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Metabolic Myopathies: "Human Knockout" Models and Translational Medicine

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Metabolic myopathies are rare diseases characterized by derangements of glycogen or lipid metabolism or mitochondrial function, caused by genetic mutations leading to defects of the main pathways of energy provision in skeletal muscle fibers.

The patients can be considered a sort of "human knockout" model, and present unique opportunities to investigate fundamental physiological mechanisms. One of the first authors to exploit this possibility was James Hagberg in the early 1980's, in a classic paper (Hagberg et al., 1982) carried out on patients with McArdle disease (McA). In McA patients the absence of myophosphorylase activity substantially impairs the flux of substrates along the glycolytic pathway (Lewis and Haller, 1986). At the end of an incremental exercise these patients typically reach exhaustion without any increase in blood lactic acid levels vs. those determined at rest (Hagberg et al., 1982), and no metabolic acidosis ensues during exercise. Hagberg et al. (1982) demonstrated that the "ventilatory threshold" (pulmonary ventilation [$\dot{V}E$] and CO_2 output [$\dot{V}CO_2$] "disproportionate increases" vs. that of O_2 uptake [$\dot{V}O_2$], occurring at about 50–70% of peak work rate) (Beaver et al., 1986) was not different in McA patients vs. controls, providing evidence against a causative role for blood lactic acid in the $\dot{V}E$ and $\dot{V}CO_2$ responses.

Mechanisms of metabolic regulation often present a redundancy, and within a complex system several factors may concur in determining a response. Thus, in strict terms the experimental "elimination" of one factor, and the observation of a normal response, does not mean that the factor, in physiological conditions, does not have a regulatory role. Nonetheless, in the example mentioned above [identification of the factor[s] responsible for the ventilatory threshold], McA patients represent indeed an ideal experimental model. Let's summarize the *scenario*. Experimental question: are the disproportionate increases in $\dot{V}E$ and $\dot{V}CO_2$, vs. that of $\dot{V}O_2$, observed during an incremental exercise, attributable to an increased elimination of CO_2 generated in tissues and blood as a consequence of H^+ buffering by bicarbonate? The experimental approach was straightforward: what happens in McA patients, in whom (without the need of any experimental intervention) no H^+ accumulation in muscles and blood occurs during exercise? The experimental data were clear: no differences for the $\dot{V}E$ or $\dot{V}CO_2$ vs. $\dot{V}O_2$ responses in the McA patients vs. the controls. Take home message: H^+ accumulation in blood is not necessary for the $\dot{V}E$ or $\dot{V}CO_2$ vs. $\dot{V}O_2$ disproportionate increases observed at 50-70% of peak work rate during an incremental exercise (Hagberg et al., 1982).

In McA patients the reduced flux of substrates along the glycolytic pathway limits the supply of substrates to the tricarboxylic acid cycle, thereby impairing oxidative metabolism, as also suggested by the slow $\dot{V}O_2$ kinetics during the transition from rest to exercise (Grassi et al., 2009) and by the slow phosphocreatine recovery kinetics during the recovery from exercise (Siciliano et al., 1995). A similar impairment, although by a different and more "downstream" cause (mutations leading to impairment[s] of enzyme[s] of the mitochondrial respiratory chain) is described in patients affected by another type of metabolic myopathy, the heterogeneous series of diseases termed "mitochondrial myopathies" (MM). Also in this respect McA and MM patients may function as human knockout models, allowing to elucidate basic physiological mechanisms.

An excellent example is represented by a set of compensatory responses occurring in the framework of a physiological adaptation, described over the years, thanks to the elegant work of different groups. In MM and McA patients the impaired oxidative metabolism [considered as whole body "peak" pulmonary $\dot{V}O_2$ (Linderholm et al., 1969; Lewis and Haller, 1986; Taivassalo et al., 2003; Grassi et al., 2007), or as muscle peak $\dot{V}O_2$ (Taivassalo et al., 2002)] is almost exclusively attributable to an impaired peak capacity of fractional O_2 extraction, i.e., to an impairment of the second term of the right-hand portion of the equation describing the Fick principle of conservation of mass (systemic arterial—mixed venous O_2 concentration difference, $C(a-\bar{v})O_2$; or "local" arterial—venous O_2 concentration difference, $C(a-v)O_2$). After putting fractional O_2 extraction in the equation expressing the Fick principle we obtain, at the whole body level:

$$\text{Pulmonary } \dot{V}O_2 = \text{cardiac output} \times C(a-\bar{v})O_2 \qquad (1)$$

On the other hand, if we consider the Fick principle of conservation of mass across a specific skeletal muscle group we obtain:

$$\text{Muscle } \dot{V}O_2 = \text{muscle blood flow} \times C(a-v)O_2 \qquad (2)$$

Several groups have documented decreased submaximal and peak $C(a-\bar{v})O_2$ in MM (Linderholm et al., 1969; Taivassalo et al., 2003) and McA (Lewis and Haller, 1986) patients, mainly by measuring cardiac output and $\dot{V}O_2$ during an incremental test. Other groups demonstrated a decreased submaximal and peak $C(a-v)O_2$ across an exercising limb (Taivassalo et al., 2002), by invasive measurements of CaO_2 and CvO_2, or indirectly by determining submaximal and peak fractional O_2 extraction in muscle by near-infrared spectroscopy (NIRS, see the review by Grassi and Quaresima, 2016) (Grassi et al., 2007).

These authors also described a highly significant and negative linear correlation between peak $\dot{V}O_2$ and the NIRS-determined peak fractional O_2 extraction (Grassi et al., 2007). Although a correlation does not imply cause-effect, within the patho-physiological context the data strongly suggest that in MM and McA patients the impaired peak fractional O_2 extraction is presumably the cause of the impaired peak $\dot{V}O_2$ (Grassi et al., 2007).

Oxidative metabolism is essential to sustain activities of exeryday life. Thus, it is reasonable to expect some compensatory mechanisms entailed by MM and McA patients in order to restore pulmonary or muscle $\dot{V}O_2$ values, or at least to attenuate the $\dot{V}O_2$ impairments attributable to the disease. How to accomplish this? The answer lies again in the equations expressing the Fick principle of conservation of mass mentioned above (Equations 1, 2): if the second term of the right-hand portion of the equations (fractional O_2 extraction) is impaired, the system tries to compensate for this impairment by increasing the first term of the right-hand portion of the equations (O_2 delivery), that is cardiac output or muscle blood flow.

This compensatory mechanism has been repeatedly demonstrated in MM and in McA patients, starting from the description, in the pioneering work by Linderholm et al.

(1969), of the "exaggerated" or "hyperkinetic" cardiovascular response to exercise. Higher O_2 delivery values vs. controls, for the same work rate, were subsequently described both in MM (see e.g., Taivassalo et al., 2002, 2003) and in McA patients (see e.g., Lewis and Haller, 1986). Grassi et al. (2007) considered the heart rate (HR) response a reasonable "proxy" of the cardiac output response, and described steeper HR vs. work rate relationships in McA and MM patients vs. controls. Interestingly, the slopes of the HR vs. work rate relationships were linearly related to the impairment of fractional O_2 extraction, as estimated by NIRS (Grassi et al., 2007). In other words, in the presence of a more severe impairment of oxidative metabolism, a more pronounced cardiovascular response was described; the signals involved in this "metaboreflex" are presumably different in MM and in McA patients. Enhanced systemic norepinephrine vasoconstriction and enhanced functional sympatholysis in working muscles have been described in MM patients (see e.g., Jeppesen and Vissing, 2019); in these patients a preminent role in peripheral vasodilation would be played by the ATP released by red blood cells (Jeppesen and Vissing, 2019).

The compensatory mechanism of enhanced O_2 delivery would manifest itself also at a morphological level. Taivassalo et al. (2012) have indeed demonstrated that in MM patients the iperkinetic cardiovascular response was associated with increased capillary and vascular angiogenic growth factor levels in skeletal muscles, the increased capillarity being more pronounced around the fibers with the most pronounced oxidative impairment. In other words, besides inducing an enhanced cardiovascular response the impaired skeletal muscle oxidative metabolism would also promote angiogenesis. Another beautiful example of physiological mechanisms uncovered by the study of the human knockout models.

Apart from the algebraic approach (see above), what would the physiological rationale be of increasing O_2 delivery to muscle fibers, which have problems in utilizing the O_2 they receive from the cardiovascular system? Porcelli et al. (2019) recently proposed an answer to this question by applying to MM patients the "Wagner's approach" (Wagner, 1996) evaluating perfusive and diffusive limitations in the O_2 pathway. In Figure 3 of that paper (Porcelli et al., 2019), peak O_2 delivery (cardiac output × CaO_2) in MM patients was considered to be normal (see Linderholm et al., 1969; Taivassalo et al., 2003). In the model peripheral O_2 diffusion is dictated by the Fick law of diffusion:

$$\dot{V}O_2 = DmO_2 \times (PmvO_2 - PiO_2) \qquad (3)$$

in which DmO_2 represents peripheral O_2 diffusive capacity, $PmvO_2$ microvascular partial pressure of O_2 and PiO_2 intracellular PO_2. In MM and McA patients $\dot{V}O_2$ at peak exercise is lower than normal because peripheral O_2 diffusion is impaired (see Figure 3 in Porcelli et al., 2019). This impairment occurs because $PiO2$ is presumably higher than normal (impaired intracellular oxidative metabolism), thereby decreasing the PO_2 gradient from the microvascular to the intracellular compartment. In this *scenario*, how can the partial pressures gradient be increased, thereby enhancing peripheral O_2 diffusion? The answer: an enhanced O_2 delivery would increase

PmvO$_2$, thereby increasing the driving pressure for the peripheral diffusion of the gas.

Whereas, at submaximal work rates the increased O$_2$ delivery can fully compensate for the impaired fractional O$_2$ extraction ($\dot{V}$O$_2$ being therefore substantially normal, for the same submaximal work rate, between patients and controls), the compensation may not be complete in "maximal" or "peak" conditions, that is when the patient reaches exhaustion. This translates into a lower peak $\dot{V}$O$_2$, with the associated impaired exercise tolerance. Peak $\dot{V}$O$_2$ values in MM and McA patients, although presenting a substantial variability among patients, are typically around 10–20 ml kg^{-1} min^{-1} (see e.g., Lewis and Haller, 1986; Taivassalo et al., 2003; Grassi et al., 2007; Porcelli et al., 2016, 2019), i.e., in a range compatible with patients belonging to NYHA class II-IV heart failure.

In conclusion, we hope we have convinced the reader that the human knockout models of MM and McA patients are ideally suited to be evaluated within a translational approach, which brings basic science methods and tools "to the bed of the patient." This can be accomplished by utilizing non-invasive experimental approaches developed over the years in the field of exercise physiology. In these patients the aims are to address specific issues related to basic pathophysiological mechanisms, to identify and quantify the impairment of skeletal muscle oxidative metabolism and the factors limiting exercise tolerance, and ultimately the patients' quality of life. The non-invasiveness of the adopted methods facilitates serial measurements, allowing clinical course of the diseases to be examined, as well as the efficacy of rehabilitation or therapeutical interventions.

Physiology and physiological research remain the essential link between genes, molecules and clinical care (Joyner, 2011; Wagner and Paterson, 2011). The –omics world may identify concepts and mechanisms, but only physiology can give a meaning to these concepts and mechanisms within the general picture of a human body, healthy or ill (Grassi et al., 2019).

AUTHOR CONTRIBUTIONS

BG, SP, and MM participated to the studies mentioned in the Opinion. BG wrote the first draft of the present manuscript. SP and MM participated in the discussion and in the editing of the manuscript.

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REFERENCES

Beaver, W. L., Wasserman, K., and Whipp, B. J. (1986). A new method for detecting the anaerobic threshold by gas exchange. *J. Appl. Physiol.* 60, 2020–2027. doi: 10.1152/jappl.1986.60.6.2020

Grassi, B., Marzorati, M., Lanfranconi, F., Ferri, A., Longaretti, M., Stucchi, A., et al. (2007). Impaired oxygen extraction in metabolic myopathies: detection and quantification by near-infrared spectroscopy. *Muscle Nerve.* 35, 510–520. doi: 10.1002/mus.20708

Grassi, B., Porcelli, S., and Marzorati, M. (2019). Translational medicine: exercise physiology applied to metabolic myopathies. *Med. Sci. Sports Exerc.* 51, 2183–2192. doi: 10.1249/MSS.0000000000002056

Grassi, B., Porcelli, S., Marzorati, M., Lanfranconi, F., Vago, P., Marconi, C., et al. (2009). Metabolic myopathies: functional evaluation by analysis of oxygen uptake kinetics. *Med. Sci. Sports Exerc.* 41, 2120–2127. doi: 10.1249/MSS.0b013e3181aae96b

Grassi, B., and Quaresima, V. (2016). Near-infrared spectroscopy and skeletal muscle oxidative function *in vivo* in health and disease: a review from an exercise physiology perspective. *J. Biomed. Optics.* 21:091313. doi: 10.1117/1.JBO.21.9.091313

Hagberg, J. M., Coyle, E. F., Carroll, J. E., Miller, J. F., Martin, W. H., and Brooke, M. E. (1982). Exercise hyperventilation in patients with McArdle's disease. *J. Appl. Physiol.* 68, 1393–1398. doi: 10.1152/jappl.1990.68.4.1393

Jeppesen, T. D., and Vissing, J. (2019). "The pathophysiology of exercise and effect of training in mitochondrial myopathies," in *Diagnosis and Management of Mitochondrial Disorders*, eds M. Mancuso, T. Klopstock (Basel; Springer Nature Switzerlamd), 331–348. doi: 10.1007/978-3-030-05517-2_20

Joyner, M. J. (2011). Giant sucking sound: can physiology fill the intellectual void left by the reductionists? *J. Appl. Physiol.* 111, 335–342. doi: 10.1152/japplphysiol.00565.2011

Lewis, S. F., and Haller, R. G. (1986). The pathophysiology of McArdle's disease: clues to regulation in exercise and fatigue. *J. Appl. Physiol.* 61, 391–401. doi: 10.1152/jappl.1986.61.2.391

Linderholm, H., Müller, R., Ringqvist, T., and Sörnäs, R. (1969). Hereditary abnormal muscle metabolism with hyperkinetic circulation during exercise. *Acta Med. Scand.* 185, 153–166. doi: 10.1111/j.0954-6820.1969.tb07314.x

Porcelli, S., Grassi, B., Poole, D. C., and Marzorati, M. (2019). Exercise intolerance in patients with mitochondrial myopathies: perfusive and diffusive limitations in the O$_2$ pathway. *Curr. Opinion Physiol.* 10, 202–209. doi: 10.1016/j.cophys.2019.05.011

Porcelli, S., Marzorati, M., Morandi, L., and Grassi, B. (2016). Home-based aerobic exercise training improves skeletal muscle oxidative metabolism in patients with metabolic myopathies. *J. Appl. Physiol.* 121, 699–708. doi: 10.1152/japplphysiol.00885.2015

Siciliano, G., Rossi, B., Martini, A., Angelini, C., Martinuzzi, A., Lodi, R., et al. (1995). Myophosphorylase deficiency affects muscle mitochondrial respiration as shown by ^{31}P-MR spectroscopy in a case with associated multifocal encephalopathy. *J. Neurol. Sci.* 128, 84–91. doi: 10.1016/0022-510X(94)00207-5

Taivassalo, T., Abbott, A., Wyrick, P., and Haller, R. G. (2002). Venous oxygen levels during aerobic forearm exercise: an index of impaired oxidative metabolism in mitochondrial myopathy. *Ann. Neurol.* 51, 38–44. doi: 10.1002/ana.10027

Taivassalo, T., Ayad, K., and Haller, R. G. (2012). Increased capillaries in mitochondrial myopathy: implications for the regulation of oxygen delivery. *Brain* 135, 53–61. doi: 10.1093/brain/awr293

Taivassalo, T., Jensen, T. D., Kennaway, N., DiMauro, S., Vissing, J., and Haller, R. G. (2003). The spectrum of exercise tolerance in mitochondrial myopathies: a study of 40 patients. *Brain* 126, 413–423. doi: 10.1093/brain/awg028

Wagner, P. D. (1996). Determinants of maximal oxygen transport and utilization. *Annu. Rev. Physiol.* 58, 21–50. doi: 10.1146/annurev.ph.58.030196.000321

Wagner, P. D., and Paterson, D. J. (2011). Physiology: found in translation. *Am. J. Physiol. Endocrinol. Metab.* 301, E427–E428. doi: 10.1152/ajpendo.00317.2011