UCDAVIS MMPC Mouse Metabolic Phenotyping Center

MMPC National Mouse Metabolic Phenotyping Center

Data Report: Comprehensive Lab Animal Monitoring System (CLAMS), Energy Metabolism
UCD-MMPC Body Composition, Thermoregulation, and Food Intake Behavior Core

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Summary of Findings: Original data and a graphical summary of primary results from the study may be found in the accompanying Excel spreadsheet. In addition to individual tabs within the spreadsheet that correspond to results from individual animals, there are colored tabs associated with consolidated data from each of the main outputs from the CLAMS instrumentation. The latter will be summarized here. For each of the 3 major readouts (tabs for Energy Expenditure/RER, Food Intake, and Activity), at the top is the summary for all animals for each parameter. Below that, there are 4 tables for the summary data defined by group (means, n, StDev and SEM.) The data described herein is derived from 48 hr of continuous data collection (2 light cycles, 2 dark cycles). As an example, a dark cycle bar as depicted is the mean value derived from the 2 dark cycle periods. In addition, there are basic t-test analyses (just below the top table- outlined in fuschia) on each of these 3 main tabs for the most relevant parameters. On the EnergyExp-BW&LM adj(ANCOVA) tab, we have performed ANCOVA using body weight or lean mass as covariate for the energy expenditure (heat) data.

The following abbreviations were used in the report: WT = mice Wildtype for B6.129S4-Myf5tm3(cre)Sor/J and Wildtype for B6-P11-beta<floxed>; HOM = Heterozygous for B6.129S4-Myf5tm3(cre)Sor/J and Homozygous for B6-P11-beta<floxed>; HET = Heterozygous for B6.129S4-Myf5tm3(cre)Sor/J and Heterozygous for B6-P11-beta<floxed>.

1. Respiratory Exchange Ratio (RER; see Energy Expenditure tab). Background: The RER is calculated as the rate of CO_2 production divided by the rate of O_2 consumption (VCO_2/VO_2), and reflects relative macronutrient combustion toward meeting the energy needs of the animal. An RER of 1.0 reflects pure carbohydrate oxidation, 0.7 pure fat oxidation, with protein oxidation yielding an intermediate value. Occasionally, in the course of active *de novo* lipogenesis, an RER value >1.0 will be observed. Differences in RER between treatment groups or in genetically modified mice would indicate a difference in tissue "fuel preference" and this difference may manifest throughout the day or only in select periods such as the light or dark cycle. For instance, it is not uncommon to observe an RER increase during the dark cycle since this is when mice typically eat most of their food.

RESULTS: ***There was no difference in average 24 hour, light cycle or dark cycle RER between genotypes. These results indicate that genotype did not have a significant influence on substrate oxidation. In all genotypes, there was a decrease in light versus dark cycle RER. This diurnal change is expected in mice and reflects that fact that carbohydrates are the primary substrate oxidized during periods of feeding which mostly occurs during the dark cycle. A decrease in RER during the light cycle is consistent with periods of fasting during this time and a switch towards reliance on fat as the primary source of energy.***

2. Energy Expenditure (EE; see Energy Expenditure tab). Background: The metabolic rate is influenced by many factors, including changes in sympathetic nervous system (SNS) tone, ambient temperature, activity level, and plane of nutrition/fed-fasted state. In mice, the activity of the brown adipose tissue (BAT) must also be considered when interpreting differences in EE between treatments or strains, since this tissue is quite abundant and is a specialized site of thermogenesis when SNS tone is high. EE, or "heat" in the CLAMS data output parlance, may be expressed several ways. Whole mouse EE (kcal/d) is useful when considering energy intake vs. expenditure (energy balance). EE expressed per BWT (kcal/h/g) or per lean body mass or "lean mass" (LM) (kcal/h/LM) provides important information on differences in thermogenesis on a "tissue" level. LBM is considered to be metabolically active relative to, e.g., fat mass, and so is most often reported. Historically, mass-specific EE has also been reported as BWT raised to the 0.75 or 0.67 power, as these describe the BWT-EE relationships typically observed when comparing across diverse species with a wide range of BWT or when adjusting for body surface area, respectively. These values are provided to you for context should a comparison to the historic literature be desirable. Since BWT and LM themselves impact EE across and within species, in order to more sensitively determine genotype- or treatment-differences in EE in mice, an analysis of covariance (ANCOVA) approach has been championed, taking BWT into account in the statistical model.

RESULTS: ***There were no significant differences in body weight or lean body mass between genotypes. However, there was an increase (P = 0.04) in average EE (kcal/24 hr) in the HOM compared to WT mice. Light (P = 0.07) and dark (P = 0.14) cycle EE values both tended to be higher in the HOM versus wild-type mice. There were no differences in average 24 hour, light cycle or dark cycle energy expenditure between the HOM and HET mice or the WT and HET mice. The increase in EE in the HOM versus WT mice remained after EE was adjusted for body weight (P = 0.046) or lean body mass (P = 0.048) using ANCOVA. These results indicate that the HOM genotype induces an increase in EE. Studies of longer duration are needed to determine if this increase in EE will be maintained and lead to differences in body weight/composition between the HOM and WT mice. There were no differences in EE adjusted for body weight or lean body mass between the HOM and HET mice or the WT and HET mice.***

2. Food Intake (see Food Intake tab). Background: Food intake behavior is complex and differences can manifest across treatment groups or genotypes as changes in meal sizes, meal frequency, light-dark cycle partitioning of food intake, etc. Energy intake typically tracks EE (neutral energy balance) except, e.g., in times of animal growth and development or obesity-promoting hyperphagia (positive energy balance), or during conditions of hypophagia/cachexia or inordinately high EE (negative energy balance). In the study spreadsheet, data is presented from the CLAMS basic meaurement of daily and light/dark cycle food intake in grams, which was then converted to kcal derived from the metabolizable energy content of the study diet.

RESULTS: ***As expected in mice, all genotypes consumed most of their food during the dark cycle. Average 24 hour food intake was greater (P = 0.03) in the HET compared to WT mice. This was entirely due to an increase (P = 0.03) in dark cycle food intake in the HET mice since no differences in light cycle food intake were observed between any of the genotypes. Dark cycle food intake was also increased (P = 0.02) in the HET compared to HOM mice, although this changes was not of sufficient magnitude to produce a significant difference in 24 hour food intake between the HET and HOM mice. There were no differences in 24 hour, light cycle or dark cycle food intake between the HOM and WT mice. Mean 24h energy (kcal) intake was greater than 24h EE (kcal/mouse) for all genotypes, indicating that the mice were in positive energy balance. This would be expected in young mice which are still undergoing some level of growth.***

3. Activity Levels (see Activity tab). Background: Activity level can be an important contributor to energy needs of the organism, and thus increase in net whole-body EE without a treatment- or genotype-specific alteration in tissue-specific EE per se. In addition, hyper- or hypophagia will typically impact foraging behavior and thus net activity level. Therefore, proper interpretation of EE, food intake behavior, and energy balance in mice is supported by a knowledge of activity. The CLAMS instrument utilizes infrared light beam-break technology to measure activity in the $X_{tot}/X_{amb}/Z$ planes that correspond to total X plane cage movements, consecutive (ambulatory) X plane cage movements, and rearing movements, respectively.

RESULTS: ***There was a decrease in total horizontal (X_{tot}), ambulatory (X_{amb}) and rearing (Z) movements in the HET compared to HOM mice. The HET mice showed decreases in both light (P = 0.032) and dark (P = 0.01) cycle Z movements compared to the HOM animals. Similarly, there were trends toward decreases in both light and dark cycle X_{tot} and X_{amb} activity in the HET versus HOM mice. The magnitude of these changes in activity, however, was not sufficient to induce overall difference in EE between the HET and HOM mice (see section 2 above). There were no differences in physical activity between the WT and HET or WT and HOM mice.***

Also, please remember the policy to acknowledge the MMPC in your publications of the work that was generated using UC Davis MMPC services. Please include a statement in the acknowledgement section: Research was supported by NIH grant U24-DK092993 (UC Davis Mouse Metabolic Phenotyping Center).