Methodologic considerations in the evaluation of adaptive thermogenesis

Dear Editor:

Even stringent adherence to a reduced-calorie diet often results in suboptimal weight loss, partly because of declines in resting energy expenditure (REE) that are disproportionately greater than would be expected from the loss of fat-free mass (FFM) and fat mass (FM) alone. This phenomenon was initially described by Keys et al. (1) and is now commonly termed "adaptive thermogenesis" (AT). In their elegantly designed quasi-replication of Keys et al.'s landmark Minnesota Starvation Experiment, Müller et al. (2) conducted 2 studies to extensively investigate anthropometric and physiologic determinants of AT in a group of 32 young men. In study 1, each participant underwent 1 wk of overfeeding, which was immediately followed by 3 wk of underfeeding. In study 2, 8 of these men returned 18 mo later for 1 wk each of over- and underfeeding. The authors found that AT became significant after just 3 d of caloric restriction in study 2 and was potentiated by the loss of FFM, declines in resting heart rate, reductions in C-peptide excretion (a marker of insulin secretion) and plasma glucagon concentrations, and water retention. Surprisingly, AT was unrelated to changes in metabolic hormone concentrations in study 1. The authors concluded that altered metabolic activities of the heart, liver, and kidneys, along with a slight reduction in body temperature, fully accounted for AT.

Perhaps equally as important as their identification of the aforementioned mediators of AT are the findings that AT was unrelated to changes in FM and/or thyroid hormone, leptin, or catecholamine concentrations in either study 1 or study 2. In contrast, changes in FM but not FFM were predictive of AT in Keys et al.'s study (3), and both Müller's group and others previously reported body composition–independent associations between weight loss–related changes in REE and triiodothyronine, leptin, and/or norepinephrine (4–6). It is of interest to note that the degree of AT can vary considerably depending on how it is calculated, and the methods used to evaluate AT should be considered given the inconsistent findings across these different studies. In the article under review, Müller et al. calculated AT as REE adjusted for FFM and REE adjusted for both FFM and FM ($REE_{adjFFM+FM}$) after caloric restriction minus the same measure after overfeeding. The authors also estimated the masses of skeletal muscle, organ, adipose, and residual tissues using whole-body MRI and applied their specific metabolic rates to predict REE ($REE_{predicted\ from\ organ\ masses}$). They then compared the difference between the measured REE and $REE_{predicted\ from\ organ\ masses}$ ($REE_{m\text{-}p}$) at each time point.

Müller et al. concluded that AT averaged 104 kcal/d in normal-weight men when using a 2-compartment model to quantify the difference between body composition–adjusted REE after over- and underfeeding (2). Although it is unclear whether the aforementioned value also included any overweight men, the difference between $REE_{adjFFM+FM}$ (i.e., a 2-compartment adjustment) after overfeeding and caloric restriction in all participants in study 1 yielded an AT of 166 ± 124 kcal/d. Although the difference between $REE_{predicted\ from\ organ\ masses}$ at baseline (rather than after overfeeding) and after caloric restriction equated to 104 ± 38 kcal/d, this calculation is inconsistent with that provided for the quantification of AT. Nevertheless, this value is similar to the 108 ± 153-kcal/d difference between measured and predicted REE ($REE_{m\text{-}p}$) after caloric restriction and overfeeding in study 2. Last, $REE_{m\text{-}p}$ after caloric restriction in study 1 showed that AT totaled 72 kcal/d after FFM composition was accounted for. In the abstract, the authors seem to have subtracted this "true" AT (from study 1) from the 108 kcal/d average degree of AT (from study 2), concluding that changes in FFM composition accounted for 36 kcal/d of the decline in REE. Because these calculations yielded different degrees of AT, it is of interest to delineate which was selected to quantify AT for the correlational analyses to draw comparisons with previous studies.

This issue also brings to light the need for a standardized approach to evaluate AT. In comparison with $REE_{adjFFM+FM}$, $REE_{predicted\ from\ organ\ masses}$ more accurately estimates REE and, in turn, AT (2, 4). It follows that detailed body-composition analyses should be used to evaluate AT whenever possible. Regardless of whether $REE_{adjFFM+FM}$ or $REE_{predicted\ from\ organ\ masses}$ is used, estimated REE should be considered in relation to the measured REE when evaluating AT. Although Müller et al. stated that AT was calculated as the difference between body composition–adjusted REE after caloric restriction and overfeeding (2), $REE_{m\text{-}p}$ after caloric restriction (rather than the difference in $REE_{m\text{-}p}$ between the over- and underfeeding periods) was used to quantify the portion of the decline in REE that could not be accounted for by changes in individual tissue masses in study 1. When considering the high costs and risks of some body-composition imaging techniques (e.g., MRI, computed tomography, and dual-energy X-ray absorptiometry), multiple assessments are not always feasible. Because the "true" AT was calculated as $REE_{m\text{-}p}$ after caloric restriction only, the comprehensive body-composition assessments before completion of the weight-loss phases did not seem to be necessary to accurately estimate AT. Taken together, the estimation of individual tissue masses at a single time point (e.g., after weight loss) may be a cost-effective approach to accurately quantifying AT.

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Matthew G Browning

From the Department of Kinesiology and Health Sciences, Virginia Commonwealth University, Richmond, VA (e-mail: browningmg2@vcu.edu).

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Reply to MG Browning

Dear Editor:

We thank Browning for his thoughtful methodologic considerations about the definition of adaptive thermogenesis (AT). Following the definition by Keys et al. (1), AT refers to changes in resting energy expenditure (REE) independent of changes in body mass or the mass of its metabolically active components. That is, the REE component of AT is due to the decrease in the average and specific metabolic activity of body cells (2). Until now, different authors have used different definitions of AT, resulting in divergent data on the magnitude of AT and some confusion about AT itself (for a review see reference 2).

In our recent study (3), AT was defined as the greater-than-expected change in REE with underfeeding, in which the expected change in REE was calculated by using a quantitative model that includes body weight, fat-free mass (FFM), fat mass (FM), and individual organ and tissue masses. Thus, detailed and accurate in vivo body-composition analysis (BCA) was necessary to calculate AT. REE was adjusted (REE_{adj}) for either FFM or FFM+FM or the sum of organ and tissue masses. Because standard techniques and models of BCA (e.g., based on measurements of body density by air-displacement plethysmography or dual-energy X-ray absorptiometry measurements) have limitations during non–steady state conditions [i.e., during short-term negative and positive energy balances (4, 5)], these issues add to adjustment of REE and thus to AT and its interindividual variance. Instead of air-displacement plethysmography and dual-energy X-ray absorptiometry, we used magnetic resonance technologies, which are robust and most precise for in vivo BCA. The recent quantitative magnetic resonance technology accurately detects small changes in FM and body water (6). Within FFM, the masses and the metabolism of high-metabolic-rate organs (brain, liver, heart, and kidneys) were assessed by whole-body MRI (5). In addition to quantitative magnetic resonance and MRI, nitrogen and fluid balance techniques in combination with in vivo BCA have been used to assess and validate short-term changes in body composition (3, 7).

In our study, AT was first calculated from the difference between REE_{adj} for FFM or FFM+FM at caloric restriction and their corresponding values at day 7 of overfeeding (3). This may be called AT_1. In addition, we calculated the difference between REE as measured by indirect calorimetry and REE as calculated from individual organ masses multiplied by their corresponding specific metabolic rate, reflecting mass-independent changes in the specific metabolic rates of organs (i.e., AT_2) (3). The latter calculation took into account the composition of FFM. Thus, we considered AT_2 as "true" AT, which was 72 kcal/d. With the use of that calculation, weight-loss–associated changes in both FFM as well as FFM composition at the organ and tissue level must be taken into account. This is what Keys et al. had proposed (1). Because, however, *1*) calculation of AT_2 was based on numerous assumptions (e.g., with regard to specific metabolic rates of individual organs and tissues, which may differ between different nutritional states; see reference 8) and *2*) MRI data had been obtained in 16 of 32 subjects only, we finally selected AT_1 for our correlational analyses.

We agree on the need for a standardized approach to evaluate AT. We feel that detailed BCA should be used. This idea is limited by the high effort and costs of whole-body MRI, which may limit at least repeated measurements. Thus, the idea to assess organ and tissue masses after weight loss only is well taken. In that case, the prediction of REE from organ and tissue masses is made after weight loss only and the (in our case, small) difference between measured and predicted REE before weight loss remains unknown. In a weight-stable situation, this difference results from a sum of technical errors and uncertain assumptions. To overcome that problem, we suggest that each group of scientists evaluating AT should know about its laboratory-specific errors. Ideally, those errors should be characterized for different groups (e.g., children, the elderly, and underweight and obese subjects). If the error is known, the estimation of individual organ and tissue masses at a single time point (e.g., after weight loss or weight gain) will provide an accurate basis to calculate AT.

As far as short-term changes in body weight are concerned, this will also change the density and hydration of FFM and its individual organs (i.e., the molecular composition of FFM). For example, in early starvation, part of the losses in muscle and liver masses is explained by glycogen breakdown and loss of tissue water. In our present study, FFM hydration (as assessed by deuterium dilution) increased by 3.3% (mean difference between overfeeding − basal) but decreased by 3.6% during caloric restriction and increased by 0.9% in response to refeeding. Thus, within a 3-d period of caloric restriction, ~2–3 L of water was lost. In this situation, changes in FFM cannot reflect changes in "functional" FFM or "functional" organ masses. This gives rise to the idea that adjustments of REE for FFM (or of REE for FFM+FM) after weight loss will affect AT. This is shown schematically in **Figure 1**. Before weight loss (T0), REE is on the REE-FFM regression line (in blue). After weight loss (T1), there is a decrease in both FFM and REE. REE decreases with FFM and a further decrease is due to AT (in orange and red). However, this is an underestimation of AT because the decrease in measured FFM exceeds the decrease in "functional" FFM (T1′, in green) due to the loss in body water. At "functional" FFM, true AT is higher than the calculated value based on FFM alone. At the organ tissue level, adjustment of REE for organ masses also underestimates AT because the decrease in masses of high-metabolic-rate organs is overestimated. It becomes obvious that to evaluate AT there is a need for more sophisticated concepts about body composition that have not been considered in previous studies on AT (9).

Neither of the authors had a conflict of interest related to this letter.

Manfred James Müller
Anja Bosy-Westphal

From the Institute of Human Nutrition and Food Science, Christian Albrechts University, Kiel, Germany (MJM, e-mail: mmueller@nutrfoodsc.uni-kiel.de), and the Institute of Nutritional Medicine, University of Hohenheim, Stuttgart, Germany (AB-W).