Review of: "Modelling Skeletal Muscle Motor Unit Recruitment Contributions to Contractile Function: Part 3 - Substrate Oxidation of Phosphagen, Lipid, and Carbohydrate Metabolism"

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Potential competing interests: No potential competing interests to declare.

In the manuscript entitled '**Modelling Skeletal Muscle Motor Unit Recruitment Contributions to Contractile Function: Part 3 - Substrate Oxidation of Phosphagen, Lipid, and Carbohydrate Metabolism**," the authors expanded a previous model of vastus lateralis (VL) motor unit recruitment to analyze specific substrate oxidation by muscle fiber type. Using variable twitch frequencies and different motor unit recruitment profiles, the authors' model estimated the contribution of various energy systems (phosphagen, glycolytic, and mitochondrial respiration) in different fiber types using data on contractile power and ATP turnover. The results show that the phosphagen system and mitochondrial carbohydrate respiration are dominant in type I motor units, while glycolysis is prevalent in type II fibers. Fatty acid oxidation is more prevalent in slower-recruiting, slow-twitch units. This study highlights the need for further research on fiber-specific metabolism during various levels of exercise.

The work is well written, and the experiments are well thought out and well done.

Minor points

The experiments I propose could enrich the work with empirical data, providing a more detailed understanding of the energetic and metabolic mechanisms associated with different levels of muscle contraction.

1. I suggest to the authors a direct measurement of oxygen consumption: since the work relies on ATP data to estimate substrate oxidation, a direct experiment measuring oxygen consumption in vivo or ex vivo on isolated muscle fibers could further validate the model, providing quantitative details on the real contribution of each energy system (such as fatty acid and carbohydrate oxidation).
2. I also suggest to the authors an experiment performed on single muscle fibers: by performing experiments on single muscle fibers, one could compare metabolic activity in individual motor units rather than on mixed muscle samples, reducing variability and obtaining more specific data on the role of different fibers (e.g., type I and IIb).
3. I suggest to the authors an analysis of the enzymatic activity: since the energy pathways are described at the theoretical level, an enzymatic analysis that quantifies the activity of specific enzymes, such as creatine kinase, phosphofructokinase, and lactate dehydrogenase, could clarify the dependence of each fiber type on oxidative phosphorylation or glycolysis at different contraction intensities.

4. To improve the lactate production data, a dynamic analysis, such as the use of spectrophotometric techniques or microdialysis, would be useful to monitor lactate production in real time during various intensities of muscle contraction.
5. The authors could also use different stimuli to test metabolic plasticity: in fact, to evaluate how the model responds to variable stimuli, it would be interesting to apply different stimulation modalities, such as isometric versus concentric contractions, to see how these affect the relative participation of each energy system.
6. Finally, I propose to the authors to integrate measures of biomarkers for muscle damage: it could be useful to monitor specific biomarkers of muscle damage (such as serum creatine kinase) to correlate the intensity of contraction and the recruitment of motor units with potential muscle damage.