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LETTER TO THE EDITOR

Uncoupling mitochondrial uncoupling from alternative substrate utilization: implications for heavy intensity exercise

In their recent report, Bartlett *et al.* (2020) used phosphorus magnetic resonance spectroscopy to measure phosphorus metabolites non-invasively during 4 min of all-out exercise. They showed convincingly that the rates of oxidative ATP synthesis decreased over time in the face of an unaltered ATP cost of force generation, i.e. that the P/O ratio is reduced during all-out exercise. The authors interpret these exciting results as an indication that mitochondrial uncoupling occurs during maximal exercise. Traditionally, mitochondrial uncoupling is described as a dissipation of the proton motive force in which the activity of the electron transport system (mitochondrial complexes I–IV) is uncoupled from the rate of ATP production at ATP synthase. Although uncoupling is one mechanism by which P/O may be reduced, there exist other mechanisms besides dissipation of the proton motive force over the inner mitochondrial membrane. We propose an alternative explanation of the findings of Bartlett *et al.* (2020) that does not involve proton leak or inner mitochondrial membrane damage.

It is unlikely that a greater proton leak occurs during high-intensity exercise for a number of reasons. Firstly, Fernström *et al.* (2004) were unable to show upregulation of mitochondrial uncoupling proteins after 75 min of heavy exercise in humans, meaning that an acute upregulation of mitochondrial uncoupling proteins seems unlikely within the 4 min of all-out exercise in the protocol used by Bartlett *et al.* (2020). Secondly, even if proton leak does contribute to the reduced P/O ratio during exercise, modelling studies predict that this accounts for <1% of oxygen consumption during heavy exercise (Korzeniewski & Zoladz, 2015). Moreover, using isolated skeletal muscle mitochondria and physiological concentrations of substrates to mimic high-intensity exercise, Goncalves *et al.* (2015) demonstrated that electron leak causing reactive oxygen species (ROS) generation was 0.01% at conditions simulating 90% of maximal oxygen consumption. This indicates that the contribution of mitochondria to ROS-mediated membrane damage during exercise is vanishingly small. Hence, alternative mechanisms are required to explain the relative reduction in oxidative ATP synthesis observed over time during maximal exercise.

What other mechanisms could explain a reduction in the P/O ratio during high intensity exercise? Electrons from the conversion of NADH to NAD^+ (produced in the Krebs' cycle) normally enter the electron transport system via mitochondrial complex I, where 4 protons are pumped across the inner mitochondrial membrane to contribute to the proton motive force. Several other sources of reducing equivalents may contribute to electron transfer via other pathways. For example, $FADH_2$ produced via fatty-acid oxidation directly feeds electrons into ubiquinone via the electron transfer flavin. Convergent electron input into ubiquinone and complex III can also occur via other mechanisms. For instance, the Krebs' cycle intermediate succinate donates electrons (via $FADH_2$) directly into ubiquinone via succinate dehydrogenase (mitochondrial complex II). These pathways bypass the proton-pumping capacity of mitochondrial complex I and immediately reduce the P/O ratio by approximately 1.

High-resolution respiration experiments show that when only NADH-stimulated substrates are provided to mitochondria, oxidative phosphorylation is constrained by the maximal activity of complex I (Wüst *et al.* 2015). A recent modelling study by Nilsson *et al.* (2019) supports this concept and suggests that mitochondrial complex I is bypassed during high-intensity exercise in order to overcome this potential constraint. This strategy allows for greater rates of oxidative phosphorylation at the expense of substrate efficiency during high-intensity exercise. A metabolic model without complex I bypass was unable to account for the observed 'excess' oxygen consumption present during ramp exercise above the lactate threshold (notionally equivalent to the slow component observed during constant power exercise) (Nilsson *et al.* 2019). Increased rates of electrons bypassing mitochondrial complex I could therefore also account for the reduced P/O ratio observed during maximal all-out exercise observed by Bartlett *et al.* (2020).

The precise mechanisms of how mitochondrial complex I bypass occurs *in vivo* are currently unknown. It is likely that a high $NADH/NAD^+$ ratio causes a build-up of electrons activating other metabolic pathways to maximize oxidative phosphorylation capacity. Certainly, increased glycolytic flux results in cytosolic NADH production during high-intensity exercise, which is dissipated both by lactate accumulation and the glycerol-3-phosphate shuttle that transfers electrons to ubiquinone and into complex III. Indeed, an increased reliance on the glycerol-3-phosphate shuttle – and a consequent reduction of the P/O ratio – was proposed previously to be a contributory mechanism to the $\dot{V}_{O_2}$ slow component (Whipp, 1987; Özyener *et al.* 2001; Grassi *et al.* 2015). It is worth noting that the reliance on the glycerol-3-phosphate shuttle is greater in fibres that express greater glycolytic flux (Schantz & Henriksson, 1987), e.g. type II fibres that are highly activated during all-out exercise.

It is currently unknown how the electron donors NADH and $FADH_2$ alter their redox balance during high-intensity exercise. Whilst the suggested contribution of the glycerol-3-phosphate pathway has not yet been demonstrated in humans *in vivo* during whole-body exercise, it serves to illustrate that a reduced P/O ratio during heavy exercise may be attributable to factors other than a (ROS-mediated) dissipation of the protonmotive force.

In conclusion, we suggest that increased dissipation of the mitochondrial proton motive force is not the sole explanation for a reduction in P/O ratio during maximal, all-out exercise. Rather, differential electron transport routes (such as those bypassing mitochondrial complex I via the glycerol-3-phosphate shuttle) are likely to be increasingly contributory during high-intensity exercise, resulting in an immediate, transient and non-destructive reduction in the P/O ratio (Nilsson *et al.* 2019). The study of Bartlett *et al.* (2020) clearly paves the way for future studies aiming to assess whether the lower P/O ratio observed during high-intensity exercise is due to alternative

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substrate utilization or dissipation of the protonmotive force.

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Additional information

Competing interests

None declared.

Author contributions

Both authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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