Practical considerations in the analysis of indirect calorimetry data when comparing lean and obese groups
Specific to TSE PhenoMaster system

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Introduction

Oxidation of carbohydrates, lipids, and proteins provide the energy necessary to maintain homeostasis in the body. Energy balance is defined as the difference between energy intake and energy expenditure (EE).[1] To maintain weight, not only must energy intake must match energy expenditure, macronutrient intake must balance macronutrient oxidation. Low levels of energy expenditure, lipid oxidation, and physical activity are conducive to weight gain.[2] Determination of energy expenditure has proven to be particularly useful in research regarding the pathogenesis of obesity and diabetes. Indirect calorimetry (IC) is a technique that provides one of the most accurate measurements of EE in an individual.[3] IC allows for the estimation of EE and determination of the type and rate of substrate oxidation based on gaseous exchange measurements. IC is considered indirect as it measures the volume of oxygen consumed (VO2) and carbon dioxide produced (VCO2) as opposed to directly measuring energy lost as heat.

IC has proven to be a valuable research tool and is increasingly used in small animals to investigate how compounds or genetic modifications alter metabolic phenotype. However, there is no standard approach for calculating metabolic activity from calorimetric data. When it comes to data analysis, various methods and equations are seen in literature. We will focus on the different ways to interpret IC data and the limitations involved. We will specifically focus on data collected from the TSE PhenoMaster (TSE Systems, Chesterfield, MO) an indirect open circuit system and the comparison of lean and obese groups.

Verifying Data Quality

Once the data is obtained from the experiment, it is critical to evaluate the quality of the results before any conclusions are drawn. Any anomalies in the behavior of the mice or complications with the metabolic cages may compromise the results. The TSE PhenoMaster reports flow rate and temperature over time; scan the data to ensure these values consistent throughout the experiment and between cages. Fluctuations in airflow or temperature have the potential to alter energy expenditure, if any of the cages display anomalies data must be discarded.

When using the TSE Phenomaster, respiratory exchange rate, activity, and uncorrected energy expenditure are calculated and reported by the system. These are denoted as RER, XT, and H(3),

respectively. Plotting these values as a function of time is a useful way to examine the behavior of the mice. Mice are generally more active at night, a normal circadian rhythm should be reflected in the graphs.

Correcting for differences in body size and composition

In order to accurately make comparisons between mice, raw data must be corrected for differences in body size and composition.

It is known that larger animals with more body mass will expend more energy than smaller ones. A major issue in IC is how to correct for differences in body size. It may seem intuitive to compare measurements of VO2 and VCO2 per gram of body weight, however this is misleading as different types of tissues vary in levels of metabolic activity.[4] This is especially important to consider when comparing lean and obese groups. Lean tissue displays significantly higher levels of metabolic activity than adipose tissue; therefore, dividing the measurements by total body mass would not accurately correct for these differences.

It is seen in literature that some researchers normalize their data by body mass to the 0.75 power. Use of allometric scaling exponents to correct for differences in body size is commonly seen but is a similarly flawed approach. Allometric scaling rests on the basis that heat loss is a function of surface area as opposed to body mass. As surface area is difficult to measure, it was expected rates would scale to the ⅔ power as a reflection of the relationship between surface area and volume.[5] However, the work of Max Kleiber demonstrates the exponent may be closer to 0.75.[6] This is known as Kleiber's law. However, this method of scaling is subject to severe criticism in the scientific community on both theoretical and empirical grounds.[5] Experts argue the exponent may lie anywhere between 0.66 and 0.75.

Regardless of the criticism surrounding Kleiber's law, normalizing by body weight to an allometric scaling exponent is ineffective when comparing fat and lean groups of the same species. When comparing obese and lean groups, body composition affects energy expenditure to a much greater extent than body size. To most effectively compare groups that differ greatly in body composition, dividing by grams of lean body is the most practical. However, this method has not been widely employed because until recently, estimating fat free mass has been difficult.[4] Lean body mass can be accurately determined using magnetic resonance imaging (MRI). Technologies for small animals such as EchoMRI (EchoMRI, Houston, TX) can be used to measure body composition with high precision.

Determining Times to Use

IC measurements are generally collected over the course of multiple days giving rise to hundreds of data points per animal. It can be difficult to discern which data points give an accurate reflection of the metabolic phenotype. The first thing to consider is that animals will show unusually high activity as they acclimate to the metabolic cages. Many researchers choose to eliminate the first 24 hours from the data set to control for this. However, the TSE PhenoMaster reports activity (XT) allowing users to see exactly how long it takes the animals to start displaying normal behavior. Plotting XT as a function of time allows for a more accurate determination of which points should be removed from the data set. In our experience, we have typically had to remove about the first 8 hours of data. However, this may be dependent on treatment or strain of mice and should be assessed for each individual experiment.

Useful Equations

Energy expenditure is most commonly reported in calories per minute as calculated by the Weir Equation (7).

EE (cal/min) = (3.94 VO2) + (1.11 VCO2).

The Weir equation is the most accurate method of calculating energy expenditure in indirect calorimetry experiments known to date. Alternative, outdated equations such as the Lusk equation are occasionally seen in literature, but is considered significantly less accurate.[8] The TSE PhenoMaster reports energy expenditure as H(3) in kilocalories per hour. We confirmed, when converted to calories per minute the values match energy expenditure as calculated by the Weir Equation.

Substrate oxidation rates also prove to be valuable measurements when determining metabolic phenotype. Using gaseous exchange measurements glucose oxidation (Gox) and lipid oxidation (Lox) can be calculated.[9] If urinary nitrogen excretion is measured, protein oxidation can also be determined. Using VO2 and VCO2 measurements collected by the TSE system we are able to calculate Gox and Lox using the following equations:

Gox= (4.55 x VO2) – (3.21 x VCO2)

Lox= (1.67 x VO2) – (1.67 x VCO2)

References

1. Campbell SC. Nutritional Recommendations for Athletes. *Nutrition in the Prevention and Treatment of Disease*. 2013:279-293. doi:10.1016/b978-0-12-391884-0.00016-0.
2. Galgani J, Ravussin E. Energy metabolism, fuel selection and body weight regulation. *International Journal of Obesity*. 2008;32(S7). doi:10.1038/ijo.2008.246.
3. Thomas N, Gupta R, Ramachandran R, Venkatesan P, Anoop S, Joseph M. Indirect calorimetry: From bench to bedside. *Indian Journal of Endocrinology and Metabolism*. 2017;21(4):594. doi:10.4103/ijem.ijem_484_16.
4. Arch JRS, Hislop D, Wang SJY, Speakman JR. Some mathematical and technical issues in the measurement and interpretation of open-circuit indirect calorimetry in small animals. *International Journal of Obesity*. 2006;30(9):1322-1331. doi:10.1038/sj.ijo.0803280
5. Niklas KJ, Kutschera U. Kleibers Law: How theFire of Lifeignited debate, fueled theory, and neglected plants as model organisms. *Plant Signaling & Behavior*. September 2015:00-00. doi:10.1080/15592324.2015.1036216.
6. Kleiber M. *The Fire of Life*. New York: John Wiley & Sons; 1961.
7. Weir JBDV. New methods for calculating metabolic rate with special reference to protein metabolism. *The Journal of Physiology*. 1949;109(1-2):1-9. doi:10.1113/jphysiol.1949.sp004363.
8. Lighton JRB. *Measuring Metabolic Rates: A Manual for Scientists*. 2nd ed. Oxford, United Kingdom: Oxford University Press; 2019.
9. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology*. 1983;55(2):628-634. doi:10.1152/jappl.1983.55.2.628.

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