A Recurring Problem With the Analysis of Energy Expenditure in Genetic Models Expressing Lean and Obese Phenotypes

Andrew A. Butler[1] and Leslie P. Kozak[2]

Defining the molecular mechanisms linking obesity with insulin resistance is important for developing new therapies against the rising incidence of type 2 diabetes in industrialized nations. Maintaining a balance between calorie intake and energy expenditure is critical for preventing insulin resistance, the precursor for type 2 diabetes (1). Mouse genetics has made enormous contributions to theoretical models explaining how organisms balance energy intake and energy expenditure. A seminal event was the positional cloning of the *obese* gene (now called the *Leptin* gene) by Friedman and colleagues in the early 1990s (2). Leptin deficiency causes severe obesity in mice and humans (3), and leptin was proposed to regulate energy homeostasis by suppressing appetite and increasing energy expenditure (4). However, it has clearly been forgotten that the interpretation of energy expenditure data from mice homozygous for the Lep^{ob} mutation was challenged shortly after the initial publication (5). Increasingly sophisticated technologies for manipulating the mouse genome are now used routinely to analyze new genes linked to energy homeostasis, resulting in many new mouse models with obese or lean phenotypes. Altered energy expenditure is frequently cited as the primary mechanism underlying the obese or lean phenotype. However, in many cases the same issues with interpretation of energy expenditure data are evident. Here, we discuss what has developed into a recurring problem in the literature with the analysis of energy balance. Specifically, we shall discuss the practice of using body weight as a denominator in analyzing energy balance to overestimate the role of energy expenditure.

The growing number of individuals with chronic metabolic diseases like type 2 diabetes provides a powerful incentive for investigating mechanisms linking obesity with insulin resistance. That a balance between food intake and energy expenditure (thermogenesis) is maintained through homeostatic mechanisms is a central tenet of obesity research. A major goal is to discover mechanisms to avoid a positive energy balance, a pathway to weight gain, increased susceptibility to insulin resistance, and diseases of the metabolic syndrome (6,7).

There has been significant progress in understanding how organisms regulate caloric intake and adiposity. Identification of factors secreted from peripheral organs, including leptin and adiponectin from adipocytes, insulin from the pancreas, ghrelin from the stomach, and fibroblast-growth factor-21 from the liver and examining how they regulate adiposity and insulin sensitivity has been essential for the evolving concepts of energy homeostasis (8–10). These factors have been found to interact with networks of specific neurons in the hypothalamus and brain stem, modulating behaviors relevant to energy homeostasis including satiety, reward, and motivation (11–13). Through regulating autonomic and neuroendocrine output, and by acting directly on peripheral tissues, these factors can regulate glucose and lipid homeostasis. Importantly, aberrant regulation and action of these molecules has been linked to the development of insulin resistance and diabetes in the obese state. These findings have given hope that therapies designed to restore or replace the normal function of these peptides will provide effective treatment for obesity and the associated metabolic disorders.

Neuroendocrine factors that regulate food intake have been reported to have reciprocal effects on thermogenesis that should, in principle, facilitate attainment of energy balance. A frequently cited example is the ability of leptin to inhibit food intake and stimulate thermogenesis (4), the latter occurring through stimulating the activity of uncoupling protein 1 (UCP1) in brown adipose tissue (BAT) (14). The central nervous melanocortin system, primarily acting through melanocortin-4 receptors (Mc4r), has also been suggested to function similarly. Mc4r-deficient mice do not exhibit diet-induced thermogenesis (15) linked to impaired regulation of BAT UCP1 expression (16). Collectively, these and other observations form the basis of a neuromolecular model linking calorie intake and diet-induced thermogenesis, a system for maintaining energy homeostasis proposed by Rothwell and Stock nearly 30 years ago (17).

While such a model linking food intake to thermogenesis is theoretically satisfying, it falls short for several reasons. The clinical relevance is unclear, as it has long been debated whether nonshivering thermogenesis is the basis for diet-induced thermogenesis in humans (18). However, recent reports on the presence of deposits of brown adipocytes in adult humans (19–21), and the discovery of molecular mechanism for transforming myocytes into brown adipocytes (22), will likely further stimulate this debate. Several studies have also failed to demonstrate that leptin stimulates thermogenesis, as determined by measuring oxygen consumption, even when administered to Lep^{ob}/Lep^{ob} mice (23). That the marked decline in leptin associated with food restriction instigates

a neuroendocrine response affecting a compensatory reduction in energy expenditure is not disputed (24). Also not disputed is the experimental evidence for systems residing in the hypothalamus responding to hormones and metabolites that, when disrupted, lead to rapid weight gain and insulin resistance (25). However, the ability of leptin per se to stimulate a compensatory thermogenic effect in the well-fed state is negligible (23), while lean animals fail to respond to pharmacological doses of leptin (4). A positive energy balance is also rapidly associated with a state of leptin resistance and deterioration in the hypothalamic control of energy homeostasis (26).

Another key point is that we have little documented knowledge for a thermogenic mechanism that has the prevention of positive energy balance as its primary function. An alternative hypothesis is that the physical effort to feed life and limb and escape predators precluded the evolution of a thermogenic system to protect those few with the luxury of having a positive energy balance (27). However, complementation of this perceived evolutionary deficiency in energy homeostasis through pharmacological stimulation of thermogenesis remains an important goal for developing drugs against obesity and diabetes.

Finally, recent data from studies investigating how calorie restriction affects mitochondrial activity also question our assumptions of a correlation between calorie intake and thermogenesis. Calorie restriction extends lifespan in rodents and is associated with reduced energy expenditure. However, calorie restriction stimulates mitochondrial biogenesis in skeletal muscle, a response involving nuclear factors that regulate mitochondrial biogenesis, such as Pgc1a (28). In other words, while a positive correlation between calorie intake and physical energy expenditure exists, it is not necessarily associated with stimulating mitochondrial respiratory activity at a molecular level.

A problem in the obesity research field lays in the interpretation of energy expenditure data. The fact remains that an urgent need exists for the development of new therapies to control body weight and improve insulin action, and modulation of energy expenditure provides one potential therapeutic target. The availability of spontaneous and targeted mutations in mice is an important resource for identifying novel genes involved in energy homeostasis. Hardly a month goes by without publication of data demonstrating novel mechanisms for regulating adipose mass or further characterizing pathways of previously identified genes involved in regulating satiety or metabolism (Table 1). Often these mutant genes are reported to affect obesity/leanness and diabetes phenotypes as a consequence of their effects on substrate oxidation and energy expenditure. However, few of these have ultimately led to the identification of the novel underlying thermogenic mechanism.

The problem facing the field is that, with the exception of UCP1/BAT thermogenesis, there is little evidence for an alternative thermogenic mechanism. A case has been made for UCP1/BAT as a thermogenic mechanism for diet-induced thermogenesis at thermoneutrality (29,30). However, mice in research colonies and in the wild and humans before World War II did not spend most of their time at thermoneutrality, and the evidence is clear that at even slightly reduced ambient temperatures (24°C) UCP1/BAT is not required for diet-induced thermogenesis (31–34). Even severely obese Lep^{ob}/Lep^{ob} mice, which exhibit clear deficits in BAT thermogenesis in response to cold

TABLE 1
Publications claiming an energy expenditure phenotype based upon erroneous estimates of oxygen consumption

Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM, Moghal N, Lubkin M, Kim YB, Sharpe AH, Stricker-Krongrad A, Shulman GI, Neel BG, Kahn BB. Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. Mol Cell Biol 2000;20:5479–5489

Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, Fumagalli S, Allegrini PR, Kozma SC, Auwerx J, Thomas G. Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. Nature 2004;431:200–205

Jiang G, Li Z, Liu F, Ellsworth K, Dallas-Yang Q, Wu M, Ronan J, Esau C, Murphy C, Szalkowski D, Bergeron R, Doebber T, Zhang BB. Prevention of obesity in mice by antisense oligonucleotide inhibitors of stearoyl-CoA desaturase-1. J Clin Invest 2005;115:1030–1038

Picard F, Géhin M, Annicotte J, Rocchi S, Champy MF, O'Malley BW, Chambon P, Auwerx J. SRC-1 and TIF2 control energy balance between white and brown adipose tissues. Cell 2002;111:931–941

Smith SJ, Cases S, Jensen DR, Chen HC, Sande E, Tow B, Sanan DA, Raber J, Eckel RH, Farese RV Jr. Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. Nat Genet 2000;25:87–90

Tansey JT, Sztalryd C, Gruia-Gray J, Roush DL, Zee JV, Gavrilova O, Reitman ML, Deng CX, Li C, Kimmel AR, Londos C. Perilipin ablation results in a lean mouse with aberrant adipocyte lipolysis, enhanced leptin production, and resistance to diet-induced obesity. Proc Natl Acad Sci U S A 2001;98:6494–6499

Hofmann SM, Zhou L, Perez-Tilve D, Greer T, Grant E, Wancata L, Thomas A, Pfluger PT, Basford JE, Gilham D, Herz J, Tschöp MH, Hui DY. Adipocyte LDL receptor-related protein-1 expression modulates postprandial lipid transport and glucose homeostasis in mice. J Clin Invest 2007;117:3271–3282

Almind K, Kahn CR. Genetic determinants of energy expenditure and insulin resistance in diet-induced obesity in mice. Diabetes 2004;53:3274–3285

Martinez-Botas J, Anderson JB, Tessier D, Lapillone A, Chang BH-J, Quast MJ, Gorenstein D, Chen K-H, Chan L. Absence of perilipin results in leanness and reverses obesity in $Lepr^{db/db}$ mice. Nature Genetics 2000;26:474–479

Plum L, Rother E, Munzberg H, Wunderlich FT, Morgan DA, Hampel B, Shanabrough M, Janoschek R, Konner AC, Alber J, et al. Enhanced leptin-stimulated PI3k activation in the CNS promotes white adipose tissue transdifferentiation. Cell Metab 2007;6:431–445

exposure, exhibit a robust increase in oxygen consumption in response to hyperphagia (15,35). While it is clear that mutations and environmental conditions that affect UCP1/BAT will impact diet-induced obesity, these conditions can affect fuel supply for BAT thermogenesis, for example, acyl-CoA dehydrogenases knockouts (36), or can inactivate transcription factors (37,38) or receptors (39,40) associated with the cold-mediated induction of thermogenesis BAT. Although these mutations primarily affect the efficiency of a thermogenic mechanism devoted to body temperature regulation, the efficiency of UCP1/BAT thermogenesis indirectly affects body weight regulation as less efficient systems for maintaining core body temperature require more calories.

That thermogenic systems for dissipation of calories have not been found does not mean that ion/substrate cycles could not serve this function (41–45). Presumably some targeted mutations with adiposity phenotypes are based upon UCP1-independent alternative thermogenic mechanisms. However, inspection of the phenotypic data for energy expenditure in the studies cited in Table 1 readily leads one to the disappointing conclusion that it is probable that none of them have a bona fides defect in energy expenditure.

What is the basis for this provocative statement? It will be obvious to anyone that has used indirect calorimetry for comparing lean and obese mice that total 24-h energy expenditure (TEE) increases as a function of body weight. Studies comparing 24-h energy expenditure in humans with a wide range of body weight also demonstrate an increase in 24-h energy expenditure (46). To compensate for the often dramatic differences of body mass in lean and obese models, and perhaps to estimate energy expenditure at a cellular level, investigators have resorted to normalizing energy expenditure reported as oxygen consumption (V_{O_2}) to body weight. This approach assumes that lean and adipose tissues contribute equally to V_{O_2}; however, while adipose tissue is not metabolically inert, it contributes relatively little to the total energy expenditure of an organism compared with lean mass. Furthermore, this distortion in estimates of energy expenditure will increase in proportion to the accumulation of lipid in the adipocyte as obesity increases.

The theoretical and experimental bases for the effect of mass on energy expenditure are complex and have been covered in depth by many physiologists for over a century. A particularly lucid account can be found in "Energy Metabolism in Animals and Man" by Kenneth Blaxter (NY, Cambridge University Press, 1989). During the past 7 years, in excess of 10 papers published in high profile journals devoted to the phenotypic characterization of new targeted gene mutations affecting body composition have calculated whole-body energy expenditure by dividing the volume of oxygen consumed by the total body weight (Table 1). This use of body weight to normalize energy expenditure data between groups of mice with often marked differences in body weight makes the assumption that all tissues have an equivalent metabolic demand. A variation in estimating energy expenditure to account for differences in the mass of mutant versus wild-type mice divides oxygen consumption by body weight to the 3/4 power. It has long fascinated biologists that body mass and energy expenditure among a range of species vary as a function of the 3/4 power (47,48). However, it is assumed that species that conform to this relationship will have normal body composition, that is, neither obesity nor insulin resistance. Therefore, including a highly variable fat mass in the body weight when the lean mass is minimally variable will similarly distort estimates of energy expenditure.

As an example, we provide data from our studies analyzing energy expenditure in genetic mutants that exhibit obesity owing to increased fat mass (Fig. 1A). Obesity associated with mutations in either the *leptin* or melanocortin-4 receptor (*Mc4r*) genes is associated with a 30–40% increase in TEE expressed as kilojoules per mouse per day (Fig. 1B) (49,50). Normalization of TEE by body weight (which includes substantial increases in the amount of adipose in the obese models, Fig. 1A) appears to indicate lower TEE per unit of body weight with extreme obesity, at least in females. Two-way ANOVA indicates significant effects of genotype ($P < 0.0001$) and sex ($P < 0.0001$), with the effect of genotype dependent on sex ($P < 0.05$). TEE per gram of body weight was significantly lower in female Lep^{ob}/Lep^{ob} mice compared with wild type ($P < 0.0001$).

When the same data are normalized using lean mass, a very different outcome is observed. TEE per gram of lean mass is significantly (and markedly) increased with obesity (effect of genotype, $P < 0.0001$, $P < 0.002$ between all genotypes). Sex also significantly affected TEE per gram of lean mass, with males having a lower value irrespective of genotype ($P < 0.0001$); however, sex had no affect on the impact of genotype. Note that the quite large differences of fat mass observed between $Mc4r^{-/-}$ and Lep^{ob}/Lep^{ob} mice (~10 g, or 15–20% of total body weight) do not correlate with significant changes in TEE (Fig. 1B). This suggests that the additional fat mass is not associated with any appreciable increase of metabolic activity.

One interpretation of this data is that severe obesity is supported by increased energy expenditure. As both genetic models of obesity may be hyperphagic relative to lean controls, the oxidation of any additional calories could contribute to increased energy expenditure. Note that while these genetic mutants obviously accumulate more mass over several weeks to a few months, these recordings were taken over a time frame of a few days where minimal or no significant change in body mass would occur. In other words, the state of energy balance (intake less expenditure) is likely near neutrality, and any additional calories ingested are likely being oxidized or used to support the maintenance of the additional mass (i.e., oxygenation and turnover of protein and lipid in the extra tissue). Similar results were observed whether the analysis used total energy expenditure or resting energy expenditure, assessed using data obtained during period of minimal activity.

The rationale for dividing V_{O_2} by body weight to calculate energy expenditure is also sometimes applied to food intake. That is, food intake is expressed as gram or calorie per gram of body weight. Of course, when applied universally the outcome makes no sense, since it would result in the conclusion that leptin-deficient Lep^{ob}/Lep^{ob} mice are hypophagic. In some instances authors will present one parameter adjusted for body weight and one expressed per animal (or per lean mass). Quite often, the presentation of data appears to reflect a desire to align the data with assumptions made a priori, as opposed to an unbiased analysis of the contribution of food intake or energy metabolism to the phenotype being investigated.

To illustrate the problem we draw attention to two recent examples, one a report by Plum et al. in *Cell Metabolism* (51), and the other by Hofmann et al. in the *Journal of Clinical Investigation* (52). Plum et al. performed a technological tour-de-force by deleting the phosphatidylinositol-3-phosphate (PIP3) kinase Pten, specifically neurons expressing the long-form of the leptin-receptor (ObRb). The deletion of Pten in ObRb neurons ($Pten^{\Delta ObRB}$) resulted in the over activation of the phosphatidylinositol-3 (PI3) kinase pathway, previously reported to be involved in the anorectic actions of leptin (53). As predicted, these mice exhibit a lean phenotype, with a marked reduction in perigonadal fat pad weight and a 10–15% (2- to 3-g) reduction in body weight. The lean phenotype was largely attributed to energy expenditure, based upon increased V_{O_2} expressed as milliliters per
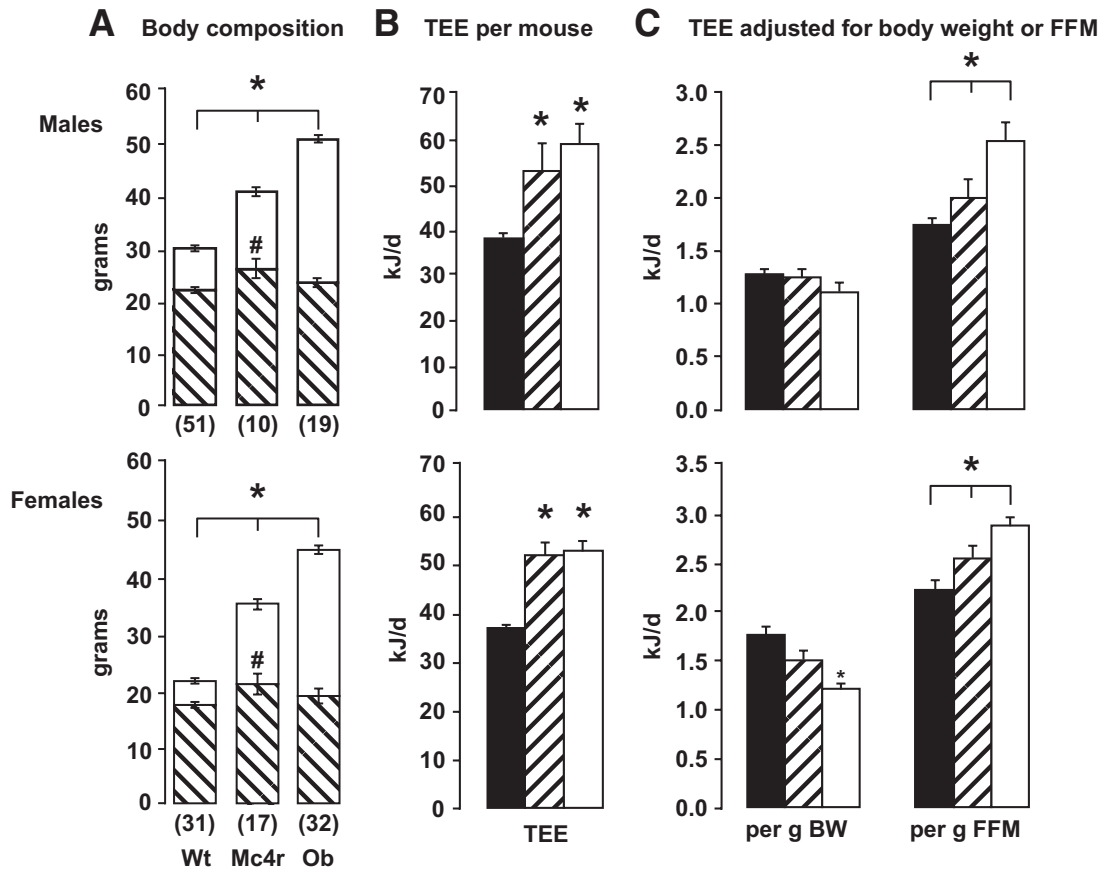
FIG. 1. Comparison of energy expenditure in genetically obese mouse strains using data expressed per mouse or adjusted for body weight (BW) or fat-free mass (FFM). The data shown are from studies performed by the Pennington Biomedical Research Center using indirect calorimetry (Columbus Instruments). The numbers in parentheses in *A* indicate the number of animals used per genotype. Data were analyzed by two-way ANOVA, testing for significance of sex, genotype, and interactions. *A*: Body composition (fat-free mass) of male and female wild-type (WT), *Mc4r*$^{-/-}$ (Mc4r), and *Lep*^{ob}/*Lep*^{ob} (Ob) mutant mice on the C57BL/6J background was measured using nuclear magnetic resonance (Bruker Minispec) (61). The effects of genotype were highly significant (*$P < 0.0001$ between all groups); sex did not alter the effect of genotype on obesity. Fat-free mass was affected by genotype ($P < 0.0001$) and sex ($P < 0.0001$), with *Mc4r*$^{-/-}$ mice having significantly more fat-free mass than wild-type and *Lep*^{ob}/*Lep*^{ob} mice (#$P < 0.01$), while male mice had greater fat-free mass irrespective of genotype. □, fat mass; ▨, fat-free mass. *B*: When expressed per animal, TEE was increased by obesity (effect of genotype, *$P < 0.0001$ vs. wild type; effect of sex, $P = 0.27$). *C*: Normalization of TEE by body weight or lean mass yielding different results. TEE expressed per gram of fat-free mass is significantly increased relative to control mice. ■, wild-type B6; ▨, B6. *Mc4r*$^{-/-}$; □, B6. *Lep*^{ob}/*Lep*^{ob}.

kilogram per hour; however, this increase in energy expenditure would disappear if expressed as VO$_2$ in milliliters per mouse per hour or milliliters per gram of lean mass per hour. Additionally, the authors confound the problem by presenting food intake accumulated over 3 weeks by adjusting to body weight (grams per grams per day). Based on the body weight data, it appears likely that there would be mild reduction in total food intake in *Pten*$^{\Delta ObRB}$ mice compared with controls, which could contribute to the lean phenotype. More surprisingly, in the same figure, food intake during a 6-h period after an overnight fast was expressed as gram per animal and was not different. No rationale is provided for the ad hoc adjustment of food intake for body weight. Their conclusion is that *Pten* ablation in leptin-sensitive neurons reduces adiposity by increasing energy expenditure.

The Hofmann et al. article is another molecular genetic tour-de-force in which adipocyte LDL receptor–related protein-1 was specifically inactivated in white adipose tissue to assess its role in diet-induced obesity. However, the authors use the same problematic experimental approach to calculate energy expenditure from indirect calorimetry, food intake, and, in addition, loss of fat mass during fasting, and they come to a similar conclusion,

namely that reduced adiposity is caused by elevated energy expenditure from enhanced muscle thermogenesis.

In both studies if the energy expenditure was calculated by normalizing to lean body mass or total energy expenditure per mouse, then no significant differences would have been observed. It is important to emphasize that these and other studies in Table 1 represent only a few of many articles published in a broad spectrum of journals that publish such research.

The complex issues faced when characterizing energy expenditure. This issue with the analysis of indirect calorimetry data was raised in a letter in *Science* in 1997 by Himms-Hagen (5). This letter was a response to the publication of the first studies on the effectiveness of recombinant leptin to correct the obese phenotype of *Lep*^{ob}/*Lep*^{ob} mice (4). The authors of that study claimed that leptin increased energy expenditure in these mice when calculating energy expenditure by dividing oxygen consumption by body weight. Himms-Hagen pointed out that if energy expenditure is estimated from oxygen consumption per mouse, it leads to the conclusion that leptin stimulates fat oxidation and reduces food intake in the *Lep*^{ob}/*Lep*^{ob} mouse, but it does not stimulate energy expenditure. The serious negative consequence of the conclu-

sion that Lep^{ob}/Lep^{ob} mice have a slow metabolism led to years of fruitless and expensive clinical studies on obese humans seeking to find obese individuals with a slow metabolism. None were found, and the blame for these fruitless studies was placed on the Lep^{ob}/Lep^{ob} mouse, claiming that its obesity is not similar to human obesity (54). However, a review of energy expenditure data in the Lep^{ob}/Lep^{ob}, $Mc4r^{-/-}$, and wild-type mice from our laboratories illustrates how Lep^{ob}/Lep^{ob} have a slow metabolism when oxygen consumption data are expressed per gram of body weight (Fig. 1) but not when oxygen consumption data are expressed per gram of lean mass or per mouse. Thus, the problem is not with the mouse model but with the inability to appreciate that a unit of fat mass has a lower level of oxygen consumption than a unit of lean mass.

The letter by Himms-Hagen should have significantly reduced the publication of studies that employed similar methods; however, the extensive list of high-profile publications since 1997 in which changes in adiposity have been erroneously attributed to modulations in energy expenditure indicates that the problem continues (Table 1). Consequently, these conclusions as to how various metabolic pathways contribute to obesity phenotypes through modulation of energy expenditure impair progress in this area. The effect of genetic manipulation on the basic adiposity phenotype is not in dispute, rather the manner in which indirect calorimetry has been used to provide a physiological mechanism based upon energy expenditure is the problem.

This point is further illustrated using a simple thought experiment showing that subtle differences in energy expenditure can account for marked differences in the accumulation of adipose over time. A difference of 0.5 g/week in the rate of accumulation of fat between a mutant and control mouse would, if constant, result in a 10-g difference in body weight over 5 months. These estimates are not unreasonable, as studies examining obesity phenotype usually involve mice in the 4- to 6-month age range (if not older); a difference of 10 g represents a marked change for the B6 mouse, which weighs 25–30 g when maintained on standard chow, increasing to 45–50 g when fed a high-fat diet. However, this number represents a difference of 18.8 kJ per week, or <2.9 kJ/day. Assuming a range of energy expenditure of 50–60 kJ/day and identical caloric intake, then this would mean a difference in daily energy expenditure of ~5%, which is at the limits of the discriminatory capacity of indirect calorimetry.

Of course, this crude estimation does not take into account the actual efficiency of the conversion of nutrients to triglyceride stores in adipose. It also does not take into account differences in the balancing of nutrient composition with substrate oxidation, indicated by the respiratory exchange ratio, which may also impact energy homeostasis. However, it does highlight the small changes in energy balance that can explain relatively large differences in the accumulation of adipose mass over time. Similar reasoning has been applied to the normally exquisite regulation of energy balance affecting adiposity over time in humans (55).

The number of studies where energy expenditure has been expressed as V_{O_2} divided by total body weight appears not to have been diminished by Himms-Hagen's admonitions. In most cases articles identifying a specific thermogenic mechanism associated with the phenotypes have not followed these initial reports. In other cases,

modest changes in $Ucp1$ mRNA expression especially in white adipose tissues have been interpreted to implicate alterations in UCP1 as the thermogenic mechanism underlying the differences in energy expenditure (51,56). However, whether a 50% change in $Ucp1$ expression affects overall adiposity is questionable given that complete deficiency of UCP1 does not affect the obese state or energy expenditure at an ambient temperature of 23°C (34). Cold tolerance has also been used by many studies as an ad hoc indicator of reduced metabolism; however, even this interpretation is complicated, since homozygous Lep and $Ucp1$ mutant mice can, given the right conditions or, in the case of UCP1-deficient mice on an F1-hybrid background, adapt to cold (57).

Some recommendations. We have approached this discussion of the problems arising from calculations of energy expenditure from V_{O_2} data based upon an oversimplified obesity model in which the only morphological variable is body fat content. Mutations in pleiotropic signaling molecules may not only affect body fat content, but also change the overall size and body composition and affect behavioral phenotypes changing physical activity and food intake. Two recent studies on the targeted inactivations of the FTO and $IKK[epsilon]$ genes have the potential of having a high impact on studies of human obesity, especially FTO, given the association of FTO alleles with human obesity. These studies also propose that differences in diet-induced obesity are due to differences in energy expenditure (58,59); however, this interpretation is uncertain because of significant differences in both lean and fat mass in the mutant versus wild-type mice. Each of these subphenotypes will have an effect on calculations of energy expenditure. Therefore, data provided by systems for assessing energy expenditure require evaluation with respect to each of the morphological and the behavioral variables. If a change in adiposity is determined by mutations to enzymes of fat metabolism or to preferential fat oxidation, then the respiratory quotient can provide additional useful information. In addition, an independent evaluation of energy expenditure can readily be acquired by determination of metabolic efficiency, that is, by measuring changes in adiposity and lean mass relative to caloric intake over the course of several weeks or months.

Finally, regression analysis of energy expenditure against body composition data, which is the method of choice in determining energy expenditure in humans (46), could be used. However, this approach is impractical for mouse studies characterizing the effects of targeted mutations on obesity and energy expenditure. In most laboratories, relatively small numbers (6–10) of age- and sex-matched mutant and wild-type mice are available for indirect calorimetry studies. However, if variation in lean mass or body size was present as revealed by duel-energy X-ray absorptiometry or nuclear magnetic resonance, then analysis by regression analysis would be justified. Alternatively, National Institutes of Health (NIH)-funded investigators in institutions without the technology for indirect calorimetry or analysis or body composition by nuclear magnetic resonance can send animals to the NIH-funded Mouse Metabolic Phenotyping Centers (http://www.mmpc.org/).

Final remarks. We have attempted to refocus attention on a significant but easily corrected problem encountered often in the literature in studies determining energy expenditure in animals with variations in adiposity, and first clearly enunciated by Jean Himms-Hagen 12 years ago (5).

Establishing physiologically meaningful phenotypes of energy balance is a challenging endeavor influenced by variations in physical activity, circadian rhythms, subtle differences of substrate utilization, and diet composition. Careful phenotyping can provide unique insights into energy balance at the whole animal level that cannot be obtained through molecular or cell culture experiments. However, major problems lie ahead as our ability to generate new knockout models has outstripped our capacity for a thorough investigation of phenotype. The technical feasibility of rapidly generating knockout gene models has led to an international consortium to knockout every gene in the mouse (http://www.genome.gov/17515708). However, we are not the first to voice concern about whether the experience, resources, and financing are available first to design physiologically meaningful gene knockouts of energy balance and second to create and support the laboratories with the experience to phenotype the mice for defects in energy homeostasis (60).

ACKNOWLEDGMENTS

REFERENCES

1. Hill JO. Understanding and addressing the epidemic of obesity: an energy balance perspective. Endocr Rev 2006;27:750–761
2. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372:425–432
3. Farooqi IS, O'Rahilly S. Mutations in ligands and receptors of the leptin-melanocortin pathway that lead to obesity. Nat Clin Pract Endocrinol Metab 2008;4:569–577
4. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. Science 1995;269:540–543
5. Himms-Hagen J. On raising energy expenditure in ob/ob mice. Science 1997;276:1132–1133
6. Flier JS. Obesity wars: molecular progress confronts an expanding epidemic. Cell 2004;116:337–350
7. Ravussin E, Kozak LP. Have we entered the brown adipose tissue renaissance? Obes Rev 2009;10:265–268
8. Rosen ED, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. Nature 2006;444:847–853
9. Reitman ML. FGF21: a missing link in the biology of fasting. Cell Metab 2007;5:405–407
10. Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. Nature 2006;443:289–295
11. Horvath TL, Andrews ZB, Diano S. Fuel utilization by hypothalamic neurons: roles for ROS. Trends Endocrinol Metab 2009;20:78–87
12. Garfield AS, Lam DD, Marston OJ, Przydzial MJ, Heisler LK. Role of central melanocortin pathways in energy homeostasis. Trends Endocrinol Metab 2009;20:203–215
13. Saper CB, Chou TC, Elmquist JK. The need to feed: homeostatic and hedonic control of eating. Neuron 2002;36:199–211
14. Scarpace PJ, Matheny M, Pollock BH, Tümer N. Leptin increases uncoupling protein expression and energy expenditure. Am J Physiol 1997;273:E226–E230
15. Butler AA, Marks DL, Fan W, Kuhn CM, Bartolome M, Cone RD. Melano-cortin-4 receptor is required for acute homeostatic responses to increased dietary fat. Nat Neurosci 2001;4:605–611
16. Voss-Andreae A, Murphy JG, Ellacott KL, Stuart RC, Nillni EA, Cone RD, Fan W. Role of the central melanocortin circuitry in adaptive thermogenesis of brown adipose tissue. Endocrinology 2007;148:1550–1560
17. Rothwell NJ, Stock MJ. A role for brown adipose tissue in diet-induced thermogenesis. Nature 1979;281:31–35
18. Hervey GR, Tobin G. Luxuskonsumption, diet-induced thermogenesis and brown fat: a critical review. Clin Sci (Lond) 1983;64:7–18
19. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P, Teule GJ. Cold-activated brown adipose tissue in healthy men. N Engl J Med 2009;360:1500–1508
20. Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerbäck S, Nuutila P. Functional brown adipose tissue in healthy adults. N Engl J Med 2009;360:1518–1525
21. Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, Iwanaga T, Miyagawa M, Kameya T, Nakada K, Kawai Y, Tsujisaki M. High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. Diabetes 2009;58:1526–1531
22. Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scimè A, Devarakonda S, Conroe HM, Erdjument-Bromage H, Tempst P, Rudnicki MA, Beier DR, Spiegelman BM. PRDM16 controls a brown fat/skeletal muscle switch. Nature 2008;454:961–967
23. Ukropec J, Anunciado RV, Ravussin Y, Kozak LP. Leptin is required for uncoupling protein-1-independent thermogenesis during cold stress. Endocrinology 2006;147:2468–2480
24. Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS. Role of leptin in the neuroendocrine response to fasting. Nature 1996;382:250–252
25. Schwartz MW, Woods SC, Seeley RJ, Barsh GS, Baskin DG, Leibel RL. Is the energy homeostasis system inherently biased toward weight gain? Diabetes 2003;52:232–238
26. Myers MG, Cowley MA, Münzberg H. Mechanisms of leptin action and leptin resistance. Annu Rev Physiol 2008;70:537–556
27. Hayes M, Chustek M, Heshka S, Wang Z, Pietrobelli A, Heymsfield SB. Low physical activity levels of modern Homo sapiens among free-ranging mammals. Int J Obes (Lond) 2005;29:151–156
28. Guarente L. Mitochondria—a nexus for aging, calorie restriction, and sirtuins? Cell 2008;132:171–176
29. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. Physiol Rev 2004;84:277–359
30. Feldmann HM, Golozoubova V, Cannon B, Nedergaard J. UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. Cell Metab 2009;9:203–209
31. Ma SW, Foster DO. Brown adipose tissue, liver, and diet-induced thermogenesis in cafeteria diet-fed rats. Can J Physiol Pharmacol 1989;67:376–381
32. Ma SW, Foster DO, Nadeau BE, Triandafillou J. Absence of increased oxygen consumption in brown adipose tissue of rats exhibiting "cafeteria" diet-induced thermogenesis. Can J Physiol Pharmacol 1988;66:1347–1354
33. Liu X, Rossmeisl M, McClaine J, Riachi M, Harper ME, Kozak LP. Paradoxical resistance to diet-induced obesity in UCP1-deficient mice. J Clin Invest 2003;111:399–407
34. Anunciado-Koza R, Ukropec J, Koza RA, Kozak LP. Inactivation of UCP1 and the glycerol phosphate cycle synergistically increases energy expenditure to resist diet-induced obesity. J Biol Chem 2008;283:27688–27697
35. Albarado DC, McClaine J, Stephens JM, Mynatt RL, Ye J, Bannon AW, Richards WG, Butler AA. Impaired coordination of nutrient intake and substrate oxidation in melanocortin-4 receptor knockout mice. Endocrinology 2004;145:243–252
36. Guerra C, Koza RA, Walsh K, Kurtz DM, Wood PA, Kozak LP. Abnormal nonshivering thermogenesis in mice with inherited defects of fatty acid oxidation. J Clin Invest 1998;102:1724–1731
37. Cederberg A, Grønning LM, Ahrén B, Taskén K, Carlsson P, Enerbäck S. FOXC2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. Cell 2001;106:563–573
38. Lin J, Wu PH, Tarr PT, Lindenberg KS, St-Pierre J, Zhang CY, Mootha VK, Jäger S, Vianna CR, Reznick RM, Cui L, Manieri M, Donovan MX, Wu Z, Cooper MP, Fan MC, Rohas LM, Zavacki AM, Cinti S, Shulman GI, Lowell BB, Krainc D, Spiegelman BM. Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. Cell 2004;119:121–135
39. Bachman ES, Dhillon H, Zhang CY, Cinti S, Bianco AC, Kobilka BK, Lowell BB. betaAR signaling required for diet-induced thermogenesis and obesity resistance. Science 2002;297:843–845
40. Valet P, Grujic D, Wade J, Ito M, Zingaretti MC, Soloveva V, Ross SR, Graves RA, Cinti S, Lafontan M, Lowell BB. Expression of human alpha

2-adrenergic receptors in adipose tissue of beta 3-adrenergic receptor-deficient mice promotes diet-induced obesity. J Biol Chem 2000;275:34797–34802

41. Dulloo AG. Regulation of fat storage via suppressed thermogenesis: a thrifty phenotype that predisposes individuals with catch-up growth to insulin resistance and obesity. Horm Res 2006;65(Suppl. 3):90–97

42. Dulloo AG, Jacquet J. An adipose-specific control of thermogenesis in body weight regulation. Int J Obes Relat Metab Disord 2001;25(Suppl. 5):S22–S29

43. Dulloo AG, Stock MJ, Solinas G, Boss O, Montani JP, Seydoux J. Leptin directly stimulates thermogenesis in skeletal muscle. FEBS Lett 2002;515:109–113

44. Simonides WS, Thelen MH, van der Linden CG, Muller A, van Hardeveld C. Mechanism of thyroid-hormone regulated expression of the SERCA genes in skeletal muscle: implications for thermogenesis. Biosci Rep 2001;21:139–154

45. Newsholme EA, Stanley JC. Substrate cycles: their role in control of metabolism with specific references to the liver. Diabete Metab Rev 1987;3:295–305

46. Ravussin E, Lillioja S, Anderson TE, Christin L, Bogardus C. Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. J Clin Invest 1986;78:1568–1578

47. Schmidt-Nielsen B. August and Marie Krogh and respiratory physiology. J Appl Physiol 1984;57:293–303

48. Porter RK, Brand MD. Body mass dependence of H+ leak in mitochondria and its relevance to metabolic rate. Nature 1993;362:628–630

49. Sutton GM, Trevaskis JL, Hulver MW, McMillan RP, Markward NJ, Babin MJ, Meyer EA, Butler AA. Diet-genotype interactions in the development of the obese, insulin-resistant phenotype of C57BL/6J mice lacking melanocortin-3 or -4 receptors. Endocrinology 2006;147:2183–2196

50. Trevaskis JL, Butler AA. Double leptin and melanocortin-4 receptor gene mutations have an additive effect on fat mass and are associated with reduced effects of leptin on weight loss and food intake. Endocrinology 2005;146:4257–4265

51. Plum L, Rother E, Münzberg H, Wunderlich FT, Morgan DA, Hampel B, Shanabrough M, Janoschek R, Könner AC, Alber J, Suzuki A, Krone W, Horvath TL, Rahmouni K, Brüning JC. Enhanced leptin-stimulated Pi3k activation in the CNS promotes white adipose tissue transdifferentiation. Cell Metab 2007;6:431–445

52. Hofmann SM, Zhou L, Perez-Tilve D, Greer T, Grant E, Wancata L, Thomas A, Pfluger PT, Basford JE, Gilham D, Herz J, Tschöp MH, Hui DY. Adipocyte LDL receptor-related protein-1 expression modulates postprandial lipid transport and glucose homeostasis in mice. J Clin Invest 2007;117:3271–3282

53. Niswender KD, Morton GJ, Stearns WH, Rhodes CJ, Myers MG Jr, Schwartz MW. Intracellular signaling: key enzyme in leptin-induced anorexia. Nature 2001;413:794–795

54. Prentice AM. Obesity and its potential mechanistic basis. Br Med Bull 2001;60:51–67

55. Friedman JM. A war on obesity, not the obese. Science 2003;299:856–858

56. Almind K, Manieri M, Sivitz WI, Cinti S, Kahn CR. Ectopic brown adipose tissue in muscle provides a mechanism for differences in risk of metabolic syndrome in mice. Proc Natl Acad Sci U S A 2007;104:2366–2371

57. Hofmann WE, Liu X, Bearden CM, Harper ME, Kozak LP. Effects of genetic background on thermoregulation and fatty acid-induced uncoupling of mitochondria in UCP1-deficient mice. J Biol Chem 2001;276:12460–12465

58. Chiang SH, Bazuine M, Lumeng CN, Geletka LM, Mowers J, White NM, Ma JT, Zhou J, Qi N, Westcott D, Delproposto JB, Blackwell TS, Yull FE, Saltiel AR. The protein kinase IKKepsilon regulates energy balance in obese mice. Cell 2009;138:961–975

59. Fischer J, Koch L, Emmerling C, Vierkotten J, Peters T, Brüning JC, Rüther U. Inactivation of the Fto gene protects from obesity. Nature 2009;458:894–898

60. Accili D. A note of caution on the Knockout Mouse Project. Nat Genet 2004;36:1132

61. Tinsley FC, Taicher GZ, Heiman ML. Evaluation of a quantitative magnetic resonance method for mouse whole body composition analysis. Obes Res 2004;12:150–160