Circulating blood markers have long been utilized clinically for disease diagnosis and prediction of prognosis due to the blood's unique property of interaction (through perfusion) with every organ system and exposure to both systemic environmental stressors and hormones released by other organs. Consistent with this concept, the use of peripheral blood cells has emerged as a viable option to measure mitochondrial function and cellular bioenergetics in human cohorts. Sufficient quantities of blood can be obtained through a simple blood draw, and leukocytes and platelets, which contain fully functional mitochondria and glycolytic machinery, can be isolated by simple ex vivo procedures. Mitochondria in each type of leukocyte as well as platelets are suited to carry out the specialized function of these cell types and thus demonstrate differential bioenergetic profiles at baseline (reviewed below) (105, 106). However, a major strength in utilizing these circulating cells is their response to systemic stimuli and contact with vasculature in all major organ systems. The following sections outline the predominant methodology utilized for blood cell bioenergetic measurement and recent advances in this area in terms of utilization of this methodology for translational research.

Methodology to Measure Mitochondrial Function and Bioenergetics in Blood Cells

Studies in the late 1980's set the stage for the use of circulating cells to measure mitochondrial function. These studies aimed to assess mitochondrial differences between healthy humans and those with diagnosed disease through the measurement of the activity or expression of isolated mitochondrial enzymes in blood cells. For example, Sangiorgi et al showed that in patients with migraine headaches, complex I, complex IV, and citrate synthase activities were decreased compared to healthy controls (107), and Parker and colleagues showed decreased complex I activity in platelet mitochondria from Parkinson's

disease patients (30). Similarly, changes in isolated platelet mitochondrial enzyme activities were observed in patients with Huntington's disease (108, 109), Alzheimer's disease (31, 110), and schizophrenia (111, 112). While these studies were important in establishing that mitochondrial alterations occur in peripheral cells during human pathogenesis, it is important to note that isolated enzymatic activities do not always translate into significant differences in OXPHOS or other facets of mitochondrial function. This is due to the fact that electron transport chain proteins are physiologically expressed in excess of what is required to maintain OXPHOS, and each complex exercises a varying degree of control over respiration, such that a threshold of inhibition must be achieved before a complex deficiency impacts ATP production (113, 114).

One of the most informative tests for mitochondrial function is the measurement of mitochondrial respiration, which reflects not only the activity of the electron transport chain complexes, but is also directly influenced by the function of the ATP Synthase and inner mitochondrial membrane potential (Ψ). Mitochondrial Respiration has classically been assessed using the Clark-type polarographic electrode, which measures oxygen consumption in cells or isolated mitochondria in real-time (115). The Oroboros high-resolution respirometric system (Oroboros Instruments, Austria), introduced in 1994, improved upon traditional Clark electrode technology to provide accurate amperometric measurement of O_2 consumption with maximal sensitivity and precision (116). The major advantages of the Oroboros system over traditional Clark-type electrodes are its temperature control system that allows measuring O_2 consumption at different temperatures ranging from 2–45 °C, and the relatively small sample volume required for respirometric analysis (117). In addition, the high-resolution detection capability of Oroboros is enhanced by low instrumental background noise, stability of the signal and the ability to supplement the system with oxygen in order to prevent hypoxic conditions in the experimental system even in the presence of high rates of mitochondrial oxygen consumption as well as measure oxygen consumption rate in a fixed range of oxygen tension if required (118–120). Combined with these advantages is the ability for the user to sequentially titrate mitochondrial substrates, modulators, and inhibitors into the system to probe multiple parameters of respiration, as outlined below.

More recently, the advent of the Seahorse XF Extracellular Flux Analyzer (Agilent) has facilitated the high throughput measurement of cellular bioenergetics (117, 121). This system utilizes novel fluorescent sensors in a multi-well plate format to simultaneously measure with high resolution, oxygen consumption rate (OCR) and extracellular acidification rate (ECAR- which is utilized to calculate glycolytic rate) in intact cells (121). In addition to that it is the only system that enables simultaneous measurement of up to 90 different samples in a single run, and requires a relatively small number of blood cells (105, 122).

Like the Oroboros, the Seahorse XF is optimized to inject multiple modulators into the system to probe different aspects of bioenergetics in the same set of cells in the same experiment. The most common series of modulators comprise an assessment known as the Mitochondrial Stress Test (MST) that includes the addition of the ATP synthase inhibitor oligomycin A, followed by the protonophore carbonyl cyanide-4-

(trifluoromethoxy)phenylhydrazine (FCCP), and lastly a mitochondrial inhibitor such as rotenone or antimycin A (123). Measurements of OCR after the addition of these modulators generate a “bioenergetic profile” from which the following parameters can be calculated (Figure 1):

Basal OCR:

This represents the basal respiration rate of the cells in the absence of any added modulators and can serve as the baseline in response to the steady state energy demand of the cell. Non-mitochondrial OCR is subtracted from this value to represent only mitochondrial OCR as described in Figure 1.

Proton-leak OCR:

This parameter is measured after the addition of oligomycin A. Inhibition of proton translocation through complex V by oligomycin A increases membrane potential, which decreases electron transport through complexes I-IV. Thus, residual OCR that is not affected by oligomycin treatment addition is due to “proton leak” across the inner membrane and is independent of ATP production. This parameter is often referred to as “inefficient respiration”. While a minimal proton leak is observed under physiological conditions, a significant increase in proton leak can indicate mitochondrial membrane damage or increased activity of mitochondrial uncoupling proteins (UCP). Importantly, subtraction of proton leak from the basal OCR allows the calculation of **ATP-linked OCR** or the OCR that contributes to ATP production. Non-mitochondrial OCR is subtracted from this value to represent only mitochondrial OCR as described in Figure 1.

Maximal OCR:

Administration of the protonophore FCCP abolishes the inner mitochondrial membrane potential by transporting protons across the mitochondrial inner membrane, thus uncoupling electron transport by complexes I-IV from ATP synthesis at Complex V. Attenuation of the membrane potential “takes the brakes off respiration” leading to an increase in OCR to the maximal capability of the respiratory chain. Decreases in maximal OCR can be indicative of respiratory chain damage or limitations in substrate availability. The subtraction of basal respiration rate from maximal respiration rate yields the **Reserve Capacity** of the cell which represents the extent to which the cell can increase mitochondrial respiration to respond to bioenergetic stress.

Non-mitochondrial OCR:

Administration of rotenone or the combination of rotenone and antimycin A completely inhibits the electron transport chain, and thus inhibits OCR due to mitochondrial respiration. The remaining OCR after administration of these compounds represents oxygen consumption due to cellular processes other than mitochondrial respiration and can include the activity of the enzymes lipoxygenase, cyclooxygenase, xanthine oxidase, or NADPH oxidases.

Notably, in addition to modulators used in the basic MST, a host of other substrates and inhibitors can be separately administered in the assay to probe specific aspects of energetics.

For example, to investigate substrate utility, palmitate and etomoxir, a substrate for fatty acid oxidation and inhibitor of fatty acid transport respectively, can be used to quantify the proportion of total respiration due to fatty acid oxidation. Similarly, 2-deoxyglucose is utilized to inhibit glycolysis and investigate glucose oxidation. Further, protocols in which cells are permeabilized and then substrates specific to each complex are administered allows for measurement of individual electron transport complex activity in the Seahorse XF analyzer (124). These protocols have successfully been utilized in healthy human platelets to quantify the utilization of glucose versus fatty acids for respiration (125, 126) as well as to measure individual complex activities (113).

Defining Baseline Physiological Blood Cell Bioenergetics

The establishment of methodology to precisely measure bioenergetics in blood cells raises a number of questions that are necessary to answer in order to utilize this methodology as potential translational research or clinical tool. Specifically, it is important to determine how bioenergetic data should be analyzed, what the characteristics are of a healthy blood cell bioenergetic profile, and how much variability exists (and what factors contribute to this variability) in the same person over time and within human populations. Ongoing studies from a number of labs have begun to answer these questions.

With respect to defining the characteristics of a healthy human bioenergetic profile, the first consideration is which circulating cell type is being utilized. Due to the variable number of mitochondria contained in each blood cell type and differential expression of mitochondrial proteins to support the specialization of the function of individual blood cell types, these cells have differential bioenergetic profiles at baseline. A comparison of bioenergetic profile in different blood cell types from the same individuals showed that monocytes, lymphocytes, and platelets have a strong response to the mitochondrial modulators utilized in the MST, while neutrophils have little change in OCR with these modulators, suggesting a minimal role for oxidative phosphorylation in neutrophils (105). Further, distinct patterns of OCR components were observed for monocytes, lymphocytes and platelets. For example, platelets have a high rate of ATP-linked respiration, with a low reserve capacity and proton leak, while lymphocytes have significantly higher proton leak with less ATP-linked respiration (105). These data demonstrate that comparisons between bioenergetic datasets should only be made using the same cell type. Additionally, the use of different cell types may provide distinctly different datasets in a given system. This is demonstrated in a study by Tyrrell and colleagues in which bioenergetics were measured in monocytes and platelets isolated from non-human primates and compared to bioenergetics of skeletal muscle fibers from the same animals (127). In this study, basal OCR in monocytes showed a significant correlation with basal OCR of the skeletal muscle fibers. While platelet basal OCR did not show such a correlation with skeletal muscle, platelet maximal OCR did correlate with the reserve capacity of the skeletal muscle (127). Similarly, comparison of the bioenergetic profiles in lymphocytes, platelets, and monocytes isolated from calcium oxalate kidney stone formers versus healthy subjects showed that maximal OCR was decreased in monocytes from stone formers compared to controls, while no significant change was observed in platelets and lymphocytes (128).

Once bioenergetics are measured, perhaps the most straightforward method of data analysis is a simple comparison of individual parameters of the bioenergetic profile. This is particularly useful if significant change is observed between two groups or treatments in one part of the profile. In order to integrate the parameters of the bioenergetic profile into a single value that provides a composite of overall mitochondrial function, Chacko and colleagues defined the Bioenergetic Health Index (BHI) (129, 130). This value is proportional to positive energetic parameters including reserve capacity and ATP-linked respiration, while inversely proportional to the non-ATP generating processes - non-mitochondrial respiration and proton leak (Equation 1) (129).

$$BHI = \log \frac{(reserve\ capacity) \times (ATP-linked)}{(non-mitochondrial) \times (proton\ leak)} \quad (\text{Equation 1})$$

According to this equation, the BHI corresponds to alterations in the electron transport chain or substrate supply and is directly proportional to the capacity for ATP production, while it is inversely proportional to inefficient oxygen consumption.(129). Consistent with this, oxidative damage to the respiratory chain decreases BHI (129, 130). Notably, the calculation of one index utilizing all the parameters measured can uncover differences in OCR that may not be apparent by simply comparing individual parameters of the bioenergetic profile. This was the case in a study of monocytes isolated from peripheral blood of postoperative cardiac surgery patients. In this study, while no single parameter of the bioenergetic profile was significantly different in monocytes isolated from the cardiac surgery patients post-operatively compared to healthy controls, the calculated BHI, which takes into account slight changes in each parameter, was observed to be lower than the BHI of monocytes from healthy control subjects (131). The advantages of the BHI are its ability to represent small changes in multiple parameters that may not otherwise appear significant if compared as an individual parameter, as well as the ease of comparing one integrated value. However, the major limitation to this analytical method is that as a stand alone value, the BHI does not provide information as to why bioenergetic health is altered. For example, a decrease in BHI may reflect an increase in either proton leak or non-mitochondrial OCR. These are two distinctly different parameters, and an increase in one versus the other has different mechanistic and downstream implications for mitochondrial function. Proton leak can attenuate mitochondrial oxidant production, while increased non-mitochondrial OCR is often due to oxygen consumption by oxidant producing enzymes such as xanthine oxidase. Thus, while comparison of BHI is useful, it is also important to consider it in the context of analysis of the individual parameters of the bioenergetic profile.

To further understand the parameters of the bioenergetic profile, Chacko and colleagues interrogated the relationship between these parameters in healthy platelets from 85 human donors (113). Multivariate analysis of six bioenergetic parameters demonstrated a wide range of variation in absolute OCR between subjects though particular relationships between bioenergetic measures were maintained in the population. For example, basal and maximal OCR values for individual subjects ranged between approximately 50–150 and 50–250 pmol O₂/min respectively. However, a strong correlation existed between basal OCR and Maximal OCR (113), suggesting these parameters are potentially regulated by a similar mechanism in

healthy subjects. Notably, this relationship between basal and maximal OCR did not exist in platelets isolated from patients with sickle cell disease, a pathology in which we have previously reported that platelets lack ATP Synthase activity (113, 122). This suggests that pathogenic modifiers can disrupt processes which mechanistically link parameters of bioenergetics. Further, this study utilized high-resolution metabolomics to quantify metabolites in platelets from a subset of the healthy cohort. The integrated analysis showed significant relationships between the basal metabolome and the six bioenergetic parameters (113). Taken together these data define healthy platelet bioenergetic relationships and demonstrate their relation to metabolism, and on a practical level suggest that this type of integrated analysis is powerful in more fully understanding changes in blood cell bioenergetic function and its relationship to altered metabolism.

While several studies have shown variability in bioenergetic parameters among healthy individuals (74, 87, 113, 122, 125, 126, 132–134), the factors that contribute to this variability are just starting to be elucidated. It is well established that mitochondrial function is influenced by both genetics and environmental stimuli. Further, mitochondrial studies in solid organs show changes in function with age (132–134), race (113), body mass index (74, 135), and gender (136, 137), and thus it is likely that these elements influence blood cell bioenergetics as well. Recent studies have started to define changes in blood cell bioenergetic profile in different natural populations (74, 113, 132–134). For example, work from our group has shown that platelets from older adults (85–93 years old) show decreased ATP-linked OCR and increased proton leak compared to younger adults (18–35 years old), and this increased proton leak is associated with an upregulation of uncoupling protein 2 (132). However, while significant differences were shown between two extreme age groups, it is yet to be determined whether these differences become significant once an age threshold is met or whether bioenergetic parameters are on a continuum of change throughout the entire lifespan. Similarly, Willig and colleagues demonstrated in a population of virologically-suppressed HIV-infected women that total body fat mass negatively correlated with the BHI (135). However, it remains to be tested whether this relationship exists in healthy cohorts. Until the exact influence of demographic characteristics on blood cell bioenergetics is elucidated, current studies consider these variables by recruitment of experimental groups with similar demographics or by performing partial correlations that take these variables into account (132–134).

Though variability exists between individuals, data from our lab suggests that platelet bioenergetic profile remains stable in the same healthy person over time. In a pilot study of twelve healthy subjects, fasting blood was drawn at several time points over the course of three months, from which platelets were isolated and subjected to extracellular flux analysis. Though absolute rates of respiration differed among individuals, bioenergetic profile in the same individual remained stable over time (Figure 2). These data demonstrate the reproducibility of the extracellular flux methodology. Further, while longer-term studies need to be performed, the stability of the bioenergetic profile in a healthy individual over time suggests that it is feasible to utilize blood cell bioenergetics for temporal studies that potentially measure disease progression or therapeutic efficacy. In this regard, studies have utilized the ability to draw multiple samples from the same subject over time to examine temporal changes in blood cell bioenergetics with pathogenesis (138) or treatment (139).

The studies highlighted herein begin to define characteristics of a healthy blood cell bioenergetic profile, demonstrate levels of variability in populations and establish methodology for data analysis and interpretation of bioenergetics in circulating cells. In the next sections we discuss the potential to utilize blood cell bioenergetics, particularly in non-healthy populations, as a tool for translational medicine and ultimately in the clinic for personalized medicine.

Are blood cell bioenergetics altered in disease?

As noted above, bioenergetic alterations are associated with the pathogenesis of most major diseases across all organ systems and accumulating data suggests that these mitochondrial changes may be systemic rather than limited to the primary organ of pathology. Thus, an obvious question is whether blood cell bioenergetics can serve as a biomarker of disease. To this end, several controlled studies now demonstrate altered blood cell bioenergetics in a number of cohorts ranging from acute pathologies such as pulmonary embolism (140) and sepsis (138, 141) to chronic diseases such as asthma and Parkinson's disease (142) (Figure 3). For instance, Sack and colleagues showed that platelets from individuals with Type 2 Diabetes display decreased basal and ATP-linked OCR, which was associated with increased markers of oxidative stress (143). Separately, in a study comparing bioenergetics in monocytes isolated from patients with porphyria, proton leak and maximal respiration were shown to be significantly lower in porphyria patients versus healthy controls. Further, these parameters were even lower in monocytes from active porphyria patients compared to those in remission (144).

While Type 2 Diabetes and porphyria both have a metabolic component, altered blood cell bioenergetics have also been shown in non-metabolic diseases and in some cases, these studies have also elucidated novel mechanisms that potentially play a role in disease progression. For example, our group demonstrated a change in bioenergetics in platelets isolated from patients with SCD, a disease in which mitochondrial function had previously not been assessed in any organ system. Utilizing Extracellular Flux analysis to screen the bioenergetic profile of 24 SCD patients and compare to healthy African American individuals, we uncovered that platelets from SCD patients showed attenuated basal OCR and proton leak, with no change in maximal OCR. Further investigation of this phenomenon demonstrated that this change in bioenergetic profile was due to the inhibition of platelet complex V activity which increased Ψ resulting in the enhanced generation of mtROS, which stimulated thrombotic activation of the platelet (122). Further, inhibition of complex V in the SCD subjects was associated with the release of hemoglobin from erythrocytes during hemolysis. Thus, these data for the first time showed that altered platelet mitochondrial function may contribute to the mechanism underlying hemolysis-associated thrombosis, which has been reported in SCD and other hemolytic diseases (122, 145).

The ability to concomitantly measure ECAR with OCR by extracellular flux analysis provides additional information to distinguish bioenergetic changes in different populations. For example, in addition to increased mitochondrial respiratory rate, platelets from lean asthmatic individuals show a significantly lower basal glycolytic rate than age and race matched healthy controls (74, 87). In contrast, platelets from patients with pulmonary

arterial hypertension (PAH) also show an increase in maximal OCR due to enhanced fatty acid oxidation, but with a concomitant increase in basal glycolytic rate (126). Further, individuals with Group 2 pulmonary hypertension also show an increase in maximal OCR, but with no significant change in glycolytic rate compared to healthy controls (125). These data demonstrate the utility of E/CAR measurements to supplement OCR profile and also highlight the ability to investigate the mechanisms by which glycolysis and mitochondrial oxidative phosphorylation are regulated.

Taken together these studies demonstrate that circulating blood cell bioenergetics are altered in a number of pathologies and can also serve as a useful tool to potentially uncover pathogenic mechanisms. As bioenergetic data from different pathologies accumulate, the specificity of particular patterns of alteration to individual diseases can be assessed to potentially develop this tool into a minimally invasive diagnostic test.

Can blood cell bioenergetics be used as a surrogate for other cell types?

While it may be useful to assess blood cell bioenergetics as a biomarker of disease, a relevant question that arises is whether these circulating cell measurements provide information about bioenergetics in solid tissues. Many studies now demonstrate that peripheral blood cells can indeed reflect bioenergetics in solid tissues both at basal conditions and during pathogenesis (Table 1). Classically, muscle biopsies are utilized and have been considered the gold standard for human mitochondrial measurements. To that end, Molina and colleagues showed in non-human primates that monocyte and platelet bioenergetics reflect skeletal muscle bioenergetics measured in the same animal (127). Specifically, monocyte basal OCR, maximal OCR, and reserve capacity showed a significant correlation with complex I and II linked OXPHOS in permeabilized skeletal muscle fibers, while platelet maximal OCR significantly correlated with maximal respiration in skeletal muscle fibers. Our group confirmed these results in older human subjects by demonstrating that platelet maximal OCR and proton leak correlated significantly with similar parameters in skeletal muscle fibers of the same individuals (132). Further, in these subjects, ATP-linked OCR showed a significant positive correlation with muscle ATP generation measured non-invasively by ^{31}P -MRS (Figure 4).

Peripheral blood cells mirror bioenergetics in tissues beyond the skeletal muscle as well. In non-human primates, platelet and monocyte basal and maximal OCR correlate significantly with the respiratory control ratio of isolated mitochondria from the heart (127). Additionally, monocyte maximal respiration has been shown to reflect maximal respiration of mitochondria isolated from the frontal cortex of the brain (110, 146). In a subset of non-human primates in this study, Tyrrell and colleagues utilized ^{18}F -fluorodeoxyglucose PET imaging to non-invasively measure glucose metabolism in various brain regions. Platelet maximal OCR and monocyte BHI was shown to significantly associate with glucose metabolism in various areas of the brain.

Beyond highly metabolic organs such as the brain and heart, a study by our group isolated primary airway epithelial cells and platelets from the same cohorts of lean and obese healthy and asthmatic individuals. Measurement of bioenergetics in these cohorts showed that

platelet bioenergetics reflect mitochondrial basal and maximal respiration as well as the basal glycolytic rate of airway epithelial cells in the same individuals (74). Prior studies have shown that due to increases in whole blood arginine metabolism, glycolytic rate is decreased and mitochondrial respiration increased in asthmatic individuals (75). We confirmed this effect in the airway epithelial cells from our cohort and further showed that platelets reflect this pathogenic change as well (74).

Taken together, these studies demonstrate that specific parameters of monocyte and platelet bioenergetics reflect mitochondrial OXPHOS and glycolytic metabolism in a variety of tissue types. At first consideration, this may appear counterintuitive to the current dogma that mitochondria in individual tissue types are highly specialized to support the energetic and signaling demands of that tissue. However, it is important to note that although strong correlations exist between cell types, the absolute values of OCR can differ dramatically between different tissues based on the energetic requirements of that tissue. For example brain mitochondrial maximal capacity was two-fold higher than that of monocytes (110, 146). Additionally, some parameters of the blood cell bioenergetic profile were more strongly correlated with other cell types suggesting that the individual parameters of the bioenergetic profile may be differentially regulated in each tissue type. These differences potentially contribute to specialization of mitochondria in different cell types while maintaining similarities systemically.

The mechanisms that account for the similarity in bioenergetics between cell types remain unclear and are likely regulated by a combination of genetic, environmental, and circulating factors. However, while these mechanisms are being elucidated, accumulating evidence suggests that peripheral blood cells can be used as a surrogate for other cell types or tissues to assess systemic mitochondrial health. Importantly, this enables measurement from a single blood draw to serve as an alternative to the more invasive tissue biopsy and can potentially act as a proxy for more expensive and complicated non-invasive methodology such as FDG-PET and ³¹P-MRS.

Do circulating cell bioenergetics relate to clinical parameters?

The fact that circulating cell bioenergetics show alterations with pathogenesis and reflect other organ systems begs the question as to whether circulating cell bioenergetics are associated with physiological or clinical parameters. Identifying relationships between blood cell bioenergetics and clinical parameters would pave the way to utilize blood-based bioenergetics as a relatively simple diagnostic or prognostic indicator. A number of studies have now tested this concept both in healthy subjects as well as in pathology, and show correlations between circulating bioenergetics and physical or clinical parameters.

In a study of overweight/obese well-functioning older adults (~mean age 69), Tyrrell and colleagues showed that maximal respiration of PBMCs isolated from these subjects positively correlated with measures of strength including knee extensor strength, grip strength and performance in a short physical function battery (134). In a similar study, gait speed in this population correlated significantly with PBMC maximal respiration as well (133). Interestingly, in this study, the correlation between gait speed and PBMC or skeletal

muscle respiration was equally strong (133). In a separate study, our group demonstrated in older adults (86–93 years old) that higher platelet proton leak was associated with faster gait speed and that platelet basal glycolytic rate showed a significant positive correlation with physical fatigability score (132). These data are compelling in that in older adults, gait speed and other measures of physical function are strong predictors of morbidity and mortality. These data suggest that blood cell bioenergetics may also have some predictive value in this regard, particularly in the aging population.

Numerous studies now provide evidence that the degree of bioenergetic dysfunction in peripheral blood cells can also be associated with tissue damage or disease severity. For example, in a cohort of twenty individuals admitted to the critical care unit with septic shock, decreased ADP-dependent mitochondrial respiration in PBMCs was associated with the Sequential Organ Failure Assessment (SOFA) Score, which numerically quantifies the severity of morbidity (147). In a separate study, PBMC respirometric profiles in African Americans with Type 2 diabetes revealed that basal and maximal OCR of PBMC positively correlated with total brain intracranial volume, a parameter related to brain atrophy, and further, basal OCR correlated with cognitive assessment scores (148). Our lab has shown that in subjects with Pulmonary Arterial Hypertension (PAH), platelet reserve capacity is significantly positively correlated with mean pulmonary artery pressure (mPAP), the diagnostic indicator of PAH, and right ventricular stroke work index (RV-SWI), a strong prognostic marker for the disease (126). Interestingly, in pulmonary hypertension patients with heart failure with preserved ejection fraction (PH-HFpEF), platelet reserve capacity was negatively correlated with RV-SWI (125) suggesting mechanistic differences that are currently being elucidated between the pathogenesis of heart failure in different classes of patients with pulmonary hypertension. In a separate study of patients with heart failure, high-intensity interval training (HIIT) enhanced platelet bioenergetic parameters and BHI, and BHI correlated positively with an increase in left ventricular ejection fraction (LVEF) and peak oxygen consumption ($VO_{2\text{ peak}}$) (149).

Summary and considerations for the use of blood cell bioenergetics as a translational and clinical tool

Herein we have reviewed the mounting evidence supporting the use of blood cell bioenergetics as a biomarker of disease as well as a tool to understand mitochondrial mechanisms. Recent advancements in methodology, optimization of data analysis, and application to clinical cohorts have propelled the field forward, but several hurdles must still be cleared prior to the implementation of this methodology as a mainstream translational research and potential clinical tool. Currently, extracellular flux analysis (Seahorse XF) provides an efficient platform for the high throughput screening of bioenergetics in peripheral blood cells. Multiple methods for the analysis of data have provided critical information on the components and characteristics of the bioenergetic profile of healthy individuals and its relation to the metabolome. These studies have also characterized variability in the human population, though the factors that influence this variability are not completely clear. While recent studies have begun to define the influence of the extremes of body mass index (74, 135) and age (132–134) on blood cell bioenergetics, more extensive

studies are required to fully determine the effect of these factors across a continuum, as well as examine the effects of other demographic variables including race, ethnicity, and gender.

Ongoing studies in the field utilize different circulating cells with monocytes, platelets, or the mixed population of PBMCs being most popular. However, all peripheral blood cells do not have the same bioenergetic profile. Understanding bioenergetic screens and potentially translating them for clinical utility will require knowledge of the best cell type with which to interrogate a particular disease process. For instance, monocytes and lymphocytes have higher levels of cytochrome c oxidase as well as more reserve bioenergetic capacity than platelets under basal conditions (106), which may make them more sensitive to complex IV defects, and thus an appropriate cell choice for diseases in which complex IV is compromised. Further, specific bioenergetic parameters of different circulating cell types were shown to correlate differently with bioenergetics of other tissue types (heart, brain, skeletal muscle, lung). Thus, it may be appropriate to focus on different circulating cells depending on the primary organ of pathology that is being investigated. Additionally, the rate of turnover of the peripheral blood cells of interest may also play a critical role in defining the bioenergetic profile in both health and disease. Each type of blood cell has a different lifespan in the circulation, with platelets living for 8 to 9 days (150), neutrophils for 5.4 to 6.3 days (151, 152), monocytes for 1.6 to 7 days (153) and lymphocytes circulating for a week to months (154). In a healthy individual, production of new blood cells and their removal from circulation is a continuous process, and thus the bioenergetic profile likely portrays the average of a mixed population of cells that have been in circulation for varying periods of time (155). However, this may be altered in pathological conditions, such as HIV infection or coronary artery disease, where there is increased turnover of monocytes and lymphocytes or platelets respectively (156–159). In these conditions, if one of these rapid turnover cell types is chosen as a marker, differences in bioenergetics between healthy individuals and those with pathology may purely be a reflection of the measurement of cells of different ages in the circulation. Likewise, in longitudinal studies, it is important to consider timing intervals of blood sampling that allow complete turnover of the cell type of interest between time points. As data accumulate from multiple groups in this arena, cell types that are appropriate in different conditions will emerge. These studies will also ideally address whether relevant information is most accurately obtained from one pure cell type (monocyte or platelet), a mixed population (PBMCs) that reports on the average of different cell types, or a panel of cells.

Cross-sectional studies demonstrating differences in bioenergetics between healthy individuals and those with pathology, as well as strong correlations between blood cell bioenergetics and physical function or clinical parameters show promise for the potential diagnostic and prognostic value of this tool. However, longer-term longitudinal studies are now required to better assess the change in blood-based bioenergetics with pathogenesis along with its predictive value. While more recent studies have tested the effect of interventions such as HIIT (149) and remote ischemic conditioning (160) on platelet bioenergetic profile, more extensive studies will be required to define blood cell bioenergetic alterations caused by common therapeutic agents.

Despite several challenges, peripheral blood cells are gaining popularity as an attractive tool for understanding human systemic mitochondrial and cellular bioenergetic changes in both health and disease. As data from a growing number of labs accumulates, we anticipate that the field will overcome the hurdles outlined above. As a translational tool, blood cell bioenergetics provide insight into mitochondrial mechanisms of human pathogenesis and enables the acquisition of human bioenergetic information in a relatively less invasive manner than muscle biopsies or skin grafts and more easily than FDG-PET or ^{13}P -MRS. This is crucial for clinical studies of large cohorts or for longitudinal studies with multiple time points. Once these studies define the standard bioenergetic parameters and values different for healthy and disease populations, measurement of circulating cell bioenergetics can potentially be translated to the clinic as a personalized medicine tool to assess disease susceptibility in healthy individuals and to determine diagnosis and prognosis in disease. In summary, blood-based bioenergetics has emerged as a powerful tool that enables the study of human mitochondrial function and may have a great impact not only on translational and mechanistic research but also in clinical practice.

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REFERENCES

1. Burke PJ. Mitochondria, Bioenergetics and Apoptosis in Cancer. *Trends Cancer*. 2017 12;3(12):857–70. [PubMed: 29198441]
2. Chandel NS. Mitochondria as signaling organelles. *BMC Biol*. 2014 5 27;12:34. [PubMed: 24884669]
3. Granatiero V, De Stefani D, Rizzuto R. Mitochondrial Calcium Handling in Physiology and Disease. *Adv Exp Med Biol*. 2017;982:25–47. [PubMed: 28551780]
4. Orkin SH, Zon LI. Hematopoiesis: an evolving paradigm for stem cell biology. *Cell*. 2008 22;132(4):631–44. [PubMed: 18295580]
5. Takubo K, Nagamatsu G, Kobayashi CI, Nakamura-Ishizu A, Kobayashi H, Ikeda E, et al. Regulation of glycolysis by Pdk functions as a metabolic checkpoint for cell cycle quiescence in hematopoietic stem cells. *Cell Stem Cell*. 2013 1 3;12(1):49–61. [PubMed: 23290136]
6. Suda T, Takubo K, Semenza GL. Metabolic regulation of hematopoietic stem cells in the hypoxic niche. *Cell Stem Cell*. 2011 10 4;9(4):298–310. [PubMed: 21982230]
7. Shyh-Chang N, Daley GQ, Cantley LC. Stem cell metabolism in tissue development and aging. *Development*. 2013 6;140(12):2535–47. [PubMed: 23715547]
8. Klimmeck D, Hansson J, Raffel S, Vakhrushev SY, Trumpp A, Krijgsvelde J. Proteomic cornerstones of hematopoietic stem cell differentiation: distinct signatures of multipotent progenitors and myeloid committed cells. *Mol Cell Proteomics*. 2012 8;11(8):286–302. [PubMed: 22454540]
9. Yu WM, Liu X, Shen J, Jovanovic O, Pohl EE, Gerson SL, et al. Metabolic regulation by the mitochondrial phosphatase PTPMT1 is required for hematopoietic stem cell differentiation. *Cell Stem Cell*. 2013 1 3;12(1):62–74. [PubMed: 23290137]
10. Tothova Z, Kollipara R, Huntly BJ, Lee BH, Castrillon DH, Cullen DE, et al. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. *Cell*. 2007 1 26;128(2):325–39. [PubMed: 17254970]
11. Xu X, Duan S, Yi F, Ocampo A, Liu GH, Izpisua Belmonte JC. Mitochondrial regulation in pluripotent stem cells. *Cell Metab*. 2013 9 3;18(3):325–32. [PubMed: 23850316]
12. Wanet A, Arnould T, Najimi M, Renard P. Connecting Mitochondria, Metabolism, and Stem Cell Fate. *Stem Cells Dev*. 2015 9 1;24(17):1957–71. [PubMed: 26134242]

13. Qian P, He XC, Paulson A, Li Z, Tao F, Perry JM, et al. The Dlk1-Gtl2 Locus Preserves LT-HSC Function by Inhibiting the PI3K-mTOR Pathway to Restrict Mitochondrial Metabolism. *Cell Stem Cell*. 2016 2 4;18(2):214–28. [PubMed: 26627594]
14. Romero-Moya D, Bueno C, Montes R, Navarro-Montero O, Iborra FJ, Lopez LC, et al. Cord blood-derived CD34+ hematopoietic cells with low mitochondrial mass are enriched in hematopoietic repopulating stem cell function. *Haematologica*. 2013 7;98(7):1022–9. [PubMed: 23349299]
15. Zhang J, Wu K, Xiao X, Liao J, Hu Q, Chen H, et al. Autophagy as a regulatory component of erythropoiesis. *Int J Mol Sci*. 2015 2 13;16(2):4083–94. [PubMed: 25689426]
16. Garcia-Souza LF, Oliveira MF. Mitochondria: biological roles in platelet physiology and pathology. *Int J Biochem Cell Biol*. 2014 5;50:156–60. [PubMed: 24569121]
17. Mehta MM, Weinberg SE, Chandel NS. Mitochondrial control of immunity: beyond ATP. *Nat Rev Immunol*. 2017 10;17(10):608–20. [PubMed: 28669986]
18. Sandoval H, Kodali S, Wang J. Regulation of B cell fate, survival, and function by mitochondria and autophagy. *Mitochondrion*. 2018 7;41:58–65. [PubMed: 29175010]
19. Caccese D, Pratico D, Ghiselli A, Natoli S, Pignatelli P, Sanguigni V, et al. Superoxide anion and hydroxyl radical release by collagen-induced platelet aggregation--role of arachidonic acid metabolism. *Thromb Haemost*. 2000 3;83(3):485–90. [PubMed: 10744158]
20. Pignatelli P, Pulcinelli FM, Lenti L, Gazzaniga PP, Violi F. Hydrogen peroxide is involved in collagen-induced platelet activation. *Blood*. 1998 1 15;91(2):484–90. [PubMed: 9427701]
21. Khare A, Raundhal M, Chakraborty K, Das S, Corey C, Kamga CK, et al. Mitochondrial H2O2 in Lung Antigen-Presenting Cells Blocks NF-kappaB Activation to Prevent Unwarranted Immune Activation. *Cell Rep*. 2016 5 24;15(8):1700–14. [PubMed: 27184852]
22. Romero MM, Basile JJ, Corra Feo L, Lopez B, Ritacco V, Aleman M. Reactive oxygen species production by human dendritic cells involves TLR2 and dectin-1 and is essential for efficient immune response against Mycobacteria. *Cell Microbiol*. 2016 6;18(6):875–86. [PubMed: 26709456]
23. Chung J, Yoon YO, Lee JS, Ha TK, Ryu SM, Kim KH, et al. Inulin induces dendritic cells apoptosis through the caspase-dependent pathway and mitochondrial dysfunction. *Biol Pharm Bull*. 2011;34(4):495–500. [PubMed: 21467635]
24. Waters LR, Ahsan FM, Wolf DM, Shirihai O, Teitell MA. Initial B Cell Activation Induces Metabolic Reprogramming and Mitochondrial Remodeling. *iScience*. 2018 7 27;5:99–109. [PubMed: 30240649]
25. Cowdry EV. The vital staining of mitochondria with Janus green and dietiylsafranin in human blood cells. . *Internat Monats f Anat u Physiol, Bd*. 1915;31(S).
26. Kropp BM R A study of mitochondria in white blood cells following gaseous inhalation. *The Anatomical Record*. 1924;27(5).
27. Fukami MH, Salganicoff L. Isolation and properties of human platelet mitochondria. *Blood*. 1973 12;42(6):913–8. [PubMed: 4759917]
28. Siegel A, Burri PH, Weibel ER, Bettex-Galland M, Luscher EF. Density gradient centrifugation and electron microscopic characterization of subcellular fractions from human blood platelets. *Thromb Diath Haemorrh*. 1971 6 30;25(2):252–67. [PubMed: 5568046]
29. Montagna P, Sacquegna T, Martinelli P, Cortelli P, Bresolin N, Moggio M, et al. Mitochondrial abnormalities in migraine. Preliminary findings. *Headache*. 1988 8;28(7):477–80. [PubMed: 3243709]
30. Parker WD Jr., Boyson SJ, Parks JK. Abnormalities of the electron transport chain in idiopathic Parkinson's disease. *Ann Neurol*. 1989 12;26(6):719–23. [PubMed: 2557792]
31. Shi C, Guo K, Yew DT, Yao Z, Forster EL, Wang H, et al. Effects of ageing and Alzheimer's disease on mitochondrial function of human platelets. *Exp Gerontol*. 2008 6;43(6):589–94. [PubMed: 18359596]
32. Xu J, Shi C, Li Q, Wu J, Forster EL, Yew DT. Mitochondrial dysfunction in platelets and hippocampi of senescence-accelerated mice. *J Bioenerg Biomembr*. 2007 4;39(2):195–202. [PubMed: 17436064]

33. Mitochondrial Medicine Society's Committee on D, Haas RH, Parikh S, Falk MJ, Saneto RP, Wolf NI, et al. The in-depth evaluation of suspected mitochondrial disease. *Mol Genet Metab*. 2008 5;94(1):16–37. [PubMed: 18243024]
34. Garcia I, Jones E, Ramos M, Innis-Whitehouse W, Gilkerson R. The little big genome: the organization of mitochondrial DNA. *Front Biosci (Landmark Ed)*. 2017 1 1;22:710–21. [PubMed: 27814641]
35. Taanman JW. The mitochondrial genome: structure, transcription, translation and replication. *Biochim Biophys Acta*. 1999 2 9;1410(2):103–23. [PubMed: 10076021]
36. Cohen BH, Gold DR. Mitochondrial cytopathy in adults: what we know so far. *Cleve Clin J Med*. 2001 7;68(7):625–6, 9–42. [PubMed: 11453081]
37. Pieczenik SR, Neustadt J. Mitochondrial dysfunction and molecular pathways of disease. *Exp Mol Pathol*. 2007 8;83(1):84–92. [PubMed: 17239370]
38. Wallace DC. Mitochondrial DNA mutations in disease and aging. *Environ Mol Mutagen*. 2010 6;51(5):440–50. [PubMed: 20544884]
39. Wallace DC. Mitochondrial DNA mutations in diseases of energy metabolism. *J Bioenerg Biomembr*. 1994 6;26(3):241–50. [PubMed: 8077179]
40. Leigh Finsterer J. and Leigh-like syndrome in children and adults. *Pediatr Neurol*. 2008 10;39(4):223–35. [PubMed: 18805359]
41. Mitochondriopathies Finsterer J.. *Eur J Neurol*. 2004 3;11(3):163–86. [PubMed: 15009163]
42. Stork C, Renshaw PF. Mitochondrial dysfunction in bipolar disorder: evidence from magnetic resonance spectroscopy research. *Mol Psychiatry*. 2005 10;10(10):900–19. [PubMed: 16027739]
43. Nierenberg AA, Kansky C, Brennan BP, Shelton RC, Perlis R, Iosifescu DV. Mitochondrial modulators for bipolar disorder: a pathophysiologically informed paradigm for new drug development. *Aust N Z J Psychiatry*. 2013 1;47(1):26–42. [PubMed: 22711881]
44. Krzywanski DM, Moellering DR, Fetterman JL, Dunham-Snary KJ, Sammy MJ, Ballinger SW. The mitochondrial paradigm for cardiovascular disease susceptibility and cellular function: a complementary concept to Mendelian genetics. *Lab Invest*. 2011 8;91(8):1122–35. [PubMed: 21647091]
45. Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet*. 2005;39:359–407. [PubMed: 16285865]
46. Fosslien E Mitochondrial medicine--molecular pathology of defective oxidative phosphorylation. *Ann Clin Lab Sci*. 2001 1;31(1):25–67. [PubMed: 11314862]
47. Kaufman BA, Li C, Soleimanpour SA. Mitochondrial regulation of beta-cell function: maintaining the momentum for insulin release. *Mol Aspects Med*. 2015 4;42:91–104. [PubMed: 25659350]
48. Dolle C, Flones I, Nido GS, Miletic H, Osuagwu N, Kristoffersen S, et al. Defective mitochondrial DNA homeostasis in the substantia nigra in Parkinson disease. *Nat Commun*. 2016 11 22;7:13548. [PubMed: 27874000]
49. Lin MT, Cantuti-Castelvetri I, Zheng K, Jackson KE, Tan YB, Arzberger T, et al. Somatic mitochondrial DNA mutations in early Parkinson and incidental Lewy body disease. *Ann Neurol*. 2012 6;71(6):850–4. [PubMed: 22718549]
50. Coxhead J, Kurzawa-Akanbi M, Hussain R, Pyle A, Chinnery P, Hudson G. Somatic mtDNA variation is an important component of Parkinson's disease. *Neurobiol Aging*. 2016 2;38:217 e1–e6.
51. Beadnell TC, Scheid AD, Vivian CJ, Welch DR. Roles of the mitochondrial genetics in cancer metastasis: not to be ignored any longer. *Cancer Metastasis Rev*. 2018 12;37(4):615–32. [PubMed: 30542781]
52. Chen EI. Mitochondrial dysfunction and cancer metastasis. *J Bioenerg Biomembr*. 2012 12;44(6):619–22. [PubMed: 22892817]
53. Zifa E, Daniil Z, Skoumi E, Stavrou M, Papadimitriou K, Terzenidou M, et al. Mitochondrial genetic background plays a role in increasing risk to asthma. *Mol Biol Rep*. 2012 4;39(4):4697–708. [PubMed: 21947849]
54. van der Walt JM, Nicodemus KK, Martin ER, Scott WK, Nance MA, Watts RL, et al. Mitochondrial polymorphisms significantly reduce the risk of Parkinson disease. *Am J Hum Genet*. 2003 4;72(4):804–11. [PubMed: 12618962]

55. van der Walt JM, Dementieva YA, Martin ER, Scott WK, Nicodemus KK, Kroner CC, et al. Analysis of European mitochondrial haplogroups with Alzheimer disease risk. *Neurosci Lett*. 2004 7 15;365(1):28–32. [PubMed: 15234467]
56. Serrano-Teruel ME, Garcia-Vieites M, Rego-Perez I, Domenech-Garcia N, Blanco-Garcia F, Cuenca-Castillo JJ, et al. Mitochondrial DNA haplogroups influence the risk of aortic stenosis. *Asian Cardiovasc Thorac Ann*. 2019 1;27(1):5–10. [PubMed: 30409026]
57. Umbria M, Ramos A, Caner J, Vega T, Lozano JE, Santos C, et al. Involvement of mitochondrial haplogroups in myocardial infarction and stroke: A case-control study in Castile and Leon (Spain) population. *Mitochondrion*. 2019 1;44:1–6. [PubMed: 29258787]
58. Cardena MM, Ribeiro-Dos-Santos AK, Santos SE, Mansur AJ, Bernardez-Pereira S, Santos PC, et al. Mitochondrial and genomic ancestry are associated with etiology of heart failure in Brazilian patients. *J Hum Hypertens*. 2016 2;30(2):120–3. [PubMed: 25947276]
59. De Benedictis G, Rose G, Carrieri G, De Luca M, Falcone E, Passarino G, et al. Mitochondrial DNA inherited variants are associated with successful aging and longevity in humans. *FASEB J*. 1999 9;13(12):1532–6. [PubMed: 10463944]
60. Rose G, Passarino G, Carrieri G, Altomare K, Greco V, Bertolini S, et al. Paradoxes in longevity: sequence analysis of mtDNA haplogroup J in centenarians. *Eur J Hum Genet*. 2001 9;9(9):701–7. [PubMed: 11571560]
61. Nishigaki Y, Fuku N, Tanaka M. Mitochondrial haplogroups associated with lifestyle-related diseases and longevity in the Japanese population. *Geriatr Gerontol Int*. 2010 7;10 Suppl 1:S221–35. [PubMed: 20590837]
62. Fetterman JL, Zelickson BR, Johnson LW, Moellering DR, Westbrook DG, Pompilius M, et al. Mitochondrial genetic background modulates bioenergetics and susceptibility to acute cardiac volume overload. *Biochem J*. 2013 10 15;455(2):157–67. [PubMed: 23924350]
63. Betancourt AM, King AL, Fetterman JL, Millender-Swain T, Finley RD, Oliva CR, et al. Mitochondrial-nuclear genome interactions in non-alcoholic fatty liver disease in mice. *Biochem J*. 2014 7 15;461(2):223–32. [PubMed: 24758559]
64. Dunham-Snary KJ, Sandel MW, Sammy MJ, Westbrook DG, Xiao R, McMonigle RJ, et al. Mitochondrial - nuclear genetic interaction modulates whole body metabolism, adiposity and gene expression in vivo. *EBioMedicine*. 2018 10;36:316–28. [PubMed: 30232024]
65. Marshall JD, Bazan I, Zhang Y, Fares WH, Lee PJ. Mitochondrial dysfunction and pulmonary hypertension: cause, effect, or both. *Am J Physiol Lung Cell Mol Physiol*. 2018 5 1;314(5):L782–L96. [PubMed: 29345195]
66. Prakash YS, Pabelick CM, Sieck GC. Mitochondrial Dysfunction in Airway Disease. *Chest*. 2017 9;152(3):618–26. [PubMed: 28336486]
67. Perez Ortiz JM, Swerdlow RH. Mitochondrial dysfunction in Alzheimer's disease: Role in pathogenesis and novel therapeutic opportunities. *Br J Pharmacol*. 2019 1 24.
68. Brown DA, Perry JB, Allen ME, Sabbah HN, Stauffer BL, Shaikh SR, et al. Expert consensus document: Mitochondrial function as a therapeutic target in heart failure. *Nat Rev Cardiol*. 2017 4;14(4):238–50. [PubMed: 28004807]
69. Zhou B, Tian R. Mitochondrial dysfunction in pathophysiology of heart failure. *J Clin Invest*. 2018 8 31;128(9):3716–26. [PubMed: 30124471]
70. Lopaschuk GD. Metabolic Modulators in Heart Disease: Past, Present, and Future. *Can J Cardiol*. 2017 7;33(7):838–49. [PubMed: 28279520]
71. Paulin R, Michelakis ED. The metabolic theory of pulmonary arterial hypertension. *Circ Res*. 2014 6 20;115(1):148–64. [PubMed: 24951764]
72. Culley MK, Chan SY. Mitochondrial metabolism in pulmonary hypertension: beyond mountains there are mountains. *J Clin Invest*. 2018 8 31;128(9):3704–15. [PubMed: 30080181]
73. Ryan J, Dasgupta A, Huston J, Chen KH, Archer SL. Mitochondrial dynamics in pulmonary arterial hypertension. *J Mol Med (Berl)*. 2015 3;93(3):229–42. [PubMed: 25672499]
74. Winnica D, Corey C, Mullett S, Reynolds M, Hill G, Wendell S, et al. Bioenergetic Differences in the Airway Epithelium of Lean Versus Obese Asthmatics Are Driven by Nitric Oxide and Reflected in Circulating Platelets. *Antioxid Redox Signal*. 2019 3 6.

75. Xu W, Ghosh S, Comhair SA, Asosingh K, Janocha AJ, Mavrikakis DA, et al. Increased mitochondrial arginine metabolism supports bioenergetics in asthma. *J Clin Invest*. 2016 7 1;126(7):2465–81. [PubMed: 27214549]
76. Trian T, Benard G, Begueret H, Rossignol R, Girodet PO, Ghosh D, et al. Bronchial smooth muscle remodeling involves calcium-dependent enhanced mitochondrial biogenesis in asthma. *J Exp Med*. 2007 12 24;204(13):3173–81. [PubMed: 18056286]
77. Haas RH, Nasirian F, Nakano K, Ward D, Pay M, Hill R, et al. Low platelet mitochondrial complex I and complex II/III activity in early untreated Parkinson's disease. *Ann Neurol*. 1995 6;37(6):714–22. [PubMed: 7778844]
78. Swerdlow RH, Parks JK, Miller SW, Tuttle JB, Trimmer PA, Sheehan JP, et al. Origin and functional consequences of the complex I defect in Parkinson's disease. *Ann Neurol*. 1996 10;40(4):663–71. [PubMed: 8871587]
79. Yu Q, Chan SY. Mitochondrial and Metabolic Drivers of Pulmonary Vascular Endothelial Dysfunction in Pulmonary Hypertension. *Adv Exp Med Biol*. 2017;967:373–83. [PubMed: 29047100]
80. Xu W, Koeck T, Lara AR, Neumann D, DiFilippo FP, Koo M, et al. Alterations of cellular bioenergetics in pulmonary artery endothelial cells. *Proc Natl Acad Sci U S A*. 2007 1 23;104(4):1342–7. [PubMed: 17227868]
81. Gomez-Arroyo J, Mizuno S, Szczepanek K, Van Tassell B, Natarajan R, dos Remedios CG, et al. Metabolic gene remodeling and mitochondrial dysfunction in failing right ventricular hypertrophy secondary to pulmonary arterial hypertension. *Circ Heart Fail*. 2013 1;6(1):136–44. [PubMed: 23152488]
82. Sutendra G, Dromparis P, Paulin R, Zervopoulos S, Haromy A, Nagendran J, et al. A metabolic remodeling in right ventricular hypertrophy is associated with decreased angiogenesis and a transition from a compensated to a decompensated state in pulmonary hypertension. *J Mol Med (Berl)*. 2013 11;91(11):1315–27. [PubMed: 23846254]
83. Rabinovitch M, Guignabert C, Humbert M, Nicolls MR. Inflammation and immunity in the pathogenesis of pulmonary arterial hypertension. *Circ Res*. 2014 6 20;115(1):165–75. [PubMed: 24951765]
84. Sutendra G, Michelakis ED. The metabolic basis of pulmonary arterial hypertension. *Cell Metab*. 2014 4 1;19(4):558–73. [PubMed: 24508506]
85. Malenfant S, Potus F, Fournier F, Breuils-Bonnet S, Pflieger A, Bourassa S, et al. Skeletal muscle proteomic signature and metabolic impairment in pulmonary hypertension. *J Mol Med (Berl)*. 2015 5;93(5):573–84. [PubMed: 25548805]
86. Batt J, Ahmed SS, Correa J, Bain A, Granton J. Skeletal muscle dysfunction in idiopathic pulmonary arterial hypertension. *Am J Respir Cell Mol Biol*. 2014 1;50(1):74–86. [PubMed: 23972212]
87. Xu W, Cardenes N, Corey C, Erzurum SC, Shiva S. Platelets from Asthmatic Individuals Show Less Reliance on Glycolysis. *PLoS One*. 2015;10(7):e0132007. [PubMed: 26147848]
88. Picado C, Deulofeu R, Lleona R, Agusti M, Casals E, Quinto L, et al. Lipid and protein metabolism in asthma. Effects of diet and corticosteroid therapy. *Allergy*. 1999 6;54(6):569–75. [PubMed: 10435470]
89. Joyce NC, Oskarsson B, Jin LW. Muscle biopsy evaluation in neuromuscular disorders. *Phys Med Rehabil Clin N Am*. 2012 8;23(3):609–31. [PubMed: 22938878]
90. Shanely RA, Zwetsloot KA, Triplett NT, Meaney MP, Farris GE, Nieman DC. Human skeletal muscle biopsy procedures using the modified Bergstrom technique. *J Vis Exp*. 2014 9 10(91):51812.
91. Kaur A, Midha S, Giri S, Mohanty S. Functional Skin Grafts: Where Biomaterials Meet Stem Cells. *Stem Cells Int*. 2019;2019:1286054. [PubMed: 31354835]
92. Hamaoka T, Iwane H, Shimomitsu T, Katsumura T, Murase N, Nishio S, et al. Noninvasive measures of oxidative metabolism on working human muscles by near-infrared spectroscopy. *J Appl Physiol* (1985). 1996 9;81(3):1410–7. [PubMed: 8889781]

93. Boushel R, Langberg H, Olesen J, Gonzales-Alonzo J, Bulow J, Kjaer M. Monitoring tissue oxygen availability with near infrared spectroscopy (NIRS) in health and disease. *Scand J Med Sci Sports*. 2001 8;11(4):213–22. [PubMed: 11476426]
94. Lanza IR, Nair KS. Mitochondrial metabolic function assessed in vivo and in vitro. *Curr Opin Clin Nutr Metab Care*. 2010 9;13(5):511–7. [PubMed: 20616711]
95. Quinn KP, Bellas E, Fourligas N, Lee K, Kaplan DL, Georgakoudi I. Characterization of metabolic changes associated with the functional development of 3D engineered tissues by non-invasive, dynamic measurement of individual cell redox ratios. *Biomaterials*. 2012 7;33(21):5341–8. [PubMed: 22560200]
96. Kolwicz SC Jr., Tian R. Assessment of cardiac function and energetics in isolated mouse hearts using ³¹P NMR spectroscopy. *J Vis Exp*. 2010 8 31(42).
97. Weiss K, Schar M, Panjath GS, Zhang Y, Sharma K, Bottomley PA, et al. Fatigability, Exercise Intolerance, and Abnormal Skeletal Muscle Energetics in Heart Failure. *Circ Heart Fail*. 2017 7;10(7).
98. Akki A, Gupta A, Weiss RG. Magnetic resonance imaging and spectroscopy of the murine cardiovascular system. *Am J Physiol Heart Circ Physiol*. 2013 3 1;304(5):H633–48. [PubMed: 23292717]
99. Valkovic L, Chmelik M, Krssak M. In-vivo(31)P-MRS of skeletal muscle and liver: A way for non-invasive assessment of their metabolism. *Anal Biochem*. 2017 7 15;529:193–215. [PubMed: 28119063]
100. Coen PM, Jubrias SA, Distefano G, Amati F, Mackey DC, Glynn NW, et al. Skeletal muscle mitochondrial energetics are associated with maximal aerobic capacity and walking speed in older adults. *J Gerontol A Biol Sci Med Sci*. 2013 4;68(4):447–55. [PubMed: 23051977]
101. Chen IY, Wu JC. Cardiovascular molecular imaging: focus on clinical translation. *Circulation*. 2011 2 1;123(4):425–43. [PubMed: 21282520]
102. Al Badarin FJ, Malhotra S. Diagnosis and Prognosis of Coronary Artery Disease with SPECT and PET. *Curr Cardiol Rep*. 2019 5 18;21(7):57. [PubMed: 31104158]
103. Aoyama R, Takano H, Kobayashi Y, Kitamura M, Asai K, Amano Y, et al. Evaluation of myocardial glucose metabolism in hypertrophic cardiomyopathy using 18F-fluorodeoxyglucose positron emission tomography. *PLoS One*. 2017;12(11):e0188479. [PubMed: 29176885]
104. Cochran BJ, Ryder WJ, Parmar A, Klaeser K, Reilhac A, Angelis GI, et al. Determining Glucose Metabolism Kinetics Using 18F-FDG Micro-PET/CT. *J Vis Exp*. 2017 5 2(123).
105. Chacko BK, Kramer PA, Ravi S, Johnson MS, Hardy RW, Ballinger SW, et al. Methods for defining distinct bioenergetic profiles in platelets, lymphocytes, monocytes, and neutrophils, and the oxidative burst from human blood. *Lab Invest*. 2013 6;93(6):690–700. [PubMed: 23528848]
106. Kramer PA, Ravi S, Chacko B, Johnson MS, Darley-Usmar VM. A review of the mitochondrial and glycolytic metabolism in human platelets and leukocytes: implications for their use as bioenergetic biomarkers. *Redox Biol*. 2014;2:206–10. [PubMed: 24494194]
107. Sangiorgi S, Mochi M, Riva R, Cortelli P, Monari L, Pierangeli G, et al. Abnormal platelet mitochondrial function in patients affected by migraine with and without aura. *Cephalalgia*. 1994 2;14(1):21–3. [PubMed: 8200018]
108. Parker WD Jr., Boyson SJ, Luder AS, Parks JK. Evidence for a defect in NADH: ubiquinone oxidoreductase (complex I) in Huntington's disease. *Neurology*. 1990 8;40(8):1231–4. [PubMed: 2143271]
109. Silva AC, Almeida S, Laco M, Duarte AI, Domingues J, Oliveira CR, et al. Mitochondrial respiratory chain complex activity and bioenergetic alterations in human platelets derived from pre-symptomatic and symptomatic Huntington's disease carriers. *Mitochondrion*. 2013 11;13(6):801–9. [PubMed: 23707479]
110. Cardoso SM, Proenca MT, Santos S, Santana I, Oliveira CR. Cytochrome c oxidase is decreased in Alzheimer's disease platelets. *Neurobiol Aging*. 2004 1;25(1):105–10. [PubMed: 14675736]
111. Ben-Shachar D, Zuk R, Gazawi H, Reshef A, Sheinkman A, Klein E. Increased mitochondrial complex I activity in platelets of schizophrenic patients. *Int J Neuropsychopharmacol*. 1999 12;2(4):245–53. [PubMed: 11285140]

112. Ben-Shachar D, Bonne O, Chisin R, Klein E, Lester H, Aharon-Peretz J, et al. Cerebral glucose utilization and platelet mitochondrial complex I activity in schizophrenia: A FDG-PET study. *Prog Neuropsychopharmacol Biol Psychiatry*. 2007 5 9;31(4):807–13. [PubMed: 17329000]
113. Chacko BK, Smith MR, Johnson MS, Benavides G, Culp ML, Pilli J, et al. Mitochondria in precision medicine; linking bioenergetics and metabolomics in platelets. *Redox Biol*. 2019 4;22:101165. [PubMed: 30877854]
114. Brookes PS, Shiva S, Patel RP, Darley-Usmar VM. Measurement of mitochondrial respiratory thresholds and the control of respiration by nitric oxide. *Methods Enzymol*. 2002;359:305–19. [PubMed: 12481582]
115. Silva AM, Oliveira PJ. Evaluation of Respiration with Clark-Type Electrode in Isolated Mitochondria and Permeabilized Animal Cells. *Methods Mol Biol*. 2018;1782:7–29. [PubMed: 29850992]
116. Pesta D, Gnaiger E. High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. *Methods Mol Biol*. 2012;810:25–58. [PubMed: 22057559]
117. Horan MP, Pichaud N, Ballard JW. Review: quantifying mitochondrial dysfunction in complex diseases of aging. *J Gerontol A Biol Sci Med Sci*. 2012 10;67(10):1022–35. [PubMed: 22459622]
118. E.G.. Polarographic oxygen sensors, the oxygraph, and high-resolution respirometry to assess mitochondrial function In: Dykens JA, YW, eds. *Drug-Induced Mitochondrial Dysfunction*: John Wiley & Sons, 2008:327–52.
119. Letellier T, Malgat M, Coquet M, Moretto B, Parrot-Roulaud F, Mazat JP. Mitochondrial myopathy studies on permeabilized muscle fibers. *Pediatr Res*. 1992 7;32(1):17–22. [PubMed: 1635840]
120. Kuznetsov AV, Wiedemann FR, Winkler K, Kunz WS. Use of saponin-permeabilized muscle fibers for the diagnosis of mitochondrial diseases. *Biofactors*. 1998;7(3):221–3. [PubMed: 9568252]
121. Mookerjee SA, Gerencser AA, Nicholls DG, Brand MD. Quantifying intracellular rates of glycolytic and oxidative ATP production and consumption using extracellular flux measurements. *J Biol Chem*. 2017 4 28;292(17):7189–207. [PubMed: 28270511]
122. Cardenes N, Corey C, Geary L, Jain S, Zharikov S, Barge S, et al. Platelet bioenergetic screen in sickle cell patients reveals mitochondrial complex V inhibition, which contributes to platelet activation. *Blood*. 2014 5 1;123(18):2864–72. [PubMed: 24677541]
123. Divakaruni AS, Paradyse A, Ferrick DA, Murphy AN, Jastroch M. Analysis and interpretation of microplate-based oxygen consumption and pH data. *Methods Enzymol*. 2014;547:309–54. [PubMed: 25416364]
124. Salabei JK, Gibb AA, Hill BG. Comprehensive measurement of respiratory activity in permeabilized cells using extracellular flux analysis. *Nat Protoc*. 2014 2;9(2):421–38. [PubMed: 24457333]
125. Nguyen QL, Wang Y, Helbling N, Simon MA, Shiva S. Alterations in platelet bioenergetics in Group 2 PH-HFpEF patients. *PLoS One*. 2019;14(7):e0220490. [PubMed: 31365585]
126. Nguyen QL, Corey C, White P, Watson A, Gladwin MT, Simon MA, et al. Platelets from pulmonary hypertension patients show increased mitochondrial reserve capacity. *Jci Insight*. 2017 3 9;2(5).
127. Tyrrell DJ, Bharadwaj MS, Jorgensen MJ, Register TC, Molina AJ. Blood cell respirometry is associated with skeletal and cardiac muscle bioenergetics: Implications for a minimally invasive biomarker of mitochondrial health. *Redox Biol*. 2016 12;10:65–77. [PubMed: 27693859]
128. Williams J, Holmes RP, Assimios DG, Mitchell T. Monocyte Mitochondrial Function in Calcium Oxalate Stone Formers. *Urology*. 2016 7;93:224 e1–6.
129. Chacko BK, Kramer PA, Ravi S, Benavides GA, Mitchell T, Dranka BP, et al. The Bioenergetic Health Index: a new concept in mitochondrial translational research. *Clin Sci (Lond)*. 2014 9;127(6):367–73. [PubMed: 24895057]

130. Chacko BK, Zhi D, Darley-USmar VM, Mitchell T. The Bioenergetic Health Index is a sensitive measure of oxidative stress in human monocytes. *Redox Biol.* 2016 8;8:43–50. [PubMed: 26748041]
131. Kramer PA, Chacko BK, George DJ, Zhi D, Wei CC, Dell'Italia LJ, et al. Decreased Bioenergetic Health Index in monocytes isolated from the pericardial fluid and blood of post-operative cardiac surgery patients. *Biosci Rep.* 2015 7 1;35(4).
132. Braganza A, Corey CG, Santanasto AJ, Distefano G, Coen PM, Glynn NW, et al. Platelet bioenergetics correlate with muscle energetics and are altered in older adults. *Jci Insight.* 2019 5 23;5.
133. Tyrrell DJ, Bharadwaj MS, Van Horn CG, Kritchevsky SB, Nicklas BJ, Molina AJ. Respiriometric Profiling of Muscle Mitochondria and Blood Cells Are Associated With Differences in Gait Speed Among Community-Dwelling Older Adults. *J Gerontol A Biol Sci Med Sci.* 2015 11;70(11):1394–9. [PubMed: 25030980]
134. Tyrrell DJ, Bharadwaj MS, Van Horn CG, Marsh AP, Nicklas BJ, Molina AJ. Blood-cell bioenergetics are associated with physical function and inflammation in overweight/obese older adults. *Exp Gerontol.* 2015 10;70:84–91. [PubMed: 26226578]
135. Willig AL, Kramer PA, Chacko BK, Darley-USmar VM, Heath SL, Overton ET. Monocyte bioenergetic function is associated with body composition in virologically suppressed HIV-infected women. *Redox Biol.* 2017 8;12:648–56. [PubMed: 28395172]
136. Keuper M, Berti L, Raedle B, Sachs S, Bohm A, Fritsche L, et al. Preadipocytes of obese humans display gender-specific bioenergetic responses to glucose and insulin. *Mol Metab.* 2019 2;20:28–37. [PubMed: 30528280]
137. Sanz A, Hiona A, Kujoth GC, Seo AY, Hofer T, Kouwenhoven E, et al. Evaluation of sex differences on mitochondrial bioenergetics and apoptosis in mice. *Exp Gerontol.* 2007 3;42(3):173–82. [PubMed: 17118599]
138. Sjoval F, Morota S, Hansson MJ, Friberg H, Gnaiger E, Elmer E. Temporal increase of platelet mitochondrial respiration is negatively associated with clinical outcome in patients with sepsis. *Crit Care.* 2010;14(6):R214. [PubMed: 21106065]
139. Dezfulian C, Taft M, Corey C, Hill G, Krehel N, Rittenberger JC, et al. Biochemical signaling by remote ischemic conditioning of the arm versus thigh: Is one raise of the cuff enough? *Redox Biol.* 2017 8;12:491–8. [PubMed: 28334684]
140. Rezaian S, Puskarich MA, Petrusca DN, Neto-Neves EM, Rondina MT, Kline JA. Platelet hyperactivation, apoptosis and hypercoagulability in patients with acute pulmonary embolism. *Thromb Res.* 2017 7;155:106–15. [PubMed: 28528289]
141. Grundler K, Angstwurm M, Hilge R, Baumann P, Annecke T, Crispin A, et al. Platelet mitochondrial membrane depolarization reflects disease severity in patients with sepsis and correlates with clinical outcome. *Crit Care.* 2014 2 12;18(1):R31. [PubMed: 24521521]
142. Michalak S, Florzak-Wypianska J, Rybacka-Mossakowska J, Ambrosius W, Osztynowicz K, Baszczuk A, et al. Mitochondrial Respiration in Intact Peripheral Blood Mononuclear Cells and Sirtuin 3 Activity in Patients with Movement Disorders. *Oxid Med Cell Longev.* 2017;2017:9703574. [PubMed: 29081897]
143. Avila C, Huang RJ, Stevens MV, Aponte AM, Tripodi D, Kim KY, et al. Platelet mitochondrial dysfunction is evident in type 2 diabetes in association with modifications of mitochondrial anti-oxidant stress proteins. *Exp Clin Endocrinol Diabetes.* 2012 4;120(4):248–51. [PubMed: 21922457]
144. Chacko B, Culp ML, Bloomer J, Phillips J, Kuo YF, Darley-USmar V, et al. Feasibility of cellular bioenergetics as a biomarker in porphyria patients. *Mol Genet Metab Rep.* 2019 6;19:100451. [PubMed: 30740306]
145. Villagra J, Shiva S, Hunter LA, Machado RF, Gladwin MT, Kato GJ. Platelet activation in patients with sickle disease, hemolysis-associated pulmonary hypertension, and nitric oxide scavenging by cell-free hemoglobin. *Blood.* 2007 9 15;110(6):2166–72. [PubMed: 17536019]
146. Tyrrell DJ, Bharadwaj MS, Jorgensen MJ, Register TC, Shively C, Andrews RN, et al. Blood-Based Bioenergetic Profiling Reflects Differences in Brain Bioenergetics and Metabolism. *Oxid Med Cell Longev.* 2017;2017:7317251. [PubMed: 29098063]

147. Japiassu AM, Santiago AP, d'Avila JC, Garcia-Souza LF, Galina A, Castro Faria-Neto HC, et al. Bioenergetic failure of human peripheral blood monocytes in patients with septic shock is mediated by reduced FIFo adenosine-5'-triphosphate synthase activity. *Crit Care Med*. 2011 5;39(5):1056–63. [PubMed: 21336129]
148. Mahapatra G, Smith SC, Hughes TM, Wagner B, Maldjian JA, Freedman BI, et al. Blood-based bioenergetic profiling is related to differences in brain morphology in African Americans with Type 2 diabetes. *Clin Sci (Lond)*. 2018 12 12;132(23):2509–18. [PubMed: 30401689]
149. Chou CH, Fu TC, Tsai HH, Hsu CC, Wang CH, Wang JS. High-intensity interval training enhances mitochondrial bioenergetics of platelets in patients with heart failure. *Int J Cardiol*. 2019 1 1;274:214–20. [PubMed: 30072155]
150. Harker LA, Roskos LK, Marzec UM, Carter RA, Cherry JK, Sundell B, et al. Effects of megakaryocyte growth and development factor on platelet production, platelet life span, and platelet function in healthy human volunteers. *Blood*. 2000 4 15;95(8):2514–22. [PubMed: 10753829]
151. Pillay J, den Braber I, Vrisekoop N, Kwast LM, de Boer RJ, Borghans JA, et al. In vivo labeling with 2H2O reveals a human neutrophil lifespan of 5.4 days. *Blood*. 2010 7 29;116(4):625–7. [PubMed: 20410504]
152. Tofts PS, Chevassut T, Cutajar M, Dowell NG, Peters AM. Doubts concerning the recently reported human neutrophil lifespan of 5.4 days. *Blood*. 2011 6 2;117(22):6050–2; author reply 3–4. [PubMed: 21636720]
153. Patel AA, Zhang Y, Fullerton JN, Boelen L, Rongvaux A, Maini AA, et al. The fate and lifespan of human monocyte subsets in steady state and systemic inflammation. *J Exp Med*. 2017 7 3;214(7):1913–23. [PubMed: 28606987]
154. Tough DF, Sprent J. Lifespan of lymphocytes. *Immunol Res*. 1995;14(1):1–12. [PubMed: 7561338]
155. Flidner TM, Graessle D, Paulsen C, Reimers K. Structure and function of bone marrow hemopoiesis: mechanisms of response to ionizing radiation exposure. *Cancer Biother Radiopharm*. 2002 8;17(4):405–26. [PubMed: 12396705]
156. Hasegawa A, Liu H, Ling B, Borda JT, Alvarez X, Sugimoto C, et al. The level of monocyte turnover predicts disease progression in the macaque model of AIDS. *Blood*. 2009 10 1;114(14):2917–25. [PubMed: 19383966]
157. De Boer RJ, Mohri H, Ho DD, Perelson AS. Turnover rates of B cells, T cells, and NK cells in simian immunodeficiency virus-infected and uninfected rhesus macaques. *J Immunol*. 2003 3 1;170(5):2479–87. [PubMed: 12594273]
158. Grove EL, Hvas AM, Mortensen SB, Larsen SB, Kristensen SD. Effect of platelet turnover on whole blood platelet aggregation in patients with coronary artery disease. *J Thromb Haemost*. 2011 1;9(1):185–91. [PubMed: 20955349]
159. Cesari F, Marcucci R, Caporale R, Panicia R, Romano E, Gensini GF, et al. Relationship between high platelet turnover and platelet function in high-risk patients with coronary artery disease on dual antiplatelet therapy. *Thromb Haemost*. 2008 5;99(5):930–5. [PubMed: 18449424]
160. Cowan DB, Yao R, Akurathi V, Snay ER, Thedsanamoorthy JK, Zurakowski D, et al. Intracoronary Delivery of Mitochondria to the Ischemic Heart for Cardioprotection. *PLoS One*. 2016;11(8):e0160889. [PubMed: 27500955]

Highlights:

1. Platelets and PBMCs have fully functional and metabolically active mitochondria
2. Blood cell bioenergetics change in disease and correlate with tissue bioenergetics
3. Circulating blood cells can serve as a minimally invasive biomarker for systemic bioenergetics

Figure 1. Mitochondrial bioenergetic profile generated by Seahorse XF.

Bioenergetic profile generated by measuring oxygen consumption rate in the basal state as well as after the sequential addition of mitochondrial modulators (Oligomycin A, FCCP, Rotenone and Antimycin A). Electron transport chain diagram in which electron transport and oxygen consumption by complexes I-IV are linked to ATP production at complex V by the protonmotiv force is shown to depict the action of each modulator that used to produce the bioenergetic profile (basal respiration, proton leak, maximal respiration and non-mitochondrial respiration). See text for more details.

Figure 2. Variability in the platelet bioenergetic profile between individuals and in the same individual over time.

(A) Variability between platelet basal OCR (pmol/min/ 5×10^7 platelets) and maximal OCR (pmol/min/ 5×10^7 platelets) in 75 individuals with no diagnosed disease. (B) Platelet basal OCR (pmol/min/ 5×10^7 platelets) and maximal OCR (pmol/min/ 5×10^7 platelets) in 12 individuals over time. Platelets were isolated from independent blood draws in each individual made at baseline then 1 week, 1 month and 3 months later. (C) Coefficient of variation for platelet basal OCR and maximal OCR describing the variability between individuals – calculated from the data in Panel A. Second two bars are the coefficient of variability of the measurements made over time from the same individual (raw data shown in Panel B). The second two bars show the mean and standard deviation of the coefficient of variability of the 12 individuals measured.

Figure 3. Blood cell bioenergetics are altered in disease.

Summary of human studies that show altered mitochondrial bioenergetics in platelets (left side) and peripheral blood mononuclear cells (PBMCs – right side) in individuals with Asthma (¹Winnica et al., 2019, and ²Xu et al., 2015), Pulmonary Hypertension with Heart Failure with Preserved Ejection Fraction (PH-HFpEF) (³Nguyen et al., 2019), Pulmonary Arterial Hypertension (PH) (⁴Nguyen et al., 2017), Type 2 Diabetes (T2D) (⁵Avila et al., 2012, and ⁶Mahapatra et al., 2018), Sickle cell disease (SCD) (⁷Cardenes et al. 2017), Sepsis (⁸Sjovall et al., 2010), Aging (⁹Braganza et al., 2019), Parkinson's disease (¹⁰Michalak et al., 2017), post-operative cardiac surgery changes in Bioenergetic Health Index (BHI) (¹¹Kramer et al., 2015), Porphyria (¹²Chacko et al., 2019) and HIV (¹³Willig et al., 2017).

Figure 4. Blood cell bioenergetics correlate with tissue energetics.

Platelet ATP-linked respiration (measured by Seahorse XF Analysis) correlates with muscle state 3 respiration (respiration in the presence of pyruvate and ADP; measured by Oxygraph 2K, $r = 0.568$, $P = 0.005$) as well as with muscle ATP synthesis (measured by ³¹P-MRS, $r = 0.643$, $P = 0.0004$) in older humans (ages >75).

Table 1:

Correlation of blood cell bioenergetics with peripheral tissue bioenergetics and clinical parameters

Population	Mitochondrial Bioenergetic Parameter Measured	Human/ Primate	Peripheral Cell Type Examined	Correlating Tissue or Organ	Correlating Clinical Parameter	Reference
Asthma; Obesity	Basal respiration; Maximal respiration	Human	Platelet	Airway Epithelium	N/A	Winnica et al. (2019) Antioxid Redox Signal
Healthy	Maximal respiration	Non-human Primate	Monocyte	Brain (frontal cortex)	N/A	Tyrrell et al (2017) Oxid Med Cell Longev
Healthy	Maximal respiration	Non-human primate	Monocyte/platelet	Skeletal Muscle Cardiac Mito	N/A	Tyrrell et al (2016) Redox Biology
Aging	Maximal respiration	Human	PBMC	Skeletal Muscle	Gait speed	Tyrrell et al. (2015) J Gerontol A Biol Sci Med Sci
Aging	Basal glycolytic rate; Proton leak	Human	Platelet	Skeletal Muscle	Gait speed; Physical Fatigability Score	Braganza et al. (2019) JCI Insight
Aging; Obesity	Maximal respiration	Human	PBMC	N/A	Grip strength, Peak knee extensor strength, Physical performance battery	Tyrrell et al. (2015) Exp Gerontol.
Sepsis	ADP-dependent mitochondrial respiration	Human	PBMC	N/A	Sequential Organ Failure Assessment (SOFA) Score	Japiassu et al. (2011) Crit Care Med
Diabetes Type 2	Basal respiration; Maximal respiration	Human	PBMC	N/A	Total brain cranial volume; Cognitive Assessment Score	Mahapatra et al. (2018) Clin Sci
Pulmonary Arterial Hypertension	Reserve capacity	Human	Platelet	N/A	Mean pulmonary arterial pressure; Right ventricular stroke work index	Nguyen et al. (2017) JCI Insight
Left Heart Failure	Maximal and Reserve OCR capacity	Human	Platelet	N/A	Graded exercise test; Peak oxygen consumption	Chou et al. (2019) Int J Cardiol