Metabolic Inflexibility during Submaximal Aerobic Exercise Is Associated with Glucose Intolerance in Obese Older Adults

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Objective: People with type 2 diabetes have reduced cardiorespiratory fitness and metabolic impairments that are linked to obesity and often occur prior to the development of type 2 diabetes. We hypothesized that obese, older adults with impaired glucose tolerance (IGT) have lower ability to shift from fat to carbohydrate oxidation when transitioning from rest to submaximal exercise than normal glucose tolerant (NGT) controls.

Design and Methods: Glucose tolerance, body composition, and substrate oxidation (measured by RER: respiratory exchange ratio) during submaximal exercise (50% and 60% VO2max) and insulin infusion (3-hour hyperinsulinic–euglycemic clamp) were assessed in 23 sedentary, overweight-obese, older men and women.

Results: Obese subjects with NGT (n = 13) and IGT (n = 10) had similar resting RER, but during submaximal exercise those with IGT had a lower RER and less transition to carbohydrate oxidation than the NGT group (P < 0.05). The IGT group also oxidized less carbohydrate during insulin infusion than NGT (P < 0.05). RER at each exercise intensity independently correlated with 120-minute postprandial glucose (r = 0.54 to 0.58, P < 0.05), but not with body composition, VO2max, or RER during insulin infusion.

Conclusions: Obese, older adults have metabolic inflexibility during exercise that is associated with the degree of glucose intolerance independent of age and body composition.

Introduction

Over 26% of older Americans have diagnosed or undiagnosed impaired glucose tolerance (IGT) (1), placing them at high risk for development of type 2 diabetes. Obesity is a major risk factor for IGT and type 2 diabetes, and is often accompanied by metabolic dysfunction such as abnormal fat and carbohydrate oxidation (2). These impairments may contribute to metabolic inflexibility, previously defined as the inability to switch from fat to carbohydrate oxidation in response to a meal or insulin administration (2). Obese, insulin-resistant individuals and those with IGT are metabolically inflexible in response to insulin infusion, while lean, insulin-sensitive subjects are metabolically flexible (2,3). The concept of metabolic inflexibility also may extend to metabolism during aerobic exercise, wherein the normal response in the fasted state is to shift from utilizing fat to carbohydrate during the transition from rest to exercise of increasing intensity. Because fat cannot be oxidized at high enough rates to supply all of the energy for moderate to vigorous exercise, this shift from fat to carbohydrate oxidation supplies the necessary energy as exercise intensity increases (4). Previous studies show lower cardiorespiratory fitness levels in type 2 diabetes (5), and this may extend to obese, older adults with metabolic inflexibility and IGT.

Middle-aged and older, overweight-obese subjects with IGT often have metabolic abnormalities such as impaired glucose uptake in response to insulin, and also have lower glycogen content in skeletal muscle and higher intramyocellular lipid levels in the postabsorptive state. These metabolic abnormalities may affect the ability to switch from fat to carbohydrate oxidation when going from rest to exercise of increasing intensity. The results of studies examining substrate oxidation during exercise in obese young and middle-aged subjects vary (6-9), but two studies in insulin-resistant subjects report lower...
carbohydrate oxidation during exercise in young insulin-resistant women (10) and middle-aged subjects with type 2 diabetes (6). While these studies indicate that abnormalities in fat and carbohydrate oxidation during exercise are related to obesity and/or insulin resistance, the metabolic response to exercise of increasing intensity has not been established in obese, older subjects with a clinically relevant designation of IGT or normal glucose tolerance (NGT) to our knowledge. Therefore, this study was designed to test the hypothesis that the ability to shift from fat to carbohydrate oxidation during submaximal exercise (metabolic flexibility during exercise) is lower in overweight-obese older subjects with IGT compared with NGT controls. To accomplish this, we assessed metabolic flexibility during aerobic exercise at 50% and 60% of maximal cardiopulmonary fitness (VO₂max) levels and used a hyperinsulinemic–euglycemic clamp to confirm metabolic inflexibility during insulin infusion in obese, older adults with IGT compared with NGT controls.

Methods

Subjects
Twenty-three sedentary (self-reported moderate-intensity activity <20 minutes on 2 or fewer days per week), overweight-obese (BMI 25.38 kg/m²) men and women between the ages of 45 and 80 years old were recruited from the Baltimore metropolitan area. All subjects were nonsmokers and had no previous diagnosis of diabetes or cardiovascular disease. Additional exclusion criteria included (i) cancer, thyroid, renal, hematological, or pulmonary diseases; (ii) taking medications such as beta-blockers, steroids, or medications normally prescribed for diabetes; and (iii) poorly controlled hypertension or dyslipidemia, anemia, or recent weight change of more than 2 kg. Prior to participation, all subjects had an asymptomatic screening treadmill exercise test. All subjects provided written informed consent. All study procedures were approved by the Institutional Review Board at the University of Maryland, School of Medicine.

Study protocol
Prior to research testing, all subjects received instruction on maintaining a weight-stable, Therapeutic Lifestyle Changes (TLC) diet (11), by a Registered Dietitian 1 day per week for 6-8 weeks. All subjects were weight-stable (±2%) for at least 2 weeks prior to research testing and were provided an isocaloric diet for 2 days before testing to control nutrient intake. Subjects were also asked to refrain from any moderate-to-vigorous physical exercise during this 2-day period.

Maximal oxygen consumption (VO₂max)
VO₂max was measured using indirect calorimetry (Quark, Cosmed, Chicago, IL, USA) during a graded exercise test on a motorized treadmill as previously described (12). Briefly, subjects walked at a constant velocity throughout the protocol and grade was initially set to 0% and increased every 2 minutes thereafter to maximal effort. VO₂max was defined as the highest oxygen consumption value obtained for a full 30-second increment. All subjects attained VO₂max as evidenced by standard physiological criteria (respiratory exchange ratio [RER] > 1.10 or a plateau in VO₂ with an increase in workload).

Oral glucose tolerance test
Subjects underwent a 2-hour oral glucose tolerance test (OGTT) after a 12-hour overnight fast. A catheter was placed in an antecubital vein and blood samples were drawn before and every 30 minutes after the ingestion of a 75-g glucose solution for 2 hours. Blood samples were centrifuged and plasma was separated and stored at -80°C until analysis. Plasma glucose levels were analyzed with a glucose analyzer (2300 STAT Plus, YSI, Yellow Springs, OH, USA). Plasma insulin levels were determined using radioimmunoassay (Millipore, St. Charles, MO, USA). Glucose (GₐUC) and insulin (IₐUC) areas under the curve during the OGTT were calculated using the trapezoidal method. The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as described by Matthews et al. (13), and the insulin sensitivity index (ISIₐ) was calculated using the method of Matsuda and DeFronzo (14). Subjects were classified as having NGT or IGT by American Diabetes Association criteria (15). Two subjects with a 120-minute postprandial glucose concentration >11.1 mmol/l were included in the IGT group because their fasting plasma glucose concentrations were <7 mmol/l (i.e., isolated post-challenge hyperglycemia).

Resting and insulin-stimulated substrate utilization (hyperinsulinemic–euglycemic clamp)
All subjects reported for testing between 7:00 and 9:00 am after a 12-hour overnight fast. Subjects underwent measurement of resting RER for the determination of resting substrate utilization prior to the hyperinsulinemic–euglycemic clamp. The subjects were instructed to lay supine and were covered with a canopy to capture expired air. VO₂ and VCO₂ were measured using indirect calorimetry (Quark, Cosmed) and recorded for 20 minutes, with the 20-minute averages used to calculate resting RER. Twenty of the 23 subjects (10 NGT and 10 IGT) underwent a 3-hour hyperinsulinemic–euglycemic glucose clamp (16,17) as implemented in our laboratory with an insulin infusion rate of 555 pmol × m⁻² × min⁻¹. Insulin-stimulated RER was measured during the last 30 minutes of the insulin infusion using the same procedure as that used for resting RER. Plasma insulin levels were determined by radioimmunoassay (Millipore, St. Charles, MO, USA). Mean insulin levels during the clamp were 1214 ± 73 pmol/l and did not differ between groups (P = 0.75).

Measurement of substrate utilization during submaximal exercise
On a subsequent visit, subjects underwent submaximal exercise testing. All subjects reported for testing between 7:00 and 9:00 am after a 12-hour overnight fast. Prior to the start of the test, RER values were confirmed to match resting RER (±2%). After a 5-minute warm-up, data were collected during two continuous, 10-minute, steady state treadmill exercise bouts at 50% VO₂max and 60% VO₂max (calculated as % VO₂ reserve) as determined by breath-to-breath VO₂ measurements. The mean intensities achieved for each bout were 49.4 ± 0.5% VO₂max and 60.2 ± 0.6% VO₂ reserve, respectively. Data collected during the last 5 minutes of each workload were analyzed when subjects were at a steady state of exercise (VO₂ mean CV = 3.4%; CV was ≤5% in all subjects). Likewise, RER was stable during the 5-minute measurement period in each workload (RER mean CV = 1.1%. CV was <2% in all subjects). Data are reported as the average of this 5-minute period and the amount of kilocalories derived from fat and carbohydrate were calculated using the Weir equation (18).

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Body mass index and body composition

Body mass index (BMI) was calculated by dividing body weight (kilogram) by height (meter square). Body weight was measured to the nearest 0.1 kg with an electronic scale and standing height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Body composition (fat mass, lean mass, and percent body fat) was determined by dual energy X-ray absorptiometry (iDXA, LUNAR Radiation Corp., Madison, WI, USA). Intra-abdominal (IAF) and subcutaneous abdominal fat (SAF) areas were determined by a computed tomography scan at the L4–L5 region using a Siemens Somatom Sensation 64 Scanner (Fairfield, CT, USA) and Medical Image Processing, Analysis and Visualization software (MIPAV v.7.0.0, NIH, Bethesda, MD, USA). Three subjects did not undergo computed tomography scans.

Statistical analysis

Data are presented as means ± SEM. All statistical analyses were performed using SPSS v12.0 (IBM, Armonk, NY, USA). Repeated measures ANCOVA was used to test for differences between subjects with IGT and NGT, using sex as a covariate where appropriate. Pearson product-moment correlations and multivariable regression analyses were used to determine relationships between metabolic variables. A type I error rate of α = 0.05 was selected and two-tailed probabilities are reported for all analyses.

Results

Subject characteristics are presented in Table 1. There were no differences in age, weight, or body composition, nor were there differences in the distribution of sex and race between the IGT and NGT groups. As expected, the 13 subjects with IGT had higher fasting plasma glucose, 120-minute postprandial glucose (G120), and Gauc compared to the 10 subjects with NGT (P < 0.001 for all). Subjects with IGT also had significantly lower ISIM values, as well as higher HOMA-IR values, fasting plasma insulin concentrations, 120-minute postprandial insulin levels, and IAUC than subjects with NGT (P ≤ 0.05 for all). VO2max was lower in subjects with IGT compared to those with NGT when expressed relative to lean body mass (P = 0.03).

Metabolic responses to submaximal exercise and insulin infusion

VO2 and heart rate responses to submaximal exercise are also shown in Table 1. There were no statistically significant differences between the IGT and NGT groups for actual VO2 and heart rate attained during submaximal exercise. At rest, RER was similar between the IGT and NGT groups. During sub-maximal aerobic exercise at 50% VO2max and 60% VO2max, the IGT group had a significantly lower RER compared with the NGT group (Figure 1B, P < 0.05). At 50% VO2max, the IGT group utilized 44% carbohydrate and 56% fat, while the NGT group utilized 57% carbohydrate and 43% fat (P = 0.04).

When the intensity was increased to 60% VO2max, the difference in carbohydrate utilization between the IGT and NGT groups was maintained (49% vs. 65% carbohydrate, respectively; P = 0.02).

In response to the insulin infusion during a hyperinsulinemic–euglycemic clamp, IGT subjects (n = 10) were metabolically inflexible compared with the NGT subjects (n = 10). RER increased in

| TABLE 1 Physical and metabolic characteristics of subjects and responses to submaximal exercise |
|---------------------------------------------------------------|
| **NGT (n = 13)** | **IGT (n = 10)** |
| Sex (men/women) | 5/8 | 3/7 |
| Race (black/white) | 5/8 | 3/7 |
| Age (yr) | 63 ± 2 | 62 ± 3 |
| **Body composition** | | |
| Weight (kg) | 29 ± 1 | 30 ± 1 |
| Body fat (%) | 159 ± 21 | 190 ± 22 |
| Lean body mass (kg) | 48 ± 2 | 51 ± 3 |
| Intra-abdominal fat area (cm²) | (n = 10) | (n = 10) |
| Subcutaneous abdominal fat area (cm²) | 223 ± 25 | 278 ± 26 |
| **Cardiorespiratory fitness** | | |
| VO2max (l/min) | 49.0 ± 1.2 | 45.0 ± 1.3³ |
| VO2max (ml/kg LBM/min) | | |
| Oral glucose tolerance test | | |
| Fasting plasma glucose (mmol/l) | 5.0 ± 0.1 | 5.6 ± 0.2³ |
| Fasting plasma insulin (pmol/l) | 83 ± 8 | 127 ± 14⁴ |
| 120-minute glucose (mmol/l) | 5.7 ± 0.3 | 9.4 ± 0.5⁵ |
| 120-minute insulin (pmol/l) | 425 ± 65 | 860 ± 147⁶ |
| GAUC (mmol/l/120min) | 817 ± 45 | 1121 ± 54⁷ |
| IAUC (pmol/l/120min) | 51247 ± 6473 | 74597 ± 9750⁸ |
| ISIM | 4.1 ± 0.5 | 2.0 ± 0.2² |
| HOMA-IR | 2.65 ± 0.30 | 4.62 ± 0.59⁹ |
| **Submaximal exercise** | | |
| Actual VO2 at 50% VO2max workload (ml/kg LBM/min) | 27.3 ± 0.8 | 26.1 ± 1.4 |
| HR at 50% VO2max workload (beats/min) | 112 ± 5 | 106 ± 3 |
| Actual VO2 at 60% VO2max workload (ml/kg LBM/min) | 32.3 ± 1.2 | 30.5 ± 1.8 |
| HR at 60% VO2max workload (beats/min) | 125 ± 5 | 116 ± 4 |
response to insulin within each group (P < 0.01 for both groups); however, the increase in the IGT group was significantly less than the increase in the NGT group (0.07 ± 0.02 vs. 0.12 ± 0.02, P < 0.05).

**Discussion**

The current study shows that glucose intolerant, obese, older adults at high risk for type 2 diabetes exhibit metabolic inflexibility in response to aerobic exercise transitioning from rest to 50% and 60% VO_{2max}, as well as metabolic inflexibility during insulin infusion when compared with older adults with NGT. We also show that the lower carbohydrate utilization during exercise of increasing intensity is related to the degree of postprandial hyperglycemia, but not to metabolic inflexibility in response to insulin infusion. Higher fat oxidation during exercise may seem advantageous to obese older adults; however, the normal response is to increase carbohydrate oxidation in order to supply sufficient energy to exercising muscle during acute aerobic exercise of increasing intensity. Thus, the inability to effectively regulate fat and carbohydrate oxidation during aerobic exercise could limit the ability to supply sufficient energy at higher levels of aerobic exercise in obese adults with IGT.
Obesity as a component of metabolic inflexibility in some subjects (19), but their resting RER. A higher resting RER was previously described exercise, the IGT and NGT groups did not differ with respect to Although our IGT subjects were metabolically inflexible during exercise at workloads higher than 50-60% VO2max because VCO2 rises dis- Our findings are concordant with previous reports of lower carbohy- drate oxidation during submaximal exercise in younger insulin-resistant subjects (6,10). The present report adds that carbohydrate oxidation is reduced during submaximal exercise in a larger group of older men and women with IGT, that the metabolic inflexibility is manifest prior to the development of type 2 diabetes, and that this abnormality persists as exercise intensity increases. Previous research shows that VO2max is lower in subjects with type 2 diabetes (5), and we find that VO2max also is lower in IGT compared with controls. In this study, we chose not to measure substrate utilization at workloads higher than 50-60% VO2max because VCO2 rises dis-proportionately and confounds the interpretation of substrate utilization from RER. However, if this inflexibility persists beyond 60% VO2max, it is possible that the lower shift to carbohydrate oxidation in subjects with IGT could contribute to the limitation of VO2max. Although our IGT subjects were metabolically inflexible during exercise, the IGT and NGT groups did not differ with respect to their resting RER. A higher resting RER was previously described as a component of metabolic inflexibility in some subjects (19), but this was not observed in all cohorts (20). Similar resting RER values in our IGT and NGT groups may indicate that IGT subjects still suppress glucose oxidation normally under fasting conditions, or that defects in muscle glucose metabolism present at rest are masked because skeletal muscle accounts for a smaller proportion of whole body metabolism at rest than during exercise.

Other studies report discordant results when examining substrate utilization during submaximal exercise in overweight or insulin-resistant and normal subjects (8-10,21,22). Goodpaster et al. (8) found that obese young men had lower rates of carbohydrate oxidation and higher fat oxidation during exercise compared with lean controls. Conversely, Hickner et al. (9) reported higher rates of carbohydrate oxidation during exercise in obese compared with lean, young, Caucasi- an women. We did not study lean subjects, but our assessment of percent body fat as a continuous variable in regression analyses indicates that within overweight-obese, older adults, the degree of obesity itself was not associated with reduced carbohydrate oxidation inde- pendent of glucose tolerance. Interestingly, the obese and lean young subjects studied by Goodpaster et al. (8) had fasting plasma glucose levels (5.5 vs. 5.0 mmol) similar to the IGT and NGT groups in this study, respectively. While Hickner et al. (9) did not report plasma glu- cose or insulin concentrations, it is possible that differences in glucose metabolism contribute to these disparate findings. Russell et al. (21) reported no difference in metabolic flexibility during passive stretching between subjects with or without type 2 diabetes and controls; however, VO2 and RER did not significantly increase during the stretching protocol. Although Numao et al. (22) reported differences between overweight men and postmenopausal women in RER during submaximal cycling exercise at 50% VO2peak, there were no differ- ences between the men and women in this study.

Similar studies have used stable isotopes to elucidate contributions of specific sources (i.e., muscle glycogen, plasma glucose, plasma fatty acids, and intramyocellular triglycerides) to substrate oxidation during exercise. Blaak et al. (23) reported higher oxidation of intra-myocellular triglycerides during cycling exercise at 50% VO2max in obese middle-aged men with type 2 diabetes compared to controls; however, oxidation of plasma-derived fatty acids was lower in type 2 diabetes and overall fat oxidation was not different between groups. In a subsequent report, Mensink et al. (24) reported numeri- cally lower RER and numerically higher fat oxidation during exercise at 50% VO2max in seven middle-aged obese men with IGT compared to controls, but these differences did not reach statistical significance in the relatively small sample. One limitation of the present study is that stable isotope tracers were not used to assess specific substrate sources; thus, we cannot comment on specific limitations from each source during exercise.

The metabolic inflexibility observed during exercise may be the result of several metabolic abnormalities present in adults with IGT, including reduced glucose uptake by exercising muscle, the presence of ectopic fat and reduced glycogen stores, and mitochondrial dys-function. Galgani et al. (20) show that reduced glucose uptake may explain the differences observed in insulin-stimulated metabolic flexibility between subjects with type 2 diabetes and normal con- trols. We showed that skeletal muscle capillarization is lower in oth- erwise healthy older adults with IGT compared to those with NGT (25,26), and levels of glucose transporter-4 (GLUT4) also may be lower in insulin-resistant older adults with IGT. While these could both limit glucose uptake by muscle during exercise (27,28),

![Image](https://via.placeholder.com/150)

**FIGURE 2** Glucose intolerance (120-minute postprandial plasma glucose) is associated with lower RER (i.e., lower carbohydrate oxidation) during aerobic exercise at (A) 50% and (B) 60% VO2max.
research shows similar levels of glucose uptake by skeletal muscle during exercise in subjects with type 2 diabetes and normal controls (29). Thus, the contribution of glucose uptake to differences in metabolic flexibility may differ between insulin-stimulated and exercise conditions. We and others report higher intramyocellular lipid (30-32), as well as reduced glycogen synthesis and content in insulin-resistant subjects (33,34), but it is unclear whether a moderate reduction in glycogen stores would limit carbohydrate oxidation as seen in this study during a relatively short bout of exercise. Mitochondrial defects are also implicated in insulin-stimulated metabolic inflexibility (for review see (35)); however, one would anticipate these mitochondrial defects would not preferentially limit fat or carbohydrate oxidation during exercise because the metabolic pathways of fatty acid oxidation and carbohydrate oxidation converge as they enter the mitochondria and are converted to acetyl-CoA.

The results of this study indicate that the strongest predictor of substrate oxidation during submaximal aerobic exercise in obese, older adults was glucose tolerance, and not age, body composition, or the degree of metabolic inflexibility during insulin infusion. While definitive conclusions cannot be drawn from our data at this time, the mechanisms underlying metabolic inflexibility during exercise may be distinct from those observed in insulin-stimulated metabolic inflexibility and could be caused by other defects in the ability to metabolize carbohydrate during exercise. One possibility is at the point of pyruvate transport into the mitochondria or at the point of pyruvate conversion to acetyl-CoA by the pyruvate dehydrogenase complex. Pyruvate dehydrogenase expression is lower, and there is increased expression of pyruvate dehydrogenase kinase-4 (PDK-4) and PDK-2 (both inhibitors of pyruvate dehydrogenase) in insulin-resistant individuals and people with type 2 diabetes (36,37). Constantin-Teodosiu et al. (38) demonstrated that PDK-4 may inhibit carbohydrate oxidation during exercise, and that the upregulation of pyruvate dehydrogenase may attenuate this inhibition. Conversely, it is possible that the present findings could simply reflect a preference for fat oxidation as a compensatory mechanism for higher intramyocellular lipid levels in IGT. In general, there are conflicting reports on mitochondrial and transport mechanisms affecting metabolic flexibility during both insulin administration and exercise. Future studies are needed to determine specific mechanisms underlying metabolic inflexibility during aerobic exercise in IGT and type 2 diabetes.

In conclusion, these findings suggest that the ability to shift from fat to carbohydrate oxidation when going from rest to submaximal aerobic exercise of increasing intensity is reduced in overweight and obese, older subjects with IGT, and is related to the degree of postprandial hyperglycemia. This limitation in obese, older adults with IGT may affect the ability to supply energy to skeletal muscle during moderate–vigorouac aerobic activities. Because regular exercise training and weight loss can improve glucose tolerance and reduce progression to type 2 diabetes (39,40), we postulate that these lifestyle interventions may improve metabolic flexibility in response to exercise in subjects with IGT, but future investigations will be required to confirm this.

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