Commentary

MOLECULAR METABOLISM

What does indirect calorimetry really tell us?

Karl J. Kaiyala*

Accurate and sensitive assessments of energy expenditure (EE) in mice are essential given their importance to research on the molecular mechanisms of energy homeostasis [1]. EE phenotyping in mice is widely available using state-of-the-art equipment in many laboratories, including those in regional centers in the United States of America (http://www.mmpc.org/, http://www.niddk.nih.gov/research-funding/research-programs/Pages/nutrition-obesity-centers.aspx) and Europe (http://www.eumodic.org/, http://www.mouseclinic.de/). These and many other laboratories worldwide employ respirometric indirect calorimetry, which estimates EE based on mathematical relationships [2] that link respiratory oxygen and carbon dioxide exchange to metabolic heat production. Direct calorimetry, the 'gold standard' method for quantifying metabolic rate [2], measures the heat generated by the test subject, and was the method employed in the research using pre-1940s technology that validated respirometry based on studies involving humans and a limited number of other species [3]. Surprisingly, despite myriad studies using respirometry in mouse models, this technique does not appear to have been validated against direct measurements of EE in mice until recent work by Burnett and Grobe [4]. Now a second study by these authors in the current issue of *Molecular Metabolism* [5] indicates that while the two methods generate similar values (probably indistinguishable if measured using the old technology), respirometry may promote erroneous conclusions regarding the impact of diet on EE.

In this work [5], respirometry was employed in conjunction with simultaneous direct calorimetry to quantify resting EE (REE) in C57BL/6J mice, the most commonly used mouse strain for metabolic research. The findings confirm and extend previous work [3,4] questioning our reliance on the indirect method. Specifically, respirometry underestimated REE by ~7% when the mice were maintained on a standard chow diet, consistent with previous work in which respirometry underestimated REE by ~10% in chow-fed C57BL/6J mice [4]. When the mice were switched to a high-fat diet, however, respirometry indicated a significant increase of REE in comparison with the chow-fed state, whereas direct calorimetry did not [5]. To further complicate matters, when the mice were switched back to the chow diet, both methods indicated a reduction of REE [5].

Discrepancies between direct and indirect measures of EE can reflect several sources. Two likely explanations are *1)* measurement error and *2)* erroneous assumptions about the mathematical relationships between metabolic rate and respiratory gas exchange. A potential explanation for *2)* involves the gut microbiota comprised of trillions of microbes dominated by *anaerobic* bacterial species [6]. The aggregate metabolic rate of anaerobic species cannot be measured by respirometry, but is captured by direct calorimetry [1–3]. Accordingly, changes in the balance of gut microbial species could differentially affect EE as measured by direct and indirect calorimetry. Studies to test this hypothesis are needed.

Additional variables include nitrogen turnover and microbial methane production, factors that can affect respirometric REE calculations [7]. These measures are rarely employed, and methane production has long been assumed to represent a minor component of energy flux in monogastric mammals. Combined with potential complications involving other intestinal gasses (principally hydrogen, hydrogen sulfide, and carbon dioxide) generated by enteric bacterial fermentation of unabsorbed carbohydrates [8], the gut microbiota may indeed have effects on indirect measures of EE that are both significant and complex. In addition to the potential role of gut flora, uncontrolled diabetes and other models of disordered energy homeostasis can cause serious violations of key assumptions of respirometry [1,2,9]. These considerations indicate that the mathematical relationships between whole body oxygen uptake, carbon dioxide release, and metabolic heat production may be more complex and less predictable than generally assumed by practitioners of respirometric indirect calorimetry. Indeed, technically rigorous research [3] disclosed large errors in respirometric EE estimates in non-standard laboratory species (dove, quail, and Kangaroo rats).

Burnett and Grobe [4,5] measured REE to preclude the need for food, water, and waste management, factors that impact the evaporative heat loss component of direct calorimetry [2]. Studies that obviate this problem will be necessary to test the validity of respirometry as it pertains to total 24-h EE. This need will require development of "live in" direct/indirect calorimeter systems.

The current study [5] raises important concerns for murine EE phenotyping. The existence of brown adipose tissue in adult humans and the discovery of inducible thermogenic adipose tissue (beige fat) have stimulated interest in the potential value of drugs that increase EE as targets for obesity drug development [10], and mice will play a pivotal role in this effort. Because even small differences of energy balance can have a marked impact on energy storage if they are sustained over time, it is imperative to use methods that accurately measure EE within groups of mice having different characteristics while also detecting small within-group changes due to pharmacological or other inducible interventions. These challenges also require valid methods to adjust EE for differences in body mass/composition [1] and http://www.mmpc.org/shared/regression.aspx. The finding that respirometry may be subject to systematic errors that vary with diet [5] indicates that the mouse metabolic phenotyping field should be mindful of the peril of the "streetlight effect", the tendency to search for one's lost keys based on where the light is best [11].

1959 NE Pacific Street, Box 7475, Seattle, WA 98195-7475, USA

*Tel.: +1 206 616 5203. E-mail: kkaiyala@u.washington.edu.

Received March 14, 2014 • Accepted March 21, 2014 • Available online 28 March 2014

http://dx.doi.org/10.1016/j.molmet.2014.03.005

ACKNOWLEDGMENTS

Grant support: National Institutes of Health (P30 DK035816, U24DK076169, R03-DA033453).

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