Handling data from indirect calorimetry experiments performed on the TSE system

When presenting or publishing data that you obtained using technical expertise or equipment from the COBRE Pathology Core or Metabolic Core, please acknowledge as follows: “Research reported in this [publication, release] was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103527.”
Please also notify Wendy Katz when you publish, so that we may include the citations in our progress reports.

Thanks.

1. **Contents of data file**
2. **Analyzing differences between light and dark periods.**
3. **Checking data quality**
4. **Abbreviations and formulas in the data**
5. **Published resources**

1. **Contents of data file**
   a. You will receive 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 usually 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 a significant 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:

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Animal No.</th>
<th>Box</th>
<th>FlowSamp</th>
<th>O2</th>
<th>CO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/7/2009</td>
<td>10:46</td>
<td>931</td>
<td>1</td>
<td>0.41</td>
<td>19.96</td>
<td>0.036</td>
</tr>
<tr>
<td>6/7/2009</td>
<td>11:16</td>
<td>931</td>
<td>1</td>
<td>0.41</td>
<td>20.03</td>
<td>0.036</td>
</tr>
<tr>
<td>6/7/2009</td>
<td>11:46</td>
<td>931</td>
<td>1</td>
<td>0.4</td>
<td>20.03</td>
<td>0.035</td>
</tr>
<tr>
<td>6/7/2009</td>
<td>10:46</td>
<td>932</td>
<td>2</td>
<td>0.41</td>
<td>19.96</td>
<td>0.036</td>
</tr>
<tr>
<td>6/7/2009</td>
<td>11:16</td>
<td>932</td>
<td>2</td>
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<td>20.03</td>
<td>0.036</td>
</tr>
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<td>932</td>
<td>2</td>
<td>0.4</td>
<td>20.03</td>
<td>0.035</td>
</tr>
<tr>
<td>6/7/2009</td>
<td>10:46</td>
<td>889</td>
<td>3</td>
<td>0.41</td>
<td>19.96</td>
<td>0.036</td>
</tr>
<tr>
<td>6/7/2009</td>
<td>11:16</td>
<td>889</td>
<td>3</td>
<td>0.41</td>
<td>20.03</td>
<td>0.036</td>
</tr>
</tbody>
</table>

**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:

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Box-1</th>
<th>Flow</th>
<th>O2</th>
<th>CO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/7/2009</td>
<td>10:46</td>
<td></td>
<td>0.45</td>
<td>19.88</td>
<td>0.421</td>
</tr>
<tr>
<td>11:16</td>
<td></td>
<td></td>
<td>0.45</td>
<td>19.92</td>
<td>0.377</td>
</tr>
<tr>
<td>11:46</td>
<td></td>
<td></td>
<td>0.45</td>
<td>19.92</td>
<td>0.344</td>
</tr>
</tbody>
</table>

2. Analyzing differences between light and dark periods.

We recommend graphing activity (XT), RER and H3 (uncorrected energy expenditure) with respect to time. This tells you if your mice are displaying normal circadian rhythms and confirms that the lights cycled correctly throughout the experiment. 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.
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 know on-off times for your experiment, you may ask Cheryl Carmichael for this information for room 059D on the dates of your experiment.

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.

3. Checking 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.

4. 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
   \( V_1 = N_2Ref \times dO₂ \)
   \( V_2 = O₂Ref \times (dO₂ – dCO₂) \)

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

\[ \text{VO2}(2) = \text{FlowML} \times \frac{(V1 + V2)}{(N2\text{Ref} \times \text{Animal Weight}\^{0.75} \times 100.0)} \]

\[ \text{VCO2}(2) = \text{FlowML} \times \frac{d\text{CO2}}{(\text{Animal Weight}\^{0.75} \times 100.0)} \]

\[ \text{H}(2) = \frac{\text{CVO2} \times \text{VO2} + \text{CVCO2} \times \text{VCO2}}{1000}; \]

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).

\[ \text{VO2}(3) = \text{FlowML} \times \frac{(V1 + V2)}{(N2\text{Ref} \times 100)} \]

\[ \text{VCO2}(3) = \text{FlowML} \times \frac{d\text{CO2}}{(100.0)} \]

\[ \text{H}(3) = \frac{\text{CVO2} \times \text{VO2} + \text{CVCO2} \times \text{VCO2}}{1000}; \]

6. Published resources


*Discusses normalizing by analysis of covariance. Also explains the importance of air flow rate.*


*Description of the equipment and principles behind its design.*


*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.*


*Referenced by Butler and Kozak. Definitions of RMR and BMR, discussion of issues related to normalizing data to body size.*