Improved insulin action following short-term exercise training: role of energy and carbohydrate balance

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Black, Steven E., Elizabeth Mitchell, Patty S. Freedson, Stuart R. Chipkin, and Barry Braun. Improved insulin action following short-term exercise training: role of energy and carbohydrate balance. *J Appl Physiol* 99: 2285–2293, 2005. First published August 4, 2005; doi:10.1152/japplphysiol.00291.2005.—Short-term exercise training improves insulin action, but the impact of replacing the energy expended during exercise to prevent energy deficit is unclear. The purpose of this study was to establish the role of an energy deficit in mediating improved insulin action after short-term exercise training. Two groups of previously sedentary, overweight/obese subjects performed 6 consecutive days of moderate-intensity walking to expend ~500 kcal/day. In one group, energy and carbohydrate expended during exercise was replaced [balance group (BAL), $n = 8$] and in the other group, energy was not replaced [deficit group (DEF), $n = 8$]. Insulin action (blood glucose uptake during glucose infusion) and selected lipids and adipokines were measured pre- and posttraining. Training increased estimated daily energy expenditure by ~500 kcal/day (DEF = 469 ± 45, BAL = 521 ± 48), generating an energy deficit in DEF (−481 ± 24 kcal/day) but not BAL (+8 ± 20 kcal/day). Insulin action increased 40% in DEF ($P = 0.032$) but not BAL (−8.4%, $P = 0.107$). Hepatic glucose production was suppressed during glucose infusion in DEF (30.2 ± 9.5%, $P = 0.037$) but not BAL (−10.0 ± 7.4%, $P = 0.417$). Fasting leptin concentrations declined in DEF but not BAL. Six days of exercise training without energy replacement significantly increased insulin action. Restoring energy balance by refeeding the energy and carbohydrate expended during exercise resulted in no change in insulin action. These findings suggest that changes in short-term energy and/or carbohydrate balance play a key role in mediating the beneficial effects of exercise on whole body and hepatic insulin action.

insulin resistance; stable isotope; glucose uptake; adipokine; leptin

PHYSICAL INACTIVITY BLUNTS the sensitivity of insulin-sensitive tissues (e.g., skeletal muscle, adipose) to the stimulatory effect of circulating insulin on uptake of glucose from the blood (14). Reduced insulin sensitivity (i.e., insulin resistance) is a central mediator of the pathophysiology, leading to Type 2 diabetes, and regular exercise enhances insulin sensitivity and reduces the risk for diabetes (30). Exercise training studies (>6 wk) result in improved insulin sensitivity but are often confounded by loss of body fat and/or visceral fat. In three long-term training studies, increased energy expenditure due to exercise was deliberately balanced by a proportionate increase in energy intake to loss of body mass as a confounding factor (42, 43, 45), and, in each study, the researchers observed no significant change in insulin sensitivity following exercise training.

These results imply that the benefits of exercise training on insulin action require maintenance of an energy deficit that results in a physiologically relevant loss of body mass. How-

ever, in several studies investigating the effects of weight loss, insulin sensitivity was enhanced during the active weight loss phase but returned toward baseline when the new lower body weight was maintained (2, 27, 39). Assali et al. (2) reported that two-thirds of the improvement in insulin sensitivity induced by a low-calorie diet occurred in the first week, before significant weight loss. Similar findings were reported from a lifestyle intervention trial in which the greatest change in glucose and insulin concentrations during an oral glucose tolerance test was noted after the first week of a low-calorie diet compared with 4 and 12 mo later when a considerable amount of body mass and body fat was lost (39). Furthermore, it was recently reported that 10-kg subcutaneous abdominal fat loss by large-volume liposuction (no energy deficit as subjects maintained presurgery diet and exercise habits) did not improve insulin sensitivity or other cardiovascular risk factors in obese women (29). Thus the energy deficit per se, rather than the weight loss that occurs in response to its repeated application, is an important factor to increase tissue sensitivity to insulin.

Short-term exercise training, consisting of 1–7 days of exercise, improves insulin sensitivity without any change in body composition (total or visceral fat loss), which suggests an independent effect of exercise on insulin action (1, 20, 41). However, the role of the acute energy deficit in these studies was not directly tested. If the impact of exercise on insulin action is mostly attributable to induction of an energy deficit rather than some more specific effect of muscle contraction, preventing an energy deficit may negate the effect of exercise on these pathways and ultimately, on insulin sensitivity. To address this question, we examined whether replacing the energy and carbohydrate (CHO) expended during exercise opposes the enhanced insulin action observed after short-term exercise training. We compared two groups of sedentary, insulin-resistant individuals engaged in 6 days of aerobic exercise while in different energy states. In one group, subjects were fed extra energy to balance their increased energy and CHO expenditure (BAL). In the other group, exercise energy expenditure was not replaced (DEF). We hypothesized that, because energy and CHO balance are important mediators of insulin action, the 6 days of exercise would be more effective to enhance insulin action in the DEF group compared with the BAL group.

MATERIALS AND METHODS

Study design. Sedentary and overweight/obese, but otherwise healthy subjects at risk for developing insulin resistance, were re-

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cruited and placed into one of two groups to assess the role of energy and CHO balance on insulin action before and after 6 consecutive days of treadmill walking. Additional calories from high-CHO foods equivalent to the amount of energy expended during exercise were added to the diet of one group but not the other, creating an exercise group in energy balance (BAL) and an exercise group in energy deficit (DEF). Because the two groups also differed in CHO balance, the DEF group was also "relatively" CHO deficient compared with BAL group.

Subjects. We recruited 16 volunteers from within the Amherst, Massachusetts area and assigned them into DEF or BAL using age, gender, body composition, and physical activity data to create similarly matched groups (Table 1). Subjects were between the ages of 30 and 60 yr, performed <0.5 h of exercise a week, and were weight stable (± 2 kg) for 6 mo. Subjects exhibited at least three of the following risk factors for the insulin-resistance syndrome, including overweight to moderately obese (body mass index: 25–35 kg/m^2), waist circumference >80 cm for women and 94 cm for men, a sedentary lifestyle defined as <0.5 h of exercise per week (e.g., brisk walking, cycling, running, aerobics, etc.), an immediate family member with Type 2 diabetes mellitus, a history of gestational diabetes, and a history of elevated blood pressure ($>130/90$ mmHg) or triglycerides (>150 mg/dl, 1.7 mM). Subjects were excluded from the study if they were not in good overall health, had diabetes or cardiovascular disease, used tobacco products, fell outside the body mass index range, performed regular endurance exercise, followed very low- or very high-CHO diets (<30 or $>70\%$ CHO, respectively), or chronically used antioxidant vitamins (e.g., vitamin E, vitamin C, and lipoic acid), anti-inflammatory medicines (e.g., aspirin, nonsteroidal anti-inflammatory drugs), or lipid-lowering drugs (e.g., statins and fibrates). The DEF group included six women and two men, whereas the group in energy balance (BAL) included five women and three men. Eight of the women were postmenopausal or posthysterectomy, none used oral contraceptives, and one used hormone replacement therapy (in BAL group). The study protocol was approved by the Institutional Review Board at the University of Massachusetts, Amherst before initiation of the study, and all subjects gave their informed consent before entering the study.

Preliminary testing. Subjects reported for placement into groups and initial familiarization with the laboratory facilities. A Physical Activity Readiness Questionnaire, a health and fitness history, and a record of recent physical activity were completed and reviewed. A diet history and 24-h dietary recall were obtained to identify typical diet patterns and screen for those on low- or high-CHO diets. Subjects were instructed to maintain their usual nutrition habits during this period to maintain their current weight. The 3-day experimental meal plan was also reviewed and altered as needed, depending on subject's

requirements. Baseline measurements of height and body mass were taken. Body weight was obtained in light workout clothes without shoes using a balance scale. Waist circumference was measured above the uppermost lateral border of the iliac crest in a horizontal plane around the abdomen by using a flexible measuring tape. One to two days before their pretraining assessment of insulin action and again on the day of their posttraining measurement (± 1 day), body composition (fat mass, fat-free mass, %body fat, and %trunk fat) was assessed by using dual-energy X-ray absorptiometry (Lunar, Madison, WI). Trunk fat was determined from anatomic landmarks and default calculations of the Lunar computer software.

Peak oxygen consumption ($\dot{V}_{O_2 peak}$) was estimated by using TREADWALK, a modified Rockport walk test adjusted for laboratory treadmill walking (37). The time required to complete the distance and average heart rate (Polar Electro, Woodbury, NY) from the last 2 min of the test were entered into a regression equation to determine $\dot{V}_{O_2 peak}$ [$\dot{V}_{O_2 peak} = 92.08 - 0.10$ (body mass in pounds) $- 0.34$ (age in yr) $+ 9.72$ (gender; male $= 1$, female $= 2$) $- 1.01$ (walk time in minutes and hundredths of a minute) $- 0.13$ (walk heart rate in beats/min) $+ 0.86$ (activity level)]. The results of the modified Rockport walk test were used to set the appropriate exercise intensity during the 6 days of training.

Energy expenditure calculations. Resting energy expenditure was measured in the morning after an overnight fast by using indirect calorimetry to determine caloric requirements for each subject. Subjects sat comfortably in a reclining chair for 30 min, and then respiratory gases were collected for a minimum of 20 min (True-Max2400 Metabolic Measurement System, Parvomedics, Salt Lake City, UT). Measured resting energy expenditure was multiplied by an activity factor of 1.3–1.5, representative of the very light to light habitual physical activity patterns of our subjects, to provide an estimate of total daily energy expenditure (32).

Subjects wore a triaxial accelerometer (CT-1 activity monitor, Stayhealthy, Monrovia, CA) to provide a quantifiable measure of physical activity and energy expenditure during weight maintenance and training (15). The CT1 triaxial accelerometer measures the changes in acceleration in the horizontal, vertical, and lateral plane in counts and uses a proprietary formula to determine 24-h energy expenditure. Monitors were worn on the left or right hip during waking hours, except during bathing, and remained consistent for the duration of the study.

Diet assessment and control. Subjects in each group consumed a controlled diet for 3 days before each test of insulin action and consumed their usual diet for at least 3 days before the pretest of insulin action and for the first 3 days of exercise training. On entry into the study, subjects received instructions on how to record food and beverage intake and provided a detailed recording of all nutrient intake for the duration of the study. Food journals were reviewed daily with study coordinators to improve completeness and accuracy and analyzed using Food Processor nutrition analysis software (ESHA Research, Salem, OR).

The DEF group received a diet calculated for weight maintenance requirements that did not account for the exercise-induced increase in energy expenditure, whereas the group in caloric balance (BAL) received a diet that included additional kilocaloriess to replace the energy expended during exercise. Three-day menus were designed for each group using commercially prepared frozen entrees and foods prepared and weighed in the energy metabolism laboratory. The diet for each day was designed to provide 55–60% CHO, 15–18% protein, 26–30% fat (8% saturated fat), and 25–30 g of dietary fiber, in accordance with the latest Dietary Guidelines for Americans (50).

All menus were reviewed with each subject, and adjustments were made based on tolerance and compliance issues. Subjects reported to the laboratory each of the 3 days before the first assessment of insulin action for daily measurements of body mass and meal pickup. Subjects were instructed to consume all provided food and drinks. If subjects were unable to finish any food item, they were told to return

Table 1. *Subject characteristics in energy deficit and energy balance groups*

	Energy Deficit (DEF)	Energy Balance (BAL)	*P* Value
n	8 (6 F, 2 M)	8 (5 F, 3 M)	
Age, yr	49.0 ± 5.1	46.4 ± 11.4	0.552
Body mass, kg	79.8 ± 10.4	85.4 ± 9.8	0.303
BMI, kg/m^2	29.4 ± 1.0	30.5 ± 3.7	0.541
$\dot{V}_{O_2 peak}$, ml·kg^{-1}·min^{-1}	27.6 ± 7.1	29.1 ± 11.5	0.769
HOMA-IR	2.4 ± 1.5	3.2 ± 1.6	0.335
Fat mass, %	40.6 ± 5.3	36.9 ± 10.3	0.361
Trunk fat, %	41.8 ± 4.9	39.2 ± 9.0	0.342
FFM, kg	45.0 ± 7.5	51.3 ± 10.3	0.190
Waist circumference, cm	99.6 ± 3.5	102.4 ± 3.5	0.512

Values are means $\pm$ SD; *n*, no. of subjects. F, female; M, male; BMI, body mass index; $\dot{V}_{O_2 peak}$, peak oxygen consumption; HOMA-IR, homeostasis model assessment of insulin resistance; FFM, fat-free mass.

it to the laboratory, where it was weighed and subtracted from the calculated amount in the diet. Compliance was high based on interviews with subjects and the small number of returned food items. Only water or other noncaloric beverages were allowed to supplement the prepared meals and drinks. Subjects noted these in their daily food journals. Subjects were instructed and agreed to abstain from alcohol and caffeinated beverages for the 24 h preceding each test.

The replacement calories for the BAL group were fed in the form of 20 oz. of a CHO-electrolyte beverage consumed during exercise and a combination of fruit, energy bar, or smoothie consumed immediately afterward in our laboratory to provide adequate kilocalories to prevent energy deficit. The macronutrient composition for the replacement calories, beverage, and snacks combined was 75% CHO, 13% protein, and 12% fat. During the first 3 days of training when subjects were self-selecting their meals and maintaining their usual eating habits, the BAL group received all replacement kilocalories immediately after exercise, whereas, during the 3 days of the prepared meals, ~80% of the caloric deficit was accounted for by these snacks consumed during and immediately following exercise, with the balance added to the prepared meals.

The diets provided to each group in the 3 days before the pretraining tests of insulin action contained identical macronutrient profiles: CHO = 56%, protein = 15%, and fat = 29%. The same macronutrient profile was maintained for the posttraining tests of insulin action in the DEF group, but, because of the higher CHO content of the replacement calories, the BAL group consumed a slightly higher percentage of CHO (59%) and lower percentage from fat (26%) (DEF = 2,246 total kcal, 1,258 CHO kcal, 651 fat kcal; BAL = 2,925 total kcal, 1,726 CHO kcal, 761 fat kcal).

Exercise training. Each exercise bout was performed under continuous supervision in the laboratory under controlled environmental conditions, and heart rate was recorded throughout all exercise sessions. Subjects performed treadmill walking (LifeFitness 9100HR, Schiller Park, IL) at 60–65% of their estimated $\dot{V}_{O_2\,peak}$ for the required time period to expend 500 cal. On the first day of exercise training, treadmill speed and grade were adjusted until subjects reached 60–65% $\dot{V}_{O_2\,peak}$ as measured by indirect calorimetry. On reaching the desired intensity, energy expenditure was measured during 20 min of steady-state exercise. Energy expended per minute was calculated from oxygen consumption, and subjects walked for the time necessary to expend 500 kcal. Subjects unable to reach the target expenditure by 70 min of exercise were stopped at that point, and actual energy expenditure was calculated. This occurred in three subjects total: DEF = 2 and BAL = 1. Replacement calories were adjusted for subjects in the BAL group who could not expend 500 kcal. Every subsequent session was conducted at the same speed, duration, and percent grade to expend an equivalent amount of energy. A second assessment of exercise energy expenditure was performed on the 3rd or 4th day of training to reassess workload and time.

All exercise training included 5-min warm-up and cooldown periods at a self-selected pace. One to two short breaks, 1–3 min in duration, were scheduled as needed during each training session. The breaks consisted of decreasing the speed and grade of the treadmill to a self-selected pace or of stepping off the treadmill and sitting before increasing the workload to the target intensity for the remainder of the workout. Because of scheduling issues, two subjects from each group performed 7 consecutive days of exercise rather than 6, so the last bout occurred 24 h before the posttraining measurement of insulin action. Subjects were encouraged to maintain their typical physical activity patterns during the training period and were shown daily accelerometer reports to encourage compliance.

Assessment of insulin action: glucose infusion and stable isotopes. Insulin action was measured the day before training and 24 h after the final bout of exercise using a continuous infusion of 20% glucose that contained a 2% stable [6,6-²H]glucose isotope tracer. Each measurement involved a 90-min infusion of the isotope tracer followed by a 60-min infusion of 20% glucose with isotope tracer added. Insulin action was determined from isotopically determined glucose uptake per unit of steady-state insulin concentrations achieved during the continuous glucose/stable isotope infusion (18).

Subjects rested quietly in a reclining chair throughout each measurement of insulin action. Indwelling catheters were placed in a superficial vein of each forearm for venous blood sampling and continuous infusion of [6,6-²H]glucose. Baseline blood samples were collected to determine background levels of isotopic enrichment. A priming bolus of 200-mg [6,6-²H]glucose was given followed by a 90-min infusion of [6,6-²H]glucose at a rate of 2.5 mg/min delivered by a peristaltic infusion pump (Harvard Apparatus Pump 22, Holliston, MA). Respiratory gases and venous blood samples were collected at 0, 75, and 90 min. At 90 min, the infusate was changed to a 20% dextrose solution containing 2.0% [6,6-²H]glucose delivered at a rate of 8.45 mg·min⁻¹·kg⁻¹ fat-free mass for 60 min. Blood samples and respiratory gases were collected at 50, 55, and 60 min of the glucose/stable isotope infusion to determine glucose rate of appearance (R_a) and disappearance (R_d), as well as plasma concentrations of glucose and insulin. Glucose and insulin concentrations from *minutes 50, 55,* and *60* were averaged to determine the steady-state plasma glucose (SSPG) and insulin (SSPI) concentrations.

Blood collection and biochemical analyses. Venous blood samples were collected in tubes containing a glycolytic inhibitor (sodium fluoride and potassium oxalate) for analysis of glucose, glucose isotopic enrichment, and triglycerides; an anticoagulant (K_3-EDTA) for analysis of insulin, adiponectin, and free fatty acids; or serum separator for high-sensitivity C-reactive protein (CRP), total cholesterol, and high-density lipoprotein. Samples were immediately centrifuged and stored at −70°C until analysis. Plasma glucose concentrations were determined by the glucose oxidase method using a GL5 Analox Analyzer (Analox Instruments, Lunenberg, MA). Plasma insulin and adiponectin concentrations were determined by using radioimmunoassay kits specific for human insulin and adiponectin (Linco Research St. Charles, MO). CRP was analyzed by using a high-sensitivity ELISA assay by ICN Pharmaceuticals (Orangeburg, NY). Plasma triglycerides were determined by using an enzymatic colorimetric assay kit (Sigma Chemical, St. Louis, MO), while serum cholesterol and HDL were analyzed by using the cholesterol oxidase method (Analox Instruments, Lunenberg, MA). Low-density lipoprotein was calculated by using the Friedewald equation (12). Measurement of fasting insulin resistance was calculated by using homeostasis model assessment (52): [fasting insulin (mU/l) × fasting glucose (mmol/l)]/22.5.

Glucose isotopic enrichment was measured by gas chromatography-mass spectrometry. Plasma was deproteinized by adding 0.3 N $ZnSO_4$ and 0.3 N $BaOH_3$. Samples were vortexed, incubated in an ice bath, and centrifuged at 4°C at 3,300 rpm. The supernatant was extracted and lyophilized (−50°C, 5 mTorr), and the dried samples were then reconstituted in a 2:1 solution of acetic anhydride and pyridine to form the pentacetate derivative of glucose. The samples were capped, heated in a water bath at 60°C for 60 min, and transferred to clean tubes. Double-distilled water and dichloromethane were added, the tubes were centrifuged, and the remaining dichloromethane phase was transferred to gas chromatography vials, evaporated under nitrogen, capped, and then reconstituted in ethyl acetate. A 25-μl sample was injected into the gas chromatography-mass spectrometry and separated on a gas chromatograph with spectra recorded on a mass spectrometer (Hewlett-Packard 6890, Palo Alto, CA). Selected ion monitoring was used to compare the abundance of the unlabeled fragment with that of the enriched isotopomer (Chemstation Software). After correcting for background enrichment, the abundance of the dideuterated isotopomer (mass-to-charge ratio = 202) was expressed as percentage of total glucose species (mass-to-charge ratio = 200 + 201 + 202).

Calculations. Isotope-derived glucose turnover was calculated as:

$$\text{Glucose } R_a = \frac{F - V[(C1 + C2)/2][(IE2 - /IE1)/(t2 - t1)]}{[(IE2 + IE1)/2]}$$

$$\text{Glucose } R_d = R_a - V[(C2 - C1)/(t2 - t1)]$$

where F is the isotope infusion rate, IE1 and IE2 are enrichments of plasma glucose with isotope label at time *t1* and *t2*, C1 and C2 are plasma glucose concentrations, and V is the estimated volume of distribution for glucose (180 ml/kg).

Whole body insulin action was defined as glucose R_d/SSPI, where SSPI is the mean plasma insulin concentration during the final stages of the infusion (4, 45).

Hepatic insulin action was defined as the percent suppression of basal hepatic glucose production (HGP; HGP_{basal}) by the glucose infusion = $1 - (HGP_{inf}/HGP_{basal}) * 100$, where HGP_{basal} is equal to the basal R_a, and HGP during the infusion (HGP_{inf}) is calculated as follows: (steady-state glucose R_a) − (glucose infusion rate).

Nonoxidative glucose disposal was calculated as follows: (glucose R_d) − (total CHO oxidation rate).

Statistical analyses. Data were analyzed by using SAS, version 8 (SAS Institute, Cary, NC). Differences in subject characteristics between groups before training were analyzed by using independent *t*-tests. Raw data are presented as means ± SD. Insulin action, glucose kinetics, and substrate and hormone variables pre- and posttraining were compared by using a two-way (time × condition) ANOVA using a mixed model. Tukey's post hoc analysis was used to detect differences when there was a significant interaction or main effect. No adjustment for multiple comparisons or for number of variables was made. Mean percent change, 95% confidence intervals, and standard error are reported. Pearson's product-moment correlation coefficient was used to examine the relations between change in insulin action and other outcome variables. A probability level of 0.05 was used to denote statistical significance for all analyses.

RESULTS

Exercise training. Training data for both groups, collected on the 3rd or 4th training day, are listed in Table 2. There was no significant difference in training intensity as measured by oxygen consumption, heart rate, or Borg's rating of perceived exertion. Exercise duration was very similar between groups. As designed, energy expenditure during exercise training, determined by indirect calorimetry, was not different between groups (DEF = 481 ± 33 kcal vs. BAL = 508 ± 40 kcal).

Energy balance. There was no significant difference between groups in the habitual diet consumed either in total kilocalories (DEF = 1,948 ± 150 kcal; BAL = 2,296 ± 179 kcal, *P* = 0.159) or macronutrient intake (DEF = 48.4 ± 2.3% CHO, 16.0 ± 0.7% protein, 32.5 ± 2.1% fat; BAL = 48.2 ±

Table 2. *Training data collected on training day 3 or 4 in energy deficit and energy balance groups*

	Energy Deficit (DEF)	Energy Balance (BAL)	*P* Value
V̇O₂, l/min	1.5±0.3	1.7±0.4	0.308
V̇O₂, ml·kg⁻¹·min⁻¹	19.0±1.9	19.5±3.4	0.775
Minutes on treadmill	65.6±4.7	61.6±7.8	0.280
Exercise energy expenditure, kcal	481.1±32.6	507.5±39.9	0.143
HR last 20 min, beats/min	135.4±1.6	136.3±0.9	0.611
RPE	13.4±0.1	13.1±0.2	0.267
METs, ml·kg⁻¹·min⁻¹	6.2±0.3	6.2±0.4	0.983

Values are means ± SE. V̇O₂, oxygen consumption; HR, heart rate; RPE, rating of perceived exertion; METs, metabolic equivalents.

Table 3. *Energy balance during 6 days of exercise training*

	Energy Deficit (DEF)	Energy Balance (BAL)
Energy ingested, kcal	2,246±97	2,925±159
CHO, g	314±14 (56%)	431±24 (59%)
Protein, g	84±4 (15%)	110±6 (15%)
Fat, g	72±3 (29%)	84±5 (26%)
Estimated energy expenditure, kcal	2,727±182	2,917±169
Energy balance, kcal	−481±24	+8±20
Body mass change, kg	−0.62±0.2	+0.03±0.2
Weight change as % of body mass	−0.8±0.2	0.04±0.1

Values are means ± SE. CHO, carbohydrates. Energy ingested reflects mean of self-recorded food journals, *days 1–3,* and meals from laboratory, *days 4–6.* Estimated energy expenditure was measured using indirect calorimetry and calculated from resting (measured during weight maintenance period) and exercise energy expenditure (measured on *day 3* or *4*).

1.7% CHO, 17.4 ± 0.8% protein, 31.8 ± 2.1% fat; *P* = 0.761, 0.207, 0.889 for CHO, protein, and fat, respectively). Analysis of food records during the first 3 days of exercise training when subjects were consuming a self-selected diet indicated that there were no significant changes in kilocalories or macronutrients in either group.

Accelerometer data indicated that, as designed, daily treadmill exercise increased physical activity and total energy expenditure from pretraining in both groups (DEF = 2,389 ± 158 kcal pretraining, 2,858 ± 182 kcal during training vs. BAL = 2,537 ± 149 kcal pretraining, 3,074 ± 169 kcal during training).

Table 3 presents a summary of overall energy balance during training. The actual energy balance achieved during training in the DEF group (−481 ± 24 kcal/day) and the BAL group (+8 ± 20 kcal/day) was very close to that specified in the study design (DEF = −500 kcal/day; BAL = 0). There was a small but significant decrease in mean body mass in DEF (−0.62 kg, *P* = 0.005) but not in BAL (0.03 kg, *P* = 0.65). Fat mass, percent body fat, and percent trunk fat did not change in either group following 6 days of exercise (data not shown). Macronutrient composition by percentage was similar between groups but, as designed, energy intake and grams of CHO, protein, and fat were all higher in the BAL group (DEF = 2,246 ± 97 kcal, 314 ± 14 g CHO, 84 ± 4 g protein, 72 ± 3 g fat; BAL = 2,925 ± 159 kcal, 431 ± 24 g CHO, 110 ± 6 g protein, 84 ± 5 g fat).

Plasma glucose and insulin. Basal glucose (DEF = 5.4 ± 0.2 mM pretraining, 5.5 ± 0.1 mM posttraining vs. BAL = 5.5 ± 0.2 mM pretraining, 5.5 ± 0.2 mM posttraining) and SSPG (DEF = 10.0 ± 0.3 mM pretraining, 10.3 ± 0.3 mM posttraining vs. BAL = 10.4 ± 0.5 mM pretraining, 10.6 ± 0.3 mM posttraining) were unchanged in both groups following 6 days of treadmill exercise. Mean fasting insulin concentrations decreased by 12.7% (not significant), and SSPI declined by 22.6% (*P* = 0.023) in the DEF group (Fig. 1). There was no change in either fasting insulin concentrations or SSPI in the BAL group.

Glucose turnover and insulin action. HGP_{basal} and glucose R_d were not different between groups before or after training (Table 4). During the infusion, glucose R_d increased by almost 20% in the DEF group following exercise training (*P* = 0.013), and there was no change in the BAL group (+3.2%, *P* = 0.219) (Fig. 2). Insulin action, defined as glucose R_d per unit of
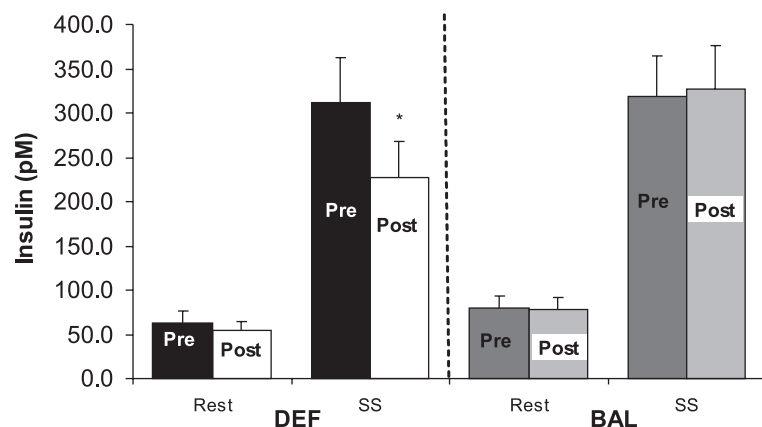
Fig. 1. Change in resting and steady-state (SS) (mean of 50, 55, 60 min) insulin concentrations in energy deficit (DEF) and energy balance (BAL) groups. Values are means ± SE. Pre, pretraining; Post, posttraining. *Significantly different from pretraining, $P < 0.05$.

SSPI, was significantly increased in the DEF group (+40.3%, $P = 0.032$) and was unchanged in the BAL group (−8.4%, $P = 0.107$). Because there was no change in the rate of glucose oxidation after training, the increase in glucose R_d in the DEF group was completely accounted for by an increase in nonoxidative glucose disposal ($P = 0.011$). There was no change in either oxidative or nonoxidative glucose disposal in the BAL group. The rate of lipid oxidation at rest or during the infusion was not altered by training in either group.

At baseline, HGP_{inf} was different between groups ($P = 0.039$). In response to training, HGP_{inf} decreased in the DEF group ($P = 0.109$) and increased in the BAL group ($P = 0.078$), so that the two groups were no longer different ($P = 0.519$). Hepatic insulin action, defined as percent suppression of HGP_{basal} during the infusion, was significantly increased only in the DEF group ($P = 0.015$) (Fig. 3).

The increased glucose R_d and decreased HGP observed in the DEF group were not reflected by concomitant changes in steady-state glucose concentrations. It is likely that the lack of change in SSPG was due to differences in the SSPI since, after training, SSPI was consistently lower in DEF relative to both their pretraining concentrations and the BAL group.

Leptin, adiponectin, CRP, and lipids. Fasting leptin concentrations declined in the DEF group ($P = 0.049$), whereas there was no change in the BAL group (Table 5). There was no statistically significant change in either group in mean plasma concentrations of CRP, triglycerides, total cholesterol, and adiponectin (Table 5), although positive trends were apparent in the DEF group (Fig. 4).

Correlations. Insulin action was inversely correlated with an increase in dietary energy (−0.597, $P = 0.015$) and dietary CHO ($r = -0.593$, $P = 0.015$). There were no significant relationships observed between change in insulin action and leptin, triglycerides, CRP, adiponectin concentrations, or any other outcome variable.

Baseline leptin concentrations were directly correlated with percent body fat ($r = 0.924$, $P < 0.001$) and age ($r = 0.814$, $P < 0.001$) but were inversely correlated with $\dot{V}O_{2\,peak}$ ($r = -0.903$, $P < 0.001$). Baseline CRP concentrations were directly correlated with age ($r = 0.580$, $P = 0.019$) and trunk fat ($r = 0.5,739$, $P = 0.020$) and inversely correlated with $\dot{V}O_{2\,peak}$ ($r = -0.687$, $P = 0.003$).

DISCUSSION

Short-term exercise training (from 1–10 days) increases insulin action, when measured within 24 h after the last exercise bout, in previously sedentary individuals, irrespective of age, ethnicity, weight, gender, or diabetes (1, 5, 8, 21, 25, 48). However, none of those studies directly assessed the role of energy balance in mediating the observed changes in insulin action. Expended energy was not deliberately replaced, and, therefore, subjects were likely in energy deficit when posttraining measurements of insulin action were made. The present

Table 4. *Glucose turnover data before and after 6 days of exercise training in energy deficit and energy balance groups*

	Energy Deficit (DEF)					Energy Balance (BAL)				
	Pre	Post	Δ	95% CI	P value	Pre	Post	Δ	95% CI	P value
HGP_{basal}, μM·kg FFM^{-1}·min^{-1}	27.2±2.8	30.5±2.8	3.29	(−3.4, 9.9)	0.283	28.3±3.7	29.2±3.7	0.9	(−4.7, 6.5)	0.721
$HGP_{infusion}$, μM·kg FFM^{-1}·min^{-1}	24.4±2.0	19.2±4.1	−5.18	(−11.8, 1.5)	0.109	18.5±1.6	22.5±2.9	4.02	(−0.6, 8.7)	0.078
Glucose$_{basal}$ R_d, μM·kg FFM^{-1}·min^{-1}	26.3±2.8	28.2±3.1	1.93	(−4.8, 8.7)	0.518	27.5±3.6	28.4±3.8	0.9	(−4.7, 6.4)	0.726
Glucose$_{infusion}$ R_d, μM·kg FFM^{-1}·min^{-1}	44.1±2.1	52.7±3.6	8.90	(2.8, 15.1)	0.013*	51.6±4.0	48.2±2.8	−3.35	(−9.2, 2.5)	0.219
Oxidative	14.8±1.9	14.0±1.8	−0.82	(−4.5, 2.9)	0.617	17.7±3.7	13.1±1.8	−4.59	(−11.2, 2.0)	0.144
Nonoxidative	29.1±3.4	38.9±4.3	9.72	(4.4, 15.1)	0.004*	33.9±6.5	35.1±3.3	1.23	(−10.4, 12.8)	0.562
Insulin action (R_d/SS insulin)	1.76±0.06	2.47±0.76	0.71	(0.19, 1.23)	0.032*	1.31±0.29	1.20±0.26	−0.11	(−2.13, 0)	0.107
RER (basal)	0.75±0.02	0.75±0.03	−0.01	(−0.03, 0.02)	0.187	0.80±0.03	0.77±0.03	−0.03	(−0.07, 0.02)	0.448
RER (during SS)	0.82±0.01	0.81±0.01	−0.01	(−0.03, 0.02)	0.585	0.85±0.01	0.82±0.01	−0.04	(−0.09, 0.01)	0.132
Energy expenditure, kcal (kcal/min during SS)	1.20±0.06	1.17±0.06	−0.02	(−0.07, 0.03)	0.546	1.30±0.09	1.25±0.08	−0.05	(−0.24, 0.14)	0.306

Values are means ± SE. Pre, pretraining; Post, postraining; Δ, change; CI, confidence interval; HGP_{basal}, basal hepatic gluose production; $HGP_{infusion}$, hepatic glucose production during infusion; R_d, rate of disappearance; SS, steady state; RER, respiratory exchange ratio. *Significantly different from pretraining, $P < 0.05$.
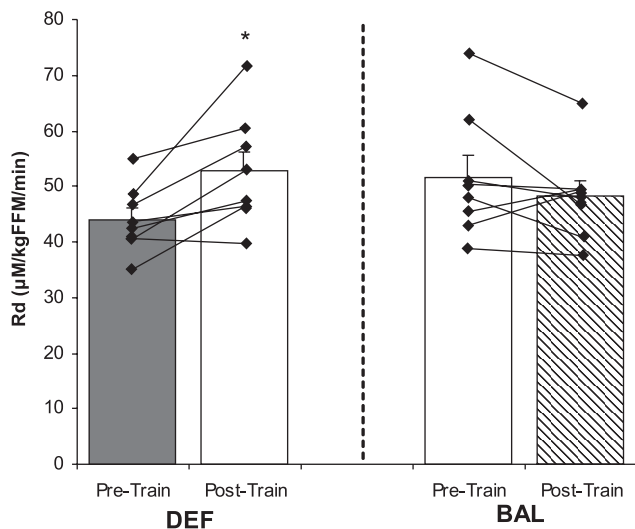
Fig. 2. Change in glucose rate of disappearance (R_d) during glucose infusion in DEF and BAL groups. Values are means ± SE. FFM, fat-free mass. *Significantly different from pretraining, $P < 0.05$.

intake matches energy expenditure with dietary composition held constant.

A plausible explanation for our observations is that differences in CHO availability, rather than energy per se, play a role in mediating the difference between the two states of energy balance. Results from several studies indicate that glycogen resynthesis accounts for much of the increased glucose uptake observed after glycogen-depleting exercise (3, 26, 53). Postexercise CHO feeding replenishes muscle glycogen stores and reverses the exercise-induced enhancement of insulin action (7, 19). The reversal is especially pronounced by a combination of glycogen-depleting exercise and dietary CHO replacement that induces glycogen supercompensation (19, 26). In contrast, glycogen-depleted animals that are fasted or fat fed after exercise do not replenish muscle glycogen, and enhanced insulin sensitivity persists, despite differences in energy balance (7, 19). The importance of CHO availability is supported by data from two recent human studies in which investigators manipulated energy replacement but held CHO intake constant after glycogen-depleting exercise (11, 44). Fox et al. (11) reported that the insulin response to oral glucose in an energy deficit condition (induced by exercise) was not different than in an energy balance condition (energy added back exclusively in the form of fat) in young, normal-weight men when CHO intake was constant. Similar results were reported by Schenk et al. (44) when CHO intake was again matched between conditions, and pure fat was infused to create an energy surplus (±1,060 kcal) that had no impact on insulin action, as measured by frequently sampled intravenous glucose tolerance test. Sparti and Decombaz (47) noted the opposite pattern however, when insulin action was estimated 36 h after either a high-CHO or low-CHO diet following a single high-intensity exercise bout in healthy men. In that study, the integrated insulin area under the curve was significantly lower than baseline after the high-CHO but not the low-CHO dietary intervention. Further support for an independent effect of perturbing energy balance on substrate metabolism was recently reported by Horowitz et al. (17), who found that CHO oxidation was lower and lipid

study was designed to test the role of restoring energy balance, by replacing expended energy and CHO, in mediating the response to short-term exercise training by systematically comparing a group in energy (and in a relative sense, CHO) deficit with a group in energy balance. The primary finding was that 6 consecutive days of exercise increased peripheral and hepatic insulin action in overweight, sedentary subjects, but this occurred only in the energy-deficient group. When dietary energy was replaced immediately postexercise using high-CHO foods to restore daily energy balance, there was no change in peripheral or hepatic insulin action. The clear distinction between the two states of energy balance suggests that adding back the energy and CHO expended during exercise negates exercise-induced enhancement of insulin action.

We anticipated that refeeding the total and CHO energy expended during exercise would attenuate the improvement in sensitivity to insulin, but the lack of any measurable change in the BAL group was striking. The results are in accordance with longer term studies (12- to 14-wk exercise training programs) that were designed to isolate the insulin-sensitizing effects of exercise from the impact of body mass (fat) loss (42, 43, 45). As in the present study, Segal et al. (45) added dietary energy, in the form of a CHO supplement, equivalent to the energy expended during each training session. Following cycle ergometry training for 12 wk, the authors reported that peripheral insulin sensitivity was not enhanced (although HGP$_{basal}$ was lower) in lean men and obese men with and without Type 2 diabetes (45). Ross et al. reported similar findings in groups of overweight men (42) and women (43) who exercised ~60 min per day, 5 days/wk for 14 wk but, by design, increased dietary intake to maintain a stable body mass. In all three studies, insulin action was measured 4 or more days after the last training session to specifically assess adaptations to training and not residual effects of the most recent bout of exercise (28). The lapse of several days between the last exercise bout and the assessment of insulin action likely accounts for at least some of the results. The data from the present study are novel, since they show that even the residual effects of exercise, measured 18–24 h postexercise, are negated when energy
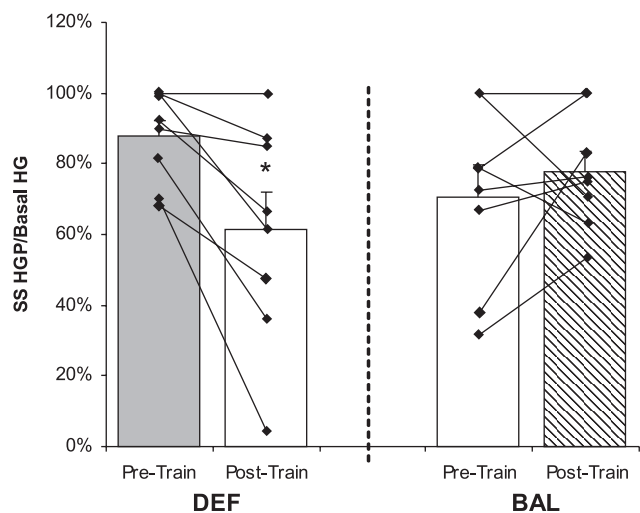

Fig. 3. Change in hepatic insulin resistance during glucose infusion in DEF and BAL groups [SS HGP: SS mean of 50-, 55-, 60-min hepatic glucose production (HGP)]. Values are means ± SE. *Significantly different from pretraining, $P < 0.05$.

Table 5. *Lipids, adipokines, and C-reactive protein*

	Energy Deficit (DEF)					Energy Balance (BAL)				
	Pre	Post	Δ	95% CI	P value	Pre	Post	Δ	95% CI	P value
Triacylglycerols, mg/dl	117±23	98±15	−19	−49, 11	0.181	133±39	126±27	−7	−36, 22	0.583
Total cholesterol, mg/dl	203±8	192±9	−11	−24, 1	0.070	178±10	173±10	−5	−21, 11	0.491
HDL, mg/dl	52±3	53±3	+1	−5, 3	0.689	51±4	46±5	−5	−9, −1	0.031*
LDL, mg/dl	127±6	120±6	−7	−19, 4	0.159	100±14	102±14	+2	−15, 19	0.770
Adiponectin, ng/ml	9.95±1.4	10.46±1.2	+0.5	−.21, 1.23	0.136	6.47±1.4	6.51±1.3	+0.04	−1.21, 1.29	0.941
CRP, mg/l	3.8±0.6	3.1±0.6	−0.7	−2.25, 0.83	0.314	4.9±1.7	5.0±1.6	+0.1	−.99, 1.24	0.801
Leptin, ng/ml	16.2±2.1	13.6±2.2	−2.6	−5.16, 0.01	0.049*	14.0±3.1	14.0±3.2	−0.1	−1.09, 0.96	0.883

Values are means ± SE. CRP, C-reactive protein. *Significantly different from pretraining, $P < 0.05$.

oxidation higher the day after glycogen-depleting exercise when in energy deficit rather than energy balance, even with CHO intake held constant.

Because of the particular question that the current study was designed to address, we cannot distinguish the relative importance of energy or CHO balance in generating the observed results. The objective was to assess how replacing energy expended during exercise, in roughly the same proportions as the typical diet, would impact insulin action in previously sedentary, insulin-resistant individuals. Since the BAL group consumed extra energy to replace exercise energy expenditure, the two groups also consumed different amounts of CHO during training (DEF = ~4 g/kg body mass; BAL = ~5 g/kg). To power the modest exercise dose of 500 kcal/day expended at $60-65\%$ $\dot{V}O_{2\,peak}$, estimated muscle glycogen use (based on total CHO oxidation from indirect calorimetry and assuming 80% of total CHO oxidation attributable to muscle glycogen) was ~80 g per daily exercise session. Although not measured directly, this glycogen output could have been replaced by the daily CHO intake in both groups ($\geq$55% of total kilocalories, DEF = 314 ± 14 g; BAL = 431 ± 24 g) (33). It is possible though that the timing of the postexercise energy intake relative to the bout of exercise may have played a role in the differential response between our two groups. We fed the replacement energy to the BAL group immediately postexercise to minimize differences in meal timing as a potential

source of interindividual variability. The immediate provision of the high-CHO replacement calories could have differentially affected the rate of glycogen repletion since glycogen synthesis is greatest $0-2$ h postexercise (38), and CHO feeding during this time period enhances glycogen synthesis (22). Without muscle biopsies to quantify skeletal muscle glycogen concentrations in each condition, we are unable to distinguish whether the results observed were due primarily to energy or CHO balance. The strong links between glycogen depletion and glucose uptake, the recent studies showing that energy replacement as pure lipid may not oppose postexercise insulin action, and the positive correlation we found between glucose R_d and CHO intake suggest that CHO availability is one of the key mediators of postexercise insulin action. Conversely, given published data showing that energy deficit modulates insulin action and the known impact of energy status on intracellular energy-sensing pathways and regulatory hormones, it also seems likely that energy status (deficit, balance, or surplus) impacts postexercise insulin action. Further systematic studies are needed to disentangle the relative roles of energy and CHO availability.

Results from our study, by virtue of the study design, are specific to energy deficit induced by exercise alone and likely not generalizable to energy deficit induced by reducing caloric intake. In broad terms, the energy deficit incurred by adding exercise without replacing dietary energy induces metabolic changes similar to those observed in response to caloric restriction (27). Studies done in rodents show that as little as 5 days of energy restriction ($60-75\%$ of ad libitum kilocalories) increases glucose uptake and insulin action (10). In several human studies, however, the effects of chronic energy restriction (resulting in measurable loss of total body mass) do not clearly match those attributable to chronic exercise training (including an energy deficit) on insulin action, possibly due to the difficulty in controlling for the myriad confounding variables (e.g., physical activity patterns, actual energy intake and diet composition, etc.) inherent to long-term human studies (9, 24, 40, 42, 43). To minimize the impact of these potential confounding variables in our short-term study of energy balance in a free-living population, we depended on a variety of methods. Subjects were provided with weighed and measured food during the critical time periods (3 days before the baseline and posttraining measures) of the study. Food records were collected and analyzed by a trained dietitian. Subjects wore previously validated physical activity monitors to maintain an estimate of free-living energy expenditure. A major investment of energy went into developing and maintaining a strong
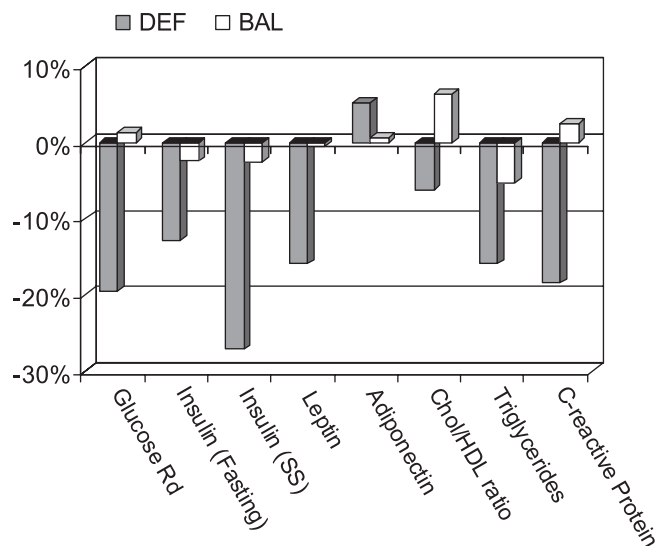

Fig. 4. Summary of changes in selected metabolic variables following exercise training.

working relationship with subjects to maximize motivation and compliance. While any one of these measures alone is insufficient to accurately and precisely measure energy balance (15, 23, 32, 35), the methods in combination bolster our confidence that, as designed, one group was in energy deficit, whereas the other was in energy balance. Results from activity monitors, dietary records, body mass (no change in BAL group, −0.7-kg change in DEF) and plasma leptin assays (decrease in DEF, no change BAL) suggest that we were successful in creating the desired physiological conditions.

In this study, insulin action was assessed by using a continuous infusion of glucose combined with a stable isotope tracer (46). While the hyperinsulinemic, euglycemic clamp offers a more quantitative measure of peripheral insulin sensitivity, the glucose infusion and stable isotope tracer can be used to assess both peripheral and hepatic insulin action because HGP is not completely suppressed. As the effects of prior exercise are manifested in both peripheral and hepatic tissues (34), there is useful information that can be gained regarding the impact of exercise on hepatic, as well as muscle, insulin action. Given the importance of elevated HGP as a primary mediator of impaired fasting glucose (6), the finding that 6 days of exercise significantly improved hepatic insulin action, but only in the DEF group, has potential clinical relevance. The data imply that energy deficit induced by exercise has insulin-sensitizing effects on the liver that are similar in magnitude to those manifested in peripheral (presumed to be mainly skeletal muscle) tissues.

We found that fasting leptin concentrations declined significantly in the DEF group but were unaltered in the BAL group. These results are concordant with other human studies, which generally show that leptin levels change with exercise only when there is a concurrent change in energy balance (36, 49, 51) or energy availability (16). The observation that leptin, a signal of a short-term energy balance as well as an indicator of adipose mass (13), was reduced in the DEF but not the BAL group also helps to confirm that our two groups were truly in different energy states. On the other hand, because increasing dietary CHO can independently result in greater leptin concentrations (31), the results may be a reflection of the differences in CHO intake between groups. In the DEF group, the decrease in circulating leptin, like the enhanced insulin action, preceded a clinically relevant loss of body weight or body fat or increase in cardiorespiratory fitness. The correlation between the two parameters was weak, however, suggesting that there was no causal relationship. The leptin results also suggest that the impact of altering energy balance or CHO availability was manifested in multiple insulin-sensitive tissues: skeletal muscle (i.e., increased glucose uptake), liver (i.e., greater suppression of HGP), and adipose (i.e., decreased fasting leptin).

In summary, 6 days of exercise training, when energy balance was maintained by refeeding high-CHO foods and beverages to match the extra energy expenditure, had no impact on peripheral and hepatic insulin action compared with pretraining baseline. These results contrasted sharply with the 40% improvement in insulin action observed after short-term exercise training when energy expenditure exceeded energy intake by 500 kcal. The divergent responses of the two groups were also evident in positive trends observed in adipokines, CRP, and the traditional risk factors of cardiovascular disease. Given the study design, these findings imply a critical role for either energy or relative CHO deficit (or more likely, both) in mediating exercise-enhanced insulin action. Future studies are needed to determine whether the composition of the postexercise meal, or the timing, alters the response to energy intake. From a practical perspective, our results suggest that preventing the replacement of exercise energy expenditure, particularly from high-CHO foods and beverages, could serve to maximize the metabolic benefits of each individual exercise session.

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