Webinar Q&A Report:
Sex, Sugar, Fat, and Heat: Factors That Affect Energy Budgets, Weight Management and Behaviors in Mice

1. **Are the differences in body composition in females attributed to hormonal changes only?**

   M. Morris: This would be hard to discern from this experimental design. We chose the 7-day feeding, in part, to ensure that each female mouse was through at little more than one estrus cycle. However, we did not stage the mice prior to these experiments, as that procedure could have added additional stress to these mice. Importantly, both sexes are 3 - 4 weeks post 'puberty.' However, we do not know if estrus or sex hormone rhythmicity is impacted by differences in housing temperature.

2. **How long does it take to invert the light/dark cycle?**

   L. Woodie: It takes about 5 days for animals to acclimate to a reversed light cycle.

3. **Could you discuss the interpretation of physiological responses to liquid sugar when it's not an ancestral part of their diets?**

   L. Woodie: Great point and even more relevant given that liquid sugar was not a part of the human ancestral diet either. Humans evolved getting their carbohydrates from solid sources (fruits, vegetables, etc.), which was not terribly different from the ancestral rodent source (berries and nuts). Thus, rodents are good models for human obesity not only because they gain weight and become metabolically unhealthy in response to the Western diet like humans, but also because their ancestral diet didn't include liquid high-sugar components either. So, the physiological responses we see in both humans and rodents are ancient reactions to a modern problem. Neither organism is well suited to metabolize *ad libitum* liquid sugar, which is part of the reason it is so detrimental to metabolic health. This also makes the EFG findings very interesting! A normally detrimental supplement (liquid sugar) can actually be metabolically helpful when timed to jumpstart metabolism, which really highlights the synergy between the circadian clock and nutrient metabolism.

4. **Should you also test sugar water only during two 6-hour periods during the light phase?**

   L. Woodie: This would indeed be interesting, although not the direction we wanted to go based on our previous solid fat TRF study in Metabolism. Since we time-restricted solid fat to the dark phase with *ad lib* sugar water and saw a mixed bag in metabolic improvements, we wanted to follow up and see how timing sugar during the dark phase could impact metabolic health in the Physiology & Behavior paper.

5. **It would be great to repeat these great experiments in castrated females and compare that group with both the intact and male mice. Weight gain in menopausal women is a particularly problematic public health problem.**

   M. Morris: Quite true. One problem with ovariectomy in mice, is how robust the weight gain is on low-fat diet. So, once the animals recovered from the surgery, the OVX mice would already weigh more and have greater adiposity. While this would complicate the outcomes during the HFHS feeding. If we looked at the change in body weight and body composition, as we did here, the data should be very revealing.

6. **In relationship to maintaining thermogenic housing temperatures and experimental outcomes, how does this really translate to human data and outcomes?**

   M. Morris: I think the biggest point to make here is the ability to make more relevant human disease models by housing mice at or near thermoneutrality. The work describing the creation of diet-induced arteriosclerosis and advanced NAFLD without genetic models or chemical lesions is very important. Further, the accumulating data that sub-thermoneutral housing is negatively impacting drug development and cancer research only adds to the importance of appropriate animal housing in research. For this study, it is important to remember that I was attempting to model a difference in energy expenditure, without a difference in physical activity. This was done in an attempt to assess how the difference of energy expenditure...say between sedentary or highly active people...impacts the acute metabolic response to overnutrition.

7. **There is a move toward having housing temperature gradients in housing systems, how can one evaluate that component when it isn't held constant?**

   M. Morris: I think one could start by analyzing the data across the appropriate cycle. A gradient housing system would cycle through one day. Keeping the data analysis to no less than this time frame would allow for the calculation of average temperature and daily indirect calorimetry outcomes.

8. **Do you think it comes down to the timing of the restricted period or the restriction itself? In other words, if you fed for a different restricted period, would you anticipate seeing the same results? Thinking of shift workers etc.**

   L. Woodie: Timing of restriction, definitely. Studies using TRF paradigms with only inactive phase feeding find even Chow fed animals are metabolically unhealthy. It is key to time nutrient intake to the time when the body is best equipped to metabolize nutrients. This is part of the reason shift workers tend to have a higher incidence of metabolic disease.

9. **Can you speculate on a mechanism for the drastic differences in total energy expenditure between the early vs. late sugar drinkers? For example, could these differences be caused by differences in activity levels or in body temperature?**

   L. Woodie: We were not able to measure temperature, but we did look at activity and VO2. The energy expenditure that we reported in the paper and that I discussed here is calculated by the Weir equation which has activity and VO2 baked in. We found that activity was not changed by liquid sugar (so the animals were not exhibiting anticipatory behaviors), but their diurnal VO2 exhibited the same pattern as energy expenditure. This suggests that liquid sugar timing is altering the metabolic rate of the animals not behaviors.

10. **Mice seem to be more dietary specialists than rats who naturally consume more sweet things. Would rats be a good model to examine these patterns?**

    L. Woodie: Rats are a great model for diet studies. Mice, however, have the advantage of being widely used. We wanted our studies to fit into the larger field of TRF studies all of which had used mice. For comparisons sake, we wished to use mice as well.

11. **What advice do you have for the 'average' researcher who is only studying male mice at 21°C for their research?**

    M. Morris: At the very least, step back from your research plan or collected data and ask yourself 'how could increased energy flux (greater energy expenditure and intake) be impacting my research question?'. It may be beyond the individual researcher's environment to alter the housing temperature...but the environment is most definitely impacting the researcher's questions.

12. **You mentioned that fecal energy losses could be useful to quantify. Do you think it is possible that differences in fecal energy losses could differ among different classes of macronutrients (e.g., lipids, fats, carbs)?**

    M. Morris: For the most part, glucose and protein loss in the feces is only due to extreme over-consumption or genetic mutations impacting uptake mechanisms. While lipid fecal loss is also due to mutations impacting uptake, increased fecal loss of lipids is observed in both animals and humans during changes from low- to medium- to high-fat diets. So, in the dietary paradigm I am utilizing, lipid loss would be my first question.

13. **The study began while mice were still growing, they were only about 5 weeks old. Could the differences seen between males and females be due to the differences in their growth rate?**

    M. Morris: Perhaps. But, the growth curve data for mice is almost always different by sex if you use pregnancy naïve females. Now, if you are talking about the differences in the body composition response to HFHS…again…maybe. However, the growth curve for both male and female c57Bl/6Js flattens at 5 weeks, right as we start these studies.

14. How would timing liquid sugar impact the metabolism of mice fed *ad libitum* Western Diet?

L. Woodie: What we would hope to observe is that timing liquid sugar could improve metabolic outcomes in the *ad lib* Western diet group. I speculate that the metabolic enhancement seen in the EFG group would be protective against some of the glucose handling deficits caused by *ad lib* Western diet feeding.

15. What are potential explanations for the EFG phenotype?

L. Woodie: We believe that early sugar water availability is working WITH the natural metabolic rhythms of the mice instead of AGAINST it. Mice exhibit a natural elevation in energy expenditure and metabolic activity during the first six hours of the day that progressively decreases as the light cycle approaches. Therefore, EFG is enhancing this natural rhythm and we believe this enhancement is behind the metabolic benefits observed in the EFG group.

16. Why did you just use male mice?

L. Woodie: We wanted to avoid the potential confounding factor of female mouse estrous cycling. It would be fantastic to perform these studies in female mice, but the controls and number of animals needed became too great for us to tackle in the present works.

17. Do you think the smaller size of the female mice was involved in the observed energy metabolism data?

M. Morris: Definitely. We published the analysis of covariance for total energy expenditure, with fat mass and fat-free mass as the co-variates. The adjusted total energy expenditure was higher in females compared to males, with fat-free mass being the primary determinant of this difference.

18. Did the difference in ambient housing temperature impact feeding behavior?

M. Morris: Not really. The 20°C mice ate more at each meal. But the meals were not longer, and they didn't have more meals. So, really, the only difference is that the 20°C mice had a faster rate of food intake.

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North America

Sable Systems International
Headquarters and North America Sales
3840 N. Commerce Street
North Las Vegas, NV 89032 U.S.A.

Tel: 1-800-330-0465 / +1 (702) 269-4445 (U.S.)
Tel: +1-866-217-6760 / +1 (702) 269-4445 (Canada)

Email: sales@sablesys.com

Europe

Sable Systems Europe GmbH
Ostendstr. 25
D-12459 Berlin, Germany

Tel: +49-30-5304-1002
Mobile: +49-176-2078-7008
Fax: +49-30-5304-1003

Email: sales@sablesys.com