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Effects of OXPHOS complex deficiencies and ESA dysfunction in working intact skeletal muscle: implications for mitochondrial myopathies

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ABSTRACT

The effects of inborn oxidative phosphorylation (OXPHOS) complex deficiencies or possible each-step activation (ESA) dysfunction on the bioenergetic system in working intact skeletal muscle are studied using a computer model of OXPHOS published previously. The curves representing the dependencies of $\dot{V}O_2$ and metabolite concentrations on single complex activity, entire OXPHOS activity or ESA intensity exhibit a characteristic threshold at some OXPHOS complex activity/ESA intensity. This threshold for $\dot{V}O_2$ of single complex activities is significantly lower in intact muscle during moderate and heavy work, than in isolated mitochondria in state 3. Metabolite concentrations and pH in working muscle start to change significantly at much higher OXPHOS complex activities/ESA intensities than $\dot{V}O_2$. The effect of entire OXPHOS deficiency or ESA dysfunction is potentially much stronger than the effect of a single complex deficiency. Implications of these findings for the genesis of mitochondrial myopathies are discussed. It is concluded that $\dot{V}O_2$ in state 3 and its dependence on complex activity in isolated mitochondria is not a universal quantitative determinant of the effect of mitochondrial dysfunctions *in vivo*. Moderate and severe mitochondria dysfunctions are defined: the former affect significantly only metabolite concentrations and pH, while the latter also decrease significantly $\dot{V}O_2$ in intact skeletal muscle during work. The dysfunction-caused decrease in $\dot{V}O_2$/oxidative ATP synthesis flux, disturbance of metabolite homeostasis, elevated ROS production and anaerobic glycolysis recruitment can account for such mitochondrial myopathy symptoms as muscle weakness, exercise intolerance (exertional fatigue) and lactic acidosis.

1. Introduction

Oxidative phosphorylation (OXPHOS) in mitochondria is responsible for most energy supply in the form of ATP in most muscles under most conditions. The rate of respiration ($\dot{V}O_2$) is proportional to the rate of aerobic ATP synthesis.

It has been demonstrated, through titration of OXPHOS complexes with specific inhibitors, that the curves representing the dependence of $\dot{V}O_2$ on the complex activity in isolated mitochondria in state 3 exhibit a characteristic threshold — above a certain threshold value of the complex activity $\dot{V}O_2$ is little affected by this activity, while below the threshold $\dot{V}O_2$ falls steeply with a decrease in complex activity (see e.g., [32,38,36]). This property was related to the genesis of inborn mitochondrial diseases, which develop below a certain threshold level of wild-type mtDNA/complex activity [32,31,36]. The threshold curves constitute also a basis for determination of flux control coefficients (fccs) defined within Metabolic Control Analysis (see Ref. [6] for review), determining the impact of a small change in complex activity on $\dot{V}O_2$. It has been demonstrated that the metabolic control over the respiration flux is distributed more or less uniformly among particular OXPHOS complexes (their fccs are of the same order of magnitude) in isolated mitochondria in state 3 [10,32,37].

Inborn OXPHOS complex deficiencies caused by mutations in mitochondrial or nuclear DNA (mtDNA and nDNA, respectively), can lead to various diseases, concerning mostly tissues with high relative energy demand (in relation to OXPHOS capacity), such as skeletal muscle or neural tissue [40,5,13,35,36,39,34]. They are characterized by a threshold value of an OXPHOS complex/whole OXPHOS activity below which the disease develops. This threshold increases with age, as mutations in mtDNA accumulate [40]. The deficiencies can potentially exert their effect through the decrease in the oxidative ATP supply flux, disturbances in metabolic homeostasis during work transitions, cytosol acidification or impact on the rate of ROS (reactive oxygen species) production. Mutations in mtDNA and nDNA (point mutations or deletions) can affect genes involved in protein synthesis in general (leading to entire OXPHOS deficiencies; for instance genes for tRNA, rRNA, genes controlling the number of mtDNA copies) as well as genes encoding particular OXPHOS complex units [40,5,36,39]. Mitochondrial dysfunctions can be somehow associated with calcium dysregulation [13] or skeletal muscle insulin resistance (restriction of glycogen deposition) [34]. Mitochondrial $\dot{V}O_2$ in state 3 correlates linearly with heteroplasmy

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of deleted mtDNA [7]. Most of mitochondrial diseases are due to a defect within the respiratory chain. Its complexes are composed of several subunits encoded in either nDNA or mtDNA. Most of the known mutations have been identified in mtDNA [36]. However, generally, the mechanisms responsible for the genesis and etiology of mitochondrial diseases are not well understood.

Brand and Nicholls proposed that $\dot{V}O_2$ in state 3 or, alternatively, RCR (respiratory control ratio = state 3 $\dot{V}O_2$/state 4 $\dot{V}O_2$) in isolated mitochondria is the best indicator of mitochondrial dysfunction [3].

It has been argued that random separation of mtDNA molecules during mitochondria divisions in organisms with some fraction of mutated mtDNA molecules (point mutations in single OXPHOS complex encoding genes) can lead to the so-called 'binary mitochondrial heteroplasmy', that is to the presence of two distinct mitochondria sub-populations, containing only wild-type or mutated mtDNA molecules [26]. The former would be fully active, the latter — fully inactive, containing only the non-functional form of a given OXPHOS complex encoded by the mutated gene, although they would contain wild-type molecules of other complexes encoded by not mutated genes. Therefore, such a situation would be equivalent to the entire OXPHOS deficiency [26]. Its impact on $\dot{V}O_2$ is much stronger than the impact of a single complex deficiency in the absence of 'binary mitochondrial heteroplasmy' [26]. Large deletions in mtDNA [5,7,35,36] or mutations in genes responsible for protein synthesis in mitochondria (mostly tRNA and rRNA genes) [40,5,13,35,36] would exert a similar effect.

Mitochondrial myopathies, affecting mostly skeletal muscle, have several symptoms, among others: 1. muscle weakness and atrophy; 2. exercise intolerance (exertional fatigue); 3. lactic acidosis (myocyte cytosol acidification related to lactate accumulation) [40,5,35,39]. The mechanisms underlying mitochondrial myopathies etiology are still not fully understood.

It has been postulated, using the computer model of OXPHOS in mitochondria and of the whole skeletal muscle bioenergetic system developed previously [25,15,27,23], that the main mechanism responsible for the regulation of OXPHOS during rest-to-work transition in skeletal muscle (and other tissues) is the so-called each-step activation (ESA) [15,16,19,21]. This mechanism is able to explain numerous system kinetic properties (see e.g., Refs. [16,19,21]). According to this mechanism, all OXPHOS complexes (complex I, complex III, complex IV, ATP synthase, ATP/ADP carrier, P_i carrier) and the NADH supply block are directly activated (probably through some mechanism involving, mostly cytosolic, Ca^{2+} and protein phosphorylation, see Refs. [15,16,19,21]) during muscle stimulation, in parallel with the activation of ATP usage (actomyosin-ATPase and Ca^{2+}-ATPase) by Ca^{2+}. In fact, it was proposed [24,18,21] that perfect ESA functions in the intact heart in vivo during low-to-high-work transition, where ATP demand is activated to the same extent as ATP supply and essentially no changes in metabolite concentrations (PCr, P_i, ADP, NADH) take place [12]. In skeletal muscle, the mixed mechanism (MM) takes place, in which OXPHOS is activated by ESA, but to a smaller extent than ATP usage, and therefore moderate changes in metabolite concentrations take place during rest-to-work transition and contribute to the regulation of OXPHOS [15,16,21]. Namely, an increase in the concentration of ATP hydrolysis products: ADP and P_i constitutes a negative-feedback signal that elevates oxidative ATP synthesis in working muscle.

A dysfunction of ESA for all OXPHOS complexes would be equivalent to a decrease in the 'effective activity' of all OXPHOS complexes (and therefore of entire OXPHOS) in working intact skeletal muscle (see below). Therefore, its effect would be formally similar (for a given work intensity) to the effect of the 'binary mitochondrial heteroplasmy' or to a decrease in mitochondria amount/entire OXPHOS activity. The difference is that ESA does not act at rest and that a complete lack of ESA during work does not mean a decrease of OXPHOS activity to zero, but to the resting value. ESA is of course absent in isolated mitochondria (at least in the absence of Ca^{2+}, see Discussion).

The computer model of OXPHOS and of the whole skeletal muscle bioenergetic system, that is also used in the present theoretical study, has been extensively validated for a broad range of variable values and system properties (see e.g., Refs. [19–21] for overview.) In particular, what is particularly important in the context of the present article, the model has been tested by comparison of computer simulations with the experimental values of flux control coefficients defined within Metabolic Control Analysis and with the whole inhibitor titration/threshold curves for OXPHOS complexes in isolated skeletal muscle mitochondria in state 3 [25,21].

In the present theoretical study the computer model of OXPHOS and of the whole skeletal muscle bioenergetic system is used to analyze the impact of a decrease in single complex/whole OXPHOS activity or in ESA intensity on the oxygen consumption flux and metabolite concentrations in isolated mitochondria in state 3 or in intact skeletal muscle during moderate and heavy work. It is hypothesized that: 1. An OXPHOS complex deficiency/inhibition that causes a huge fall in $\dot{V}O_2$ (and thus in oxidative ATP production) in isolated mitochondria in state 3 can have only a minor effect on $\dot{V}O_2$ in working intact skeletal muscle. This would be related to a higher value of the complex activity threshold, below which $\dot{V}O_2$ drops steeply, in isolated mitochondria than in intact muscle; and 2. In working intact skeletal muscle metabolite (especially ADP, PCr, P_i and Δp) concentrations and pH start to change significantly at much higher OXPHOS complex activities/ESA intensities, than those below which $\dot{V}O_2$ begins to fall significantly (such changes can disturb metabolism, in particular cause muscle fatigue). For both of these reasons, $\dot{V}O_2$ in state 3 or RCR (respiratory control ratio, state 3 respiration/state 4 respiration) in isolated mitochondria would not be universal determinants of the effect of mitochondria dysfunctions *in vivo*. Also the threshold values of single OXPHOS complex/entire OXPHOS activities for $\dot{V}O_2$ in isolated mitochondria in state 3 would not correspond quantitatively to the threshold values for $\dot{V}O_2$ and metabolite concentrations in working skeletal muscle. Additionally, the isolated mitochondria system cannot serve as a model for studying the effect of possible ESA dysfunctions in intact skeletal muscle (it can simply demonstrate what would happen, when ESA is absent at all). Finally, it is proposed to roughly classify mitochondrial dysfunctions as: 1. moderate dysfunctions that affect significantly metabolite and pH levels, but not $\dot{V}O_2$ in working intact skeletal muscle; 2. severe dysfunctions that affect significantly both metabolite concentrations (and pH) and $\dot{V}O_2$ in working intact skeletal muscle. Generally, it is argued that not only (the threshold in) $\dot{V}O_2$/oxidative ATP synthesis, but also (disturbance of) metabolite and pH homeostasis are strictly relevant for the genesis of mitochondrial myopathies.

2. Theoretical methods

2.1. Computer model

The theoretical model of OXPHOS in mitochondria and of the whole skeletal muscle cell bioenergetic system including anaerobic glycolysis developed previously [25,15,27,23] was used in the present study. This model comprises particular oxidative phosphorylation (OXPHOS) complexes (complex I, complex III, complex IV, ATP synthase, ATP/ADP carrier, P_i carrier), anaerobic glycolysis, creatine kinase (CK), ATP usage, NADH supply and proton efflux/influx.

The model has been broadly validated by comparison of its predictions with experimental data and used for numerous theoretical studies (see e.g., Refs. [19–21] for overview). Among others, what is particularly important in the context of the present study, the model has been tested for the values of flux control coefficient and the shapes of whole inhibitor titration/threshold curves of OXPHOS complexes in isolated skeletal muscle mitochondria in state 3 [25,21]. The complete model description