Muscle glycogen content in type 2 diabetes mellitus

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Muscle glycogen content in type 2 diabetes mellitus (T2DM) have stimulated increased interest in understanding potential mechanisms for nutrient-induced insulin resistance (IR) (24). A principal focus has been on intramyocellular lipid (IMCL) (25, 28, 32). Mitochondrial dysfunction in skeletal muscle has been described in T2DM, in those at risk for this disease, and in obesity (17, 22, 26, 30) and may relate to IR by disposing to IMCL accretion (16, 29). Furthermore, muscle oxidative enzyme capacity (OX-Enz) may modulate the relation between IMCL and IR. Highly trained athletes manifest heightened insulin sensitivity and OX-Enz and maintain high IMCL (12), and recent studies, including interventions, indicate that OX-Enz might be more important than IMCL in relation to IR (4, 15, 20, 38). Thus it would seem that the relationship between IMCL and IR is more precisely delineated when considered in relation to muscle OX-Enz.

Whether there is an analogous interaction between OX-Enz and glycogen (Glyc) content in skeletal muscle in modulating insulin sensitivity has not, to our knowledge, been examined. Whereas IMCL is just a minute fraction of systemic lipid stores, skeletal muscle contains the largest reservoir of Glyc, approximately fourfold that of postprandial liver. Glyc content can regulate insulin sensitivity of muscle (9, 11). Increases in muscle Glyc induced by overfeeding and inactivity rapidly induce IR, whereas depletion of Glyc by activity rapidly improves insulin sensitivity, even in those with IR (3, 23, 27), effects that persist until Glyc is replete (11).

It has been firmly established that insulin-stimulated rates of Glyc synthesis in muscle are markedly reduced in T2DM (2, 8, 18, 19, 40, 44). This impairment has been directly imaged by use of NMR spectroscopy during insulin infusions (40) and after meal ingestion (6). There is no evidence that IR of muscle in T2DM is caused by increased muscle Glyc. Instead, most studies indicate that muscle Glyc is decreased or not different in T2DM. Roch-Norlund et al. (34) measured muscle Glyc in a large cohort of patients with diabetes mellitus. In those with type 1 diabetes mellitus, among a cohort of patients in generally poor metabolic control, a 65% reduction in muscle Glyc was observed, whereas in those with T2DM treated with diet or oral medications, muscle Glyc was reduced by ~20% (34). Interestingly, this was a nonsignificant reduction, as just one-quarter of T2DM subjects had muscle Glyc below the normal range. A similarly mild deficit was observed by Carey et al. (6) using NMR despite a marked reduction in rates of postprandial Glyc synthesis in muscle. Consistent with the above findings, in several animal models of obesity, T2DM, and IR, muscle Glyc is equivalent to control animals or only modestly reduced, despite prominent defects in activity of glycogen synthase and other components of the Glyc synthesis pathway (5, 10, 39, 43). Thus there is an intriguing paradox, with marked IR in rates of Glyc accretion in T2DM yet only a moderate decrease in muscle Glyc.

The current study was undertaken to examine muscle Glyc content in T2DM, taking into account the potential effects of muscle fiber type, and to explore interactions among muscle Glyc, IMCL, and OX-Enz in relation to IR. These objectives were addressed using a quantitative, fiber type-specific, historical method to assess muscle Glyc, IMCL, and OX-Enz. It has been recognized that mitochondrial diseases can dispose to Glyc accumulation in muscle in addition to accumulation of IMCL (41), and that increased Glyc disposes to increased fat balance (37). Thus we undertook these studies to test the novel hypothesis that with regard to mitochondrial dysfunction in T2DM and Ob, muscle Glyc might contribute to nutrient sensing mechanisms that modulate IR (9, 24).

METHODS

Subjects. The clinical characteristics of the 16 lean nondiabetic (L), 15 obese nondiabetic (Ob), and 14 research participants with T2DM...
Blood samples were obtained for measurement of Hb A1c and fasting plasma glucose and were insulin resistant; the participants with T2DM were matched for body mass index (BMI), and, with respect to L, were newly sectioned and analyzed for Glyc in conjunction with the current hypothesis concerning muscle Glyc, the muscle tissue blocks previously described (14). In this study, to address our hypothesis, 100–150 fibers were analyzed for parameters of fiber type, Glyc, IMCL, and OX-Enz, divided approximately equally among type I, type IIa, and type IIb fibers. The mean value for Glyc (and for other parameters) within each fiber type was calculated and used to calculate group mean values. The within-subject coefficient of variation for assessment of Glyc was 6 ± 2% for each fiber type. Images were captured by an optical microscope (Microphot-FX; Nikon, Tokyo, Japan) and image analyses were performed using Optimas (Media Cybernetics, Silver Spring, MD) as previously described (14). Representative images are shown in Fig. 1, including as a negative control a muscle section incubated in 1% amylase for 45 min at 37°C to hydrolyze Glyc before proceeding to PAS staining.

Table 1. Clinical characteristics of research volunteers

<table>
<thead>
<tr>
<th></th>
<th>Lean (9 F/7 M)</th>
<th>Obese (5 F/10 M)</th>
<th>T2DM (8 F/6 M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>35.2 ± 0.7</td>
<td>42.2 ± 0.9</td>
<td>52 ± 2</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.5 ± 0.6*</td>
<td>32.0 ± 0.8†</td>
<td>31.8 ± 1.7</td>
</tr>
<tr>
<td>Hb A1c, %</td>
<td>5.2 ± 0.3*</td>
<td>5.3 ± 0.3*</td>
<td>8.0 ± 0.2</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>86 ± 2.2*</td>
<td>96 ± 2.2†</td>
<td>213 ± 18</td>
</tr>
<tr>
<td>Fasting insulin, μU/ml</td>
<td>7.0 ± 0.7*</td>
<td>11.8 ± 1.1†</td>
<td>16.0 ± 4.8</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.5 ± 0.2*</td>
<td>2.8 ± 0.3†</td>
<td>8.4 ± 2.7</td>
</tr>
</tbody>
</table>

BMI, body mass index; HOMA, homeostasis model assessment; IR, insulin resistance; T2DM, type 2 diabetes mellitus; F, female subjects; M, male subjects. *P < 0.05, obese vs. lean volunteers. †P < 0.05, T2DM vs. lean or obese.

are shown in Table 1. Data on IMCL and OX-Enz have previously been reported on these individuals (14). In this study, to address our hypothesis concerning muscle Glyc, the muscle tissue blocks were newly sectioned and analyzed for Glyc in conjunction with fiber type, IMCL, and OX-Enz by use of the single-fiber analytic approach previously described (14). Research participants were recruited by public advertisement, and written, informed consent was obtained. A medical examination was performed before participation. Those on treatment with insulin were excluded, and oral agents were discontinued 4 wk before muscle biopsy. Those with T2DM had fasting hyperglycemia and higher Hb A1c and had more severe IR. T2DM and Ob were matched for body mass index (BMI), and, with respect to L, the Ob group had higher fasting insulin and higher fasting plasma glucose and were insulin resistant; the participants with T2DM were slightly older. Values for fasting insulin and plasma glucose were used to calculate homeostasis model assessment (HOMA)-IR (fasting plasma glucose × insulin/22.5 (33). This research was approved by the University of Pittsburgh Institutional Review Board, and volunteers gave written informed consent before participation.

Muscle biopsy. The research participants fasted overnight and had been instructed to not exercise on the day before the muscle biopsy. Blood samples were obtained for measurement of Hb A1c and fasting plasma glucose and insulin. After bed rest for 30 min, a percutaneous muscle biopsy was performed in the vastus lateralis. Samples were immediately dissected free of adipose and connective tissue, embedded in Cryomatrix freezing compound (Shandon, Pittsburgh, PA), and frozen in isopentane cooled in liquid nitrogen. Biopsy samples were maintained within sealed containers at −70°C until microscopy studies.

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RESULTS

Muscle Glyc. Muscle Glyc was lower in type 1 than in type IIa and IIb fibers (P < 0.001), regardless of group, as shown in Fig. 2. With regard to L, Ob, and T2DM groups, Glyc did not differ in type I fibers (P = 0.2). Also, group differences for Glyc were not different for type IIb fibers (P = 0.4). In type IIa fibers, there was a nearly significant difference for lower Glyc in T2DM (P = 0.06), a reduction of ~14% vs. Ob and of ~18% compared with L. In L, type IIa fibers accounted for the largest percentage of muscle fiber types (41, 50, and 9% for fiber types I, IIa, and IIb, respectively); the percentages were
similar for Ob (34, 56, and 10%) and T2DM (39, 52, and 9%). An overall value for muscle Glyc was calculated on the basis of the percentage of each fiber type and its respective Glyc. Muscle Glyc was lowest in T2DM [77.8 ± 5.5, 94.6 ± 4.6, and 99.5 ± 4.4 arbitrary units (AU) in T2DM, Ob, and L, respectively; *P < 0.01] with significant differences between T2DM and L (*P = 0.02) and between T2DM and Ob (*P = 0.03). There was not an effect of gender on Glyc within any muscle fiber type (*P = 0.3–0.4; data not shown).

The potential relation of muscle Glyc to fasting hyperglycemia was examined. There was a significant negative correlation for all three fiber types (type I, r = −0.37, *P = 0.01; type IIa, r = −0.50, *P < 0.001; and type IIb, r = −0.45, *P < 0.01), indicating that fasting hyperglycemia was associated with lower Glyc, as has been previously reported by Carey et al. (6). This negative correlation was particularly robust when taking into account overall Glyc (r = −0.57, *P < 0.01). The relation of muscle Glyc to HOMA-IR was also examined. There was a negative correlation of moderate statistical significance for type IIa (r = −0.31, *P = 0.04) and type IIb fibers (r = −0.35, *P = 0.02), but the relation to Glyc in type I fibers was not significant (r = −0.21, *P = 0.17). The relationship between overall muscle Glyc and HOMA-IR was of borderline significance (r = −0.32, *P = 0.052). These findings indicate that reduced Glyc is associated with greater IR.

**Muscle Glyc in relation to OX-Enz.** There were strong group differences in OX-Enz (*P < 0.01). OX-Enz was lower in T2DM and Ob compared with L for all fiber types, as shown in Table 2. Furthermore, OX-Enz was negatively correlated with HOMA-IR, although more strongly for oxidative fiber types: type I (r = −0.52, *P < 0.001), type IIa (r = −0.38, *P < 0.05), and type IIb (r = −0.31, *P = 0.06).

Values for the ratio of Glyc normalized to OX-Enz (Glyc-to-OX-Enz ratio) are shown in Table 2 and Fig. 3. These values differed significantly across fiber types: type IIb > type IIa > type I (*P < 0.001). There were not significant group differences for Glyc-to-OX-Enz ratio in type I fibers; however, the ratio of Glyc to OX-Enz did correlate significantly with HOMA-IR (r = 0.68, *P < 0.001) and more strongly than OX-Enz alone (as shown above). For type IIa and type IIb fibers, the lowest values for Glyc-to-OX-Enz ratio were in L (*P < 0.05). The ratio of Glyc to OX-Enz for both type IIa and type IIb fibers was highest in Ob. HOMA-IR was greatest in T2DM, and therefore correlation with Glyc-to-OX-Enz ratio was not statistically significant across all groups. However, the association between Glyc-to-OX-Enz ratio and HOMA-IR was statistically significant among L and Ob with respect to type IIa (r = 0.39, *P < 0.05) and type IIb fibers (r = 0.50, *P < 0.01). Muscle IMCL and IMCL-to-OX-Enz ratio. IMCL was higher in T2DM and Ob compared with L in each fiber type (*P < 0.01). IMCL was correlated with HOMA-IR for type I fibers (r = 0.32, *P = 0.04), but the correlation with type IIa and IIb fibers was not statistically significant. There was not a significant correlation between IMCL and Glyc within any of the types.

**Table 2. Muscle SDH and Oil Red O**

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Obese</th>
<th>T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>50.7 ± 2.3*</td>
<td>40.7 ± 3.2†</td>
<td>37.8 ± 3.5</td>
</tr>
<tr>
<td>Type IIa</td>
<td>40.2 ± 2.4*</td>
<td>25.1 ± 3.9†</td>
<td>30.3 ± 3.0</td>
</tr>
<tr>
<td>Type IIb</td>
<td>31.2 ± 2.9*</td>
<td>18.8 ± 4.2†</td>
<td>20.5 ± 2.5</td>
</tr>
<tr>
<td>Glycogen-to-SDH ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>1.71 ± 0.11</td>
<td>2.18 ± 0.17†</td>
<td>2.22 ± 0.40</td>
</tr>
<tr>
<td>Type IIa</td>
<td>2.72 ± 0.24†</td>
<td>6.02 ± 1.27</td>
<td>3.23 ± 0.52</td>
</tr>
<tr>
<td>Type IIb</td>
<td>3.49 ± 0.51†</td>
<td>11.88 ± 2.83</td>
<td>5.85 ± 0.86</td>
</tr>
<tr>
<td>Oil Red O-to-SDH ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>12.2 ± 1.1*</td>
<td>16.7 ± 1.3†</td>
<td>18.2 ± 2.0</td>
</tr>
<tr>
<td>Type IIa</td>
<td>9.5 ± 1.1*</td>
<td>11.4 ± 1.1</td>
<td>14.9 ± 2.2</td>
</tr>
<tr>
<td>Type IIb</td>
<td>7.0 ± 1.0</td>
<td>9.1 ± 1.33</td>
<td>10.6 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. SDH, succinate dehydrogenase; Oil Red O, Oil Red O stain. *P < 0.05, T2DM vs. lean or obese. †P < 0.05, obese vs. lean volunteers.

![Fig. 2. Glyc content within type I, type IIa, and type IIb muscle fibers, shown for vastus lateralis muscle obtained by percutaneous biopsy from lean nondiabetic, obese nondiabetic, and T2DM volunteers. Units of Glyc content are arbitrary absorbance units (AU) measured from PAS staining. There were no significant differences across groups in Glyc content for any of the 3 fiber types.](image-url)
three muscle fiber types; values for $r$ ranged from 0.05 to 0.20. We next assessed the ratio of IMCL to OX-Enz for each fiber type and the potential association with HOMA-IR. In muscle from L volunteers, the ratio of IMCL to OX-Enz is strikingly similar regardless of fiber types and approximately one-half to one-third of the values in Ob and T2DM. These differences between groups were highly significant for each fiber type ($P < 0.001$), as shown in Table 2. The ratios of IMCL to OX-Enz were strongly correlated with HOMA-IR for type I ($r = 0.79, P < 0.001$) and type IIA fibers ($r = 0.64, P < 0.001$) but were more modest in relation to type IIB fibers ($r = 0.38, P < 0.05$).

Because both Glyc-to-OX-Enz and IMCL-to-OX-Enz ratios correlated with HOMA-IR, these relationships were further assessed with the use of stepwise regression analysis. For type I fibers, for all volunteers or for L and Ob volunteers separately, after IMCL-to-OX-Enz ratio, inclusion of Glyc-to-OX-Enz ratio did not add significantly to the association with HOMA-IR. A similar pattern was observed for type IIA fibers among L and Ob nondiabetic volunteers. However, for type IIB, or glycolytic, fibers, an opposite pattern emerged wherein Glyc-to-OX-Enz ratio was more strongly correlated with HOMA-IR than IMCL-to-OX-Enz ratio, and inclusion of IMCL-to-OX-Enz ratio did not add significantly after adjusting for Glyc-to-OX-Enz ratio.

**DISCUSSION**

The current study was undertaken to examine the relation of muscle content of Glyc in T2DM and Ob subjects to IR and hyperglycemia and with specific regard to muscle fiber type, IMCL, and OX-Enz. A diminished rate of insulin-stimulated Glyc formation in skeletal muscle is highly characteristic of IR in T2DM and Ob (18, 19, 40, 44). The mechanisms that account for IR of Glyc synthesis include impaired glucose transport, elevated plasma fatty acids and IMCL, impaired insulin signaling, and reduced activation of glycogen synthase (1, 7, 18, 36, 44). Because of the severe impairment in rates of insulin-stimulated Glyc formation in skeletal muscle in T2DM and Ob subjects, it seems logical to postulate an equally severe reduction in muscle Glyc. Yet prior studies indicate a relatively small, but more modest in relation to type IIb fibers. This differs from the pattern observed for IMCL, which is increased in all fiber types in T2DM and Ob subjects, and by similar amplitude. Furthermore, the reductions in OX-Enz in T2DM and Ob subjects are quite proportionate across fiber types (14). Therefore, to consider our findings on muscle Glyc from these additional perspectives, we examined muscle Glyc in relation to muscle OX-Enz and IMCL.

Considerable research in recent years has focused on the relationship of IMCL to IR, and recent progress has served to emphasize the role of muscle OX-Enz in modulating the relationship between IMCL and IR (13). Intervention studies of physical activity in Ob and lean sedentary individuals suggest that increased OX-Enz might be more important than IMCL per se in assessing effects of training on IR (4, 15, 20, 38). Muscle OX-Enz is diminished (17), related to severity of IR in Ob and T2DM subjects (42), and likely caused by underexpression of nuclear genes encoding oxidative enzymes in T2DM (22, 26). Mechanistically, reduced OX-Enz might dispose to IMCL accumulation in muscle. Too, OX-Enz capacity might modulate the relationship of IMCL with IR. Considered as separate parameters, IMCL and OX-Enz were correlated with HOMA-IR; higher IMCL and lower OX-Enz each related to IR, and these associations were most robust for type I fibers. Nevertheless, for all fiber types, and most especially for type I fibers, the correlation with IR was even stronger for the IMCL-to-OX-Enz ratio. We posit that the IMCL-to-OX-Enz ratio provides an index of proportionality between stored lipid and the maximal capacity of a myocyte to oxidize substrate and, in this regard, provides an index potentially germane to nutrient sensing in modulating insulin sensitivity.

Might a similar perspective pertain to muscle Glyc and IR in T2DM and Ob subjects? Mott et al. (23) showed that even very slight increases in the absolute concentration of muscle Glyc induced marked IR in human volunteers. Glyc depletion, caused by physical activity, rapidly but transiently improves IR (3, 27). Thus there is certainly a conceptual basis for examining muscle Glyc as exerting feedback on IR. Despite lower Glyc in T2DM, the more striking deficit in OX-Enz yielded values for the Glyc-to-OX-Enz ratio that were similar among lean, Ob, and T2DM subjects for type I fibers and marginally increased in T2DM for type IIA and IIB muscle fibers. In Ob subjects, this ratio in type IIA and IIB fibers is even more elevated. Higher values of Glyc-to-OX-Enz ratio were associated with more severe IR. An interpretation we would like to put forward for consideration is that increased Glyc-to-OX-Enz ratio, like...
increased IMCL-to-OX-Enz ratio, serves as an index of intramyocyte nutrient surplus relative to cellular capacity for substrate oxidation. Certainly this concept remains to be more rigorously tested. For example, we would speculate that elevated Glyc-to-OX-Enz ratio and IMCL-to-OX-Enz ratio may denote disposition for accumulation of malonyl-CoA, acyl-CoA, hexosamine, or ceramides, nutrient-induced signals that have been shown to modulate IR (21, 35).

When the relative strength of association of these indexes with IR was compared, for type I muscle fibers the relation between IR and IMCL-to-OX-Enz ratio was considerably stronger than that of Glyc-to-OX-Enz ratio. This applied as well to type Ila fibers. Given that type I and type Ila fibers comprise a large majority, this would indicate that, of the two indexes, IMCL-to-OX-Enz ratio is more important for IR. For type Iib fibers, the Glyc-to-OX-Enz ratio was a stronger correlate of IR than was the IMCL-to-OX-Enz ratio. Indeed, for type Iib fibers, neither IMCL nor OX-Enz had significant independent correlation with IR. These patterns, wherein lipid stores are better correlated with IR in oxidative fibers and Glyc stores are more strongly correlated with IR in glycolytic fibers, are consistent with the known metabolic dispositions of oxidative and glycolytic fibers (31).

In summary, in the current study we assessed muscle Glyc in T2DM and Ob subjects and found a significant but modest deficit in T2DM. However, considered in relation to OX-Enz, there is correlation of increased Glyc-to-OX-Enz ratio with IR. We postulate that evaluation of lipid and Glyc storage in relation to capacity for oxidative metabolism might be a useful approach for examining nutrient-induced IR in T2DM and Ob subjects.

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