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.

2. Make sure your final data file is formatted like this which the same headers as well:

Time	XT	RER	H3	EE cal/min	EE cal/min/kg lbm	VO2(3)	VCO2(3)	VO2 norm	VCO2 norm	Gox	Lox	Gox normalized	Lox normalized
19:06	78	0.75	0.609	10.15	4337.606838	128	96	54700.8547	41025.64103	274.24	53.44	117196.5812	22837.61
19:15	8	0.736	0.584	9.733333	4159.54416	123	90	52564.10256	38461.53846	270.75	55.11	115705.1282	23551.28
19:24	218	0.731	0.596	9.933333	4245.014245	125	92	53418.80342	39316.23932	273.43	55.11	116850.4274	23551.28
19:33	471	0.753	0.656	10.93333	4672.364672	137	104	58547.00855	44444.44444	289.51	55.11	123722.2222	23551.28

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.