Metabolic fate of plasma glucose during hyperglycemia in impaired glucose tolerance: evidence for further early defects in the pathogenesis of type 2 diabetes

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Bokhari S, Emerson P, Israeli Z, Gupta A, Meyer C. Metabolic fate of plasma glucose during hyperglycemia in impaired glucose tolerance: evidence for further early defects in the pathogenesis of type 2 diabetes. *Am J Physiol Endocrinol Metab* 296: E440–E444, 2009. First published January 13, 2009; doi:10.1152/ajpendo.90505.2008.—We examined the intracellular metabolic fate of plasma glucose during a hyperglycemic clamp in impaired glucose-tolerant (IGT; *n* = 21) and normal glucose-tolerant subjects (10) using a combination of [3-3H]glucose infusion with measurement of [3H]water formation and indirect calorimetry. IGT was associated with ~35% reduced first-phase insulin responses, normal second-phase insulin response, and 25–30% reduced insulin sensitivity, resulting in ~35% reduced plasma glucose disposal. This was coupled with ~55% reduced storage of plasma glucose (*P* < 0.01) and ~15–20% reduced glycolysis of plasma glucose (*P* < 0.03), accounting for ~75 and 25% of the reduction in glucose disposal, respectively. Decreased glucose oxidation accounted for virtually all the decrease in glycolysis. Therefore, nonoxidative glycolysis of plasma glucose in IGT was similar to that in NGT (*P* > 0.9) and accounted for an increased proportion of systemic glucose disposal (*P* < 0.05). We conclude that, in IGT, decreased disposal of plasma glucose involves mainly decreased glycogen synthesis and to a lesser extent decreased glycolysis, which is accounted for by decreased glucose oxidation. An increased proportion of plasma glucose hence undergoes nonoxidative glycolysis, representing a novel early abnormality in the pathogenesis of T2DM.

glucose oxidation; glycogen synthesis; glycolysis

CARBOHYDRATE INTOLERANCE IS LARGELY THE RESULT of reduced insulin-stimulated glucose disposal due to decreased insulin secretion and a decreased response of glucose uptake by skeletal muscle to insulin. Factors that have been implicated in the latter include impaired insulin-induced glucose transport, glycogen synthesis, and glucose oxidation, owing to an impaired ability of muscle to switch between fat and carbohydrate as fuel sources, referred to as metabolic inflexibility.

Simoneau et al. (25) have shown that muscle from obese, insulin-resistant individuals is characterized by significantly increased activities of glycolytic enzymes such as phosphofructokinase and glyceraldehyde phosphate dehydrogenase but slightly reduced enzyme activities of oxidative metabolism such as cytochrome c oxidase and citrate synthase. Moreover, in women with normal glucose tolerance (NGT) (26) and in a group of lean and obese normal glucose-tolerant subjects and type 2 diabetes mellitus (T2DM) subjects (27), the ratio of glycolytic to oxidative enzyme activities in skeletal muscle correlated strongly with insulin resistance. These findings suggest that, in addition to decreased glycogen synthesis, an increased proportion of insulin-stimulated plasma glucose disposal may undergo nonoxidative glycolysis in insulin-resistant humans prior to the development of overt hyperglycemia.

In humans, insulin-stimulated rates of whole body glycolysis of plasma glucose and whole body glucose oxidation can be quantified by measuring the formation of tritiated water from [3-3H]glucose (23) and indirect calorimetry (9), respectively. Since glucose taken up by tissues either is essentially stored as glycogen or undergoes glycolysis (23), rates of whole body glycogen synthesis and rates of whole body nonoxidative glycolysis from plasma glucose can be calculated as the difference between systemic glucose uptake and glycolysis and the difference between glycolysis and glucose oxidation, respectively. Indeed, using this approach, Woerle et al. (29) found that, in postprandial T2DM subjects, nonoxidative glycolysis was increased ~30%, accounting for ~27% of systemic glucose disposal. This abnormal flux was due to decreased rates of postprandial glucose oxidation in the face of quantitatively normal rates of postprandial glycolysis (29), consistent with the above-mentioned human in vitro data (25–27).

In the present study, we used the same approach in conditions of standardized hyperglycemia in subjects with impaired glucose tolerance (IGT) and show that a relative increase in nonoxidative glycolysis of plasma glucose disposal is independent of concurrent or prior increased plasma glucose concentrations, hence representing an early abnormality in the pathogenesis of T2DM.

RESEARCH DESIGN AND METHODS

Subjects. We studied 31 subjects, 21 with IGT and 10 with NGT, after the study had been approved by the Institutional Review Board of the Carl T. Hayden Veterans Affairs (VA) Medical Center and informed written consent had been obtained. Subjects were classified into IGT and NGT by a standard, 75-g, 2-h oral glucose tolerance test using American Diabetes Association criteria (1). IGT subjects (17 male, 4 female) and NGT subjects were matched for age (57.9 ± 1.2 vs. 54.6 ± 1.5 yr), sex (17/4 vs. 8/2 males/females), body weight (95 ± 4 vs. 92 ± 3 kg), and body composition (31 ± 2 vs. 32 ± 1% body fat) (all not significant). Body composition, including body water mass (vide infra), was measured by bioelectric impedance. None of the subjects were taking any medications known to affect carbohydrate metabolism or participated in regular exercise. All

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subjects had been on a weight-maintaining diet containing \( \geq 200 \) g of carbohydrates for 3 days prior to the study. Sixteen of the 21 subjects participated in a placebo-controlled randomized crossover study to determine the effects of 6 wk of treatment with the angiotensin receptor blocker valsartan on insulin sensitivity and \( \beta \)-cell function (2). The results of this study (no effect on insulin sensitivity and \( \beta \)-cell function) have been published (2). In the present report, only data from the 16 IGT subjects that were obtained after 6 wk of placebo treatment are included.

**Protocol.** All subjects were admitted to the Clinical Research Center at the Phoenix VA Medical Center the evening before the experiment and were given a standard dinner (10 kcal/kg: 50% carbohydrate, 35% fat, 15% protein) between 6:30 and 7:00 PM. Subjects fasted thereafter except for water ad libitum until the study procedures were completed.

At \( \sim 7:00 \) AM the following morning, an antecubital vein was cannulated and a primed, continuous infusion of \([3-3H]\)glucose (30 \( \mu \)Ci, 0.3 \( \mu \)Ci/min) was started. At \( \sim 8:00 \) AM, a dorsal hand vein was cannulated in a retrograde fashion and maintained in a thermoregulated box at 65°C to obtain arterialized venous blood (16). At \( \sim 9:00 \) AM, after allowing \( \geq 2 \) h to achieve isotopic steady state, three arterialized venous blood samples were collected at 30-min intervals for baseline measurements of plasma glucose, insulin, glucagon, lactate, alanine, and free fatty acid (FFA) concentrations as well as \([3-3H]\)glucose specific activities (SA) and \([3H]\)water SA (60–30, and 0 min). Baseline whole body \( CO_2 \) production and \( O_2 \) consumption were determined using a canopy indirect calorimetry system (Delta Trac II; Sensormedics, Yorba Linda, CA) placed from \(-60 \) to \(-40 \) min and from \(-20 \) to 0 min. To allow for equilibration, only data during the last 10 min of each measurement period were used for analyses.

After the 60-min baseline period, plasma glucose concentrations were acutely increased to 180 mg/dl (10 mM) and subsequently maintained at this level for 180 min by infusion of 20% glucose using the glucose clamp technique. The 20% glucose infusion was enriched with \([3-3H]\)glucose (“hot-GINF technique”) to minimize changes in plasma glucose SA that might lead to an underestimation of glucose rates of appearance (31). During the clamp, arterialized venous blood was obtained at 2.5, 5.0, 7.5, 10, 15, 30, 60, 90, 120, 140, 160, and 180 min. Measurements by indirect calorimetry were carried out from 120 to 140 min and from 160 to 180 min.

**Analytical procedure.** Plasma glucose and lactate concentrations were determined by a glucose analyzer (YSI 2300 STAT Plus Glucose & Lactate Analyzer; Yellow Springs Instruments). Plasma insulin and glucagon (both from Linco Research) were measured by standard radioimmunoassay. Plasma alanine concentrations were measured by a fluorimetric method (17), and plasma FFA levels were measured by an enzymatic colorimetric method (NEFA C; Wako Pure Chemical Industries, Osaka, Japan). For determination of plasma \([3-3H]\)glucose SA, plasma was deproteinized with barium and zinc and the protein-free supernatant after and before evaporation to dryness (22). Urinary nitrogen was measured by the Kjeldahl procedure (12).

**Calculations.** First-phase insulin secretion was calculated as the mean plasma insulin concentration from 2.5 through 10 min during the hyperglycemic clamp. Second-phase insulin release was calculated as the mean plasma insulin concentration during the last hour of the hyperglycemic clamp, when plasma insulin concentrations were expected to plateau (18). Whole body insulin sensitivity was determined by dividing the average glucose infusion rate during the last hour of the hyperglycemic clamp (\( M \) value) by the average plasma insulin concentration during the same interval (M/I). Systemic glucose appearance (\( R_a \)) and systemic glucose disposal (\( R_d \)) at baseline were calculated by standard steady-state equations (30); during the hyperglycemic clamp, \( R_a \) and \( R_d \) were calculated by the non-steady-state equations of DeBodo et al. (5), modified for the “hot-GINF technique” as described by Finegood et al. (8).

Endogenous glucose release was calculated as the difference between glucose \( R_a \) and the exogenous glucose infusion rate. Rates of glycolysis from plasma glucose were calculated from the increment per unit time of \([3H]water \) (disintegrations\( \cdot \)min\(^{-1} \)\cdot ml\(^{-1} \)\cdot min\(^{-1} \)) multiplied by body water mass (ml) per \([3-3H]\)glucose SA (disintegrations\( \cdot \)min\(^{-1} \)\cdot \mu\)mol\(^{-1} \)) (22, 23). Rates of glycogen synthesis from plasma glucose were calculated as the difference between glucose \( R_a \) and glycolysis from plasma glucose (22, 23). Whole body glucose oxidation was calculated from \( O_2 \) consumption and \( CO_2 \) production using the equations of Frayn (9); protein oxidation was estimated from nitrogen excretion in the urine collected during the study. During the hyperglycemic clamp, during which oxidation of glucose derived from intracellular glycogen can be assumed to be negligible, rates of nonoxidative glycolysis from plasma glucose were calculated as the difference between the rates of glycolysis and glucose oxidation.

**Statistical analyses.** Unpaired Student’s \( t \)-tests were used for comparisons of data from the IGT and NGT subjects. All data are given as means \( \pm \) SE. \( P \) values <0.05 were considered statistically significant.

**RESULTS**

Baseline plasma glucose concentrations were slightly but significantly higher in IGT than in NGT (\( P < 0.05 \)). Baseline plasma concentrations of insulin, glucagon, lactate, alanine, and FFA were similar in both groups; baseline rates of systemic glucose turnover, glycolysis of plasma glucose, storage of plasma glucose, and whole body glucose oxidation were also comparable (Table 1).

During the hyperglycemic clamp, plasma glucose concentrations increased to equivalent levels in both groups (\( P > 0.7 \); Fig. 1). In IGT, first-phase plasma insulin responses were \(-35\% \) reduced (69.8 \( \pm \) 7.7 vs. 106.1 \( \pm \) 12.0 pM, \( P < 0.02 \)), second-phase plasma insulin responses were slightly but not significantly reduced (255 \( \pm \) 28 vs. 314 \( \pm \) 41 pM, \( P > 0.2 \)), and the \( M \) value was \( >40\% \) reduced (20.0 \( \pm \) 1.5 vs. 35.2 \( \pm \) 5.1 \( \mu\)mol\( \cdot \)kg\(^{-1} \)\cdot min\(^{-1} \), \( P < 0.002 \)) (Fig. 1). As a result, M/I was \(-25\% \) to \(-30\% \) reduced in IGT (0.065 \( \pm \) 0.006 vs. 0.089 \( \pm \) 0.009 \( \mu\)mol\( \cdot \)kg\(^{-1} \)\cdot min\(^{-1} \), \( P < 0.04 \)).

<table>
<thead>
<tr>
<th></th>
<th>IGT</th>
<th>Controls</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose, mM</td>
<td>5.8 ( \pm ) 0.1</td>
<td>5.3 ( \pm ) 0.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Plasma lactate, ( \mu )M</td>
<td>1.105 ( \pm ) 0.105</td>
<td>961 ( \pm ) 160</td>
<td>&gt;0.4</td>
</tr>
<tr>
<td>Plasma alanine, ( \mu )M</td>
<td>380 ( \pm ) 45</td>
<td>328 ( \pm ) 15</td>
<td>&gt;0.3</td>
</tr>
<tr>
<td>Plasma FFA, ( \mu )M</td>
<td>668 ( \pm ) 44</td>
<td>642 ( \pm ) 74</td>
<td>&gt;0.7</td>
</tr>
<tr>
<td>Plasma insulin, pM</td>
<td>95 ( \pm ) 11</td>
<td>84 ( \pm ) 9</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Plasma glucagon, ng/l</td>
<td>80 ( \pm ) 7</td>
<td>66 ( \pm ) 5</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>Systemic glucose turnover, ( \mu)mol( \cdot )kg(^{-1} )\cdot min(^{-1} )</td>
<td>10.7 ( \pm ) 0.4</td>
<td>10.4 ( \pm ) 0.4</td>
<td>&gt;0.6</td>
</tr>
<tr>
<td>Glycolysis of plasma glucose, ( \mu)mol( \cdot )kg(^{-1} )\cdot min(^{-1} )</td>
<td>9.3 ( \pm ) 0.7</td>
<td>9.1 ( \pm ) 0.8</td>
<td>&gt;0.8</td>
</tr>
<tr>
<td>Storage of plasma glucose, ( \mu)mol( \cdot )kg(^{-1} )\cdot min(^{-1} )</td>
<td>1.4 ( \pm ) 0.8</td>
<td>1.3 ( \pm ) 0.6</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td>Glucose oxidation, ( \mu)mol( \cdot )kg(^{-1} )\cdot min(^{-1} )</td>
<td>7.0 ( \pm ) 0.7</td>
<td>7.3 ( \pm ) 0.8</td>
<td>&gt;0.7</td>
</tr>
</tbody>
</table>

IGT, impaired glucose tolerant; NGT, normal glucose tolerant; FFA, free fatty acid.
During the last 60 min of the hyperglycemic clamp, plasma glucose specific activities did not significantly change in both groups (Fig. 1), indicating that a new near-steady state has been achieved. Endogenous glucose production was slightly less suppressed in IGT than in NGT (2.9 ± 0.9 vs. 0.2 ± 0.1 µmol·kg⁻¹·min⁻¹), but this difference did not reach statistical significance (P = 0.07). Therefore, the decreased requirement of exogenous glucose during the hyperglycemic clamp in IGT was due primarily to their decreased systemic glucose disposal (23.2 ± 1.5 vs. 35.5 ± 4.8 µmol·kg⁻¹·min⁻¹, P < 0.004). This was associated with ~15–20% reduced glycolysis of plasma glucose (15.4 ± 0.6 vs. 18.5 ± 1.4 µmol·kg⁻¹·min⁻¹, P < 0.03) and ~55% reduced storage of plasma glucose (7.7 ± 1.3 vs. 17.0 ± 3.8 µmol·kg⁻¹·min⁻¹, P < 0.01), accounting for ~25 and 75% of the reduced glucose disposal, respectively (Fig. 2). Of the plasma glucose undergoing glycolysis, 12.3 ± 0.4 µmol·kg⁻¹·min⁻¹ was oxidized in NGT, but only 9.4 ± 0.4 µmol·kg⁻¹·min⁻¹ was oxidized in IGT (P < 0.01) so that decreased glucose oxidation accounted for essentially all of the IGT’s decrease in glycolysis. Therefore, nonoxidative glycolysis of plasma glucose was similar in both groups (6.1 ± 0.7 vs. 6.2 ± 1.2 µmol·kg⁻¹·min⁻¹, P > 0.9) but accounted for a significantly greater proportion of plasma glucose disposal in IGT (27 ± 3 vs. 17 ± 2%, P < 0.05) (Fig. 2).

Plasma concentrations of glucagon (53.4 ± 5.3 vs. 50.0 ± 6.4 ng/l, P > 0.7), lactate (1,208 ± 86 vs. 1,210 ± 153 µM, P > 0.9), alanine (374 ± 36 vs. 351 ± 24 µM, P > 0.5), and FFA (180 ± 20 vs. 171 ± 27 µM, P > 0.7) during the hyperglycemic clamp did not differ between IGT and NGT.

**DISCUSSION**

Consistent with a decrease in oxidative capacity relative to glycolytic capacity in muscle in insulin-resistant and T2DM subjects (25–27), Woerle et al. (29) have recently shown that an increased proportion of plasma glucose disposal undergoes glycolysis in T2DM subjects following a mixed meal. This altered metabolic fate of plasma glucose might have been due to differential resistance of individual metabolic pathways to stimulation by insulin, impaired insulin secretion, increased mass action effects of plasma glucose due to postprandial hyperglycemia, prior chronic hyperglycemia, or a combination thereof. In the present study, we sought to determine whether the metabolic fate of plasma glucose is altered prior to the development of T2DM in high-risk individuals and independent of effects of differences in concurrent glycemia and prior chronic hyperglycemia. To this end, we used a relatively large number of IGT’s subjects and performed hyperglycemic clamps in combination with [3-¹H]glucose infusion and indirect calorimetry.

In agreement with previous reports (7, 10, 11, 15, 20, 28), we found that the IGT subjects were characterized by ~25–30% reduced insulin sensitivity, ~35% reduced first-phase plasma insulin responses, and ~20%, albeit not significantly, reduced second-phase plasma insulin responses compared with normal glucose-tolerant control subjects matched for demographic and physical characteristics. These abnormalities resulted in ~35% reduced systemic glucose disposal during the last hour of the hyperglycemic clamp, equating to 12.3

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**Fig. 1.** Plasma concentrations of glucose and insulin, glucose infusion rates, and plasma glucose-specific activities (SA) during the hyperglycemic clamp in impaired glucose-tolerant (IGT) subjects and normal glucose-tolerant (NGT) control subjects.

**Fig. 2.** Intracellular metabolic fate of systemic glucose disposal during the hyperglycemic clamp in IGT subjects and NGT control subjects.
µmol·kg\(^{-1}\)·min\(^{-1}\). Of this reduction in systemic glucose disposal, the majority (~75%) was accounted for by ~55% decreased glycogen synthesis, consistent with measurements of muscle glycogen synthesis by \(^{13}\)C-nuclear magnetic resonance spectroscopy (\(^{13}\)C-NMR) during hyperinsulinemic hyperglycemic conditions in insulin-resistant T2DM subjects (24); ~25% was accounted for by reduced glycolysis of plasma glucose, which in turn was virtually fully accounted for by decreased glucose oxidation. In contrast, nonoxidative glycolysis of plasma glucose in the IGT subjects was similar to that in the control subjects so that it accounted for a greater proportion of overall plasma glucose disposal. This finding is consistent with the comparable concentrations of plasma lactate and alanine, the main products of nonoxidative glycolysis, during the hyperglycemic clamp in both groups.

In the present study, the metabolic fate of plasma glucose was assessed during standardized hyperglycemic conditions. These differ from postprandial conditions, in which whole body plasma glucose disposal is quantitatively normal in IGT individuals due to the compensatory mass action effects of increased plasma glucose concentrations. Therefore, our finding of a relative increase in nonoxidative glucose disposal during hyperglycemic clamp conditions may turn into an absolute increase during postprandial conditions in IGT individuals, and the resultant increased release of gluconeogenic substrates may contribute to increased postprandial endogenous glucose production by stimulating gluconeogenesis. In fact, Woerle et al. (29) found that nonoxidative glycolysis was on average ~30% increased in T2DM subjects following ingestion of a mixed meal and that the increase in nonoxidative glycolysis correlated significantly with increased postprandial endogenous glucose production via gluconeogenesis.

In the normal glucose-tolerant subjects of the present study, ~50% of plasma glucose taken up by tissues was stored as glycogen, and ~50% was glycolyzed as determined by the \(^{3}H\)water technique. Of the plasma glucose undergoing glycolysis, approximately two-thirds was oxidized and approximately one-third underwent nonoxidative glycolysis as determined by indirect calorimetry. Using \(^{13}\)C-NMR in conjunction with indirect calorimetry during hyperinsulinemic hyperglycemic conditions in normal glucose-tolerant tolerant women that were similarly obese as our subjects, Petersen et al. (19) found that nonoxidative glucose disposal accounted for ~58% of whole body glucose disposal, of which ~67% was due to whole body muscle glycogen synthesis. Assuming that the 33% difference between whole body nonoxidative glucose disposal and muscle glycogen synthesis reflects nonoxidative glycolysis, it can be estimated that whole body glycolysis (nonoxidative glycolysis + oxidative glucose disposal) accounted for ~60% of whole body plasma glucose disposal and that nonoxidative glycolysis accounted for ~30% of whole body glycolysis. Thus, the proportions of intracellular metabolic fluxes of plasma glucose of the present study appear comparable with those determined by \(^{13}\)C-NMR in conjunction with indirect calorimetry under similar experimental conditions in similar individuals (19). In this context, it is of note that in the \(^{13}\)C-NMR study by Petersen et al. (19), muscle glycogen synthesis accounted for a greater proportion of whole body nonoxidative glucose disposal in lean women than in the obese women who were more insulin resistant (~99 vs. 67%). Although this difference did not reach statistical significance, it suggests that there may already be a tendency toward an increased contribution of nonoxidative glycolysis in obese insulin-resistant but normal glucose-tolerant individuals. It is of interest to note that Kelley et al. (14) found no increased release of lactate and alanine by the leg during hyperinsulinemic-euglycemic clamps in lean T2DM subjects, who had normal insulin sensitivity. These findings, together with those by Petersen et al. (20) and ours, suggest that increased nonoxidative glycolysis is linked to insulin resistance but not insulin deficiency.

In summary, the present study demonstrates that decreased glycogen synthesis and to a lesser extent decreased glycolysis account for the decreased glucose disposal during standardized physiological hyperglycemia in IGT individuals. The decrease in glycolysis is virtually fully accounted for by decreased glucose oxidation so that nonoxidative glycolysis of plasma glucose is normal. An increased proportion of plasma glucose hence undergoes nonoxidative glycolysis in IGT, representing a novel early abnormality in the pathogenesis of T2DM.

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