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Exercise reduces appetite and traffics excess nutrients away from energetically efficient pathways of lipid deposition during the early stages of weight regain

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Steig AJ, Jackman MR, Giles ED, Higgins JA, Johnson GC, Mahan C, Melanson EL, Wyatt HR, Eckel RH, Hill JO, MacLean PS. Exercise reduces appetite and traffics excess nutrients away from energetically efficient pathways of lipid deposition during the early stages of weight regain. *Am J Physiol Regul Integr Comp Physiol* 301: R656–R667, 2011. First published June 29, 2011; doi:10.1152/ajpregu.00212.2011.—The impact of regular exercise on energy balance, fuel utilization, and nutrient availability, during weight regain was studied in obese rats, which had lost 17% of their weight by a calorie-restricted, low-fat diet. Weight reduced rats were maintained for 6 wk with and without regular treadmill exercise (1 h/day, 6 days/wk, 15 m/min). In vivo tracers and indirect calorimetry were then used in combination to examine nutrient metabolism during weight maintenance (in energy balance) and during the first day of relapse when allowed to eat ad libitum (relapse). An additional group of relapsing, sedentary rats were provided just enough calories to create the same positive energy imbalance as the relapsing, exercised rats. Exercise attenuated the energy imbalance by 50%, reducing appetite and increasing energy requirements. Expenditure increased beyond the energetic cost of the exercise bout, as exercised rats expended more energy to store the same nutrient excess in sedentary rats with the matched energy imbalance. Compared with sedentary rats with the same energy imbalance, exercised rats exhibited the trafficking of dietary fat toward oxidation and away from storage in adipose tissue, as well as a higher net retention of fuel via de novo lipogenesis in adipose tissue. These metabolic changes in relapse were preceded by an increase in the skeletal muscle expression of genes involved in lipid uptake, mobilization, and oxidation. Our observations reveal a favorable shift in fuel utilization with regular exercise that increases the energetic cost of storing excess nutrients during relapse and alterations in circulating nutrients that may affect appetite. The attenuation of the biological drive to regain weight, involving both central and peripheral aspects of energy homeostasis, may explain, in part, the utility of regular exercise in preventing weight regain after weight loss.

energy balance; obesity; weight loss; dietary fat oxidation; de novo lipogenesis

TWO OUT OF EVERY THREE AMERICAN ADULTS are considered to be overweight or obese (60), and at any given time, 46% of women and 33% of men in the United States are trying to lose

weight (3). Unfortunately, less than 20% of those attempting to lose weight are able to achieve and maintain a 10% reduction in body weight after one year (38). The inability to sustain weight loss contributes to this poor therapeutic success rate. Over 35% of the lost weight tends to return within in the first year after a structured weight loss program, and the majority is gained back within 3 to 5 years (2, 81). A number of reasons have been proposed for the high incidence of weight regain (16, 81), and several implicate the biological response to weight loss.

The biological response to dieting involves an integrative set of adaptations in neuroendocrine and metabolic systems involved in energy homeostasis (15, 44, 67). Peripheral signals describing adipose tissue stores send an “energy-depleted” message to neural networks in the brain, which increases the drive to eat and suppresses energy expenditure. The caloric difference between elevated appetite and suppressed energy requirements, or energy gap, necessitates forced or cognitive energy restriction to maintain energy balance at the reduced weight. In rodents, this energy gap can be used to estimate the magnitude of the biological drive for weight regain, as it describes in energetic equivalents the biological pressure to go off a calorie-restricted weight maintenance diet (34, 45, 48). Weight reduction also improves insulin sensitivity and enhances metabolic regulation (12, 26, 52, 64, 73). While beneficial for overall health, this adaptive response prepares the body to clear, metabolize, and store excess energy in an efficient manner (45, 77). The resulting decline in nutrient availability is sensed through peripheral and central systems, which convey a “nutrient-deficient” signal that converges with and reinforces the drive to overeat. When overeating occurs, carbohydrate (CHO) oxidation is enhanced, fat oxidation is suppressed, and dietary fat is trafficked to adipose tissue (34).

Regular exercise is thought to counter a number of these adaptations to weight loss. In rodent postobese models, both volitional wheel running and regimented treadmill exercise during weight maintenance after weight loss reset the homeostatic system to defend a lower body weight (40, 48). Treadmill exercise appears to be more potent, affecting both appetite and expenditure to reduce the energy gap driving weight regain (48). Beyond effects on appetite and expenditure, regular exercise is known to induce a training adaptation in peripheral tissues, enhancing the capacity to oxidize fat and to store

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glycogen (5, 23, 39, 61). This effect on peripheral nutrient metabolism may be important after weight loss for two reasons. First, changing fuel preference may alter the rapidity and energetic efficiency of depositing excess nutrients when overfeeding occurs. Second, it could change the nutrient and neuroendocrine signals that feed back to the neural circuits in the brain that regulate energy balance.

While exercise can increase fat oxidation during and after an exercise bout, the impact on fat balance is negligible over the day when overall energy balance is maintained (54, 55). We have suggested that exercise may increase 24-h fat oxidation during the positive energy imbalance associated with weight regain (48, 54). Under these conditions, the overfeeding-induced suppression of fat oxidation that we have previously shown (34) would be in direct contrast to these putative effects of exercise and may minimize any impact on fuel utilization. To date, no study has shown that regular exercise enhances dietary fat oxidation over the day during weight regain after weight loss or that it affects substrate balance beyond attenuating the energy imbalance.

In the present study, we hypothesized that regular exercise would increase 24-h dietary fat oxidation during relapse from weight loss and would alter substrate balance and the neuroendocrine and nutrient signals in circulation known to regulate energy balance. We tested this hypothesis with a study of energy balance and nutrient metabolism in a rodent paradigm modeling weight regain after long-term weight loss. To distinguish peripheral effects that extend beyond the exercise-induced reduction of the energy gap, we included relapsing, sedentary animals that were provided just enough food, such that their energy imbalance reflected those that exercised regularly ("gap-matched" rats). Our observations provide evidence of both central and peripheral effects of exercise that coordinately work together to attenuate weight regain and highlight the inherent interplay between energy balance and the utilization of ingested energy in the relapse process. The integrated impact of exercise on the regulation of energy balance and fuel utilization may explain why regular exercise has emerged as an important strategy for long-term weight maintenance after weight loss in humans (35, 53, 62).

METHODS

Experimental paradigm of weight regain. Obesity-prone rats ($n = 59$) were selected from a cohort of 170 male Wistar rats (125–150 g, Charles River Laboratories, Wilmington, MA), based upon their propensity to gain weight in response to ad libitum access to a high-fat diet (HFD, 46% kcal fat, Research Diets, New Brunswick, NJ; RD# D12344) (31). For 12 subsequent weeks, the selected rats matured into adult obese rats with free access to this diet. One cohort of obese rats ($n = 13$) was switched to ad libitum feeding of a diet lower in fat (LF, 25% kcal fat, Research Diets, New Brunswick, NJ; RD# 07091301) for the duration of the study (obese, Fig. 1). The remaining rats underwent weight reduction by placing them on restricted provisions of LF equivalent to ~60% of the calories they had eaten ad libitum. This weight loss regimen was maintained for 2 wk (Fig. 1), causing a 14–18% loss in total body mass. The animals were maintained at this reduced weight for 6 wk by providing a limited provision of the LF diet at the beginning of each dark cycle (14:00). Rats were weighed every 3rd day and energy intakes were adjusted accordingly to maintain a stable weight (Fig. 1). During weight maintenance, 16 of the 46 rats were exercised daily during weight maintenance. All rats were individually housed in metabolic cages in the University of Colorado Denver (UCD) Center for Comparative Medicine and the Center for Human Nutrition Satellite Facility (22–24°C; 12:12-h dark-light cycle; dark at 3 PM, light at 3 AM) with free access to water for the entire study. All procedures were approved by the UCD Animal Care and Use Committee.

Study design. The a priori design objective was to begin the study with groups that were similar in body weight and % weight loss, as we have found that these are strong predictors of weight regain. Weight-reduced sedentary and exercising rats were stratified by body weight and then randomly assigned to experimental groups. Before proceeding, we examined % weight loss in the groups to ensure no group differences emerged by random chance. The exercising rats were then divided into two groups (Fig. 1). One was studied under energy balance, weight maintenance conditions (RED-EX, $n = 8$), and the other during the first day of relapse (REL-EX, $n = 8$), on which they were given free access to the LF diet. Sedentary rats were divided into three groups (Fig. 1), two of which were similar to the weight maintenance (RED-SED, $n = 10$) and relapsing (REL-SED, $n = 8$) groups of the exercised animals. The other sedentary group was given a food provision during the tracer experiment, such that it experienced the same positive energy imbalance as the REL-EX rats (gap-matched, REL-GM, $n = 12$). REL-GM rats allowed us to examine the

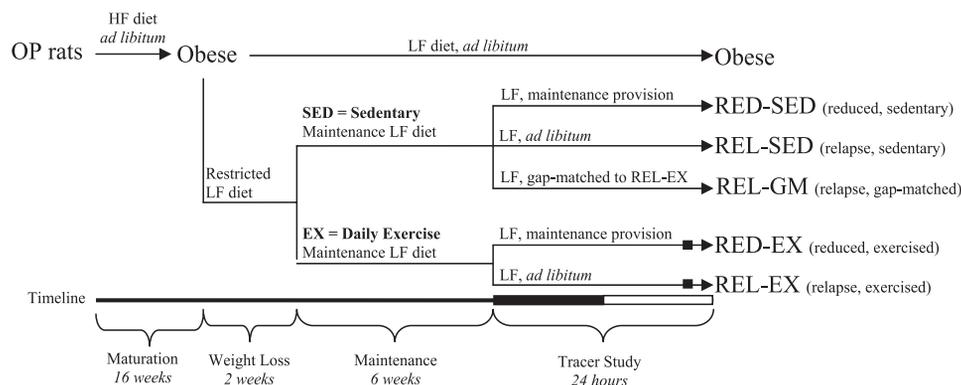


Fig. 1. Experimental groups and tracer study design. Study design generating the groups to look at the effect of exercise [sedentary (SED) vs. exercise (EX)] in weight-reduced (RED) and relapsing (REL) conditions. The REL-GM (relapsing, gap-matched) group was a sedentary group of weight-reduced rats that were given a specific amount of food on the tracer day, so that their positive energy imbalance matched that of the REL-EX group. Rats were placed in the metabolic monitoring system for the final 4 days of the study. The 12:12-h dark-light cycle of the final tracer day is indicated on the timeline (black bar and white bar, respectively). The RED-EX and REL-EX groups performed their regular bout of exercise on the final tracer day (black box), within 3 h of the end of their final light cycle.

effects of exercise on peripheral fuel metabolism, given the same caloric excess as the relapsing, exercised animal.

Exercise regimen. RED-EX and REL-EX rats exercised on a three-lane Exer-6M Treadmill (Columbus Instruments, Columbus, OH) during weight maintenance. Rats were acclimated to the treadmill by ramping the exercise intensity from 5 to 15 m/min during the 1st week of training and then increasing the exercise duration from 10 to 60 min during the 2nd week. For the remainder of the study, rats performed regular exercise bouts for 1 h a day, 6x days a week, at an intensity of 15 m/min (48). The exercise bout was performed around the same time of day (between 12:00 PM and 3:00 PM), within the 3 h prior to the start of the dark cycle. Rats were encouraged to perform the daily exercise bouts using one or more of the following methods: 1) placing food pellets or dangling a novel play item at the head of the treadmill so it was just out of reach of the animal; 2) providing a short shock from an electric grid at the rear of the treadmill (10 V, 0.5 A, 0.75 Hz); 3) applying a bristle brush to the animal's feet on the rear treadmill grid; or 4) providing intermittent air puffs to the hind-quarters. The type and combination of motivation used varied depending on the rat's response to the different motivational strategies. The bout was also performed at the regularly scheduled time on the tracer day.

Energy balance and fuel utilization. Rats were placed in a metabolic monitoring system for the final 4 days of the study (Oxymax CLAMS-8M; Columbus Instruments), modified for performing in vivo tracer studies in combination with indirect calorimetry (34). Total, ambulatory, and nonambulatory activity was measured with infrared beam-break sensors. Metabolic rate (MR) was measured every 16 min and calculated by using the Weir equation ($MR = 3.941 \cdot \dot{V}O_2 + 1.106 \cdot \dot{V}CO_2 - 2.17 \cdot N$), where N is urinary nitrogen. MR averaged over the day was then extrapolated throughout the 24-h testing period to acquire estimates of total energy expenditure (TEE), and component analysis of TEE was used to acquire estimates of nonresting (NREE) and resting (REE) energy expenditure, described previously (46). For relapsing animals, an average of the previous day (while in energy balance) and the tracer day (positive energy imbalance) resting MR was used for the component analysis. Respiratory exchange ratio (RER, $\dot{V}CO_2/\dot{V}O_2$) was acquired from the gas exchange data via indirect calorimetry, and whole body fuel utilization was calculated from $\dot{V}O_2$, $\dot{V}CO_2$, and from measurements of urinary nitrogen, using the following equations (17): CHO disappearance = $(4.57 \cdot \dot{V}CO_2) - (3.23 \cdot \dot{V}O_2) - (2.6 \cdot N)$, lipid disappearance = $(1.69 \cdot \dot{V}O_2) - (1.69 \cdot \dot{V}CO_2) - (2.03 \cdot N)$, and protein disappearance = $6.25 \cdot N$.

In vivo, 24-h dual-tracer study. A dual-tracer study was performed, with a design similar to our previous work in sedentary relapsing rats (34), to assess dietary fat oxidation and the net retention of dietary fat and de novo derived lipid in tissues over 24 h. The rats were acclimated to the metabolic chambers for 2 days, and then spent another full day in the chambers for lead-in measurement of energy balance and fuel utilization, with the same treatments experienced throughout the weight maintenance period. Energy expenditure data from this weight maintenance lead-in day were used to determine the food provision for RED and REL-GM rats. A 250 μ Ci intraperitoneal injection of 3H_2O was given 2 h prior to start of the final dark cycle (1 PM). Previous studies have shown that this tracer equilibrates with total body water within 2 h and remains relatively steady over the next 24-h period (11). Subsequent incorporation of tritium into lipid pools provided an estimate of the net retention of carbons via lipogenesis, the vast majority of which is de novo derived.

To reflect the dietary fat within the LF diet, 1-[^{14}C]palmitate and 1-[^{14}C]oleate tracers were blended into the diet (1:3 ratio, reflecting their ratio in the diet) at a specific activity of 0.8 μ Ci/kcal of dietary fat. The labeled diet was given at the start of the final dark cycle (3:00 PM). Although different groups were provided different amounts of food, and thus different amounts of the ^{14}C tracer, the specific activity of the diet was constant. Therefore, the absolute amount of tracer

consumed reflected the amount of dietary lipid ingested over the subsequent 24-h period. Energy intake (EI) was assessed every 3 h, while $\dot{V}O_2$, $\dot{V}CO_2$, and activity were monitored every 16 min by indirect calorimetry. Cumulative estimates of EI were adjusted for spillage at each 3-h interval. Total spillage was weighed for the dark cycle (hours 0–12) and light cycle (hours 12–24) of the tracer study, and portions of this weight were assigned to each 3-h interval during the preceding 12 h based upon visual estimates of cumulative spillage at each time point. Spillage over the 24-h period was less than 7% of the total EI with the vast majority arising during the dark cycle (hours 0–12), when the majority of ingested energy was consumed. The chamber flow rate was rechecked after each EI measurement, and gas exchange and activity data were only included in the analysis after the chamber had once again equilibrated. Every 3 h, effluent CO_2 from each chamber was collected over timed intervals (~3 min). The CO_2 was collected in 3.0-ml aliquots of a 2:1 mixture of methanol and methylbenzethonium hydroxide (Sigma Chemical #B2156). The ^{14}C content of these samples was then measured with a Beckman LS6500 scintillation counter.

At the end of the 24-h tracer study, rats were anesthetized with isoflurane. A sample of blood was obtained from the vena cava for tracer measurements, and tissues were collected for the determination of ^{14}C and 3H retention in the lipid fraction of gastrocnemius muscle, liver, and retroperitoneal (RP) adipose tissue (34). Dietary fat absorption was assessed by subtracting the tracer content remaining in the gastrointestinal tract from the amount ingested (46). Serum and urine tracer content was determined in both fresh (wet) and dehydrated samples. Total serum tracer content was calculated as the measured ^{14}C activity/ml of serum $\times 0.0385 \times$ body mass (grams) (6). The specific activity of the diet was used to convert the ^{14}C measurements into kilocalories of dietary fat oxidized or retained in lipid fractions of tissues. Tritium counts were expressed in units of radioactivity (μ Ci or nCi).

Exercise energetics. The energetics of the tracer day exercise bout were assessed using an indirect calorimetry treadmill chamber (Columbus Instruments), as we have previously described (48). 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. Steady-state measurements were extrapolated to estimate the energetic cost of the hour-long bout. Steady-state nonprotein RER (NP-RER) was used to estimate CHO and fat oxidation rates during the bout (48). On the tracer day, a sample of the chamber exhaust flow was collected, and the trapped CO_2 was used to estimate dietary fat oxidation during exercise.

Body composition analysis. Body composition was measured 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. Corrected fat mass and fat-free mass (FFM) were calculated from DXA data and carcass weights (18). All weight-reduced animals were scanned within a week of entering the metabolic chambers for the end of study tracer experiment.

Skeletal muscle gene expression. Total RNA was extracted from powdered gastrocnemius tissue collected at the end of the 24-h tracer study using the EaZy nucleic acid isolation kit (Omega Bio-Tek, Norcross, GA). Quantification was determined with a Nanodrop 1000 Spectrophotometer (Thermo Scientific) system software version 3.2.1. RNA integrity was verified with an Agilent 2100 Bioanalyzer (Agilent Technologies, CA). Relative transcript levels were detected by real-time RT-PCR using SYBR Green technology. Reverse transcription was performed using 1 μ g total RNA with iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). Quantitative PCR was performed using primer sets for genes of interest and a reference gene and iQSupermix or iQ SYBR Supermix (Bio-Rad) following manufacturer's protocols. Reactions were run in duplicate on an iQ5 real-time PCR detection system (Bio-Rad) along with a no-template control per gene. The cycling conditions consisted of 3-min initial denaturation at 95°C and 45 cycles of comparative threshold cycle method. Validation experi-

ments were performed to demonstrate that efficiencies of target and reference genes were approximately equal. Data were normalized to ubiquitin C mRNA.

Metabolite and endocrine analyses. The plasma was assayed for endocrine factors with the Rat Endocrine Lincoplex Kit (RENDO-85K; Linco Research/Millipore, St. Charles, MO). Plasma glucose, free fatty acids (FFA), total cholesterol, and triglycerides (TG) were measured via colorimetric analysis (49). Urinary creatinine, urea, and corticosterone were measured by colorimetric (Thermoelectron, Melbourne, Australia) and enzyme immunoassays (Diagnostic Systems Laboratories, Webster, TX). Glycogen content in muscle and liver was determined using the method described by Chan and Exton (8).

Statistical analysis. Data were analyzed using SPSS software version 18.0 by ANOVA with the planned comparisons examining whether exercise alters energy homeostasis in weight-reduced animals (RED-SED vs. RED-EX), during the first day of relapse (REL-SED vs. REL-EX), and during relapse if the energy imbalance is controlled (REL-EX vs. REL-GM). In some instances, ANCOVA was used to examine differences after adjusting for the variation imposed by specified parameters. Duncan's post-hoc analysis for homogeneous groups was used to present homogeneous groups within the entire data set in tables and figures, unless otherwise specified. It should be noted that this general comparison applied to the entire data set does not control for the possibility of type I errors resulting from multiple comparisons. Statistical significance was assumed when $P < 0.05$.

RESULTS

Body weight and composition. The development of obesity in obesity-prone rats reflected the body weight and composition characteristics that we have published extensively elsewhere (34, 45–48). Calorie restriction resulted in a body weight loss of $16.5 \pm 0.4\%$, or 103 ± 5 g. At the time of the tracer study, obese rats weighed 677 ± 14 g and had a body fat of $38.4 \pm 1.2\%$. Sedentary and exercised weight-reduced rats did not differ in body weight (518 ± 6 vs. 508 ± 9 g), FFM (351 ± 7 vs. 341 ± 6 g), fat mass (167 ± 4 vs. 168 ± 7 g), or % body fat (32.1 ± 0.6 vs. $32.8 \pm 1.1\%$) at the time of the tracer study.

Energy balance and gap-matching. EI, TEE, and energy balance during the 24-h tracer study are shown in Fig. 2. RED-SED and RED-EX rats were given a provision of the low-fat (LF) diet to maintain energy balance. REL-SED and REL-EX rats were allowed free access to the LF diet, and REL-EX consumed 28% less food (Fig. 2A, $P < 0.001$). TEE was higher in exercised rats during weight maintenance (RED-SED vs. RED-EX, $P < 0.05$) but was similar for REL-SED vs. REL-EX rats on the first day of relapse. On the basis of their lead-in TEE, REL-GM was given a provision of food that resulted in a positive energy imbalance similar to that found in REL-EX rats (Fig. 2B). Because TEE was lower in REL-GM rats than REL-EX rats ($P < 0.001$), the food provision tended to be slightly lower in REL-GM rats, but this was not statistically different (Fig. 2A).

Feeding patterns during the tracer study are shown in Fig. 2C. All weight-reduced animals finished the restricted food provision within the first 6 h of the 24-h testing day, but consumption was delayed in exercised animals (RED-EX vs. RED-SED, $P < 0.001$). The exercise-associated attenuation of EI in relapsing animals was readily apparent at 3 h and primarily occurred during the first 9 h of the tracer study. The REL-GM feeding pattern was similar to that of REL-SED rats until all of their food was gone (between 6 and 9 h).

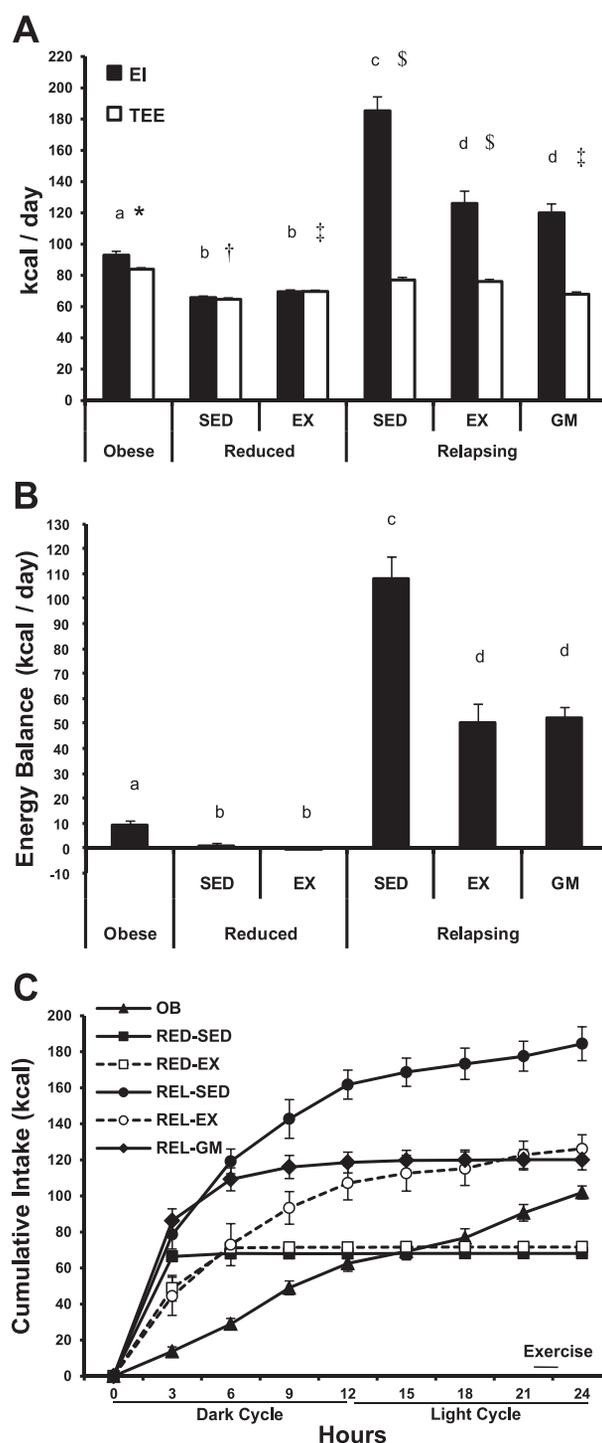


Fig. 2. Energy balance and pattern of food intake. A: energy intake (EI) and total energy expenditure (TEE), as measured by indirect calorimetry, is shown. Obese and relapsing SED and EX rats ate ad libitum. Reduced rats were given an energy balance provision of the diet. Relapsing gap-matched (REL-GM) were given just enough food to match the energy imbalance of the relapsing EX group. Groups designated by the same letter (a,b,c,d for EI) or symbol (*, †, ‡, § for TEE) are not significantly different from one another ($P < 0.05$). B: energy balance (EI-TEE) of the groups is shown. a,b,c,d Groups designated by the same letter (for EI) are not significantly different from one another ($P < 0.05$). C: cumulative food intake, measured every 3 h, is presented for the six groups of rats. In a repeated-measure analysis, exercise significantly delayed consumption of the food provision during weight maintenance (hour 3, $P < 0.05$) and attenuated ad libitum consumption during the first 9 h of relapse ($P < 0.001$). The dark and light cycles are shown, as is the timing of the final exercise bout.

Table 1. Component analysis of energy expenditure

		Reduced			Relapsing		
		Obese	SED	EX	SED	EX	GM
measured days (<i>n</i>)		39	57	23	14	8	12
NREE	unadjusted	26.8 ± 0.5 ^{a,b}	20.9 ± 0.4 ^c	25.4 ± 0.5 ^b	28.5 ± 0.1.7 ^{b,d}	30.8 ± 1.1 ^d	24.2 ± 0.9 ^b
	adjusted*	27.0 ± 0.5 ^{a,b}	21.2 ± 0.4 ^c	25.7 ± 0.6 ^b	27.2 ± 1.0 ^{a,b}	29.8 ± 1.1 ^a	23.4 ± 0.9 ^{b,c}
	EAT	0	0	3.1 ± 0.1	0	3.5 ± 0.3	0
Activity	Total	13.9 ± 0.3 ^{a,b}	13.1 ± 0.4 ^a	13.3 ± 0.5 ^a	17.9 ± 0.8 ^c	17.1 ± 0.8 ^c	16.3 ± 0.7 ^{b,c}
	Ambulatory	6.1 ± 0.2 ^a	5.8 ± 0.2 ^a	5.9 ± 0.3 ^a	9.4 ± 0.5 ^b	8.8 ± 0.5 ^b	8.4 ± 0.5 ^b
REE	unadjusted	58.6 ± 0.8 ^a	43.4 ± 0.8 ^b	44.4 ± 0.7 ^b	48.6 ± 1.4 ^c	45.2 ± 0.9 ^{b,c}	44.2 ± 1.4 ^b
	adjusted**	57.6 ± 1.3 ^a	46.1 ± 1.1 ^b	46.9 ± 0.7 ^b	48.9 ± 1.2 ^b	46.7 ± 1.5 ^b	44.9 ± 1.1 ^b
PAL		1.47 ± 0.01 ^a	1.49 ± 0.01 ^a	1.57 ± 0.01 ^b	1.56 ± 0.03 ^a	1.65 ± 0.02 ^b	1.53 ± 0.02 ^a

n represents the number of measured days, and includes several lead-in days prior to the tracer for obese and weight reduced groups. Nonresting energy expenditure (NREE), resting energy expenditure (REE), and exercise activity thermogenesis (EAT) are expressed as kilocalories per day. Components of activity are expressed as counts × 10³/day. Physical activity level (PAL) is calculated as total energy expenditure (TEE)/REE. *ANCOVA, adjusted for ambulatory activity (*P* = 0.011). **ANCOVA, adjusted for fat-free mass (FFM) (*P* = 0.009). ^{a,b,c,d}Groups with the same letter designation are not significantly different (*P* < 0.05). SED, sedentary; EX, exercise; GM, gap-matched.

Component analysis of TEE is shown in Table 1. The exercise bout increased TEE by ~5%, resulting in a higher NREE for the weight-reduced rats (RED-EX vs. RED-SED, *P* < 0.05). In relapsing rats, NREE was higher in REL-EX rats than in REL-GM rats (*P* < 0.001), but it was not different from REL-SED rats that ate substantially more food. This effect of exercise on NREE remained significant after adjusting for ambulatory activity, which was also a significant predictor of NREE. While relapsing rats were generally more active than weight-reduced rats, exercise did not affect spontaneous activity in either metabolic state. The energetic cost of the exercise bout (~3 kcal) did not account for the ~8-kcal difference in TEE between REL-EX and REL-GM rats. Taken together, these data show that regular exercise reduces the energy gap after weight loss, both by reducing appetite and by increasing expended energy beyond the cost of the exercise bout. The lower energy gap resulted in an attenuated positive energy imbalance during the first day of relapse (Fig. 2B).

Whole body fuel utilization. Whole body substrate disappearance in obese and weight-reduced rats is shown in Fig. 3A. When in energy balance, substrate disappearance provides a reasonable estimate of whole body substrate oxidation. Accompanying the higher TEE was a modest increase in both CHO and lipid disappearance over the day in RED-EX vs. RED-SED rats. All groups exhibited a diurnal fluctuation in CHO disappearance, reflecting a higher level of CHO oxidation during the dark cycle (when they ate and were awake) than in the light cycle (when they were generally asleep) (*P* < 0.001). While lipid disappearance remained relatively constant over the diurnal cycle in obese rats, it was suppressed during the dark cycle and enhanced during the light cycle for both RED-SED and RED-EX groups (*P* < 0.001). During the dark cycle, CHO disappearance was higher in RED-EX than in RED-SED. During the light cycle, lipid disappearance was higher in RED-EX rats than in RED-SED rats (*P* < 0.01). Exercise did not affect protein disappearance in the weight reduced or the relapsing condition (Fig. 3A).

These data indicate that while in weight maintenance, exercise increases both CHO and fat oxidation over the entire day. This has little effect on 24-h substrate balance but allows RED-EX rats to eat more than RED-SED rats in an amount that extends above the actual cost of the exercise bout.

Dietary fat oxidation. In weight-reduced rats, exercise increased the oxidation of dietary fat during the light cycle (Fig. 3B, *P* < 0.01), which increased total dietary fat oxidation over the day (*P* < 0.01). This difference in 24-h fat oxidation remained significant when adjusted for fat-free mass (Fig. 3C). This increase in dietary fat oxidation can account for the majority of the increase in whole body fat oxidation observed with exercise, while in energy balance (Fig. 3A).

Regardless of being sedentary or exercised, relapsing animals consumed substantially more dietary fat, but oxidized less in absolute terms (Fig. 3B, *P* < 0.001) and as a percentage of total ingested (data not shown). However, exercise attenuated this relapse-induced suppression of dietary fat oxidation in both the dark and light cycle (*P* < 0.01). REL-GM rats exhibited lower levels of dietary fat oxidation than REL-EX rats, even though they experienced the same energy imbalance. Approximately 48% of the difference in dietary fat oxidation between REL-SED and REL-EX rats could be explained by the attenuated energy imbalance, while the remainder can be attributed to metabolic regulation in the periphery (Fig. 3C). These data indicated that the exercise-induced increase in dietary fat oxidation persists during the early stages of weight regain and that both the attenuated energy imbalance and adaptations in peripheral tissues contribute to this shift in fuel utilization.

Nutrient retention in peripheral lipid pools. The net retention of dietary fat and de novo derived fat in lipid extracts from muscle, liver, and retroperitoneal adipose tissue are shown in Table 2 and Fig. 4. In weight-reduced rats, exercise did not significantly affect the retention of dietary fat in these lipid depots, although RED-EX rats tended to have lower levels in adipose tissue (Fig. 4A, *P* = 0.09). REL-EX rats exhibited the same retention of dietary fat in muscle and hepatic lipid pools as REL-SED rats (Table 2), but they had a 30% lower retention of dietary fat in adipose tissue (Fig. 4A, *P* < 0.05). The REL-GM rats, given the same energy excess, retained less in muscle (*P* < 0.01) and tended to retain less in hepatic lipid pools (*P* = 0.08), compared with REL-EX rats. At the same time, more dietary fat was trafficked to adipose tissue in REL-GM rats than in REL-EX rats (Fig. 4A).

Exercise did not significantly affect the net retention of de novo derived lipid in the muscle or liver of weight-reduced rats

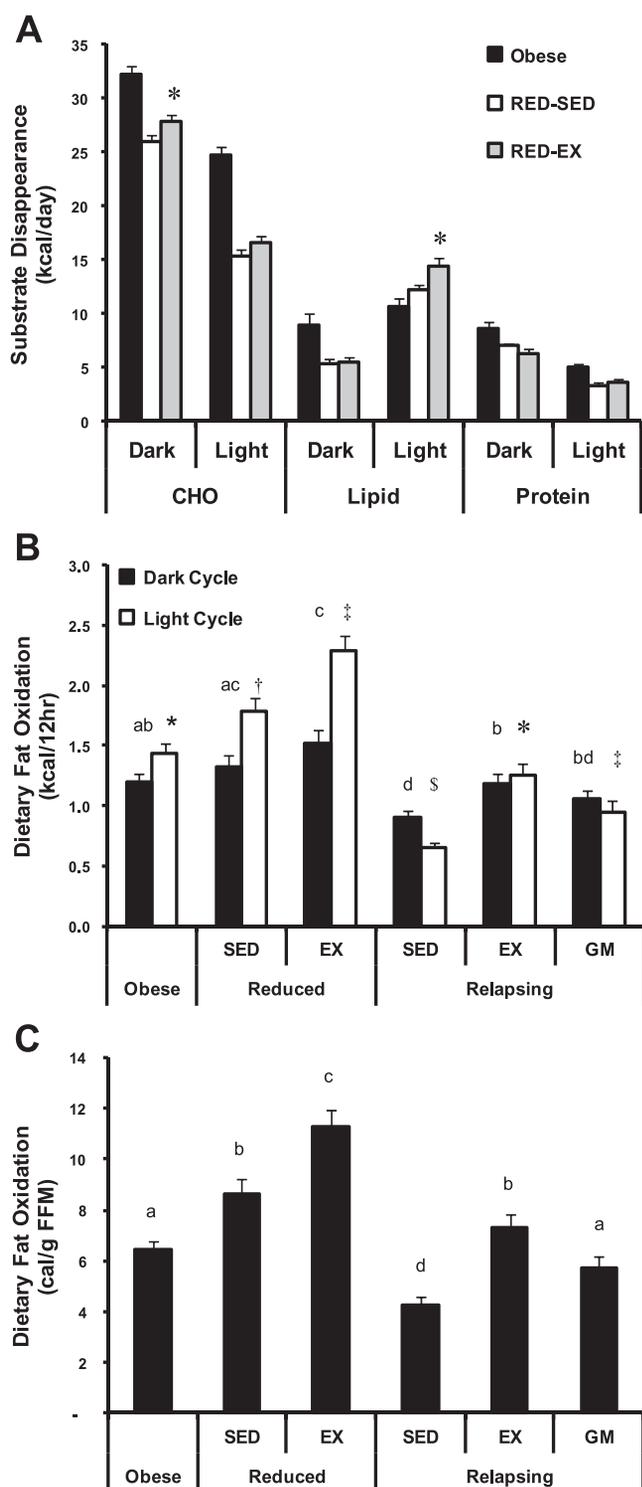


Fig. 3. Whole body and dietary fat oxidation. A: substrate disappearance for carbohydrate (CHO), lipid, and protein, assessed during the dark and light cycles, is shown for obese and weight-reduced rats in energy balance. Significant difference between RED-SED and RED-EX is shown ($*P < 0.05$). B: diurnal dietary fat oxidation is shown for the six groups. Groups designated by the same letter (a,b,c,d for dark cycle) or symbol (*, †, ‡, \$ for light cycle) are not significantly different from one another ($P < 0.05$). C: 24-h fat oxidation, normalized to lean body mass is shown. FFM, fat-free mass. ^{a,b,c,d}Groups with the same letter designation are not significantly different ($P < 0.05$).

(Table 2), but retention tended to be higher in the RP adipose lipid in RED-EX than in RED-SED (Fig. 4B, $P = 0.07$). In relapsing rats, hepatic retention of de novo derived lipid was similar for REL-SED and REL-EX rats but was significantly lower in REL-GM rats (Table 2). Retention in adipose tissue was 38% lower in REL-EX rats than in REL-SED rats, and REL-EX rats had over twice as much de novo lipid in adipose tissue than the REL-GM rats that experienced the same energy imbalance (Fig. 4B).

Summary of tracer and energy balance data. Taken together, exercise-reduced the energy imbalance on the first day of relapse by 50% and enhanced dietary and whole body fat oxidation and trafficked excess dietary fat to short-term lipid storage depots (muscle, liver) rather than to long-term storage in adipose tissue. At the same time, exercise increased the trafficking of other nutrients (CHO and protein) to long-term storage depots (adipose tissue) via de novo lipogenesis. This shift in nutrient trafficking is more energetically expensive and resulted in REL-EX rats expending ~ 5 more kcal over the day than REL-GM rats to deposit the same amount of excess energy.

Skeletal muscle gene expression. Because exercise induced a change in dietary fat oxidation beyond its impact on the energy balance, we investigated the exercise-related adaptive response to weight reduction in skeletal muscle metabolic gene expression. Skeletal muscle peroxisome proliferator-activated receptor- γ coactivator 1 alpha (PGC1 α) mRNA was elevated with exercise during weight maintenance and after the first day of relapse (Fig. 5A). This exercise-induced elevation in PGC1 α mRNA was still apparent when REL-EX rats were compared with REL-GM rats. Peroxisome proliferator-activated receptor- δ , sirtuin 1, and adiponectin receptor 2 (AdipoR2) mRNA were also elevated with exercise during weight maintenance, but these differences were eliminated after a day of relapse.

The expression of genes involved in lipid uptake, mobilization, and transport, is shown in Fig. 5B. Lipoprotein lipase (LPL), hormone-sensitive lipase (HSL), and CD36 mRNA levels were upregulated by exercise during weight maintenance. This effect of exercise was no longer apparent after the first day of relapse. Genes related to fuel preference are shown in Fig. 5C. Hexokinase II (HKII) mRNA levels were elevated with exercise only during weight maintenance. The expression of pyruvate dehydrogenase kinase 4 (PDK4) was increased almost three-fold with regular exercise in the weight-reduced state. While 1 day of relapse led to a dramatic reduction in PDK4 mRNA levels, they remained higher in REL-EX than in REL-SED rats. This effect during relapse appeared to be related to the magnitude of the energy imbalance, as PDK4 levels were similar for REL-EX and REL-GM rats.

Measured genes related to mitochondrial function are shown in Fig. 5D. β -hydroxyacyl dehydrogenase (β -HAD) mRNA levels were elevated with exercise during weight maintenance, but not after the day of relapse. During relapse, REL-GM rats had a higher level of carnitine palmitoyl transferase I and β -HAD mRNA levels than REL-EX or REL-SED rats. Citrate synthase activity was significantly higher with exercise in both weight-reduced and relapsing animals, confirming the well-established adaptive response in oxidative capacity with exercise training reflected in the expression of metabolic genes (data not shown).

Table 2. Nutrient retention in lipid pools

	Obese	Reduced			Relapsing	
		SED	EX	SED	EX	GM
Tissue weights						
Gastrocnemius	6.87 ± 0.16	6.22 ± 0.18	6.46 ± 0.24	6.46 ± 0.12	6.40 ± 0.19	6.44 ± 0.20
Liver	19.7 ± 0.4 ^a	12.3 ± 0.4 ^b	12.2 ± 0.7 ^b	21.0 ± 0.6 ^a	15.0 ± 0.3 ^c	16.0 ± 0.5 ^c
RP Adipose	24.1 ± 1.3 ^a	9.1 ± 1.2 ^b	8.1 ± 1.6 ^b	11.1 ± 1.0 ^b	9.9 ± 0.9 ^b	8.5 ± 0.9 ^b
Dietary fat retention						
Gastrocnemius	2.57 ± 0.45 ^{a,b}	2.04 ± 0.20 ^a	1.95 ± 0.19 ^a	3.37 ± 0.26 ^{b,c}	3.59 ± 0.25 ^c	2.67 ± 0.25 ^a
Liver	61.1 ± 9.1 ^{a,b}	36.7 ± 3.1 ^c	34.5 ± 2.3 ^c	71.5 ± 4.2 ^a	64.9 ± 5.0 ^{a,b}	52.6 ± 4.5 ^b
De novo fat retention						
Gastrocnemius	0.16 ± 0.02	0.19 ± 0.03	0.14 ± 0.02	0.18 ± 0.03	0.24 ± 0.03	0.20 ± 0.02
Liver	7.5 ± 1.3 ^{a,b}	4.6 ± 0.8 ^{b,c}	2.8 ± 0.2 ^c	7.8 ± 0.5 ^a	7.3 ± 0.7 ^a	4.3 ± 0.5 ^{b,c}

Tissue weights expressed in grams. Dietary fat retention is expressed in lipid calories per gram of tissue. De novo fat retention is expressed in lipid nanocalories per gram tissue. ^{a,b,c}Groups with the same letter designation are not significantly different ($P < 0.05$). RP, retroperitoneal.

Endocrine factors and metabolites. Metabolites and endocrine factors were measured in blood samples collected at the end of the 24-h tracer study. The obese group had significantly higher insulin, leptin, and cholesterol levels than the weight-

reduced and relapsing animals (Table 3). In the weight-reduced state, RED-EX rats had higher glucose levels than RED-SED rats, but the rest of the parameters measured were not different between the two groups. After the first day of relapse, REL-EX rats had lower TGs and higher nonesterified fatty acids (NEFAs) than REL-SED rats. The effect on TGs was recapitulated in REL-GM rats, suggesting that this effect was related to the exercise-induced attenuation in the energy imbalance. REL-GM rats had lower levels of NEFAs than REL-EX rats, reflecting those of REL-SED rats. This observation implies that the effects of exercise on circulating NEFAs during relapse cannot simply be explained by the exercise-induced attenuation of the energy imbalance. Among the weight-reduced rats, urinary corticosterone levels did not differ between groups (data not shown), indicating that this exercise program was not accompanied by a stress-related elevation in this parameter at the end of weight maintenance.

DISCUSSION

This is the first study to show that regular exercise increases 24-h dietary fat oxidation during a positive energy imbalance after weight loss and that this shift in fuel utilization impacts the energy imbalance associated with the relapse to obesity. Exercise reduced the energy gap after weight loss, not only by reducing appetite, but also by increasing energy expenditure with the cost of the exercise bout and the reduced efficiency of depositing excess energy. By enhancing the oxidation of dietary fat during overfeeding, more of the excess energy was trafficked to storage depots via de novo lipogenesis. The enhanced oxidative capacity and increased expression of genes in skeletal muscle favoring the uptake, mobilization, and oxidation of fat are likely to have contributed to this preferential utilization of ingested fuels early in relapse. While the present study cannot identify the specific mechanisms that mediate the exercise-induced suppression of appetite, the differential meal patterns of sedentary and exercised animals in Fig. 2C would suggest that relevant signals from the periphery and/or the neural responses in the brain may be found in the first 9 h of relapse in this model. We would hypothesize that the impact of exercise on peripheral metabolism and nutrient availability may contribute to this signal in some way. Regardless, the favorable effects of exercise on energy balance, fuel utilization,

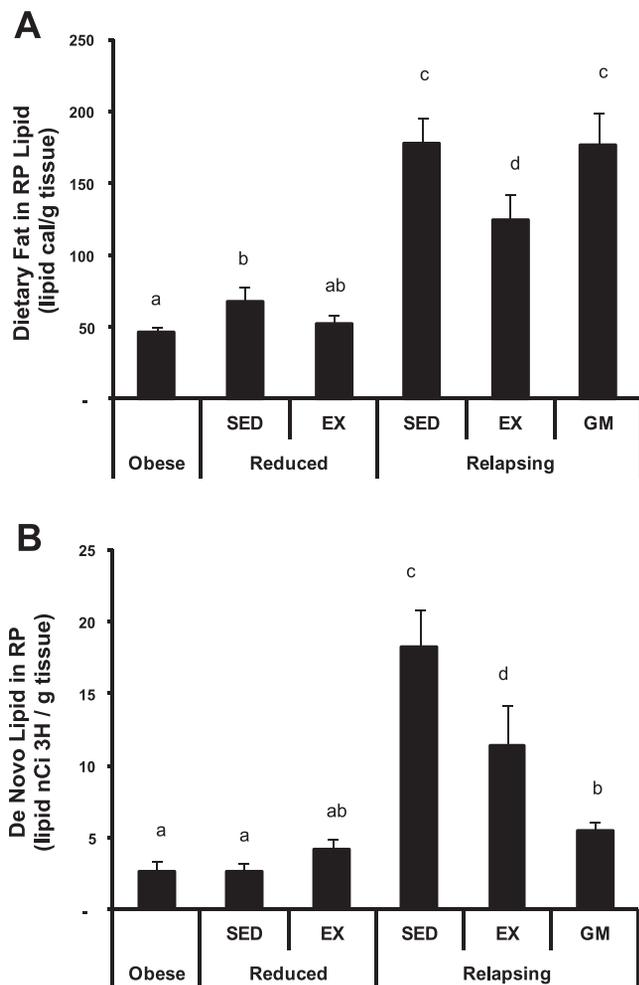


Fig. 4. Net retention of dietary fat and de novo-derived lipid in retroperitoneal adipose tissue. The retention of dietary fat (A; measured with a ¹⁴C-free fatty acid dietary tracer) and de novo-derived lipid measured by the incorporation and retention (B) of ³H into tissue lipid is shown. RP, retroperitoneal. ^{a,b,c,d}Groups with the same letter designation are not significantly different ($P < 0.05$).

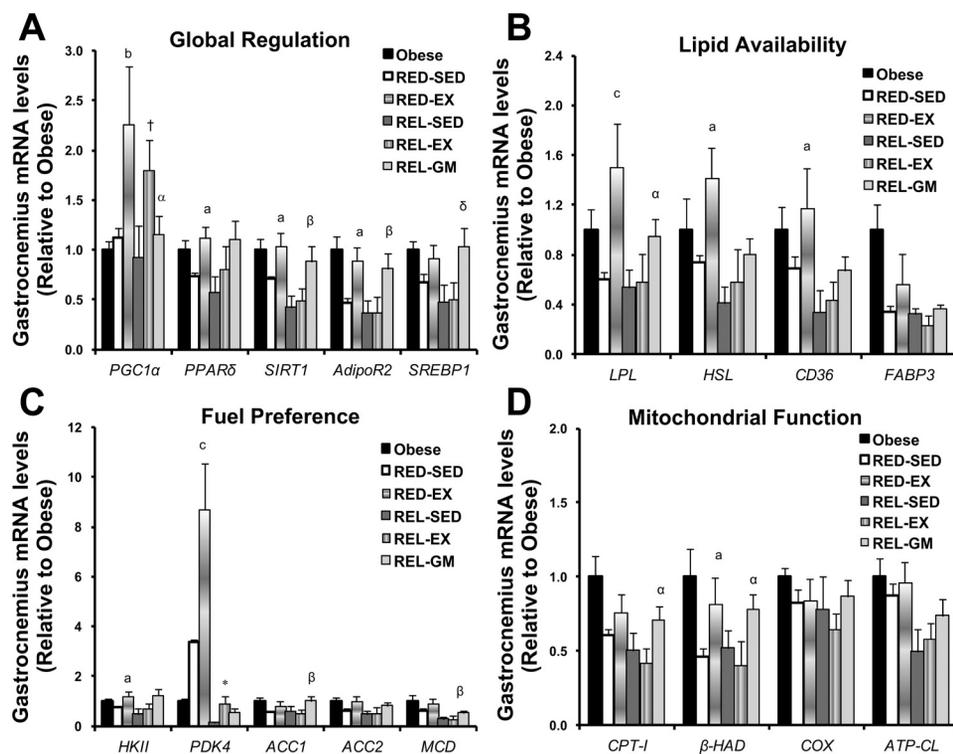


Fig. 5. Skeletal muscle gene expression. The expression of genes in the gastrocnemius known to be involved in global metabolic regulation [peroxisome proliferator-activated receptor- γ coactivator 1 α (PGC1 α), peroxisome proliferator-activated receptor- δ (PPAR δ), sirtuin 1 (SIRT1), adiponectin receptor 2 (AdipoR2), and sterol response element binding protein 1 (SREBP1)] (A), lipid availability (B) [lipoprotein lipase (LPL), hormone sensitive lipase (HSL), CD36, and fatty acid binding protein 3 (FABP3)], fuel preference (C) [hexokinase II (HKII), pyruvate dehydrogenase kinase 4 (PDK4), acetyl co-A carboxylase 1 (ACC1), and 2 (ACC2), malonyl-CoA decarboxylase (MCD)], and mitochondrial function (D) [carnitine palmitoyl transferase I (CPT-1), β -hydroxyacyl dehydrogenase (β -HAD), cytochrome-c oxidase (COX), ATP citrate lyase (ATP-CL)], is shown for the six groups. Data were normalized to ubiquitin C mRNA, and results from the planned comparisons examining the effect of exercise are shown. An effect in weight-reduced rats indicated by lower case letters (RED-SED vs. RED-EX; $^{\alpha}P < 0.05$; $^{\beta}P < 0.01$; $^{\gamma}P < 0.001$). An effect in relapsing rats is indicated by symbols (REL-SED vs. REL-EX; $^*P < 0.05$; $^{\dagger}P < 0.01$). An effect in relapse, when the energy imbalance is controlled is indicated by Greek letters ($^{\alpha}P < 0.05$; $^{\beta}P < 0.01$; $^{\gamma}P < 0.001$).

and nutrient availability, may facilitate long-term weight reduction after calorie-restricted weight loss.

The observations in this study exhibit the inherent interdependence between energy balance and fuel utilization in energy homeostasis (Fig. 6). The transition to relapse increases the amount of nutrients that need to be absorbed, metabolized, and stored, all of which require energy (56, 80). The increase in the thermic effect of food (TEF) elevates the total amount of energy expended throughout the day. Consistent with our previous reports in this model, the transition to relapse in sedentary animals results in suppressed fat oxidation, enhanced oxidation of ingested CHO, and the trafficking of dietary fat to adipose tissue, with recruitment of de novo pathways on the first day of relapse occurring during the latter part of the light cycle (*hours 12–24*) (34, 48). The timing of this adaptive response presumably serves the purpose of depositing CHO and protein into lipid pools once glycogen stores have been restored and energy needs have been met. The preferential storage of dietary fat and utilization of CHO for energy

production is the most efficient way to restore depleted energy reserves. The energetic expense of depositing dietary fat is minimal ($<2\%$ of the nutrient excess), but is more substantial ($\sim 25\%$ of the nutrient excess) for glucose and protein that must be converted to fat through de novo lipogenesis. As such, REL-EX rats expend more energy over the day to store the same excess as REL-GM rats and expend about the same energy over the day to store less than half of the extra energy consumed by REL-SED rats.

While the increase in the energy expenditure above the energetic cost of the exercise bout was relatively modest (~ 5 kcal), this amount of energy, if directed in total toward de novo lipogenesis, could have been responsible for depositing as much as 30% (~ 16 kcal) of the ~ 50 kcal excess. One limitation of this study, however, is that our component analysis of TEE in relapsing rats cannot definitively distinguish between the increase in TEF and the facultative thermogenesis related to the overfeeding-induced sympathetic tone (74). Exercise potentiates the sympathetic response to a meal after diet-induced weight loss (13), and

Table 3. Endocrine factors and metabolites

	Obese	Reduced		Relapsing		
		SED	EX	SED	EX	GM
<i>n</i>	8	9	8	8	8	11
Insulin, ng/ml	150 \pm 19 ^a	67 \pm 10 ^b	52 \pm 7 ^b	105 \pm 8 ^c	92 \pm 8 ^{c,d}	68 \pm 9 ^{b,d}
Leptin, ng/ml	906 \pm 104 ^a	296 \pm 43 ^b	308 \pm 51 ^b	475 \pm 28 ^c	436 \pm 47 ^{b,c}	447 \pm 60 ^{b,c}
Amylin, ng/ml	27.3 \pm 2.7 ^a	16.8 \pm 3.8 ^b	13.5 \pm 2.7 ^b	20.2 \pm 1.9 ^b	16.0 \pm 1.8 ^b	16.3 \pm 2.1 ^b
Glucagon, ng/ml	131 \pm 20	110 \pm 15	92 \pm 12	131 \pm 32	88 \pm 13	102 \pm 14
Glucose, mM	10.8 \pm 0.5 ^{a,b}	9.0 \pm 1.0 ^a	11.1 \pm 0.8 ^{b,c}	12.3 \pm 0.7 ^{b,c}	12.9 \pm 0.4 ^c	10.5 \pm 0.8 ^b
Triglycerides, mg/dl	146 \pm 16 ^{a,b}	28 \pm 7 ^c	34 \pm 3 ^c	186 \pm 20 ^b	102 \pm 16 ^d	107 \pm 13 ^d
NEFAs, nM	462 \pm 61 ^a	646 \pm 48 ^b	600 \pm 75 ^{a,b}	267 \pm 21 ^c	513 \pm 75 ^{a,b}	295 \pm 46 ^c
Cholesterol, mg/dl	87.9 \pm 10.0 ^a	59.3 \pm 7.7 ^b	49.0 \pm 6.0 ^b	50.4 \pm 4.0 ^b	34.0 \pm 8.6 ^b	54.5 \pm 1.8 ^b

^{a,b,c,d}Groups with the same letter designation are not significantly different ($P < 0.05$).

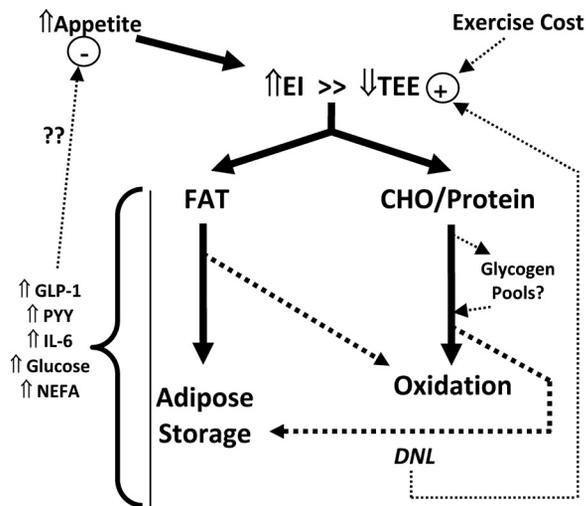


Fig. 6. Impact of regular exercise on fuel utilization during weight regain. The energy gap and general flow of energy during weight regain are indicated with bold lines. The impact of exercise on nutrient partitioning and energy expenditure (TEE) is denoted by dashed lines. Exercise increases the oxidation of dietary fat and traffics excess nutrients to storage via de novo lipogenesis (DNL). Exercise may also increase diversion of de novo lipogenic precursors to glycogen pools, and the carbohydrate (CHO)-sparing effect may delay subsequent bouts of feeding. TEE is increased both from the energetic cost of the exercise bout and by the energetic expense incurred from de novo lipogenesis. Several factors, at the left of the diagram, have been implicated as the peripheral signal mediating the effect of exercise on appetite. EI, energy intake; PYY, peptide YY; NEFA, nonesterified fatty acids; GLP, glucagon-like peptide.

this effect may contribute to the increased TEE in REL-EX rats independent of the de novo lipogenic pathway. The increase in TEE from de novo pathways of lipid deposition should increase NREE, while the sympathetic activation may increase both basal energy expenditure (REE) and components of NREE. Basal metabolism is traditionally measured under fasted conditions, which could not be accurately assessed in relapsing rats. The practical consequence of this technical limitation is that TEF and facultative thermogenesis may impact both NREE and REE with excessive overfeeding. However, the increased net retention of de novo derived lipid in peripheral tissues and the elevated NREE, in combination, suggests that an increase in TEF at least contributes to the higher TEE.

On its own, energy-restricted weight loss in obese individuals fails to increase skeletal muscle oxidative capacity (72, 75, 76), unless the calorie restriction is accompanied by regular exercise (30, 69, 70). Without exercise, mitochondrial respiratory capacity declines (64), glycolytic capacity appears to decline (29, 72), and the expression of enzymes associated with β -oxidation remain low and, if anything, decline (14, 64, 72, 75). The relapse-associated positive energy imbalance creates discordance between the nutrient overload and the metabolic capacity of muscle. The classic training response, mediated at least in part by increased PGC1 α expression (5, 61), ameliorates this discordance to some extent, by sustaining the metabolic capacity of muscle to dissipate the excess energy (58). Obesity-resistant rats respond to overfeeding, in part, by increasing TEE and the oxidation of dietary fat (32, 33), and regular exercise adjusts the relapse-associated overfeeding response in a similar manner. These adaptations enhance the

response to other metabolic challenges as well (27, 51). Increasing skeletal muscle oxidative capacity with the overexpression of LPL, rather than with regular exercise, yields a similar improvement in the metabolic response to cold exposure (37). Even so, most of the exercise-induced changes in gene expression are eliminated by overfeeding on the first day of relapse (Fig. 5). It is unclear whether this overfeeding-associated reversal of skeletal muscle gene expression affects oxidative capacity and the persistence of the exercise-induced shift in fuel utilization as the relapse to obesity continues. The role of adipose and hepatic metabolic gene expression adaptations in facilitating the observed nutrient trafficking and clearance also deserves further study.

Weight loss establishes a hypoleptinemic state and, because of the improved insulin sensitivity and lower nutrient intake, reduces circulating insulin (45, 68). The low levels of these adiposity signals are known to feed back to neural circuits in the hypothalamus and other areas of the brain to increase appetite and suppress energy expenditure. Yet, we show here and in previous studies (34) that these signals resolve for the most part after only 1 day of overfeeding, and neither appears to be mediating the exercise effect on appetite. As observed previously, treadmill exercise and volitional wheel running do not affect the low leptin and insulin levels during weight maintenance (40, 48), and the levels in the satiated REL-EX rats of the present study were similar to that found in unsatiated REL-GM rats. Rather than increasing the levels of these signals, there is growing evidence to suggest that exercise enhances the brain's sensitivity to them (20, 65, 66). Consistent with this assertion, exercised rats defend a lower level of adiposity with similar levels of leptin and insulin, after the high rate of gain in relapse subsides (48).

A number of recent reports have shown that the transient increase in IL-6 that occurs with acute bouts of exercise may mediate this sensitizing effect via AMPK-activated protein kinase (20, 65) and NF- κ B signaling in the hypothalamus (66). Exercise also reportedly potentiates the postabsorptive response to the satiety signals PYY, GLP-1, and PP (50). Our measures of IL-6 and GLP-1 in the present study failed to identify an effect of exercise (data not shown), but the timing of our blood collection would likely have missed the transient postexercise increase in IL-6 or changes in GLP-1 during the early hours of relapse when the animals were feeding. Further studies investigating their levels and recapitulating or blocking their response at critical times of the day are needed to clarify their role in the exercise-associated decline in appetite on the first day of relapse.

An alternative or complementary signal may be found in circulating nutrients. Glucose inhibits food intake and/or hunger whether infused centrally (7, 25, 82) or peripherally (28, 57, 71). Dietary fat can impact hypothalamic FFA levels (63), and FFAs reduce subsequent food intake when infused into the gut (22, 43), into circulation (78), or directly into the brain (10, 59). Unlike glucose and triglycerides, FFAs in postobese subjects decline postprandially (Table 1) and during weight regain (34). Diet-induced weight loss is known to enhance postprandial suppression of FFAs (1, 9, 36, 79) and postprandial excursions in glucose (1, 9, 79). We have hypothesized that the increased clearance rates and suppressed levels of FFA and glucose may relieve the suppressive effects these nutrients have on appetite, whether it be via sensing the nutrients

themselves or neuroendocrine signals that reflect their levels in circulation. Exercise may counter this effect on circulating nutrients in two ways. The sympathetic response to the exercise bout mobilizes stored energy, directing it through the circulation to working muscle for energy production during and after the bout. Second, recovery from exercise is accompanied by glycogen supercompensation when carbohydrate is ingested in plentiful amounts (4), and our previous studies suggest that this may sustain CHO availability in weight-reduced rats after their energy balance provision has been consumed (48). In the present study, glucose levels were higher in weight-reduced rats that exercised, and both glucose and FFAs were higher in relapsing rats that exercised (Table 3). Further studies are needed to see whether these modest differences observed before and after a full day of relapse reflect larger differences aligned with the differential meal patterns of sedentary and exercised rats in the early hours of relapse. Moreover, we do not yet understand how changing the meal pattern or the timing of the bout will affect the metabolic response during weight regain.

While the bout of exercise was regularly performed, modest in intensity, and relatively short in duration, this intervention undoubtedly imposed some level of chronic stress. The present study is consistent with our previous reports in that exercised rats required some level of external motivation to ensure compliance with the exercise protocol, even after they had become well acclimated to the daily event for several weeks (48). The lack of corticosterone changes with the exercise regimen does not preclude the involvement of some other aspect of chronic stress in the impact of exercise. We suspect that the exercised rats at the very least experience a daily sympathetic response to the exercise, which facilitates mobilization of stored energy from the periphery to meet the increased energy demand. Obesity-prone rats do have a preexisting impairment in responding to chronic stress (41), and overfeeding and consumption of highly palatable foods are also known to impair the response to chronic stress (21, 24). These characteristics may impart an impaired ability for or aversion to physical activity in weight-reduced and relapsing rats. Postobese obesity-prone rats do exhibit a decline in spontaneous wheel running (40) and reduced compliance to regular exercise during the relapse to obesity (48). As such, the role of chronic stress in both the beneficial effects on energy balance and in the poor compliance to exercise programs deserves further study.

Perspectives and Significance

The results of this study demonstrate how regular exercise counters some of the biological responses to dieting that drive weight regain. By reducing the energy gap between the elevated appetite and suppressed energy needs, regular exercise may facilitate long-term weight loss by making it easier to stay on a calorie-restricted diet. Regular exercise may also blunt the detrimental impact of transient excursions off of the weight maintenance diet by increasing the energetic cost of depositing excess nutrients and, by affecting both appetite and expenditure, reducing the magnitude of overfeeding when relapse occurs. The experimental paradigm employed in the present study reflects the patterns of weight loss/weight regain that is commonly seen in humans (2), only with a compacted timeline consistent with the shorter life span of rats (41, 42, 45, 46, 48).

Weight changes during this first day of overfeeding are not likely to be observed in 1 day of relapse in a human but could be spread over several days or weeks. More studies are needed to examine whether these beneficial effects of exercise persist further into relapse. Our previous studies show that the impact on food intake and energy balance persists throughout the entire relapse process, but we do not know whether the effects on nutrient trafficking are sustained. The exercise-induced gene expression favoring fat oxidation is substantially minimized with 1 day of refeeding in the present study. Regardless, the key consideration when looking for human correlates to these studies, however, is that obesity-prone humans are also subject to environmental and behavioral pressures that may affect weight regain (19, 50). Humans are not likely to maintain a low-fat diet composition once they go off their energy-restricted diet, and relapse on a high-fat diet may minimize the benefits of exercise during weight regain. In addition, compensatory increases in food intake can be driven by hedonic predispositions, justified by compliance to exercise regimens, or result from the lack of cognitive restraint in the face of an elevated biological drive to eat in excess. Such compensation can and will undermine the utility of exercise in weight regain prevention. With this knowledge and the cognitive pursuit to avoid such compensation, regular exercise remains the most potent strategy for successful long-term weight maintenance after calorie-restricted weight loss.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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