Measuring mitochondrial protein synthesis to assess biogenesis

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Submitted 9 March 2012; accepted in final form 12 March 2012

IN THEIR PERSPECTIVES ARTICLE, Miller and Hamilton (6) propose that the term “mitochondrial biogenesis” be limited to the measurement of the synthesis rate of mitochondrial proteins. For support, they cite evidence that changes in the abundance of transcription factors for mitochondrial genes or the mRNAs encoding mitochondrial proteins may not result in corresponding changes in mtDNA or protein synthesis, while the number of mitochondria or concentration of proteins may not reflect the rate of protein turnover. This challenge is faced by investigators studying the regulation of many cell types and structures and highlights the complex layers of control between the genome and the proteome. The authors posit that measuring the synthesis rate of mitochondrial proteins is an optimal approach to determining whether a condition or intervention has an impact on mitochondrial biogenesis. Rather than focus only on protein synthesis rate, however, this outcome should be part of a more comprehensive assessment of mitochondrial function and regulation than implied by Miller and Hamilton.

Mitochondrial protein synthesis rate measurements are important. It should be recognized that a method to isolate mitochondria in human skeletal muscle and measure the in vivo incorporation of [1-14C]leucine to determine the fractional synthesis rate (FSR) was described by Rooyackers et al. (8) several years ago. In that seminal study, it was reported that the FSR of muscle mitochondrial proteins declined with aging in healthy men and women who were not active exercisers. This same methodology was used by our research group to reveal that skeletal muscle mitochondrial protein FSR is acutely increased during an infusion of insulin and amino acids in humans or pigs, supporting a hypothesis that insulin in combination with amino acids enhances mitochondrial biogenesis (2, 10). The pig model also demonstrated that mitochondrial protein FSR was not stimulated by insulin and amino acids in the liver or heart, highlighting the differential control of protein metabolism of the same organelle among unique tissues (2).

One might expect that muscle mitochondrial FSR would be altered by type 2 diabetes (T2D) because of the chronic exposure to hyperinsulinemia or the altered expression patterns in many mitochondrial gene transcripts that have been reported (1), but we found that mitochondrial protein FSR in adults with T2D was not different from that of age- and sex-matched people without T2D (3). Likewise, we found that when healthy humans were given an overnight infusion of growth hormone there was an increase in the activity of mitochondrial oxidative enzymes, the ATP production rate, and the abundance of some transcripts for mitochondrial transcription factors and oxidative enzyme proteins in skeletal muscle, but these events occurred in the absence of a change in mitochondrial protein FSR (9). The examples are cited to emphasize that mitochondrial protein synthesis is an important process to investigate but that it should not be studied alone without associated outcomes to put the results in context. Hopefully, Miller and Hamilton agree that well-designed studies should include complementary measurements of mitochondrial functions like oxidative phosphorylation, activities of individual enzymes or transporters, abundance of gene transcripts and proteins, and even morphological studies of mitochondrial size, number, cellular distribution, and connectivity. Although mitochondrial protein synthesis rate may be the best assessment of mitochondrial biogenesis, as the authors assert, it should be measured as part of a toolkit of methods used to understand the complex process of mitochondrial regulation.

As a word of caution about data interpretation, when differences in mitochondrial protein FSR do not correspond with gene transcripts or specific proteins, such observations do not exclude the possibility of coordinated regulation that operates on a time scale beyond the measurement period. In the case of acute or short-term perturbations in homeostasis, it is plausible to expect that changes in the abundance of many gene transcripts and regulatory proteins precede eventual changes in protein synthesis rate, although this may require many hours or days to manifest. Because of the complexity of the methodologies used to measure these outcomes, it is often difficult or impractical to perform repeated measures over time to map the time course of the many steps in mitochondrial regulation. However, there are examples using animal models that demonstrated, for example, that skeletal muscle contractile activity leads to sequential changes in genes and proteins that ultimately result in increased mitochondrial size, number, and/or function (4). Performing any one measurement of protein FSR or mRNA abundance at a single point in time would not likely reveal the complex but apparently coordinated regulation in the adaptive response to muscle contraction or other types of stimuli. However, if viewed in a broader context, one could assert that early changes in transcription factors and key regulatory proteins really should be considered part of the mitochondrial biogenesis pathway if those changes ultimately result in increased mitochondrial protein synthesis.

Another challenge in the measurement of mitochondrial protein FSR is timing of the measurement. In cross-sectional studies (e.g., comparisons of younger and older people, or people with a disease versus healthy controls), or in longitudinal interventions (e.g., exercise or weight reduction) a critical decision must be made about when to perform measurements because of the likelihood that protein synthesis rate fluctuates with amino acid availability, hormones, physical activity, or other factors. Protein synthesis and breakdown are very dynamic metabolic processes and often the FSR measurement represents only a snapshot in time that may not be generalizable beyond the specific conditions under study. In the case of skeletal muscle, for example, protein synthesis rates increase considerably when amino acids increase (e.g., following a
meal) (7). It is possible that the stimulatory effect of nutrients reveals (or obscures) differences in mitochondrial FSR between groups or intervention times when compared to the same measurements performed under basal (e.g. overnight fasting) conditions. Viewed in this light, it is also plausible to expect that mitochondrial FSR may not follow the same temporal patterns as the various gene transcripts and proteins that constitute and regulate mitochondria. Thus, investigators should be encouraged to measure mitochondrial protein synthesis as promoted by Miller and Hamilton but should give careful consideration to the experimental conditions and measurement timing so that the results acquired are most meaningful for their experimental question. This concern extends to studies performed under non-steady-state conditions (e.g., following a meal or shortly after exercise) but analyzed using steady-state stochastic models that may not be appropriate.

Finally, it should be noted that for some studies it may not be sufficient to measure the FSR of the mitochondrial protein fraction isolated as a whole from a tissue. Just as it is now appreciated that individual gene transcripts are expressed at different levels of abundance, the recent study by Jaleel et al. (5) revealed that within the mitochondrial protein fraction from skeletal muscle the FSR of individual proteins, even those with related functional roles, can vary by ~5-fold. How this occurs, the implications for protein function, and whether there is coordinated synthesis of the many proteins within mitochondria in response to various conditions are new challenges awaiting discovery.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: K.R.S. drafted manuscript; K.R.S. edited and revised manuscript; K.R.S. approved final version of manuscript.

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