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

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Simoneau, 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 vastus 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 glycolgenolytic 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 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 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 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.

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
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), greater than mild background retinopathy, known coronary or peripheral vascular disease, or insulin treatment 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 N$_2$ and shipped on dry ice to Laval University (J.-A. Simoneau) for analysis of enzyme activity. Small pieces of the muscle sample (~10 mg) were homogenized in a glass- and glycolytic 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 ($R_g$) 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 ± 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 intrapersonal correlations. Among all subjects, there was a broad range of enzyme activity levels, as shown in Table 2. 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 and individualswith NIDDM muscle in lean and obese nondiabetic subjects and oxidative enzyme activity in vastus lateralis.

Range and mean values for glycolytic and oxidative enzyme activities the group rankings for mean values in general conformed to 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.

Table 2. Range and mean values for glycolytic and oxidative enzyme activity in vastus lateralis muscle in lean and obese nondiabetic subjects and individuals with NIDDM

<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>35–65</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.

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.

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).
Seven skeletal muscle enzymes were assayed for the

tion of fast-twitch, glycolytic fibers in NIDDM (17).

consistent with recent data on an increased distribu-

tive enzyme capacity was specifically addressed. The

issue of proportionality between glycolytic and oxida-

neither the relationship to insulin sensitivity nor the

findings of the present study support both hypotheses.

Subjects with NIDDM had the highest ratio of glyco-

lytic 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 exer-

cise, aerobic fitness was not determined, and it is

possible that some of the differences in oxidative capac-

ity 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

muscle 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 resis-

tance. This pattern seems to be one characterized by an

increased reliance on glycolytic capacity and dimin-

ished 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

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. Func-
tional differentiation of muscle was shown to be strongly

related to differences in glycolytic and oxidative en-

zyme 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 observa-
tions 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 en-
zyme 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 oxida-
tive 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 distribu-
tion 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 en-
zymes (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 pat-
terns confirm prior observations regarding coordinated
regulation of enzyme activity levels within each path-
way yet independent regulation of glycolytic compared
with oxidative pathways (19). These findings under-
score the conceptual validity of examining proportion-
ality 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 activ-
ity has been reported as well (7, 22).

Fig. 2. Relationship between rate of glucose disposal (Rd) during
insulin-stimulated conditions and ratio of HK to CS enzyme activities
in subjects with NIDDM and in obese and lean nondiabetic subjects.
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 glycolysis 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 mRNA 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), a 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.


