

An introduction to the COCVD Metabolic Phenotyping Core Capabilities and procedures

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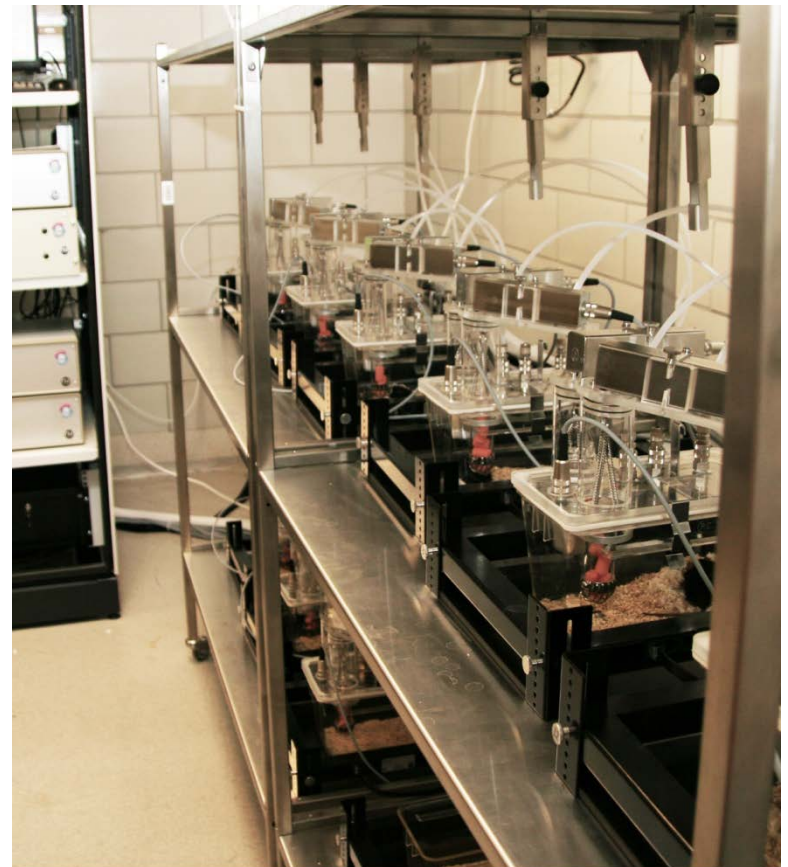
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An introduction to the COCVD Metabolic Phenotyping Core

- The TSE LabMaster Indirect Calorimetry System monitors energy expenditure, activity, and food and water consumption of mice in individual chambers.



Standard Protocol - acclimation

1. We recommend that lean and fat mass be determined by EchoMRI at the beginning and end of the experiment.
2. Investigator weighs mice and places them in acclimation chambers prepared by core personnel.
3. Food and water bottles are weighed at the start of acclimation and the following 1-2 days to confirm that mice are eating and drinking.
4. Mice remain in acclimation chambers for 1-2 weeks, depending on age and obesity. Investigator checks mice daily during acclimation.



Standard protocol - recording

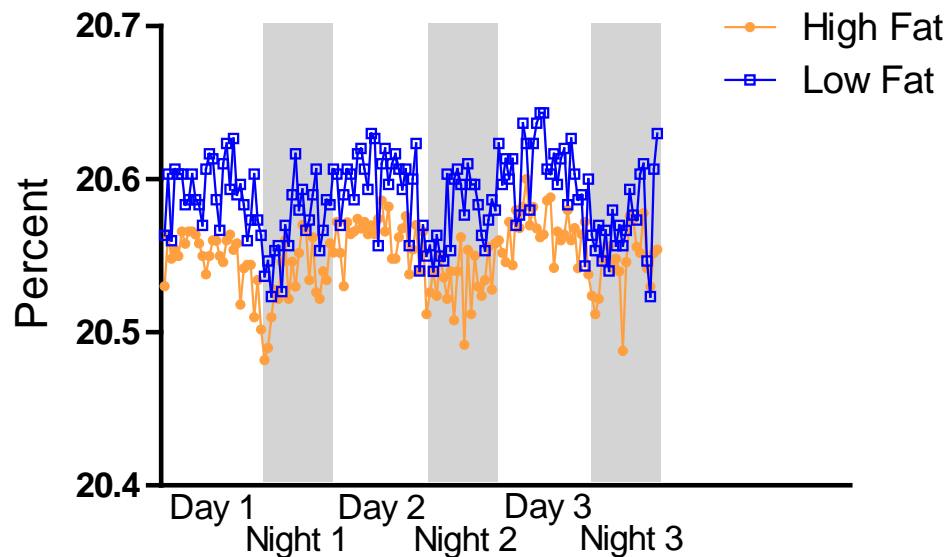
1. Core personnel weigh mice and transfer them to recording platforms on Monday morning.
2. Core personnel check mice twice daily during recording period.
3. On Friday, investigators and core personnel weigh mice and return them to standard cages.
4. Investigators assist with cleanup and perform EchoMRI.



Parameters recorded

1. Each chamber is sampled in succession, at 30 minute intervals. An air sample is drawn from the cage, oxygen and carbon dioxide levels in the sample are measured, and compared to the values measured in an unoccupied reference chamber.

O₂ Concentration



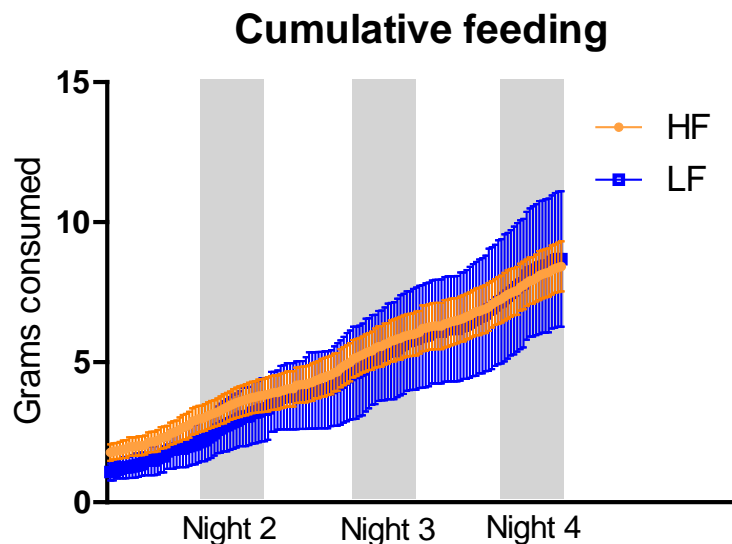
This timecourse, from a pilot experiment performed by the Core using male C57/BL6 mice, shows that oxygen concentrations normally are lowest during the dark period, when the mice are most active.

Parameters recorded

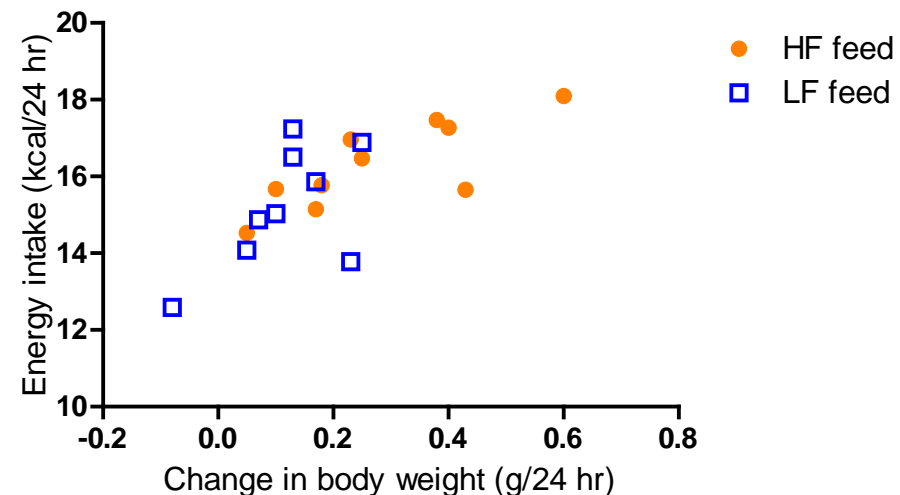
- Food and water dispensers hang from weight sensors that continuously record removal of food or water from each dispenser. Removal is reported cumulatively every 30 minutes, with the option of reporting smaller time increments.

Our male C57/BL6 mice typically eat more during the dark period but also eat a significant amount during the light period.

Investigators typically convert the data to net energy intake (kcal per period), e.g.:

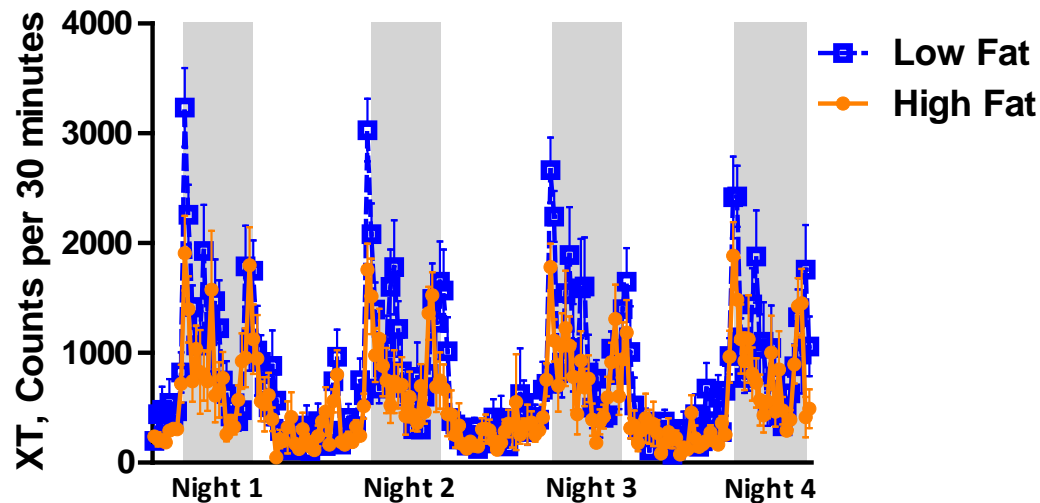


Food consumption vs change in body weight



Parameters recorded

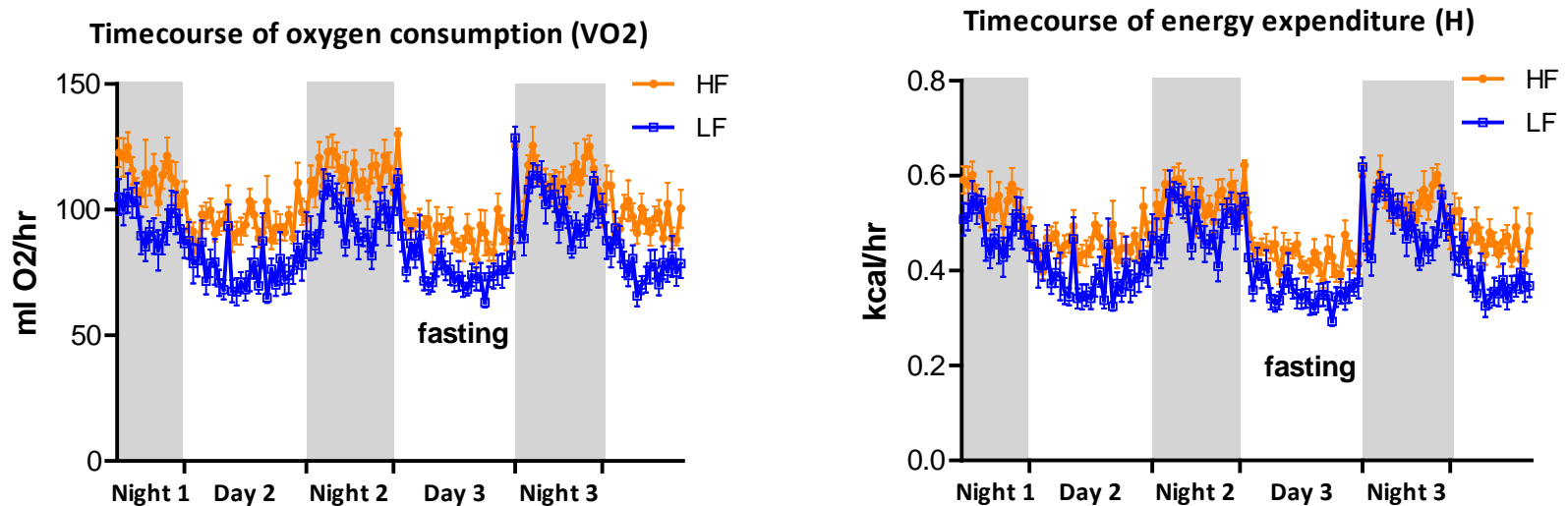
- Activity on the long axis of the cage is measured by an array of infrared beams and sensors. Counts are recorded continuously and reported at 30 minute intervals, with the option of displaying activity in smaller time increments.



Activity data are reported as
XA (ambulatory): mouse crosses two adjacent beams;
XF (fine): mouse crosses the same beam twice;
XT (Total activity): $XA + XF$.

Values calculated by the software

1. VO_2 (oxygen consumption) and VCO_2 (carbon dioxide production) in ml/hour.
2. Energy expenditure (H) in kcal/hour, calculated using VO_2 , VCO_2 and physiological constants.



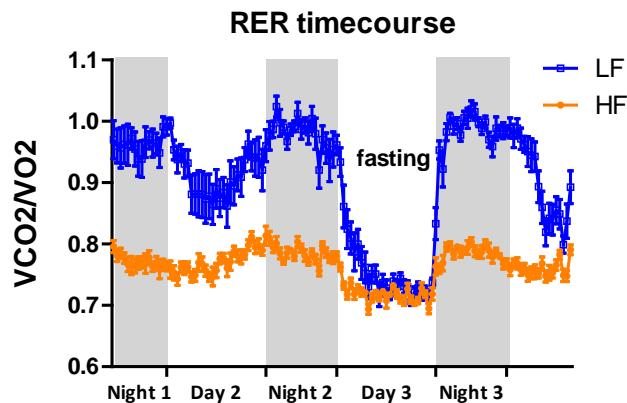
The curves for VO_2 , H and XT normally have very similar shapes

Values calculated by the software

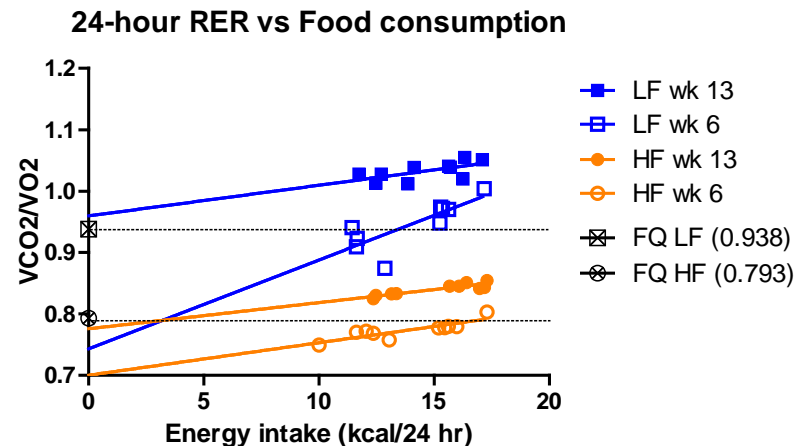
3. Respiratory exchange ratio (RER), the ratio of VCO_2/VO_2 .

RER is a function of macronutrient utilization, molecular interconversion (e.g. lipogenesis), and blood chemistry.

Observed RER can be compared to the Food Quotient, which is a theoretical RER based on the macronutrient composition of the diet.



RER normally displays a circadian rhythm. When the mice are fasted, RER approaches 0.7, the value for utilization of lipid as energy source.



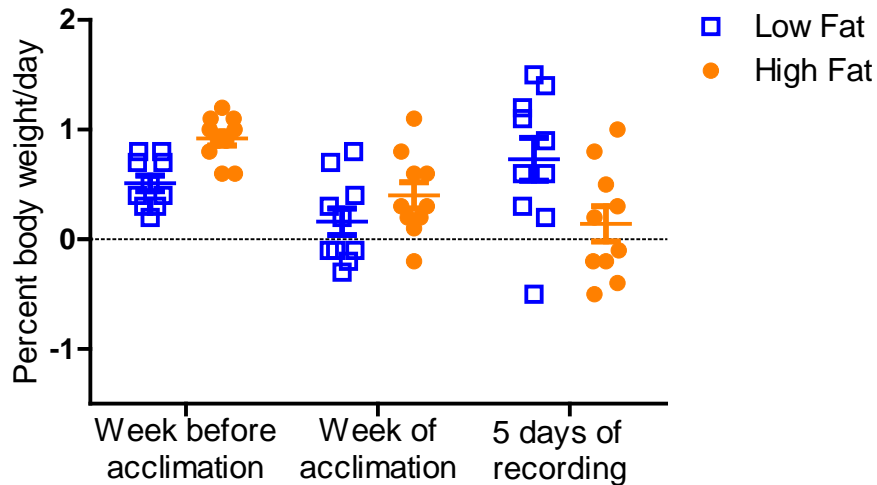
If RER depended only on macronutrient utilization, we would expect the RER vs energy intake plot to regress to a Y-intercept of 0.7

Other Parameters analyzed

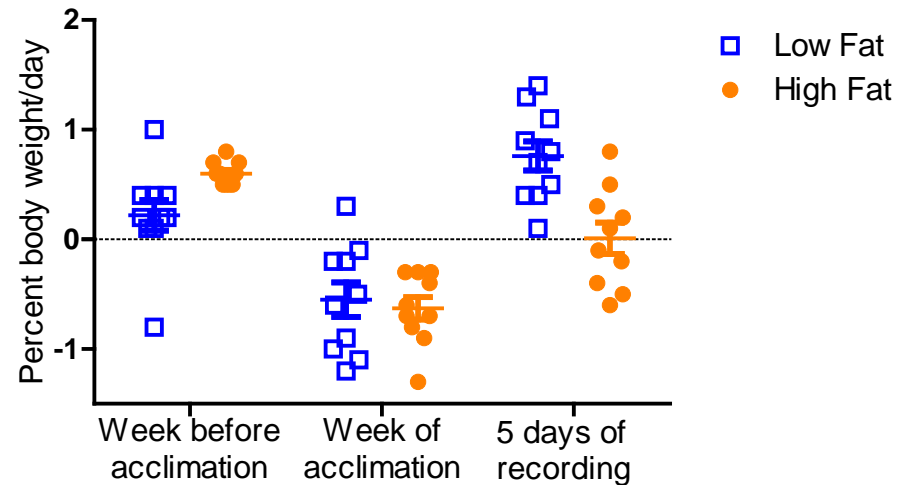
1. Body weight

Rate of body weight change in the chambers is compared to rate of change in standard housing to validate interpretation of calorimetry data.

**Weight change after 6 weeks on diet
(percent of initial body weight per day)**



**Weight change after 13 weeks on diet
(percent of initial body weight per day)**

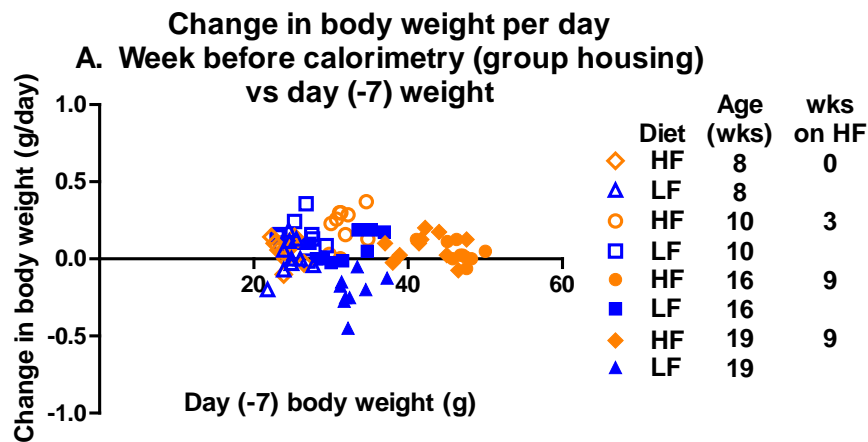


We have observed that weight gain may slow or reverse when mice are transferred to the chambers. The groups may recover at different rates. Weight gain in the chambers must be the same as in standard housing in order for calorimetry data to reflect normal metabolic values.

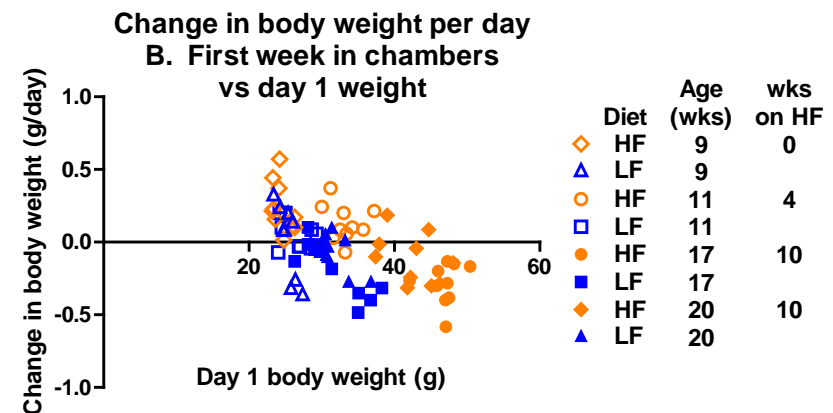
Other Parameters analyzed

1. Body weight

Core personnel conducted pilot experiments to determine optimum times of acclimation and measurement for mice made obese by consumption of a high fat (HF) diet.



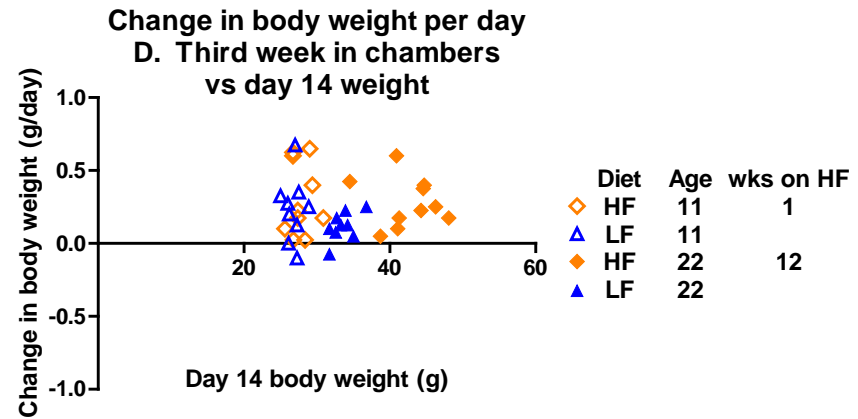
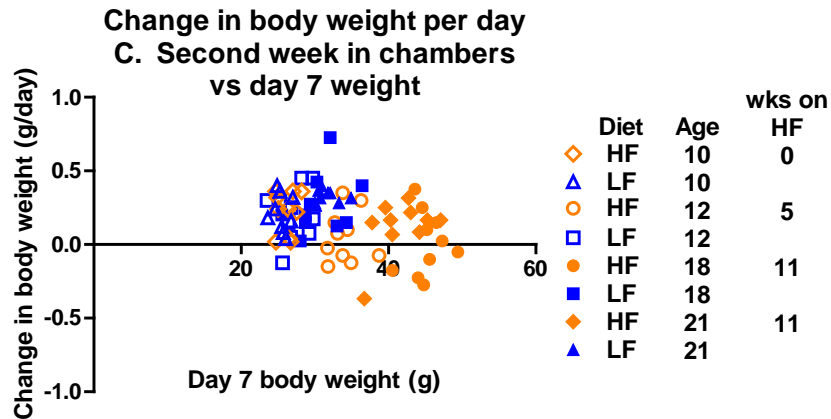
A, Mice in group housing displayed variable changes in body weight that were unrelated to diet. Low fat (LF), high fat (HF).



B, During the first week in the chambers, older and heavier mice showed greater decrease in weight gain than younger and lighter mice.

Other Parameters analyzed

1. Body weight



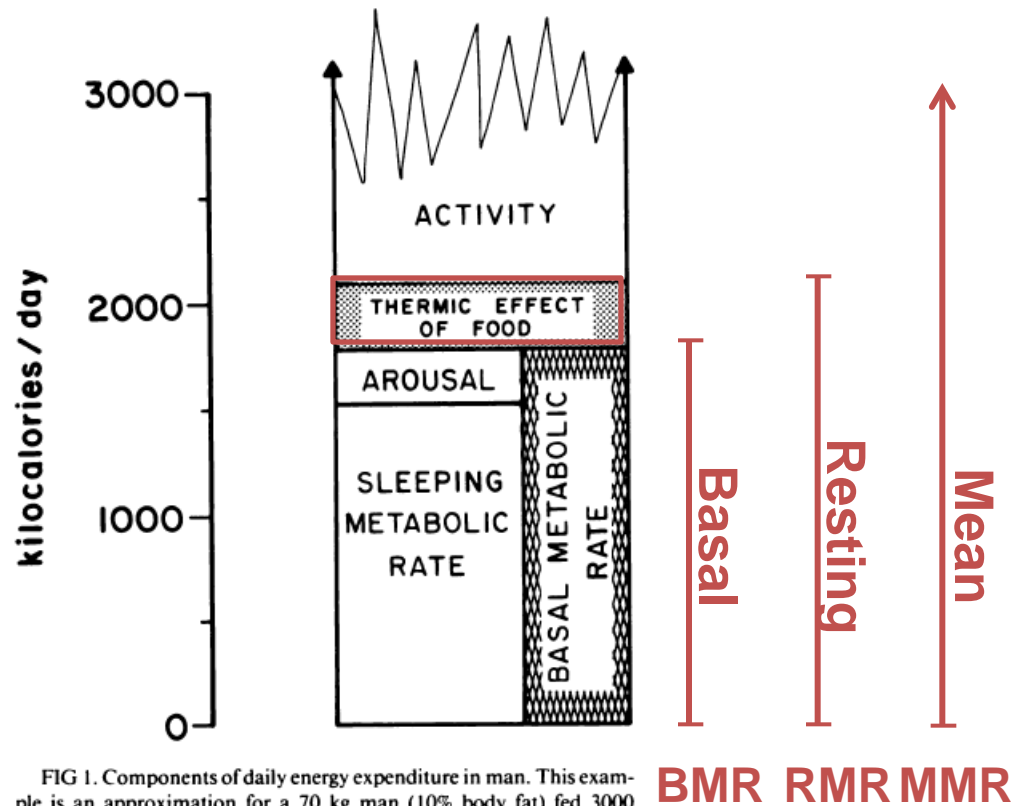
C, Fewer mice lost weight during the second week in chambers.

D, During the third week in chambers, some mice showed an additional increase in rate of weight gain, regardless of diet.

Based on these observations, we recommend that body weight be monitored, and that mice be given one to two weeks of acclimation to the chambers before recording calorimetry data, until weight change is representative of that in standard housing.

Other parameters analyzed

2. resting energy expenditure



Energy expenditure can be reported in three forms:

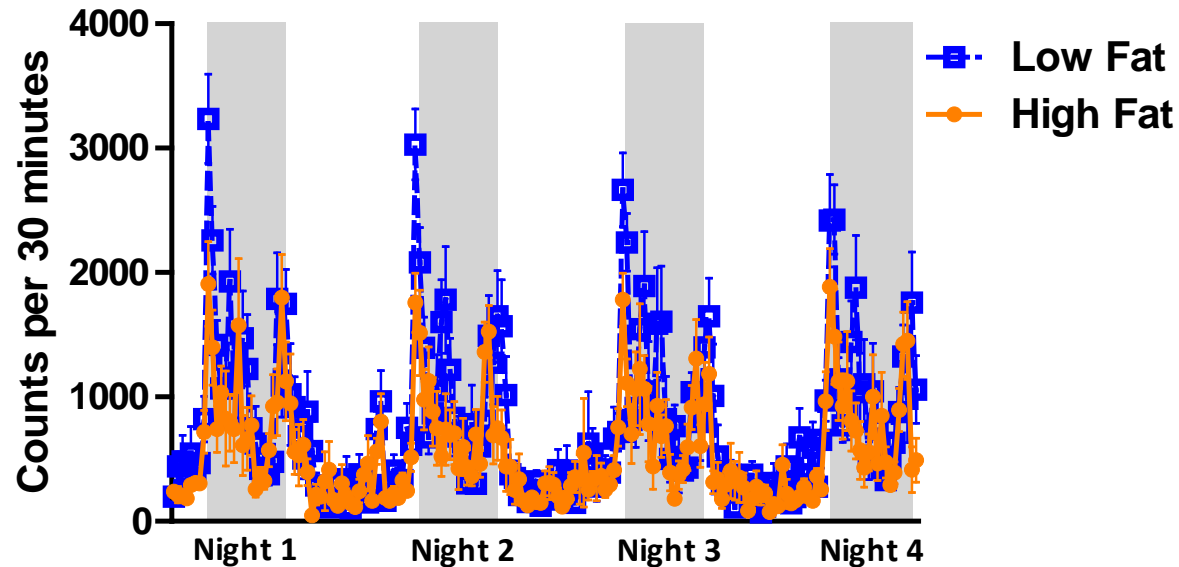
1. Mean Metabolic Rate (MMR) = awake, active, fed (rarely used in human studies but found in mouse papers)
2. Resting Metabolic Rate (RMR) = quiet, fed
3. Basal Metabolic Rate (BMR) = quiet, fasted

FIG 1. Components of daily energy expenditure in man. This example is an approximation for a 70 kg man (10% body fat) fed 3000 kcal/d.

Other parameters analyzed

2. resting energy expenditure

Normal mice display a circadian rhythm of activity and feeding, with lowest mean activity during the light period:

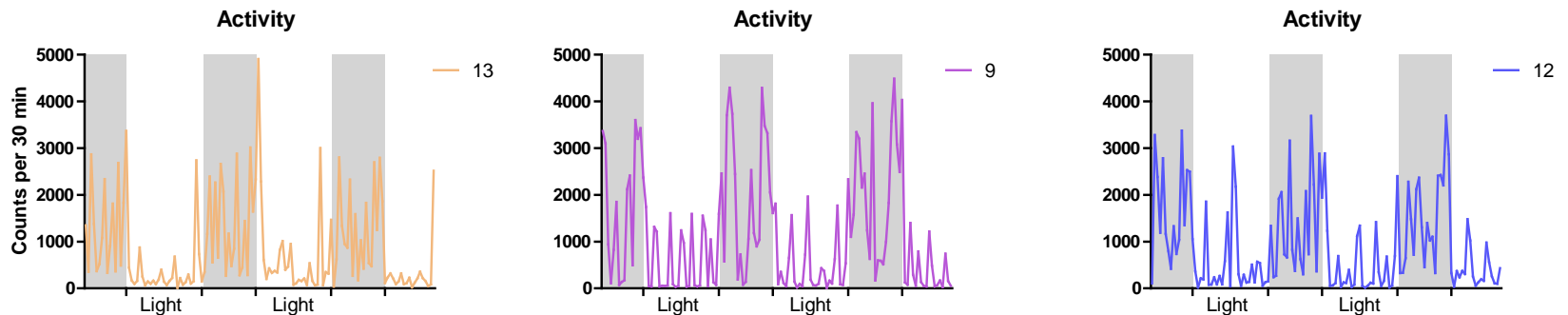


Therefore, energy expenditure during this period of minimal activity approximates the RMR.

Other parameters analyzed

2. resting energy expenditure

However, while mean activity of a group is low, individual mice are variably active during the light period when “resting” metabolism is measured.



One can use the filtering capabilities of Excel to analyze time points during the light period at which activity of a given mouse is minimal.



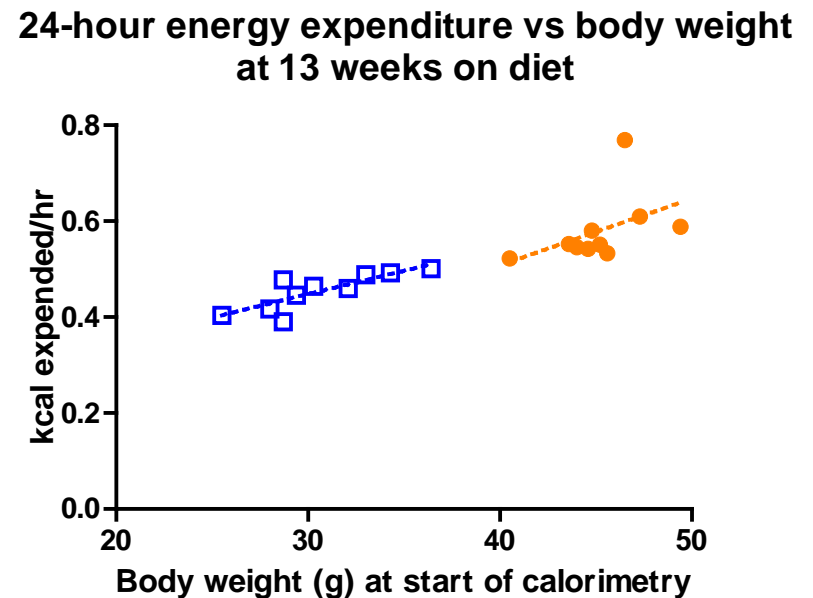
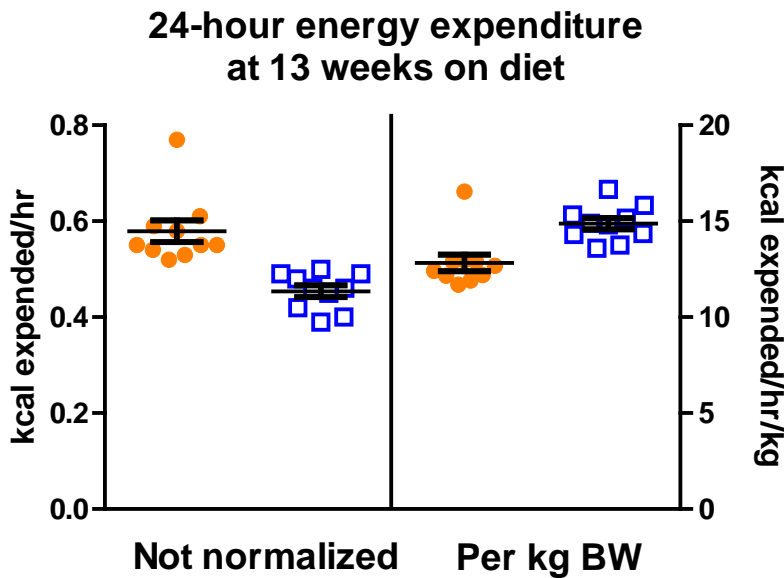
Comparing energy expenditures

- Tschop et al (Nature Methods 9, 57–63, 2012) advise applying analysis of covariance (ANCOVA) to calorimetry data rather than comparing mean energy expenditure.
 - ANCOVA is just a fancy way of saying linear regression including both dichotomous and continuous predictor variables.
- Mean values can be misleading because differences directly attributable to genotype can, in effect, “cancel out” differences attributable to imbalances of body mass.
- The figures on the next slide show pilot experiments performed by the Metabolic Core, which illustrate the potential for misinterpretation when mean values are compared:

Comparing energy expenditures

A. When mean EE was not normalized (left panel), levels appeared higher in HF- than LF-fed mice, because the HF-fed mice are bigger. When normalized to total body mass (right panel), mean EE appeared lower in HF-fed mice.

B. When energy expenditure was plotted with respect to total body weight (ANCOVA), we observed no difference in slopes or intercepts of diet groups (i.e., EE here is a function of body weight, not diet).



Recommendations for designing and analyzing calorimetry experiments

1. Track weight change in your mice during standard housing, acclimation and while in chambers. If relative weight changes of groups is different in the chambers than when in standard housing, you shouldn't compare their energy expenditure.
2. Track daily EE while in calorimetry, if it goes down each day you may want to consider acclimating and/or recording for longer durations to eliminate stress-related elevations in energy expenditure.
3. Pilot experiments performed by the Core indicated that filtering physical activity may increase differences in Resting Metabolic Rate between LF and HF groups.
4. EE data should be compared by ANCOVA rather than by comparing mean values, whether or not you normalize to body weight, lean body mass, or any variation thereof.

Recommendations for statistical analysis

1. Unless the scatterplot exhibits a clear and strong signal of a nonlinear pattern *within* one of the treatment groups, we recommend using linear regression analysis.

(Note that an *overall* nonlinear pattern may appear in the scatterplot if two different treatment groups have two linear patterns with different slopes, but this is not a nonlinear pattern.)

2. In the following circumstances we recommend that a researcher consult with the friendly neighborhood statistician:
 - (a) the scatterplot shows a nonlinear pattern within one of the treatment groups
 - (b) each specimen contributes two or more observations to the scatterplot
 - (c) there are power/sample size questions
 - (d) the researcher is uncertain whether he/she is proceeding appropriately.

Pilot experiment: acute vs chronic HF feeding

The effects of acute and chronic HF feeding were compared to determine longitudinal effects of obesity on metabolic parameters.

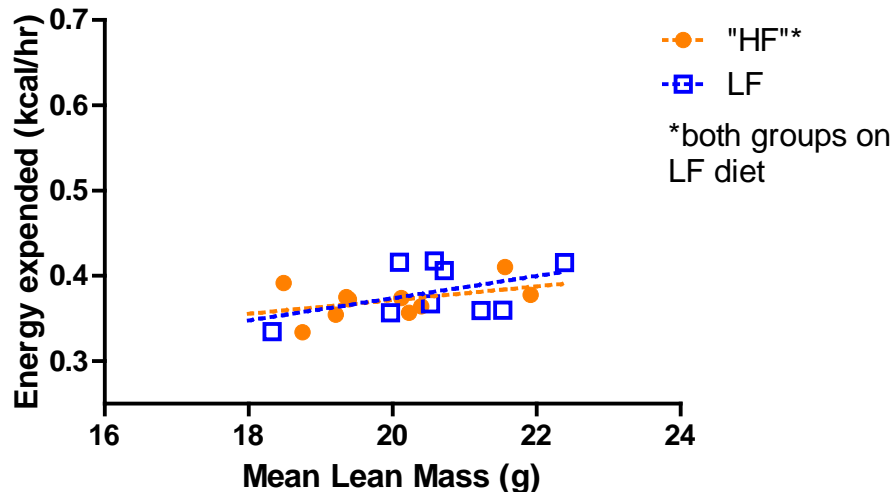
- *C57/BL6 male mice, 8 weeks of age, were placed on LF diet one week prior to day 1 (“day -7”).*
- *On day 1, lean mass was measured by EchoMRI before the mice were placed in chambers. Mice were acclimated to chambers for one week (days 1-7).*
- *Calorimetry recording was begun on day 7.*
- *HF diet was introduced on day 14, at the start of the third week in chambers.*
- *On day 18, calorimetry was ended and lean mass was measured again before the mice were returned to group housing.*
- *Resting energy expenditure was plotted with respect to lean mass for analysis of covariation (ANCOVA).*

Pilot experiment: acute vs chronic HF feeding

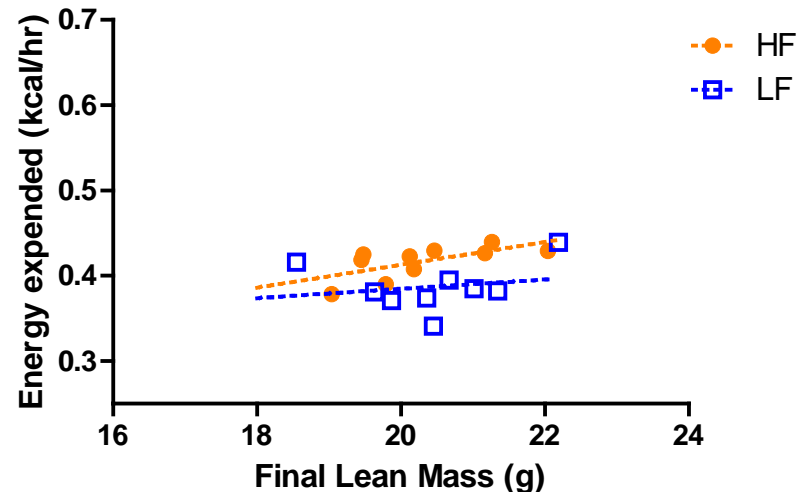
A, Before introduction of the HF diet, the two groups of mice had indistinguishable resting energy expenditure with respect to lean mass.

B, After three days of HF feeding, the groups displayed a significant difference in resting energy expenditure with respect to lean mass ($P=0.0087$).

Resting energy expenditure vs lean mass
10th day in chambers, prior to high fat feeding



Resting energy expenditure vs lean mass
17th day in chambers, 3rd day of HF diet



Pilot experiment: acute vs chronic HF feeding

At the end of the third week, the mice were returned to group housing and maintained on HF or LF diets. In the 10th week, they were returned to chambers and recorded during the 12th week of HF feeding to observe the effects of chronic high fat feeding.

C, In the twelfth week of HF feeding, the groups displayed a significant difference in resting energy expenditure with respect to lean mass ($P=0.0003$).

Resting energy expenditure vs lean mass
17th day in chambers, 12th week of HF feeding

