

## Regular exercise attenuates the metabolic drive to regain weight after long-term weight loss

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**MacLean PS, Higgins JA, Wyatt HR, Melanson EL, Johnson GC, Jackman MR, Giles ED, Brown IE, Hill JO.** Regular exercise attenuates the metabolic drive to regain weight after long-term weight loss. *Am J Physiol Regul Integr Comp Physiol* 297: R793–R802, 2009. First published July 8, 2009; doi:10.1152/ajpregu.00192.2009.—Weight loss is accompanied by several metabolic adaptations that work together to promote rapid, efficient regain. We employed a rodent model of regain to examine the effects of a regular bout of treadmill exercise on these adaptations. Obesity was induced in obesity-prone rats with 16 wk of high-fat feeding and limited physical activity. Obese rats were then weight reduced (~14% of body wt) with a calorie-restricted, low-fat diet and maintained at that reduced weight for 8 wk by providing limited provisions of the diet with (EX) or without (SED) a daily bout of treadmill exercise (15 m/min, 30 min/day, 6 days/wk). Weight regain, energy balance, fuel utilization, adipocyte cellularity, and humoral signals of adiposity were monitored during eight subsequent weeks of ad libitum feeding while the rats maintained their respective regimens of physical activity. Regular exercise decreased the rate of regain early in relapse and lowered the defended body weight. During weight maintenance, regular exercise reduced the biological drive to eat so that it came closer to matching the suppressed level of energy expenditure. The diurnal extremes in fuel preference observed in weight-reduced rats were blunted, since exercise promoted the oxidation of fat during periods of feeding (dark cycle) and promoted the oxidation of carbohydrate (CHO) later in the day during periods of deprivation (light cycle). At the end of relapse, exercise reestablished the homeostatic steady state between intake and expenditure to defend a lower body weight. Compared with SED rats, relapsed EX rats exhibited a reduced turnover of energy, a lower 24-h oxidation of CHO, fewer adipocytes in abdominal fat pads, and peripheral signals that overestimated their adiposity. These observations indicate that regimented exercise altered several metabolic adaptations to weight reduction in a manner that would coordinately attenuate the propensity to regain lost weight.

fat oxidation; energy balance; indirect calorimetry; postobese; adipocyte cellularity

WEIGHT REGAIN AFTER WEIGHT loss has been repeatedly shown in both obese animals (3, 4, 6, 9, 21–23, 25, 26, 30) and humans (14, 18, 32, 42, 43). A meta-analysis of a large number of U.S. weight loss studies summarized the propensity to regain after a wide variety of weight loss programs (1). Not only does lost weight tend to return, but the rate of return is highest immediately after the cessation of the structured weight loss program. Over 35% of the lost weight returns in the first year, and the majority is gained back within five years. Most people view their weight loss program as a transient change in their lifestyle

and dietary habits or have difficulty in sustaining the changes that they have made to lose the weight (7, 11).

The proposed reasons for the high rate of weight regain are many (11), but there is substantial evidence of an important role for metabolic adaptations that occur in response to weight loss (10, 13). The reductions in leptin and insulin (21, 23–25), in combination with a number of other neural, nutrient, and endocrine signals, convey an “energy deficit” signal to energy balance control centers within the brain. The result is an increased drive to eat (8, 15, 21, 23, 25) and a suppressed energy expenditure (9, 14, 25–27, 33, 35, 44). To maintain the reduced weight, food intake must be proactively limited to the level that expenditure is suppressed. This large energy gap between appetite and expenditure does not dissipate and may even become more profound with time in weight maintenance (26). Peripherally, the increase in insulin sensitivity and reversal of metabolic inflexibility primes tissues to utilize and store the impending caloric excess in an energetically efficient manner, in part because the ability to regulate fat oxidation is restored (2, 15, 38, 41). Additionally, studies in both humans and animals suggest that weight loss can lead to an increase in the number of adipocytes, which could facilitate the repletion and expansion of adipose tissue depots (15, 24, 25). Taken together, these observations provide evidence that the metabolic adaptations to weight reduction not only make it very difficult to sustain the necessary level of calorie restriction, but they also facilitate rapid, efficient weight regain when the inevitable bout of overfeeding occurs.

In light of this metabolic response, it is not surprising that successful weight loss maintenance is a challenging prospect. Ninety percent of those who are successful, as reported from the National Weight Control Registry, include regular exercise as part of their weight maintenance program (20). Poor adherence to exercise prescription, however, has made it difficult to show in randomized, controlled trials that regular exercise is an effective strategy for weight regain prevention (5). Regardless, studies in both animals and humans provide evidence that being more physically active results in less regain (16, 17, 21). The mechanisms that underlie this beneficial effect of physical activity are not clearly understood, but there is evidence to suggest that physical activity counters the biological adaptations to weight loss that facilitate weight regain (21).

In the present study, we employed a well-established rodent model of weight regain to examine the impact of a regular bout of aerobic, treadmill exercise on the homeostatic system that controls body weight. Our approach with this analysis was to provide an integrative picture of how regular endurance exercise affects energy balance, fuel utilization, lipid accretion, and peripheral adiposity signals, all of which are key components

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of the homeostatic system controlling body weight. We hypothesized that several aspects of this system would be coordinately altered in a manner that would facilitate maintenance of the weight-reduced state. Our observations indicate that, in male rats, regular exercise abrogates much of the biological drive to overeat that initiates the relapse to obesity and alters peripheral fuel metabolism and adipocyte cellularity in a manner that would reduce the rate and energetic efficiency of weight regain.

## METHODS

**Experimental paradigm of weight regain.** Male Wistar rats (125–150 g) were purchased from Charles River Laboratories (Charles River Laboratories, Wilmington, MA) and individually housed for the duration of the study in the University of Colorado Denver Center for Comparative Medicine and the Center for Human Nutrition Satellite Facility (22–24°C; 12:12-h light-dark cycle) with free access to water. All procedures were approved by the University of Colorado Denver Institutional Animal Care and Use Committee.

Obesity-prone and obesity-resistant rats were identified by their weight gain response to a high-fat diet (HF, 46% kcal fat, RD no. 12344; Research Diets, New Brunswick, NJ) as previously described (26, 27). These rats were then matured in an obesogenic environment (with free access to the HF diet; individually housed in cages that limit physical activity) for 16 wk to yield obese and lean rats. Twenty-one obese rats were then placed on an energy-restricted low-fat diet (LF, 13% kcal fat, RD no. 11724; Research Diets) for 2 wk, equivalent to ~55% of the calories eaten ad libitum by obese rats to yield a 10–15% reduction in their body weight. The remaining obese and lean rats were switched to ad libitum access of the LF diet for the remainder of the study.

The weight-reduced rats were stratified according to weight and weight loss, and then divided into sedentary (SED) and exercise (EX) groups for the duration of the study. For eight subsequent weeks, the reduced weight was maintained by providing a limited provision of the LF diet at the beginning of each dark cycle (3:00 P.M.), with (EX) or without (SED) a bout of treadmills exercise occurring within the first 3 h of the dark cycle. The relapse to obesity in SED and EX rats was followed for eight subsequent weeks of ad libitum feeding on the LF diet, while their respective daily regimens of inactivity or daily treadmill exercise continued.

**Treadmill exercise.** At the start of the 8 wk of weight maintenance, EX rats were acclimated to 15 m/min for 30 min/day on 6 days/wk. Two Exer-6M Treadmill (Columbus Instruments), each with three individual lanes, were used for the training regimen. This was accomplished by ramping up the speed from 9 to 15 m/min during a 5-min bout over a 1-wk period, and then ramping up the time from 5 to 30 min over a 2-wk period. Rats were motivated to complete their daily training by using one or more of the following stimuli: 1) positioning food pellets just out of reach or dangling a novel play item at the head of the treadmill lane; 2) shock from an electric grid at the rear of the treadmill (10 volts, 0.5 amps, 0.75 Hz); 3) application of a bristle brush to the feet on the rear grid; and/or 4) intermittent air puffs to the hindquarters. The type and combination of motivation used varied within the same rat and between rats, depending on the response to the motivation. One rat that did not respond to any motivational tool refused to perform any treadmill exercise. This rat was removed from the study. The best technique on a given day for each individual rat was chosen to complete their training session. Rats were scored on a graded scale by the technician for their performance (1 = poor; 10 = outstanding), based on the amount of oversight and encouragement required to ensure compliance. This regimen of exercise continued to the end of the study in EX rats, which included both the weight-loss maintenance and relapse phases of the protocol.

**Intake, expenditure, and fuel utilization.** A metabolic monitoring system with indirect calorimetry, urine collection, and 24-h food intake was used to assess energy balance and fuel utilization before and during the relapse to obesity. Metabolic rate (MR) was calculated from gas exchange measurements acquired every 6 min over the 24-h monitoring period, using the Weir equation ( $MR = 3.941 \times \dot{V}O_2 + 1.106 \times \dot{V}CO_2 - 2.17 \times N$ , where N is urinary nitrogen), as previously described (26, 27). Average MR, extrapolated over 24 h provided an estimate of total energy expenditure (TEE). Resting energy expenditure (REE) was estimated in the latter part of the light cycle as previously described (26), and the difference between TEE and REE yielded an estimate of nonresting energy expenditure (NREE). Respiratory exchange ratio (RER) was calculated as the ratio of CO<sub>2</sub> production to O<sub>2</sub> consumption ( $\dot{V}CO_2/\dot{V}O_2$ ). In a select cohort of animals that were in energy balance ( $\pm 5$  kcal/day), substrate oxidation was calculated from  $\dot{V}O_2$ ,  $\dot{V}CO_2$ , and total urinary nitrogen, using the following equations: CHO disappearance =  $(4.57 \cdot \dot{V}CO_2) - (3.23 \cdot \dot{V}O_2) - (2.6 \cdot N)$ ; lipid disappearance =  $(1.69 \cdot \dot{V}O_2) - (1.69 \cdot \dot{V}CO_2) - (2.03 \cdot N)$ ; protein disappearance =  $6.25 \cdot N$ .

The energetics of the exercise bout was assessed in an indirect calorimetry treadmill chamber (Columbus Instruments, Columbus, OH) during the final week of the weight-loss maintenance. Gas exchange data were collected every 30 s during the first 15 min of the bout or until a relative steady state of metabolism was achieved. Measurements during this steady state were extrapolated to estimate the energetic cost and fuel utilization during the remainder of the bout. The energetic cost of the exercise bout was added to the 24-h calculations of TEE within the NREE component, and the estimates of fuel utilization were added to the calculations of substrate oxidation. During exercise, the contribution of protein oxidation was assumed to be ~8 cal/min, extrapolated from 24-h urinary nitrogen measurements. Previous studies have shown that protein oxidation may increase by 10 to 15% during exercise, which would have little impact on the estimates of substrate oxidation during the bout. The energetic measurements suggested that this activity was more consistent with an aerobic or endurance training activity.

**Tissue specific analyses.** Body composition was determined by dual-energy X-ray absorptiometry as previously described (25). At the end of relapse, rats were fasted for 6 h, blood was collected, and isolated plasma was stored at –80°C for analysis of humoral metabolites and endocrine factors. At the end of the 8-wk relapse period, the rats were killed, and fat pads were excised and weighed.

Plasma glucose, triglycerides (TG), free fatty acids, and total cholesterol were assayed as previously described (28). Urinary nitrogen was estimated from measurements of urea and creatinine in 24-h urine collections (25, 27), and these samples were also used to estimate 24-h corticosterone release (25). Concentrations of insulin, leptin, amylin, and glucagon were simultaneously measured in plasma samples using the Rat Endocrine LINCoplex Kit 96-Well Plate Assay (RENDO-85K; Linco Research/Millipore, St. Charles, MO).

A portion of retroperitoneal (RP) adipose tissue (~250 mg) was suspended in 2 ml of cold Krebs-Ringer phosphate (KRP) buffer (in mM): 129 NaCl, 5.15 KCl, 1.72 CaCl<sub>2</sub>, 2.65 MgSO<sub>4</sub>, 1.47 KH<sub>2</sub>PO<sub>4</sub>, and 0.84 Na<sub>2</sub>HPO<sub>4</sub> (pH 7.4). Each sample was minced and mixed with 5 mg of collagenase (198 U/mg) and 5 μl of methylene blue. Following light vortexing, the sample was incubated at 37°C for 10 min. The sample was washed three times in 5 ml of cold KRP, and the fat cells were transferred to a glass slide. Adipocyte cells were immediately viewed and imaged by a blinded microscopist with a 0.01-mm stage micrometer using an Olympus Max U-CMAD3 microscope and a C-mounted Canon Power Shot G5 digital camera. Images were analyzed with a Cell Counting Analysis Program (Mayo Clinic, Rochester, MN) to obtain the diameter of 100–200 cells in each sample, and the number of cells per fat pad was calculated with the average diameter, a density conversion factor (0.915 g/ml), and the mass of the fat pads (25).

**Statistical analyses.** Data were expressed as means  $\pm$  SE and analyzed with SPSS (version 16.0) by ANOVA, with Fisher's least-significant difference or Duncan's post hoc test when a significant effect was observed. Morphometric and energy balance data were examined in a repeated-measures model, testing for an effect of exercise, time, and their interaction. In some cases, ANCOVA was used to examine differences that remained after adjusting for a relevant covariate [i.e., REE, adjusted for fat-free mass (FFM)]. Pearson and partial correlation coefficients were calculated to examine the relationships between parameters of interest. Statistical significance was assumed when  $P$  was  $<0.05$ .

## RESULTS

**Exercise attenuates weight regain after weight loss.** Regular exercise attenuated the rate of weight regain early in the relapse period and reduced the defended body weight (Fig. 1A). The percent of lost weight regained was substantially reduced ( $120 \pm 8$  vs.  $77 \pm 7\%$ ,  $P < 0.05$ ) with regular exercise after the 8 wk of relapse (Fig. 1B). Regular exercise had no impact on body composition during the weight maintenance phase of the study (data not shown), and the attenuated weight gain in the EX rats during relapse was fat mass (Table 1). EX rats exhibited a reduction in tissue weights of the liver, RP, and mesenteric fat pads compared with SED rats at the end of relapse. In addition, relapsed EX rats had fewer adipocytes than relapsed SED rats when cellularity characteristics were assessed in the RP pads (Fig. 1C).

**Energy balance during weight regain.** Energy balance data are shown in Fig. 2. Energy intake (EI) was significantly lower in weight-reduced rats than in obese ad libitum-fed controls (Fig. 2A), reflecting their calorie-restricted diet regimen. When this restriction was ended, ad libitum intake increased dramatically in SED rats during the first few days of relapse and remained elevated throughout the remainder of the study. Regular exercise prevented this hyperphagia during the early stages of relapse. EI in EX rats was significantly lower than in SED rats throughout most of relapse. SED rats ate more food than obese controls during relapse ( $P < 0.05$ ), whereas EX rats ate less than obese controls over that time ( $P < 0.05$ ).

The energetic cost of the 30-min exercise bout of aerobic exercise was extrapolated from steady-state measurements during the first 15 min of the bout in an exercise calorimetry chamber (Fig. 3A). This estimate [exercise activity thermogenesis (EAT)], excluding basal energy requirements, was  $\sim 2.0$  kcal/30-min bout, or  $\sim 4\%$  of total daily expenditure. EAT was dependent on body weight ( $r^2 = 0.59$ ,  $P < 0.001$ ), which allowed us to crudely estimate EAT as weight was regained during relapse [EAT =  $(0.0083 \times \text{body wt}) - 0.604$ ]. Although the cost of the bout increased by  $\sim 15\%$  as the lost weight returned, the impact of this gradual elevation on total expenditure was  $<1\%$ . EAT was included in the NREE of TEE in subsequent component analyses of energy metabolism.

As expected, the calorie-restricted weight loss reduced TEE (Fig. 2B), which primarily reflected a reduction in REE (Fig. 4A). Average TEE increased during the first several days of relapse in both SED and EX rats ( $P < 0.05$ ; Fig. 2B), and this effect was more profound in SED rats (interaction,  $P < 0.05$ ). During the relapse period, TEE gradually increased in both SED and EX rats throughout relapse ( $P < 0.05$ ). On the final day of measurements, TEE was significantly lower in the EX-relapsed than in the SED-relapsed rats by  $\sim 7$  kcal (Fig.

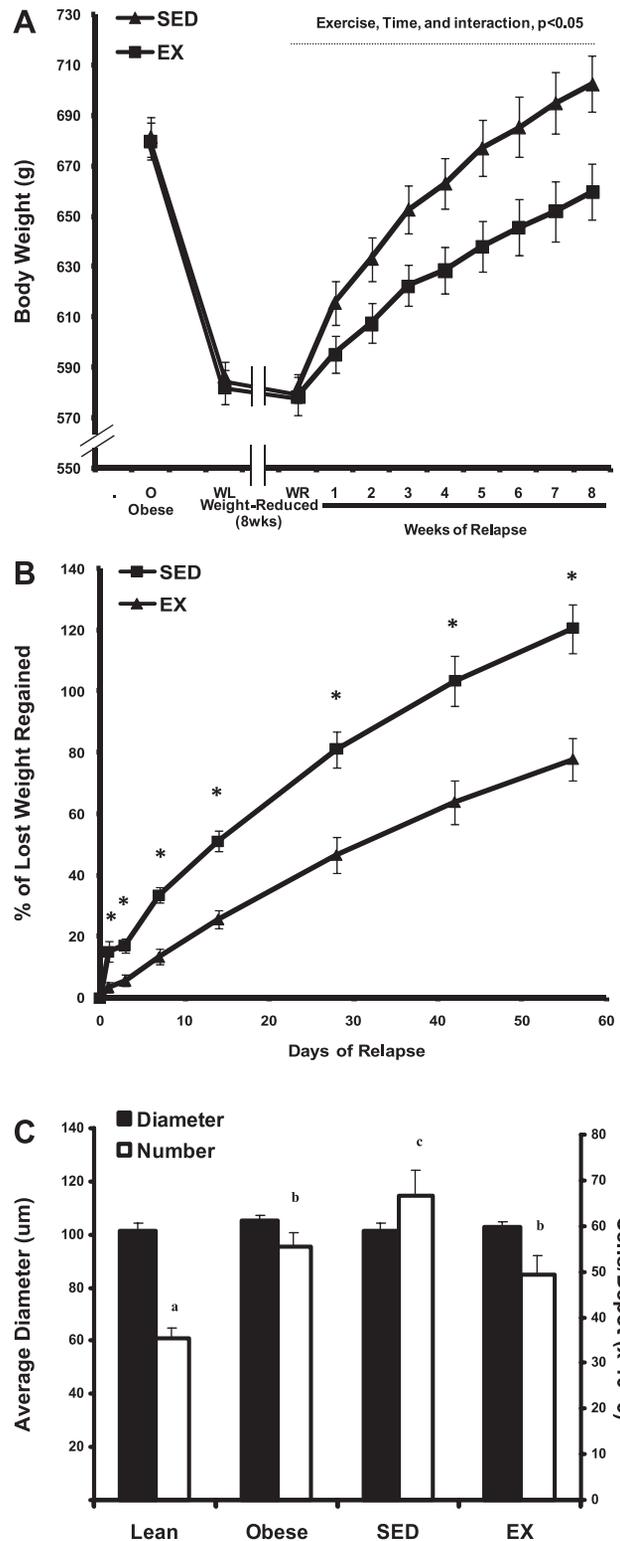


Fig. 1. Weight regain after weight loss. A: body weight is shown for obese, weight-reduced, and relapsing rats, which were either sedentary (SED,  $n = 10$ ) or performed a daily bout of treadmill exercise (EX,  $n = 11$ ) during weight maintenance and relapse. B: data expressed as %lost weight that was regained during the 8-wk period of relapse ( $*P < 0.05$ ). C: cellularity characteristics (average cell size and cell number) for the retroperitoneal fat pads are shown for lean ( $n = 10$ ) and obese ( $n = 9$ ) rats, as well as for SED (8) and EX (9) rats after relapse. <sup>a,b,c</sup>Groups with the same letter designation are not significantly different ( $P < 0.05$ ).

Table 1. *Body composition and adiposity after relapse*

	Lean	Obese	SED	EX
<i>n</i>	14	12	10	11
Body wt, g	579 ± 15*	735 ± 20†	709 ± 14†	641 ± 11‡
Fat-free mass, g	422 ± 14	468 ± 17	452 ± 15	456 ± 11
Fat mass, g	157 ± 7*	267 ± 16†	257 ± 17†	184 ± 13*
Body fat, %	27.2 ± 1.2*	36.3 ± 1.8†	36.1 ± 2.1†	28.6 ± 1.4*
Bone, g	11.6 ± 0.4	11.8 ± 0.5	12.3 ± 0.4	12.1 ± 0.6
Bone density, g/cm <sup>3</sup>	0.320 ± 0.002	0.322 ± 0.002	0.325 ± 0.002	0.317 ± 0.003
Liver, mg/dl	15.7 ± 0.3*	18.0 ± 0.4†	18.2 ± 0.6†	15.4 ± 0.5*
RP, g	17.0 ± 1.2*	31.1 ± 1.7†	32.4 ± 1.6†	26.2 ± 1.3‡
EPI, g	13.4 ± 1.2*	20.4 ± 0.8†	18.4 ± 1.2‡	15.5 ± 0.8*‡
MES, g	12.7 ± 1.0*†	17.6 ± 1.2‡	14.9 ± 1.1‡	11.2 ± 0.7*
Gastrocnemius, g	3.15 ± 0.17	3.07 ± 0.09	3.25 ± 0.10	3.09 ± 0.08

Data are expressed as means ± SE and examined by ANOVA; *n*, no. of rats. SED, sedentary; EX, exercised; RP, retroperitoneal; EPI, epididymal fat pad; MES, mesenteric. Body composition was determined by dual-energy X-ray absorptiometry. When a significant effect was observed, Duncan's post hoc test for homogeneous groups was performed. Groups with similar symbol designations are not significantly different,  $P < 0.05$ .

4A). This difference was found to be due to a lower REE in the EX rats. These observations remained significant after adjusting REE for the variation in FFM (Fig. 4B).

Energy balance data during the first few days of relapse quantify the effective energy gap between the rat's appetite and its expenditure requirements in the weight-reduced state. Taken together, the energy gap was significantly reduced with regular exercise (Fig. 2C). The peak energy imbalance occurred on the first day of relapse in SED rats, but was delayed by several days in EX rats. Thereafter, the positive energy imbalance remained lower in EX by ~40%. After weight reduction, SED rats were subjected to persistent desire to eat more than they ever had, while their expenditure was reduced to a level reflective of lean rats. Regular exercise reduced this energy gap primarily by reducing the magnitude and delaying the onset of this drive to overfeed.

**Fuel utilization.** As has been observed previously, lean rats exhibited a diurnal fluctuation in RER that suggests a shift in fuel utilization favoring the oxidation of carbohydrate (CHO) during the dark cycle and fat oxidation during the light cycle, whereas obese rats did not exhibit the diurnal shift in fuel utilization (interaction,  $P < 0.05$ ; Fig. 5A). After weight reduction, the diurnal shift in fuel utilization returned (Fig. 5A), and both CHO and fat oxidation were lower than they were before the weight was lost (Fig. 5B). Regular exercise blunted this diurnal shift after weight reduction, because 1) the exercise bout led to a rapid decline in RER that gradually recovered over a 3-h period at the beginning of the dark cycle; and 2) RER was higher in the EX rats during the middle hours of the light cycle (Fig. 5C). The rate of fat oxidation increased fivefold during the first 7 min of the exercise bout, before reaching a relative steady state (Fig. 3B). Extrapolating this data to the full 30-min bout suggested that this bout of exercise led to an additional ~1.2 kcal of fat oxidation over resting energy requirements.

On the first day of relapse, the diurnal shift in metabolism observed in weight-reduced rats in energy balance was eliminated, as RER increased in both the dark and light cycles (Fig. 5A). RER was significantly lower in EX rats throughout most of the day, but this difference was particularly apparent during and after the exercise bout and during the light cycle in the latter part of the 24-h monitoring period (Fig. 5D). The effect of exercise on RER remained significant throughout the first

week of relapse. At the end of the 8-wk relapse period, exercise did not significantly affect the diurnal fluctuation in RER (Fig. 5A), but it did lead to a significant reduction in 24-h CHO oxidation (Fig. 5B). In SED rats, during the early stages of the relapse to obesity, CHO is the preferred fuel for energy needs, fat is trafficked to lipid depots, and a significant amount of de novo lipogenesis occurs (15). Regular aerobic exercise abrogates one or more aspects of this shift in fuel utilization.

**Training performance.** An immediate and persistent decline in the training index was observed in EX rats at the initiation of relapse (Fig. 3C). EX rats became less volitionally compliant to the exercise regimen and required technicians to be more proactive in their oversight of the training bout. This aversion to compliance did not appear to be a function of the increasing body weight, as the average training index did not decline while ~80% of the lost weight returned.

**Endocrine and metabolite profiles.** As has been shown previously, weight loss led to a reduction in plasma insulin, leptin, amylin, and glucagon. Regular exercise did not significantly affect the levels of these hormones measured at the end of the weight maintenance period, and the changes that occurred in these parameters after the first day of relapse also were unaffected by exercise (data not shown). The two groups of weight-reduced rats also did not differ in free fatty acids, TG, or cholesterol. Glucose tended to be higher in EX rats than in SED rats at the end of weight maintenance ( $11.1 \pm 0.8$  vs.  $8.8 \pm 1.2$ ), but this comparison did not reach statistical significance ( $P = 0.1$ ). After relapse, the adiposity signals of insulin, leptin, and amylin were not significantly different between SED and EX rats (Table 2). In addition, glucose, TGs, free fatty acids, and 24-h urinary corticosterone were similar for the two groups (Table 2).

## DISCUSSION

The novel observations from this study are that regular, aerobic, treadmill exercise in male rats countered several metabolic adaptations to weight loss that are known to facilitate weight regain and the eventual relapse to obesity. These effects reduced the rate of regain early in the relapse process and ultimately lowered the defended body weight and fat mass. During weight maintenance, regular endurance exercise reduced the energy gap between the strong drive to eat and the

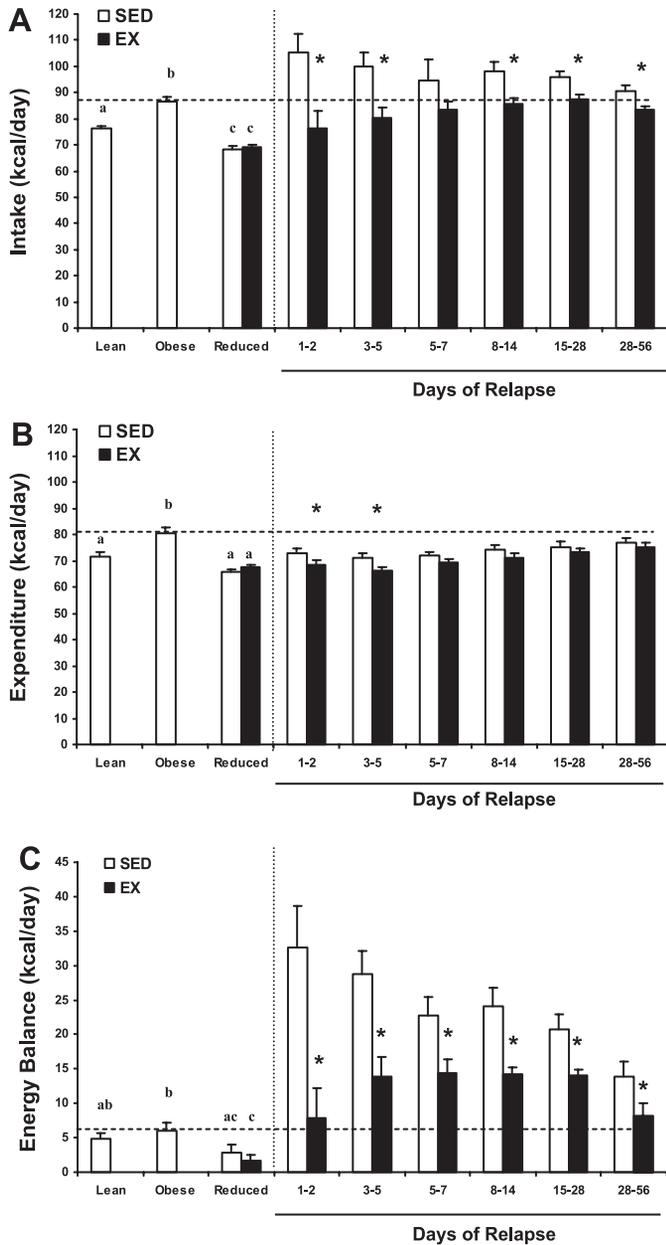


Fig. 2. Intake, expenditure, and energy balance. Energy balance data are shown for lean ( $n = 14$ ), obese ( $n = 12$ ), and obese rats after weight reduction and during relapse ( $\pm$  treadmill exercise; SED/EX,  $n = 10/11$ ). Average daily intake (A), daily expenditure (B), and energy balance (energy intake-total energy expenditure; C), for the groups are shown for several time periods during relapse. Two separate analyses were run on these data, separated by the vertical broken line. The effects of obesity and weight loss were examined by ANOVA, and Duncan's post hoc test was used to identify homogeneous subsets (<sup>a,b,c</sup>groups with the same letter designation are not significantly different). In a repeated-measures design, the effect of exercise was examined on these parameters at several time points during relapse (\*Difference between SED and EX rats during that time period,  $P < 0.05$ ).

suppressed level of expenditure. On the first day of relapse, exercise attenuated the positive energy imbalance and blunted the shift in fuel utilization that favored rapid, efficient weight regain. At the end of the 8-wk relapse, regular exercise established a different homeostatic balance between intake and expenditure than what was found in the obese or relapsed SED rats. In defense of their lower weight, relapsed EX rats exhib-

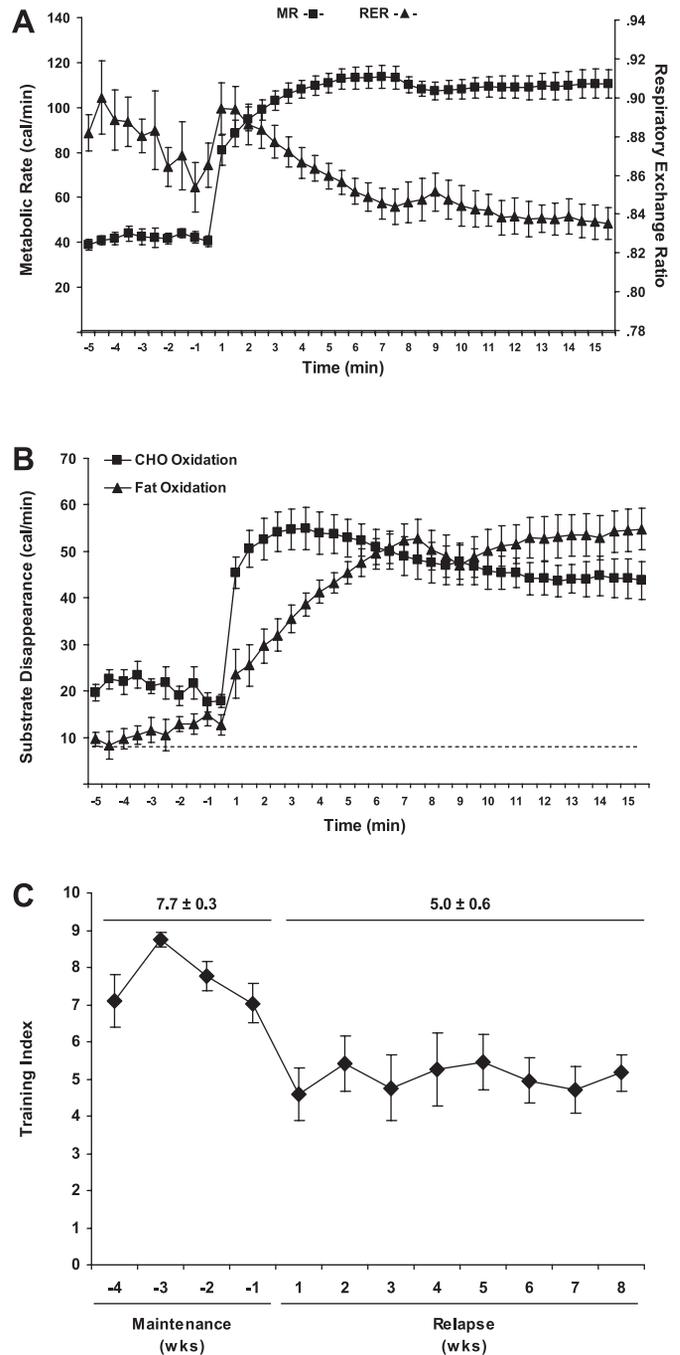


Fig. 3. Exercise energetics and training performance. A: metabolic rate (MR) and resting energy expenditure (RER) during the first 15 min of the exercise bout were calculated from the gas exchange data acquired from an exercise indirect calorimetry chamber. B: substrate disappearance was calculated for the exercise bout to provide an estimate of carbohydrate (CHO) and fat oxidation. A steady level of protein oxidation (broken line), derived from urinary nitrogen measurements, was assumed. MR and substrate utilization data were extrapolated from steady state to estimate the exercise activity thermogenesis (EAT) and substrate oxidation for the entire 30-min bout. After 15 min, the animals were removed from the chamber and placed in a standard treadmill, where training technicians could implement motivational stimuli to ensure compliance. C: performance score was recorded for each rat based on their volitional compliance to the exercise regimen (1 = poor; 10 = outstanding). The index showed a sharp reduction at the transition from weight maintenance to relapse ( $n = 8$  EX rats,  $P < 0.05$ ).

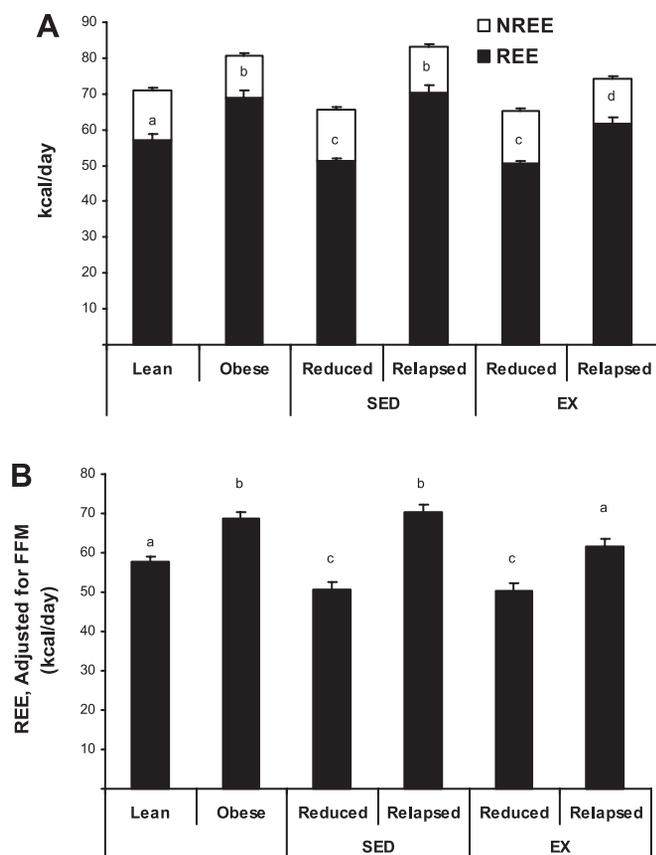


Fig. 4. Components of total energy expenditure (TEE) and metabolic efficiency. *A*: TEE was divided into resting (REE) and nonresting (NREE) components. The groups differed with respect to REE (main effect,  $P < 0.05$ ), and those with a similar letter designation were not significantly different ( $P < 0.05$ ). *B*: REE was examined by ANCOVA with fat-free mass as a covariate, and the adjusted values are presented. The groups differed with respect to REE (main effect,  $P < 0.05$ ). <sup>a,b,c,d</sup>Groups with a similar letter designation were not significantly different ( $n = 10\text{--}14/\text{group}$ ,  $P < 0.05$ ).

ited an energy balance and fuel utilization profile that more reflected the lean controls. Taken together, these observations indicate that the regular aerobic treadmill exercise in male rats, which accounted for  $\sim 4\%$  of the day's total energy requirements, countered the metabolic adaptations to weight loss and lessened the metabolic drive to regain the lost weight. These effects may explain, in part, the therapeutic efficacy of regular aerobic exercise for weight regain prevention.

Our observations are likely to be specific to the regular treadmill regimen employed in this study. A study in a similar paradigm of weight regain examined the effects of volitional running wheel use during the weight loss maintenance and weight regain. Both approaches to increase physical activity reset the homeostatic balance between intake and expenditure at a lower defended body weight and a reduced level of visceral fat. Treadmill exercise, however, imparted the additional benefit of reducing the rate of weight regain at the beginning of relapse, when the metabolic drive to regain was at its peak for sedentary animals. Beyond this additional benefit of the regimented intervention, a general head-to-head comparison would suggest that treadmill exercise primarily affected appetite and fuel preference, while running wheel access primarily affected the expenditure side of the energy balance equation (21). In the

present study, we went to great lengths to acquire the energetic cost and fuel preference of the exercise bout. This regimen of exercise did not represent a large contribution to TEE. More surprisingly, there appeared to be an adaptive response with exercise to reduce the other components of TEE during weight maintenance such that TEE was similar for SED and EX rats. Finally, neither running wheel access nor the treadmill exercise used in the present study reflects a regular strength training intervention, which may also affect energy balance regulation. This experimental paradigm may provide a good model to explore the differential impact of intervention that varies the type and intensity of the intervention.

The effects of exercise could not be explained by the well-known adiposity signals, leptin and insulin (13, 46). Neither intervention, running wheels or treadmill exercise, ameliorated the exceptionally low levels of these hormones that convey the energy deficit message from the periphery. Rather, it is likely that this regimen of daily exercise affected how this message from the periphery was received. There is growing evidence to suggest that increasing physical activity might enhance hypothalamic sensitivity to these adiposity signals (12, 34) and/or potentiate the postabsorptive response to the satiety signals peptide YY, glucagon-like peptide 1, and pancreatic polypeptide (29). These effects of exercise are thought to be more acute, being mediated by the hypothalamic nutrient sensor, AMP-activated protein kinase (AMPK). Recent studies have shown that acute bouts of intense exercise blunt AMPK's response to peripheral signals of acute energy deprivation, which in turn reduces the subsequent drive to overfeed (29, 34). In the present study, weight-reduced rats exhibit a diurnal cycle of two metabolic extremes: an unsatisfying period of gorging followed by a prolonged period of deprivation (15). If the acute effects of exercise on this hypothalamic nutrient sensor are recapitulated on a daily basis, it may account for the reduced energy gap during maintenance and the attenuated positive energy imbalance at the beginning of relapse.

This same nutrient sensor, AMPK, is found in the peripheral tissues, responding to low nutrient availability and reduced energy charge by mobilizing fuel stores and enhancing energy production (19). Like peripheral insulin sensitivity, nutrient sensing by AMPK in the periphery appears to be impaired with obesity (15, 37). Both insulin resistance and impaired AMPK sensing contribute to a more global state of metabolic inflexibility (38) that prevents the appropriate adjustment in metabolic regulation in response to a number of metabolic challenges, like fasting, exercise, and overfeeding. The normal response to a large influx of calories is suppressed fat oxidation, enhanced glucose oxidation, and the trafficking of dietary fat to adipose tissue. This shift in fuel utilization provides the quickest, most efficient way to utilize, clear, and deposit excess fuels. This response to a meal or overfeeding is blunted in the obese but reemerges after weight loss (2, 15). In the present study, metabolic inflexibility in the obese manifests as a relatively static state of metabolism that persists throughout the day, with little or no diurnal fluctuation in RER. Weight loss ameliorates some aspects of this metabolic impairment, since both insulin sensitivity and AMPK responsiveness are improved (15, 25). This reversal of metabolic inflexibility contrasts what is seen in younger animals undergoing catch-up growth after food restriction. Catch-up growth in immature

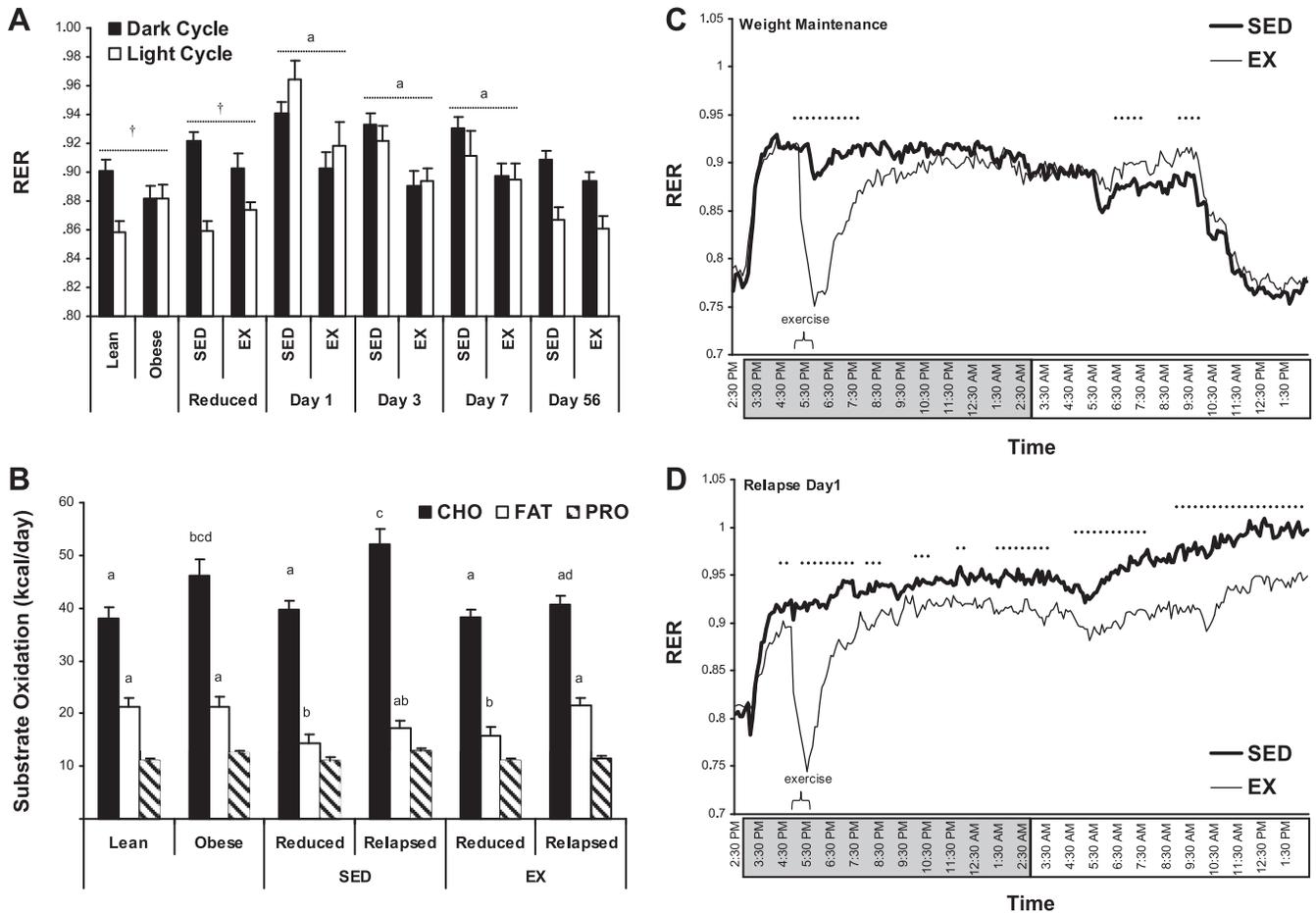


Fig. 5. Fuel utilization before, during, and after the relapse to obesity. The effect of exercise on the respiratory exchange ratio (RER) and substrate oxidation is presented. *A*: RER is separated into 12:12-h dark-light cycles. The diurnal pattern was affected by obesity ( $\dagger P < 0.05$ ) and differed between SED and EX rats at the end of weight maintenance. Exercise reduced RER during the 1st wk of relapse ( $^*P < 0.05$ ). *B*: substrate oxidation was measured in animals that were in energy balance before and at the end of relapse. For each macronutrient [CHO, fat, or protein (PRO)], a,b,c,d groups with the same letter designation were not significantly different ( $P < 0.05$ ). RER measured every 6 min over the 24-h monitoring period is shown for SED ( $n = 8$ ) and EX ( $n = 8$ ) rats at the end of weight maintenance (*C*) and on the first day of relapse (*D*). The meal was delivered at the beginning of the dark cycle (3:00 P.M.). The shaded time on the x-axis indicates the range for the dark cycle. Broken lines indicate extended periods of time during which RER was significantly different between the two groups ( $P < 0.05$ ).

animals is associated with impaired insulin sensitivity and AMPK signaling and a different consequence on metabolic regulation (39). In the case of the postobese, the enhanced metabolic regulation that occurs after weight loss is thought to establish a biological vulnerability to weight regain by providing the regulatory means to better clear, utilize, and store excess fuels (2, 38, 41). The diurnal fluctuation in RER of

weight-reduced SED rats is a manifestation of this improved response to ingested calories.

In a previous study, we provided a practical demonstration of this difference in metabolic flexibility by challenging obese and weight-reduced (SED) rats with the exact same caloric excess over a 24-h period (15). The SED rats rapidly consumed, utilized, and stored the meal provision, exhibiting a

Table 2. Humoral characteristics and urinary corticosterone

	Lean	Obese	SED	EX
Insulin, pM	585 ± 75*	794 ± 81†	580 ± 74*‡	657 ± 67†‡
Leptin, pM	677 ± 223*	1,308 ± 233†	799 ± 148‡	1,119 ± 197†‡
Amylin, pM	13.3 ± 2.1	20.7 ± 6.1	13.7 ± 1.7	17.4 ± 3.2
Glucagon, pM	43.4 ± 4.3*	65.7 ± 6.7†	50.6 ± 4.2*	49.7 ± 7.8*
Glucose, mM	7.4 ± 0.3	7.7 ± 0.3	7.1 ± 0.2	7.3 ± 0.6
Triglycerides, mg/dl	128 ± 11*	185 ± 13†	154 ± 22*†	177 ± 20†
Free fatty acids, μM	562 ± 80	656 ± 45	680 ± 40	657 ± 76
Cholesterol, mg/dl	100 ± 10	108 ± 5	105 ± 7	97 ± 7
Corticosterone, μg/day	3.75 ± 0.42	3.78 ± 0.68	2.91 ± 0.43	3.23 ± 0.26

Data are expressed as means ± SE and examined by ANOVA. Insulin and leptin were assessed by enzyme-linked immunosorbent assays, and plasma metabolites were determined by colorimetric assays. When a significant effect was observed, Duncan's post hoc test for homogeneous groups was performed. Groups with similar symbol designations are not significantly different,  $P < 0.05$ .

predictable fluctuation in fuel preference. By the end of the 24-h period, the metabolic state of the weight-reduced rat essentially reflected that of a fasted animal. In stark contrast, obese rats showed little change in fuel preference through the day and used the entire day to consume the meal provision. Our assertion is that the enhanced metabolic response to the over-feeding challenge after weight reduction accelerates the transition to the postabsorptive state and may contribute to the insatiable desire to continue eating if food was available. The collection of nutrient, hormonal, and neural signals that conveys this short-term homeostatic signal of "energy deprivation" may be complex, but it likely involves hypothalamic nutrient sensing by AMPK. In the present study, daily aerobic exercise blunted the diurnal extremes in fuel utilization that are associated with this alternating cycle of gorging and deprivation. Our observations provide evidence that this intervention increasing physical activity promotes the oxidation of fat and spares/ stores CHO when they feed, and then increases the availability and utilization of glucose during the deprivation phase of this cycle. As such, the fluctuation of fuel availability and the associated signal of extreme energy deficit occurring during the latter part of the light cycle may be blunted. This exercise-associated shift in fuel utilization is subtle during maintenance, without necessarily affecting 24-h substrate oxidation when the rats are in energy balance. The impact on whole body fuel utilization only emerges when the weight-reduced rats are faced with the metabolic challenge of unlimited food availability at the start of relapse.

An alternative explanation for our observations is that EX rats may have been just as hungry as SED rats, but the bout of exercise, occurring 2 h after meal delivery, may have interrupted their gorging meal pattern. This delay in continued feeding may have allowed more time to generate feedback signals that would satiate the animal before it could overeat to the same extent as the SED rat. Calorie-restricted rats with access to running wheels perform much of their activity in the hours before the delivery of the daily food provision (21). Unlike the present study, little or no effect on food intake during relapse is observed with running wheel use. Interrupting this gorging meal pattern after calorie-restricted weight loss has been shown to affect weight regain, even when the total daily caloric food intake is standardized (45). Furthermore, proactively altering meal patterns can affect daily EI in humans (36). Additional studies are needed to examine how the timing and intensity of the exercise intervention, in relation to meal delivery, impacts energy balance and fuel utilization.

At the end of the study, relapsed EX rats established a new homeostatic steady state at a lower body weight and fat mass. In this respect, the effects of regular treadmill exercise are somewhat similar to the effects of volitional wheel use (21). As in that study, the effects of exercise on adiposity may have been emphasized on visceral fat pads. What is particularly interesting is that the energy balance and fuel utilization profile of relapsed EX rats is more akin to a lean rat. In contrast to rats with running wheels, EX rats defend this lower level of fat mass with the same level of adiposity signals found in the obese. This would suggest that the sensitivity to these peripheral signals of adiposity is greater in EX rats than in the obese. These long-term homeostatic signals tend to be lower in relapsed SED rats, but the energy balance data (Fig. 2C) would suggest that these rats were not finished with the relapse-

related weight gain. We have observed in previous studies that SED rats overshoot their pre-weight-loss weight to an extent that reflects the length of time in weight maintenance (25, 26). This expansion of adipose storage capacity is accompanied by an increase in the number of adipocytes reported here and in previous studies of both rodents and humans (15, 24, 25).

In this same paradigm of weight regain, we have observed the abrupt appearance of small, presumably new, adipocytes on the first day of relapse (15). Consistent with our previous reports, the small cells are no longer distinguishable at the end of relapse (data not shown) even though the hyperplasia persists (25). We suspect, based on the persistence of hypercellularity and the normalization of average diameter at the end of relapse (15, 25), that these small adipocytes preferentially accumulate fat and hypertrophy to the point that they become indistinguishable from the preexisting pool of larger adipocytes. Smaller adipocytes have a reduced rate of basal and catecholamine-stimulated lipolysis (24) and are more sensitive to the antilipolytic effect of insulin (31). Our observations that regular aerobic exercise prevents the development and/or persistence of this hypercellularity may represent another beneficial effect of increased physical activity. Preventing the formation of small adipocytes and the subsequent hypercellularity may contribute to the reduced rate of weight gain early in relapse, as well as the different homeostatic steady state that defends a lower body weight and fat mass.

One final, intriguing observation, which may be relevant to the poor compliance to exercise prescription in humans (5), was an abrupt decline in volitional compliance to the exercise regimen at the start of relapse. This relapse-induced aversion to compliance of the regimented exercise program was not surprising, but the lack of dependence of this affect on the increasing body weight was. We expected that, as the animals became heavier during relapse, the work load would increase, making it more difficult for the animals to complete the bout. The fact that volitional compliance changed abruptly at the start of relapse would support the alternative suggestion that some aspect of neural control involved in the propensity to be physically active may be altered with the transition from weight maintenance to relapse. The propensity for physical activity is highly regulated and can significantly impact body weight and adiposity (40). What we find interesting is that previous studies with this paradigm of weight regain did not show a decline in volitional wheel running during relapse. Instead, relapse caused a dramatic change in the diurnal pattern of wheel use (21). The reasons for this differential impact on the two approaches to increase physical activity are unclear, but the disconcerting message from both is that the initiation of relapse may have a detrimental effect on the motivation to continue a regular exercise program.

### *Perspectives and Significance*

In summary, regular aerobic treadmill exercise attenuated the metabolic propensity to regain weight after long-term weight loss, and did so by reducing the rate of regain early in relapse and reestablishing a new homeostatic steady state between intake and expenditure at a lower body weight. The implications of our findings are that exercise may be critical to abrogating the overwhelming hunger pains and/or insatiable desire to eat that plagues individuals after calorie-restricted

weight loss. For most individuals, these signals are too strong to ignore, and inevitably lead to the failure to stay on their dietary regimen. Because tissues in the periphery are primed to metabolize and store any caloric excess in a rapid, energetically efficient manner, even minor or temporary excursions off the restricted diet can lead to rapid regain and, potentially, a reduction in the motivation to be physically active. Preventing the relapse-induced increase in adipocytes may limit the capacity to store excess fuels during relapse and could have substantial effects on the rate of regain early in relapse and in the defended body weight at the end of relapse. The new homeostatic steady state achieved after relapse is characterized by an energy balance and fuel utilization profile that is more reflective of lean animals, rather than obese or relapsed SED rats. The fact that regular endurance exercise after calorie-restricted weight loss has such profound effects on energy balance, fuel utilization, lipid accretion, and peripheral homeostatic signals may explain why exercise is so critical to weight regain prevention. Understanding the mechanisms by which exercise reduces that rate of regain early in relapse, as well as how it reestablishes the homeostatic balance that defends a lower weight and adiposity level, is likely to be actively pursued in future studies.

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