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Enhanced metabolic efficiency contributes to weight regain after weight loss in obesity-prone rats

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MacLean, Paul S., Janine A. Higgins, Ginger C. Johnson, Brooke K. Fleming-Elder, William T. Donahoo, Edward L. Melanson, and James O. Hill. Enhanced metabolic efficiency contributes to weight regain after weight loss in obesity-prone rats. Am J Physiol Regul Integr Comp Physiol 287: R1306–R1315, 2004. First published August 26, 2004; doi:10.1152/ajpregu.00463.2004.—Metabolic adjustments occur with weight loss that may contribute to a high rate of weight regain. We have previously observed in obesity-prone, obese rats that weight reduction is accompanied by a suppression in resting metabolic rate beyond what would be predicted for the change in metabolic mass. In the present study, we examine if this adjustment in metabolic efficiency is affected by the length of time in weight maintenance and if it contributes to the propensity to regain after weight loss. Twenty-four-hour, nonresting, and resting energy expenditure (REE) were obtained by indirect calorimetry and normalized to metabolic mass estimated by dual-energy X-ray absorptiometry. A 10% loss in body weight in weight-reduced rats was accompanied by a 15% suppression in adjusted REE. This enhancement in metabolic efficiency was not altered with either 8 or 16 wk of weight maintenance, but it did resolve when the forced control of intake was removed and the weight was regained. The rate of weight regain increased with the time in weight maintenance and was exceptionally high early during the relapse period. During this high rate of weight gain, the suppression in REE persists while consumption increases to a level that is higher than when they were obese. In summary, an enhanced metabolic efficiency and an elevated appetite both contribute (60% and 40%, respectively) to a large potential energy imbalance that, when the forcible control of energy intake is relieved, becomes actualized and results in an exceptionally high rate of weight regain.

There is growing evidence to suggest that weight loss may be accompanied by metabolic adjustments that serve to promote weight regain. Among these metabolic adjustments is a reduction in total energy expenditure (TEE), which would require a similar compensatory reduction in energy intake (EI) to maintain the reduced weight. While it is generally accepted that weight loss is accompanied by decreased energy expenditure, the controversy lies with 1) whether this decrease is simply due to the reduction in mass that would have been expending energy or whether there is a reduction in the basal metabolic rate in one or more of the metabolically active tissues; and 2) whether this adjustment persists in weight maintenance and contributes to the metabolic propensity to regain weight after weight loss. This suppressed metabolic rate has been alternatively referred to as an enhanced metabolic efficiency (12, 36). This term, metabolic efficiency, is a relative expression that refers to the conservation of expended energy by metabolically active tissues. Several studies in humans have suggested that metabolic rate after weight loss is reduced to a greater extent than would be expected for the reduction in metabolic mass (2, 10, 15, 20, 27, 31, 32, 46, 50), thereby increasing metabolic efficiency. In contrast, there are a similar number of studies that have failed to observed such an adaptation in metabolism or suggest that it resolves during weight maintenance and does not contribute to the propensity to regain weight (1, 8, 30, 48, 52, 53, 56). This disparity has led to much discussion and differing opinions regarding the normalization of metabolic mass, the methods and duration of weight loss and weight maintenance, and the selection and control of research subjects (42, 51). More important than a lack of consensus on the issue, this controversy has led some researchers to discount the relevance or the existence of an adjustment in metabolic efficiency with weight reduction and question its contribution to the metabolic propensity to regain weight.

We have studied this issue in a rat model of diet-induced obesity that is similar in many ways to human obesity (22–24). The advantage of this model is that obesity-prone rats can be selected, placed in a well-controlled obesigenic environment, and followed throughout the development, treatment, and relapse of obesity (5, 36, 37). Furthermore, unlike humans, the behavior of rats is not influenced by peer pressures, the concept...
of an ideal physique, or other societal factors that provide the motivation to alter behavior. They are subject solely to the internal metabolic pressures and the external environmental stresses that we apply by design. In a recent study, we observed a reduction in TEE and an enhancement in metabolic efficiency in weight-reduced obesity-prone rats that 1) persists throughout 8 wk of weight maintenance and 2) resolves after the weight is regained with 8 wk of ad libitum feeding (36). In the present study, we have employed this model to examine if the metabolic adjustment that we have observed is affected by the length of time in weight maintenance and whether or not it contributes to the propensity to regain weight. Our findings would suggest that, in this model of obesity, the suppression of energy expenditure and the enhancement in metabolic efficiency does not change with prolonged weight maintenance but that it plays a significant role in the propensity to regain weight after weight loss. Furthermore, the inclusion of Preobese, Obese, and Never-Obese controls in our analysis provides insight into the relative utility of these groups in our understanding the metabolic propensity to regain weight.

METHODS

Animal care and use. Male Wistar rats (125–150 g) were purchased from Charles River Laboratories (Charles River Laboratories, Wilmington, MA). Obesity-prone and obesity-resistant rats were identified by a dietary screening process that predicts future weight gain (5, 36). In short, the rats were standardized to facility and to the consumption of a low-fat diet (12% kcal fat, Research Diets, Madison, WI; RD 11724) for 2 wk. They were then switched to a high-fat diet (46% kcal fat, Research Diets, Madison, WI; RD 12344) for 1 wk, while weight gain was monitored. The rats were then switched back to the low-fat diet for another week before entering into the study. The rats were ranked by their rate of weight gain during the high-fat dietary challenge. The top tertile was classified as obesity prone and the lower tertile was classified as obesity resistant. Rats from the middle tertile were not used for this study. All rats were individually housed in the University of Colorado Health Sciences Center (UCHSC) for Laboratory Animal Care (22–24°C; 12:12-h light-dark cycle) with free access to water. All procedures were approved by the UCHSC Animal Care and Use Committee.

Experimental design. Our experimental approach employed three groups of control rats: Preobese, Obese, and Never Obese. Fourteen obesity-prone rats, designated “Preobese,” were examined before the development of obesity, immediately after the screening period and being returned to a low-fat diet for 1 wk. Another group of obesity-prone rats were placed in an obesogenic environment that included housing that limited the amount of physical activity, and free access to a high-fat diet. These rats, designated “Obese,” were examined after the development of obesity, either at 16 wk in this obesogenic environment or after continued ad libitum access to a low-fat diet through 42 wk of the study. The final control group consisted of obesity-resistant rats that were placed in similar housing with free access to the low-fat diet. These rats, designated “Never Obese,” were examined after 16 wk under these conditions.

In addition to the control animals, we employed two sets of experimental rats. The first, designated “Weight Reduced,” consisted of a group of obesity-prone rats that were placed in the obesogenic environment for 16 wk followed by 2 wk of a calorie-restricted, low-fat diet designed to induce weight loss. These rats were then examined after 0, 8, or 16 wk on an intake-regulated, low-fat diet, designed to maintain the reduced weight. The second set of experimental animals, designated “Relapsed-Obese,” were selected and treated identically to the Weight-Reduced rats, except that they were examined after 8 wk of weight regain subsequent to the 0, 8, or 16 wk of weight maintenance. Weight loss in these animals was targeted to achieve a 10–15% loss in body weight that would be reflective of targeted adjustments in human weight loss programs. To achieve this objective, weight loss was induced by limiting calories to ~60% of TEE, with a daily monitoring and adjustment of EI throughout the 14-day period to ensure a negative energy balance. Weight was maintained by restricting daily provisions of the low-fat diet such that daily body weight estimates remained stable. Weight regain in the Relapsed-Obese rats was induced by providing ad libitum access to the low-fat diet. In a followup study, four Obese rats were followed prospectively through weight reduction, weight maintenance, and the first 2 wk of weight regain.

Metabolic monitoring. Energy balance was examined with a metabolic monitoring system developed by the Energy Balance Core Laboratory at the University of Colorado Clinical Nutrition Research Unit, as described previously (7, 36, 37). This monitoring system is composed of a four-chamber indirect calorimeter designed for the continuous monitoring of up to four rats simultaneously, containing measurements of VO2 and VCO2 from each chamber every 6 min. Chambers are also equipped for the collection of urine, feces, and food spillage. Rats destined for metabolic monitoring were acclimated to the system for 2–3 days before data collection periods. The rats were then monitored for 23 h, during which time EI was measured. Urine volume was recorded and a portion of it was collected for the measurement of urinary nitrogen levels (ThermoDMA, Louisville, CO). The remaining 1 h (occurring in the middle of the light cycle) was used to clean the chamber and prepare for the next monitoring period.

Metabolic rate (MR) was calculated with the Weir equation (MR = 3.941 × VO2 + 1.106 × VCO2 − 2.17 × N). An example of metabolic rate and respiratory quotient (RQ = VCO2/VO2) data acquired from an animal during an extended monitoring period can be observed in a previous report (36). TEE was calculated as the average of all metabolic rate measurements taken (every 6 min) throughout the 23-h period and was extrapolated for presentation purposes to reflect that amount of energy expended through 24 h. In addition, the data were used to acquire 1) resting metabolic rate, extrapolated throughout a 24-h period to obtain resting energy expenditure (REE); and 2) nonresting energy expenditure (NREE), as the difference between TEE and REE. Resting metabolic rate was estimated as an average metabolic rate over a 1-h period occurring in the latter part of the light cycle as we have previously reported (36). The 1-h period is selected during a time in which metabolic rate and RQ indicates minimal physical activity and food intake for the three previous hours. In previous studies, we have observed that this coincides with a period of time in which the rat is or has been asleep and shows no activity. Energy balance was calculated from the difference between EI and TEE throughout the monitoring period.

Body composition analysis. Body composition analyses were performed by dual-energy X-ray absorptiometry (DXA) using the Lunar DPX-IQ (GE Lunar, Madison, WI), with Lunar’s Small Animal Software Version 1.0 as we have previously described (36). Corrected fat mass (FM) and fat-free mass (FFM) were calculated from DXA data and pan weights according to the recommendations of Feely et al. (16), who have standardized this approach to chemical analyses of body composition. In another cohort of rats (n = 165), we found DXA-FM calculated in this fashion accurately reflected the variation in fat pad weights (r2 = 0.94, P < 0.001).

Statistical analysis. Data were analyzed with the SPSS software version 12.0 by ANOVA with Duncan’s post hoc test when a significant effect was observed. Analysis of covariance was used to analyze NREE and REE with Fisher’s least significant difference post hoc test to examine differences between groups. In some examinations a repeated-measures ANOVA model was employed. In the prospective study of weight regain, data were analyzed in a repeated-measures ANOVA model. Statistical significance was assumed when P < 0.05.
Fig. 1. Body weights and the rate of weight regain. A: obesity-prone rats became obese with 16 wk of high-fat feeding. These Obese rats were examined metabolically before or after 26 subsequent weeks on the low-fat diet. Obesity-resistant rats, designated “Never Obese,” were examined metabolically after 16 wk on a low-fat diet. B: Weight-Reduced rats were obesity-prone rats that had developed obesity with 16 wk of high-fat feeding but then underwent weight loss on an intake-regulated low-fat diet and were maintained at this reduced weight for 0, 8, or 16 wk before metabolic monitoring. C: Relapsed-Obese rats were obesity-prone rats that had developed obesity, underwent weight loss, maintained at this reduced weight for 0, 8, or 16 wk, and then were allowed free access to the low-fat diet for 8 subsequent weeks before metabolic monitoring. D: the rate of weight regain for Relapsed-Obese rats is represented as the average for the 1st wk, the 2nd wk, and the final 4 wk of the relapse period. Data are expressed as means ± SE, with Duncan’s post hoc analysis to examine homogeneous groups. With each time period, groups that are not significantly different are represented by having the same letter designation.

RESULTS

Anthropometric data. Body weights for the different groups were followed regularly throughout the time course of the study (Fig. 1, A–C), and body composition was assessed immediately after metabolic monitoring (Table 1). As expected, Preobese rats were relatively small and lean. Compared with the Never-Obese rats (low-fat fed, obesity resistant), the Obese rats (high-fat fed, obesity prone) had a greater rate of weight gain with a preferential accumulation in FM. The weight loss regimen induced a 10% loss in body weight and a 32% reduction in FM, with little detectable change in FFM. As we have previously reported (36), the subsequent weight maintenance period (intake-regulated consumption of a low-fat diet for either 8 or 16 wk) was not accompanied by any changes in body composition. Unrestricted access to the low-fat diet for 8 wk after maintenance led to a significant amount of weight regain in Relapsed-Obese rats that was primarily FM, apparently returning them to the normal growth curve of the obesity-prone rat (compare Fig. 1, A and C). The rate of weight regain in Relapsed-Obese rats was influenced by both the length of weight maintenance and the time of weight regain (Fig. 1D). As the length of time in weight maintenance was increased, the rate and total amount of weight regained also increased (P < 0.001), and this weight was preferentially regained soon after

Table 1. Anthropometric and energy balance data

<table>
<thead>
<tr>
<th></th>
<th>Preobese</th>
<th>Obese</th>
<th>Never Obese</th>
<th>Weight-Reduced: Time in Weight Maintenance After Reduction</th>
<th>Relapsed-Obese: Time in Weight Maintenance Before Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>0 wk</td>
<td>8 wk</td>
<td>16 wk</td>
<td>0 wk</td>
</tr>
<tr>
<td>Anthropometric data</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body wt, g</td>
<td>317 ± 7</td>
<td>749 ± 22</td>
<td>559 ± 17</td>
<td>634 ± 23</td>
<td>629 ± 22</td>
</tr>
<tr>
<td>FFM, g</td>
<td>260 ± 6</td>
<td>428 ± 12</td>
<td>387 ± 10</td>
<td>416 ± 15</td>
<td>435 ± 14</td>
</tr>
<tr>
<td>FM, g</td>
<td>58 ± 3</td>
<td>320 ± 16</td>
<td>172 ± 8</td>
<td>218 ± 20</td>
<td>195 ± 19</td>
</tr>
<tr>
<td>SBF</td>
<td>18.2 ± 1.2</td>
<td>42.6 ± 1.3</td>
<td>30.5 ± 0.3</td>
<td>34.3 ± 2.0</td>
<td>30.8 ± 2.0</td>
</tr>
<tr>
<td>Energy balance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EI</td>
<td>96.1 ± 2.3</td>
<td>94.3 ± 2.5</td>
<td>81.7 ± 2.7</td>
<td>55.9 ± 5.6</td>
<td>78.5 ± 4.5</td>
</tr>
<tr>
<td>TEE</td>
<td>76.1 ± 1.6</td>
<td>91.3 ± 1.7</td>
<td>82.1 ± 1.8</td>
<td>78.4 ± 2.6</td>
<td>83.1 ± 2.4</td>
</tr>
</tbody>
</table>

Body weight, fat-free mass (FFM), fat mass (FM), percent body fat (%BF), energy intake (EI, kcal/day) and total energy expenditure (TEE, kcal/day) are expressed as means ± SE. EI represents the average daily intake over the 3 days before metabolic monitoring and the 1 day in the monitoring system. TEE was measured over a 23-h period in the metabolic chamber and extrapolated for the 24-h period. Data were analyzed by ANOVA with Duncan’s post hoc analysis to identify homogeneous groups (P < 0.05). Groups with similar letters for a variable are not significantly different from one another.
the rats were given free access to food (P < 0.001). In particular, all Relapsed-Obese rats exhibited an exceptionally high rate of weight regain in the first week that returns to an age-matched, diet-matched control by the end of the 8 wk of regain period.

**Energy balance.** Energy balance data shown in Table 1 are similar to what we have previously reported for obesity-prone rats throughout the different stages of obesity development, treatment, and relapse (36). In this cohort we addressed two additional questions in this model: 1) how a never-obese group, commonly used in human studies, would compare; and 2) how the length of weight maintenance after weight reduction would influence the metabolic adjustments and weight regain. The Preobese group exhibited a considerable energy imbalance that explains, in large part, their susceptibility to the development of obesity. With the development of obesity, Obese rats continued to eat a similar amount but their TEE increased to a level that brought them closer to energy balance. In contrast, the Never-Obese rats consumed less than their Preobese and Obese counterparts, but matched this with a lower TEE that was similar to that of Preobese rats. With weight reduction, EI was forcibly reduced, and TEE decreased to a level that was similar to Preobese and Never-Obese rats. This adjustment in TEE did not change significantly with 8 or 16 wk of intake-regulated weight maintenance. However, both TEE and EI in the Relapsed-Obese rats had returned to levels found before weight loss.

**Metabolic adjustments in energy expenditure.** As with human dieting, those rats that lost weight due to forcibly reduced EI showed a compensatory adjustment in the level of TEE. The next question was whether this adjustment was due to an adjustment in the thermic effect of food, in activity, or in basal metabolism. To address this issue, we divided TEE into NREE (activity and thermic effect of food) and REE (our best estimate of basal metabolism) components. As with TEE, there was no detectable effect of the length of time in weight maintenance on any component of TEE, so we grouped the Weight-Reduced rats together and Relapsed-Obese rats together to increase the power of our analysis (Fig. 2A). With NREE, we did not observe a significant change in obesity-prone rats throughout the development, treatment, and relapse of obesity. We did, however, observe that NREE was significantly higher by 2 to 3 kcal in Never-Obese rats (P < 0.05). This difference remained significant after the statistical adjustment for the variation in caloric intake (to control for the potential differences in thermic effect of food, Fig. 2B), suggesting that these animals were generally more physically active than their obesity-prone counterparts. In contrast, absolute REE was significantly lower by ~10 kcal/day in Weight-Reduced rats, reflective of a level similar to that found in Preobese and Never-Obese rats (P < 0.01). With weight regain in Relapsed-Obese rats, this difference disappeared (Fig. 2A). These observations suggest that the metabolic adjustment that occurs with weight loss and persists throughout an extensive period of weight maintenance is the result of a suppression in REE.

The next question we addressed was whether the adjustment in REE that occurs with weight reduction is due to 1) a change in basal MR of those tissues that significantly contribute to REE, 2) a reduction in the amount of tissue that significantly contribute to REE, or 3) both. To address this issue, we reanalyzed the REE data with ANCOVA using two approaches: 1) controlling for the variation in FFM, or 2) controlling for both FFM and FM. With the former adjustment, we observed that REE in both Never-Obese and Weight-Reduced rats remained lower than the Preobese, Obese, and Relapsed-Obese groups (Fig. 3A). To examine the magnitude of this effect, we chose the Preobese group as the standard from which to calculate the relative metabolic efficiency or the amount of resting energy conserved in the other groups expressed as a percentage of the standard. From this calculation, we observed that metabolic efficiency was 12 and 15% higher in the Never-
Obese and Weight-Reduced rats, respectively (Fig. 3B). With the adjustment for both FM and FFM, the REE in the Weight-Reduced rats remained significantly lower than that of all of the other groups (Fig. 3C). In contrast to the former approach, the adjusted REE in Never-Obese rats was similar to that found in the Obese and Relapsed-Obese rats, and the adjusted REE was considerably higher in Preobese rats than in all other groups. Again, we used the Preobese group as the standard to calculate the relative metabolic efficiency in the other groups of rats (Fig. 3D). Controlling for the variation in FM did lead to a dramatic reduction in the magnitude of the effect compared with the Obese, Relapsed-Obese, and Never-Obese groups, but the Weight-Reduced rats remained more metabolically efficient.

**Contribution of enhanced metabolic efficiency to weight regain.** One final question was how much the adjustment in metabolic efficiency contributes to the high rate of weight regain that occurs when external control of EI is terminated. To address this issue, we monitored four Obese rats prospectively through weight reduction and through the first 2 wk of weight regain, when the propensity to regain weight is the most profound (Fig. 4A). These rats were 800 ± 13 g in body weight and 41 ± 4% body fat before weight loss. The weight-reduction phase led to a 16% drop in body weight that was...
primarily FM, and this reduced weight was maintained for 8 subsequent weeks before the rats were given free access to the low-fat diet. By the end of the 2-wk period of regain, the rats had regained 49% of their lost weight. We examined the rats with indirect calorimetry in the obese state before weight loss (Obese), after weight reduction with 8 wk of weight maintenance (Weight Reduced), and on days 1, 3, 7, and 14 of relapse. Figure 4B shows the EI and TEE of the rats at these time points. On the first day of weight regain, EI returned to levels observed before weight loss, while TEE did not significantly change. While EI remained high and did not change significantly through the relapse period, TEE gradually increased over the 2-wk period. These levels of intake and expenditure translated into a significant energy imbalance in the relapse phase (Fig. 4C) that tended to decline with time. The gradual resolution in energy expenditure was primarily the result of an increase in NREE (Fig. 4D). Adjusting for caloric intake normalized for some, but not all of these differences, suggesting that both the thermic effect of food and the level of physical activity contributed to the elevated NREE during weight regain.

Because FFM did not significantly change and there were only four rats, FFM was not a significant predictor of REE. The rats served as their own control for FFM in this repeated-measures design, and a small increase in REE that was significant by day 3 was observed for REE (Fig. 5A). To examine the magnitude of the change in REE, we used the Obese measurement as the standard from which to calculate the relative metabolic efficiency of the other groups (Fig. 5B). This expression of the data provides a better indication that the enhanced metabolic efficiency that occurs with weight loss does not resolve immediately, but gradually over time during the relapse phase. While close to 50% of the lost weight had been regained after 2 wk, only 23% of the adjustment in metabolic efficiency had resolved (Fig. 5C). These data would suggest that metabolic efficiency remains elevated and contributes to a lower energy expenditure, a greater energy imbalance, and the high rate of weight regain early in relapse. From these data, we can estimate, based upon the energy balance data observed before weight loss, that both adjustments, in appetite and REE, contribute significantly to the energy imbalance early in relapse (40% and 60%, respectively).

DISCUSSION

In this examination of obesity-prone rats through the development, treatment, and relapse of obesity, we report several novel findings. First, the enhanced metabolic efficiency and the subsequent suppression in TEE that occur with weight reduction in obesity-prone rats is not influenced by the amount of time in weight maintenance. This adjustment, therefore, does not alone explain the increasing rate of weight regain as the length of time in weight maintenance is extended. Second, regardless of the length of time in weight maintenance using this model, the enhanced metabolic efficiency resolves after the weight is regained and the growth rate returns to an age-matched control. The third observation, representing the key contribution of this report, is that the resolution of this adjustment in metabolic efficiency is not immediate. While there is a gradual attenuation during the early part of relapse, the enhanced metabolic efficiency persists to the extent that it has a significant impact on the expenditure of energy. Despite an increase in NREE during the relapse phase, the suppressed REE has a profound impact on energy balance when the external regulation of intake is removed. Finally, while the Never-Obese rats appear to have a similar level of REE and
metabolic efficiency compared with Weight-Reduced rats, there is a differential impact on energy balance that is due to the drive to consume calories. From these data, we conclude that, in obesity-prone rats that have become obese, the metabolic propensity to regain weight after weight loss includes a suppression in metabolic rate and an enhanced metabolic efficiency that is sustained throughout weight maintenance and, when the external control of EI is removed, contributes to an exceptionally high rate of weight gain. This metabolic propensity to regain weight can be represented as a “potential energy imbalance” that exists when energy intake is being externally controlled (Fig. 6). In this model, the “potential energy imbalance” becomes more profound with prolonged weight maintenance and promotes a high rate of weight regain.

Our data are consistent with the extensive evidence in both humans (25, 38, 41, 45, 53) and rodents (6, 13, 28, 36) that weight loss induced by caloric restriction reduces TEE by suppressing REE and enhancing metabolic efficiency. In addition, weight regain after weight loss has been repeatedly shown in both rodents (4, 13, 33–36) and humans (21, 47), and our rodent model of obesity and weight loss is no different after removal of the restricted dietary regimen. However, there is more controversy as to whether the adjustment in metabolic efficiency persists with prolonged weight maintenance and whether this adjustment predisposes to the high rate of weight regain. Several studies in humans have suggested that a metabolic adaptation persists in weight maintenance after weight loss and may play an important role in promoting weight regain (2, 10, 15, 20, 27, 31, 32, 46, 50), while others would argue that the metabolic adjustment is not sustained after maintenance is achieved and does not contribute to the propensity to regain weight (1, 8, 30, 48, 52, 53, 56). In contrast to studies in humans, studies in rodents have provided consistent evidence that weight reduction is accompanied by a suppression in TEE via an enhancement in metabolic efficiency (suppressed REE adjusted for FFM), that this adjustment is sustained in weight maintenance, and that it has a significant impact on the propensity to regain weight (4, 6, 11–14, 33, 35, 36). Why are the observations in rodents so consistent while studies in humans remain mired in controversy? There are a number of issues to consider when formulating an opinion on the matter.

Normalization of metabolic data. One of these considerations is how to normalize the TEE data. With regression analysis one can find several correlates to TEE, and choosing the most appropriate presentation can be challenging. We would agree with Toth (44) who asserts that once a difference in TEE has been established, it is best to break TEE into its NREE and REE components and employ well-defined reasoning, based on the parameter and the question posed, to adjust for a specific variable. While this approach may appear to be fairly intuitive, there are some complicating factors that have clouded data interpretation and prevented a consensus as to the concept of metabolic efficiency. For example, a number of early studies that suggested an enhancement in metabolic efficiency with weight reduction were criticized because the data were normalized by dividing REE by FFM (15, 32, 50), an approach that has the tendency to magnify the adjustment in metabolic efficiency. It is generally accepted, therefore, that the more appropriate way to normalize metabolic rate for the amount of metabolic mass is to use covariance in the model to factor out the variation explained by FM. Studies in both human and rodents, including the present one, have used this approach and observed an enhancement in metabolic efficiency that persists well after weight maintenance has been achieved (8, 27, 31, 36, 46).

A more controversial issue is whether to include FM in the estimation of metabolic mass. While the basal metabolic rate of adipose tissue, measured both in vivo and in vitro, is very small (44), we and others have suggested that the development of obesity may make it a considerable contributor to REE and thereby provide reason to include it as a covariate when controlling for metabolic mass. Controlling for FM has subsequently been widely used for this purpose (2, 8, 10, 30, 52, 53). We have expressed the data in the present study with and without including FM as a covariate for the purpose of comparing the two approaches. We would raise the likelihood that the inclusion of FM as a covariate when controlling for metabolic mass may bias the analysis. There is considerable evidence to support the hypothesis that the body has a homeostatic feedback system for controlling fat stores and that the energy efficiency of metabolic processes would be adjusted to maintain and/or replenish fat stores and body weight (3, 14). Many of the proposed signals of this system are adipose-derived hormones, like leptin and adiponectin, and/or are linked to the amount of FM. As a consequence, using FM as a covariate may not only factor out what little the adipose tissue contributes to basal metabolic rate, but it may also factor out the role of FM and its signals have in the adjustment of this homeostatic feedback system. Separating these two components of the influence of FM on metabolic rate presents a challenging technical problem. However, if the link were only a contribution to the pool of metabolic mass, we should expect a 50% resolution in the adjustment of REE and metabolic efficiency after 2 wk of relapse, when ~50% of the weight is

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**Fig. 6.** Metabolic propensity to gain weight: potential vs. actual imbalance of energy. A representation of how the actual energy imbalance compares with the potential energy imbalance throughout the development, treatment, and relapse of obesity. The actual and potential energy imbalance deviate from each other during and after weight loss because REE is suppressed, appetite is elevated, and the food provision does not match the appetite. Even after weight maintenance is achieved, the potential energy imbalance persists because of a suppressed metabolic rate and an elevated appetite that is not met. With prolonged weight maintenance, the potential energy imbalance becomes more profound as metabolic rate remains suppressed in the face of an increasing appetite. Once the external control of intake is removed, the actual and potential energy imbalance converge and a high rate of weight regain is observed. The metabolic rate and appetite resolve after weight regain such that energy balance is achieved without controlled intake.
regained. Instead, we observed <25% resolution. It is for this reason that we would agree with other researchers who assert that FFM alone would be the better covariate to use as an estimate of metabolic mass (44). In the present study, including FM does not eliminate the effect, but it does attenuate the magnitude of the observed metabolic adjustment. Our data are consistent with most prospective studies that observe an effect when only controlling for FFM (8, 15, 20, 27, 31, 32, 46), but little, if any, difference when controlling for both FFM and FM (8, 10, 53).

Selection of control subjects. Another critical consideration has to deal with the selection of control subjects. Our data are consistent with most studies in humans that employ a “never-obese” group as a control in their design (1, 30, 50, 52, 56) in that the relative metabolic efficiency is similar for Weight-Reduced and Never-Obese rats. This observation has been used to conclude by us and others that there is no adjustment in metabolic efficiency that would contribute to a high rate of weight regain. Furthermore, the observation that adjusted REE does not predict future weight gain in humans has been used to provide support for this assertion (1, 52, 53). However, our results have compelled us to question the relevancy of using a “never-obese” group as a control when studying the adjustment in metabolic efficiency with weight loss and its contribution to weight regain. Metabolic efficiency increases when an Obese rat loses weight and becomes a Weight-Reduced rat. While a Weight-Reduced rat may have a similar relative metabolic efficiency to a Never-Obese rat, the metabolic context for this level of efficiency differs dramatically for the two rats. The drive to consume calories for the Weight-Reduced rat has not decreased from what it was when the rat was Obese. In fact, our observations would suggest that the drive to consume actually increases as the length of time in weight maintenance is increased even though the relative metabolic efficiency remains constant, an effect that has been observed in humans (9). In contrast, the drive to consume in Never-Obese rats essentially matches expenditure. Therefore, Weight-Reduced rats are primed to experience an extensive energy imbalance when external control of EI is removed. Without the adjustment in metabolic efficiency and TEE, Weight-Reduced rats would not exhibit as high of a rate of weight regain when given free access to food. Instead, they would remain in a small positive energy imbalance but consuming and expending energy at a higher level than their Never-Obese counterparts. Based on this reasoning, it is our assertion that the most appropriate control for a weight-reduced subject, whether it be a rat or a human, is that same subject or representative subject before weight loss or after weight regain. Studies that have used this approach using an appropriate statistical consideration for only FFM as an estimate of metabolic mass consistently support the concept of an adjustment in metabolic efficiency with weight loss (8, 27, 31, 32, 36, 46).

If this assertion is true, then why is it that humans do not consistently display the dramatic rate of weight regain that we observe in rodents? We believe that the answer lies in the primary reason why nonhumans may be more appropriate for studies that focus on metabolic adjustments. Humans are conscious of their weight and are motivated, with varying degrees of success, to proactively modify their behavior to counteract the metabolic adjustments. Nonhumans have no such motivation and are driven primarily by their metabolic state. A self-imposed control of intake would certainly blunt regain, but a self-imposed regimen of physical activity may be as important to successful weight maintenance (29, 33, 49). Given the difficulties in controlling or even standardizing human motivation and behavior, it is not surprising that REE does not always predict weight regain in weight-reduced subjects (1, 52). Furthermore, it is difficult to accurately measure the drive to consume calories or acquire accurate estimates of physical activity in humans. Studies from our group and others that have suggested that an enhanced metabolic efficiency does not contribute to the metabolic propensity to regain weight either did not acquire accurate estimates of the drive to consume calories and/or did not control for physical activity levels (30, 48, 53, 56). In the present study, one interesting observation regarding this issue is that NREE adjusted for caloric intake, assumed to be an estimation of activity levels, is higher in obesity-resistant, Never-Obese rats (Fig. 2B).

Selection of experimental subjects. Beyond controlling the levels of physical activity, there are other issues with regard to the selection of experimental subjects that must be considered. Some of the studies that make the argument against a sustained adjustment in metabolic efficiency tend to be biased toward the use of only those subjects who were successful with weight loss or weight maintenance (1, 30, 56), excluding those individuals who may exhibit the most profound adjustment and, as a result, could not successfully maintain the weight-reduced state. Other studies are not inclusive of subjects that were obese or severely obese before their weight loss (53). Inclusion of only mildly overweight people may exclude those individuals who are genetically prone to obesity and who may exhibit the most dramatic metabolic adjustment, or the magnitude of the metabolic adjustment may be linked to the amount of weight loss. On the other hand, it should be noted that our approach of selecting obesity-prone rats may limit the relevance of our observations to a subset that is prone to severe obesity. It is unclear whether these metabolic adjustments are the result of 1) purely the environmental pressures applied by design, 2) purely the genetic background that is predisposed to obesity, or 3) the interaction between the environmental pressures and this obesity-prone genetic background. We plan to address this by applying similar environmental pressures to Never-Obese rats and compare their metabolic propensity to regain weight with that of Obese animals.

Limitations of the study. The estimation of basal metabolism is generally done in the postabsorptive state under resting conditions. Our approach in this study was to examine metabolism under free-living conditions, and, therefore, it is unlikely that we achieved a true postabsorptive state. Furthermore, it should be noted that no direct measurements of motor activity were made to confirm that animals were at rest over the 1-h period in which REE was estimated. In the light cycle, the rats spend a good portion of their time sleeping, and oxygen consumption decreases and remains low at a steady level for sustained periods of time (approaching 3 h). We have observed that even small amounts of activity in the cage can be detected by a spike in the oxygen consumption, allowing us to be selective when choosing an hour block in which we are confident there is little, if any, activity (unpublished results). Even so, this assertion would be more credible if we had simultaneous activity-monitoring data to accompany our analysis. Thus the REE measurements and their relevance to basal
metabolism in this study should be considered in light of the metabolic state and our indirect estimation of motor activity.

Summary and conclusions. In summary, the observations from this study are generally consistent with studies in both rodents and humans with regard to an adjustment in REE resulting from weight loss. The concept of relative metabolic efficiency is a mathematical expression that, by itself, does not necessarily predict weight gain. The effect of a certain level of efficiency on weight gain depends on the metabolic context. In the metabolic context of a Never-Obese rat, the relatively low REE and high metabolic efficiency does not contribute to a severe energy imbalance when there is free access to food, whereas in the metabolic context of a Weight-Reduced rat, it does. REE declines and metabolic efficiency increases as the Obese rat loses weight, and this adjustment persists regardless of the length of time in weight maintenance. Most importantly, this adjustment in metabolic efficiency persists well into the relapse period. When the forcible control of consumption is removed, the increased intake and suppressed REE contribute to the expansive energy imbalance and the high rate of weight regain.

In this model of obesity-prone rats, an enhanced metabolic efficiency with a progressively increasing appetite appear to be the hallmark of the metabolic propensity to regain weight after weight loss. From our prospective analysis, the persistent suppression in REE explained 60% of the potential energy imbalance, while the elevated appetite explained 40%. It is likely that these metabolic responses, along with other interrelated adjustments, including an energetically favorable shift in fuel utilization, improved insulin sensitivity, and adipocyte hyperplasia, may explain why successful weight reduction is so challenging. Our observations with this model support the majority of evidence that weight-reduced humans face a metabolic propensity to regain weight. However, we need to further characterize the physiological basis of this metabolic propensity in this model to understand if similar mechanisms occur in the human condition. In humans, counteracting this biological propensity may require 1) adjusting environmental pressures, 2) redirecting conscious behavioral and nutritional decisions, and/or 3) pharmaceutical strategies. It is reassuring to know that modification of this biological propensity is possible. In a similar model of obesity-prone rats, Levin and Dunn-Meynell (33) recently reported that regular physical activity favorably altered the potential energy imbalance and lowered the defended level of adiposity. These observations may explain why exercise appears to be critical to successful weight maintenance in humans (29, 33, 49). We are currently using this rodent model of obesity development, treatment, and relapse as a tool to identify the strategies that best counteract the metabolic propensity to regain weight after weight loss, with the ultimate aim of translating our observations to facilitate successful weight maintenance in humans.

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Energy expenditure and prolonged weight reduction


