Glucosamine Infusion in Rats Mimics the β-Cell Dysfunction of Non–Insulin-Dependent Diabetes Mellitus

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Sustained hyperglycemia can cause peripheral insulin resistance and pancreatic β-cell dysfunction and has been termed glucose toxicity or glucose-induced desensitization. Glucosamine, a product of glucose flux through the hexosamine biosynthetic pathway (HBP), causes insulin resistance in peripheral tissues and has been shown to cause abnormal glucose-insulin secretion coupling, and thus has been implicated in the pathogenesis of glucose toxicity. Here, we investigate whether glucosamine-induced insulin secretory dysfunction is specific to glucose or also extends to nonglucose secretagogues such as arginine. Two groups of 12 weight-matched Sprague-Dawley rats underwent hyperglycemic clamp studies (steady-state blood glucose, ~220 mg · dL^{-1}) during infusion of normal saline or glucosamine 3.5 mg · kg^{-1} · min^{-1} over a 100-minute period. Insulin levels were measured at baseline and between 90 and 100 minutes. One hundred minutes into the hyperglycemic clamp, subgroups of seven rats each (saline- and glucosamine-infused rats) received a bolus of arginine (100 mg · kg^{-1}) while the glucose infusion rate was unaltered. Glucose and insulin levels were measured at 1, 3, 5, 10, 15, and 30 minutes after the arginine bolus. Both groups had similar fasting glucose and insulin levels. At steady state (60 to 100 minutes), glucose levels were almost identical in both groups (223.58 ± 3.94 *v* 224.58 ± 4.34 mg · dL^{-1}), but the glucose infusion rate (26.55 ± 1.60 *v* 8.83 ± 1.35 mg · kg^{-1} · min^{-1}, $P < .0001$) and insulin level (41.36 ± 6.47 *v* 18.04 ± 2.95 mU · mL^{-1}, $P < .0001$) were markedly reduced in animals receiving glucosamine. Peak insulin levels 1 minute after the arginine bolus were lower in rats infused with glucosamine versus saline (274.00 ± 30.38 *v* 176.25 ± 20.12 μU · ml^{-1}, $P = .0319$). Total insulin secretion in response to arginine was significantly lower in the glucosamine group as determined by the area under the curve (1,268.09 ± 142.27 *v* 706.77 ± 84.79 μU · mL^{-1} · min, $P = .0054$). In conclusion, glucosamine causes severe impairment in glucose-induced insulin secretion. Further, glucosamine-induced β-cell secretory dysfunction extends to nonglycemic stimuli like arginine. This pattern of insulin secretory dysfunction is similar to that observed in patients with non–insulin-dependent diabetes mellitus (NIDDM). These data suggest that glucosamine may participate in the pathogenesis of glucose toxicity at the level of the β cell in NIDDM patients.

NON-INSULIN-DEPENDENT diabetes mellitus (NIDDM) is a complex metabolic disease characterized by insulin resistance in peripheral tissues[1,2] and defects in pancreatic β-cell function.[3] In the pathogenesis of NIDDM, β-cell dysfunction is critical in the development of hyperglycemia and is characterized by impaired glucose-stimulated insulin secretion,[4] also referred to as "glucose blindness." β-Cell dysfunction could be the result of inherited genetic factor(s) and/or be induced by the metabolic consequences of the insulin-resistant or diabetic state.

Glucose toxicity induced by sustained hyperglycemia (the hallmark of NIDDM) can worsen both insulin resistance and the glucose-induced insulin secretory response.[5,6] While the mechanisms of glucose toxicity are not fully understood, mounting evidence suggests that the hexosamine biosynthetic pathway (HBP) is instrumental in mediating the toxic effects of glucose. Indeed, glucosamine (a product of the HBP) was more potent than glucose in inducing insulin resistance in primary cultured adipocytes.[7] Moreover, inhibitors of glutamine-fructose-6-phosphate amidotransferase, the rate-limiting enzyme in hexosamine biosynthesis, prevent glucose-induced insulin desensitization in these cells.[7] Infusion of glucosamine has also been shown to induce in vivo insulin resistance in skeletal muscle of normal rats,[8] suggesting that products of the HBP could be mediators of glucose-induced insulin resistance in vivo.

Two studies have addressed the effects of glucosamine on insulin secretion.[9,10] In one, glucosamine-induced insulin secretory dynamics in response to a glucose stimulus were impaired during infusion of a single dose of glucosamine (0.895 mg · kg^{-1} · min^{-1}).[9] In another, insulin secretion in response to a glycemic stimulus was demonstrated to be blunted after an intravenous bolus of glucosamine.[10] Both reports indicated that the pancreatic β-cell response to the nonglucose secretagogue L-arginine was normal with glucosamine administration.[9,10] Untreated hyperglycemic patients with NIDDM appear to have a normal β-cell response to nonglucose secretagogues.[11] However, when tested at matched glycemic levels, NIDDM patients display impaired insulin secretion in response to nonglycemic stimuli when compared with controls.[12] The normal arginine response during glucosamine infusion would suggest that products of the HBP alone cannot account for the abnormal β-cell function in NIDDM subjects.

Because glucosamine induces insulin resistance in peripheral tissues in a dose-dependent fashion, it is possible that higher glucosamine doses (>0.895 mg · kg^{-1} · min^{-1}) can induce β-cell secretory defects to both glucose and L-arginine stimulation under matched glycemic conditions. Confirmation of this proposition would support the notion that products of the HBP may mediate β-cell dysfunction in patients with NIDDM. To this end, we studied the effect of glucosamine infusion at a dose that induces maximal insulin resistance[8] in male Sprague-

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Dawley rats under conditions of matched glycemic stimulus on insulin secretion in response to both glucose and nonglucose secretagogues.

MATERIALS AND METHODS

Animals and Catheterization Procedure

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing between 250 and 350 g were studied while awake and unstressed after overnight food deprivation. Three to 4 days prior to study, each rat underwent surgery for implantation of both carotid artery and jugular vein catheters (described later). On the day of study, each rat had fully recovered as evidenced by progressive weight gain that, at a minimum, led to the recovery of presurgical weight. On the morning of study, the arterial and venous catheters were connected to withdrawal and infusion syringes, respectively.

Catheterization was performed under ketamine hydrochloride anesthesia (10 mg/100 g animal weight, intramuscularly). Polyethylene tubing (ID 0.23 in × OD 0.038 in; Becton Dickinson, Parsippany, NJ) was inserted into the left carotid artery, and silastic tubing (ID 0.20 in × OD 0.037 in; Dow Corning, Midland, MI) was inserted into the right jugular vein. Both catheters were tunneled under the skin and exteriorized in the nuchal area. Animals were allowed to recover over the following 3 to 4 days, during which time normal behavior and progressive weight gain were observed. Catheters were kept patent by flushing with a heparin solution on alternate days. Except during flushing and during infusion studies, catheters were kept capped at all times.

Experimental Design

The control group (saline, n = 12; weight, 268 ± 6 g) was studied during infusion of normal saline (0.018 ml · min^{-1}), while the experimental group (glucosamine, n = 12; weight, 254 ± 7 g) was studied during glucosamine infusion (3.5 mg · kg^{-1} · min^{-1}, 0.018 ml · min^{-1}). Subgroups of rats in each group (saline, n = 7, 263 ± 9 g; and glucosamine, n = 7, 242 ± 8 g) underwent arginine stimulation during a hyperglycemic clamp.

Hyperglycemic clamps were performed in awake, chronically catheterized rats. The protocol is summarized in Fig 1. After a 20-minute baseline period during which fasting glucose and insulin levels were measured in duplicate, a variable infusion of 20% dextrose solution (Abbott Laboratories, North Chicago, IL) via an adjustable Razel pump (model A-99; Razel Scientific Instruments, Stamford, CT) was used to achieve blood glucose levels of approximately 220 mg · dL^{-1}. Blood glucose was determined every 10 minutes (Yellow Springs Instruments, Yellow Springs, OH). When blood glucose was maintained at the desired level with minimal changes in the glucose infusion rate (<10%) for at least 30 minutes, we assumed that steady-state was achieved. The steady-state glucose infusion rates were maintained from 60 to 130 minutes. Insulin levels were determined at 90 and 100 minutes, after which a 100 mg · kg^{-1} bolus of L-arginine (100 mg · dL^{-1}) was infused (arginine stimulation). Insulin and glucose levels were determined 1, 3, 5, 10, 15, and 30 minutes following the arginine bolus.

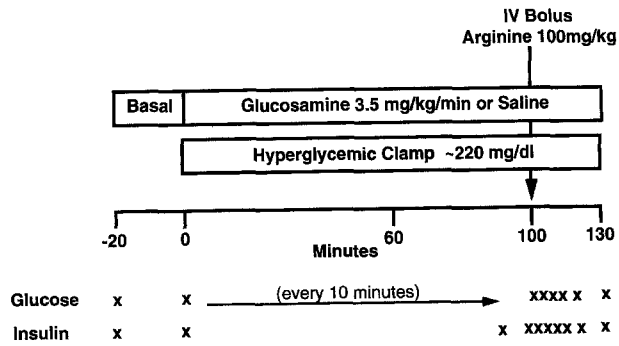

Fig 1. Two groups of rats (n = 12 per group) underwent hyperglycemic clamp (glucose ~220 mg · dL^{-1}) studies during concomitant infusion of either glucosamine (3.5 mg · kg^{-1} · min^{-1}) or saline for up to 130 minutes. Serum insulin and glucose levels were measured at baseline and after 2 hours of infusion. After 100 minutes of the hyperglycemic clamp with concomitant infusion of glucosamine or saline, subgroups of 7 rats from each group underwent an arginine tolerance test (L-arginine 100 mg · dL^{-1} bolus).

Assays

Blood glucose levels were measured by the glucose oxidase method using a glucose analyzer (model 2500; Yellow Springs Instruments).

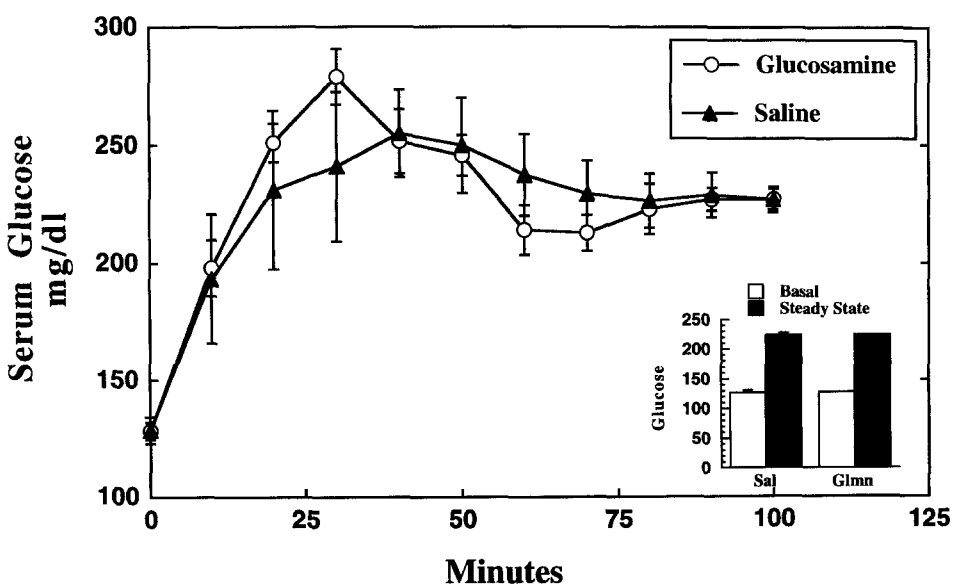

Fig 2. Hyperglycemic clamp (glucose ~220 mg · dL^{-1}) in rats receiving a coinfusion of glucosamine (3.5 mg/kg/min) or normal saline. Insert, baseline and steady-state serum glucose (~60 to 100 minutes).

Serum insulin levels were measured by a double-antibody radioimmunoassay (Linco, St Louis, MO) using rat insulin antibody.

Statistical Methods

The data are expressed as the mean ± SEM. All comparisons were made between saline and glucosamine groups. Results were analyzed using the Statview 4.5 software package (Abacus Concepts, Berkeley, CA) on a Power Macintosh 7600 computer (Apple, Seattle, WA). The two groups were compared using ANOVA followed by Fisher's protected least-significant difference test or Student's *t* test where applicable. Significance was accepted at P less than .05. The area under the curve for insulin secretion after arginine stimulation was calculated using the trapezoidal rule.

RESULTS

Glucose-Stimulated Insulin Secretion

Steady-state blood glucose levels (between 60 and 100 minutes) were achieved in the saline and glucosamine groups and were almost identical (223.58 ± 3.94 *v* 224.58 ± 4.34 mg · dL^{-1}, respectively, ANOVA and *t* test, $P > .05$; Fig 2). However, glucose infusion rates at steady state were markedly different in the two groups (26.55 ± 1.60 *v* 8.83 ± 1.35 mg · kg^{-1} · min^{-1}, respectively, ANOVA and *t* test, $P < .0001$; Fig 3).

Insulin levels were comparable in the saline and glucosamine groups at baseline (9.95 ± 3.32 *v* 12.29 ± 3.91 μU · mL^{-1}, respectively, $P > .05$, *t* test; Fig 4). In contrast, at steady-state, insulin levels in glucosamine-infused animals were less than half those of the saline-infused controls (41.36 ± 6.47 *v* 18.04 ± 2.95 μU · mL^{-1}, $P < .0001$, *t* test).

Arginine-Stimulated Insulin Secretion

The prevailing glucose concentrations were comparable in the saline and glucosamine groups during the arginine stimulation test (data not shown). The pattern of insulin release following the arginine bolus was identical in both groups (Fig 5). The peak insulin level (274 ± 30.78 *v* 176.25 ± 20.12 μU · mL^{-1}, $P = .0209$, *t* test), increment above the prevailing insulin level before the arginine bolus (232.64 ± 24.9 *v* 158 ± 17.9 μU · mL^{-1}, $P = .0319$, *t* test), and area under the curve (1,268.09 ± 142.27, *v* 706.77 ± 84.79 μU · min^{-1} · min, $P = .0054$, *t* test) were significantly reduced in the glucosamine versus saline groups.

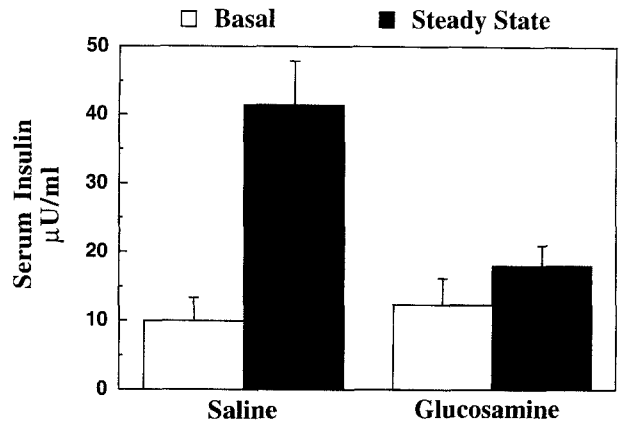

Fig 4. Baseline and steady-state (~120 minutes) serum insulin during a hyperglycemic clamp (glucose ~220 mg · dL^{-1}) in rats receiving a coinfusion of glucosamine (3.5 mg · kg^{-1} · min^{-1}) or normal saline.

DISCUSSION

This study was designed to test the hypothesis that an elevated circulating glucosamine concentration can recreate in normal nondiabetic rats the insulin secretory defects observed in patients with NIDDM. The current data are consistent with this formulation.

In cultured rat adipocytes, glucosamine, a product of the hexosamine pathway, was found to mediate glucose-induced insulin resistance—"glucose toxicity."[7,8] This led to studies assessing the role and mechanism of glucosamine in inducing insulin resistance. Our group and others have demonstrated that a continuous infusion of glucosamine can result in peripheral and hepatic insulin resistance in nondiabetic rats.[8,9,13] We have further documented that the mechanism of glucosamine-induced insulin resistance in skeletal muscle is maximal at a dose of 3.5 mg · kg^{-1} · min^{-1} and is due to an impairment of the insulin-induced translocation of GLUT-4, the insulin-responsive glucose transporter in muscle and fat, from a cytosolic location to the plasma membrane.[8] This has led to the proposi-

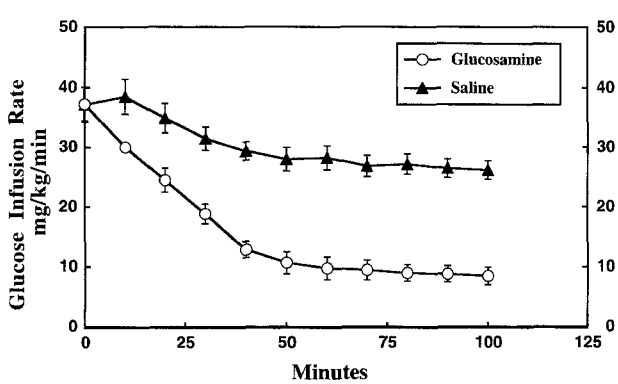

Fig 3. Glucose infusion rate (mg · kg^{-1} · min^{-1}) during hyperglycemic clamp (glucose ~220 mg · dL^{-1}) in rats receiving a coinfusion of glucosamine (3.5 mg · kg^{-1} · min^{-1}) or normal saline.

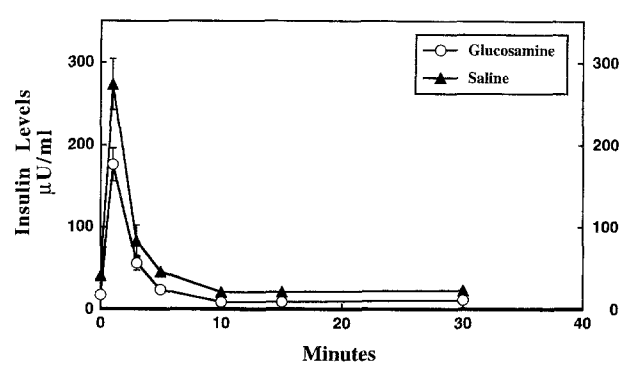

Fig 5. Serum insulin levels in a subgroup of animals (n= 7 from each group) during a hyperglycemic clamp after an arginine bolus of 100 mg/dL, during either glucosamine (3.5 mg · kg^{-1} · min^{-1}) or saline infusion.

tion that in patients with uncontrolled NIDDM, hyperglycemia leads to increased glucose flux through the HBP, resulting in increased intracellular levels of glucosamine and other HBP products, which in turn worsen the insulin resistance characteristic of this disorder.

In animals and humans, sustained hyperglycemia has been shown to impair insulin secretion,[14-17] but the mechanism is not clear. Glucosamine has been shown to impair insulin secretion in response to glycemic stimuli in perfused rat pancreata.[18] Few recent studies have addressed this issue in vivo.[9,10] In one study, a 200-mg intravenous bolus of glucosamine or saline was injected into adult Sprague-Dawley rats,[10] and insulin secretion in response to subsequent infusion of glucose and arginine was compared in both groups. The investigators reported that after the glucosamine bolus, insulin secretion in response to a glucose challenge was markedly reduced, but insulin levels achieved after arginine infusion were not statistically different in the two groups. They concluded that glucosamine induces a glucose-specific β-cell abnormality in insulin secretion. Although the studies were well performed, the conclusions were weakened by the fact that prevailing blood glucose levels were not matched in the experimental groups, and thus glucosamine-infused animals exhibited higher blood glucose values. It is arguable that the insulin level in the saline group would have been higher if the blood glucose level had been matched to that of the glucosamine group. Therefore, a role for glucosamine to impair non–glucose-mediated insulin secretion was not ruled out by this study.

In another study, Giaccari et al[9] found that at matched prevailing blood glucose concentrations, glucosamine infusion ($0.805\ mg \cdot kg^{-1} \cdot min^{-1}$) induced defects in glucose-induced but not arginine-induced insulin secretion in normal adult Sprague-Dawley rats. Given that this study controlled for the prevailing blood glucose levels, the difference in the results versus our study cannot be attributed to differences in glycemic stimulus. The higher dose of glucosamine infused in our study ($3.5\ mg \cdot kg^{-1} \cdot min^{-1}$) could explain the difference in the results obtained. Because circulating and intra-β-cell levels of glucosamine in patients with uncontrolled diabetes mellitus are not known, it is difficult to ascertain which infusion rate most closely reproduces the pathophysiological state of NIDDM. Although we did not test lower doses of glucosamine, our data and those of Giaccari et al[9] are consistent with the formulation that the effect of glucosamine on insulin secretion is dose-dependent, as is its effect to induce insulin resistance.[8] It is also possible that glucosamine is more potent in inducing defects in glucose-mediated insulin secretion versus nonglucose secretagogues.

The exact mechanisms of glucosamine-induced β-cell dysfunction have not been addressed in this study. With in vitro studies, glucosamine has been shown to inhibit glucokinase in the β cell.[10] Glucokinase, one of the rate-limiting enzymes of the glycolytic pathway, phosphorylates glucose and functions as a glucose sensor in the pancreatic β cell. Decreased activity of this enzyme results in a higher threshold for glucose-induced insulin release.[4] It is not clear how a defect in glucokinase activity impairs arginine-induced insulin secretion, but in individuals with an inherited defect in the glucokinase gene (a form of maturity-onset diabetes of the young), both glucose- and arginine-induced insulin secretion appear to be impaired.[19] Another in vitro study suggests that glucosamine inhibits β-cell phospholipase C activity, resulting in a decrease in the hydrolysis of phosphoinositol, which is then responsible in part for β-cell desensitization to glycemic stimuli.[18] Similar changes have been described in pancreata isolated from rats subjected to chronic in vivo hyperglycemia.[20] Both an impairment of glucokinase and a reduced phospholipase C activity may be instrumental in the glucosamine-induced β-cell dysfunction.

In conclusion, glucosamine induces β-cell insulin secretory defects very similar to the defects noted in patients with NIDDM. Therefore, it is possible that the HBP is instrumental in causing β-cell dysfunction in hyperglycemic patients. If this is the case, it is possible that postprandial hyperglycemia which precedes fasting hyperglycemia in the pathogenesis of NIDDM could contribute to further β-cell failure by increasing glucose flux through the HBP. If this proposition is correct, strategies for early treatment of postprandial hyperglycemia or its toxicity could delay or prevent the onset of NIDDM in populations at risk. This hypothetical construct remains to be tested.

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