2023 IMPACT CIRCLE

Project Title: **How fit are your mitochondria?**

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Unmet Need/Primary Question: There is an ever-evolving need for improved diagnosis, assessing disease susceptibility and monitoring interventions from blood. Can testing how live cells function from blood contribute to this, complementing state-of-the-art multiomic approaches? Mitochondrial dysfunction is an early and central component of multiple age-related diseases, and therefore it is a potential biomarker allowing early detection of disease. In basic research, complex tools are available to precisely define and measure mitochondrial function, some developed by us. Here we aim to standardize and translate to the clinic such a novel technology.

Novel Hypothesis:

We will use a novel electrical power term to define mitochondrial fitness. We hypothesize that measuring peak bioenergetic power in mitochondria will be a better standardizable and more sensitive approach to determining mitochondrial fitness in blood, than other existing mitochondrial metrics. Furthermore, we hypothesize that peak bioenergetic power, measurable in blood, declines with age.

Project Proposal:

Mitochondrial function in blood cells is broadly assumed to reflect mitochondrial properties of the whole body, and specifically to reflect metabolic changes observed during aging, because of the intimate relationship of blood with all organs. The overall project objective is to develop a novel biomarker that provides a better assessment of an individual's mitochondrial fitness from blood than with currently available technologies possible, and to test its applicability in tracking age-related conditions. Our novel biomarker candidate is peak bioenergetic power. Mitochondria are indeed like power plants, and – we put forward here that – how powerful they are, as for electrical power, can be expressed in Watts, as the product of current and voltage. We define bioenergetic power as the product of cell respiration and mitochondrial membrane potential. These are two bioenergetic quantities that are commonly measured in laboratory settings and we have previously developed novel methods to calibrate and precisely measure them in small samples. We define peak power in a physiological-like condition that maximizes bioenergetic output of mitochondria in intact cells. This is very novel, because mitochondria are currently evaluated during a non-physiological, short-circuited operation. Furthermore, only current terms have been used as a blood-based metric before, measured as cell respiration that is equivalent to a flow of electrons in the mitochondria. Thus, half of the equation (the voltage term) has been overlooked so far, and therefore bioenergetic power aims to provide a more complete picture on

mitochondrial fitness. The Impact Circle will enable us to optimize peak bioenergetic power assaying in human blood samples, develop protocols, validate and test for sensitivity and repeatability. Specifically, we will determine that which blood cell type within the mitochondria-containing white blood cells is the most suitable for assaying, and then we will include a purification step for this cell type in the assay. Then we will work out cryogenic storage of samples for assaying, supporting transporting and pooling samples. Preliminary data indicates feasibility of performing this assay in cryo-preserved samples. To validate and benchmark the technology, we will repeatedly assay stored, identical batches of blood cells, use a small extent of inhibition with mitochondrial poisons to model disease, and compare bioenergetic power to existing, cell respirometry-based metrics and indices. Finally, we will survey a small cohort of individuals, spanning varying ages, in samples available from blood banks. Funds from the Impact Circle will enable us performing these experiments by paying for part effort of a postdoc and the PI, cost of blood bank samples, reagents and lab consumables and microscopy beam time.

Description of Potential Impact:

The immediate impact of this study will be that proof of principle experiments, protocols, and data on assay sensitivity and repeatability of peak bioenergetic power assaying will be generated allowing designing a study on a specific cohort of human subjects, such as individuals suffering in Alzheimer's disease or type 2 diabetes and matching control subjects. Future applications of blood-based peak bioenergetic power assaying may include intervention testing or screening at-risk population for age-related conditions, for example in cases where metabolic exercise tests, such as ones performed on a treadmill are contraindicated. A peak bioenergetic power blood test could be valuable for personalized medicine or for personalizing dietary supplement intake related to energy metabolism, such as Coenzyme Q10, nicotinamide riboside or mononucleotide, lipoic acid and carnitine, by tracking if desired mitochondrial changes take place.