Handling data from indirect calorimetry experiments performed on the TSE system

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Please also notify Wendy Katz when you publish, so that we may include the citations in our progress reports.
Thanks.

1. Quick overview

a. Graphs are provided for body weight and fat mass-lean mass changes over the course of the experiment. It is not unusual for mice to lose up to 10% of body weight during the acclimation period. Ideally we would like the weights to be stable or back in growth mode by the time recording begins. It's not unusual for lean mass to decrease and fat mass to increase over the course of an experiment.

b. Timecourse graphs provide basic quality control:

- Circadian rhythms displayed for RER, energy expenditure, activity.
- No discontinuities that might indicate environmental disturbance, or, in the case of cumulative food and drink graphs, a leaked-out water bottle or error in taring a food bottle after refilling.
- Are the peaks and troughs equal to one another, indicating the mice are acclimated, or are they gradually rising or falling, indicating the mice may still be adjusting to the system?

c. Linear regression plots permit comparison of experimental groups (see accompanying file on ANCOVA).

- Mean RER (ratio of CO2 production to O2 uptake) is highly influenced by macronutrient intake, but is also affected by intermolecular conversions ,exercise and anything that affects blood pH (notably hyperventilation).
- Mean H3 is energy expenditure measured at 30 minute intervals and reported as kcal/hr

- Mean XT is activity as measured by crossings of the infrared beams in the recording platform. XT is measured continually and reported in 30 minute increments.
- Food and water removal are measured cumulatively, and reported in ml or grams. Net food consumption is calculated per 30-minute interval and reported in kcal.

2. Contents of data file

a. Data are contained in an Excel file containing several tabs:

- Your data in format 1 (explained below). **Before making any changes or calculations, make a copy of the data sheet you want to work with, then lock the original sheet (on "Review" tab, click "Protect Sheet" option and enter a password), so your raw data are preserved**.

- Activity and drinking-feeding data recorded at smaller time intervals (the tabs labeled "Act" and "DFT").

    - Note: some mice, especially with crumbly diets, will gnaw chunks of food out of the tube and then eat it off the floor over a period of time.

- Events, which notifies you of any unusual occurrences. If there is note of a water bottle refilled, that sometimes means the bottle leaked and had to be replaced.

- If a feeder is noted as being refilled, the food may have been stuck in the tube. Check the interval since the mouse last ate to see if it went without food for an unusual amount of time, which might change activity and energy values for that interval.

b. Data are saved in 3 formats.

- **Format 1**: time points are organized in columns. Values are organized in a single column for each value. We use this format to make pivot tables for sorting and filtering the data.

Example:

Date	Time	Animal No.	Box	FlowSamp	O2	CO2
				l/min	%	%
6/7/2009	10:46	931	1	0.41	19.96	0.036
6/7/2009	11:16	931	1	0.41	20.03	0.036
6/7/2009	11:46	931	1	0.4	20.03	0.035
6/7/2009	10:46	932	2	0.41	19.96	0.036
6/7/2009	11:16	932	2	0.41	20.03	0.036
6/7/2009	11:46	932	2	0.4	20.03	0.035
6/7/2009	10:46	889	3	0.41	19.96	0.036
6/7/2009	11:16	889	3	0.41	20.03	0.036

- **Format 2**: Time points are organized in columns. Values are organized in a separate column for each box. Values are grouped for each box. *(We will provide this format on request, but to date nobody has been using it, so we stopped downloading it).*

Example:

Date	Time	Box-1			Box-2			Box-3		
		Flow	O2	CO2	Flow	O2	CO2	Flow	O2	CO2
		[l/min]	[%]	[%]	[l/min]	[%]	[%]	[l/min]	[%]	[%]
6/7/2009	10:46	0.45	19.88	0.421	0.45	19.93	0.352	0.45	19.93	0.416
	11:16	0.45	19.92	0.377	0.45	19.94	0.34	0.45	19.95	0.335
	11:46	0.45	19.92	0.344	0.45	19.95	0.305	0.45	19.96	0.31

- **Format 3**: Time points are organized in rows; values are organized in a separate row for each box. Boxes are grouped for each value. This format is useful for making graphs in Excel for a quick overview of feeding and activity of individual mice. *(We will provide this format on request, but to date nobody has been using it, so we stopped downloading it).*

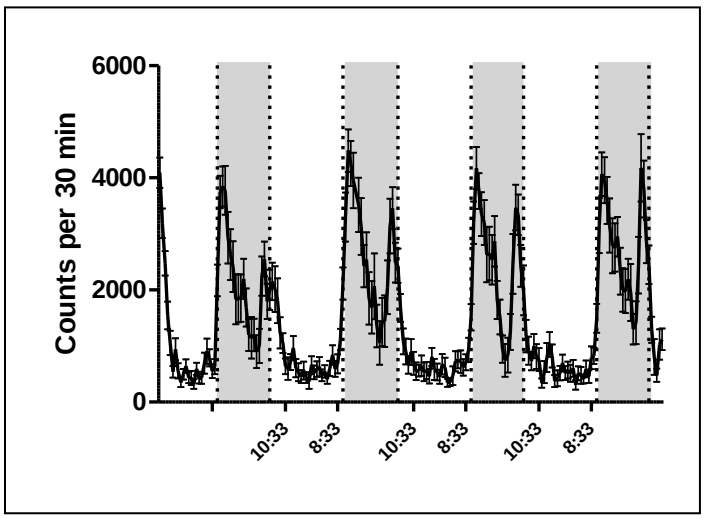
Example:

Box	Paramete	6/7/2009 10:46	6/7/2009 11:16	6/7/2009 11:46		
Box1	O2	19.88	19.92	19.92		
Box2	O2	19.93	19.94	19.95		
Box3	O2	19.93	19.95	19.96		
Box1	CO2	0.421	0.377	0.344		
Box2	CO2	0.352	0.34	0.305		
Box3	CO2	0.416	0.335	0.31		

3. Analyzing differences between light and dark periods.

We graph activity (XT), RER, H3 (uncorrected energy expenditure), feeding and drinking with respect to time. Activity and feeding are typically higher at night, with the mice resting during the day. Many mice show peaks of activity at dawn and dusk. The most sensitive energy expenditure readings are obtained when activity (XT) is lowest. Mice snack during the resting period, so resting RER doesn't drop as low as would be expected in the fasted state.

The mice are on a 10 hour dark/14 hour light cycle. During Daylight Saving time, lights come on between 07:00 and 7:30 and go off between 21:00 and 21:30. During standard time (winter), lights come on between 06:00 06:30 and go off between 20:00 and 20:30. Exact on-off times change occasionally because lights in the facility come on one room at a time, starting at 7:00 AM (daylight saving time). Our room is near the end of the sequence. DLAR monitors light intensity at 15 minute intervals, so if you need to check on-off times for your experiment, you may ask Cheryl Carmichael for this information for room 059D on the dates of your experiment.



In this example, activity started to drop when the lights came on at 7:15 (the data point was recorded at 7:33). However, the mice weren't well settled till after 10 am. Time points between 10 am and 8 pm would be the closest to a resting state.

You may find it useful to calculate Resting Metabolic Rate. Since most mice are variably active during the day, you can use the filtering capabilities of Excel to select data points where activity is below a certain value (see the accompanying pivot table instructions).

The system discriminates between ambulatory activity (XA) and fine activity (XF), depending on whether the mouse breaks two adjacent beams in succession, or breaks the same beam twice. The times I've looked at this, the curves for XT, XA and XF were qualitatively similar, so I've always analyzed XT. You might want to check this for your genotypes and treatment conditions, however.

4. Additional checks on data quality

We typically discard the first day and night of activity because it is a partial period and also the mice are agitated. We discard the last day because it is a partial period. That leaves 3 days and nights to analyze (Tuesday light period through Thursday dark period).

- Scan down the "Flow Sample" and "Flow" columns to make sure the air flow rates are stable for all boxes at all time points. **NOTIFY WENDY IF YOU DISCOVER ANY ABERRANT FLOW RATES**.
- Graph a time course of food and water consumption for individual mice to make sure each started eating and drinking within a reasonable period of time, and that there are no anomalies (such as a leaky water bottle or stuck food bottle) that would throw off your group averages.
- Before taking multiple day averages, calculate each period separately to make sure values aren't progressively rising or falling. Some mice acclimate over several days and you may end up using only the last full period.

Sorting the data

We recommend using pivot tables in Excel to sort and filter your data (see the accompanying document).

5. Abbreviations and formulas used by the software

a) Calorimetric parameters that the system **measures directly** and displays:

O2 (%), CO2 (%), Flow (l/min)

These values are measured for each chamber, and for an unoccupied reference chamber (Ref).

b) Calorimetric parameters that the system **calculates** and reports (details of calculations are described below)

dO2 (%), dCO2 (%), VO2 (ml/h/kg), RER, H (kcal/h/kg)

c) Values calculated but not displayed:

FlowML (ml/h) = Flow x 1000.0 x 60

V1 = N2Ref x dO2

V2 = O2Ref x (dO2 – dCO2)

d) Constants entered into the system for calculating H:

CVO2 = 3.941

CVCO2 = 1.106

e) Equations used to calculate parameters displayed in the data table:

(i) weight-independent

dO2 = O2Ref – O2

dCO2 = CO2 – CO2Ref

RER = VCO2 / VO2

(ii) results for VO2, VCO2 and H come in three forms

(1) Takes weight 100% into account

VO2(1) = FlowML x (V1 + V2)/(N2Ref x Animal Weight x 100.0)

VCO2(1) = FlowML x dCO2/(Animal Weight x 100.0)

H(1) = (CVO2 x VO2 + CVCO2 x VCO2)/1000;

(2) Takes Lean Body mass into account, using a user-defined power of the animal weight in the weight position of the calculation. This power can be set to any value in the range 0.001 to 0.999. In our system it is normally set to 0.750

VO2(2) = FlowML x (V1 + V2)/(N2Ref x Animal Weight$^{0.75}$ x 100.0)

VCO2(2) = FlowML x dCO2/(Animal Weight$^{0.75}$ x 100.0)

H(2) = (CVO2 x VO2 + CVCO2 x VCO2)/1000;

Note: we don't recommend using (1) and (2), the normalized forms of the data, because the outcomes can be misleading. Instead, the non-normalized form (3) should be plotted against body weight, lean mass and/or fat mass to test for covariance (Tschop et al., 2012).

(3) The weight of the animal is not taken into account (power of the animal weight = 0).
VO2(3) = FlowML x (V1 + V2)/ (N2Ref x 100)
VCO2(3) = FlowML x dCO2/(100.0)
H(3) = (CVO2 x VO2 + CVCO2 x VCO2)/1000;

6. Published resources

John R. Speakman. (2013). Measuring energy metabolism in the mouse – theoretical, practical, and analytical considerations. Front Physiol. 2013 Mar 14;4:34. doi: 10.3389/fphys.2013.00034. eCollection 2013.
An outstanding review of the classical and current literature. As the title indicates, the author discusses important points to consider when designing and analyzing calorimetry experiments.

Tschop et al. (2012). A guide to analysis of mouse energy metabolism. Nature Methods 9(1): 57–63.
Up-to-date guidance, authored by a consortium of leaders in the field. Explains the importance of analyzing data by covariance, rather than by comparing mean values.

Kenneth A. Longo, S. Charoenthongtrakul, D. J. Giuliana, E. K. Govek, T. McDonagh, P. S. DiStefano and B. J. Geddes. (2010). The 24-hour respiratory quotient predicts energy intake and changes in body mass. Am. J. Physiol. Regu. Integr. Comp. Physiol. 298:R747-R754
They see weight loss in chambers even when mice are individually raised. Tests acute effect of high fat diet. Describes an approach to adjusting data for weight loss.

Andrew A. Butler and Leslie P. Kozak (2010). A Recurring Problem With the Analysis of Energy Expenditure in Genetic Models Expressing Lean and Obese Phenotypes. Diabetes 59, February, pp 323-329.
Recommended by our advisory panel at the 2010 annual COBRE program review. Discusses problems with normalizing metabolic values to total body weight or to lean mass.

JRS Arch, D Hislop, SJY Wang & JR Speakman (2006). Some mathematical and technical issues in the measurement and interpretation of open-circuit indirect calorimetry in small animals. International Journal of Obesity 30:1322-1331.
Discusses normalizing by analysis of covariance. Also explains the importance of air flow rate.

Dalan R. Jensen, et al. (2001). A self-correcting indirect calorimeter system for the measurement of energy balance in small animals. J Appl Physiol 90:912-918.
Description of the equipment and principles behind its design.

Patrick C. Even, Asghar Mokhtarian and Agnes Pele (1994). Practical Aspects of Indirect Calorimetry in Laboratory Animals. Neuroscience and Biobehavioral Reviews, Vol. 18, No. 3, pp. 435-447,
Operating principles of open-circuit calorimetry (the system we use) are explained starting on page 438. Discussion of principles for discriminating between maintenance metabolism and activity-related metabolism begins on page 441.

Ravussin & Bogardus (1989). Relationship of genetics, age, and physical fitness to daily energy expenditure and fuel utilization. Am J. Clin. Nutr. 49:968-975
Referenced by Butler and Kozak. Definitions of RMR and BMR, discussion of issues related to normalizing data to body size.