

Open peer review and authors' responses

Platelet bioenergetics are associated with resting metabolic rate and exercise capacity in older adult women

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Reviewer 2

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*Only major points from review and responses included.

Reviewer 2

Section 2.5

(1) I recommend mentioning, in a separate section prior to this, how (and in what tubes) blood was drawn and how the platelets were isolated (or refer to a previously published method).

(2) I strongly recommend indicating the concentration of cells in the O₂k chambers, and the chamber volume (2mL?). Which buffer was used, and at what temperature? (and referring to a previously published protocol/method for further details).

(3) The concentrations of injected chemicals seem to be those of the working stocks in the syringes, and the volumes are not indicated; I recommend stating the final concentrations in the chambers instead.

Authors

We have improved our methods by indicating that blood was collected in Acid citrate dextrose (ACD) tubes (Vacutainer; Becton Dickinson, Franklin Lakes, NJ), and we have added a more detailed protocol for how our platelets were isolated. Please see the new section, Section 2.3, titled "Platelet isolation."

In section 2.4 "High-resolution respirometry," we have included that 2×10^8 cells per 2mL chamber were used. We further clarified the buffer used (MiR05) and temperature (37°C).

We have included the final concentrations for each chemical used within the chamber, instead of the stock concentration. The submitted manuscript contained a typo; the concentrations written were meant to be M, not mM. Please see section 2.4 "High-resolution respirometry" for the updated values of final concentrations per chemical per chamber. Additionally, it is also noted in this section that the concentrations listed are final concentrations per chemical per chamber.

Reviewer 2

Figure 1, 2 and 3, Table 1, including related text:

(1) Mitochondrial respiration in cells is typically presented as O₂ flow per cell (or million cells), not per volume. I recommend converting the values (changing the Layout in DatLab).

(2) Are the values presented corrected for non-mitochondrial respiration?

Authors

First, we have updated the graphs in the figures and table to reflect mitochondrial respiration as O₂ flow per cell (amol).

Second, no, we utilized absolute values without subtracting non-mitochondrial respiration. This has now been noted in section 3.1 "Participant characteristics and platelet respiration."

Reviewer 2

Discussion:

(1) Has normalization to mitochondrial content been considered, e.g. citrate synthase activity or mtDNA copy number, in the platelets of your study participants? Could the correlation to RMR and peak RER at least in part be due to differences in the number of mitochondria or mitochondrial components per platelet? Or something else? Something around this could be added to the discussion.

(2) In addition to absolute values of mitochondrial respiration, respiratory control ratios could also be considered in further analyses.

Authors

First, thank you for bringing up this great point on considering normalization to mitochondrial content. We have considered normalization to mitochondrial content as we have done in other studies, however we no longer have access to biological samples from this cohort, so performing these experiments is not possible at this point. This is a secondary data analysis manuscript, and therefore we are unable to perform the additional experiments suggested.

Second, we appreciate the feedback that respiratory control ratios could be considered in further analyses. In our study, we decided not to focus on respiratory control ratios, which are often used to report on the quality of the sample preparation. Instead, we have used cytochrome *c* response as an internal quality control measurement, which can report on membrane damage and integrity. The use of ratios, such as RCR are also useful when comparing data across different studies or laboratories. In this study, all

experiments were performed at the same site using the same machines by the same technician. For these reasons, we were confident that we could complete our analyses using absolute respiration values, rather than ratios, which can complicate standard statistical approaches.