Effects of insulin resistance on substrate utilization during exercise in overweight women

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Braun, Barry, Carrie Sharoff, Stuart R. Chipkin, and Francesca Beaudoin. Effects of insulin resistance on substrate utilization during exercise in overweight women. J Appl Physiol 97: 991–997, 2004. First published May 7, 2004; 10.1152/japplphysiol.00231.2004.—During exercise, obese individuals oxidize less glyco- gen and more fat than their lean counterparts, but the shift in substrate use may be mediated by insulin resistance rather than body fat per se. In addition, individuals with Type 2 diabetes are not resistant to contraction-mediated glucose uptake during exercise, but in vivo studies uncomplicated by hyperglycemia are lacking. The purpose of this study was to compare blood glucose uptake and the balance between carbohydrate and fat utilization during exercise in insulin-resistant (IR) and insulin-sensitive (IS) women of equivalent body fat, spares muscle glycogen and shifts substrate oxidation toward less carbohydrate use during exercise. Insulin-resistant individuals with normoglycemia appear to have no defect in blood glucose uptake during exercise.

OBESITY ALTERS THE REGULATION of metabolic pathways that mediate energy storage and expenditure (1, 7, 17, 24, 28). In the postabsorptive state, obese individuals oxidize less fat and more carbohydrate compared with their lean counterparts (7, 17, 28). The impact of obesity on substrate utilization during exercise has been less clearly demonstrated. Researchers have reported that obesity biases exercise substrate use toward greater carbohydrate oxidation (40), no change in carbohydrate oxidation (14), or less carbohydrate oxidation (6, 17). Colberg et al. (6) reported that, compared with lean men, the rate of fat use tended to be higher and the rate of glycogen use tended to be lower in men who were obese but not diabetic. Recently, Goodpaster et al. (17) directly addressed the role of obesity in exercise substrate use by comparing lean men with a group of obese men matched for age, maximal aerobic capacity, and habitual physical activity. Relative to lean subjects, obese individuals oxidized 50% less muscle glycogen during submaximal exercise and the portion of energy derived from fatty acids was significantly higher. These results imply that obesity shifts exercise substrate use in the direction of less muscle glycogen oxidation and greater oxidation of fatty acids. It is likely that the obese group in that study was somewhat insulin resistant (fasting plasma insulin concentrations were 3-fold greater than in the lean group). Therefore, the two groups differed on two potentially important variables: obesity and insulin resistance. It is possible that insulin resistance, not body fat per se, is responsible for the shift toward less glycogen and more fat use during exercise in obese individuals.

Insulin resistance is characterized by a reduced capacity for tissues (mainly skeletal muscle) to take up glucose from the blood at a given physiological insulin concentration (9). Because skeletal muscle can take up glucose from the blood via an alternate (contraction-mediated) pathway, it is a promising mode of treatment for individuals who are insulin resistant (18, 19, 22, 28). A series of studies, mainly attributable to the Goodyear research group, demonstrate that the two pathways involve distinctly different molecular signaling events (18, 19, 38). Studies in vitro indicate that exercise-mediated glucose transport is normal in muscle isolated from insulin-resistant humans with diabetes (29, 41). Studies in vivo are less clear. In obese individuals with Type 2 diabetes, whole body glucose uptake during exercise was higher in men with Type 2 diabetes than in obese and lean men without diabetes (6). Similarly, Blaak et al. (2) found that total carbohydrate oxidation was higher in obese men with Type 2 diabetes compared with equally obese men who were normoglycemic. The diabetic subjects in these two studies were hyperglycemic, and blood glucose movement from blood to tissues could have been enhanced by mass action. The impact of insulin resistance, not confounded by concomitant hyperglycemia, on blood glucose uptake during exercise in vivo has not been directly assessed.

Therefore, the present project was designed to assess whether insulin resistance, uncomplicated by differences in body composition or hyperglycemia, would alter blood glucose uptake and substrate utilization during exercise. To that end, exercise substrate use was measured in a group of untrained, overweight or obese, insulin-resistant women and a group of women of similar age, weight, body composition, habitual physical activity, and maximal aerobic capacity who were insulin sensitive.
Body composition, habitual physical activity, and cardiovascular fitness (Table 1).

\( V_{O_2 \text{ peak}} \) was determined by an incremental treadmill walking test. Women started walking at a self-selected pace, and the grade of the treadmill was increased every 2 min until subjects could no longer maintain the required pace. Results were used to set the appropriate level of physical work during the submaximal exercise protocol.

Control of diet, activity, and menstrual cycle phase. To control for potentially confounding effects of vastly different estrogen and progesterone concentrations (10), subjects were tested in the early to mid follicular phase (days 5–10) of the menstrual cycle. Subjects refrained from exercise for 24 h before testing. Although diet was not rigidly controlled in the days before the study, all subjects consumed the same preexercise snack the evening before each test (35% of estimated daily energy requirements, composed of 55% carbohydrate, 15% protein, and 30% fat). The Harris-Benedict equation was used to calculate resting metabolic rate, and this value was multiplied by an activity factor of 1.4 (assuming women in this study were relatively sedentary) to estimate daily energy requirements (3). Subjects were instructed to fast after this meal until testing was completed.

Testing procedures. Subjects reported to the laboratory in the morning, after an overnight fast, and a catheter was inserted into an antecubital vein for infusion of glucose stable isotope. A second catheter was placed in a forearm or wrist vein of the contralateral arm for blood sampling. A venous blood sample was collected before infusion for determination of background isotopic enrichment, and a priming bolus of 200 mg [6,6\(^2\)H]glucose in 0.9% sterile saline was then rapidly infused into the venous catheter. To reach and maintain isotopic equilibrium, [6,6\(^2\)H]glucose was then continuously infused at 2.5 mg/min by a peristaltic infusion pump (Harvard Apparatus, South Natick, MA). Venous blood samples and 5-min collections of expired oxygen and carbon dioxide were taken at rest 75 and 90 min after the start of the infusion.

Immediately after the last resting measurement, the subject began submaximal exercise by walking on a motorized treadmill (LifeStride 9100, LifeFitness, Chicago, IL). To maintain a steady isotopic enrichment of blood glucose, [6,6\(^2\)H]glucose infusion rate was increased to 6.0 mg/min. During the first 15 min of exercise, the intensity was adjusted by manipulating the treadmill speed and grade until oxygen consumption reached a steady state at \( \sim 45\% \) of the previously measured \( V_{O_2 \text{ peak}} \). Blood and breath samples (5-min) were collected at 15, 30, 40, and 50 min of exercise (see Fig. 1).

Biochemical assays. Samples of venous blood for analysis of glucose, lactate, insulin, and glucose isotopic enrichment were collected in heparinized syringes and then transferred to vacutainers containing sodium fluoride (to inhibit glycolysis). Samples for analysis of free fatty acids were collected in heparinized syringes and then transferred to vacutainers containing sodium fluoride. All samples were immediately centrifuged, and the plasma was transferred to cryogenic vials and frozen at \(-80^\circ\text{C}\) until analysis. Plasma glucose and lactate concentrations were determined enzymatically by using a glucose-lactate analyzer (GLS Analyzer, Analox Instruments, Lunenberg, Massachusetts).
was extracted, and water was removed via freeze-drying (10 min, and centrifuged at 4°C for 20 min at 2,000 rpm. The supernatant was deproteinized by adding 1.2 ml of 0.3 N Zn(SO)₄ and 1.2 ml of a 2:1 acetic anhydride-pyridine mixture, capped, heated in a water bath at 60°C for 60 min, and transferred to clean 13 × 100-mm borosilicate tubes. Double-distilled water (1.5 ml) and 0.4 ml of dichloromethane were added in that order, and the tubes were centrifuged at 2,000 rpm for 10 min. The dichloromethane phase was transferred to a 1-ml gas chromatography vial and evaporated under nitrogen. Tubes were capped, and 25 μl of ethyl acetate were added by use of a gas-tight syringe. A 1-μl sample of the penta-acetate derivative was injected and separated on a gas chromatograph, and spectra were 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 we corrected for background enrichment, the abundance of the deuterated isotopomer (m/z = 202) was expressed as percentage of total glucose species (m/z = 200 + 201 + 202).

Calculations. Standard equations were used to calculate the rate at which glucose is taken up from the blood (rate of disappearance, R_d) and replaced by the liver (rate of appearance, R_a):

\[
glucose R_a (mg/min) = \frac{F - V[(C_1 + C_2)/2][(IE_2 - IE_1)/(t_2 - t_1)]}{[(IE_2 + IE_1)/2]}
\]

\[
glucose R_d (mg/min) = R_a - V[(C_1 - C_2)/(t_2 - t_1)]
\]

F represents the isotopic infusion rate, IE_1 and IE_2 are the enrichments of plasma glucose with deuterated glucose at time points t_1 and t_2, respectively, C_1 and C_2 are the concentrations of plasma glucose at t_1 and t_2, and V is the estimated volume of distribution for glucose (180 ml/kg).

Percent of energy from carbohydrate was determined from total respiratory exchange ratio (RER) as

\[
[\text{RER} - 0.71]/0.29 \times 100
\]

Carbohydrate (CHO) oxidation rate (in mg/min) was determined by

\[
[\%\text{CHO}/100] \times (V_{O_2} \text{ in l/min}) \times (5.05 \text{ kcal/l})/4.0 \text{ g/kcal}
\]

An estimate of muscle glycogen utilization was determined by

\[
(\text{total carbohydrate oxidation}) - (\text{blood glucose } R_d)
\]

This estimate is based on the assumption that 100% of blood glucose taken up from the blood is oxidized. This assumption is unlikely to be true; i.e., the percent of R_a oxidized is probably 70–90% (12, 23) but may vary across the conditions used in this study; therefore, the calculation underestimates glycogen use and is best described as minimal muscle glycogen utilization.

Statistical analysis. All data in tables and figures are presented as group means and 95% confidence intervals except where noted. Descriptive data characterizing the two subject groups were compared by unpaired t-tests. The summary data were analyzed as raw data by ANOVA with repeated measures using the PROC-MIXED univariate analysis for all variables (SAS Institute, Cary, NC). Statistical significance was defined as α < 0.05. Post hoc analyses with planned comparisons were made using Fischer’s protected least square differences.
The contribution of non-blood glucose carbohydrates (i.e., muscle glycogen) to EEE was considerably lower (by almost 50%) in the IR group (23.3% of total) compared with the IS group (35.7% of total). Lipid contribution to EEE was 28% higher in the IR group (56.1% of total) compared with the IS group (44.0% of total).

DISCUSSION

The present study was designed to gain additional insight into factors mediating the obesity-related shift in the balance of fat and carbohydrate use during exercise. Specifically, we addressed the hypothesis that underlying insulin resistance, rather than excess body fat per se, would reduce dependence on non-plasma-derived glucose (i.e., muscle glycogen). Consistent with this hypothesis, the primary finding was that the proportion of EEE derived from nonplasma glucose (i.e., muscle glycogen) was lower in obese or overweight, insulin-resistant individuals compared with equally obese, insulin-sensitive individuals. In addition, on the basis of in vitro studies (29, 38, 41) and in vivo studies of humans with Type 2 diabetes (2, 6), we predicted that insulin resistance would not impair blood glucose uptake during exercise. Supporting that hypothesis, we found that blood glucose use in the IR group was almost identical to that of an IS group matched for body fat, lean body mass, aerobic capacity, and habitual physical activity.

A potential interpretation of the present findings is that the shift in exercise substrate use in obese individuals is entirely attributable to underlying insulin resistance and not the presence of excess body fat. A problem with reaching for this conclusion is that, although the two groups of subjects were
relatively well matched on several important variables, other potentially key characteristics were not as carefully controlled. There are ample data showing that body fat stored in the abdominal region is more metabolically "active" and much more closely associated with insulin resistance than subcutaneous fat stored gluteofemorally (5, 16, 24). The metabolic effects of visceral adiposity could have contributed to the observed shift in substrate use indirectly, via insulin resistance, or directly, with insulin resistance as a "symptom" rather than as part of the causal pathway. It is possible that the insulin-resistant subjects in the present study had more visceral adiposity than those who were insulin sensitive. In a manner similar to Carey et al. (5), we used regional information from the dual-energy X-ray absorptiometry scans to make an indirect estimate of body fat distribution. In our study population, the proportion of total body fat contained in the abdominal area was similar between the IR (53.9%) and IS (51.5%) groups. Without a direct measurement of visceral fat using computed tomography or MRI scans, however, we cannot conclude that there were no between-group differences in visceral adiposity.

Even if some aspect of insulin resistance does explain, at least partially, the observed shift in exercise substrate use, it is not possible to determine whether the effect is direct (e.g., insulin resistance impacts regulation of glycolytic and/or lipolytic pathways during exercise) or an indirect consequence of altered nutrient storage. Researchers have repeatedly shown that the size of intramyocellular lipid stores is positively correlated with insulin resistance (15, 27, 30, 33, 35). Conversely, insulin resistance has been associated with decreased muscle glycogen stores (30). Therefore, the pattern of substrate use that we observed (increased fat use, reduced muscle glycogen use) could be a downstream consequence of initial muscle substrate levels. Because the rate of glycogen use is directly related to the glycogen concentration (37), if our insulin-resistant subjects began exercise with lower initial glycogen stores than the IS group, a lower rate of glycogen use would not be surprising. We attempted to minimize individual differences in muscle glycogen stores by providing each sub-
ject with a standardized meal containing 35% of estimated daily energy requirements with 55% of energy from carbohydrate (>120 g of carbohydrate) the night before testing. Without measurement of glycogen stores via muscle biopsy or 13C-magnetic resonance spectroscopy, however, the possibility that the impact of insulin resistance was indirectly mediated by low initial muscle glycogen stores cannot be discounted.

The potential role played by intramyocellular lipid (IMCL) in generating the observed results is complicated by the probability that elevated levels of IMCL are both a cause and a consequence of insulin resistance (16, 20, 21, 33; but see Refs. 4 and 13 for conflicting evidence). The likelihood that IMCL concentrations were higher in the IR group could be manifested in the pattern of substrate use we observed. It has been shown several times that reliance on plasma-derived fatty acids is compromised in the obese compared with the lean (1, 2, 17), suggesting that our IR group may have been more reliant on IMCL. Goodpaster et al. (17) reported that plasma fatty acid use in their obese and lean groups was the same, implying that higher rates of fat oxidation during exercise in the obese were entirely attributable to increased utilization of intramyocellular lipid. Similarly, Blaak et al. (2) showed that obesity induced a change in the balance between oxidation of blood-borne fatty acids vs. other lipids. If, like glycogen, elevated initial stores of IMCL translate to increased use during exercise, acetyl-CoA derived from IMCL could displace acetyl-CoA derived from glycogen (akin to an intracellular “glucose-fatty acid cycle”) and reduce reliance on muscle glycogen.

An effect of insulin resistance to directly alter metabolic pathways to bias exercise substrate use toward greater lipid oxidation might appear paradoxical, given that the opposite pattern is observed at rest (16, 28). It has been postulated that, in the postabsorptive state, impaired fat oxidation is directly or indirectly linked with excess storage of intramyocellular fat (20, 28). During exercise, however, those metabolic restrictions may not be operational. Without direct measurements of the activity of key energy-sensing enzymes (e.g., AMP-activated protein kinase, acetyl CoA carboxylase, etc.) and/or concentrations of critical metabolites (e.g., malonyl CoA), the effects of insulin resistance on those pathways during exercise remain relatively unexplored (but see Ref. 8). Regardless of mechanism, one of the great benefits of exercise in obese, insulin-resistant individuals may be to oppose the impaired ability to oxidize fat in the resting state, promoting utilization of excess lipid stores and partially restoring normal sensitivity to insulin.

The finding that individuals with insulin resistance have no impairment in the rate of blood glucose uptake during exercise conforms in vitro work strongly suggesting that the insulin-mediated and contraction-mediated pathways of glucose uptake are separate. Colberg et al. (6) found that obese individuals with Type 2 diabetes had elevated, not reduced, rates of blood glucose uptake during exercise. These results may be partially explained by a potentiation of blood glucose uptake in response to a mass action effect of hyperglycemia in this population. Group differences in body composition, fitness, or habitual training could also obscure the effect of insulin resistance per se. In the present study, insulin-resistant but normoglycemic individuals showed no impairment in blood glucose uptake compared with individuals with similar body composition and fitness. Although several potentially confounding covariates (relative exercise intensity, body composition, aerobic capacity, habitual activity, lean body mass) were controlled in the present study, there was no way to account for the possible impact of hyperinsulinemia in the IR group. Plasma insulin levels were twofold higher in the IR group throughout exercise compared with the IS group. Because insulin and exercise have additive effects on skeletal muscle glucose transport (18, 19, 38), it is possible that any impairment in exercise-mediated glucose transport in the resistant group was masked by greater insulin-mediated transport. Although the elevated plasma insulin levels in the IR group do not accentuate glucose transport at rest or in response to an oral or intravenous glucose challenge (by definition), it is possible that skeletal muscle cells become less resistant to insulin action during exercise so that the added insulin effectively stimulates greater glucose transport. It is well known that insulin action is enhanced in previously exercised muscle (3, 21, 22, 26, 32), and there is no question that the addition of exogenous insulin (e.g., in Type 1 diabetes) will potentiate glucose uptake during exercise (18, 19, 38). Two observations suggest that the higher plasma insulin levels in the resistant group were not important contributors to blood glucose uptake during exercise. First, if the extra insulin was effectively stimulating cellular responses, total carbohydrate oxidation would be elevated, not depressed in this group. Second, free fatty acid levels would be expected to decline in the resistant group if their elevated insulin levels were becoming more “effective,” at least in adipose tissue. But plasma concentrations of free fatty acids and the pattern of change over time were very similar in both groups.

The observation that insulin resistance shifts exercise substrate use away from glycogen utilization and toward greater fat use may serve to explain prior results in which exercise carbohydrate utilization has been higher (25, 34), lower (39), and the same (1, 39) after exercise training in obese individuals. As insulin sensitivity increases in response to an intervention (but, despite some weight loss, subjects are still obese), the pattern of substrate use would shift toward what we observed in the obese, IS group. As training continues and subjects gain the adaptations (e.g., greater mitochondrial mass and activity of enzymes regulating B-oxidation) associated with long-term endurance training (36), substrate use might be expected to shift back to a greater reliance on fat and thrifter use of glycogen. Depending on the length and intensity of exercise training, the magnitude of weight loss, and the initial degree of insulin resistance and fitness in the study population, the relative balance between the two opposing forces could generate almost any outcome in terms of a shift in carbohydrate and fat oxidation.

In addition to interest as a basic research question, there is potential clinical relevance to the reported results. Exercise, along with dietary modification and pharmacology, is considered a “cornerstone” of treatment for individuals with diabetes mellitus, but its utility is somewhat limited by a poor understanding of its mechanism of action. The effectiveness of exercise in the prevention and management of Type 2 diabetes depends, to some extent, on the degree to which insulin-resistant individuals are able to utilize alternate pathways to stimulate glucose uptake. Our findings, in conjunction with work done by others, strongly suggest that effect of exercise to stimulate clearance of glucose from the blood is completely unimpaired in people with insulin resistance. In addition, our
findings suggest that the enhanced fat oxidation during exercise may favorably affect the balance between lipid storage and oxidation and help to partially restore insulin sensitivity in obese, insulin-resistant individuals.

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