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Mitochondrial psychobiology: foundations and applications

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Mitochondrial psychobiology is the study of the interactions between psychological states and the biological processes that take place within mitochondria. It also examines how mitochondrial behavior influence neural, endocrine, and immune systems known to transduce psychological experiences into health outcomes. Unlike traditional biological outcomes and mediators, mitochondria are dynamic and multifunctional living organisms. By leveraging a variety of laboratory tools including omics, scientists can now map mitochondrial behavior at multiple levels of complexity – from isolated molecular markers to dynamic functional and signaling outcomes. Here, we discuss current efforts to develop relevant measures of mitochondrial behavior in accessible human tissues, increase their biological specificity by applying precise measurements in defined cell populations, create composite indices reflecting mitochondrial health, and integrate these approaches with psycho-neuro-endocrino-immune outcomes. This systematic inter-disciplinary effort will help move the field of mitochondrial psychobiology toward a predictive science explaining how, and to what extent, mitochondria contribute to the biological embedding of stress and other psychological states.

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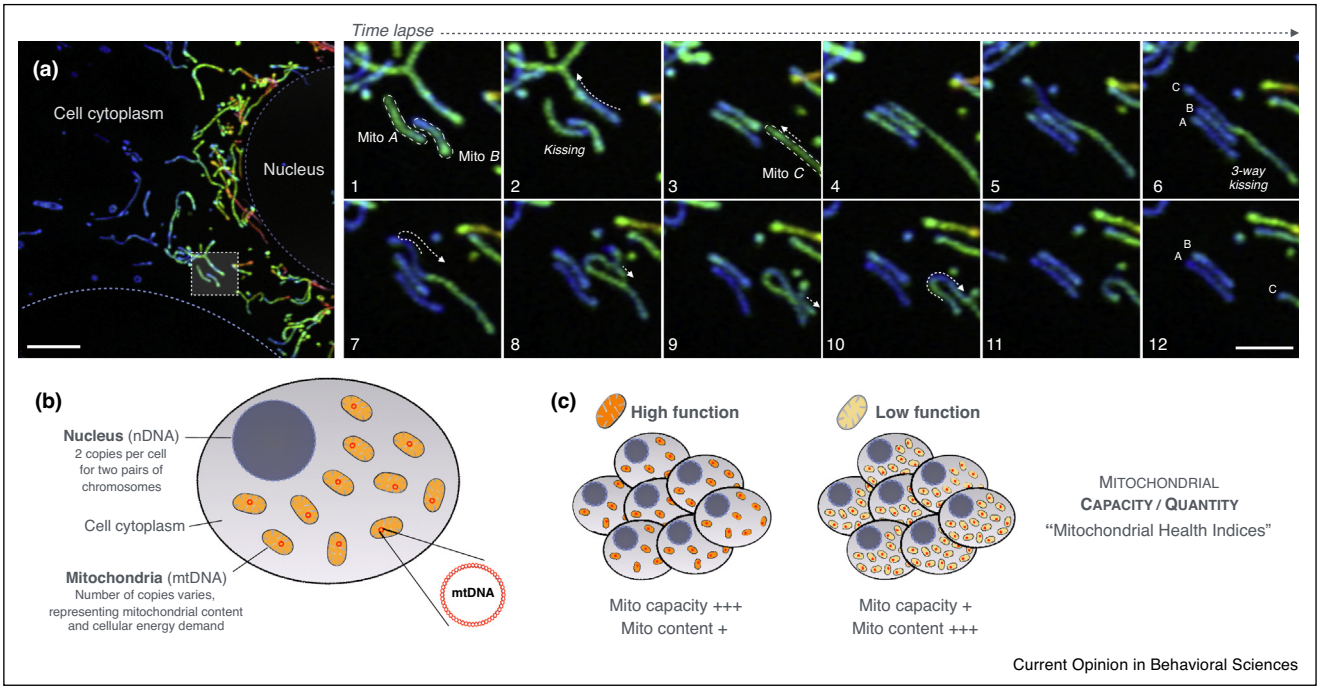
Introduction

Mitochondria are dynamic living microorganisms, unlike traditional molecular biomarkers used in medicine and psycho-neuro-endocrino-immunology (PNEI). Hundreds to thousands of small interacting mitochondria populate the cytoplasm of every cell across the human body (Figure 1) – except in red blood cells, which transport the oxygen needed to sustain energy flow within mitochondria. They are the only cellular organelle to contain their own genome – the mitochondrial DNA (mtDNA). Together, the mitochondrial and nuclear genomes produce components of the respiratory chain, a multi-protein system embedded within the mitochondrial membranes where the oxygen we breathe and the food we eat are consumed to generate a transmembrane charge, similar to batteries. Humans and other animals breathe to sustain respiration and energize their mitochondria. Energized mitochondria then produce the cellular energy currency (adenosine triphosphate, ATP) and regulate essential cellular processes including gene expression, cell differentiation, immunity, steroid hormone biosynthesis, oxidative stress, and cell death [1,2].

In response to signals from the environment – including stressors of various nature – mitochondria rapidly undergo changes in shape and function, and produce signals that influence cellular behavior and physiological stress responses systemically [3,4]. In the clinic, genetic mitochondrial defects cause multisystem disease, and functional alterations in mitochondria are involved in common metabolic, neurodegenerative, immune, and neoplastic disorders [5]. Given their pervasive role in human disease, mitochondria now stand as the most studied organelle in biomedical research [6]. Developing suitable methods and approaches to examine their behavior in accessible human tissues, including blood, would constitute a powerful tool to explore the inter-disciplinary processes that shape human health and disease trajectories.

A challenge for biomedical and behavioral sciences is to understand the interaction among psychological and biological processes that jointly contribute to health and disease. This field – *psychobiology* – focuses on the bidirectional exchange of information between social/individual-level experiences and molecular processes. The cross-talk between these levels has historically been established and explored through the brain, endocrine, and immune systems – the domain of PNEI [7]. Landmark studies have documented the effects of mental/emotional states at the molecular level including but not limited to inflammation [8], gene expression [9,10], and telomere length [11]. This integration of concepts and methods across fields has revealed biological plausible pathways for the transduction of life events and psychological states into disease-relevant biological mechanisms [12], which

Figure 1



Mitochondria are dynamic living organisms that populate the cell cytoplasm and vary in content and function.
(a) Top view of a living cell with colored mitochondria visualized by confocal microscopy. Mitochondria move, change shape, and interact with each other *via* rapid reversible fusion (i.e. kissing) that allows molecular exchanges while remaining separate mitochondria. Pictures 1–12 are higher magnifications (4,250 times larger than life) of the boxed area from the picture on the left (magnified 1,600 times). Image were captured approximately every 20 s, revealing the movement of specific mitochondria *A*, *B*, and *C*. See Video S1 for the animated version. Scale bars are 5 μm for whole cell (*left*) and 2 μm for magnified areas (*right*). Color scheme indicates the position (depth) of each mitochondrion in the cell where blue is at the bottom and red is at the top of the cell. **(b)** Schematic of a human cell containing two major compartments: the nucleus with the nuclear genome (nDNA) and the cytoplasm that houses mitochondria and their maternally inherited mitochondrial DNA (mtDNA). **(c)** Mitochondria can differ both in their *energy production capacity* and in their *content* (i.e. cells can have more or less mitochondria). The ratio of capacity to quantity reflects mitochondrial functional capacity, which can be quantified as mitochondrial health indices (MHIs).
Abbreviations: mtDNA, mitochondrial DNA; nDNA, nuclear DNA.

can eventually be targeted to promote successful adaptation across the lifespan.

In this article, we briefly discuss the scientific rationale for examining the role of mitochondria in human psychobiology. The resulting research field – mitochondrial psychobiology – aims to map the interactions between psychological states and the biological processes that take place within mitochondria. We describe available methods and approaches to assess mitochondrial health in human blood and other tissues and discuss the advantages, limitations, and technical considerations in interpreting genomic, molecular, functional, and physiological data. We end by outlining opportunities for developing integrative indices of mitochondrial health.

Mitochondrial psychobiology: an emerging field

Two main lines of work suggest that mitochondria play a mediating role in the stress-disease cascade. First, mitochondria respond to induced stress in animal models [13] and to stress hormones in cellular *in vitro* studies [14]. Mitochondria are sensitive to stress mediators and can accumulate mitochondrial allostatic load (MAL) [15••]. Second, mitochondrial dysfunction triggers symptoms and physiological alterations upon the brain, immune, and endocrine systems, including inflammation that overlap with the symptoms of stress pathophysiology [16]. Dysfunctional mitochondria are thus capable of triggering cellular and physiological consequences similar to those of chronic stress. Mitochondria's dual role as i) *target* of stress and ii) *source of signals*, leads to the hypothesis that they contribute to the transduction and biological embedding of stressful and positive psychosocial experiences [16].

This hypothesis has only been partially supported by animal research and indirectly by a small number of human studies (see Ref. [13] for a systematic review). As a field, mitochondrial psychobiology is early in its development. Nevertheless, available preliminary evidence in humans suggest that chronic psychological stress and psychopathology are associated with molecular and functional alterations

in specific aspects of mitochondrial biology, including whole blood mtDNA copy number (mtDNAcn) [17,18], enzymatic activities [19••], and cellular respiration [20,21,22••]. Recent findings also suggest that chronically depressed individuals [23], suicidal patients [24••], and healthy adults acutely exposed to laboratory psychological stress [25,26] exhibit elevated levels of serum and plasma circulating cell-free mitochondrial DNA (ccf-mtDNA) – likely actively extruded secondary to stress signals [26]. Notably, ccf-mtDNA levels may increase with age and stimulate pro-inflammatory cytokine production [27,28], suggesting that ccf-mtDNA could mediate the effects of psychological stress on immune activation, although this remains to be demonstrated.

This preliminary evidence calls for further human mitochondrial psychobiology studies, while also emphasizing the need for accurate and specific methods to establish to validity and generalizability for the influence of psychological states on mitochondria. As we discuss below, known confounds limit the interpretations of results from studies using whole blood as source material, of measures obtained from cell mixtures such as peripheral blood mononuclear cells (PBMCs), and of existing methods that do not enable to isolate mitochondrial function(s) from other cellular factors.

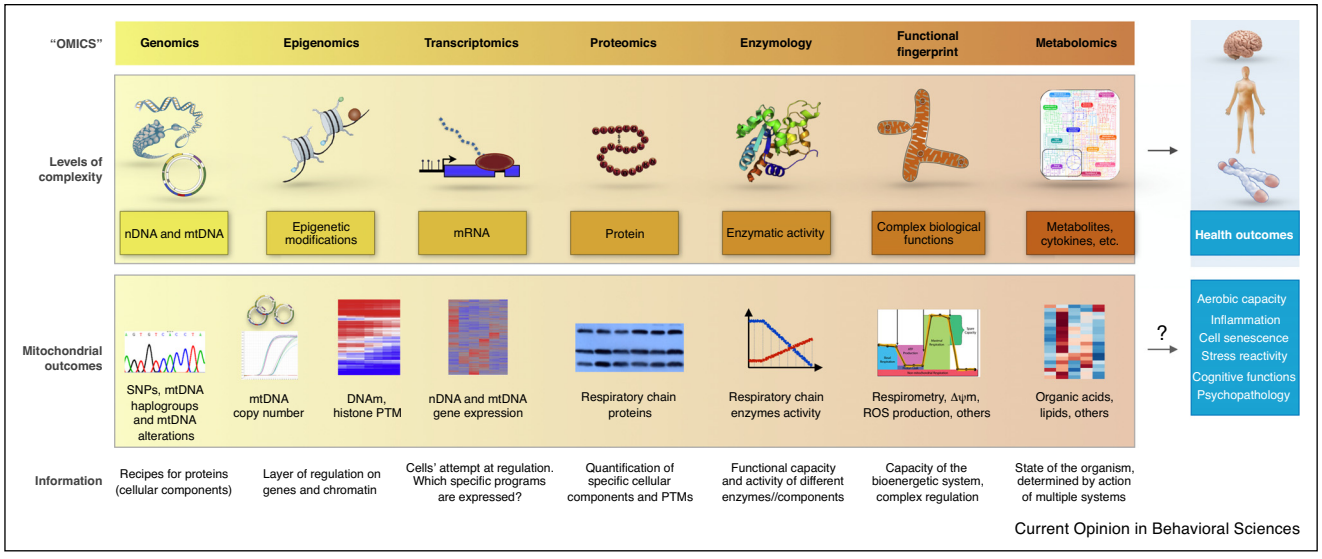
Conceptually, there are important implications to recognizing mitochondria as dynamic living organisms whose complex behavior can be disrupted in different ways (Figure 1 and Video S1). Consider, as an analogy, the complexity of psychological and behavioral processes in humans: Individuals exhibit a range of normal and abnormal neuropsychological functions, personality types, and behaviors that present as a spectrum and cannot be captured by a single measure. In the same way, mitochondrial psychobiology will need to adapt robust methods to quantify key domains of mitochondrial behavior, and to develop a framework that encompasses the spectrum of normal and abnormal mitochondrial phenotypes, or *mitotypes*.

The hypothesis that mitochondria contribute to the biological embedding of psychological states – both positive and negative – can only be tested using robust PNEI methods and approaches directly informed by knowledge of cellular and mitochondrial biology. The next section 'Mitochondrial omics' explores the current toolkit available to scientists to probe different facets of mitochondrial behavior.

Mitochondrial omics

This section describes various facets of mitochondria, ranging from the most fundamental to the most complex level of biological organization. We structure the presentation of each level of analysis by addressing the following issues: the type of biological material used; how each measure is collected; how the data are interpreted; how each level is regulated (plasticity); and the major caveats. Figure 2 shows additional information about the scientific value derived from each level.

Figure 2



Overview of the types of methods and levels of biological complexity available to study mitochondrial behavior.
The diagram is arranged from left to right according to the flow of biological information from the genomic material (*left*) toward more complex levels of organization and function (*right*). But information also flows in the reverse direction; for instance, metabolites influence epigenetic regulation and gene expression. Measures not shown include ccf-mtDNA and live cell imaging of mitochondrial movement. How specific dimensions of mitochondrial behavior, alone or in combination, influence whole-organism physiological functions and health outcomes remain to be established (*right*).
Abbreviation: DNAm, DNA methylation; mRNA, messenger RNA; mtDNAcn, mitochondrial DNA copy number; PTM, post-translational modifications; ROS, reactive oxygen species; Δψm, mitochondrial membrane potential.

Genomics

Genomic investigation is accomplished through genotyping or DNA sequencing, which can be performed on DNA extracted from any cellular material. The outcome measured is the identity (for genotyping) and precise order (for sequencing) of nucleotides within the nuclear or mitochondrial genomes, both of which encode mitochondrial components. The measured sequence is aligned and compared to a reference human genome, which yields information about naturally occurring single-nucleotide polymorphisms (SNPs) and pathogenic variants (i.e. mutations) susceptible to influence the function of mitochondrial proteins [29]. The genomic material is mostly inherited and thus not subject to acute regulation, but new mutations can occur within years to decades, particularly in the mtDNA [30]. The main caveat of this approach is that the functional significance of SNPs is difficult to establish and several levels of regulation described below in sections b) through h) modulate and buffer the influence of genetic information on actual mitochondrial behavior.

Epigenomics

Epigenomics refers to the process of mapping the reversible modification of the DNA or its associated proteins. The existence of a mtDNA epigenome is controversial but there is epigenetic regulation of mitochondrial proteins encoded in the nDNA [31]. Epigenomic data, such as cytosine methylation or histone acetylation, are collected from isolated DNA or cellular material by measuring the presence of specific chemical modifications in specific genomic locations. Data on specific epigenetic marks are classically interpreted relative to a control group or by combining multiple CpG methylation values into composite scores such as epigenetic age [32], which can be correlated with an outcome of interest (e.g. stress exposure). The human epigenome is believed to be regulated within weeks to decades by environmental factors, with a contribution of inherited patterns. Major caveats include the high dimensionality of the data (850,000 data points for the most common DNA methylation array) and that DNA methylation analysis alone does not provide information about the direction of change in gene expression.

mtDNA analysis

The mitochondrial genome can vary in integrity (deletions, mutations) and in its abundance or number of copies per cell (mtDNAcn). mtDNA measurements can be done on isolated genetic material from any biological tissue. Quantitative PCR or gel-based methods are used to quantify mtDNA integrity detected as genomic damage, and mtDNAcn is easily determined by quantifying the number of mtDNA molecules relative to the nuclear genome (mtDNA/nDNA). Various factors, including higher cellular energy demand or decreased mitochondrial energy production capacity (i.e. mitochondrial dysfunction), can both cause compensatory elevations in mtDNAcn [33]. Thus, a major caveat of mtDNAcn is that it does not reflect the downstream functional state or behavior of mitochondria and requires other measures for its biological interpretation. Moreover, in blood, circulating platelets do not contain a nucleus (nuclear DNA) but contain mtDNA (Figure 1a), which skews the mtDNA/nDNA ratio in isolated PBMCs with platelet contamination, and particularly in whole blood where mtDNAcn is minimally, if at all informative [34]. The mitochondrial genome is also found in cell-free plasma and serum as ccf-mtDNA, which does not contribute to cellular energetics and likely plays a signalling role [23,24••,25,26]. Further caveats to the measurements and interpretation of mtDNAcn are discussed elsewhere [35].

Transcriptomics

Gene expression produces transcripts (RNAs) whose levels illustrate the extent to which different genes are active or inactive. Because transcripts are particularly labile, they are detected from cellular and non-cellular (e.g. extracellular particles) fractions generally treated with a special additive to preserve RNA integrity at the time of collection. Transcript numbers are quantified either in a targeted way by qPCR or microarray [36], or in an unbiased way by RNA sequencing (RNAseq) [37]. The data are interpreted on a per-gene basis or by gene families grouped by functional significance, compared across individuals or over time. The transcriptome is under partial genomic and epigenomic regulation but is mostly under the control of transcription factors (e.g. the glucocorticoid receptor) and other regulatory events that acutely change transcript levels within minutes to days. The major caveats with transcriptomic analysis are the dimensionality and complexity of the resulting datasets, and the lack of direct relation to mitochondrial function since transcript levels alone do not determine protein abundance nor their eventual activities.

Proteomics

Proteins are the building blocks that enable mitochondria to perform hundreds of specific biochemical reactions; each protein performs a specific function. Proteins are detected and quantified from cells and plasma/serum. Quantification is based on either immune-based detection (e.g. Western blotting or ELISA for single or few proteins) or higher throughout methods such as mass spectrometry-based proteomics (for hundreds of proteins) [38]. The data for individual proteins or families of proteins are interpreted based on the known function of each protein, with an elevation in a given protein generally assumed be associated in elevated activity or process associated with this protein(s). Protein amount is regulated within minutes to days by the translation of RNA into proteins and by quality control process that actively degrade dysfunctional proteins. The major caveat with proteomics is the lack of sensitivity for proteins present in very low abundance (including key mitochondrial proteins) and certain mitochondrial proteins embedded within lipid membranes (i.e. hydrophobic) that makes them difficult to detect [39].

Because the activity of proteins is post-translationally regulated by many factors, protein abundance detected by proteomics may also not directly translate into functional mitochondrial changes.

Enzymatic activities

Enzymatic activity reflects the actual biochemical reactions performed by single proteins (e.g. citrate synthase, a marker of mitochondrial content) or complexes of proteins, such as cytochrome c oxidase, a large 13-protein complex that consumes oxygen among the respiratory chain and enables energy transformation. Enzymatic activity is measured from fresh or frozen cells. Specific activities are quantified dynamically through colorimetric or light-based detection systems on freshly homogenized samples and ideally normalized on a per-cell basis [19••]. Isolated activities for different enzymes reflect their maximal activity within known biochemical pathways (e.g. energy production capacity) and reflect the capacity of a given process or behavior. Enzymatic activities are regulated within seconds to minutes by changes in the biochemical and metabolic environment that induce post-translational modifications, and by the total amount of the protein. The major caveat with enzymatic activities is that it may degrade over time under suboptimal storage conditions [40], unlike the inert biomolecules like DNA described above. Samples destined for functional measurements should be stored in liquid nitrogen ($<-150°C$). Moreover, the throughput of enzymatic assessments is intermediate relative to high-throughput molecular omics (above) and low-throughput functional methods (below).

Mitochondrial functions and dynamics

Mitochondrial functions include oxidative phosphorylation (OXPHOS), calcium uptake, reactive oxygen species (ROS) production, mitochondrial dynamics of fusion and fission, and many others that are of functional relevance to cellular and organismal behavior. These functional readouts are measured from freshly collected cells and mitochondria. When whole intact cells are used, measures reflect the cellular demand and constraints that cells pose on mitochondria [20], whereas measures on permeabilized cells and isolated mitochondria reflect the isolated maximal capacity of the organelle [41]. Respiration rate is quantified using either Clark-type oxygen sensors or extracellular flux analysis, which assesses how much oxygen is consumed over time [42]. Other functions are assessed *via* fluorescence-based methods and microscopy. Mitochondrial functions and dynamics are regulated within seconds to hours by a large number of factors including the metabolic state of the cell, neuroendocrine factors, and other molecular regulatory changes [43]. Attempts have been made to cryopreserve PBMCs for later functional measurements but although mitochondria mostly preserve the ability to respire, cryopreservation can alter mitochondrial respiratory coupling efficiency and possibly other mitochondrial properties due to membrane damage. The main caveat with functional measures is the need to perform measurements within minutes to hours on freshly collected cells, limiting throughput, increasing technical variability between samples, and requiring exceptional standardization of procedures.

Metabolomics

The metabolome is the sum of metabolites (e.g. glucose, lactate, lipids) that reflect the sum of biochemical activities in a given system. Metabolites are measures in cells or in cell-free plasma or serum. Each metabolite is quantified either in a targeted or untargeted manner, for example by mass spectrometry-based methods. Metabolite levels are interpreted based on their relation to one another, and their belonging and position within specific biochemical pathways. The metabolome can change within seconds to minutes under the influence of mitochondrial metabolism and other cellular metabolic pathways, and can be used to link known mitochondrial pathways to symptoms (e.g. fatigue) [44]. The main caveats of metabolomics relate to the technical complexity that require the use of core services for most laboratories, and to the specificity of changes in the metabolome, which do not directly reflect mitochondrial behavior in any specific organ. Different organs have different metabolomes [45] and the signal from a given population of mitochondria (e.g. brain mitochondria) is diluted in the circulation with the signal emanating from other tissues, making changes in plasma or serum difficult to interpret.

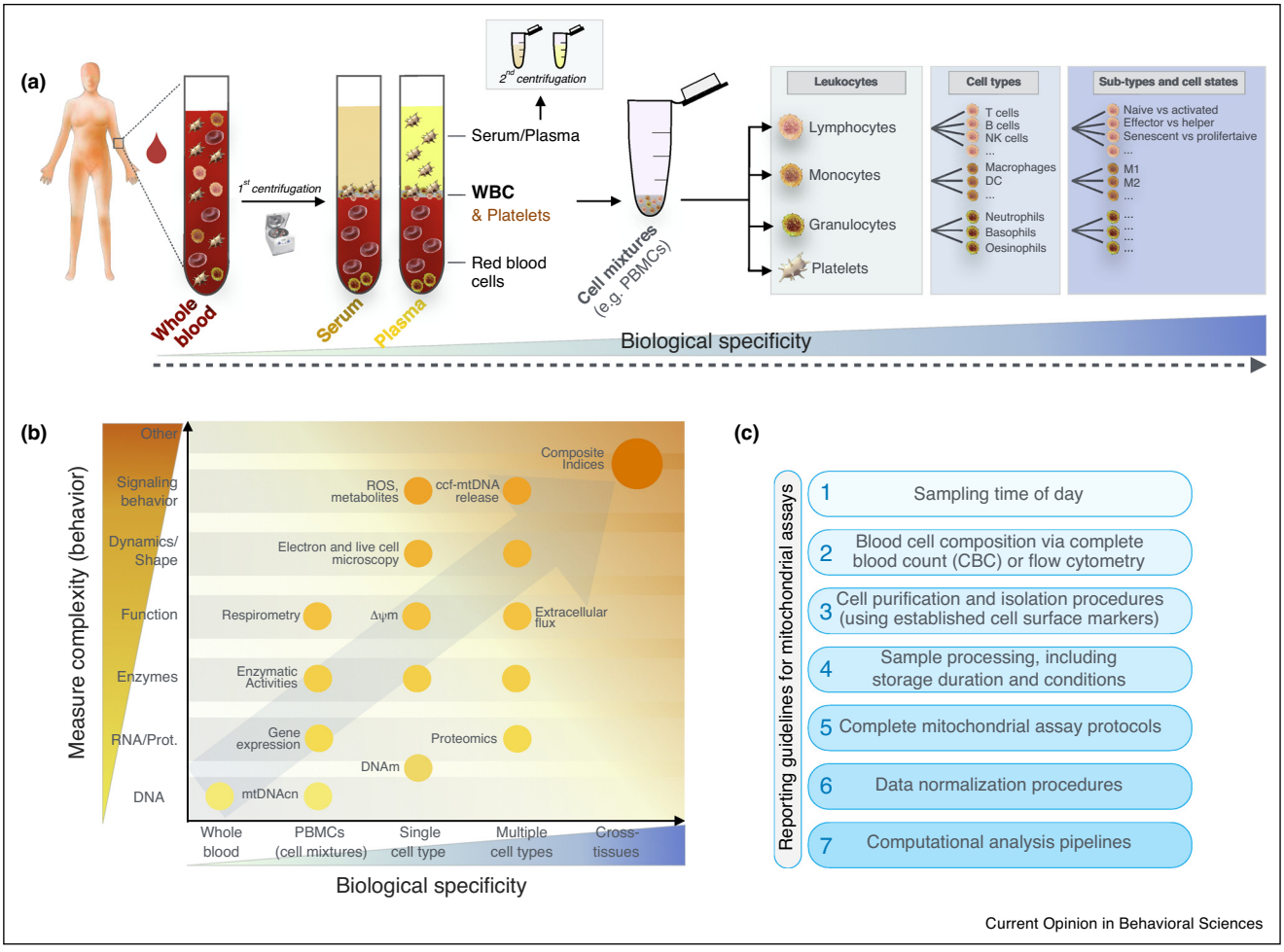
A roadmap that illustrates the application of these methods and approaches to different cellular and liquid fractions derived from human blood is shown in Figure 3. A list of mitochondrial outcomes, the biological tissues required to measure them, and other considerations are provided in Table 1.

Integrative indices and composites of mitochondrial health

Mitochondria are multifaceted organelles but our methods to study them are necessarily reductionist. We measure individual elements of mitochondrial composition and activity in an effort to understand their integrated functions and overall behavior. Data-driven multivariate functional indices will likely be necessary to capture interpretable signatures of mitochondrial health or dysfunction.

This is analogous to the situation in functional neuroimaging where individually measured brain regions are integrated into multivariate patterns to derive meaningful activity signatures reflecting the state of a person's brain [46]; or with epigenetic aging where DNA methylation levels measured at dozens or hundreds of positions throughout the genome are integrated within multivariate algorithms (e.g. clocks) to predict age [47]. This capturing of complex states from more simple measures integrated *via* machine learning and related computational approaches is an increasingly common

Figure 3



Different blood fractions and cellular compartments that can be queried with mitochondrial function assays.
(a) Biological measure on human blood can be performed at different level of biological specificity. The least specific is whole blood, which contains the liquid fraction of blood plus various cell types (mainly red blood cells, white blood cells, and platelets). Centrifugation of whole blood allows to separate liquid (serum and plasma) and cellular fractions. Additional centrifugation steps with density gradients (Ficoll 1077, 1119, etc.) allow to separate cell mixtures. Additional purification steps based on antibodies recognizing specific cell surface markers can isolate with a greater degree of specificity specific leukocyte cell types, and subtypes of cells in different states from which mitochondrial functions and behavior can be measured. Note that serum is the liquid fraction recovered after coagulation, whereas coagulation inhibitors are used to obtain plasma. A secondary centrifugation step at high speed (5,000–10,000 g, >10 min) can ensure that serum/plasma are free of cellular components.
(b) Diagram illustrating the relative measure complexity and biological specificity of various mitochondrial measures of mitochondrial behavior currently available. Higher values on both axes increase the scientific strength of the conclusions that can be derived. The use of composite indices including multiple measures high on both domains is expected to increase scientific insight. **(c)** Recommended guidelines to design and report mitochondrial psychobiology studies.
Abbreviations: ccf-mtDNA, circulating cell-free mitochondrial DNA; DC, dendritic cell; DNAm, DNA methylation; mtDNAcn, mitochondrial DNA copy number; NK, natural killer; PBMCs, peripheral blood mononuclear cells; WBC, White blood cells; Δψm, mitochondrial membrane potential.

practice in the biomedical and psychological sciences [48]. Similarly, we envision that individual mitochondrial measures can be integrated into quantitative signatures or indices that will most faithfully capture mitotypes and their relation to PNEI processes (Figure 4). Within study designs, such customized mitochondrial health indices can then be operationalized as either i) downstream outcomes from psychosocial/neuroendocrine exposures, ii) mediators, or iii) predictors of health outcomes.

Two initial approaches have been attempted to develop integrative mitochondrial health indices. The first, known as the bioenergetic health index (BHI) [49], integrated four measures of cellular respiration obtained during a protocol where mitochondria are sequentially inhibited and stimulated to obtain respiratory capacity under different conditions; it has not been studied in the context of psychobiology or PNEI. Another index integrated two enzymatic

Table 1

Mitochondrial outcomes accessible from human blood

Outcome	Tissue	Method	mtDNA[e]	Core facilities[f]	Cost per sample[g]
mtDNA and nDNA genotype	Whole blood, PBMCs, purified cell types[a]	Genotyping arrays or sequencing	Yes	Yes	$$
mtDNA copy number (mtDNAcn)	PBMCs, purified cell types[a]	qPCR, dPCR	Yes	Yes	$
Circulating cell-free mtDNA (ccf-mtDNA)	Plasma/serum	qPCR, dPCR	Yes	Yes	$
Epigenetic modifications					
- DNA (hydroxy)methylation	PBMCs, purified cell types[a]	DNA methylation array, bisulfite sequencing, pyrosequencing	No	Yes	$$
- Histone modifications and DNA accessibility	PBMCs, purified cell types[a]	ChIP, AttackSeq	No	Yes	$$$
Gene expression	PBMCs, purified cell types[a]	qRT-PCR Microarray, RNA-Seq	Yes[h]/no	Yes	$ $$–$$$
Protein level	PBMCs, purified cell types [a]	Proteomics Western blotting	Yes[h]/no	Yes	$$$ $
Enzymatic activities					
- Complex I (NADH dehydrogenase)	PBMCs, purified cell types[a]	Colorimetric assays	Yes	No	$
- Complex II (succinate dehedrogenase, SDH)			No		
- Complex IV (cytochrome c oxidase, COX)			Yes		
- Citrate synthase			No		
Respiratory capacity	Fresh PBMCs, purified cell types	Respirometry[c], extracellular flux[d]	Yes	No	$–$$$
Reactive oxygen species (ROS) production	Fresh PBMCs, purified cell types	Flow cytometry, fluorometry	Yes/no	No	$
Membrane potential	Fresh PBMCs, purified cell types	Flow cytometry, fluorometry	Yes/no	No	$
Metabolomics	Plasma/serum (PBMCs, purified cell types[a])	Mass spectrometry	No	Yes	$$–$$$
Morphology and size	Fresh purified cell types[b]	EM, Live cell microscopy	No	No	$–$$
Movement/dynamics	Live purified cell types	Live cell microscopy	No	No	$–$$

Abbreviations: ChIP, chromatin immunoprecipitation (followed by DNA sequencing or qPCR); *dPCR*, digital quantitative polymerase chain reaction (PCR); *EM*: electron microscopy; *qPCR*, quantitative PCR (same as real-time PCR, qRT-PCR); RNAseq, RNA sequencing.

[a] Frozen.

[b] Cells for EM have to be fixed immediately.

[c] Respirometry can be performed in intact cells or permeabilized cells; measures in permeabilized cells more directly isolates mitochondrial function from cellular energetic demands and metabolic constraints on respiratory states.

[d] Extracellular flux analysis for oxygen consumption rate and extracellular acidification rate provides information about cellular energetic requirements and maximal mitochondrial respiratory capacity.

[e] Indicates whether the outcome measure is biologically downstream (i.e. under direct influence) of the mitochondrial genome (mtDNA).

[f] Indicates whether these measurements and analysis platforms are typically available in centralized core facilities or commercial laboratories.

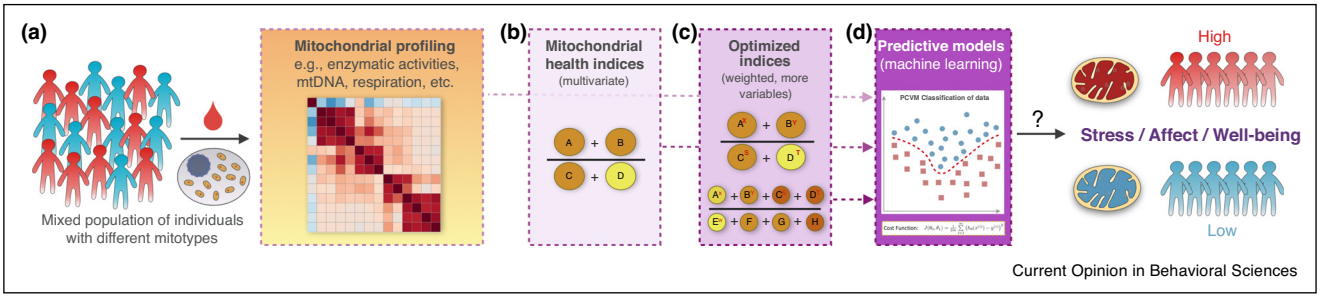
[g] Indicates the approximate cost per sample, $: $10–100, $$: $100−400, $$$ >$400.

[h] For mtDNA-encoded genes.

activity measures of energy production capacity and two markers of mitochondrial content to yield the mitochondrial health index (MHI) [19••] (see Figure 4). In this study of 91 women (half of whom were caregivers), MHI measured in total PBMCs was correlated with positive mood 1–2 days preceding measurement only, suggesting that mood influences mitochondrial function [19••].

As we move toward a predictive science for mitochondrial psychobiology, a foundational step will consist in determining, both cross-sectionally and longitudinally, how novel mitochondrial health indices interact with established markers of PNEI-related biological processes including inflammation, cellular aging, and immunosenescence (e.g. in Ref. [50]). In keeping with the concept of allostatic load, which views physiological perturbations as adaptive recalibrations [51], structural and functional recalibrations in mitochondrial measures could similarly be interpreted as mitochondrial allostatic load (MAL) [15••]. Although recalibrations and changes in mitotypes can be adaptive, chronically they can lead to wear-and-tear and pathophysiology.

Figure 4



Development of mitochondrial health indices and composites.
(a) To better capture mitochondria behavior, multiple functional outcomes can be integrated into indices that reflect complex mitotypes and can distinguish groups of individuals (e.g. high versus low stress). (**b**) Simple biologically-informed mitochondrial health indices (MHIs) can be built to reflect mitochondrial functional capacity on a per mitochondrion basis (e.g. in Ref. [19••]). **(c)** Indices can further be improved by weighing each measured parameters and/or by including additional relevant mitochondrial measures across levels of complexity (see Figure 2); darker color indicates higher biological complexity as per Figure 3. **(d)** Individual measures, indices, and optimized indices can be used to build predictive models whose performance can be compared and cross-validated using machine learning-based approaches. The diagram illustrates a decision boundary (red) established by optimizing the cost function in a probabilistic classification vector machines (PCVM) model to separate two hypothetical groups of individuals, each represented by a datapoint (e.g. blue, low stress; red, high stress).
Abbreviations: MHI, Mitochondria Health Index; mtDNA, mitochondrial DNA; PCVM, Probabilistic Classification Vector Machines.

Conceptual and technical considerations

Mitochondrial measurements from blood cells reflect their function in the immune system. But a potential limitation to their extrapolation and relevance to human health at large is that they may not reflect mitochondria in other relevant target tissues. However, some but not all [52] studies of mitochondrial respiratory capacity support the notion that immune cell mitochondrial function reflect mitochondria in other tissues, including skeletal muscles, [53] and the brain [54,55], suggesting the existence of mechanisms to harmonize the function mitochondria across organ systems.

To maximize the potential benefits of investigating mitochondrial outcomes in human blood, some technical and conceptual factors should be considered. Factors influencing the measurements of blood cell mitochondrial functions include the potential selection bias between types of leukocytes (monocytes, neutrophils, and lymphocytes [56••]), platelets contamination of leukocytes [34], delays and temperature for blood processing [57], and composition of assay buffers including glucose concentration [58]. Unlike DNA, storage temperature and duration can affect functional measures, including enzymatic activities [40]. Technically, we recommend the systematic and complete reporting of key procedures and analyses (see Figure 3c). Implementing these recommendations will help us not only reach robust interpretation of the data for single studies but also enhance reproducibility and consistency across laboratories.

Conclusions

Overall, existing evidence suggests that mitochondrial mechanisms may contribute to the transduction of psychological states into biological changes relevant to human health and disease. Rigorously testing this hypothesis in humans will require methods informed by principles of mitochondrial biology, developed for specific purified cell types to enable reliable interpretations. On the analysis side, computational approaches leveraging multi-omic and integrative indices will also likely play a substantial role in moving us from overly reductionist to more holistic and predictive models. Successfully bridging the gap between mitochondrial cell biology, the psychosocial sciences, and PNEI has the potential to inform us not only about how adverse and positive psychological experiences influence disease risk, but also to uncover fundamental mechanisms that sustain normal states of health.

Conflict of interest statement

Nothing declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cobeha.2019.04.015.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- •• of outstanding interest

1. Nunnari J, Suomalainen A: **Mitochondria: in sickness and in health**. *Cell* 2012, **148**:1145-1159.

2. Martínez-Reyes I, Diebold LP, Kong H, Schieber M, Huang H, Hensley CT, Mehta MM, Wang T, Santos JH, Woychik R: **TCA cycle and mitochondrial membrane potential are necessary for diverse biological functions**. *Mol Cell* 2016, **61**:199-209.

3. Eisner V, Picard M, Hajnóczky G: **Mitochondrial dynamics in adaptive and maladaptive cellular stress responses**. *Nat Cell Biol* 2018, **20**:755-765.

4. Picard M, McManus MJ, Gray JD, Nasca C, Moffat C, Kopinski PK, Seifert EL, McEwen BS, Wallace DC: **Mitochondrial functions modulate neuroendocrine, metabolic, inflammatory, and transcriptional responses to acute psychological stress**. *Proc Natl Acad Sci U S A* 2015, **112**:E6614-E6623.

5. Gorman GS, Chinnery PF, DiMauro S, Hirano M, Koga Y, McFarland R, Suomalainen A, Thorburn DR, Zeviani M, Turnbull DM: **Mitochondrial diseases**. *Nat Rev Dis Primers* 2016, **2**:16080.

6. Picard M, Wallace DC, Burelle Y: **The rise of mitochondria in medicine**. *Mitochondrion* 2016, **30**:105-116.

7. McEwen BS, Mirsky AE: **From serendipity to clinical relevance: how clinical psychology and neuroscience converged to illuminate psychoneuroendocrinology**. *Psychoneuroendocrinology* 2019 http://dx.doi.org/10.1016/j.psyneuen.2018.1009.1011. (in press).

8. Marsland AL, Walsh C, Lockwood K, John-Henderson NA: **The effects of acute psychological stress on circulating and stimulated inflammatory markers: a systematic review and meta-analysis**. *Brain Behav Immun* 2017, **64**:208-219.

9. Fredrickson BL, Grewen KM, Coffey KA, Algoe SB, Firestine AM, Arevalo JM, Ma J, Cole SW: **A functional genomic perspective on human well-being**. *Proc Natl Acad Sci U S A* 2013, **110**:13684-13689.

10. Snyder-Mackler N, Sanz J, Kohn JN, Voyles T, Pique-Regi R, Wilson ME, Barreiro LB, Tung J: **Social status alters chromatin accessibility and the gene regulatory response to glucocorticoid stimulation in rhesus macaques**. *Proc Natl Acad Sci U S A* 2019, **116**:1219-1228.

11. Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD, Cawthon RM: **Accelerated telomere shortening in response to life stress**. *Proc Natl Acad Sci U S A* 2004, **101**:17312-17315.

12. Cohen S, Murphy MLM, Prather AA: **Ten surprising facts about stressful life events and disease risk**. *Annu Rev Psychol* 2018, **70**:577-597.

13. Picard M, McEwen BS: **Psychological stress and mitochondria: a systematic review**. *Psychosom Med* 2018, **80**:141.

14. Du J, Wang Y, Hunter R, Wei Y, Blumenthal R, Falke C, Khairova R, Zhou R, Yuan P, Machado-Vieira R: **Dynamic regulation of mitochondrial function by glucocorticoids**. *Proc Natl Acad Sci U S A* 2009, **106**:3543-3548.

15. •• Picard M, Juster R-P, McEwen BS: **Mitochondrial allostatic load puts the 'gluc' back in glucocorticoids**. *Nat Rev Endocrinol* 2014, **10**:303-310.

This review introduced the concept of mitochondrial allostatic load (MAL) which defines the structural and functional recalibrations that mitochondria undergo in response to metabolic and psychosocial stressors. MAL was proposed as a cellular mechanism by which stressful life experiences and their associated primary neuroendocrine mediators cause biological damage and increase disease risk.

16. Picard M, McEwen BS: **Psychological stress and mitochondria: a conceptual framework**. *Psychosom Med* 2018, **80**:126-140.

17. Tyrka AR, Parade SH, Price LH, Kao HT, Porton B, Philip NS, Welch ES, Carpenter LL: **Alterations of mitochondrial DNA copy number and telomere length with early adversity and psychopathology**. *Biol Psychiatry* 2015, **79**:78-86.

18. Cai N, Chang S, Li Y, Li Q, Hu J, Liang J, Song L, Kretzschmar W, Gan X, Nicod J *et al.*: **Molecular signatures of major depression**. *Curr Biol* 2015, **25**:1146-1156.

19. •• Picard M, Prather AA, Puterman E, Cuillerier A, Coccia M, Aschbacher K, Burelle Y, Epel ES: **A mitochondrial health index sensitive to mood and caregiving stress**. *Biol Psychiatry* 2018, **84**:9-17.

This study described a high-throughput index of mitochondrial health (MHI) for frozen human leukocytes. It provided initial evidence that daily positive mood predicts mitochondrial health in PBMCs on subsequent days but found no evidence for the reverse association, suggesting that mitochondria may respond to proximate emotional states within days.

20. Boeck C, Gumpp AM, Calzia E, Radermacher P, Waller C, Karabatsiakis A, Kolassa I-T: **The association between cortisol, oxytocin and immune cell mitochondrial oxygen consumption in postpartum women with childhood maltreatment**. *Psychoneuroendocrinology* 2018, **96**:69-77.

21. Karabatsiakis A, Bock C, Salinas-Manrique J, Kolassa S, Calzia E, Dietrich DE, Kolassa IT: **Mitochondrial respiration in peripheral blood mononuclear cells correlates with depressive subsymptoms and severity of major depression**. *Transl Psychiatry* 2014, **4**:e397.

22. •• Boeck C, Koenig AM, Schury K, Geiger ML, Karabatsiakis A, Wilker S, Waller C, Gündel H, Fegert JM, Calzia E: **Inflammation in adult women with a history of child maltreatment: the involvement of mitochondrial alterations and oxidative stress**. *Mitochondrion* 2016, **30**:197-207.

This study found that childhood trauma history was associated with changes in PBMC cellular respiration measures using respirometry. Specific mitochondrial respiratory parameters were also found to be positively correlated with IL-6, TNF-α, and interleukin 1β levels, providing initial evidence for a potential implication of cellular metabolism in systemic inflammation associated with adversity.

23. Lindqvist D, Wolkowitz OM, Picard M, Ohlsson L, Bersani FS, Fernström J, Westrin Å, Hough CM, Lin J, Reus VI: **Circulating cell-free mitochondrial DNA, but not leukocyte mitochondrial DNA copy number, is elevated in major depressive disorder**. *Neuropsychopharmacology* 2018, **43**:1557-1564.

24. •• Lindqvist D, Fernström J, Grudet C, Ljunggren L, Träskman-Bendz L, Ohlsson L, Westrin Å: **Increased plasma levels of circulating cell-free mitochondrial DNA in suicide attempters: associations with HPA-axis hyperactivity**. *Transl Psychiatry* 2016, **6**:e971.

This study found that suicide attempters had significantly higher plasma levels of circulating cell-free mtDNA (ccf-mtDNA), providing evidence linking psychological distress to mitochondrial signaling.

25. Hummel E, Hessas E, Müller S, Beiter T, Fisch M, Eibl A, Wolf O, Giebel B, Platen P, Kumsta R: **Cell-free DNA release under psychosocial and physical stress conditions**. *Transl Psychiatry* 2018, **8**:236.

26. Trumpff C, Marsland AL, Basualto-Alarcón C, Martin JL, Carroll JE, Sturm G, Vincent AE, Mosharov EV, Gu Z, Kaufman BA *et al.*: **Acute psychological stress increases circulating cell-free mitochondrial DNA**. *Psychoneuroendocrinology* 2019, **106**:268-276.

27. Pinti M, Cevenini E, Nasi M, De Biasi S, Salvioli S, Monti D, Benatti S, Gibellini L, Cotichini R, Stazi MA: **Circulating mitochondrial DNA increases with age and is a familiar trait: implications for "inflamm-aging"**. *Eur J Immunol* 2014, **44**:1552-1562.

28. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, Brohi K, Itagaki K, Hauser CJ: **Circulating mitochondrial DAMPs cause inflammatory responses to injury**. *Nature* 2010, **464**:104.

29. Kraja AT, Liu C, Fetterman JL, Graff M, Have CT, Gu C, Yanek LR, Feitosa MF, Arking DE, Chasman DI *et al.*: **Associations of mitochondrial and nuclear mitochondrial variants and genes with seven metabolic traits**. *Am J Hum Genet* 2018, **104**:112-138.

30. Zhang R, Wang Y, Ye K, Picard M, Gu Z: **Independent impacts of aging on mitochondrial DNA quantity and quality in humans**. *BMC Genomics* 2017, **18**:890.

31. Sun X, Johnson J, St John JC: **Global DNA methylation synergistically regulates the nuclear and mitochondrial genomes in glioblastoma cells**. *Nucleic Acids Res* 2018, **46**:5977-5995.

32. Field AE, Robertson NA, Wang T, Havas A, Ideker T, Adams PD: **DNA methylation clocks in aging: categories, causes, and consequences**. *Mol Cell* 2018, **71**:882-895.

33. Grady JP, Pickett SJ, Ng YS, Alston CL, Blakely EL, Hardy SA, Feeney CL, Bright AA, Schaefer AM, Gorman GS: **mtDNA heteroplasmy level and copy number indicate disease burden in m.3243A> G mitochondrial disease**. *EMBO Mol Med* 2018, **10**:e8262.

34. Urata M, Koga-Wada Y, Kayamori Y, Kang D: **Platelet contamination causes large variation as well as overestimation of mitochondrial DNA content of peripheral blood mononuclear cells**. *Ann Clin Biochem* 2008, **45**:513-514.

35. Han LKV, Josine E, Tyrka Audrey, Penninx Brenda WJH, Wolkowitz Owen M, Månsson Kristoffer NT, Lindqvist Daniel, Vinkers Christiaan H, Boks Marco P, Révész Dóra *et al.*: **Accelerating research on biological aging and mental health**. *Psychoneuroendocrinology* 2019, **106**:293-311.

36. Lutgendorf SK, Thaker PH, Arevalo JM, Goodheart MJ, Slavich GM, Sood AK, Cole SW: **Biobehavioral modulation of the exosome transcriptome in ovarian carcinoma**. *Cancer* 2018, **124**:580-586.

37. Wang Z, Gerstein M, Snyder M: **RNA-Seq: a revolutionary tool for transcriptomics**. *Nat Rev Genet* 2009, **10**:57-63.

38. Lopes S, Teplytska L, Vaz-Silva J, Dioli C, Trindade R, Morais M, Webhofer C, Maccarrone G, Almeida OFX, Turck CW *et al.*: **Tau deletion prevents stress-induced dendritic atrophy in prefrontal cortex: role of synaptic mitochondria**. *Cereb Cortex* 2017, **27**:2580-2591.

39. Chandramouli K, Qian PY: **Proteomics: challenges, techniques and possibilities to overcome biological sample complexity**. *Hum Genomics Proteomics* 2009, **2009**.

40. Ronsoni MF, Remor AP, Lopes MW, Hohl A, Troncoso IH, Leal RB, Boos GL, Kondageski C, Nunes JC, Linhares MN: **Mitochondrial respiration chain enzymatic activities in the human brain: methodological implications for tissue sampling and storage**. *Neurochem Res* 2016, **41**:880-891.

41. Tyrrell DJ, Bharadwaj MS, Van Horn CG, Kritchevsky SB, Nicklas BJ, Molina AJ: **Respirometric 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* 2014, **70**:1394-1399.

42. Kuznetsov AV, Veksler V, Gellerich FN, Saks V, Margreiter R, Kunz WS: **Analysis of mitochondrial function in situ in permeabilized muscle fibers, tissues and cells**. *Nat Protoc* 2008, **3**:965-976.

43. Mishra P, Chan DC: **Metabolic regulation of mitochondrial dynamics**. *J Cell Biol* 2016, **212**:379-387.

44. Naviaux RK, Naviaux JC, Li K, Bright AT, Alaynick WA, Wang L, Baxter A, Nathan N, Anderson W, Gordon E: **Metabolic features of chronic fatigue syndrome**. *Proc Natl Acad Sci U S A* 2016, **113**:E5472-E5480.

45. Ma S, Yim SH, Lee SG, Kim EB, Lee SR, Chang KT, Buffenstein R, Lewis KN, Park TJ, Miller RA *et al.*: **Organization of the mammalian metabolome according to organ function, lineage specialization, and longevity**. *Cell Metab* 2015, **22**:332-343.

46. Kragel PA, Koban L, Barrett LF, Wager TD: **Representation, pattern information, and brain signatures: from neurons to neuroimaging**. *Neuron* 2018, **99**:257-273.

47. Karamercan MA, Weiss SL, Villarroel JP, Guan Y, Werlin E, Figueredo R, Becker LB, Sims C: **Can peripheral blood mononuclear cells be used as a proxy for mitochondrial dysfunction in vital organs during hemorrhagic shock and resuscitation?** *Shock* 2013, **40**:476-484.

48. Yarkoni T, Westfall J: **Choosing prediction over explanation in psychology: lessons from machine learning**. *Perspect Psychol Sci* 2017, **12**:1100-1122.

49. 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**:43-50.

50. Reed R: **Stress and immunological aging**. *Curr Opin Behav Sci* 2019, **28**:38-43.

51. Juster R-P, McEwen BS, Lupien SJ: **Allostatic load biomarkers of chronic stress and impact on health and cognition**. *Neurosci Biobehav Rev* 2010, **35**:2-16.

52. Hedges CP, Woodhead JST, Wang HW, Mitchell CJ, Cameron-Smith D, Hickey AJR, Merry TL: **Peripheral blood mononuclear cells do not reflect skeletal muscle mitochondrial function or adaptation to high-intensity interval training in healthy young men**. *J Appl Physiol (1985)* 2018, **126**:454-461.

53. Wang PY, Ma W, Park JY, Celi FS, Arena R, Choi JW, Ali QA, Tripodi DJ, Zhuang J, Lago CU *et al.*: **Increased oxidative metabolism in the Li-Fraumeni syndrome**. *N Engl J Med* 2013, **368**:1027-1032.

54. Yoshino H, Nakagawa-Hattori Y, Kondo T, Mizuno Y: **Mitochondrial complex I and II activities of lymphocytes and platelets in Parkinson's disease**. *J Neural Transm Park Dis Dement Sect* 1992, **4**:27-34.

55. Mosconi L, Berti V, Guyara-Quinn C, McHugh P, Petrongolo G, Osorio RS, Connaughty C, Pupi A, Vallabhajosula S, Isaacson RS *et al.*: **Perimenopause and emergence of an Alzheimer's bioenergetic phenotype in brain and periphery**. *PLoS One* 2017, **12**:e0185926.

56. •• Chacko BK, Kramer PA, Ravi S, Johnson MS, Hardy RW, Ballinger SW, Darley-Usmar VM: **Methods for defining distinct bioenergetic profiles in platelets, lymphocytes, monocytes, and neutrophils, and the oxidative burst from human blood**. *Lab Invest* 2013, **93**:690.

This study quantified various aspects of mitochondrial respiration and glycolysis in isolated leukocyte cell types to reveal substantial differences in bioenergetic profiles and respiratory chain proteins between cell types. This work led to the development of the bioenergetic health index (BHI), an integrative index based on cellular respiration that remains to be evaluated in relation to psychosocial factors.

57. Sjovall F, Ehinger JK, Marelsson SE, Morota S, Frostner EA, Uchino H, Lundgren J, Arnbjornsson E, Hansson MJ, Fellman V *et al.*: **Mitochondrial respiration in human viable platelets–methodology and influence of gender, age and storage**. *Mitochondrion* 2013, **13**:7-14.

58. Shenouda SM, Widlansky ME, Chen K, Xu G, Holbrook M, Tabit CE, Hamburg NM, Frame AA, Caiano TL, Kluge MA *et al.*: **Altered mitochondrial dynamics contributes to endothelial dysfunction in diabetes mellitus**. *Circulation* 2011, **124**:444-453.