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Metabolic adjustments with the development, treatment, and recurrence of obesity in obesity-prone rats

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MacLean, Paul S., Janine A. Higgins, Ginger C. Johnson, Brooke K. Fleming-Elder, John C. Peters, and James O. Hill. Metabolic adjustments with the development, treatment, and recurrence of obesity in obesity-prone rats. Am J Physiol Regul Integr Comp Physiol 287: R288–R297, 2004. First published March 25, 2004; 10.1152/ajpregu.00010.2004.—Obesity is reaching epidemic proportions and predisposes afflicted individuals to several comorbidities. For these individuals, losing weight has proven to be an easier feat than maintaining a reduced weight. In obesity-prone rats, we examined if there is a metabolic propensity to regain weight after a period of significant weight loss. Twenty-four-hour energy expenditure (EE), sleeping metabolic rate (SMR), and nonprotein respiratory quotient (NPRQ) were obtained by indirect calorimetry with urinary nitrogen analysis and normalized to fat mass (FM) and fat-free mass (FFM) acquired by dual-energy X-ray absorptiometry. Obesity-prone rats were examined after free access to a high-fat diet for 16 wk to establish the obese state. They were then examined after 2 wk of calorie restriction, which reduced body weight (14%) and FM (32%). Rats were then examined after a further 8 wk of intake-regulated weight maintenance or ad libitum feeding that led to weight regain. Metabolic data were compared with preobese and age-matched controls. Weight loss suppressed EE and SMR beyond what was expected for the change in metabolic mass. This elevated metabolic efficiency persisted throughout weight maintenance but resolved after 8 wk of regain. Adjusted NPRQ values were elevated in weight-maintained and weight-regaining rats, suggesting a preference for carbohydrate utilization. These data support the concept that weight reduction in obesity is accompanied by metabolic adjustments beyond the drive to consume calories that predispose to weight regain, and some aspects of this adjustment persist with prolonged weight maintenance and during weight regain.

obesity is rapidly becoming a global epidemic, affecting both developed and undeveloped countries (25, 64) and inducing significant effects on health and life expectancy (26). The most common strategy to treat obesity involves two stages: 1) producing weight loss by creating negative energy balance, and 2) preventing weight regain by preventing positive energy balance. There are several examples of success in producing weight loss in obese subjects (27, 49, 52, 63). In fact, if the outcome measure is weight loss, most diet programs work very well, particularly when they combine a reduction in energy intake (EI) with regular physical activity. However, only a small proportion of subjects can maintain this weight loss over time (39, 58), regardless of the method of weight loss (37, 48).

A major question about prevention of weight regain after weight loss is the extent to which the problem is metabolic or behavioral. A common perception is that the metabolic state after weight loss is such as to promote weight regain. However, data relevant to this question have been controversial and equivocal. Several studies have focused on the idea that weight loss may lead to an adjustment in metabolic efficiency. This term, metabolic efficiency, refers to the conservation of energy expenditure (EE) by metabolically active tissues. Several studies in humans have suggested that metabolic rate (MR) after weight loss is reduced to a greater extent than would be expected for the reduction in metabolic mass (2, 13, 19, 28, 29, 41, 59), thereby increasing metabolic efficiency. In contrast, there are a similar number of studies that have failed to observe such an adaptation in metabolism (1, 12, 40, 57, 61, 65). The disparity between these studies has been attributed to the way metabolic mass is estimated, the selection of research subjects, and the methods of weight loss and weight maintenance. Furthermore, the debate as to which controls are most appropriate continues, with human studies being unable to examine the metabolic state before and after the development of obesity in the same individual. We do not have a reliable method to identify individuals predisposed to the development of obesity, and, if we could identify them, it would be difficult to follow them throughout the protracted course of the development, treatment, and recurrence of their obese state.

Because of these limitations, we have pursued an understanding of these issues in a rat model of diet-induced obesity that displays several correlates to human obesity. There is considerable evidence that the etiology of obesity involves both genetic and environmental factors (5, 34). Our model of dietary obesity is produced by allowing male Wistar rats ad libitum access to a high-fat diet in singly-housed cages that limit physical activity (31–33). The resultant weight gain at 1 wk is not only variable but also predictive of weight gain in the long term (8, 46). Therefore, with this model we can identify out the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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those rats that are genetically predisposed to develop obesity under the environmental conditions that promote obesity in humans. Because of the ability to select obesity-prone rats, the shorter lifespan of rats, and a better ability to control environmental conditions, we have employed this rodent model of obesity to examine the metabolic state at several stages throughout the development, treatment, and recurrence of obesity.

The purpose of the present study was to examine, with indirect calorimetry, the balance of energy and fuel utilization in obesity-prone rats throughout five stages of obesity development, treatment, and recurrence: 1) preobesity, 2) established obesity, 3) weight reduced, 4) weight reduced after a period of weight maintenance, and 5) weight reduced after a period of regaining the lost weight. Our data provide insight into the metabolic adjustments that occur in response to weight loss and maintenance that make successful weight maintenance a challenging prospect.

METHODS

Animal selection. Male Wistar rats (125–150 g) were purchased from Charles River Laboratories (Wilmington, MA). Obesity-prone rats were identified by a screening process that we have previously described (8). In short, all rats were acclimatized to the University of Colorado Health Sciences Center (UCHSC) for Laboratory Animal Care for 1 wk, being housed individually (20–22°C; 12:12-h light-dark cycle) with free access to a low-fat diet and water. The rats were placed on a high-fat diet (46% kcal fat, Research Diets, New Brunswick, NJ; RD no. 12344) for 1 wk while they were monitored for weight gain. The rats with the most weight gain (top tertile, average/wk) during this period of time were identified as obesity prone and were returned to a low-fat diet (12% kcal fat, Research Diets, RD no. 11724) for 1 wk before being entered into the study. The lower two tertiles were removed from the study. All procedures were approved by the UCHSC Animal Care and Use Committee.

Experimental design. The experimental design and study groups are summarized in Fig. 1. Thirteen obesity-prone rats were placed on a high-fat diet for a period of 16 wk to promote the development of obesity. These obese rats were examined metabolically and represent a group of rats with established obesity (EO group). These rats were then placed on a low-fat, energy-restricted diet that would produce a 10–15% loss in body weight during 2 wk of caloric restriction and again underwent metabolic examination [weight loss (WL) group]. Weight loss was targeted to achieve a 10–15% loss in body weight that would be reflective of target adjustments in human weight loss programs. To achieve this objective, weight loss was induced by limiting calories to ~60% of EE, with a daily monitoring and adjustment of EI throughout the 14-day period to ensure a negative energy balance. Six of the 13 calorically restricted rats were then assigned to a weight maintained (WM) group, while the other seven were randomly assigned to a weight regain (WR) group. The assignment of the rats to WM and WR groups was based on having the two groups stratified by body weight and total weight loss. WM rats were subsequently maintained at this reduced weight with restricted EI for 8 wk before again being examined, while WR rats were allowed to regain weight with ad libitum access to a low-fat diet for 8 wk before being examined. Three other separate groups were employed as controls. One group of seven rats was examined immediately after the screening process [preobesity (PO) group]. Another group of seven rats was fed a high-fat diet for 16 wk and then examined after being switched to a low-fat diet for 10 wk to represent an age-matched diet-matched control [low-fat diet control (LFC) group]. The last group of eight rats was fed a high-fat diet for 16 wk, as with the other groups, but examined after being continued with this regimen for another 10 wk [high-fat diet control (HFC) group]. Body weight was monitored regularly throughout the entire study. Energy balance, fuel utilization, and body composition were determined at specified time points corresponding to the various stages of the development, treatment, and recurrence of obesity (Fig. 1, black boxes).

Examination of energy balance and fuel utilization. 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 (11, 46). An approved animal satellite facility is exclusively devoted to this monitoring system, allowing control of temperature, humidity, and light-dark cycle. This monitoring system is composed of a four-chamber indirect calorimeter designed for the continuous monitoring of up to

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**Fig. 1.** Experimental design and summary of study groups. A: progression of the study can be broken down into 4 time periods. Period 1 is the screening process to identify rats that are predisposed to the development of obesity. In period 2, obesity-prone rats are allowed ad libitum access to a high-fat diet and progress to a well-established state of obesity (EO group). Period 3 is the first stage of obesity treatment in which rats were calorically restricted to induce a 10–15% loss in body weight [weight loss (WL) group]. Period 4 represents 2 outcomes of the second stage of obesity treatment: 1) successful weight maintenance with restricted energy intake of a low-fat diet (WM group); or 2) unsuccessful weight maintenance in which the rats are allowed ad libitum access to a low-fat diet [weight regain (WR) group]. Black boxes represent times when energy expenditure, fuel utilization, and body composition were assessed. B: 3 additional groups were added to the study as controls, and the progression of their treatment can be divided into 3 time periods. Period 1 was the same screening process to select obesity-prone rats. The first control group was examined immediately after the screening process, representing a preobese state (PO group). In period 2, obesity-prone rats are allowed ad libitum access to a high-fat diet and progress to a well-established state of obesity. In period 3, one group of obese rats switched to a low-fat diet to represent an age-matched, diet-matched control for WM and WR groups (LFC group). The other group of obese rats was continued on a high-fat diet to represent an age-matched, obesity control group (HFC group). Black boxes represent times when energy expenditure, fuel utilization, and body composition were assessed.
four rats simultaneously. Sampling lines from each chamber have separate condensation tubes, flow controllers, and pumps, so that each line has separate but identical air flow pathways from the chamber to the analyzers (Siemens OXYMAT and ULTRAMAT 5E). A multichannel flowmeter provides separate flow rate control to each chamber. Measurements of O₂ production (V₀₂) and CO₂ production (VCO₂) are acquired from each chamber every 6 min and sent to a computer, which collects and processes the data continuously. Chambers are also equipped for the collection of urine, feces, and food spillage. Rats destined for metabolic monitoring were acclimatized 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 hour (occurring in the middle of the light cycle) was used to clean the chamber and prepare for the next monitoring period.

MR was calculated with the Weir equation (MR = 3.941 × V₀₂ + 1.106 × VCO₂ - 2.17 × N). An example of MR and respiratory quotient (RQ = VCO₂/V₀₂) data acquired from a rat during an extended monitoring period is shown in Fig. 2. EE was calculated as the average of all MR 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) 12-h EE and RQ separately for light and dark cycles, 2) sleeping MR (SMR) and RQ (SRQ), and 3) 24-h nonprotein RQ (NPRQ). SMR was estimated in the latter part of the light cycle during a time in which MR and RQ indicated minimal physical activity and food intake for the three previous hours. While we have confirmed that this period of data collection does reflect a period of time in which the rat has been asleep, it should be noted that under most circumstances, some of the allotted food for the monitoring period does remain available. Energy balance was calculated from the difference between EI and EE throughout the monitoring period.

Under most circumstances, indirect calorimetry can provide the disappearance rate of a nutrient via the calculation of RQ, regardless of any metabolic interconversions that the nutrient undergoes before its disappearance (22, 53). However, the presumption that this disappearance is equivalent to oxidation is disrupted when the net rates of gluconeogenesis, ketogenesis, or lipogenesis become significant. In the present study, these metabolic pathways may play an important role during weight loss or under conditions of a high rate of weight gain, and it is important to consider these limitations when interpreting the data presented in this study. In addition, net carbohydrate (CHO) oxidation (from RQ) is highly related to EI, and more particularly, to CHO intake (23). While fat has little impact over the short term in regulating CHO or fat oxidation, CHO stimulates its own oxidation. Therefore, some portion, if not all, of the elevation in RQ and NPRQ may be due to the greater EI and CHO consumption in these rats. It was for this reason that we adjusted the data for the variation in both energy balance and CHO intake, attempting to eliminate the effects of the deviation of EI from EE. While this approach is not ideal with respect to examining lipid and CHO utilization, we have employed it to ask the question of whether there is a shift in fuel utilization, beyond the effects of the variation in energy consumption, during the development, treatment, and recurrence of obesity.

**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. The DPX-IQ employs a pencil-beam X-ray collimator at 76 kVp, and cerium k-edge filters to produce energy peaks at 38 keV and 70 keV. The scans were all completed in the HiRes (0.6 x 1.2 mm) medium-speed mode, with daily system calibration. All scans were initiated from the same position on the scanning table. Rats were weighed, anesthetized with intraperitoneal injections of pentobarbital sodium (45 mg/kg), and placed prone on the scanning table. Anesthesia was sufficient to immobilize the rats for the duration of the scan, which varied between 22 and 40 min according to the size of the rat. 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. (20), 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 SPSS software version 11.5. Data were analyzed by ANOVA with Fisher’s least significant difference post hoc test when an effect was observed. In some examinations (i.e., light and dark cycles of a parameter), a repeated-measures ANOVA model was employed. Under certain circumstances, analysis of covariance was performed for the adjustment for certain variables that were found to significantly influence the dependent variable. Linear relationships between variables were examined with the calculation of Pearson’s correlation coefficients. Statistical significance was assumed when P < 0.05.
to achieve weight loss and weight maintenance, respectively. When allowed free access to food, EI in WR rats was dramatically elevated to the level of that found in EO and to a level that tended to be higher than in LFC and HFC controls. PO rats, which exhibited the highest rate of weight gain, also showed the highest level of EI.

Table 2 also shows the 24-h EE and SMR for the obesity-prone rats at various stages of obesity development, treatment, and recurrence before and after statistical adjustment for the variation in FFM, FM, and EI. We have adjusted for these factors because we have found in the present study and in a separate, larger cohort of animals \((n = 166);\) body weight 250–950 g, using a multivariate linear regression model, that the combined factors of FFM, FM, and EI were the best predictors of MR, explaining 78% of the variation in EE and 73% of the variation in SMR (data not shown). Caloric restriction in WL rats led to a reduction in both EE and SMR that remained significant after adjustment compared with EO rats (the closest group to their age, i.e., being 2 wk apart). WM rats exhibited a lower EE and SMR that remained lower after the adjustment for FFM, FM, and EI compared with all obese controls, including age-matched, diet-matched LFC rats. Free access to a low-fat diet in WR rats increased EE and SMR to levels that were similar to PO, LFC, and HFC controls. Adjustment of these data for FFM, FM, and EI gave similar results, except that PO exhibited a much higher EE and SMR than the other groups.

Energy balance (EI – EE) expressed in the amount of energy gained or lost per day is shown in Fig. 4. Despite a lower EE in WL rats, EI was reduced to the extent that a negative energy imbalance and the consequent weight loss were evident. EI in WM rats was manually adjusted so that it was fairly reflective of EE, and the reduced weight in these animals was maintained. WR rats that were allowed free access to a low-fat diet exhibited an increase in their EI such that a positive energy imbalance, reflective of EO rats, was observed despite their higher EE. This imbalance tended to be more positive than what was found in LFC and HFC controls \((P = 0.06 \text{ and } 0.09, \text{ respectively});\) PO rats, with an exceptionally high EI, but with an EE slightly less than EO, WR, and the other obese control groups, exhibited the largest positive energy imbalance.

Fuel utilization. NPRQ before and after adjustment for energy balance and CHO intake is shown in Table 2. Caloric restriction in WL rats led to a reduction in NPRQ and a respective shift in the disappearance of the absolute amount of

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Table 1. Anthropometric data at different stages of obesity development, treatment, and recurrence

<table>
<thead>
<tr>
<th></th>
<th>EO (^a)</th>
<th>WL (^b)</th>
<th>WM (^c)</th>
<th>WR (^d)</th>
<th>PO(^e)</th>
<th>LFC(^f)</th>
<th>HFC(^g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>628±12</td>
<td>542±13(^a)</td>
<td>547±19(^a)</td>
<td>646±18(^b,c)</td>
<td>307±12(^a)</td>
<td>668±17(^b,c,e)</td>
<td>713±18(^e)</td>
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<tr>
<td>FFM, g</td>
<td>410±6</td>
<td>393±6</td>
<td>405±9</td>
<td>426±10(^b)</td>
<td>252±8(^c)</td>
<td>434±10(^c,e)</td>
<td>417±12(^c)</td>
</tr>
<tr>
<td>FM, g</td>
<td>218±11</td>
<td>149±12(^a)</td>
<td>142±18(^a)</td>
<td>220±17</td>
<td>54±17(^a)</td>
<td>234±16(^b,c,e)</td>
<td>296±17(^e)</td>
</tr>
<tr>
<td>%BF</td>
<td>34.5±1.3</td>
<td>27.1±1.4(^a)</td>
<td>25.7±2.1(^a)</td>
<td>33.6±2.0(^b)</td>
<td>17.7±2.0(^d)</td>
<td>34.8±1.8(^b,c,e)</td>
<td>41.6±1.5(^f)</td>
</tr>
</tbody>
</table>

Data are means ± SE. Body composition estimated by dual-energy X-ray absorptiometry was obtained in obesity-prone rats at various stages of obesity development, treatment, and recurrence. The stages are established obesity (EO), weight loss (WL), weight maintenance (WM), and weight regain (WR). Data for other control groups are also included: pre-obesity (PO), low-fat-fed control (LFC), and high-fat-fed control (HFC). Experimental and control groups are described in more detail in Fig. 1. FFM, fat-free mass; FM, fat mass; BF, body fat. Each group is designated with a letter that is used to denote a significant difference \((P < 0.05);\) from other groups when indicated with the variables’ descriptive statistics.

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Fig. 3. Body weights of the rats throughout the experiment. Body weights were regularly recorded throughout the study. Data are presented as means ± SE.
CHO and lipid (data not shown). Unrestricted intake in WR rats induced a dramatic increase in NPRQ that was reflective of values found in PO rats and higher than age-matched, diet-matched LFC controls. The absolute amount of lipid disappearance in WR rats was half of that found in LFC controls (data not shown). After the adjustment, the NPRQ for WM and WR rats was higher than that found in age-matched LFC rats, suggesting a preference for the use of CHO over lipid in these rats that was independent of the variation in EI. The relatively low NPRQ values in EO and HFC rats are primarily the consequence of the high-fat diet these rats were consuming. It is interesting, however, that HFC rats had a lower NPRQ than EO rats with only eight more weeks on the same diet, even after the adjustment for EI.

Because of the variation in EI across the groups, we have presented fuel utilization as a percentage of EI (Fig. 5). Switching to a low-fat diet led to an increase in the percentage of CHO expended in WL rats, despite being in a negative energy imbalance. Because WL rats expended more energy than what their EI could provide, a large portion of their calories, assumed to come primarily from endogenous fat, is shown as horizontal hatched bars above and beyond what EI could provide. When EI was increased after the weight loss period to a level that would maintain the reduced weight, an expected increase in the disappearance of CHO was observed, and the percentage of lipid contributing to expended energy was slightly reduced. Because we observed a slight, nonsignificant increase in the percentage of lipid expended in EO rats, we have determined fuel utilization as a percentage of EI. This adjustment for energy balance and carbohydrate (CHO) intake.

Table 2. Metabolic data at different stages of obesity development, treatment, and recurrence

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Control Groups</th>
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<tbody>
<tr>
<td>EO&lt;sup&gt;a&lt;/sup&gt; (n = 13)</td>
<td>PO&lt;sup&gt;a&lt;/sup&gt; (n = 7)</td>
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<tr>
<td>WL&lt;sup&gt;b&lt;/sup&gt; (n = 13)</td>
<td>LFC&lt;sup&gt;c&lt;/sup&gt; (n = 7)</td>
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<tr>
<td>WM&lt;sup&gt;c&lt;/sup&gt; (n = 6)</td>
<td>HFC&lt;sup&gt;c&lt;/sup&gt; (n = 8)</td>
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<tr>
<td>WR&lt;sup&gt;d&lt;/sup&gt; (n = 7)</td>
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Intake and expenditure of energy

<table>
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<tr>
<th></th>
<th>EI, kcal/day</th>
<th>EE, kcal/day</th>
<th>SMR, cal/min</th>
<th>SMR, cal/min</th>
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<tbody>
<tr>
<td>EO</td>
<td>92.6±2.6</td>
<td>83.1±1.5</td>
<td>46.5±0.7</td>
<td>44.4±0.9</td>
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<tr>
<td>WL</td>
<td>39.6±2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.2±0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.9±0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.5±0.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>WM</td>
<td>59.0±4.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>64.5±1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.4±1.1&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>35.5±1.3&lt;sup&gt;a,b&lt;/sup&gt;</td>
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<tr>
<td>WR</td>
<td>92.5±3.9&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>78.1±1.4&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>42.9±1.1&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>40.1±1.2&lt;sup&gt;a,c&lt;/sup&gt;</td>
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Fuel utilization

<table>
<thead>
<tr>
<th></th>
<th>NPRQ</th>
<th>NPRQ&lt;sup&gt;†&lt;/sup&gt;</th>
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<tr>
<td>EO</td>
<td>0.855±0.009</td>
<td>0.899±0.005</td>
</tr>
<tr>
<td>WL</td>
<td>0.798±0.009&lt;sup&gt;p&lt;/sup&gt;</td>
<td>0.862±0.009</td>
</tr>
<tr>
<td>WM</td>
<td>0.895±0.010&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.918±0.008&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>WR</td>
<td>0.958±0.009&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.905±0.008&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

Data are means ± SE. Metabolic data were obtained from indirect calorimetry in obesity-prone rats at various stages of obesity development, treatment, and recurrence. Experimental and control groups are described in more detail in Fig. 1. Data were analyzed by ANOVA or by analysis of covariance. EE, energy expenditure; SMR, sleeping metabolic rate; NPRQ, nonprotein respiratory quotient. Each group is designated with a letter that is used to denote a significant difference (P < 0.05) from other groups when indicated with the variables’ descriptive statistics. *Adjusted for FFM, FM, and energy intake (EI). †Adjusted for energy balance and carbohydrate (CHO) intake.

Fig. 4. Energy balance in obesity-prone rats at various stages of obesity development, treatment, and recurrence. Metabolic data were obtained from obesity-prone rats under conditions of EO, WL, WM, and WR. Data for other control groups are also included: PO, LFC, and HFC. Experimental and control groups are described in more detail in Fig. 1. Energy balance was calculated as the difference between energy intake (EI) and energy expenditure (EE). Each group is designated with a letter that is used to denote a significant difference (P < 0.05) from other groups when indicated with the variables’ descriptive statistics.

Fig. 5. Fuel utilization as a percentage of EI. Data from the metabolic monitoring system were used to estimate fuel utilization. Because EI varied among the groups, fuel utilization was expressed as a percentage of EI. For groups in which EI > EE, the excess EI is shown as calories that are stored (black bars). For groups in which EI < EE, the excess EE is shown as endogenous calories, the majority of which are assumed to be lipid, that are expended (horizontal hatched bars).
significant negative imbalance in energy in WM rats (Fig. 4), this is also reflected in a small number of calories (assumed to be from endogenous fat stores) represented as horizontal hatched bars. The groups that were in a positive energy imbalance (EO, WR, PO, LFC, HFC) ingested more energy than they expended, and this excess of calories is shown in Fig. 5 as black bars. Unrestricted intake in WR rats dramatically increased CHO disappearance and decreased lipid disappearance compared with WL and WM rats. WR rats exhibited a preference for CHO over lipid utilization compared with age-matched, LFC controls, similar to what is found with PO rats. HFC rats that continued on a high-fat diet for eight more weeks exhibited a greater preference for the utilization of lipid than EO rats.

**DISCUSSION**

We have provided a comprehensive picture of the states of energy and macronutrient balances that occur with the development, treatment, and recurrence of obesity in obesity-susceptible rats. Because of the susceptibility of weight-reduced subjects to regain lost weight, it has been suggested that an adjustment in metabolic parameters contributes to this predisposition. This is based on 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 achieve, maintain, and/or replenish fat stores and body weight (3). Such an adjustment that would promote weight gain would include 1) an elevated drive to consume calories; 2) an adaptive lowering of the MR relative to metabolic mass; and/or 3) a preference for CHO use that would spare fat from oxidation and make it available for fat deposition. This hypothesis remains controversial and is clouded by differing opinions as to which controls are most appropriate, how to best normalize the metabolic data, and how to interpret the data. In the present study, we have addressed this issue by examining, in rats that are predisposed to obesity, the free-living metabolic state at various stages of obesity development, treatment, and recurrence. We provide evidence that, while the energy consumption has great influence, adjustments in both metabolic efficiency and fuel utilization accompany the changes in EI and may significantly affect the propensity to gain weight. Our findings provide some insight into the difficulties in successfully achieving and maintaining a weight-reduced state. The data from the present study are summarized in Table 3 and will be discussed according to each of the stages of obesity development, treatment, and recurrence.

**Metabolic changes with the development and progression of obesity.** There has been a longstanding debate as to which side of the energy balance equation is most important in the development of obesity (7, 51, 60, 62). This debate still has no satisfactory conclusion, as there is general disagreement with a number of methodological, study design, data interpretation, and general paradigm issues, and we have no ambition of resolving this issue with the present study. Instead, we can provide insight as to how the metabolic state of an obesity-prone rat changes from the preobese state to the obese state. Our first conclusion from these data is that the transition from the preobese to the obese state is accompanied by an increase in MR and metabolic efficiency but little alteration in the utilization of fuels outside of that explained by the variation in the type and amount of diet consumed.

| Table 3. Summary of how metabolic parameters change with the development, treatment, and recurrence of obesity compared with the preobese and obese states |
|---|---|---|---|---|---|---|---|
| | Compared with Preobese | | | Compared with Obesity | | |
| | EI | EE† | NPRQ‡ | | EI | EE† | NPRQ‡ |
| Obesity | ↓ | ↓ | | N/A | N/A | N/A |
| Weight loss | * | * | | * | * | |
| Weight maintenance | ↓ | ↓ | | ↓ | ↓ | |
| Weight regain | ↓ | ↓ | | ↑ | ↑ | |

↓, Decrease; ↑, increase; —, no change; N/A, not applicable; *A forced reduction by calorie restriction; †adjusted for FFM, FM, and EI; ‡adjusted for energy balance and CHO intake.

The obesity-prone rats in the present study do reflect obesity-prone humans in a number of aspects. We have observed that, compared with obesity-resistant counterparts, obesity-prone rats 1) eat more, 2) have a lower 24-h EE under obesigenic conditions, and 3) have an elevated 24-h NPRQ on both low- and high-fat diets (46). It is then no surprise that we observed a large positive energy balance in PO rats and that these rats were destined to become obese. With the development of obesity (compare PO with EO), this energy imbalance was attenuated, but it still remained positive. The rate of weight gain slowed because the rats ate less while having an elevated MR. However, they were also more metabolically efficient in that their adjusted EE was lower than in the preobese state. It is difficult to relate these observations to human studies, as few actually examine the transition from a preobese to an obese state. Because of feasibility issues, most human studies employ a “postobese” model, equating a weight-reduced obesity-prone individual to one who is in the preobese state (60) or compare obese individuals to lean or never-obese subjects. Generally, when compared with never-obese or lean counterparts, EI, EE, and SMR are high in the obese, but these effects are eliminated when examined with respect to the variation in body mass, and few have reported a change in metabolic efficiency (6, 9, 21). This is clearly a different observation of the preobese-to-obese transition in this rodent model. In any case, with the further progression of obesity (compare PO with LFC and HFC), both EI and EE declined and energy balance tended to be less positive. It is interesting, however, that these changes were exhibited in a further increase in metabolic efficiency (see adjusted EE and SMR). As with the development of obesity, a decline in intake was observed with a concomitant increase in metabolic efficiency. As it is not uncommon to find EI and metabolic efficiency concomitantly change, it is not surprising that a mechanistic link between the two has been proposed (54).

Beyond the differences in diet composition and energy balance that were adjusted for, there was no unexpected change in NPRQ that would suggest a shift in fuel utilization with the development or progression of obesity. This finding is different from the observations in human studies that compare obese subjects with never-obese or lean controls, where whole body and/or limb RQ is higher in the obese (6, 44, 66). It is unclear whether these effects on MR and NPRQ represent an effect of age or some difference between human and rodent models of obesity. Examining obesity-prone individuals before and after the onset of obesity is required to resolve this issue.
Metabolic changes with intake-regulated weight loss. We observed that a 14% loss in body weight (32% reduction in FM) induced by calorie restriction is accompanied by a reduction in EE (17%) and SMR (18%) and a suppressed NPRQ. Both EE and SMR declined to a greater extent than was expected from the combination of loss of body mass and reduction in EI, suggesting an increase in metabolic efficiency. NPRQ remained lower after the adjustment of energy balance and CHO intake, suggesting a preference for lipid use. Thus our second major conclusion is that weight loss from caloric restriction is accompanied by an increase in metabolic efficiency and a preference for the use of lipids as opposed to CHO.

Several studies in humans (35, 47, 50, 55, 61) and rodents (10, 17, 38) have indicated that weight loss induced by caloric restriction reduces both EE and resting metabolic rate (RMR) compared with lean or obese controls. We have shown that this is also true when obesity-prone rats that are energy restricted after the development of obesity are compared with energy-restricted rats in both the preobese and obese state, even after the adjustment for the variation in EI, FFM, and FM. While such changes have been linked to changes in the expression of uncoupling protein in peripheral tissues (47) and a transient hypothyroid state (61), the general finding of a reduced MR beyond what would be expected for the change in metabolic mass has provided evidence of a metabolic adjustment that favors weight regain in the energy-restricted state.

It is not surprising that NPRQ was lower in WL rats, primarily in that there was little available CHO for oxidation, and endogenous lipid stores became a major contributor to the pool of oxidized fuels. This is consistent with what others have observed in both humans and rodents. Yet, after adjusting for CHO intake and energy balance, NPRQ remained lower than all of the groups, suggesting a metabolic adjustment beyond what could be explained by these factors that would spare the use of CHO as a fuel. Whether this observation reflects a real metabolic adjustment or whether it is an artifact of the negative energy imbalance that existed in these animals requires further studies with other techniques that can follow the use of fuels more directly.

Metabolic changes with intake-regulated weight maintenance after weight loss. While the metabolic adjustment with weight loss has been observed consistently in both human and rodent studies, there is considerable controversy as to whether this metabolic adjustment remains after energy balance is achieved and weight is maintained for a period of time. We found virtually no change from the weight-reduced state in EE or SMR when the rats were maintained at a constant, reduced body weight for eight subsequent weeks, suggesting that metabolic efficiency was still higher after a prolonged period of weight maintenance. We also found that CHO disappearance was proportionally higher. Thus our second major conclusion is that continued maintenance of a reduced body weight was accompanied by an elevation in metabolic efficiency and shift in fuel utilization such that there was a preference for CHO use more so than what was expected from the diet consumed.

A reduction in both EE (13%) and SMR (22%) was maintained after a subsequent period of weight maintenance, compared with age-matched obese controls (LFC and HFC), and about half of this difference was explained by the differences in EI, FFM, and FM. Several previous studies in humans and rodents have suggested a metabolic adaptation persists in weight maintenance after weight loss (2, 19, 28, 29, 41, 43, 59). One such study from Leibel and colleagues (41) showed that RMR, after statistical consideration for FFM, remained suppressed after the maintenance of 10% weight loss in obese subjects, and this effect was more profound after maintenance of a 20% loss in weight. However, most of these studies that have suggested an adaptation in MR have come under criticism for the way in which the data were normalized for metabolic mass, either because RMR-to-FFM ratio was used or that FM was not included in the regression model for statistical adjustment (36, 45). Reports from other researchers would argue that no such adaptation in MR is sustained (1, 40, 57, 61, 65). In a recent report that examined both the immediate effects of caloric restriction and sustained weight loss, Weinsier et al. (61) reported that both the suppression in RMR and the hypothyroid condition that they observed in response to caloric restriction was normalized within 10 days of achieving energy balance. The studies that make the argument against a metabolic adjustment, however, are not free from criticism. These studies are frequently biased toward the use of only those subjects who were successful with weight loss or weight maintenance (1, 40, 65), excluding those individuals who could not lose weight or successfully maintain the weight-reduced state. If a metabolic adjustment does exist, it would be more likely found in these excluded subjects rather than in those who are included in these studies. Some studies are not inclusive of subjects that were obese or severely obese before their weight loss (61). 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. Finally, for most studies, regular physical activity was either not reported or indicated as a component of the maintenance program (40, 57, 65). Physical activity is particularly important, as there is evidence to suggest that physical activity can blunt weight regain after weight loss (58), and individuals who are involved in regular physical activity are more successful in maintaining a reduced weight (39). In humans, it is difficult to achieve long-term maintenance of a weight-reduced state without regular physical activity, but with rodent models there are some aspects of environmental conditions that can be better controlled. In rodent models, we can better monitor and adjust caloric intake and be more proactive in limiting physical activity. Several studies in rodent models of obesity have suggested there is an adjustment in metabolic efficiency with weight reduction that persists after weight maintenance (15, 16, 43). When controlling for lean body mass, Dulloo and colleagues observed an 8% reduction in 24-h EE in rats (16) and a 10% reduction in 24-h EE in mice (15) after a period of intake-regulated weight maintenance period (1–3 mo) in a weight-reduced state. In the present study, we confirmed these observations in a model of obesity-prone rats in that we observed a similar reduction in EE and SMR after 2 mo of weight maintenance with more rigorous estimations of FM and FFM.

One explanation for the disparity between human and rodent studies is that this metabolic adjustment does not occur in humans. However, we are more inclined to suggest that physical activity, even in moderate levels, may prevent this increase in metabolic efficiency in an energy-restricted state. We cur-
rently have studies in progress that incorporate an exercise regimen in the treatment phase to see if this adjustment in metabolic efficiency during weight maintenance is eliminated. If regular physical activity does have such an effect, it could provide some explanation for conflicting observations in the literature and would provide one metabolic reason as to why regular physical activity is so important to successful weight maintenance (39).

While the issue of increased metabolic efficiency in the weight-reduced state remains controversial, there is more evidence that would indicate that the weight-reduced state is accompanied by shift in fuel utilization characterized by an increased preference for CHO oxidation at the expense of lipid oxidation (14, 28, 40). Both fasting and postprandial lipid oxidation are suppressed after weight loss and remain so during prolonged weight maintenance. We observed an elevation in adjusted NPRQ values in WM that would suggest such a shift in fuel utilization in this rodent model of obesity and would promote a high rate of weight regain under obesigenic conditions.

**Metabolic changes with weight regain after weight loss.** Weight regain after weight loss has been repeatedly shown in both rodents (4, 17, 42, 43) and humans (30, 56), and our rodent model of obesity and weight loss is no different. There are metabolic adjustments with weight loss and maintenance that would promote regaining the lost weight if the rat were again placed in obesigenic conditions. We were interested in whether these metabolic adjustments would be normalized after the lost weight was regained or whether they would linger to promote further weight gain. For this purpose, one group of rats was allowed free access to food immediately after the weight loss period, which led to a significant amount of weight regain. Our final conclusion in this study is that after weight regain, WR rats no longer exhibited any evidence of an elevated metabolic efficiency, at least in relation to obese controls, but there is some evidence to suggest that they may continue to have a shift in fuel utilization with a preference for CHO. By increasing the proportion of CHO utilized, dietary fat would be spared for deposition, resulting in a more energetically efficient storage of excess calories as fat.

In rodents, several studies have shown that weight regain after weight loss leads to an increased EI that matches or surpasses controls and that “food efficiency” (weight gained/kcal food consumed) is elevated throughout weight regain (4, 10, 17, 43). This efficiency has been partly attributed to an elevated metabolic efficiency that has been shown to persist in other rodent models during weight regain (17). The data in the present study would suggest that this adjustment in metabolic efficiency is normalized after a significant amount of weight regain. However, it is possible that the hyperphagia and accelerated growth in WL rats may be masking a residual increase in metabolic efficiency. While we have attempted to control for these effects by adjusting for the variation in EI, FFM, and FM, a better design to address this issue would be to pair-feed the weight-regaining rats to weight-matched, weight-maintained control rats. Dulloo and Jacquet (18) would argue that an enhanced metabolic efficiency persists until adipose stores are replenished. Alternatively, the adjustment in metabolic efficiency may be an important contributor to rapid weight gain earlier on in the weight regain process, while the drive to consume calories persists until the set point for the body weight is again reached. Further studies are needed to better characterize how quickly this normalization occurs in this model of obesity-prone rats.

An alternate explanation for the increased feed efficiency is a shift toward a preferential use of CHO that would shunt fat to storage depots. This concept is based on the idea that when there is a preferential oxidation of CHO, ingested lipid will be stored in a manner that will avert the energy required to convert CHO to fat. Flatt and Tremblay (24) estimate that ~25% of the energy from CHO is used in the conversion. While the adjustment in MR was resolved after 8 wk of feeding, WR rats did show a proportionally greater disappearance of CHO than age-matched control rats fed the same diet. However, we cannot be certain from the data in this study whether this is actually due to preferential CHO oxidation. Given the level of CHO in the diet and the lipogenic potential in rats, lipogenesis is occurring in WL rats to an extent that would elevate RQ, and we do not have a good estimation of this pathway in these animals. However, a substantial portion of this effect is likely the result of a higher CHO intake and a more positive energy balance than in the control groups. CHO is more effective at driving its own oxidation than fat (23), and the increased CHO intake in WR could alone preferentially divert lipids to the storage depots. However, a portion of the difference did remain after the adjustment for energy balance and CHO intake that may be linked to some lingering effect on fuel use related to their fluctuation in weight and drive to defend a higher body weight and/or fat mass. Further studies employing more direct methods to follow the utilization of fuels will be needed to clearly identify what pathways are more prominent in WR rats.

In summary, our data suggest that in obese, obesity-prone rats, weight loss induced by caloric restriction is accompanied by metabolic adaptations that predispose to regain the lost weight. In rats that are losing weight, this is exhibited by a significant reduction in MR, measured as both 24-h EE and SMR, that is independent of metabolic mass and EI. This adaptation persists after 8 wk of intake-regulated weight maintenance but is no longer present with eight subsequent weeks of ad libitum feeding where rats are regaining lost weight. While rats that are regaining weight may have a shift in fuel preference that would contribute to their high rate of weight regain, it should be stressed that the drive to increase EI remains the most critical factor in the predisposition to regain lost weight. This adjustment clearly weighs more on the energy balance equation than the metabolic adjustment on EE observed in this or any other study. Additionally, the effect that EI, or more particularly CHO intake, has on RQ is much more dramatic than the metabolic adjustment we observed from weight reduction. This drive to increase food intake likely involves environmental stimuli (diet composition, food palatability, physical activity) influencing motivational and metabolic components of a genetically determined set of central systems (42). While our data suggest that these metabolic adaptations might hinder successful weight maintenance, it should not imply that successful weight maintenance is unachievable. The physical activity of the obesity-prone rats in this study was purposefully limited throughout the entire study. Regular physical activity appears to be an essential element of successful weight loss in humans (39), and it may be the key factor that counteracts these metabolic adaptations to weight loss.
REFERENCES


