Effects of Age on Food Entrainment in Mice

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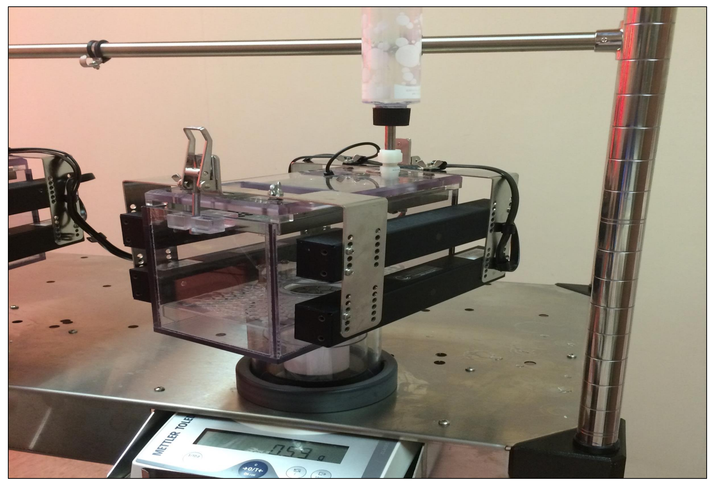
Introduction

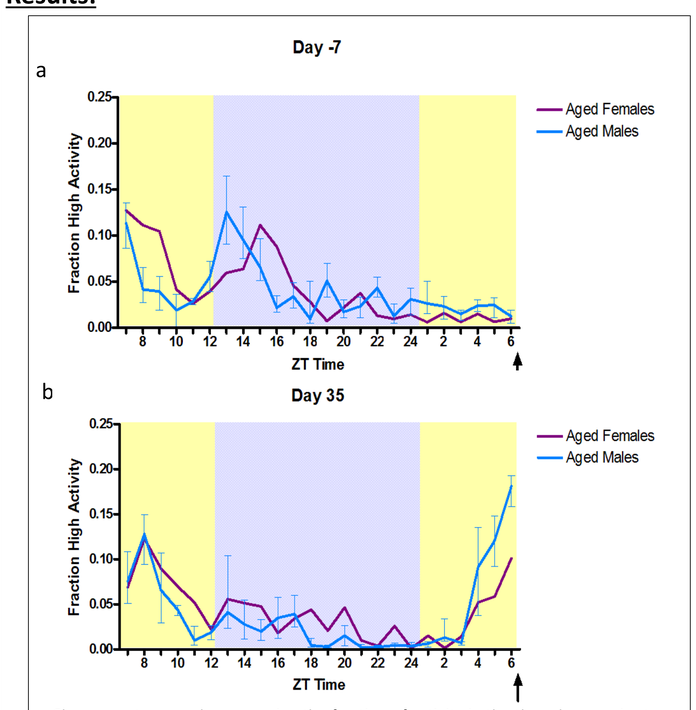
The effects of age on the body in many instances are detrimental to overall health and well-being. One such adverse side effect is a change in metabolism and response to food, resulting in loss of lean muscle mass and an increase in fat mass as we age (1). It has been suggested in previous studies that the changes in body composition as one ages could perhaps be attributed to a decrease in the resting metabolic rate (RMR), or changes in macronutrient oxidation (1). There is insufficient evidence to support change in macronutrient oxidation as a primary cause for change in body composition, and while there is a correlation between aging and a decrease in RMR, this relationship is not necessarily causal. In general, the mechanism underlying the connection between aging, body composition, and nutrient intake has yet to be elucidated. Alternatively, circadian rhythms have been implicated in aging, as they are responsible for coordinating a number of physiological and behavioral patterns in mammals. Circadian rhythms are controlled by a primary pacemaker—the suprachiasmatic nucleus—as well as numerous peripheral clocks throughout the body (2). Proper functioning of this system is essential for maintaining normal activity levels, sleep patterns, and food anticipatory activity. Previous studies have shown that aged mice generally exhibit shorter and more advanced circadian periods and have some arrhythmic tissues, all of which could possibly be attributed to a loss of coordination between the SCN and peripheral tissue clocks, although the molecular mechanism has not been elucidated (3). Currently, no study has been conducted to determine the effect of aging on circadian rhythm entrainment mediated feeding; we hypothesized that age would weaken the circadian entrainment to scheduled feeding. This may provide a model for determining the neuronal circuitry of entrainment to feeding.

Methods:

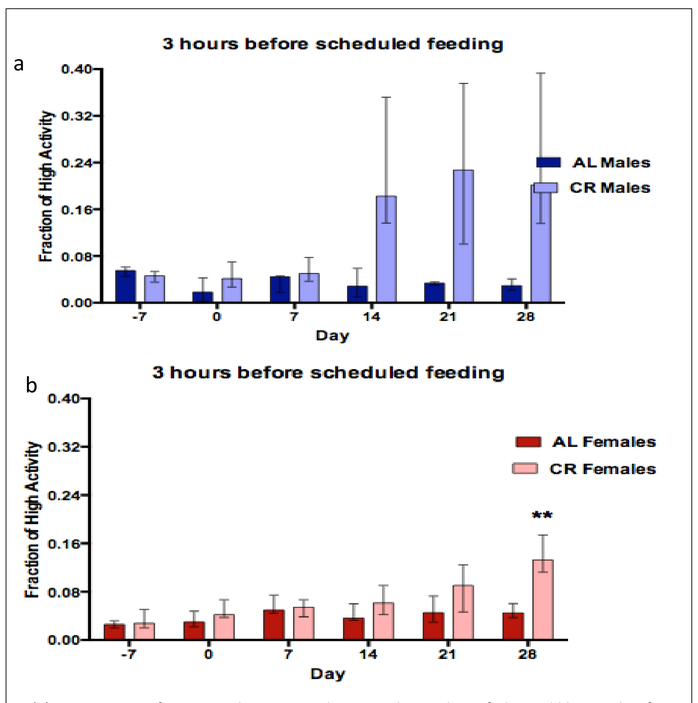
- 12 month old (aged) and 3 month old (young) C57BL/6 mice were used. Sample groups were 3 aged females, 4 aged males, 8 young females, 4 young males
- Ad libitum food intake was measured over several days, while monitoring baseline activity levels with video recognition software
- Mice were introduced to a calorie-restricted diet, in this case 60% of daily food consumption across the group.
- Feedings scheduled to 1 pm, during the light cycle.
- Video recognition used for 35 days of CR diet to measure changes in behavior associated with anticipation of a meal. Behavioral patterns could include walking, jumping, rearing, or hanging.
- The proportion of food anticipatory activity was calculated as a fraction of total daily activity.
- Animals were euthanized after 35 days of CR diet and behavioral monitoring.



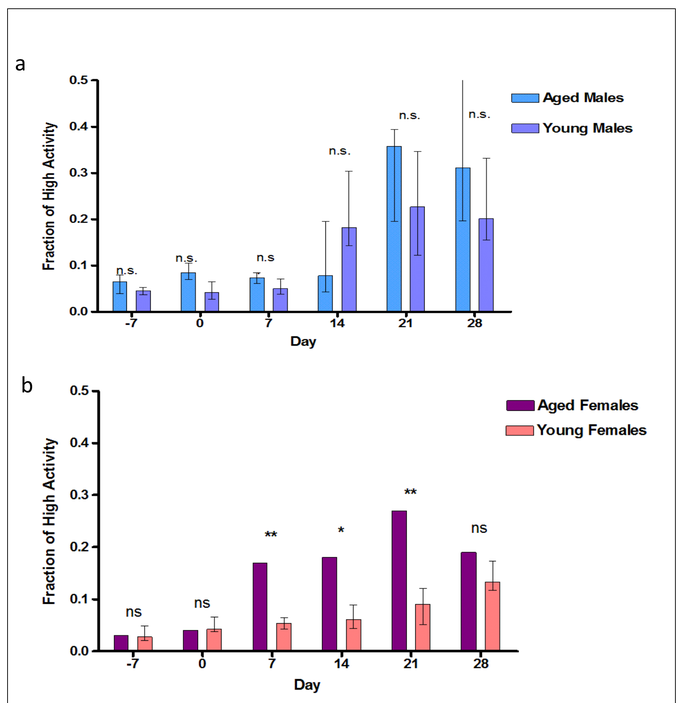
Results:



Shown are two graphs comparing the fraction of activity in the three hours prior to scheduled feeding of total daily activity between aged males and females. (a) Day -7 is recorded prior to placing mice on a CR diet, whereas (b) day 35 is the final day of the experiment. The purple shaded region is the dark cycle, where mice are typically most active.



(a) Comparison of young males on a CR diet to males to those fed an ad libitum diet for the course of the experiment. CR males show a significantly higher proportion of food anticipatory activity that AL counterparts. (b) Young females on CR diet compared to those fed AL.



(a) Comparison of food anticipatory activity in aged males to young males over 28 days. (b) Comparison of FAA in aged females to young females over 28 days. ns= not significant, * significant, ** very significant.

Discussion and Conclusion:

Our initial hypothesis in this project was that aged mice would exhibit less entrainment to scheduled feedings than their younger counterparts, across both genders; our null hypothesis was simply that there would be no difference between the groups. However, statistical analysis between the young and aged groups, as well as between aged males and females has disproved our initial hypothesis. Interestingly, aged females appear to entrain better to scheduled feeding times when compared to young females before day 28. At day 28, the difference is no longer significant. These results indicate that not only do aged females devote a higher fraction of total daily activity to food anticipatory behaviors, they also begin to entrain to scheduled feeding earlier in the experiment. It should be noted that the sample size of aged females used in this experiment; three aged females were compared to eight young females. This could result in disproportionately high values of food entrainment, and therefore a larger sample size should be used in future studies.

When comparing the aged males to young male mice, we found no significant difference in fraction of food anticipatory activity across the 35 day experiment. This was also unexpected, as we had anticipated that this percentage of activity would be at least somewhat less than that of the young mice. These results indicate that males do not lose the ability to entrain to scheduled feedings as they age.

Finally, we compared aged females to aged males. We did anticipate some difference between genders, as levels of food anticipatory activity were significantly different between young males and females. Food anticipatory activity as a fraction of total activity was significantly different between genders on days 7 and 35, but not significant on days 14-28. Females exhibited greater levels of FAA on day 7, whereas males exhibited significantly more activity on day 35. This implies that females entrain to scheduled feedings more rapidly than males, but do not exhibit increasing levels of FAA over time as do male; instead, females remain somewhat active through the circadian cycle rather than expending the majority of energy in anticipation of food.

The premise of this study was that changes in circadian rhythms and food anticipatory behavior as we age could account for unfavorable changes in metabolism and body composition. Given that food entrainment appears to improve or at least remain constant with age, we can speculate that there is likely another component of this system that plays a greater role in the aforementioned age-related changes. If we were to extend these results to humans, it could be that the human body not only entrains better to oscillating rhythms, but requires adherence to a more rigid feeding rhythm. Given that humans do not necessarily follow rhythms such as this strictly, perhaps this could deviance between will and bodily requirement could account for metabolic and compositional changes. There is also the possibility that neural circuitry has been rewired such that those circuits responsible for food entrainment remain intact, but metabolic and biochemical pathways dependent on these circuits change, therefore resulting in physical differences but no change in the ability to entrain to food.

Future studies should utilize larger samples groups to confirm or deny these results. Additionally, brain sections should be analyzed for differences in neural circuitry. Real-time PCR could also be utilized to assess differences in biochemical pathways between young and aged mice. The results of the present study, although not anticipated, may help to bring us closer to comprehending the underlying mechanisms of aging.