Altered glycolytic and oxidative capacities of skeletal muscle contribute to insulin resistance in NIDDM

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Simoneau, J. Jean-Aimé, and David E. Kelley. Altered glycolytic and oxidative capacities of skeletal muscle contribute to insulin resistance in NIDDM. J. Appl. Physiol. 83(1): 166–171, 1997.—The insulin resistance of skeletal muscle in glucose-tolerant obese individuals is associated with reduced activity of oxidative enzymes and a disproportionate increase in activity of glycolytic enzymes. Because non-insulin-dependent diabetes mellitus (NIDDM) is a disorder characterized by even more severe insulin resistance of skeletal muscle and because many individuals with NIDDM are obese, the present study was undertaken to examine whether decreased oxidative and increased glycolytic enzyme activities are also present in NIDDM. Percutaneous biopsy of vatus lateralis muscle was obtained in eight lean (L) and eight obese (O) nondiabetic subjects and in eight obese NIDDM subjects and was assayed for marker enzymes of the glycolytic [phosphofructokinase, glyceraldehyde phosphate dehydrogenase, hexokinase (HK)] and oxidative pathways [citrate synthase (CS), cytochrome-c oxidase], as well as for a glycogenolytic enzyme (glycogen phosphorylase) and a marker of anaerobic ATP resynthesis (creatine kinase). Insulin sensitivity was measured by using the euglycemic clamp technique. Activity for glycolytic enzymes (phosphofructokinase, glyceraldehyde phosphate dehydrogenase, HK) was highest in subjects with NIDDM, following the order of NIDDM > O > L, whereas maximum velocity for oxidative enzymes (CS, cytochrome-c oxidase) was lowest in subjects with NIDDM. The ratio between glycolytic and oxidative enzyme activities within skeletal muscle correlated negatively with insulin sensitivity. The HK/CS ratio had the strongest correlation (r = −0.60, P < 0.01) with insulin sensitivity. In summary, an imbalance between glycolytic and oxidative enzyme capacities is present in NIDDM subjects and is more severe than in obese or lean glucose-tolerant subjects. The altered ratio between glycolytic and oxidative enzyme activities found in skeletal muscle of individuals with NIDDM suggests that a dysregulation between mitochondrial oxidative capacity and capacity for glycolysis is an important component of the expression of insulin resistance.

Methods

Subjects. The clinical characteristics and insulin sensitivity of lean and obese nondiabetic subjects and subjects with non-insulin-dependent diabetes mellitus (NIDDM) and obesity; non-insulin-dependent diabetes mellitus; muscle enzymes; hexokinase; citrate synthase; phosphofructokinase

A GOOD DEAL OF THE FUNCTIONAL DIVERSITY of skeletal muscle derives from differences of fiber type distribution, and this also helps determine metabolic diversity of skeletal muscle. Insulin-stimulated metabolism in skeletal muscle is influenced by fiber type (10). In regard to pathophysiology, insulin resistance in men and women correlates with reduced proportions of slow-twitch, oxidative fibers and increased proportions of fast-twitch, glycolytic fibers (15). Similarly, aging and physical inactivity, which are recognized to lead to insulin resistance, are also associated with diminished oxidative capacity of skeletal muscle (18). Conversely, increased physical activity, which improves insulin sensitivity, enhances expression of oxidative enzymes while reducing expression of glycolytic enzymes (18). Thus relationships between fiber type distribution and insulin resistance quite likely arise from patterns of oxidative enzymes and glycolytic enzymes, although capillary density may also contribute. In support of this postulate, diminished oxidative enzyme capacity of skeletal muscle is a stronger correlate of obesity than is fiber type per se (21). Also, in a recent collaborative study between our laboratories (22), a strong relationship was detected in obese women between insulin resistance of skeletal muscle and the combination of increased glycolytic and reduced oxidative enzyme activity. These findings gave impetus for the present investigation, which was undertaken to examine the hypothesis that proportionality between enzyme activity of the glycolytic pathway is perturbed in relation to activity of oxidative enzymes within skeletal muscle of individuals with non-insulin-dependent diabetes mellitus (NIDDM). Insulin resistance of skeletal muscle in individuals with NIDDM is typically more severe than in simple (glucose-tolerant) obesity, and, additionally, most patients with NIDDM are obese. Thus it is logical to postulate a similar or more severe altered ratio of glycolytic to oxidative enzyme capacities in NIDDM. The relatively few prior studies to examine this issue did indeed find reduced oxidative enzyme capacity in skeletal muscle of individuals with NIDDM (2, 13, 16, 26). However, neither the relationship of enzyme activity to insulin sensitivity nor the proportionality between glycolytic and oxidative capacities was explicitly addressed. Pette and Hofer (19) were among the first investigators to articulate the concept that proportionality between glycolytic and oxidative pathways is a key determinant of the metabolic potential of skeletal muscle and can be modulated by physical exercise. The glycolytic-to-oxidative ratio connotes potential for coordinating glycolytic flux of substrate with capacity for oxidative phosphorylation. Therefore, the ratio of activities, perhaps more strongly than the activities of individual enzymes, reflects metabolic capabilities of skeletal muscle. In the present study, an increased glycolytic-to-oxidative ratio was observed in skeletal muscle of individuals with NIDDM and was more severe than the perturbation found in obesity.
NIDDM are shown in Table 1. Subjects were recruited by advertisement. Obese nondiabetic and NIDDM subjects had similar body mass index, and the groups were matched for age and gender. Three of the NIDDM subjects were previously treated by diet only, and the other five were treated with sulfonylureas and these medications were withdrawn at least 2 wk before the studies. NIDDM subjects had moderate fasting hyperglycemia and a known duration of NIDDM of \(3 \pm 1\) yr. Lean and obese nondiabetic subjects had normal glucose tolerance. Potential volunteers had a medical examination before participation, and those with medical illness other than NIDDM were excluded. Also, NIDDM volunteers with diabetic complications of symptomatic neuropathy, >1+ proteinuria (by dipstick measurement), or other clinical signs of renal dysfunction, were excluded. The protocol was approved by the University of Pittsburgh Institutional Review Board, and subjects gave written, informed consent before their participation.

Study design. Subjects were admitted to the University of Pittsburgh General Clinical Research Center on the morning of a study, having been instructed to fast overnight, refrain from exercise on the day before these studies, and maintain a carbohydrate intake of at least 200 g daily for 3 days preceding admission. To obtain skeletal muscle for measurement of glycolytic and oxidative enzyme activities, a percutaneous muscle biopsy of the vastus lateralis muscle was done after 60 min of bed rest and before insulin infusion was started. Muscle samples were immediately frozen in liquid nitrogen for later assay of enzyme activity. Insulin sensitivity was then determined by using the euglycemic insulin infusion method (14). A catheter was placed in an antecubital vein for insulin and glucose infusion, and another catheter, in a retrograde direction, was placed in a dorsal vein of the hand. A heating pad was used to warm this hand for arterialization of blood samples. A primed (20 µCi), continuous (0.20 µCi/min) infusion of \(\beta\)-[3-3H]glucose was started after 90 min before insulin infusion so that systemic rates of glucose utilization could be determined during the final 30 min of a 3-h insulin infusion (40 µU·m\(^{-2}\)·min\(^{-1}\)). After insulin infusion was started, a variable-rate 20% dextrose infusion was used to maintain euglycemia; in NIDDM subjects plasma glucose was allowed to decrease to 90 mg/dl. \(\beta\)-[3-3H]glucose was added to the 20% dextrose infusion to maintain stable plasma glucose specific activity (6). In three of the NIDDM subjects, it was necessary to extend the clamp to maintain at least 60 min of euglycemia. During the final 30 min of insulin infusion, blood was sampled at 10-min intervals for plasma glucose radioactivity. Data on collateral positron-emission tomography studies of glucose transport have been separately reported (12).

Enzyme activity in skeletal muscle. Muscle samples were kept at \(-80^\circ\)C and shipped on dry ice to the Laboratory of J.-A. Simonneau for analysis of enzyme activity. Small pieces of the muscle sample (\(-10\) mg) were homogenized in a glass-steel Dall homogenizer with 39 vol of ice-cold extracting medium (0.1 M Na-K-phosphate, 2 mM EDTA, pH 7.2). The suspension was magnetically stirred on ice for 15 min. For hexokinase (HK; EC 2.7.1.1) determination, Triton X-100 was added to an aliquot of muscle homogenate to give a final concentration of 1%. After another 15 min of being stirred on ice, this homogenate was used for assaying HK activity. The major portion of the initial aliquot was cooled with ice and sonicated five times for 5 s at 20 W, with pauses of 85 s between pulses, and the resulting homogenate was used for determination of activity levels (maximum velocity) of six enzymes. Spectrophotometric techniques were conducted at 30°C, according to methods previously used (11, 24). The enzymes were citrate synthase (CS; EC 4.1.3.7), cytochrome-c oxidase (COx; EC 1.9.3.1), phosphofructokinase (PFK; EC 2.7.1.11), glycerol dehydrogenase phophotransferase (GAPDH; EC 1.2.1.12), glycogen phosphorylase (Phos; EC 2.4.1.1), and creatine kinase (CK; EC 2.7.3.2). Values of the enzyme activities are expressed in units of micromoles of substrate per minute per gram of wet weight tissue (U/g). The intraindividual reproducibility for these measurements has been established (7, 23).

Substrate and hormone assays. Plasma glucose was measured by using a Yellow Springs Instruments glucose analyzer, (Yellow Springs, OH). Plasma glucose radioactivity was determined with liquid-scintillation spectrometry after deproteinizing plasma and evaporating supernatant to dryness to remove tritiated water. Rates of glucose appearance and utilization (R\(\text{a}\)) were calculated by using the equations of Finegood (6). Plasma insulin was measured by radioimmunoassay by using a commercial kit (Insulin RIA 100, Pharmacia Diagnostics, Uppsala, Sweden).

Statistics. Data are expressed as means \(\pm\) SE, unless otherwise indicated. Analysis of variance was used to examine for significant differences across groups (lean, obese, and NIDDM). To test the hypothesis that there was a consistent rank order in the four sets of glycolytic-to-oxidative ratios across the three groups (in the order of NIDDM > obese > lean), the nonparametric test of Terpstra and Jonckheere, which tests for a consistent pattern of rank order across multiple parallel sets of data, was utilized (14). To examine the relationship between enzyme activity and insulin sensitivity, linear regression and stepwise multiple regressions were performed by using statistical software (BBN, Cambridge, MA).

RESULTS

Range of enzyme activity and intraindividual correlations. Within subject correlative analysis revealed that despite a nearly twofold range for intersubject differences between the lowest and highest values for each of the seven enzymes, there was substantial within-subject correlation for glycolytic markers (\(r \approx 0.7\)) and also
NIDDM group ranking of these ratios was consistently enzymes expressed in relation to CS activity, the across-order of these ratios, in that for each of the glycolytic (P and individuals with NIDDM muscle in lean and obese nondiabetic subjects and oxidative enzyme activity in vastus lateralis.

Table 2. Range and mean values for glycolytic and oxidative enzyme activity in vastus lateralis

<table>
<thead>
<tr>
<th></th>
<th>Lean Non-diabetic Subjects</th>
<th>Obese Non-diabetic Subjects</th>
<th>NIDDM Subjects</th>
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<tbody>
<tr>
<td>PFK</td>
<td>35.1–65</td>
<td>42.9 ± 3.4</td>
<td>46.8 ± 3.2</td>
</tr>
<tr>
<td>GAPDH</td>
<td>187–440</td>
<td>277 ± 18</td>
<td>330 ± 27</td>
</tr>
<tr>
<td>HK</td>
<td>1.30–2.80</td>
<td>1.89 ± 0.17</td>
<td>1.95 ± 0.11</td>
</tr>
<tr>
<td>Phos</td>
<td>11.4–24.6</td>
<td>15.5 ± 1.05</td>
<td>17.2 ± 1.47</td>
</tr>
<tr>
<td>CS</td>
<td>5.30–11.00</td>
<td>8.58 ± 0.85</td>
<td>8.13 ± 0.34</td>
</tr>
<tr>
<td>COx</td>
<td>2.30–6.30</td>
<td>4.72 ± 0.55</td>
<td>4.55 ± 0.37</td>
</tr>
<tr>
<td>CK</td>
<td>209–353</td>
<td>283 ± 13</td>
<td>302 ± 16</td>
</tr>
</tbody>
</table>

Mean values are ± SE. Values are given in µmol substrate·min⁻¹·g wet wt tissue⁻¹. PFK, phosphofructokinase; GAPDH, glyceraldehyde phosphate dehydrogenase; HK, hexokinase; Phos, glycogen phosphorylase; CS, citrate synthase; COx, cytochrome-c oxidase; CK, creatine kinase.

Fig. 1. Ratios of phosphofructokinase (PFK) to citrate synthase (CS) activities (A), glyceraldehyde phosphate dehydrogenase (GAPDH) to CS activities (B), hexokinase (HK) to CS activities (C), and glycogen phosphorylase (Phos) to CS activities (D) in lean nondiabetic, obese nondiabetic, and non-insulin-dependent diabetes mellitus (NIDDM) subjects. Rank order for each of these 4 ratios (NIDDM > obese > lean) was highly significant (P < 0.001).

for oxidative markers (r = ~0.8). However, there was not significant within-subject correlation between oxidative and glycolytic enzyme activities markers.

Differences between lean, obese, and NIDDM subjects in glycolytic and oxidative enzyme activities. Mean values for glycolytic and oxidative enzyme activities of each group are shown in Table 2; ratios for glycolytic to oxidative enzyme activities are shown in Fig. 1. There was a highly significant ranking (P < 0.001) in the order of these ratios, in that for each of the glycolytic enzymes expressed in relation to CS activity, the across-group ranking of these ratios was consistently NIDDM > obese nondiabetic > lean nondiabetic. The pattern for the ratios of each glycolytic enzyme activity expressed relative to COx activity was also significant (P < 0.01). For glycolytic enzyme activities, the group rankings for mean values in general conformed to the pattern of NIDDM > obese > lean, whereas for oxidative enzyme activities the group rankings for mean values were oppositely directed: NIDDM < obese < lean. However, these rank orders did not achieve statistical significance for the set of four glycolytic enzyme activities or for the two oxidative enzyme activities.

Relationship of glycolytic and oxidative capacities to insulin sensitivity. There were significant group differences for insulin-stimulated utilization of glucose, oxidation of carbohydrate, and nonoxidative glucose metabolism, as shown in Table 1. Significant and negative relationships (r = about −0.5) were found between insulin sensitivity (Rd) and the ratios of glycolytic to oxidative enzyme activities (PFK/CS, GAPDH/CS, HK/CS, and Phos/CS). In a stepwise, multiple-regression model, containing individual marker enzymes as well as each glycolytic-to-oxidative ratio, the HK/CS ratio was the strongest predictor of insulin resistance (r = −0.60; P < 0.01) as shown in Fig. 2. Indeed, after inclusion of the HK/CS ratio, no additional variance in Rd was explained by stepwise inclusion of other enzyme activities or other enzyme ratios. Obesity (body mass index) was positively related to glycolytic enzyme markers (r = 0.33–0.63) and to the glycolytic-to-oxidative ratios (r = 0.52–0.64) and was negatively associated with oxidative enzyme markers (r = −0.24 to −0.39).

DISCUSSION

Insulin resistance within skeletal muscle is a key metabolic perturbation of obesity and NIDDM. The etiology of insulin resistance in obesity and NIDDM is multifactorial, involving interaction among impairments in hormonal signaling, enzyme and transporter activity, substrate availability and competition, modulation of blood flow, and other influences, such as recent physical activity, weight, and diet composition. In recent years, there has been a renewed interest in potential relationships between insulin sensitivity and muscle fiber type distribution (15, 17). In many respects, this interest in muscle morphology and its relationship to substrate metabolism derives from much...
earlier seminal studies by Pette and Hofer (19) and others (9), who articulated the important regulatory role that is exerted by differences in the expression of energy-producing pathways in skeletal muscle. Functional differentiation of muscle was shown to be strongly related to differences in glycolytic and oxidative enzyme capacities, and plasticity of muscle metabolism was related to changes within enzyme pathways, shown to be capable of more robust change than fiber type distribution per se. The focus of the present study was to test the hypothesis that a disproportionality exists between glycolytic enzyme activities (postulated to be increased) and oxidative enzyme activities (postulated to be decreased) in skeletal muscle of individuals with NIDDM. A corollary was that these perturbations are linked to the expression of insulin resistance, and the findings of the present study support both hypotheses.

Impetus for the present study derives considerably from a prior study in which it was found that glucose-tolerant women with visceral obesity had an increased ratio of glycolytic to oxidative enzyme capacity in skeletal muscle and that this was a strong marker of insulin resistance (22). The findings in obese, glucose-tolerant individuals of the present study reaffirm these earlier results. The findings also extend these observations because the disproportionality between glycolytic and oxidative enzyme activity was more marked in NIDDM than in obese glucose-tolerant individuals. There have been some prior investigations that have examined skeletal muscle glycolytic and oxidative enzyme activity in NIDDM (2, 13, 16, 26). These studies found that glycolytic capacity was higher, whereas oxidative capacity was reduced, in NIDDM. However, neither the relationship to insulin sensitivity nor the issue of proportionality between glycolytic and oxidative enzyme capacity was specifically addressed. The results of the present study are, therefore, consistent with these previous studies and also can be regarded as consistent with recent data on an increased distribution of fast-twitch, glycolytic fibers in NIDDM (17).

Seven skeletal muscle enzymes were assayed for the present study, and for each of these, there was at least a twofold variance in activity across the subjects, who were lean and obese nondiabetic individuals and obese individuals with NIDDM. There were strong within-subject relationships among the four glycolytic enzymes (PFK, GAPDH, HK, and Phos) and a correlation of similar strength between CS and COX; however, no significant correlation (within subject) was observed between glycolytic and oxidative activities. These patterns confirm prior observations regarding coordinated regulation of enzyme activity levels within each pathway yet independent regulation of glycolytic compared with oxidative pathways (19). These findings underscore the conceptual validity of examining proportionality between glycolytic and oxidative enzyme activities as a separate, yet integrative, parameter of skeletal muscle metabolic potential. A high level of within-subject repeatability for determination of enzyme activity has been reported as well (7, 22).

Subjects with NIDDM had the highest ratio of glycolytic to oxidative enzyme activities, with obese and lean nondiabetic subjects manifesting stepwise decrements in these ratios. This pattern for ratios of glycolytic to oxidative enzyme activities emerged so clearly because there were oppositely directed patterns for glycolytic and oxidative enzyme activities. NIDDM subjects had the highest mean value for glycolytic activity and the lowest mean value for oxidative capacity, with obese nondiabetic subjects manifesting intermediate values for each pathway.

The ranges for the oxidative markers, CS and COX, were consistent with levels typically found in sedentary subjects (7). The reduced oxidative capacity of skeletal muscle in NIDDM is consonant with low values for aerobic fitness in many individuals with NIDDM (20). Although none of the participants in the present study reported strenuous or even regular programs of exercise, aerobic fitness was not determined, and it is possible that some of the differences in oxidative capacity observed do indeed reflect differences in fitness or physical activity levels among our volunteers. Capacity for oxidative phosphorylation within skeletal muscle appears to decline with aging but can be improved with physical training. Physical activity, which enhances insulin sensitivity, has the effect of increasing oxidative capacity and reducing glycolytic enzyme activity (9). This type of response is additional, albeit indirect, evidence for a linkage between regulation of insulin sensitivity and proportionality between glycolytic and oxidative pathways. The response to training suggests that as the capacity to replete ATP through oxidative phosphorylation is enhanced, there is less reliance on ATP generation via anaerobic glycolysis. The findings of the present study and those of an earlier study in obese glucose-tolerant women (22) suggest that an opposite pattern occurs in the setting of insulin resistance. This pattern seems to be one characterized by an increased reliance on glycolytic capacity and diminished ability to utilize the higher yielding pathways of oxidative phosphorylation. Additional studies are needed to address the relative contribution of these fundamental aspects of the bioenergetics of skeletal muscle.
muscle to the various disorders associated with insulin resistance.

Relatively strong correlations were found in the present study between glycolytic-to-oxidative ratios and insulin sensitivity. The mechanisms by which an elevated ratio of glycolytic to oxidative enzyme capacities contributes to insulin resistance are not well established. However, some hypotheses can be proposed, perhaps usefully taking the direction proposed by Gerbitz et al. (8) in their recent review on mitochondrial metabolism and its relationship to insulin resistance. The ratio between glycolytic and oxidative enzyme activities reflects proportionality between cytosolic and mitochondrial capacities for ATP resynthesis. Because, during insulin-stimulated conditions, replenishment of ATP in skeletal muscle is nearly exclusively derived from oxidative phosphorylation, then an impediment within this pathway, or an increased reliance on cytosolic ATP resynthesis, might negatively influence steps that require ready provision of ATP such as glycogen formation or trapping of transported glucose via its phosphorylation. Thus it seems plausible to postulate that alterations within glycolytic and oxidative pathways form a “stage” on which defects in insulin regulation of substrate transport and metabolism are more readily manifest. Alterations in the glycolytic-to-oxidative ratio may dispose skeletal muscle toward lipid accumulation in and around muscle fibers, thereby creating a milieu for substrate competition and contributing to insulin resistance. In a prior study, positive correlation was observed between glycolytic-to-oxidative ratio and muscle attenuation determined by computed tomography, which is a noninvasive parameter of fat accumulation within skeletal muscle (22). Certainly additional research is needed to better understand the mechanisms that account for the associations between the glycolytic-to-oxidative ratio and insulin sensitivity.

In the present study, the HK/CS ratio emerged as the strongest correlate of insulin resistance. An impairment of insulin-stimulated glucose phosphorylation has recently been described to be a key defect within skeletal muscle of patients with NIDDM (12). HK serves a pivotal role in glucose transport and metabolism, by trapping glucose through phosphorylation. In the great majority of patients with NIDDM, the structure of HK II is normal (5). Reduced expression of skeletal muscle HK II mRNA in NIDDM has been reported (25). Nevertheless the mechanism of impaired glucose phosphorylation in NIDDM remains uncertain, and one consideration should be that functional capacity of HK is reduced due to diminished efficiency in providing ATP (27). This inefficiency in supplying ATP to HK might be due to altered mitochondrial binding of HK (1, 3), diminished supply of ATP due to reduced oxidative enzyme capacity, or combined impairments impeding glucose phosphorylation. These are intriguing possibilities and particularly pertinent to the present study because these are potential mechanisms by which mitochondrial dysfunction, or poor coordination of cytosolic and mitochondrial metabolism, could adversely affect insulin-stimulated glucose metabolism.

The present findings that the HK/CS ratio is a marker of insulin resistance suggests that the functional and physical coupling between HK capacity and mitochondrial oxidative capacity may be important aspects to examine as mechanisms of impaired glucose phosphorylation and insulin resistance in NIDDM.

In summary, skeletal muscle of patients with NIDDM, and, to a milder degree, skeletal muscle of obese glucose-tolerant individuals, has been found to manifest an increased ratio of glycolytic to oxidative enzyme capacities, an imbalance that is correlated to insulin resistance.

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