Webinar Q&A Report:
Thermal Physiology: The Effects of Environmental Temperatures on Energy Expenditure in Mice

1. **How does the home cage environment, with bedding vs. a center-feeder cage, with plastic floor, impact energy expenditure?**

In our hands the data produced using the center-feeder chambers and the home cage chambers with wood chip bedding are remarkable similar (doi.org/10.1016/j.celrep.2020.03.065). However, using a different type of bedding or nesting material may affect the result.

2. **How does the TNZ/TNP depend on the assay conditions?**

We have tried to minimize TNZ/TNP dependence on the assay conditions and have compared 24h vs 2-4h at a specified ambient temperature and do not see assay dependence. We do avoid appx 2h before to 2h after a light cycle change.

3. **Some of us "rebels" feel that indirect calorimetry is fundamentally flawed, e.g. if the O_2 to ATP assumptions are incorrect. Might this explain the surprising Tb-TNP-TNZ relationship you found?**

We do not think so. The Tb-TNP-TNZ relationship is fundamentally based on EE vs Ta analysis. So, changes in how EE is calculated should not change the analysis.

4. **Do you think that mice housed at thermoneutrality are truly physiologically humanized?**

No. Our conclusion is that mice have fundamentally, qualitatively different thermal physiology from humans. As discussed, we can adjust how we study mice to minimize these effects. But I would be reluctant to say that mice housed at thermoneutrality are truly physiologically humanized.

5. **Is TNP/TNZ similar in different strains of mice?**

The TNP/TNZ is similar across multiple types of mice: male/female, chow/high fat diet, C57BL/6J vs 129. It is different in ob/ob mice, which behave physiologically as if they are starving and defend a lower body temperature in both the light and dark phases. The TNP/TNZ are changed in ob/ob due to the lowered body temperature set points (doi.org/10.1016/j.celrep.2020.03.065).

6. **Will the TNP change when the humidity changes? Will the mouse's temperature sensations change?**

The humidity is important for evaporative heat loss, but evaporative heat loss is not a major mechanism of heat loss in the mouse (discussed briefly in doi:10.1152/ajpendo.00133.2020). We do not expect much of an effect, but we have not studied this.

7. **Do you think differences in light and dark TNP is related to differences in RER (using more fat during the light cycle)?**

No. The differences in TNPD/TNPL are due to the different body temperature set points.

8. **Do you agree that if the RER increases over 1 it indicates increased DNL?**

Aside from technical problems, that is the likely reason. You should see an RER over 1 when refeeding a CHO diet after a 24h fast.

9. **What is the meaning of an RER less than 0.7?**

A nice discussion of RQ less than 0.70 is in Am J Clin Nutr 1980 Vol. 33 Pages 1317-9.

10. **Chris Gordon showed mice will choose a cooler spot (in dark) in a thermal gradient. How does this relate to your suggestion to house mice at 28/29°C?**

We advocate 28-29°C when the purpose of studying the mice is to model more human-like conditions. Such conditions need not be what the mice would choose, if given a choice. The 28-29°C conditions allow the mouse to control its Tb and be in the zone where the Tb is not increased, while having less need for cold-induced thermogenesis.

11. **Since the TNP of mice are different between day and night, do you suggest to change Ta between day and night (if your calorimetry system allows it) to accurately study mice at a thermoneutral point?**

If one wants to study mice 'at thermoneutrality' a compulsively rigorous scenario would be: 1) Determine the EE vs Ta (Scholander) plot for your mice and calculate the TNPD and TNPL (33°C and 29°C, respectively in our hands). 2) Study the mice while synchronizing the ambient temperature inside the mouse cages to the the TNPD during the dark cycle and TNPL during the light cycle. These conditions are difficult to achieve in most mouse holding facilities.

12. **How can we calculate the TEE if the metabolism changes significantly during the long period recording?**

TEE is the average over the time period studied. If the TEE is changing, it is useful to show the graph of TEE vs time. You can also prespecify a specific time interval and then statistically compare your groups. Note that you get different information from acute (hrs-days) vs long term (weeks-months) metabolic rate studies, and we use indirect calorimetry for the former. For the latter we use a mass balance approach, see doi:10.1038/ijo.2012.105.

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