Metabolic regulation protocol v1

This is a protocol for measurement of metabolic regulation in mice during treadmill running, The purpose of the metabolic regulation test is for mice to run at steady state for 30 min at 60% of $VO2_{max}$ to determine differences in substrate utilization when intensity is clamped. which was modified from Columbus Instruments' manual and Jeppesen et al. 2013. *Diabetes*. The calculation of CHO and FAT is based on Perez-Martin et al. Diabetes & Metabolism 2001, 27:466-74.

Procedures

1. Perform $VO2_{max}$ at least 2 days prior.
2. Acclimate the mice to the treadmill by running for 10 min at 10 m/min for 3 consecutive days at 9 am, prior to performing an analysis.
3. Check Drierite (should be blue). If purple, change it and SAVE purple Drierite for reuse.
4. Turn on the power (surge protector) closest to the tank to turn on the entire system.
5. Turn on the computer and login. Password is: yanlab.
6. Weigh mice. This factors into the VO2 data since units are ml/kg/hr.
7. Open **CLAMS** software.
8. Go to **Setup Experiment** tab.
   - **a.** Calibrate oxymax
     - **i.** Turn gas tank valve to open. Pressure should be ~10-15 psi. DO NOT adjust the adjustment valve unless the pressure is not at the target pressure.
     - ii. Input certified gas values on the label of the gas tank. The values should be around 20.5% O2 and 0.5% CO2.
     - iii. Click "Start Calibration" in the CLAMS software when all lights of the equipment are stable (ready). This process will take approximately 8 minutes. You can set Treadmill Controller parameters during this time.
   - **b.** Select the protocol in "Treadmill Controller" - **See Below.**
   - c. Select 4 cages and make sure reference interval is set to 4. DO NOT UNSELECT A CHANNEL FOR THIS PROTCOL. Unselect in the **Experiment setup** tab on the right side on the screen for the oxymax to sample gases. **Important**, if 4 cages are selected, it will rotate through all 4 cages and reference cage (5 minute increment).
   - d. Click "Edit" in the setup Experiment Tab.
     - i. Click "Add subject". Enter a "Name" (Mouse number), "Mass" (weight), Strain (WT,AMPK KI, etc), Gender, Date of Birth
     - ii. Click and drag to a specific cage or treadmill channel
9. Place mice into designated treadmill channels. Make sure the latches (top and bottom) are secure and the treadmill channels are completely sealed.
10. Turn on stimulators at 0.3 mA and 2 Hz (lights will flash).
11. Click "Data Log Location". Select location of file and name file.
12. Click "start" to start the experiment from **Setup Experiment or Run Experiment** tabs.
13. Monitor the running activities closely. Turn off the electrical stimulator when a mouse stays on the electrical grid for more than 5 seconds,.
14. Click "stop" 3 min after the last mouse stops running (allow for recording for all mice).
15. Click "Export CSV".

a. Select cages used during the test
b. Select the tests/experiments to export
c. Save data file and excel files to: DESKTOP → CLAMS → DATE

Metabolic Regulation Protocol 4 Channel

Time (min)	Speed (m/min)	Incline (%)
-9	0	5
8	16	5
30	25*	5
3	0	5

*This speed may change depending on the speed mice.

Calculations

Calculate CHO and FAT utilization based on VO2 and RER from the excel output using the following equations:

i. RER = VCO2/VO2. This is calculated for us by Oxymax
ii. % FAT = ((1-RER)/0.29)*100
iii. % CHO = ((RER-0.71)/0.29)*100
iv. FAT (mg/min) = (1.6946* VO2) – (1.7012*VCO2). The values of VO2 and VCO2 should be in ml/min.
v. CHO (mg/min) = (4.585 * VCO2) – (3.2255 *VO2). The values of VO2 and VCO2 should be in ml/min.

Please note that our VO2 and VCO2 values are in ml/kg/h. If we use the number as it is, the number we get will be mg/kg/h. The final number of FAT and CHO oxidation may be presented as J/kg/h by multiplying the number by 0.001 (convert to g) and 9 or 4 (1 g CHO = 4 cal and 1 g FAT = 9 cal) and 4.185 (Convert to Joules). A normal mouse at baseline oxidize about 0.1 mg/min FAT and 1.69 mg/min CHO (Even and Nadkarni 2012, AJP 303:R459).