Articles in PresS. J Appl Physiol (August 4, 2005). doi:10.1152/japplphysiol.00291.2005

final accepted version **JAP-00291-2005 – R3**

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

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Running Head: Insulin action, energy balance & exercise

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ABSTRACT:

Background: 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. **Design:** 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 (BAL, n=8) and in the other group, energy was not replaced (DEF, n=8). Insulin action (blood glucose uptake during glucose infusion) and selected lipids and adipokines were measured pre- and post-training. **Results:** Training increased estimated daily energy expenditure by about 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. **Conclusions:** Six days of exercise training without energy replacement significantly increased insulin action. Restoring energy balance by re-feeding 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.

KEY WORDS: insulin resistance, stable isotope, glucose uptake, adipokine, leptin.

INTRODUCTION

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 weeks) 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. However, 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 were noted after the first week of a low-calorie diet as compared to 4 and 12 months later when a considerable amount of body mass and body fat was lost (39). Further, it was recently reported that 10 kg

subcutaneous abdominal fat loss by large-volume liposuction (no energy deficit as subjects maintained pre-surgery 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 expended during exercise opposes the enhanced insulin action observed after short-term exercise training. We compared 2 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 carbohydrate expenditure (BAL). In the other group, exercise energy expenditure was not replaced (DEF). We hypothesized that, because energy and carbohydrate balance are important mediators of insulin action, the 6 days of exercise would be more effective to enhance insulin action in the DEF group as 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 recruited and placed into one of 2 groups to assess the role of energy and carbohydrate 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 2 groups also differed in carbohydrate balance, the DEF group was also "relatively" carbohydrate 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, sex, body composition and physical activity data to create similarly matched groups (Table 1). Subjects were between the ages of 30-60, performed less than ½ hour of exercise a week, and were weight stable (±2 kgs) for 6 months. Subjects exhibited at least 3 of the following risk factors for the insulin resistance syndrome, including overweight to moderately obese (BMI 25-35), waist circumference >80 cm for women and 94 cm for men, a sedentary lifestyle defined as < 0.5 hour 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 BMI range, performed regular endurance exercise, followed very low or very high-carbohydrate diets (<30% or >70% carbohydrate, respectfully), or chronically used antioxidant vitamins (e.g: vitamin E, vitamin C and lipoic acid), anti-inflammatory medicines (e.g: aspirin, non-steroidal anti-inflammatory drugs) or lipid-lowering drugs (e.g: statins and fibrates). The DEF group included 6 females and 2 males while the group in energy balance (BAL) included 5 females and 3 males. Eight of the women were post-menopausal or post-hysterectomy; none used oral contraceptives and 1 used hormone replacement therapy (in BAL group). The study protocol was approved by the Institutional Review Board at the University of Massachusetts, Amherst prior to 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 lab facilities. A Physical Activity Readiness Questionnaire (PAR-Q), a health and fitness history and a record of recent physical activity were completed and reviewed. A diet history and 24-hour dietary recall was obtained to identify typical diet patterns and screen for those on low or high carbohydrate 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 using a flexible measuring tape. One to two days prior to their pre-training assessment of insulin action and again on the day of their post-training measurement (±1 day), body composition (fat mass, fat-free mass, % body fat and % trunk fat) was assessed using dual energy X-ray absorptiometry (DEXA) (Lunar, Madison Wisconsin). Trunk fat was determined from anatomical landmarks and default calculations of the Lunar computer software.

Peak oxygen consumption (VO_2 peak) was estimated 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, Inc., Woodbury, NY) from the last 2 minutes of the test were entered into a regression equation to determine VO_2 peak (VO_2 peak = 92.08 – 0.10 (body mass in pounds) – 0.34 (age in years) + 9.72 (gender; male = 1, female = 2) – 1.01 (walk time in minutes and hundredths of a minute) – 0.13 (walk heart rate in bpm) + 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 (REE) was measured in the morning after an overnight fast using indirect calorimetry to determine caloric requirements for each subject. Subjects sat comfortably in a reclining chair for 30 minutes and then respiratory gases were collected for a minimum of 20 minutes (TrueMax2400 Metabolic Measurement System, Parvomedics, Salt Lake City, UT). Measured REE 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 tri-axial accelerometer (CT-1 activity monitor, Stayhealthy, Inc., Monrovia, CA) to provide a quantifiable measure of physical activity and energy expenditure during weight maintenance and training (15). The CT1 tri-axial accelerometer measures the changes in acceleration in the horizontal, vertical and lateral plane in counts and uses a proprietary formula to determine 24-hour 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 prior to the pre-test of insulin action and for the first 3 days of exercise training. Upon 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, Oregon).

The energy deficit group (DEF) received a diet calculated for weight maintenance requirements that did not account for the exercise-induced increase in energy expenditure

while the group in caloric balance (BAL) received a diet that included additional kcals to replace the energy expended during exercise. 3-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% carbohydrate, 15-18% protein, 26-30% fat (8% saturated fat) and 25-30 grams of dietary fiber, in accordance with the latest Dietary Guidelines for Americans.

All menus were reviewed with each subject and adjustments were made based on tolerance and compliance issues. Subjects reported to the lab each of the 3 days prior to the first assessment of insulin action for daily measurements of body mass and meal pick-up. Subjects were instructed to consume all provided food and drinks. If subjects were unable to finish any food item, they were told to return it to the lab 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 non-caloric 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 hours preceding each test.

The replacement calories for the BAL group were fed in the form of 20 ounces of a carbohydrate-electrolyte beverage consumed during exercise and a combination of fruit, energy bar or smoothie consumed immediately afterwards in our lab to provide adequate kcal to prevent energy deficit. The macronutrient composition for the replacement calories, beverage and snacks combined, was 75% CHO, 13% Prot, and 12% Fat. During

the first 3 days of training when subjects were self-selecting their meals maintaining their usual eating habits, the BAL group received all replacement kcals immediately after exercise whereas during the 3 days of the prepared meals, approximately 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 pre-training tests of insulin action contained identical macronutrient profiles: CHO = 56%, Prot = 15%, Fat = 29%. The same macronutrient profile was maintained for the post-training tests of insulin action in the DEF group but because of the higher carbohydrate content of the replacement calories, the BAL group consumed a slightly higher percentage of carbohydrate (59%) and lower percentage from fat (26%) (DEF = 2246 total kcal, 1258 CHO kcal, 651 Fat kcal; BAL = 2925 total kcal, 1726 CHO kcal, 761 Fat kcal).

Exercise Training:

Each exercise bout was performed under continuous supervision in the lab 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 VO_2 peak for the required time period to expend 500 calories. On the first day of exercise training, treadmill speed and grade were adjusted until subjects reached 60-65% VO_2 peak oxygen consumption as measured by indirect calorimetry. Upon reaching the desired intensity, energy expenditure was measured during 20 minutes of steady-state exercise. Energy expended per minute was calculated from VO_2 and subjects walked for the time necessary to expend 500 kcals. Subjects

unable to reach the target expenditure by 70 minutes of exercise were stopped at that point and actual energy expenditure was calculated. This occurred in 3 subjects total: DEF=2; BAL=1. Replacement calories were adjusted for subjects in the BAL group who could not expend 500 kcals. 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 re-assess workload and time.

All exercise training included 5-minute warm-up and cool-down periods at a self-selected pace. One to 2 short breaks, 1-3 minutes 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, 2 subjects from each group performed 7 consecutive days of exercise rather than 6 so that the last bout occurred 24 hours before the post-training 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 & stable isotopes:

Insulin action was measured the day before training and 24 hours 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-minute infusion of the isotope

tracer followed by a 60-minute 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 minute 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'. At 90-minutes, 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/kg FFM for 60 minutes. Blood samples and respiratory gases were collected at 50, 55, and 60 minutes of the glucose/stable isotope infusion to determine glucose rate of appearance and disappearance 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 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 using radioimmunoassay kits specific for human insulin and adiponectin (Linco Research Inc. St. Charles, MO). CRP was analyzed using a high-sensitivity ELISA assay by ICN Pharmaceuticals (Orangeburg, NY). Plasma triglycerides were determined using an enzymatic colorimetric assay kit (Sigma Chemical, St. Louis, MO) while serum cholesterol and HDL were analyzed using the cholesterol oxidase method (Analox Instruments, Lunenberg, MA). Low-density lipoprotein was calculated using the Friedewald equation (12). Measurement of fasting insulin resistance was calculated using homeostasis model assessment (HOMA) (51), (fasting insulin [mU/l] × fasting glucose [mmol/l]) / 22.5.

Glucose isotopic enrichment was measured by gas chromatography/mass spectrometry (GC/MS). Plasma was deproteinized by adding 0.3 N Zn(SO)4 and 0.3 N Ba(OH)3. Samples were vortexed, incubated in an ice bath, and centrifuged at 4°C at 3300 rpm. The supernatant was extracted and lyophilized (-50 C, 5mTorr) 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 minutes and transferred to clean tubes. Double-distilled water and dichloro methane were added, the tubes were centrifuged and the remaining dichloro methane

phase was transferred to GC vials, evaporated under nitrogen, capped and then reconstituted in ethyl acetate. A 25ul sample was injected into the GCMS 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 unlabelled fragment with that of the enriched isotopomer (Chemstation Software). After correcting for background enrichment, the abundance of the dideuterated isotopomer (m/z = 202) was expressed as percentage of total glucose species (m/z = 200+201+202).

Calculations:

Isotope-derived glucose turnover:

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

$$\text{Glucose rate of disappearance (Rd)} = Ra - V[(C2 - C1) / (t2 - t1)].$$

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, V is the estimated volume of distribution for glucose (180 ml/kg).

Whole body insulin action was defined as Glucose Rd/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) by the glucose infusion = 1- (HGP_{inf}/HGP_{basal})* 100. HGP_{basal} is equal to the basal rate of appearance while HGP_{inf} during the infusion is calculated as: (steady state glucose Ra) – (glucose infusion rate).

Nonoxidative glucose disposal was calculated as: (glucose rate of disappearance) - (total carbohydrate oxidation rate).

Statistical Analyses:

Data were analyzed using SAS, version 8 (SAS Institute Inc, Cary, NC). Differences in subject characteristics between groups prior to training were analyzed using independent *t*-tests. Raw data are presented as means ± sd. Insulin action, glucose kinetics, substrate and hormone variables pre and post-training were compared using a two-way (time x condition) analysis of variance (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 third or fourth 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 kcals vs. BAL 508±40 kcals).

Energy Balance: There was no significant difference between groups in the habitual diet consumed either in total kcal (DEF = 1948±150 kcals; BAL = 2296±179 kcals, p=0.159) or macronutrient intake (DEF = 48.4±2.3% carbohydrate, 16.0±0.7% protein, 32.5±2.1% % fat; BAL = 48.2±1.7% carbohydrate, 17.4±0.8% protein, 31.8±2.1% % fat, p= 0.761, 0.207, 0.889 for carbohydrate, 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 there were no significant changes in kcals or macronutrients in either group.

Accelerometer data indicated that as designed, daily treadmill exercise increased physical activity and total energy expenditure from pre-training in both groups (DEF = 2389±158 kcals pre, 2858±182 during training vs. BAL 2537±149 kcals pre, 3074±169 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 kcals/day) and the BAL

group (+8±20 kcal/day) was very close to that specified in the study design (DEF = -500 kcals/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, body fat % and 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 carbohydrate, protein and fat were all higher in the BAL group (DEF = 2246 ± 97 kcal, 314±14 gm carbohydrate, 84±4 gm protein, 72±3 gm fat; BAL = 2925±159 kcal, 431±24 gm carbohydrate, 110±6 gm protein, 84±5 fat gm CHO).

Plasma Glucose & Insulin: Basal glucose (DEF = 5.4±0.2 mM pre, 5.5±0.1 mM post vs. BAL 5.5±0.2 mM pre, 5.5±0.2 mM post) and steady-state glucose (SSPG) (DEF = 10.0±0.3 mM pre, 10.3±0.3 mM post vs. BAL 10.4±0.5 mM pre, 10.6±0.3 mM post) 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 (Figure 1). There was no change in either fasting insulin concentrations or SSPI in the BAL group.

Glucose Turnover and Insulin Action: Basal hepatic glucose production (HGP_{basal}) and glucose rate of disappearance (Rd) were not different between groups before or after training (Table 4). During the infusion, glucose Rd 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) (Figure 2). Insulin action, defined as glucose Rd per unit of SSPI, was significantly increased in the energy deficit group (+40.3%, p=0.032) and was unchanged

in the BAL group (-8.4%, p = 0.107). Since there was no change in the rate of glucose oxidation after training, the increase in glucose Rd in the DEF group was completely accounted for by an increase in non-oxidative glucose disposal (p=0.011). There was no change in either oxidative or non-oxidative 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, hepatic glucose production during the infusion (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 % suppression of basal HGP during the infusion, was significantly increased only in the energy deficit group (p=0.015) (Figure 3).

The increased glucose Rd 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 pre-training concentrations and to 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 energy deficit group (Figure 4).

Correlations: Insulin action was inversely correlated with an increase in dietary energy (-0.597, p=0.015) and dietary carbohydrate (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 body fat %, r= 0.924, p<0.001 and age, r=0.814, p< 0.001 but inversely correlated with VO_2peak, r= -0.903, p< 0.001. Baseline CRP concentrations were directly correlated with age (r=0.580, r= 0.019) and trunk fat (r=0.5739, p= 0.020) and inversely correlated with VO_2peak (r=-0.687, p=0.003).

DISCUSSION:

Short-term exercise training (from 1-10 days) increases insulin action, when measured within 24 hours 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 post-training measurements of insulin action were made. The current study was designed to test the role of restoring energy balance, by replacing expended energy and carbohydrate, in mediating the

response to short-term exercise training by systematically comparing a group in energy (and in a relative sense, carbohydrate) 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 only in the energy deficient group. When dietary energy was replaced immediately post-exercise using high-carbohydrate foods to restore daily energy balance, there was no change in peripheral or hepatic insulin action. The clear distinction between the 2 states of energy balance suggests that adding back the energy and carbohydrate expended during exercise negates exercise-induced enhancement of insulin action.

We anticipated that re-feeding the total and carbohydrate 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 (12-14 week 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 current study, Segal et al. added dietary energy, in the form of a carbohydrate-supplement, equivalent to the energy expended during each training session (45). Following cycle ergometry training for 12 weeks, the authors reported peripheral insulin sensitivity was not enhanced (although basal hepatic glucose production 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 approximately 60 minutes per day, 5 days per week for 14 weeks but, by design, increased dietary intake to maintain a stable body mass. In all 3 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 current study are novel since they show that even the residual effects of exercise, measured 18-24 hours post-exercise, are negated when energy intake matches energy expenditure with dietary composition held constant.

A plausible explanation for our observations is that differences in carbohydrate availability, rather than energy per se, play a role in mediating the difference between the 2 states of energy balance. Results from several studies indicate that glycogen re-synthesis accounts for much of the increased glucose uptake observed after glycogen-depleting exercise (3, 26, 52). Post-exercise carbohydrate 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 carbohydrate 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 carbohydrate availability is supported by data from 2 recent human studies in which investigators manipulated energy replacement but held carbohydrate intake constant after glycogen-depleting exercise (11, 44). Fox et al. 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 carbohydrate intake

was constant (11). Similar results were reported by Schenk et al. when carbohydrate 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 IVGTT (44). Sparti and Decombaz noted the opposite pattern however, when insulin action was estimated 36 hours after either a high-carbohydrate or low-carbohydrate diet following a single high-intensity exercise bout in healthy males (47). In that study, the integrated insulin area under the curve was significantly lower than baseline after the high-carbohydrate but not the low-carbohydrate dietary intervention. Further support for an independent effect of perturbing energy balance on substrate metabolism was recently reported by Horowitz et al. who found that carbohydrate oxidation was lower and lipid oxidation higher the day after glycogen-depleting exercise when in energy deficit rather than energy balance, even with carbohydrate intake held constant (17).

Because of the particular question the current study was designed to address, we cannot distinguish the relative importance of energy or carbohydrate 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 in order to replace exercise energy expenditure, the two groups also consumed different amounts of carbohydrate during training (DEF = ~4 g/kg body mass; BAL = ~5 g/kg). To power the modest exercise dose of 500 kcals/day expended at 60-65% VO2 peak, estimated muscle glycogen use (based on total carbohydrate oxidation

from indirect calorimetry and assuming 80% of total carbohydrate oxidation attributable to muscle glycogen) was approximately 80 grams per daily exercise session. Although not measured directly, this glycogen output could have been replaced by the daily carbohydrate intake in both groups (>55% of total kcal, DEF= 314±14 g; BAL = 431±24 g) (33). It is possible though that the timing of the post-exercise energy intake relative to the bout of exercise may have played a role in the differential response between our 2 groups. We fed the replacement energy to the BAL group immediately post-exercise to minimize differences in meal timing as a potential source of interindividual variability. The immediate provision of the high-carbohydrate replacement calories could have differentially affected the rate of glycogen repletion since glycogen synthesis is greatest 0-2 hours post-exercise (38) and carbohydrate 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 carbohydrate balance. The strong links between glycogen depletion and glucose uptake, the recent studies showing that energy replacement as pure lipid may not oppose post-exercise insulin action, and the positive correlation we found between glucose Rd and carbohydrate intake, suggest that that carbohydrate availability is one of key mediators of post-exercise 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 post-exercise insulin action. Further systematic studies are needed to disentangle the relative roles of energy and carbohydrate 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 kcals) 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 post-training 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 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 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 hepatic glucose production 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 hepatic glucose production 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, 50) 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 2 groups were truly in different energy states. On the other hand, since increasing dietary carbohydrate can independently result in greater leptin concentrations (31), the results may be a reflection of the differences in carbohydrate 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 2 parameters was weak however, suggesting that there was no causal relationship. The leptin results also suggest that the impact of altering energy balance or carbohydrate availability was manifested in multiple insulin-sensitive tissues: skeletal muscle (i.e.: increased glucose uptake), liver (i.e.: greater suppression of hepatic glucose production) and adipose (i.e.: decreased fasting leptin).

In summary, 6 days of exercise training, when energy balance was maintained by re-feeding high-carbohydrate foods and beverages to match the extra energy expenditure, had no impact on peripheral and hepatic insulin action when compared to pre-training 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 2 groups were also evident in positive trends observed in adipokines, C-reactive protein and the traditional risk factors of cardiovascular disease. Given the study design, these findings imply a critical role for either energy or relative carbohydrate deficit (or more likely, both) in mediating exercise-

enhanced insulin action. Future studies are needed to determine if the composition of the post-exercise 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-carbohydrate foods and beverages, could serve to maximize the metabolic benefits of each individual exercise session.

Acknowledgments

We are grateful to the devoted research subjects, to Carrie Sharoff, Todd Hagobian, M.S., Laura Gerson, Rebecca Hasson and Sayosola Shasanya for their critical technical support, to Miki Takeda, RN and Christine Pikul, RN for their superb care of the subjects and to Steven Petsch, PhD, for assistance with the gas chromatography/mass spectrometry analysis. Thank you to Tracy Horton, Ph.D. and Michael Pagliassotti, Ph.D. for helpful discussions. This work was supported by a grant from the Glass Family Trust.

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Tables

Table 1: Subject characteristics in energy deficit (DEF) and energy balance (BAL) groups (mean ± SD)

	Energy Deficit (DEF) n = 8 (6 F, 2 M)	**Energy Balance (BAL)** n = 8 (5 F, 3 M)	**p value =**
Age (y)	49.0 ± 5.1	46.4 ± 11.4	0.552
Body Mass (kg)	79.8 ± 10.4	85.4 ± 9.8	0.303
BMI	29.4 ± 1.0	30.5 ± 3.7	0.541
VO_2peak (ml/kg/min)	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

Table 2: Training data collected on training day 3 or 4 in energy deficit (DEF) and energy balance (BAL) groups (mean ± SE).

	Energy Deficit (DEF)	Energy Balance (BAL)	p value =
VO_2 (L/min)	1.5 ± 0.3	1.7 ± 0.4	0.308
VO_2 (ml/kg/min)	19.0 ± 1.9	19.5 ± 3.4	0.775
Minutes on Treadmill	65.6 ± 4.7	61.6 ± 7.8	0.280
Exercise EE (kcal)	481.1 ± 32.6	507.5 ± 39.9	0.143
HR last 20' (bpm)	135.4 ± 1.6	136.3 ± 0.9	0.611
RPE	13.4 ± 0.1	13.1 ± 0.2	0.267
METS (ml/kg/min)	6.2 ± 0.3	6.2 ± 0.4	0.983

Table 3: Energy balance during 6 days of exercise training (mean ± SE). Energy ingested reflects mean of self-recorded food journals, days 1-3, and meals from laboratory, days 4-6. Estimated energy expenditure measured using indirect calorimetry and calculated from resting (measured during weight maintenance period) and exercise energy expenditure (measured on day 3 or 4).

	Energy Deficit (DEF)	**Energy Balance (BAL)**
Energy Ingested (kcal)	2246 ± 97	2925 ± 159
CHO g (%)	314 ± 14 (56%)	431 ± 24 (59%)
Prot g (%)	84 ± 4 (15%)	110 ± 6 (15%)
Fat g (%)	72 ± 3 (29%)	84 ± 5 (26%)
Estimated Energy Expenditure (kcal)	2727 ± 182	2917 ± 169
Energy Balance (kcal)	-481 ± 24	+8 ± 20
Body Mass Change (kg)	-0.62 ± 0.2	+0.03 ± 0.2
Wt Change as % of Body Mass	-0.8 ± 0.2%	0.04 ± 0.1%

Table 4: Glucose turnover data before and after 6 days of exercise training in energy deficit (DEF) and energy balance (BAL) groups (mean ± SE)

	Energy Deficit (DEF)					**Energy Balance (BAL)**				
	Pre	Post	Δ	95% CI	p value	Pre	Post	Δ	95% CI	P
HGP_{basal} (uM/kgFFM/min)	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
$HGP_{infusion}$ (uM/kgFFM/min)	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
$Glucose_{basal}$ Rd (uM/kgFFM/min)	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
$Glucose_{Infusion}$ Rd (uM/kgFFM/min)	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
Non-oxidative	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 *(Rd/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
EE 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

Table 5: Lipids, Adipokines and C-reactive protein (mean ± SE)

	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 Chol (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, .01	0.049*	14.0±3.1	14.0±3.2	-0.1	-1.09, 0.96	0.883

Figures

Figure 1:

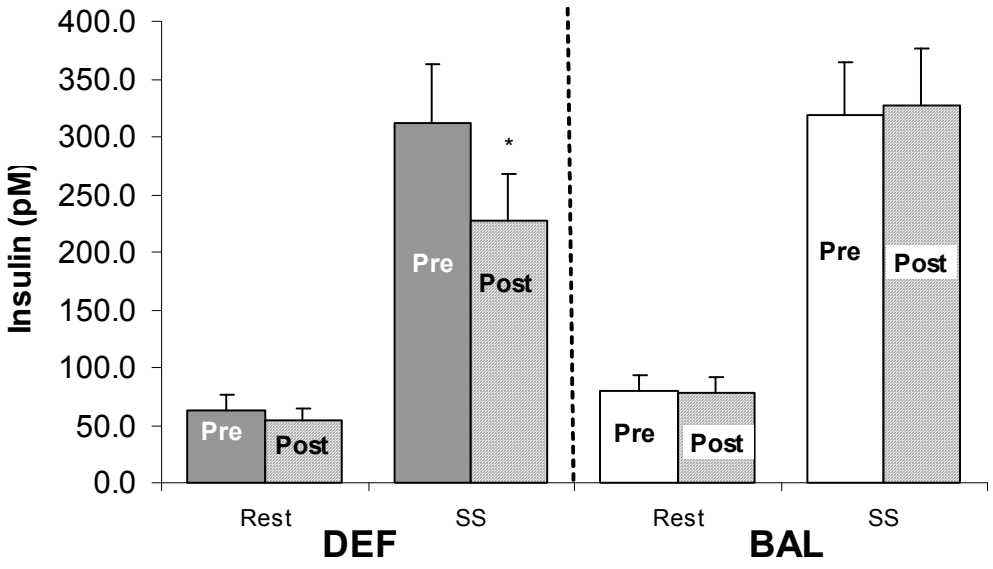
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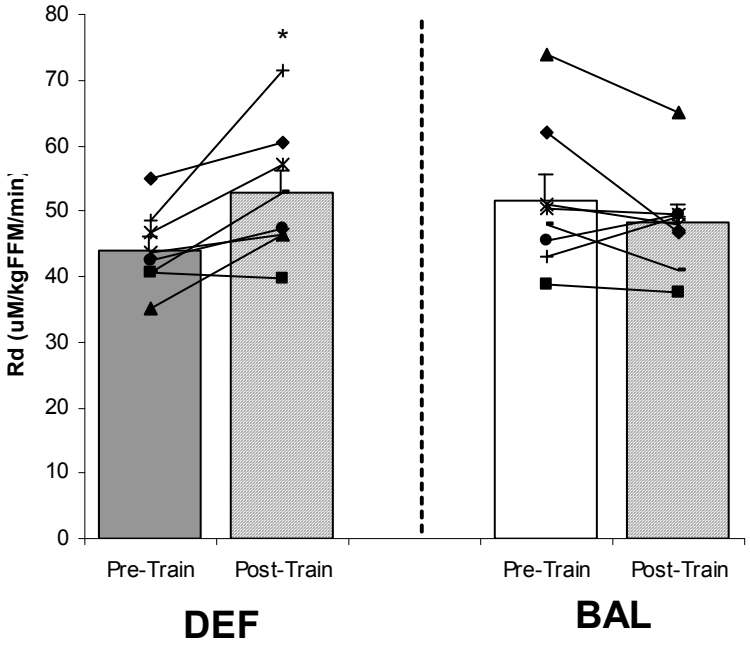
Figure 2:



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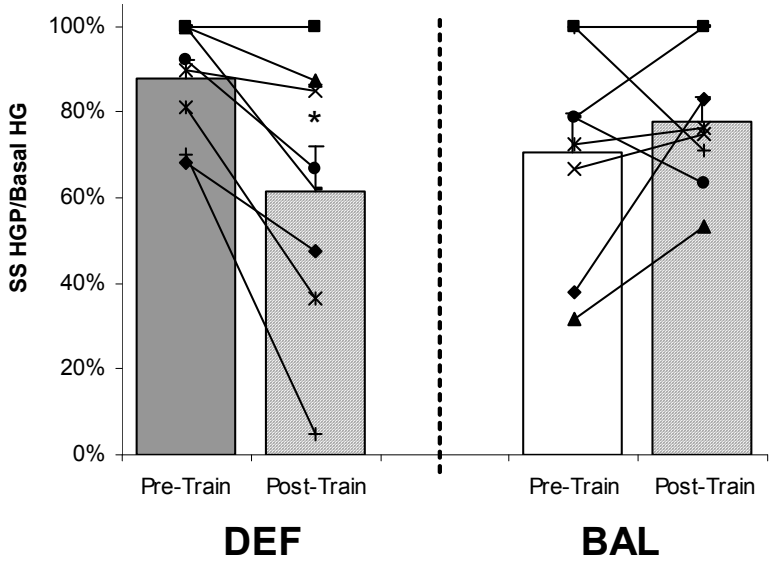

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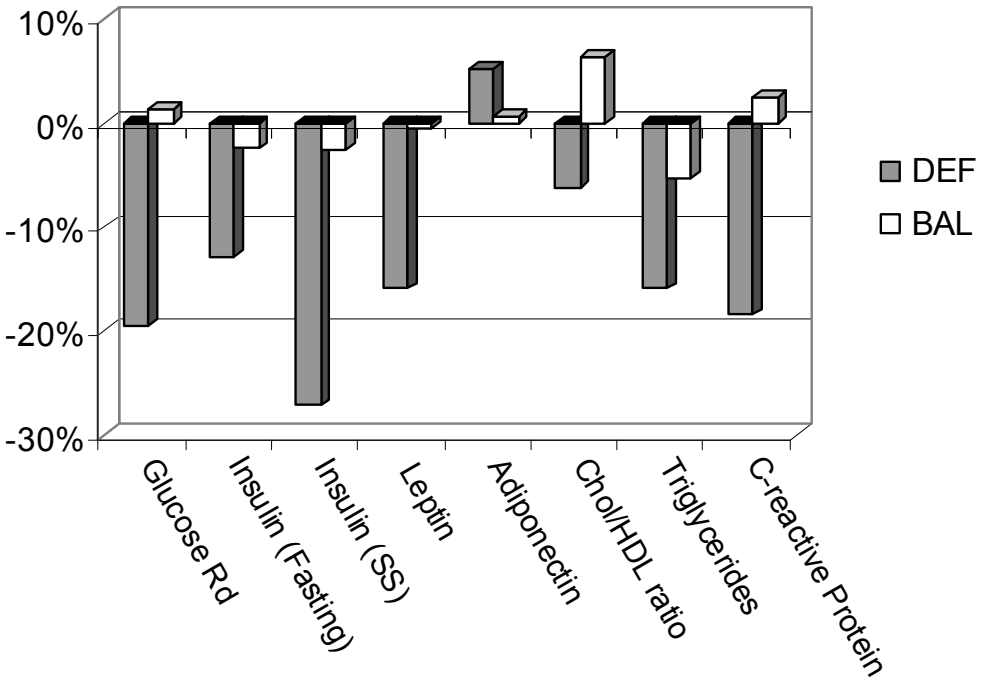
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DEF
BAL
Glucose Rd
Insulin (Fasting)
Insulin (SS)
Leptin
Adiponectin
Chol/HDL ratio
Triglycerides
C-reactive Protein