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

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Reviewer 1

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*Only major points from review and responses included.

Reviewer 1

The authors should aim to harmonized the definition of the study cohort within the manuscript: Title: "older adult women"; Abstract: "in women over 60 years of age"; Introduction: "healthy adult women (mean age = 70.2)"; Results: "healthy older adult women (mean age 70.2 ± 1.1)"; Discussion: "in healthy women over 60 years of age". Why specifically ">60" when the mean age is 70.2 [1.1]? Does this refer to a certain age-related threshold or is it necessary to state "over 60"?

Authors

Thank you; we have harmonized the description of the study cohort to say: "older adult women" in each section mentioned above. This description now matches the title. There is no age-related threshold. The reason we stated "over 60" in the first version of the manuscript was to describe the minimum age of participants enrolled in this study.

Reviewer 1

Materials and Methods section: A detailed description of isolation and preparation protocol for human platelets from whole blood (?) is missing, but would be very helpful/important to harmonize protocols and allow comparison of the respiratory data generated within other laboratories. This is important because the prevention of platelet activation during the procedure is important and sometimes platelet inhibitors are used,

which is not clear here (Siewiera, K. et al. Sample Preparation as a Critical Aspect of Blood Platelet Mitochondrial Respiration Measurements—The Impact of Platelet Activation on Mitochondrial Respiration. Int. J. Mol. Sci. 2021, 22, 9332. https://doi.org/10.3390/ijms22179332).

Authors

Thank you for suggesting this improvement. We have included this relevant information in our methods section of the manuscript. Please see the new section, Section 2.3, titled "Platelet Isolation" which describes the following: Acid citrate dextrose (ACD) tubes (Vacutainer; Becton Dickinson, Franklin Lakes, NJ) were used to collect venous blood from overnight-fasted participants. Samples were processed immediately to isolate platelets using previously described methods (Chacko 2014). Briefly, whole blood was centrifuged (500×g, 15 min, room temperature). Platelet-rich plasma was removed and centrifuged (1500×g, 10 min) to isolate platelets, washed in PBS with 1μM prostaglandin E1 (PGE1; Cayman Chemical, Ann Arbor, MI), centrifuged (1500×g, 7 min), and resuspended in Mir05 before high resolution respirometry. Cells were counted using the Coulter AC.Tdiff2 machine (Beckman Coulter, USA).

Reviewer 1

Furthermore, it's not stated how many platelet cells were used per chamber and why the oxygen consumption rate given as pmol/(s*ml) is not normalized to cell number (per Million cells)? This should be explained and added by the authors.

Authors

Thank you for pointing this out. In section 2.4 "High-resolution respirometry," we have included that 2 x10^8 cells per 2mL chamber were used. Regarding the oxygen consumption rate, we have changed these values to report flow per cell (amol) instead of flow per chamber (pmol). It makes more sense conceptually to report flow per cell based on our focus on cellular bioenergetics.

Reviewer 1

Materials and Methods section: (1) the concentrations for each chemical used for High-resolution Respirometry are representing the stock solution concentrations but not the final concentrations per chamber, e.g "ADP (0.5M)" or "FCCP, in 1mM steps" (it's actually 1μM step). This should be corrected and final concentrations within the chamber stated here. (2) Also, which medium was used for respiration assays (Mir06), and why not Mir05 (without Catalase)? According to Figure 1, there's no need for re-oxygenation. At least this should be explained in more detail. (3) Finally, why did the authors choose that specific SUIT protocol including FAO via octanoylcarnitine? (4) What is the physiological readout of each individual respiratory state, because they all correlate in the very same way with RMR and Peak RER. This method description and discussion could be improved by the authors.

Authors

First, 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 listed as M, not mM. Please see methods

section 2.4 "High-resolution respirometry" for the updated values of final concentrations per chemical per chamber.

Second, MiR05 (without catalase) was used for these experiments, instead of MiR06. Catalase was injected as the first step of this SUIT protocol in case reoxygenation was necessary. We have noted in our methods in section 2.4 that although catalase was added to MiR05 as a precaution, reoxygenation was not needed in this protocol.

Third, we use octanoylcarnitine because it is a medium chain fatty acid, and medium chain fatty acids are mostly obtained from dietary triglycerides. Also, for the measurement of FAO, we used a combination of octanoylcarnitine and malate to induce respiration from FAO. This combination bypasses the rate-limiting step of conversion of acyl-CoAs to acylcarnitines by CPT1, because octanoylcarnitine can freely cross the mitochondrial membrane. This has now been added to methods section 2.4.

Fourth, each of these respiratory states correspond to a distinct entry point into the ETS, e.g., F_p refers to Fatty Acid Oxidation and FN_p refers to Fatty Acid Oxidation + Complex I, etc. These respiratory states have been described in our previous work as FAO, FAO+Complex I, FAO+Complex I+Complex II, Maximal Uncoupled Respiration, and Max ETS (Mahapatra 2018). We have stated this explanation for our former nomenclature in Section 3.1 "Participant characteristics and platelet respiration."

Reviewer 1

It still remains unclear, why in particular platelets were investigated here and whether other types of blood cells (PBMCs, lymphocytes etc.) show a similar relationship with RMR and Peak RER. Here, the authors could further improve the discussion with an outlook or limitations section.

Authors

Thank you for pointing out this opportunity for clarity and explanation. Please see the Discussion section outlining why we have chosen to use platelets for this study and how this might relate to other blood cell types. We have added the following to the discussion: "We focused our attention on platelets in this study as previous data has indicated that platelet bioenergetic capacity is correlated to the bioenergetic capacity of peripheral tissues and is indicative to physical function. In particular, prior work has indicated that maximal and ATP-linked respirometry, specifically in older adults, has been significantly correlated with muscle maximal respiration that differs based on age (Braganza et al 2019). This, and other work, suggests that investigating platelet bioenergetic capacity may serve as a supplement and/or surrogate for measurements derived from muscle biopsies. This also provides evidence that platelets from an older adult population are a good indicator of muscle bioenergetics and could also be indicative of differences in clinical measurements of physical function and health."

We would also like to note that while we did analyze PBMCs, and found this fraction to be contaminated with platelets and potentially other cell types. The level of contamination was variable; thus we chose to only investigate the clinical relationships with platelets for this study as we are confident about the purity of this cell population. While other circulating cell types were not evaluated in this study, it is possible that similar relationships may exist. This would need to be confirmed by future studies.

Reviewer 1

(1) The authors conclude that blood cells are able to recapitulate skeletal muscle bioenergetics; however, this study is not designed to answer this question or contribute further evidence as the individual state of muscle bioenergetics was not investigated/is unknown. This should be rephrased by the authors. (2) Also, what does "systemic bioenergetic capacity" refer to and how does it interrelate with platelet mitochondrial function?

Authors

First, we agree that this study is not designed to arrive at the conclusion originally stated, and this has been removed from the conclusions section.

Second, "systemic bioenergetic capacity" refers to the ability of blood cell bioenergetic capacity to report on the bioenergetic capacity of peripheral cell types, such as brain, skeletal and cardiac muscle (Nguyen et al 2019; Mahapatra et al 2018; Sjövall et al 2014; Tyrrell et al 2016; Braganza et al 2019).

In Introduction section 1.3 "Blood-based bioenergetics and study goals," we state: "There is mounting evidence that blood-based bioenergetic profiling can be utilized to report on systemic bioenergetic capacity, and is related to mitochondrial function measured in other tissues (Nguyen et al 2019; Mahapatra et al 2018; Sjövall et al 2014). Our group has shown that blood cell respirometry correlates with skeletal and cardiac muscle respirometry (Tyrrell et al 2016). In particular, platelet mitochondrial function has been reported to be correlated with skeletal muscle mitochondrial function and exhibit bioenergetic changes associated with age in humans (Braganza et al 2019). Thus, these data suggest that systemic bioenergetic measurements can potentially be utilized to elucidate mitochondrial mechanisms underlying physical performance and decline."