Glutamine:fructose-6-phosphate amidotransferase activity in cultured human skeletal muscle cells: relationship to glucose disposal rate in control and non-insulin-dependent diabetes mellitus subjects and regulation by glucose and insulin.

M C Daniels, … , R R Henry, D A McClain


We examined the activity of the rate-limiting enzyme for hexosamine biosynthesis, glutamine:fructose-6-phosphate amidotransferase (GFA) in human skeletal muscle cultures (HSMC), from 17 nondiabetic control and 13 subjects with non-insulin-dependent diabetes. GFA activity was assayed from HSMC treated with low (5 mM) or high (20 mM) glucose and low (22 pM) or high (30 microM) concentrations of insulin. In control subjects GFA activity decreased with increasing glucose disposal rate (r = -0.68, P < 0.025). In contrast, a positive correlation existed between GFA and glucose disposal in the diabetics (r = 0.86, P < 0.005). Increased GFA activity was also correlated with body mass index in controls but not diabetics. GFA activity was significantly stimulated by high glucose (22%), high insulin (43%), and their combination (61%). GFA activity and its regulation by glucose and insulin were not significantly different in diabetic HSMC. We conclude that glucose and insulin regulate GFA activity in skeletal muscle. More importantly, our results are consistent with a regulatory role for the hexosamine pathway in human glucose homeostasis. This relationship between hexosamine biosynthesis and the regulation of glucose metabolism is altered in non-insulin-dependent diabetes.

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Glutamine:Fructose-6-Phosphate Amidotransferase Activity in Cultured Human Skeletal Muscle Cells

Relationship to Glucose Disposal Rate in Control and Non–Insulin-dependent Diabetes Mellitus Subjects and Regulation by Glucose and Insulin

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Abstract

We examined the activity of the rate-limiting enzyme for hexosamine biosynthesis, glutamine:fructose-6-phosphate amidotransferase (GFA) in human skeletal muscle cultures (HSMC), from 17 non-diabetic control and 13 subjects with non–insulin-dependent diabetes. GFA activity was assayed from HSMC treated with low (5 mM) or high (20 mM) glucose and low (22 pM) or high (30 μM) concentrations of insulin. In control subjects GFA activity decreased with increasing glucose disposal rate (r = −0.68, P < 0.025). In contrast, a positive correlation existed between GFA and glucose disposal in the diabetics (r = 0.86, P < 0.005). Increased GFA activity was also correlated with body mass index in controls but not diabetics. GFA activity was significantly stimulated by high glucose (22%), high insulin (43%), and their combination (61%). GFA activity and its regulation by glucose and insulin regulate GFA activity in skeletal muscle. More importantly, our results are consistent with a regulatory role for the hexosamine pathway in human glucose homeostasis. This relationship between hexosamine biosynthesis and the regulation of glucose metabolism is altered in non–insulin-dependent diabetes. (J. Clin. Invest. 1996. 97:1235–1241.) Key words: hexosamine biosynthesis • human skeletal muscle culture • glutamine:fructose-6-phosphate amidotransferase • glucose • non–insulin-dependent diabetes mellitus

Introduction

Hyperglycemia is now recognized to be the chief causative factor in the complications of diabetes mellitus. This is true both for the chronic vascular complications of diabetes (1) as well as more acute metabolic abnormalities including insulin resistance, impaired insulin secretion, and impaired glucose uptake (2, 3). The latter have been termed “glucose toxic” effects, although there is the recognition that such effects may simply be the exaggeration of normal regulatory responses to glucose. The insulin resistance resulting from hyperglycemia is seen in both insulin-dependent and non–insulin-dependent diabetes mellitus (IDDM and NIDDM), although insulin resistance is felt to play a more fundamental pathogenic role in NIDDM.

It has been recently proposed that some of the adverse effects of hyperglycemia may be secondary to glucose metabolism to hexosamines. For example, inhibition of insulin-stimulated glucose transport in rat adipocytes exposed to high glucose has been shown to be mediated by the hexosamine biosynthesis pathway (4). Hexosamines have also been shown to play a role in growth factor expression in rat vascular cells (5, 6) and in the regulation of insulin-stimulated glycogen synthase activity (7, 8), glycogen synthesis (9), glucokinase (10), and pyruvate kinase (11). The rate-limiting step for hexosamine biosynthesis involves the transfer of an amide group from glutamine to fructose-6-phosphate to form glucosamine-6-phosphate (GlcN-6-P), catalyzed by the enzyme glutamine:fructose-6-phosphate amidotransferase (GFA). GFA activity is subject to feedback inhibition by the end product of the pathway, UDP-N-acetylgalactosamine (UDP-GlcNAc) and has also been shown to be regulated by glucose in cultured rat adipocytes (12). The cDNA for GFA has been cloned (7, 13), and the gene localized to chromosome 2 (p13) (14).

The possible relation of hexosamine biosynthesis to glucose toxicity and insulin resistance have led us to characterize GFA activity in human insulin-responsive cells. We use a previously characterized model system of primary cultures of differentiated human skeletal muscle cells derived from biopsies (15, 16). We have examined the effects of glucose and insulin on human GFA enzyme activity in such cells derived from controls and subjects with NIDDM. We find that glucose and insulin increase GFA enzyme activity from cultured human skeletal muscle cells. Basal GFA enzyme activity and its stimulation by glucose and insulin are not altered in NIDDM. Basal GFA activity measured in the cells from nondiabetic controls is inversely related to whole-body glucose disposal rates (GDR) measured in vivo at the time of the biopsy. However, there is a significant alteration in the relationship between GFA activity and glucose disposal in NIDDM; in that GFA is positively correlated with GDR. These results, seen as a stable characteristic of cells removed from the diabetic milieu and cultured under defined conditions in vitro, suggest that the

Abbreviations used in this paper: BMI, body mass index; GDR, glucose disposal rate; GFA, glutamine:fructose-6-phosphate amidotransferase; GlcN-6-P, glucosamine-6-phosphate; HGH, high glucose high insulin; HGLI, high glucose low insulin; HSMC, human skeletal muscle cultures; LGHI, low glucose high insulin; LGLI, low glucose low insulin; NC, nondiabetic control; NIDDM, non–insulin-dependent diabetes mellitus; UDP-GlcNAc, UDP-N-acetylgalactosamine.

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Received for publication 23 June 1995 and accepted in revised form 13 December 1995.

The Journal of Clinical Investigation
Volume 97, Number 5, March 1996, 1235–1241

Hexosamine Synthesis in Control and Diabetic Cultured Muscle Cells
sensing of hyperglycemia or the responses of skeletal muscle to hyperglycemia occur through the hexosamine biosynthesis pathway and may be fundamentally altered in NIDDM.

**Methods**

**Materials.** Enhanced culture growth media (SkGM BulletKit) was purchased from Clonetics Corp. (San Diego, CA). This media includes: hEGF (10 ng/ml), insulin (100 µg/ml), dexamethasone (0.39 µg/ml), BSA (0.5 µg/ml), fetuin (0.5 µg/ml), gentamicin (50 µg/ml), and amphotericin (50 ng/ml). FBS was obtained from Gemini (Calabasas, CA), α-MEM, trypsin/EDTA, and Ham’s F-10 media were purchased from Irvine Scientific (Santa Ana, CA). Creatine kinase was from Boehringer Mannheim Biochemicals (Indianapolis, IN). Protein assay reagent was purchased from Bio-Rad Laboratories (Hercules, CA). All other reagents and chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

**Human subjects.** The clinical characteristics of nondiabetic and NIDDM subjects (17) who provided muscle tissue for these studies are summarized in Table I. All subjects studied were male and were 73% Caucasian, 17% African-American, and 10% Hispanic. The experimental protocol was approved by the Committee on Human Investigation of the University of California, San Diego (UCSD). After explanation of the protocol, written informed consent was obtained from all subjects.

**Glucose disposal rate.** Of the 30 subjects who had needle muscle biopsies, 28 (17 nondiabetic and 11 NIDDM) also had in vivo rates of glucose disposal determined on a separate day using the hyperinsulinemic glucose clamp technique (18). [3–H]glucose was infused in a continuous manner throughout the entire study, beginning at least 3 h before the infusion of insulin. Insulin was infused in a primed continuous manner at 720 pmol · M⁻² · min⁻¹ and plasma glucose was held constant at 5.0–5.5 mM for 4–5 h. GDR (in mg · kg⁻¹ · min⁻¹) was calculated during the final 40 min of each clamp study from the isotopically determined rate of glucose disappearance (RD) corrected for changes in plasma glucose within its distribution space.

**Skeletal muscle biopsy and culture procedure.** The cell culture technique used has been characterized in detail previously (15, 16). 250–400 mg of muscle was obtained by percutaneous needle biopsy from the lateral portion of the quadriceps femoralis from 17 nondiabetic and 13 NIDDM subjects and placed in 15 ml of Ham’s F-10 media at 4°C. The biopsies were carefully dissected, minced, and washed three times with F-10 media at 4°C and once at 37°C to aid in the removal of connective tissue. The tissue was dissociated by three successive treatments of 20 min each in 25 ml of 0.05% trypsin/EDTA at room temperature. Dissociated cells were centrifuged at 600 g for 4 min at 37°C, and resuspended in fully supplemented human skeletal growth media (SKGM BulletKit) with 2% FBS and no added insulin. 5,000–10,000 cells were plated on 100-mm dishes and grown in SkGM-insulin with 2% FBS at 37°C. All other reagents and chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). 5,000–10,000 cells were plated on 100-mm dishes and grown in SkGM-insulin with 2% FBS at 37°C until 70–80% confluence was reached (average 3–4 wk). Each needle biopsy yielded 1–2 × 10⁶ myoblasts.

Differentiation into myotubes was achieved by 4 d of fusion in α-MEM with 2% FBS, 1% penicillin (200 U/ml) / streptomycin (200 µg/ml) and one of the four following study conditions: (a) low (5 mM) glucose and low (22 pM) insulin, (b) low glucose and high (30 µM) insulin, (c) high (20 mM) glucose and low insulin, and (d) high glucose and high insulin. All experiments were performed on 4-d fused myotubes of first passage cells. Myotube formation was verified by histologic evidence of multinucleation using fluorescence microscopy, from induction of sarcomeric α-actin protein on Western blot, and

**Table I. Subject Characteristics**

<table>
<thead>
<tr>
<th>Group</th>
<th>Age</th>
<th>BMI</th>
<th>Insulin</th>
<th>FPG</th>
<th>2 h OGTT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>yr</td>
<td>kg/m²</td>
<td>µU/ml</td>
<td>mM</td>
<td>µU/ml</td>
</tr>
<tr>
<td>Nondiabetic (n = 17)</td>
<td>42±2</td>
<td>28.0±1.3</td>
<td>11.1±2.6</td>
<td>5.1±0.1</td>
<td>11.1±1.7</td>
</tr>
<tr>
<td>NIDDM (n = 13)</td>
<td>52±2*</td>
<td>32.1±0.8</td>
<td>57.8±26</td>
<td>10.0±1.4*</td>
<td>17.6±1.4*</td>
</tr>
</tbody>
</table>

Data are means±SEM. FPG, fasting plasma glucose; OGTT, 75 g oral glucose tolerance test. *P < 0.0005; †P < 0.025.

**Table II. Skeletal Muscle Differentiation in Muscle Cell Cultures**

<table>
<thead>
<tr>
<th>Media glucose:</th>
<th>Myoblasts</th>
<th>Myotubes</th>
<th>20 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media insulin:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22 pM</td>
<td>22 pM</td>
<td>30 µM</td>
<td>22 pM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 µM</td>
</tr>
<tr>
<td>Control subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatine kinase activity mU/mg protein (n = 5)</td>
<td>335±95</td>
<td>2,068±100*</td>
<td>1,915±124*</td>
</tr>
<tr>
<td>Cellular protein µg/plate (n = 8)</td>
<td>872±99</td>
<td>681±65</td>
<td>992±68</td>
</tr>
<tr>
<td>NIDDM subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatine kinase activity mU/mg protein (n = 5)</td>
<td>248±55</td>
<td>1,477±121*</td>
<td>1,638±158*</td>
</tr>
<tr>
<td>Cellular protein µg/plate (n = 8)</td>
<td>670±70</td>
<td>689±66</td>
<td>859±75</td>
</tr>
</tbody>
</table>

Data are means±SEM. *P < 0.01 vs. myoblasts. ND, not determined.
from examination of creatine kinase mRNA by slot blot analysis (15). Table II verifies that muscle cultures differentiated equally into myotubes for both control and NIDDM subjects and under all culture conditions. Also, no significant difference was observed in the degree of differentiation between nondiabetic and NIDDM muscle cultures. Creatine kinase activity and protein estimation were done as described (15).

**GFA activity assay.** Cultured myotubes were incubated for 1 h with serum and insulin free α-MEM containing 5 mM (low) glucose before cell extraction. After rinsing three times with 4°C PBS, cells were scraped into 1.5-ml Eppendorf tubes in 700 μl of extraction buffer (50 mM Hepes, 10 mM EDTA, 100 mM NaF, 5 mM DTT, 1 μg/ml pepstatin, 2 μg/ml leupeptin, and 0.2 mM PMSF, pH 7.5), and sonicated (50 Sonic Dismembrator; Fisher Scientific, Tustin, CA) at setting 7 for 30 s at 4°C. GFA activity was assayed in vitro using once-thawed cell extracts. 25 μg of crude cell extract was incubated with 12 mM fructose-6-phosphate, 12 mM glutamine, 40 mM NaH₂PO₄, 1 mM EDTA, and 1 mM DTT in the presence and absence of 500 μM UDP-GlcNAc (final vol 100 μl), for 45 min at 37°C. The reaction was stopped, and protein was precipitated by the addition of 50 μl 1 M perchloric acid and incubation on ice for 10 min. After centrifugation at 16,000 × g for 10 min, 145 μl of supernatant was extracted with 258 μl of tri-n-octylamine:1,1,2-trichloro-trifluoroethane (1:4), and 120 μl aqueous phase was then ready for HPLC analysis. GlcN-6-P generated was detected by derivatization of the sample with 2 volumes o-phthalaldehyde (OPA) reagent (4 mg OPA in 50 μl ethanol added to 5 ml 0.1 M sodium borate, pH 9.7, and 10 μl 2-mercaptoethanol) for 1 min, followed by neutralization with 120 μl 0.1 M sodium phosphate (monobasic). After filtration through a 0.45-μM syringe filter, the sample was loaded onto a Spherisorb ODS-2 reverse phase column (3 μm, 25 cm × 4.6 mm, C₁₈ mh Phase Separations Inc., Norwalk, CT) and separated using a single gradient (0–6 min buffer A: 15 mM sodium phosphate, pH 7.2, 2.5% 2-propanol, 2.5% acetonitrile; 6–12 min gradient to buffer B: 15 mM sodium phosphate, pH 7.2, 6% 2-propanol, 6% acetonitrile). Derivatized products were detected using a fluorescence detector (Jasco FP-920; Jasco, Easton, MD) set at 340-nm emission and 460-nm excitation wavelength with gain = 10. Peak area was integrated using an HPLC system employing System Gold software (Beckman Instruments Inc., Fullerton, CA). OPA-derivatized GlcN-6-P standards were run separately to determine peak retention time, and sample peak areas were plotted against a standard curve using linear regression analysis to correlate area to activity. Using this system, as little as 5 pmol glucosamine-6-phosphate could be detected, and detection was linear up to the highest standard tested (1 nmol). Generation of GlcN-6-P was linear over time (15–60 min) and protein concentration (10–110 μg) using this procedure. GFA activity so assayed was found to be fully substrate dependent and subject to feedback inhibition by UDP-GlcNAc (Fig. 1), verifying the specificity of the assay. GFA enzyme activity is expressed as picomoles GlcN-6-P formed per min per milligram extract protein.

**Results**

GFA activity from cultured human skeletal muscle increases with chronic (4 d) glucose and insulin treatment. GFA activity was measured in vitro from human skeletal muscle cultures treated during fusion and differentiation with various combinations of glucose (5 or 20 mM) and/or insulin (22 pM or 30 μM). As shown in Table II, similar degrees of differentiation were obtained from nondiabetic and NIDDM muscle cultures. Glucose treatment also did not result in any change in differentiation state, while insulin treatment led to a slight increase in cellular protein but no change in overall differentiation. GFA activity from cultured muscle cells was increased by glucose and insulin treatment. As shown in Fig. 2, GFA activity increased significantly by 22% in cells treated with high glucose (434.07±44.10 U of activity in 20 mM glucose and 22 pM insulin, compared to 354.37±41.51 U in 5 mM glucose and 22 pM insulin, *P < 0.05 by paired t test), and 43% in cells treated with high insulin (507.35±47.75 U in 5 mM glucose and 30 μM insulin)

![Figure 1](image1.png)

**Figure 1.** HPLC determination of glucosamine-6-phosphate. GFA activity was assayed in vitro (see Methods). GlcN-6-P generation was quantitated by fluorescent derivatization and HPLC separation. GlcN-6-P production was completely inhibited in the presence of 500 μM UDP-GlcNAc (dashed line).

![Figure 2](image2.png)

**Figure 2.** Glucose and insulin treatment of HSMC increases GFA activity. HSMC cultures were treated for 4 d during fusion and differentiation with 5 mM glucose and 22 pM insulin (low glucose-low insulin, LGLI), 20 mM glucose and 22 pM insulin (high glucose-low insulin, HGLI), 5 mM glucose and 30 μM insulin (LGGHI), or 20 mM glucose and 30 μM insulin (HGGHI), and their extracts were assayed for in vitro GFA activity. GFA activity from HSMC of all subjects is shown as mean±SEM under each treatment. *P < 0.05, **P < 0.005 compared to LGLI, and ***P < 0.005 compared to HGLI by Student’s paired t test.
Insulin treatment also led to a dose-dependent increase in GFA activity (Fig. 3). GFA activity was increased from cultures treated with 1.5 nM insulin, and activity was maximal at 30 nM insulin.

**GFA activity in NIDDM and nondiabetic control (NC) subjects.** GFA activity and its regulation by glucose and insulin did not differ significantly between control and NIDDM HSMC (Fig. 4). Basal GFA activities at low glucose and low insulin were similar: $362.85 \pm 56.06$ U in NC and $344.84 \pm 61.50$ U in NIDDM cells. No significant differences were seen in the degree of stimulation of GFA activity by glucose and insulin between control and diabetic cultures.

**In vitro GFA activity is correlated with in vivo glucose disposal rate.** Hexosamine biosynthesis has been demonstrated to play a role in the regulation of glucose uptake in both cultured cells and in insulin resistant states (3, 6–8). We therefore examined the relationship between GFA activity and GDR measured in vivo by the euglycemic hyperinsulinemic clamp technique. In vitro GFA activity in the HSMC obtained from nondiabetic control subjects was inversely correlated with the GDR (Fig. 5A). GFA activity in control HSMC treated with low glucose and low insulin decreased linearly with increasing GDR ($r = -0.68, P < 0.025$). This relationship also held true and was significant (high glucose and low insulin: $r = -0.769, P < 0.005$; low glucose and high insulin: $r = -0.462$; high glucose and high insulin: $r = -0.729, P < 0.005$) for GFA activity assayed from cells cultured in the other conditions (Fig. 5B).

Although overall GFA activity did not differ between control and NIDDM HSMC, the relationship between in vitro GFA activity and in vivo glucose disposal was altered. In contrast to NC, GFA activity from cells treated with low glucose and low insulin was significantly and positively correlated with GDR in NIDDM subjects ($r = 0.86, P < 0.005$) (Fig. 6A). This relationship was also true for GFA assayed from cells cultured in the other conditions (high glucose and low insulin: $r = 0.543$; low glucose and high insulin: $r = 0.387$; high glucose and high insulin: $r = 0.93, P < 0.005$) as shown in Fig. 6B.

**GFA activity and obesity.** Because the nondiabetic control group was slightly leaner than the NIDDM group, and because insulin resistance and NIDDM are correlated with obesity, we

![Figure 3. Insulin treatment of HSMC leads to a dose-dependent increase in GFA activity. HSMC were treated for 4 d during fusion and differentiation with 5 mM glucose and varying concentrations of insulin. Shown are the mean and SEM from HSMC from six subjects.](image)

![Figure 4. GFA activity and regulation by glucose and insulin is similar in HSMC from NC and NIDDM muscle. The data from Fig. 2 are divided into nondiabetic control (NC) and NIDDM cultures. HSMC GFA activities of all subjects is shown as means±SEM. *P < 0.025, **P < 0.01 compared to LGLI, and **P < 0.005 compared to HGLI by Student’s paired t test.](image)
next examined GFA activity as a function of weight in our study population. The two groups were therefore divided into two subsets, those with body mass indices (BMI) above and below 30 kg/m². This allowed us to analyze comparable groups, namely less obese controls (average BMI = 25.0±0.8 kg/m², n = 12) compared to less obese NIDDM subjects (average BMI = 28.7±0.8 kg/m², n = 4), and more obese controls (average BMI = 35.1±1.6 kg/m², n = 5) compared to more obese NIDDM subjects (average BMI = 33.5±0.6 kg/m², n = 9).

In the less obese group, controls did not differ significantly from NIDDM subjects in the GFA activities measured in the HSMC (Fig. 7, NC 388.9±42.1 U/mg, NIDDM 471.5±36.9, averaged for all culture conditions). In the nondiabetic controls, the GFA activity measured in the HSMC was increased by 63% in the more obese group (633.8±60.8 U/mg, P=0.005 by Student’s paired t test). In contrast, GFA activities were not higher in the more obese NIDDM subjects (455.4±70.9 U/mg) compared to the less obese NIDDM subjects. Thus, the more obese nondiabetics have significantly higher GFA activities than their NIDDM counterparts (P < 0.05).

Discussion

We have examined the activity and regulation of the rate-limiting enzyme in hexosamine biosynthesis, GFA, in cultures of skeletal muscle cells derived from nondiabetic subjects and subjects with NIDDM. Hexosamine biosynthesis may be one of the chief mechanisms by which cells sense and respond to high concentrations of glucose. For example, it is the metabolism of glucose to hexosamine products that is responsible for the down regulation of glucose transport seen in cultured adipocytes exposed to excessive glucose (4). Hexosamine metabolism is also responsible for regulatory effects of glucose on glycogen synthase in cultured cells (7, 8) and muscle (9) and the stimulation of TGF alpha by glucose (5, 6). Overexpression of GFA in cultured cells and in skeletal muscle of transgenic animals results in insulin resistance and enhanced sensitivity to regulation by glucose (7, 8, 19). These observations have led to the hypothesis that hexosamines may mediate so-called “glucose toxicity” and could even be more fundamentally involved in the pathogenesis of insulin resistance and NIDDM.

In the nondiabetic subjects, we observed a strong negative correlation between in vitro GFA activity and glucose disposal rates measured in vivo under conditions of hyperinsulinemia and euglycemia. This relationship would be expected if flux through the hexosamine pathway determined the level of insulin-stimulated glucose uptake, as is suggested by the in vitro studies mentioned above (4). It is very interesting, therefore, that a stable characteristic of the cultured muscle cells measured after ~4–6 wk in defined culture conditions is so well re-
related to in vivo glucose disposal. The maintenance of this relationship after culture lends credence to the hypothesis that hexosamine synthesis may be a major regulator of glucose metabolism. These results are also consistent with the effect of increased GFA expression in skeletal muscle of transgenic mice. Namely, these mice also exhibit insulin resistance and a decreased glucose disposal rate (19) and suggest a causal relationship between GFA and glucose disposal.

In contrast to the nondiabetic subjects, there is a significant positive correlation between GFA activity and glucose disposal in individuals with NIDDM. Why this is the case is not clear. However, the loss of the normal relationship between GFA and GDR would argue that the linkage between the sensing of and response to high glucose through the hexosamine pathway is somehow altered in NIDDM.

Age and obesity, like impaired glucose disposal, are linked to insulin resistance and NIDDM. Because the control patients were younger than the diabetics (Table I), this was a factor which might have complicated our results. In this study, however, age did not correlate at all with GFA activity in either the nondiabetic controls ($r = 0.01$; data not shown) or the NIDDM subjects ($r = 0$; data not shown). Our NIDDM subjects were also slightly more obese than control subjects. We therefore separated both groups into two subgroups, BMI $> 30$ (more obese) and BMI $\leq 30$ (less obese). HSMC from the more obese nondiabetic subjects exhibited higher levels of GFA activity. This may simply be another way of stating the relationship between glucose disposal rate and GFA; obese individuals tend to have lower GDRs, and that in turn is correlated with higher GFA activities. A similar relationship between obesity and GFA was absent in the NIDDM subjects, probably a reflection of the alteration in the relationship between glucose disposal and GFA activity. The causal connections among these related variables remain to be determined.

These studies made use of primary cultures of isolated skeletal muscle cells. Such cells provide us with several advantages in the analysis of glucose metabolism and the pathophysiologic underpinnings of NIDDM. These cells retain expression of the fat and muscle-specific glucose transporter GLUT4 as well as insulin responsiveness of glucose transport and glycogen synthase (15, 20, 21). Most importantly, they can be cultured under defined metabolic conditions to allow dissection of the primary (genetic) abnormalities of NIDDM from those that are secondary to the diabetic milieu. For example, these cells have recently been used to demonstrate that the glucose transport abnormalities of cells from NIDDM subjects are retained stably in their cultured muscle cells (16).

We find that exposure of cultured skeletal muscle cells for both nondiabetic and NIDDM subjects to high glucose and/or insulin results in increased GFA activity, occurring well within the physiologic range of concentrations for both agents. This increased activity is observed with 24 hours of glucose treatment and is dose-dependent for both glucose (data not shown) and insulin (Fig. 3). These data correlate well with data from cultured rat aortic smooth muscle, fibroblast, and mesangial cells in which we have observed regulation of GFA activity by glucose being dose dependent and occurring over a time-course of 6–24 h (unpublished observations).

Control and NIDDM cultures did not differ in their basal and stimulated levels of GFA activity. We have observed similar results in intact muscle, in that we have shown in fresh skeletal muscle biopsy specimens that GFA activity is correlated with glycated hemoglobin levels (22). That is, just as high concentrations of glucose in the culture medium increase GFA in cultured cells, so do high concentrations of plasma glucose increase GFA in intact muscle. At this time we cannot say whether the effect of insulin is independent of the effect of glucose or is secondary to insulin’s ability to promote glucose uptake. Also, why the activity of the GFA enzyme is 10- to 100-fold higher in the cultured muscle (this study) compared to the biopsied muscle (22) is unknown. Previous studies have found increased GFA activity in differentiating rat and chick tissues

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Figure 7. GFA activity as a function of body mass index. GFA activity was determined in HSMC from nondiabetic control (A) and NIDDM (B) subjects treated with the indicated concentrations of glucose and insulin for 4 d during fusion and differentiation. Mean GFA activity $\pm$ SEM is shown for subjects with BMIs $\leq 30$ (open bars [NC: LGLI, $n = 7$; HGLI, $n = 8$; LGHI, $n = 9$; HGHI, $n = 7$; NIDDM: LGLI, $n = 4$; HGLI, $n = 3$; LGHI, $n = 4$; HGHI, $n = 3$]) compared to subjects with BMIs $> 30$ (filled bars [NC: LGLI, $n = 2$; HGLI, $n = 4$; LGHI, $n = 4$; HGHI, $n = 4$; NIDDM: LGLI, $n = 4$; HGLI, $n = 6$; LGHI, $n = 8$; HGHI, $n = 7$]). $^*P < 0.05$, $^*P < 0.005$ compared to GFA activity in HSMC from subjects with BMIs $\leq 30$ treated with the same concentrations of glucose and insulin (Student’s $t$ test).
Acknowledgments

Our results differ from the down regulation of GFA activity that has been reported in adipocytes exposed to high glucose (12). This could be due to the differing responses and needs of these tissues in the face of a carbohydrate load. Lipo-protein lipase, for example, is differentially regulated by insulin in these two tissues (26). Alternatively, it is possible that the different assays used for GFA activity in these two reports are responsible for the divergent results. We have found the HPLC-based assay used in our studies to be completely dependent on both enzyme substrates (glutamine and fructose-6-phosphate) and to be nearly 100% sensitive to inhibition by glutamine analogues (diazo-oxo-norleucine) and by feedback from UDP-GlcNAc (Fig.1). The enzyme-linked assay used in the studies of the adipocytes, however, did not, in our hands, reproducibly fulfill these criteria for specificity.

In summary, we have shown that GFA activity in human skeletal muscle cultures is positively regulated by glucose and insulin. In nondiabetic subjects, GFA activity measured after culture of the cells in defined media for prolonged periods correlated extremely well with whole-body glucose disposal rates measured during hyperinsulinemia and euglycemia. This suggests an important relationship between GFA activity and the regulation of glucose homeostasis and supports the hypothesis that hexosamine biosynthesis is a major pathway used by tissues to sense and respond to changes in glucose flux. The enzyme GFA is an excellent candidate for a sensor of “cellular satiety”: The affinities of the enzyme for both fructose-6-phosphate and the amido-donor glutamine are relatively low, with Km’s in the near millimolar range (27, 28), so in theory both cellular carbohydrate and amino acid levels could be reflected in the level of hexosamine synthesis. In cells from subjects with NIDDM the basal activity and regulation of GFA was similar to that seen in controls. However, the relationship between GFA and glucose disposal rate was reversed, suggesting abnormalities in the sensing of or response to hexosamine flux in NIDDM.

Acknowledgments

This work was supported by the Research Services of the Veterans Administration Medical Centers in Jackson, Mississippi and San Diego, California.

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