Webinar Q&A Report:
Comfort Food: Effects of Stress and High-Fat Diets on Neuronal Activity and Mitochondrial Remodeling in Mice

1. **Is R_q affected by diurnal cycle?**

   J. Ayala: Since R_q is heavily influenced by feeding patterns, it can have a diurnal cycle depending on the diet the mouse is consuming. Chow fed mice eat little during the day and mostly at night, so their R_q will be close to 0.7 during the day and will increase at night as they eat. Since mice fed a high fat diet will mostly oxidize fat, their R_q will remain relatively flat throughout the day (unless there are carbs in the diet, and then the R_q will go up slightly at night).

2. **Have you found any sex-specific differences in the effects of stress?**

   J. Ayala: Yes, we have. Interestingly, female mice do not display hypophagia in response to restraint stress. We are also finding that, unlike male mice, female mice do not activate their LS Glp1r neurons in response to restraint stress.

3. **Could you clarify how the increased promotion of fat oxidation is different between HFD and exercise?**

   M. Robinson: HFD, particularly the 60% fat diet as we used, is a robust stimulus for greater fat oxidation in mouse model which seems driven by greater abundance of lipid oxidation protein of mitochondria. The mitochondrial adaptation to exercise appears more broad (not just fat oxidation). In our study, exercise had a mild increase in fat oxidation beyond the already large increase during HFD versus LFD, which largely disappear when we adjust respiration rates for mitochondrial protein abundance.

4. **Why did you choose Octanoylcarnitine, a medium-chain acylcarnitine, as the main substrate to test lipid oxidation in the mitochondria?**

   M. Robinson: We chose Oct-car because it has no diffusion limitations (being shorter) or transport limitations (being in acylcarnitine form). The data should be interpreted as an upper limit, since respiration has little hinderance. A good comparison for CPT activity is using non-carnitine forms.

5. **If we consider exercise as a form of stress, is it possible that what you showed could make also obese mice keep the same patterns of food intake even after intense sessions of exercise?**

   J. Ayala: Exercise is a very complicated intervention. Typically, we think of "stress" in the negative sense. While exercise is certainly "strenuous", it is not necessarily "stressful". Exercise also tends to have the opposite effect of stress in that it tends to promote food intake (as a feedback to the increased energy expenditure).

6. **Do you have some data on feeding behavior of mice submitted to a chronic stress model? If not, would you briefly comment on what you would expect?**

   J. Ayala: Yes, I presented results from our studies that repeated stress promotes overt hyperphagia in obese mice. In lean rodents, acute stress tends to reduce food intake and chronic stress appears to maintain that lowered food intake. Over time with chronic stress, there can be habituation that actually makes the effect of stress on food intake smaller.

7. **How can we translate these mouse studies to human brain responses?**

   J. Ayala: It is possible to do functional imaging in people to look at differences in activity of specific brain regions in lean vs. obese individuals in response to stress. There are some groups actually doing this.

8. **Is there evidence that intermittent fasting affects the mechanisms discussed in the webinar?**

   M. Robinson: Intermittent fasting and caloric restriction seem to have beneficial improvements, or at least sparing declines, in mitochondrial quality (respiration per unit) in part through targeted remodeling of mitochondria. Key questions remain regarding mechanisms and long-term response of how restrictions protect against loss of function over time, and may vary between substrates.

9. **In your obesity model, regular C57Bl6 mice are fed with high fat diet for weeks or months. I guess your animals are housed with environmental and social enrichment. How about enrichment during the stress experiment and the metabolic measures? Can the stress-induced hypophagia be exacerbated in normal mice because of poor housing conditions?**

   J. Ayala: Our mice have no enrichment, so the housing conditions during the stress session are the same as during the maintenance period. It is very possible that poor housing conditions can exacerbate the stress response. Indeed, one way to provoke chronic stress in rodents is to use chronic variable stress in which the rodents are exposed to different daily stressors. Some of these stressors include what would be considered poor housing conditions, such as wet bedding and different ambient temperatures.

10. **Were there any racial differences in your O2k studies using PC or OC/mal?**

    M. Robinson: We have not compared racial differences in fat oxidation, but this is a great consideration as others have indicated ATP production capacity can vary between people groups and diabetes status.

11. **What temperature were the animals housed at? Since its known that housing animals at room temperature rather than their thermoneutral zone vastly impacts food intake (increases), do you think this behavior may be altered at the different temperatures?**

    J. Ayala: Good question. Our mice were housed at below thermoneutral (23°C). Since feeding patterns can be affected by temperature, it is possible that stress responses are as well.

12. **Are there mitochondrial diseases that have obesity phenotype?**

    M. Robinson: Several mitochondrial diseases have impaired lipid oxidation, buildup of lipid intermediates etc. (such as MCAD deficiencies). The causal relationship between disease and obesity needs further consideration since mitochondrial disorders often include exercise intolerance and therefore susceptibility to obesity (in mouse and human models). We will need to separate the specific impairments on physical activity from obesity risk.

13. **How does your study differentiate gut and brain-produced GLP-1?**

    J. Ayala: Since gut secreted Glp1 has such a short half life, it is very unlikely that this is the source of Glp1 involved in the stress response. Stephan Trapp's group in the UK has shown that inhibiting brain Glp1 producing neurons blocks the hypophagic effect of restraint stress, so we believe that to be the relevant source of Glp1.

14. **What is the role of glucocorticoids (corticosterone in rodents) in these stress-induced obesity models since it is a hormone released under stress and has known effects on promoting obesity?**

    J. Ayala: This is something we are looking into. James Herman's group in Cincinnati has shown that glucocorticoids can affect Glp1 secretion and mRNA processing in the hindbrain, so it is very possible that this plays a role in what we are studying. Glucocorticoids themselves have been shown to promote the consumption of palatable substances. Whether this is via direct mechanisms or indirectly by adjusting Glp1 tone is not clear to me.

15. **Have you measured ROS production in the oxygraph with either substrate?**

    M. Robinson: We have measured ROS in the oxygraph and consistently have greater electron leak to H_2O_2 during lipid respiration compared to complex I or II substrates. These substrate differences are much larger than, and not modified much by, diet or exercise effects.

16. **Is there a difference in the energy behavior of mitochondria in different adipose tissues?**

    M. Robinson: Tissue differences is an excellent consideration. We have not respired adipose tissues, but other groups demonstrate lower total respiration in fat than skeletal muscle, yet appear to have greater reliance on lipids (as a percentage of total capacity). Such differences align with nutrient differences between tissues. Future considerations will likely consider the organ cross talk for nutrient turnover.

17. **Is the attenuated effect of stressor on obese mice specific to the feeding regulatory circuitry or more generally due to their sensitivity to stressors?**

    J. Ayala: While our data would suggest that at least some feeding related circuitry is affected by obesity, it is difficult to know whether this is due to a difference in the sensitivity to stressors. We would like to know what it is about stress that regulates these circuits in the first place in order to begin to address that question.

18. **Is it possible that mitochondrial capacity differs among major tissue groups (e.g., mitochondria in muscle versus neurons versus hepatic tissue)?**

    M. Robinson: Indeed yes, and the variance between tissues should be considered as we look at the appearance and clearance of substrates from circulation, and the energetic demands of each tissue. Skeletal muscle has high ATP demands to support contraction, and capacity is often high, while liver respiration appears linked to TCA cycle flux and support of gluconeogensis etc. Adipose tissue respiration is lower per tissue weight, but often higher lipid oxidation relative to total capacity. Such tissue differences mirror the energetic demands, and future directions have exciting possibilities for cross talk in response to changing supply and demands.

19. **The natural 'exercise regime' of mice is to run full speed for several seconds and then take a break and then run full speed again. Is that similar enough to be considered analogous to HIIT (High Intensity Interval Training) in humans?**

    M. Robinson: Great observation on running behavior. We chose an interval style training approach to mimic their natural running approach. Although similar to interval training in humans (high then lower intensity), the mice are not exceeding maximal workload achieved at VO2 peak, while interval training in humans often exceeds work capacity at VO2peak.

20. **Are these finding similar in male and female mice?**

    M. Robinson: Sex differences are very pertinent; thank you for rising this critical point. There seem to be divergent responses between males and females regarding mitochondrial adaptations, possibly related to females having higher baseline respiration and possibly greater protein turnover (fewer studies, but protein synthesis and degradation is often higher in females). We recently completed similar feeding and training studies in female mice, so this is ongoing consideration. Work from Dr. John Thyfault's group indicates exercise adaptations in females are dependent on BNIP activation, emphasizing sex differences in degradation pathways.

    J. Ayala: Interestingly, female mice do not display hypophagia in response to restraint stress. We are also finding that, unlike male mice, female mice do not activate their LS Glp1r neurons in response to restraint stress.

21. **Is Exendin affect NAPE-PLD (used for production of endogenous endocannabinoid)?**

    J. Ayala: We know that Ex4 decreases 2-AG levels, but at this point we don't know how. 2-AG is produced by DAG lipase, so it is different than other endocannabinoids produced by NAPE-PLPD.

22. **In the Promethion cages, is it possible to link the amount of wheel running and the food access?**

    Yes, this is possible. See Lark DS. Diabetes. 2018 67(5):831-840

23. **Can this study solve the problem of mitochondrial cytopathies? And what about maternal inheritance of mitochondria in mice?**

    M. Robinson: We have not considered maternal inheritance in our group, although this is a great consideration given that impaired respiration, and insulin resistance, can occur in off-spring of parents with insulin resistance or type 2 diabetes.

24. **Was there any structural remodeling of the mitochondria?**

    M. Robinson: This is great consideration. Unfortunately, we have not analyzed samples for structural considerations (such as via microscopy). Dr. Marco Sandri's group reported autophagy null mice (Atg7 KO) have evidence of disrupted fibers (2009). We cannot rule out alterations in structural remodeling with high-fat feeding (indicated by strong remodeling of proteome and respiratory function).

25. **How are the mitochondrial respiration normalized to be able to compare between different individuals' biopsies or with mitochondria from different animals?**

    M. Robinson: Good question regarding normalization parameters across people or animals. Normalizing respiration to protein abundance (either mitochondrial or tissue weight weight) helps account for variations in isolation yield or tissue size. We also use total abundance measures (such as western blot or proteomics) to account for potential differences in absolute mitochondrial abundance. Together, these data help identify if changes or differences in respiration are due to total abundance or intrinsic remodeling of mitochondria.

26. **How certain it is that the neuronal activity is reduced in obese animals? Can it be reduced GCaMP3 expression, or some other trivial explanation?**

    J. Ayala: We verify GCaMP expression in all of our animals and do not see a difference between lean and obese mice.

27. **Does stress correlate with inflammatory cytokine levels? Does age affect it the stress response?**

    J. Ayala: Yes, there is a link between stress and various pro- and anti-inflammatory cytokines. We have not looked at different ages, so I am not sure.

If you have additional questions for Sable Systems International regarding content from their webinar or wish to receive additional information about their products and laboratory services, please contact them by phone or email:

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