

Perspectives in Diabetes

Hexosamines and Insulin Resistance

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Glucose is an important regulator of cell growth and metabolism. Thus, it is likely that some of the adverse effects of hyperglycemia are reflections of normal regulation by abnormal concentrations of glucose. How the cell senses glucose, however, is still incompletely understood. Evidence has been presented that the hexosamine biosynthesis pathway serves this function for regulation of aspects of glucose uptake, glycogen synthesis, glycolysis, and synthesis of growth factors. Excess hexosamine flux causes insulin resistance in cultured cells, tissues, and intact animals. Further evidence for the possible role of this pathway in normal glucose homeostasis and disease is that the level of activity of the rate-limiting enzyme in hexosamine synthesis, glutamine:fructose-6-phosphate amidotransferase, is correlated with glucose disposal rates (GDRs) in normal humans and transgenic mice. *Diabetes* 45:1003-1009, 1996

ADVERSE EFFECTS OF HYPERGLYCEMIA: TOXICITY VERSUS DYSREGULATION

The results of several clinical studies, most recently the Diabetes Control and Complications Trial, convincingly demonstrate that hyperglycemia is the cause of most if not all of the chronic complications of diabetes (1). In addition to these mainly vascular problems, hyperglycemia can also have adverse consequences for glucose homeostasis itself (2,3). These changes are part of a vicious cycle that worsens the diabetic state and makes glycemic regulation more difficult. At the level of the pancreatic β -cell, there is evidence that hyperglycemia itself can lead to many of the defects in insulin secretion that are observed in NIDDM (4-6). Hyperglycemia also worsens insulin resistance (2,7,8), and resistance improves upon attaining tight control of diabetes (6). In vitro, adipocytes exposed to high concentrations of glucose develop impaired insulin signaling and responsiveness and recruit fewer glucose transporters to the plasma membrane in response to insulin (9,10). Muscle

glycogen synthase activity can also be affected by hyperglycemia (11,12). Thus, hyperglycemia interferes widely with cellular metabolism and the mechanisms for insulin-induced glucose disposal.

Such adverse metabolic consequences of hyperglycemia have been referred to as glucose toxicity (6). There have been several hypotheses proposed for the biochemical basis for glucose toxicity, and any of the several proposed mechanisms may contribute to pathology in different cells or tissues. For example, high concentrations of glucose might damage cells through nonenzymatic glycation of proteins and the accumulation of advanced glycation end products (13,14). Other theories on the mechanism of glucose toxicity have considered the accumulation in cells of normal products of glucose metabolism, but at higher than normal concentrations. Sorbitol accumulates in diabetic nervous tissue (15,16), and excess glucose can also lead to the accumulation in cells of diacylglycerol, an activator of protein kinase C (PKC) that could have wide-ranging effects on cellular regulation (17,18).

Glucose is also known to be an important regulator of normal cell growth and metabolism. Therefore, it may be useful to distinguish "toxic" effects from normal regulatory or desensitizing effects, as has been pointed out by Robertson et al. (19). Some of the consequences of hyperglycemia can be well understood as toxic in the classic sense, such as the nonenzymatic glycation of proteins. On the other hand, some of the adverse results of hyperglycemia might be caused by normally functioning regulatory pathways. The fact that excessive glucose flux through its normal metabolic pathways rather than hyperglycemia per se can have adverse consequences has been demonstrated with mice overexpressing the GLUT1 glucose transporter (20). Increased glucose flux into skeletal muscle leads to insulin resistance in these mice despite the fact that they have somewhat lower than normal serum glucose levels. In the presence of excess glucose, protective mechanisms should exist that prevent cellular overfeeding and shunt glucose toward chronic storage pathways. Such changes—blunting of insulin-stimulated glucose uptake and glycogen synthesis, downregulation of glucose transporters in sensitive tissues, and increases in the synthesis of fatty acids and triglycerides, for example—might be protective of cells and tissues over periods of hours to days but maladaptive to the organism in conditions of chronic hyperglycemia or caloric excess.

How cells sense glucose flux so that they may regulate their metabolism according to the availability of fuel is largely unknown, although it is generally agreed that glucose metabolism is required for such effects. At the simplest level, some glucose metabolites act as allosteric regulators of key

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DON, diazo-oxo-norleucine; F6P, fructose-6-phosphate; GDR, glucose disposal rate; GFA, glutamine:fructose-6-phosphate amidotransferase; GlcNAc, *N*-acetylglucosamine; PKA, protein kinase A; PKC, protein kinase C; PP1, protein phosphatase 1; TGF- α , transforming growth factor α ; UDP, uridine diphosphate; UTP, uridine triphosphate.

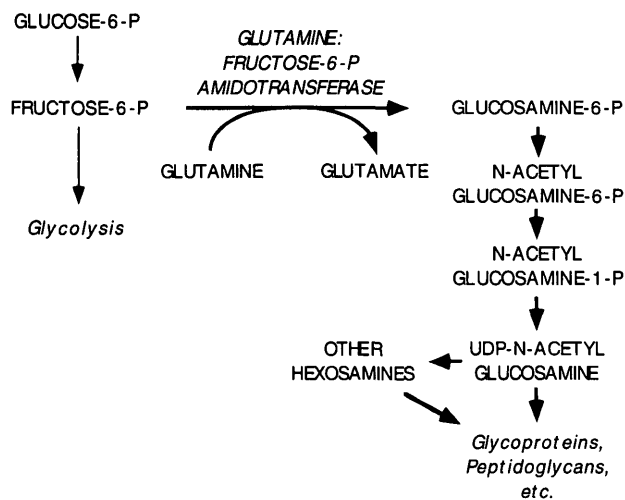


FIG. 1. The hexosamine biosynthesis pathway.

enzymes in glycolytic, glycogenic, and gluconeogenic pathways. The stimulation of insulin secretion by glucose has been hypothesized to be the result of changes in ADP/ATP ratios brought about by graded flux down the glycolytic pathway, ultimately controlled by the relatively low affinities for glucose of the glucose transporter GLUT2 and glucokinase in the β -cell (21,22). Nevertheless, other evidence is difficult to reconcile with the ADP/ATP ratio hypothesis, and the more subacute sensing of glucose flux such as is responsible for the regulation of insulin gene transcription probably operates through a different mechanism (23–25). The identification of carbohydrate response elements in several genes should ultimately lead to the clarification of these mechanisms (26). At the present time, however, the identity of the metabolic pathways through which cells are made aware of and respond to fuel availability remains unknown. Given the importance of glucose for cell growth and metabolism from prokaryotes to humans, it is not unlikely that glucose sensing and regulation may have evolved to operate through more than one mechanism.

THE HEXOSAMINE PATHWAY AND INSULIN RESISTANCE: IN VITRO STUDIES

It has recently been shown that at least some of the regulatory effects of glucose are mediated by the hexosamine biosynthesis pathway, in which fructose-6-phosphate (F6P) is converted to glucosamine-6-phosphate, with glutamine acting as the donor of its amido group (Fig. 1). The final products of the pathway are uridine diphosphate (UDP)-N-acetyl-glucosamine (GlcNAc) and other nucleotide hexosamines. The amination of F6P is rate limiting—except in cases of very high flux, in which case uridine triphosphate (UTP) may be limiting (27)—and is catalyzed by glutamine: F6P amidotransferase (GFA or GFAT). This enzyme in eukaryotes is subject to feedback inhibition by UDP-GlcNAc and can be experimentally inhibited by glutamine analogs such as azaserine or diazo-oxo-norleucine (DON).

The first to implicate the hexosamine pathway in cellular regulation were Marshall et al. (28), who were studying glucose transport in cultured adipocytes exposed to high concentrations of glucose. A serendipitous finding that glutamine was a required cofactor for glucose-induced desensitization of the insulin-stimulated glucose transport system prompted a series of systematic and elegant experiments

that resulted in the hypothesis that hexosamine metabolism might be involved. Glucosamine was found to be many times more potent than glucose in inducing insulin resistance and decreased insulin responsiveness. Marshall was able to block the ability of glucose to induce insulin resistance by inhibiting GFA, and glucosamine was able to bypass that blockade. He therefore hypothesized that hexosamine metabolism may be the pathway by which cells sense and respond to the ambient glucose levels and, when glucose flux is excessive, downregulate glucose transport and become insulin resistant (28). Later studies from Marshall's laboratory strengthened the hypothesis and currently support the idea that glucosamine flux results in transcriptional regulation of a number of genes relevant to glucose homeostasis (see below).

Several other laboratories have subsequently examined the effects of hexosamines on glucose homeostasis. Robinson et al. (29) showed that preexposure to glucosamine induced insulin resistance in skeletal muscle, the tissue responsible for the majority of insulin-dependent glucose utilization. Incubating rat hemidiaphragms in 5–22 mmol/l glucosamine resulted in a 20–60% reduction in basal glucose transport and a significant reduction in the ability of insulin to increase glucose transport (29). They hypothesized that the decrease in glucose transport was secondary to altered translocation of the GLUT4 transporter because the total GLUT4 pool was not affected by glucosamine. Moreover, they showed that preexposure to glucosamine abolished the ability of insulin to stimulate glycogen synthesis but that insulin stimulation of glycogen synthase and insulin receptor number/activation were not affected. In contrast to these results, in L_6 myotubes, hexosamine biosynthesis had no effect on glucose regulation of glucose transport (30); the different results of these studies may be explained by the fact that glucose transport in L_6 myotubes is mediated by GLUT1 rather than GLUT4.

To avoid the possible side effects of treating cells with high concentrations of glucosamine and to implicate the hexosamine pathway more directly in glucose homeostasis, our laboratory has taken a transgenic approach to modifying intracellular hexosamine biosynthesis. Initially, the yeast cDNA for GFA was isolated and transfected into Rat-1 fibroblasts by electroporation. Cells overexpressing GFA were insulin resistant, as demonstrated by a rightward shift in the dose-response curve for insulin-stimulated glycogen synthase activity (31). The cells that overexpressed GFA did not exhibit changes in total glycogen synthase activity (an indirect measure of enzyme mass), maximal insulin-stimulated activity (insulin responsiveness), or insulin binding and receptor number. Thus, the defect in insulin signaling appeared to be a postreceptor one. Glucose uptake, mediated mainly by GLUT1 in these cells, was also unaffected by overexpression of GFA.

Subsequently, we were able to stably overexpress the human cDNA for GFA (32,33) in Rat-1 fibroblasts to facilitate further mechanistic studies of how hexosamine metabolism regulates glycogen synthesis (34). The increase in the levels of GFA that we were able to achieve in our transfectants was modest, on the order of twofold. Despite this, cells stably overexpressing GFA were insulin resistant for the stimulation of glycogen synthase activity. Basal glycogen synthase activity and insulin sensitivity were both decreased by treatment of the cells with high concentrations (10–20 mmol/l) of glucose, and this decrease in basal synthase activity was

observed at lower glucose concentrations in cells overexpressing GFA. GFA overexpression also accentuated the effects of high glucose on insulin sensitivity (35). These results support the hypothesis that glucose sensing for the regulation of insulin-stimulated glycogen synthase does operate through the hexosamine biosynthesis pathway.

Glycogen synthase, the rate-limiting enzyme in glycogen synthesis, is regulated through a complex cascade of protein kinases and phosphatases. The activity of glycogen synthase is determined by the phosphorylation state of the enzyme and is under hormonal control (36). The enzyme can be phosphorylated at multiple sites by >10 protein kinases (37) that in general inhibit enzyme activity (38). Insulin activates glycogen synthase by stimulating its dephosphorylation (39–41). An insulin-stimulated protein kinase has been shown in vitro to phosphorylate and activate PP1G (41), the glycogen-bound form of type-1 protein phosphatase. In cells overexpressing GFA, we found PP1 to be downregulated by glucose. Glucosamine downregulates basal PP1 activity with greater potency than glucose, and both glucosamine and high glucose significantly reduce insulin's ability to stimulate PP1 (35). In contrast, mitogen-activated protein (MAP) kinase and S6 kinase, intermediates in the insulin signaling cascade, have been shown not to be affected by glucosamine in rat fibroblasts (29). Similarly, we have seen no alterations in S6 kinase activity in cells overexpressing GFA (E. Crook, unpublished observations). Taken together, these data show that hexosamines regulate glycogen synthase by regulating its phosphorylation state. This regulation appears to occur more distally in the insulin signaling cascade, and the relatively slow time course of the regulation suggests a transcriptional mechanism.

Other enzymes and proteins involved in glucose disposal have also been shown to be regulated by hexosamine metabolism in vitro. These include pyruvate kinase (42), glycogen synthase in rat adipocytes (43), and GLUT1 in bovine retinal capillary pericytes (44). An important goal for future research, therefore, is to define the extent and generality of the regulation of metabolism through this pathway.

HEXOSAMINES AND INSULIN RESISTANCE IN VIVO

Diabetes is a disease of the whole animal, and although there are in vitro models for aspects of diabetes, ultimate proof of any mechanism in that disease requires its demonstration in the intact organism. The effects of excess hexosamines in intact animals were first studied by Rossetti et al. (45). Rats were infused for 7 h with glucosamine, resulting in plasma glucosamine concentrations of ~1.2 mmol/l. Euglycemic-hyperinsulinemic glucose clamp studies were then performed to measure GDRs under conditions in which hepatic glucose output was suppressed. Glucosamine infusion resulted in a 31% decrease in GDRs in normal animals, but glucosamine led to no further reduction in the suppressed glucose disposal observed in partially pancreatectomized diabetic rats. The latter fact demonstrates that hyperglycemia and glucosamine are nonadditive; that is, they probably operate through the same pathway to cause decreased glucose disposal. Muscle glycogen synthase activity was unaffected by the glucosamine infusion, in contrast to the results obtained by Crook et al. (31) in cultured fibroblasts. Whether this difference is due to the cell type examined or to the relatively short-term glucosamine infusion is not known.

More recently, Baron et al. (46) observed similar results in rats infused with glucosamine at a rate of $0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, or 1/70th of the molar rate of glucose uptake (46). They were able to demonstrate that glucosamine impaired the translocation of the insulin-stimulated glucose transporter GLUT4 similarly to what is observed in human insulin-resistant states. As was the case in the previous study, these experiments were performed in animals infused with maximal concentrations of exogenous insulin, an important point because glucosamine is an inhibitor of glucokinase and has been shown to interfere with β -cell glucose sensing and endogenous insulin secretion (47).

The metabolic fate of infused glucosamine is important to consider in the interpretation of these studies. Glucosamine enters the cell through the glucose transporters and is then phosphorylated by hexokinase. The K_m for uptake of glucosamine is approximately three times that of glucose (E. Crook, unpublished observations), and the affinity of hexokinase for glucosamine is decreased to a similar degree compared with glucose. Thus, the concentrations of glucosamine achieved in the blood of infused animals are probably not sufficient to cause their effects by competing for glucose uptake or metabolism. However, glucosamine has negligible blood concentrations in animals, diabetic or not, and in order to force enough glucosamine through the hexosamine biosynthesis pathway, clearly nonphysiological concentrations of glucosamine are required. When exposed to concentrations of glucosamine in the millimolar range, cellular levels of UTP can be depleted because of increased rates of nucleotide-hexosamine formation (27). This could lead to marked changes in intracellular glucose utilization, for example if UTP became no longer available for UDP-glucose and subsequent glycogen synthesis (29). At these concentrations of glucosamine, protein glycosylation is also inhibited.

For these reasons, we have performed analogous experiments in a situation where there would less likely be large shifts in substrate fluxes. Namely, we have overexpressed GFA in transgenic animals at approximately twofold increased levels. Thus, the hexosamine pathway that normally accounts for perhaps 2% of total cellular glucose flux (28) might now account for 4–6%, a level that should not significantly alter glucose availability for oxidative or nonoxidative metabolism. In cultured cells, these levels of chronic GFA overexpression did not alter nucleotide triphosphate concentrations but did result in an approximately twofold increase in the levels of UDP-hexosamines. GFA was targeted to the two principal tissues for insulin-mediated glucose disposal, striated muscle and fat, using the promoter for the glucose transporter GLUT4. Two independent founder lines with 1.5- to 2.3-fold increased levels of GFA activity in extracts of both fat and muscle were analyzed. Fasting glucose and insulin levels were not different from the controls, the predicted result based on the specific targeting of the gene to muscle and fat and not to the liver. That is, hepatic glucose output is presumably normal, and only insulin-mediated glucose disposal into its target tissues should have been affected. Indeed, random-fed animals were hyperinsulinemic, and the insulin-to-glucose ratio was significantly elevated in the fed transgenics (48). The hyperinsulinemia was age- and weight-dependent, becoming statistically significant at 6 months and in animals >30 g, a phenotype reminiscent of NIDDM. The suggestion of insulin

resistance based on the elevated insulin-to-glucose ratios was confirmed by the use of the euglycemic-hyperinsulinemic clamp technique ($20 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ insulin, glucose levels clamped at $125 \pm 15 \text{ mg/dl}$). Transgenic animals exhibited a significant 48% decrease in GDR compared with age- and weight-matched littermate controls. The 20 mU insulin concentration resulted in maximal glucose disposal and near total suppression of hepatic glucose output. Whether these mice can serve as an accurate model for human diabetes or glucose toxicity is currently under investigation.

HEXOSAMINES AND REGULATION OF CELL GROWTH

Other evidence also links the hexosamine pathway to glucose-induced changes in cell growth. It has been shown that glucose, through its metabolism to glucosamine, can affect the regulation of the gene for transforming growth factor α (TGF- α) in cultured vascular smooth muscle cells (49,50). Glucose was shown to stimulate the level of TGF- α mRNA in primary cultures of rat aortic smooth muscle cells approximately twofold, whereas glucosamine at lower concentrations stimulated mRNA levels six- to sevenfold. GFA overexpression mimicked the effects of high glucose on TGF- α expression (50), and these effects were abolished by inhibitors of GFA. Importantly, the effects of sugars and of GFA overexpression on TGF- α were nonadditive, arguing that glucose and glucosamine did exert these effects through the hexosamine pathway. Studies with phorbol esters to pharmacologically stimulate or downregulate PKC and with various stimulators of cAMP-dependent pathways gave no evidence to support PKC or cAMP as mediators of these effects.

More recent work has focused on the growth factor TGF- β . TGF- β , which is not structurally related to TGF- α , has been implicated in the pathogenesis of diabetic nephropathy. TGF- β can cause increased cell matrix synthesis in vitro and glomerulosclerosis in vivo, and the protein is known to be upregulated by glucose (51). The question has therefore been asked whether this glucose regulation might also be based on hexosamine flux, and the preliminary indications are that it would appear to be so. Namely, glucosamine has been shown to be more potent than glucose in stimulating TGF- β transcription in cultured renal glomerular and proximal tubule cells (52). Such results may therefore link the hexosamine pathway not only to the metabolic abnormalities of hyperglycemic states but to chronic vascular complications of diabetes as well. Besides the involvement of TGF- β in diabetic nephropathy, growth factors such as platelet-derived growth factor, fibroblast growth factor, and TGF- β have also been implicated in the development or progression of atherosclerosis. Glucose-induced stimulation of these growth factors may be part of the explanation of the increased risk of vascular disease in diabetes.

POSSIBLE MECHANISMS FOR EFFECTS OF HEXOSAMINES

How alterations in hexosamine flux might regulate metabolism is unknown. The effects of glucosamine infusions into rats to cause insulin resistance and defects in glucose uptake occur very rapidly, suggesting posttranslational mechanisms. On the other hand, the effects of glucosamine on TGF- α are clearly transcriptional (49,50), and indirect pharmacological

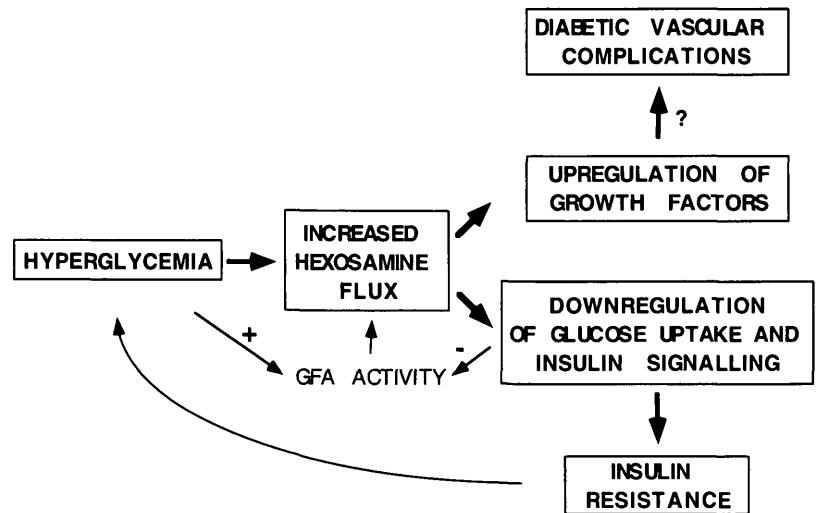
data on the desensitization of the glucose transport system in adipocytes suggested the same (53).

Production of the substrates for protein glycosylation by the hexosamine pathway suggests a possible mechanism for the regulatory effects of hexosamines. Recently, a pathway in which intracellular proteins are modified by the O-linked addition of single GlcNAc residues has been described (54). This cytosolic O-glycosylation is a widespread event that occurs reciprocally with phosphorylation and is highly dynamic and regulated, occurring with mitogenic stimulation of lymphocytes and in G_1 phase of the cell cycle (55). All of the proteins that have been found to be modified by O-linked addition of GlcNAc are multimeric, leading to the possibility that this modification may be important in regulating the assembly of large protein complexes such as occur in cytoskeletal and transcriptional assemblies. Several transcription factors are O-glycosylated (56), and the functional consequences of O-glycosylation to their assembly into active complexes are currently being studied in several laboratories. *c-myc*, for example, has been recently shown to be O-glycosylated at a known phosphorylation site, lending credence that glycosylation may be a regulatory modification (57). Glucose-responsive elements from several mammalian genes have been identified, and interestingly, these include *myc*-like response elements (26). Our current hypothesis, which is still speculative, is based on the studies of insulin-stimulated glycogen synthase in cells overexpressing GFA. It is that hexosamines will act as glucose sensors by transcriptionally modulating key regulators of cellular metabolism such as the serine/threonine phosphatases involved in insulin signaling of glycogen synthesis. Such action at one or more key nodes in the pathways regulating metabolism in cells would result in global changes in glucose flux that would allow a cell to adapt to satiety, e.g., with downregulation of glucose uptake and coordinated changes in glycogen synthesis, glycolysis, and fat metabolism.

REGULATION OF HEXOSAMINE BIOSYNTHESIS

Taken together, the data reviewed above support the hypothesis that metabolism of glucose through the hexosamine pathway has a number of effects relevant to cellular growth and metabolism. It is therefore important to understand in detail the regulation of the hexosamine pathway and its influence on other metabolic pathways. The bacterial, yeast, and human cDNAs for GFA have been cloned, and structure-function analysis of the bacterial and yeast enzymes has begun. It is known that in eukaryotes GFA activity is allosterically inhibited through feedback by the downstream product of hexosamine metabolism, UDP-GlcNAc (58). In fungi, the ability of the enzyme to be feedback inhibited is developmentally regulated (59). During germination, when uninhibited synthesis of cell wall constituents including UDP-GlcNAc would be desirable, GFA loses its feedback inhibition. During sporulation, the enzyme regains its feedback inhibition, correlated with increased phosphorylation of a protein that copurifies with GFA activity. In vitro, a similar change in feedback inhibition could be induced with cAMP-dependent kinase (protein kinase A [PKA]). Thus, there is evidence that posttranslational regulation of GFA occurs in an important physiological setting. Human GFA has two consensus PKA phosphorylation sites in the "hinge" region between the NH_2 -terminal amidohydrolase and the

FIG. 2. Proposed role of the hexosamine biosynthesis pathway in mediating effects of chronic hyperglycemia. Excess flux through the pathway has been shown to result in insulin resistance in a number of systems, both *in vitro* and *in vivo*. This insulin resistance (and impaired β -cell function) triggered by hyperglycemia has been termed glucose toxicity and results in a vicious cycle of higher levels of glycemia leading to worse insulin resistance. The hexosamine pathway has also been implicated in the regulation of various growth factors by glucose. One of these, TGF- β , has been proposed to be directly related to the expansion of extracellular matrix and pathogenesis of diabetic nephropathy. The rate-limiting enzyme in hexosamine synthesis, GFA, is subacutely upregulated by glucose and insulin. This upregulation may serve to counter the decreased glucose flux resulting from the insulin resistance and downregulation of glucose transport. Thus, in chronic hyperglycemia, pressure would be maintained on the system to keep glucose uptake downregulated even after net glucose flux into the cell had been normalized.



COOH-terminal aldose isomerase domains, and pharmacological data suggest that human GFA is modulated by cAMP-dependent pathways (60).

Both insulin and glucose upregulate GFA activity modestly (approximately twofold) in cultured human muscle cells (61), and prolonged treatment with epidermal growth factor upregulates GFA transcription in a human breast cancer cell line (62). GFA activity in freshly obtained muscle biopsy specimens is higher in NIDDM patients compared with control subjects, and the increase in activity was correlated with glycohemoglobin levels (63). This difference between diabetic and control subjects was not seen in muscle cells cultured *ex vivo* in conditions of controlled glucose and insulin concentrations (61), suggesting that the difference is secondary to hyperglycemia and/or hyperinsulinemia in the diabetic subjects. In rats, acute hyperglycemia did not affect GFA activity, whereas in chronic hyperglycemia—streptozotocin diabetic animals—GFA activity decreased (64). Insulin reversed those changes that were not associated with changes in GFA mRNA levels. The reasons for the partial discordance between the rat and human data are not clear. It must be remembered, however, that if GFA is regulated by glucose, it is intracellular glucose flux that is responsible; hyperglycemia will not correlate directly with the rates of total glucose entry into cells because of concomitant downregulation of glucose transport. It is this fact that may explain the somewhat surprising upregulation of GFA by glucose and insulin: The upregulation may serve as a compensatory mechanism to maintain total hexosamine flux in the face of the downregulation of glucose uptake (Fig. 2 and below).

RELATION OF HEXOSAMINE METABOLISM TO HUMAN DISEASE

The finding that increased GFA activity in transgenic mice leads to insulin resistance and decreased insulin-stimulated glucose uptake suggests a possible glucose-sensing role for the hexosamine pathway in the control of glucose homeostasis. This hypothesis is supported by the study of GFA levels in human muscle cell cultures. GFA levels were measured *in vitro* in these explanted cultured cells after 2–3 months in controlled culture conditions. GFA activity was significantly and negatively correlated with GDRs measured *in vivo* during a euglycemic-hyperinsulinemic clamp (61). The direc-

tion of the correlation of higher GFA being associated with lower GDR—is consistent with the transgenic mouse data and suggests a causal connection between hexosamines and glucose homeostasis. GFA activity is also correlated with obesity (61); this in part may simply restate the correlation between GFA and GDR, since obesity is so well correlated with GDR.

In NIDDM subjects, a different picture emerged (61). Namely, GFA is *positively* correlated with GDR in this group. This suggests that although basal GFA activity may be normal in NIDDM, the link between the generation of glucosamine-6-phosphate and the response to the hexosamine flux (downregulation of glucose transport, for example) may be altered in that disease. Future studies of the activity of this pathway in NIDDM are clearly indicated.

CONCLUSION

All of these studies suggest that hexosamine flux is related to glucose homeostasis and may be used for sensing extracellular glucose so that the cell can respond pleiotropically and adaptively to satiety (Fig. 2). The fact that the hexosamine pathway also utilizes glutamine as a substrate and that the K_m values for both F6P and glutamine are relatively high (in the millimolar range [58]) would allow this pathway to serve not only as a carbohydrate sensor but perhaps as a more general nutrient sensor as well. The regulatory changes signaled by this pathway may be adaptive in the short term, perhaps protecting muscle cells from excessive glucose entry or shunting surplus fuel to storage. In cases of chronic hyperglycemia or chronic caloric excess, however, these same adaptations may be reflected in some of the abnormalities of metabolism associated with the diabetic state, especially insulin resistance. The upregulation of growth factors through this pathway might also contribute to diabetic vascular complications. Finally, the altered relationship between GFA activity and glucose homeostasis in NIDDM suggests that the pathway might contribute to the underlying cause of insulin resistance as well. Future studies will be aimed at understanding the generality of metabolic regulation through the hexosamine pathway, the mechanisms by which hexosamines exert their regulatory effects, and their relation to disease states.

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