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bio-protocol http://www.bio-protocol.org/e1602 Vol 5, Iss 18, Sep 20, 2015

Measurement of Resting Energy Metabolism in Mice Using Oxymax Open Circuit Indirect Calorimeter

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[Abstract] Indirect calorimeter is a powerful tool to monitor resting energy metabolism through the measurement of oxygen (O_2) consumption and carbon dioxide (CO_2) production. From the measurement of VO_2 and VCO_2, the respiratory exchange ratio (RER) can be calculated to assess energy fuel utilization and energy expenditure (Evan *et al.*, 2012). Previously, indirect calorimeter has been widely used in metabolic disease research in mice to reveal the potential roles of specific genes or treatments in regulating energy metabolism (for example: Bi *et al.*, 2014; Feng *et al.*, 2014). Here, we described a protocol to evaluate the resting energy metabolism of C57BL/6 mice during dark and light cycles using the Oxymax Open Circuit indirect calorimeter.

Materials and Reagents

1. Adult mice (C57BL/6 male mice at 3-month old were used for data acquisition in this protocol, but male or female mice of other genetic backgrounds or strains, at different ages can be used)
2. Food (normal chow diet or high fat diet) and water (ad lib)
3. Compressed gas mixture with the components of 4,929 PPM CO_2, 20.47% O_2 and Balance N_2

Equipment

1. Oxymax Open Circuit Indirect Calorimeter (Columbus Instruments, model: Open Circuit Indirect Calorimeter) (Figure 1)

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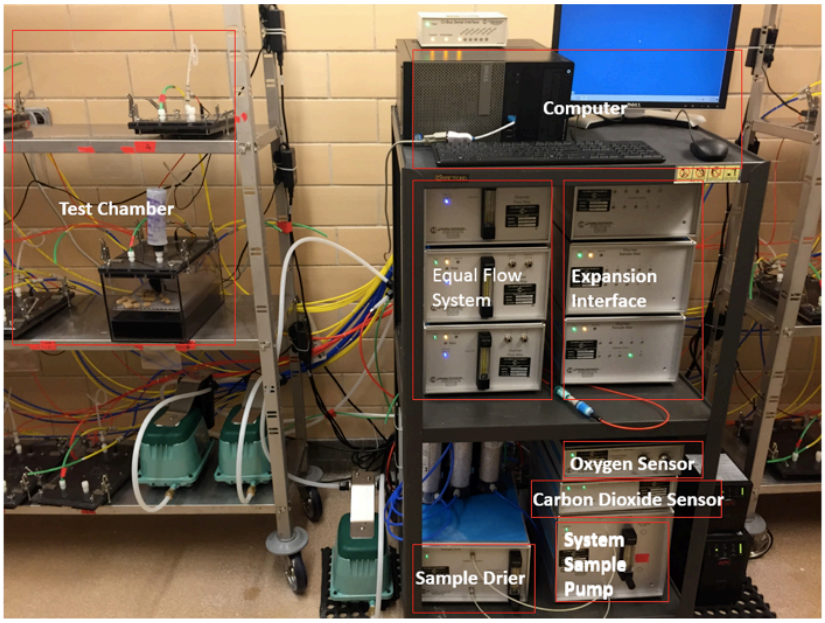

Figure 1. Open circuit indirect calorimeter components

2. Computer with software provided by the manufacture (Columbus Instruments, model: Oxymax v4.91)

Procedure

1. Turn on the Oxymax instrument and computer, allowing the system to warm up for 2 h;
2. Start the Oxymax v4.91 program;
3. Perform CO_2 calibration;
   a. Select calibration in the program, turn on the gas tank and set it to 5-10 psi;
   b. Press the CO_2 button in “calibration” window (Figure 2a). Gain should be close to 1 after calibration (Figure 2b);
4. Perform O_2 calibration;
   a. Press the O_2 button in “calibration” window (Figure 2a);
   b. Desired O_2 level is listed in the first prompt window (Figure 2d). Use the fine and coarse knobs (Figure 2c) to adjust the O_2 level to desired level;
   c. Turn off the gas tank after finishing the O_2 calibration.

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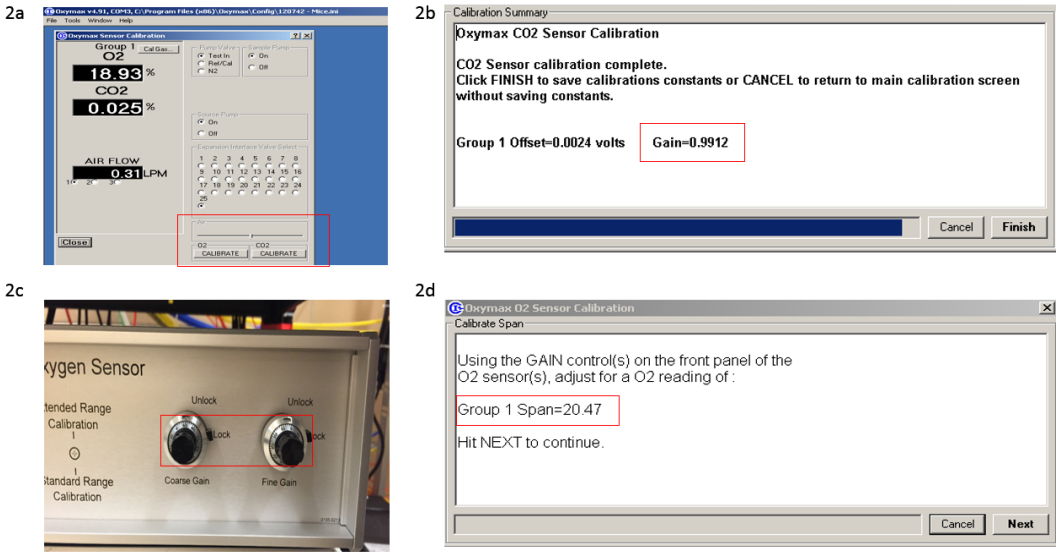

Figure 2. CO_2 and O_2 calibration

5. Setup a new experiment, choose the chambers to be used, input the Identification number and weight of mice to be measured (Figure 3), only one mouse is allowed per chamber;

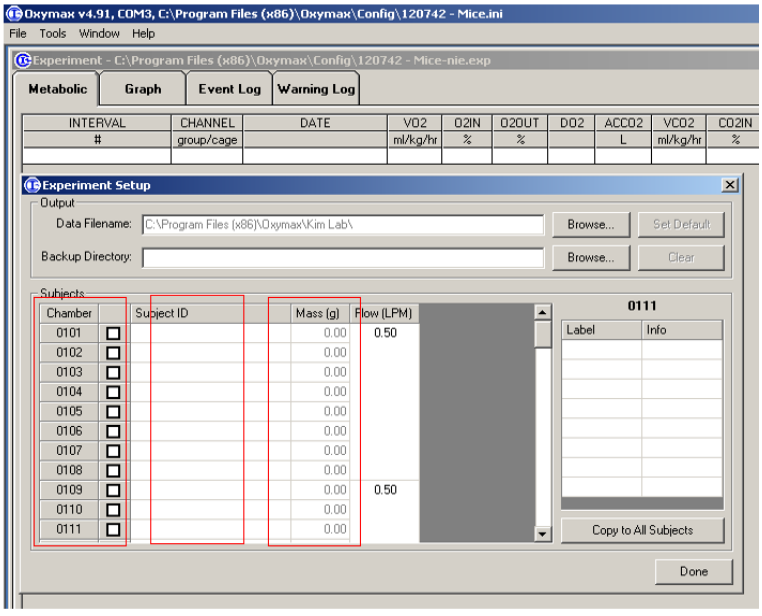

Figure 3. Input of mouse information

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6. Setup the measurement schedule including number of Intervals, time of cage settle, cage measure, reference settle, reference measure, and reference method (Figure 4).

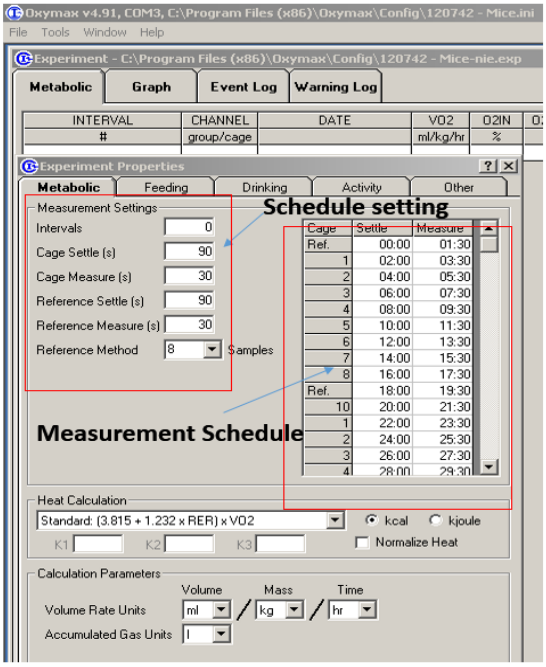

Figure 4. Setup schedule for measurement. "0" for intervals means the experiment will run indefinitely until stopped by the user. "8" for reference methods means the experiment will measure the reference air after every 8 subjects.

7. Place the mice in the corresponding calorimetric chambers, standard cages are used (Figure 5);

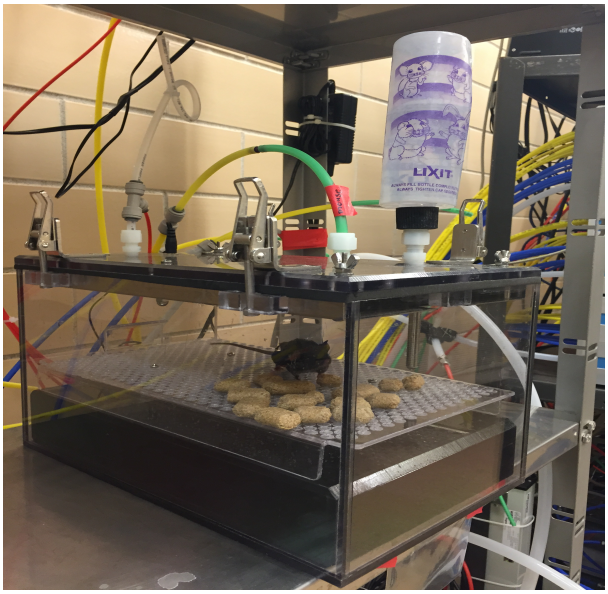

Figure 5. Test chamber for mice (standard cage)

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8. Fill the cage with sufficient food and water for a period of 24 h. Ensure that food and water are available *ad libitum* (Figure 5);
9. Check if there is any leakage in the system with software as follows:

   Open *Tools* ⟶ Select *Sample Pump* from *Oxymax utility* ⟶ Ensure *Test in* Valve open and *N2* and *Ref Air/Cal* Valve closed ⟶ Turn *Sample Pump* ON ⟶ Select the chamber to be tested in *Expansion Interface* Disconnect drier input tubing from tested chamber ⟶ put the finger over it, If there is no system leaks, the ball on the front of the system sample pump will drop to 0; If not, check all air fittings to assure an air-tight connection and test it again (Figure 6).

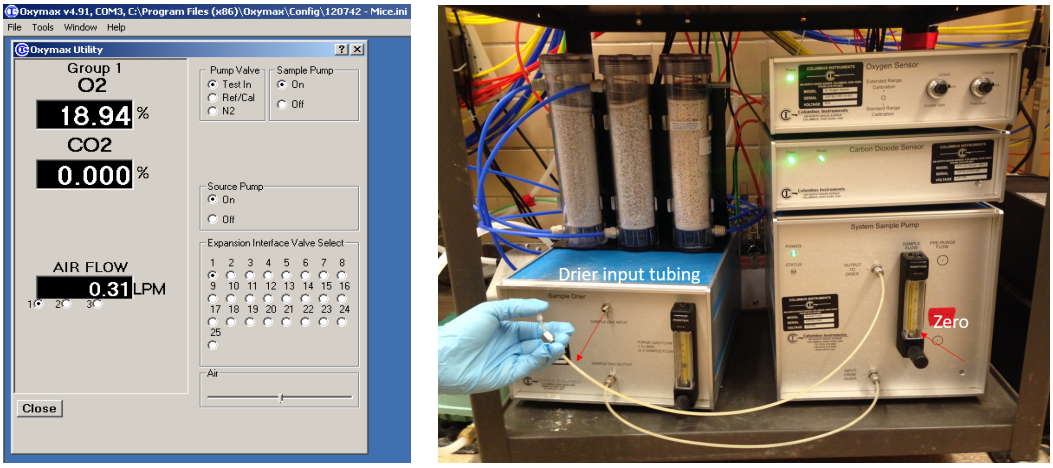

Figure 6. Gas leakage check

10. Run the experiment for 24 h and export the data in Excel file format, which include the data listed as below:
    a. O_2 consumption = (VO_2 input) – (VO_2 output), ml/kg/h;
    b. CO_2 production = (VCO_2 output) – (VCO_2) input, ml/kg/h;
    c. Respiratory exchange ratio (RER) = VCO_2/VO_2 Ratio;
    d. Energy Expenditure (Heat production) = calorific value (Cv) x VO_2 =(3.815 + 1.232 x RER) x VO_2, cal/h;
11. Close the experiment and return the mice to their home cages;
12. Turn off the system and clean the calorimetry with water and appropriate disinfectant.

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Representative data

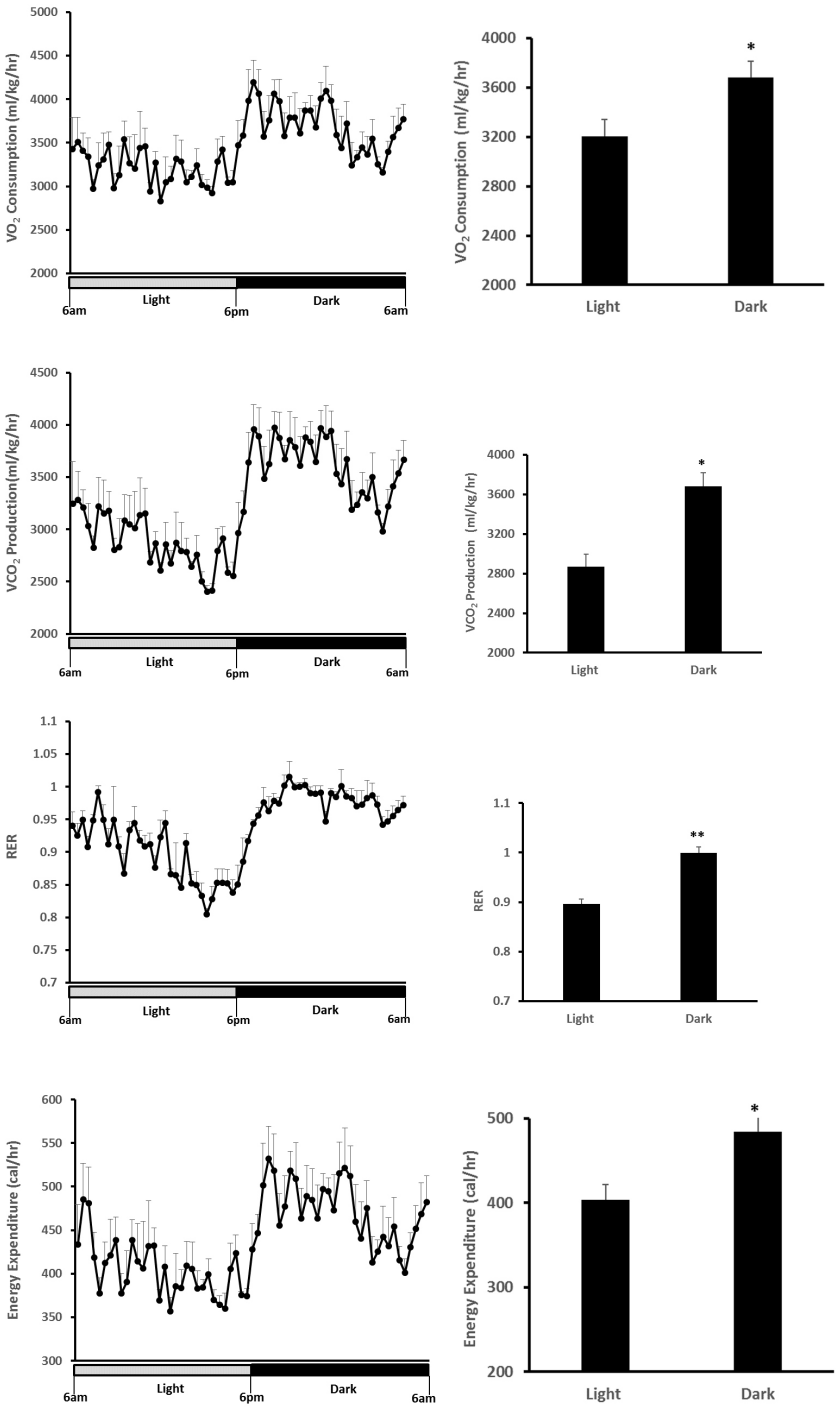

Figure 7. O_2 consumption, CO_2 production, RER and Energy Expenditure of 3-month old C57BL6 mice during light and dark cycle. The daily rhythms of metabolic parameters were recorded under a 12 h-light (open bar) and 12 h-dark cycle (black bar) (Left). Data were presented as Means ± SE (n = 5) during light and dark cycle (right). *, p < 0.05; **, p < 0.01 analyzed by the Student's t-test (comparison of mean values between the light and dark cycles).

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Notes

1. Begin data collection of mice after 1-day of acclimation in the metabolic chambers;
2. Install the Oxymax system under a constant environmental temperature (22 °C) and 12 h light (6 am-6 pm), 12 h dark cycle (6 pm-6 am);
3. VO_2 and VCO_2 were increased by approximately 15% and 28% in dark cycle, respectively;
4. RER was increased from 0.90 (light cycle) to 0.99 (dark cycle), suggesting a shift in macronutrient source from a mix of fat + carbohydrates to predominant carbohydrates in the dark cycle (the VCO_2/VO_2 ratio of fatty acid oxidation is 0.7 and carbohydrates oxidation is 1.0);
5. Energy expenditure was 20% greater in dark than light cycle, with the most active phase of mice being between 7 pm-12 pm;
6. Indirect calorimetry is a versatile system to investigate alternations of metabolic rate under different conditions. For example:
   a. To compare metabolic homeostasis and energy expenditure in wild type and mutant mice fed with normal chow diet or high-fat-diet;
   b. To investigate changes in metabolic rate with aging, a potential indicator of improved health status.
   c. Oxymax open circuit indirect calorimeter can also be incorporated with other chamber systems, such as activity, body mass, feeding, drinking, food access control, running wheel, urine collection, sleep detection, body core temperature and heart rate to fulfill different experimental designs.

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