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OXPHOS capacity in human muscle tissue and body mass excess – the MitoEAGLE mission towards an integrative database

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Introduction

"Diseases that are strongly related to a sedentary life style are spreading world-wide at an epidemic scale. Mitochondrial dysfunction is increasingly associated with the progression of such pathologies: cause or consequence? There is currently no regimented, quantitative system, or database organized to routinely test, compare and monitor mitochondrial capacities within individuals, populations, or among populations. This reflects the need for scientific innovation and represents a shortcoming in the health system of our modern, rapidly aging society" (MitoEAGLE COST Action application). The working groups of the COST Action CA15203 have made substantial progress towards meeting the mission of Mitochondrial Fitness Mapping (**Fig. 1**). The present communication (*1*) provides an example of harmonization of datasets published by different research laboratories on OXPHOS capacity in isolated mitochondria and permeabilized fibers obtained from biopsies of human skeletal muscle (vastus lateralis); (*2*) emphasizes the importance of comparative protocol harmonization projects and reproducibility studies; (*3*) illustrates the necessity and difficulty of defining objective exclusion criteria and applying quality assessment of published data; (*4*) links muscle mitochondrial fitness to whole body aerobic fitness; (*5*) discusses the extension of tissue-specific to systemic mitochondrial fitness from muscle to brain; and (*6*) documents the added value of Open Access data repositories.

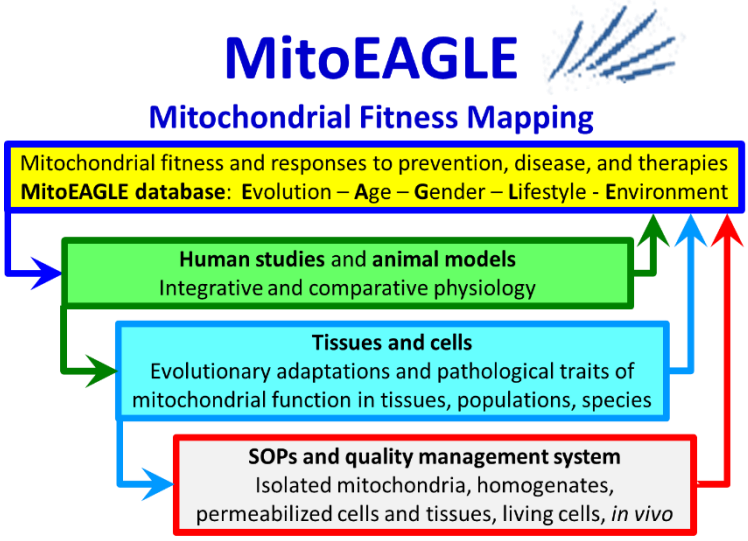

Figure 1: Challenges for initiation of a data repository designed to ultimately describe the linkage between the mt-phenotype and anthropometric variables. Modified after MitoEAGLE COST Action application.

Methods

Analogous to ergometric measurement of V_{O2max} on a cycle or treadmill, cell ergometry is based on measurement of OXPHOS-capacity, $J_{O2,P}$ [pmol $O_2 \cdot s^{-1} \cdot mg^{-1}$] equivalent to [µmol $O_2 \cdot s^{-1} \cdot kg^{-1}$], at the mitochondrial level. The main datasets on OXPHOS capacity of isolated mitochondria or permeabilized muscle fibers, harmonization algorithms, and exclusion criteria applied in the present analysis have been reviewed ten years ago [1]. Only a few more studies based on high-resolution respirometry published since then were integrated, exclusively on Caucasian healthy controls [2,3]. This 'MitoEAGLE BME database 1' is intended to initiate a comprehensive review by the MitoEAGLE Working Group 2 (skeletal muscle). Harmonization introduces potential biases with a scope of improvement based on updated evaluation of (*1*) wet/dry mass ratios applicable to studies reporting dry mass only; (*2*) flux control ratios applied to calculate combined NADH- and succinate-linked OXPHOS capacities from data limited to the NADH-pathway or succinate-pathway capacities measured separately; (*3*) temperature adjustment for measurements at temperatures different from 37 °C [4]; (*4*) oxygen limitation of measurements with

permeabilized fibers that are performed at or below air saturation [5]; (*5*) OXPHOS capacities reported without evaluation of saturating concentrations of ADP, P_i, and fuel substrates, or without concern of stable steady-state fluxes; and (*6*) potential bias when results are reported without details on instrumental O_2-background tests, calibrations, and corresponding corrections.

Results and discussion

Recent trends of an increasing body mass index (BMI) of the human population indicate an epidemic prevalence of obesity in many countries despite the fact that underweight remains the dominant problem in the world's poorest regions [6]. Extending the concept of the 'Reference Man' [7], a healthy reference population (HRP) is defined with a large range of body height (standing height, *H*) and corresponding reference body mass, *M*°, reference $V°_{O2max/M}$, and mitochondrial fitness parameters (**Fig. 2**). The reference mass/height relationship constitutes a basic component of the concept of the HRP, obtained from >17.000 measurements on healthy people reported between 1931 and 1944 before the fast food and soft drink epidemic, with about half of the reported measurements ranging from 1.2 to 1.8 m corresponding to *M*° of 22 to 68 kg and $H/M^{0.35}$ [8] (**Fig. 2a**).

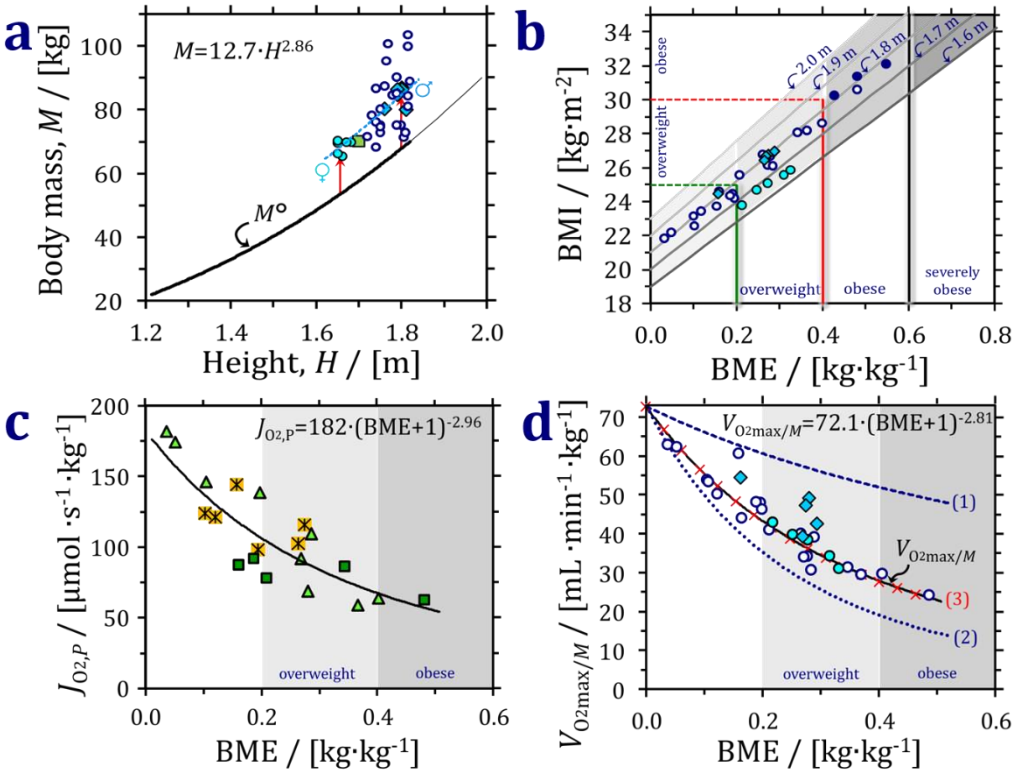

Figure 2: Anthropometrics of the healthy reference population, mitochondrial fitness and aerobic capacity. Full circles and diamonds are the averages of females and males with average height of 1.66 and 1.80 m, respectively, in the 2nd to 6th decade of life (from the Norwegian HUNT 3 fitness study [9]). Open circles are averages of healthy cohorts with average height of 1.77 m (MitoEAGLE 1 database). **(a)** Healthy reference population, HRP: thick line extrapolated by a thin line beyond the range of measurement, $H = 0.411 \cdot M°^{0.35}$ [8]. *M*° is the reference body mass corresponding to body height on the *X*-axis. The dashed line is the fit through the male and female HUNT 3 data. Vertical arrows indicate weight gain at constant body height. The green square is the Reference Man [7]. **(b)** The BMI with an exponent of 2 (instead of 2.86; Fig. 2b) increases with body mass in the HRP, from 18.9 to 22.9 with height increasing from 1.6 to 2.0 m. The body mass excess with respect to the HRP is defined as BME $\stackrel{def}{=}$ $(M-M°)/M°$. A balanced BME is BME°=0.0. Considering a height of 1.7 m (dashed horizontal lines), overweight (BMI=25) is reached at a weight gain of 20 % (BME=0.2); obesity and severe obesity (BMI=30 and 35) are reached at a weight gain of 40 % and 60 % (BME=0.4 and 0.6, respectively). **(c)** Mitochondrial fitness, $J_{O2,P}$ declines as a function of BME (MitoEAGLE BME database 1). $J_{O2,P}$ is the OXPHOS capacity of the convergent NADH- and succinate-linked pathway expressed

per wet mass of muscle tissue. **(d)** $V_{O2max/M}$ declines as a function of BME (MitoEAGLE BME database 1; a power function is fitted through the open circles, shown by the full line and extrapolated to BME=0; see equation). The females of the HUNT 3 study are on the line, whereas the males tend to have a higher aerobic capacity. Dashed line (1): Aerobic capacity modelled by adding metabolically inactive body mass to the reference $V°_{O2max/M}$=71.9 mL·min^{-1}·kg^{-1}. Dottel line (2): Diminishing muscle aerobic capacity according to the decline of mitochondrial fitness in Fig. 2c. Red crosses (3): A constant 'weight-lifting' factor is fitted to account for an increasing fraction of muscle mass as a function of BME. Modified from [10].

The body mass excess, BME, is defined as the excess of the actual body mass, *M*-*M*°, relative to the reference body mass, *M*°, at the same height (**Suppl. Tab. S1**). Deviations of *M* versus *M*° are due to weight gain without height gain. The similar displacement of men and women (Norwegian HUNT 3 study [9]) from the HRP line is consistent with the increase of average BMI in Norway during the past decades [6]. (**Fig. 2a**). BME>0 (excess) yields a more consistent index of overweight and obesity across a large range of body heights compared to the BMI (**Fig. 2b**). Similarly, BME<0 (not shown) indicates a body mass deficit which is insufficiently reflected by the BMI at different body heights. Mitochondrial OXPHOS capacity per mass of vastus lateralis declines as a power function of BME (**Fig. 2c**). $V_{O2max/M}$ can be modeled as a function of (*1*) the metabolically inactive (compared to V_{O2max}) body mass added to a person at height *H*, (*2*) the decline of mitochondrial capacity per muscle mass as a consequence of an inactive lifestyle and increased body mass, and (*3*) a slight increase of muscle mass with increasing BME as a 'weight-lifting' effect (**Fig. 2d**).

Taken together, the BME has a strong conceptual foundation on the level of large scale population statistics and is linked to lifestyle and mitochondrial fitness. Importantly, the BME has a straightforward understandable meaning that is easy to communicate to the general public on the personal level: you are overweight if your body mass is increased by 20 % relative to the reference body mass determined by your height. The consequences of mitochondrial control on $V_{O2max/M}$ will be discussed in terms of mechanistic explanations of a large range of neurodegenerative diseases related to the passive lifestyle with an increased BME [10].

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