Calorimetry Data Analysis – Running the R Program "Calorimetry Analysis.R"

1. To use the **Calorimetry Analysis** R program, you must preprocess your data file.

The data can be viewed and preprocessed as an excel file. The TSEsystems Phenomaster reports the following measurements every 9 minutes for as long as the system is running.

TSE Calorimetry Parameters	
Parameter	Description
S.Flow	Sample box flow (l/min)
Ref.O2	Reference O2 (%)
Ref.CO2	Reference CO2 (%)
Flow	Flow per box (l/min)
Temp	Temperature (Celsius)
O2	O2 concentration (%)
CO2	CO2 concentration (%)
dO2	Difference in O2 concentration (%) [O2Ref-Box O2]
dCO2	Difference in CO2 concentration (%) [CO2Ref-Box CO2]
VO2(1)	O2 consumption (full body weight considered) [ml/h/kg] or [ml/h]
VO2(2)	O2 consumption (Exponent LBM allocated to body weight considered) [ml/h/kg] or [ml/h]
VO2(3)	O2 consumption (body weight not considered) [ml/h]
VCO2(1)	CO2 consumption (full body weight considered) [ml/h/kg] or [ml/h]
VCO2(2)	CO2 consumption (Exponent LBM allocated to body weight considered) [ml/h/kg] or [ml/h]
VCO2(3)	CO2 consumption (body weight not considered) [ml/h]
RER	Respiratory Exchange Rate
H(1)	Heat production [kcal/h/kg] or [kcal/h] (full body weight considered)
H(2)	Heat production [kcal/h/kg] or [kcal/h] (Exponent LBM allocated to body weight considered)
H(3)	Heat production [kcal/h] ((body weight not considered)

XT+YT	Total beam interruptions
XT	X-beam interruptions, total counts
XA	X-beam interruptions, ambulatory movement
XF	X-beam interruptions, fine movement
YT	Y-beam interruptions, total counts
YA	Y-beam interruptions, ambulatory movement
YF	Y-beam interruptions, fine movement
Z	Z-beam interruptions, total counts
CenT	Light beam interruptions, central counts
CenA	Light beam interruptions, central ambulatory movement
CenF	Light beam interruptions, central fine movement
PerT	Light beam interruptions, peripheral counts
PerA	Light beam interruptions, peripheral ambulatory movement
PerF	Light beam interruptions, peripheral fine movement
DistK	Cumulative distance with reference to the sample interval
DistD	Differential distance with reference to the sample interval
Speed	Actual speed
Drink	Drink consumed
Feed1	Feed consumed

a. The first step is to make sure there are no anomalies in the reported data or unusual behaviors in the mice. To do this, make sure flow rate and temperature are consistent throughout the experiment and between cages. Next, put the data from each mouse into separate excel files. Plot H3, RER, and XT as a function of time for each mouse to ensure normal behavior. You should see higher activity during the night hours. DLAM facilities have lights on from 7:00am to 7:00pm.

b. Mice will show unusually high activity as they acclimate to the new environment. To control for this disregard the first daytime period (approximately 8 hours). If activity appears abnormally high for longer than 8 hours in the XT vs time graph, you may have to disregard the entire first day and night period. You can also disregard the last partial day period, as it will not be a full 12 hours.

c. For determining metabolic phenotype we are only interested in Time, XT, RER, H3, VO2(3), and VCO2(3). For file preprocessing, remove all other columns from each individual mouse file.

d. H3 represents Energy Expenditure in kcal/hour. Create another column titled EE cal/min and covert the data from kilocalories per hour to calories per minute. Verify that the data in this column matches energy expenditure calculated by the Weir equation.
Metabolic rate (cal/min) = $3.94 \, VO_2 + 1.11 \, VCO_2$

e. Make two new columns to calculate glucose oxidation and lipid oxidation, titled Gox and Lox, respectively.

$$Gox = (4.55*VO2)-(3.21*VCO2)$$
$$Lox= (1.67*VO2)-(1.67*VCO2)$$

    f.   Next, the data must be normalized to account for differences in body size and composition between mice. When comparing obese and lean mice, normalize data by kilograms of lean body mass to account for the metabolic differences between fat and lean tissues. Create new columns for normalized EE, VO2, VCO2, Gox, and Lox and divide by lean body mass determined by EchoMRI.

    g.   Lastly, add a "Cycle" column where you define the 2 night cycles and 2 day cycles in your dataset (delete any odd timings that don't fall into either cycle category). Night1 should be the first 7pm-7am cycle and night2 would be the second 7pm-7am cycle. Day1 should be the first 7am-7pm cycle and day2 is the second 7am-7pm cycle. 7am & 7pm are approximate, cutoffs may be at 6:55pm for example, or the cycle may start at 7:10am, just make sure the start and end times are within the range of 7am & 7pm.

2. Make sure your final data file is formatted like this which the same headers as well (make sure night/day are not capitalized):

Cycle	Time	XT	RER	H3	EE cal/min	EE cal/min,	VO2(3)	VCO2(3)	VO2 norm	VCO2 norn	Gox (mg)	Lox (mg)	Gox norma	Lox normalized
night1	19:06	990	0.694	0.554	9.233333	393.5777	118	82	5029.838	3496.802	273.68	60.12	11670.79	2562.66
night1	19:15	811	0.718	0.571	9.516667	405.655	121	87	5157.715	3710.021	271.28	56.78	11568.44	2420.29
night1	19:24	906	0.716	0.55	9.166667	390.736	116	83	4944.587	3539.446	261.37	55.11	11145.84	2349.105
night1	19:33	1880	0.715	0.564	9.4	400.682	119	85	5072.464	3624.733	268.6	56.78	11454.16	2420.29
night1	19:42	1842	0.704	0.58	9.666667	412.0489	123	86	5242.967	3667.377	283.59	61.79	12093.39	2633.845
night1	19:51	1526	0.689	0.611	10.18333	434.0722	130	89	5541.347	3795.309	305.81	68.47	13040.94	2918.585

3. Next, make sure that each mouse is saved into a separate CSV file with the mouse ID and any other relevant information in the file name. For example, if you have mice with a specific genotype & diet make sure to include the mouse ID, genotype, and diet all in the file name.

4. Go into the program where the line begins with **setwd** and place your folder directory that contains ONLY your input files for the program.

5. Next go to the variable **all_params** and make sure that the first 3 column names listed in the setNames function are the correct column names you wish to include for each mouse (mouse identifiers) – all the calculation column names should remain the same.

6. Go under the comment "**#extracting mouse identifiers from file name (mouse ID, genotype, diet)**". For your mouse identifiers alter the start & stop integers to reflect the location of the characters that contain your mouse's info in the file name. For example, if your mouse ID is between the 7th and 9th letter in the file name, then place 7 and 9 as your start and stop characters respectively.

7. Scroll down to the very last line in the code and change your output file name (in the **write.csv** function) to the file name of interest.