Glucosamine-induced Activation of Glycogen Biosynthesis in Isolated Adipocytes

EVIDENCE FOR A RAPID ALLOSTERIC CONTROL MECHANISM WITHIN THE HEXOSAMINE BIOSYNTHESIS PATHWAY

Received for publication, December 1, 2004, and in revised form, December 29, 2004
Published, JBC Papers in Press, January 12, 2005, DOI 10.1074/jbc.M413499200

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Enhanced flux through the hexosamine biosynthesis pathway (HBP) induces insulin resistance and facilitates lipid storage through the up-regulation of enzyme mRNA levels. Both actions occur over several hours and require gene expression. We now identify a regulatory arm of the HBP that involves rapid allosteric activation of glycogen synthase (GS) and stimulation of glycogen biosynthesis (GBS). When insulin-pretreated adipocytes were exposed to 2 mM GlcN, incorporation of [14C]glucose into glycogen doubled by 10 min (t1/2 of <5 min), whereas UDP-glucose levels were concomitantly decreased by 100% (t1/2 of <5 min), whereas UDP-glucose levels were concomitantly decreased during this time (t1/2 of 1.4 min; >90% depletion). Stimulation of GBS and depletion of UDP-glucose both correlated with an early and rapid rise in the levels of glucosamine-6-phosphate (GlcN-6-P), a known activator of GS. The lowering of GlcN-6-P levels by removing extracellular GlcN (>80% reduction by 45 min) was accompanied by the restoration of UDP-glucose levels. Prolonged GlcN treatment (20 min to 2 h) inhibited GBS, which corresponded to a massive intracellular accumulation of GlcN-6-P (t1/2 of ~32 min; >1,400 nmol/g). From these data, we conclude the following. 1) GlcN treatment elevated intracellular GlcN-6-P levels within minutes, resulting in allosteric activation of GS, stimulation of GBS, and a reduction in steady-state levels of UDP-glucose due to increased precursor utilization. 2) Prolonged treatment with high concentrations of GlcN caused massive accumulation of GlcN-6-P that adversely affected cellular metabolism and reduced GBS. 3) The biphasic actions of GlcN on GBS may explain many of the discrepant reports on the role of the HBP in glucose metabolism.

Because a major function of this pathway is the generation of hexosamine metabolites, we postulated that under hyperglycemic conditions the enhanced flux of incoming glucose through the hexosamine biosynthesis pathway (HBP) culminates in the induction of insulin resistance (1, 2). Desensitization is likely mediated by altered gene expression, because desensitization occurs relatively slowly over several hours and because transcriptional inhibitors completely block glucose-induced desensitization (3). Numerous studies, both in vitro and in vivo, have now confirmed the role of the HBP in glucose-induced desensitization and extended the scope of this regulatory system to muscle tissue and other cell types (4–9).

In the current study, we have used GlcN and isolated adipocytes to examine the hypothesis that GlcN-6-P can allosterically enhance glycogen biosynthesis (GBS) through in vitro stimulation of glycogen synthase (GS). This idea originated from earlier studies showing that GlcN-6-P can allosterically activate GS in vitro (10, 11). The choice of adipocytes as our cellular model system derives from the fact that adipocytes represent a classical insulin-responsive target tissue. Moreover, adipocytes have proven useful in elucidating the mechanism(s) underlying glucose-induced insulin resistance (1, 4, 5, 12) and in identifying various genes regulated through the HBP (13). Our experimental approach entailed treating adipocytes with GlcN and then measuring the subsequent changes in glycogen metabolism (GBS and UDP-glucose levels) and the intracellular levels of two key hexosamine products (GlcN-6-P and UDP-GlcNAc). The rationale for this pharmacological approach is based on the finding that GlcN is rapidly transported into adipocytes through the insulin-responsive GTS, where it undergoes phosphorylation to GlcN-6-P, the first product of the HBP (1, 14). Thus, GlcN is relatively specific, because it directly enters the HBP and bypasses the first and rate-limiting enzyme of this pathway, which is glutamine:fructose-6-phospho amidotransferase. GlcN action was evaluated as a function of the treatment time (5 min to 4 h) and the GlcN dose (20 μM to 10 mM) by using insulin-pretreated cells, insulin- and glucose-pretreated cells, and control cells (no insulin or glucose pretreatment).

The obtained results support the hypothesis that relatively small increases in GlcN-6-P can rapidly stimulate GBS (within minutes) through allosteric activation of GS. Moreover, enhanced formation of glycogen appears to result in a decrease in the steady-state levels of UDP-glucose through precursor utilization. In general, our findings are conceptually important because they add a new regulatory dimension to the HBP that encompasses very rapid control of glucose metabolism through an allosteric mechanism. Thus, we postulate that enhanced flux within the HBP orchestrates both short term (allosteric) and long term (transcriptional) regulatory actions in response to glucose.
to nutritional information derived from the internal and external environments.

**EXPERIMENTAL PROCEDURES**

**Materials**—Sources of materials were as follows. Porcine insulin was from Sigma, collagenase was provided by Worthington Biochemicals (Freehold, NJ), bovine serum albumin came from the Armour Company (Kankakee, IL), and penicillin-streptomycin, Dulbecco’s modified Eagle’s medium (DMEM), and DMEM formula were formulated without α-glucose were purchased from Invitrogen. All other reagents were from Sigma or Fisher unless otherwise specified.

**Preparation and Treatment of Isolated Adipocytes**—Isolated adipocytes were obtained from the epididymal fat pads of male Sprague-Dawley rats (180–225 g) by collagenase digestion as described previously (15). After digestion, cells were washed three times in Heps-buffered balanced saline solution consisting of 25 mM Heps, 120 mM NaCl, 0.8 mM MgSO\textsubscript{4}, 2 mM CaCl\textsubscript{2}, 5.4 mM KCl, 1 mM NaH\textsubscript{2}PO\textsubscript{4}, 1 mM sodium pyruvate, 100 units/ml penicillin, 100 μg/ml streptomycin, and 1% bovine serum albumin, pH 7.6. Cells were then diluted to a final concentration of 30% KOH. From a common pool of cells, 400 μl of adipocytes were aliquoted into 17 × 100-mm sterile polystyrene tubes containing 1.6 ml of Heps-buffered saline solution. Unless otherwise indicated, insulin was added (25 ng/ml, final concentration), and the adipocytes were incubated at 37 °C for either 4 h (dose-response experiments) or for various time periods (time course experiments).

**Measurement of UDP-N-acetylglucosamine and UDP-glucose Levels**—After treatment the adipocytes were transferred to 1.5-ml microfuge tubes, and the cells were washed three times with ice-cold, bovine serum albumin-free Heps-buffered saline solution. During the final wash the cell volume was reduced to 150 μl, and the adipocytes were further incubated at 37 °C for either 4 h (dose-response experiments) or for various time periods (time course experiments).

**Measurement of GlcN-6-P Levels**—We have developed a fluorometric assay to measure GlcN-6-P levels based on the same enzymatic principles used to measure G-6-P levels. At high concentrations we found that G-6-P dehydrogenase can act on GlcN-6-P and NADP, resulting in equimolar production of NADPH, whereas at low concentrations (used to measure G-6-P) NADPH was not generated. Thus, the low affinity of G-6-P dehydrogenase for GlcN-6-P allows for the resolution of G-6-P (used to measure G-6-P) NADPH. Both assay methods yielded similar results, but the fluorometric enzymatic assay was faster, less expensive, and allowed for the measurement of numerous samples (using a 96-well assay format).

**Assessment of the Glycogen Biosynthesis Rate**—GBS was measured using a modified method derived from Smith and Lawrence (17). Briefly, isolated adipocytes were added to 17 × 100 polystyrene tubes and preincubated for 30 min at 37 °C with 25 ng/ml insulin (total volume of 200 μl). GlcN was then added for various time periods. After treatment, the rate of GBS was determined by adding 10 μl of [U-14C]glucose for 5 min at 37 °C. At the end of 5 min, the reaction was terminated by transferring 180 μl of adipocytes to a 400-μl microfuge tube and centrifuging the cells through silicone oil to separate cells from extracellular buffer and free the [14C]glucose. Adipocytes were then lysed, and glycogen was extracted by boiling cells for 10 min in 250 μl of 30% KOH. The mixture was centrifuged for 2 min at 8,200 g at 4 °C, and the deproteinated metabolite extract (420 μl) was transferred to a new microfuge tube. After the extract was neutralized by adding a small amount of K\textsubscript{2}CO\textsubscript{3} (5 μl), extracts were frozen and stored at −20 °C. The concentration of UDP-GlcNAc was measured by high performance liquid chromatography as described previously (16) by first passing the cell extract through a strong anion-exchange Supelclean LC-SAX column (Supelco, Bellefonte, PA). Analysis was performed using a high performance liquid chromatography system (Waters Associates, Milford, MA) and two reverse phase LC18 columns in series (Supelco). Metabolite concentration was quantitated by UV light absorption (260 nm), and levels were determined from a standard calibration curve.

**RESULTS**

**Biphasic Actions of GlcN on Glycogen Biosynthesis**—As shown in the time course study depicted in Fig. 1A, the addition of 2 mM GlcN to insulin-pretreated adipocytes rapidly enhanced the rate at which [14C]glucose was incorporated into glycogen. By 10 min, maximal stimulatory effects were seen (an ~2-fold increase; t\textsubscript{1/2} of <5 min). The rapidity of GlcN action is further illustrated by the fact that stimulatory effects were observed when GlcN was added immediately before the short 5-min assay (at time 0). As can also be seen in Fig. 1A, GlcN treatment longer than 20 min resulted in a progressive decrease in GBS, culminating in a GBS rate that was 50% below control levels at 2 h. In Fig. 1B, insulin-pretreated adipocytes...
From the dose-response curve depicted in Fig. 2D, it can be seen that GlcN-6-P levels were minimally affected when cells were incubated for 4 h with GlcN concentrations <250 μM. At 500 μM GlcN the levels were only slightly elevated, whereas GlcN-6-P levels were maximally elevated at 4 mM GlcN (ED$_{50}$ of 620 μM). Measuring UDP-GlcNAc levels as a function of GlcN concentration (Fig. 2E) revealed a very different pattern. Low doses of GlcN progressively increased UDP-GlcNAc levels (4-fold increase, ED$_{50}$ of 60 μM), whereas higher GlcN doses (750 μM to 4 mM) diminished the maximal levels of UDP-GlcNAc. When UDP-glucose levels were measured under these conditions (Fig. 2F) we found that GlcN concentrations <100 μM had little or no effect on UDP-glucose levels, but as the concentration was increased from 100 μM to 1 mM a progressive decrease was observed (ED$_{50}$ of 300 μM). Several conclusions can be drawn by comparing the dose-response curves in panels D, E, and F of Fig. 2. First, by comparing panels D and E in Fig. 2, it is apparent that low concentrations of GlcN can markedly elevate UDP-GlcNAc levels without measurably affecting GlcN-6-P levels. From this finding, we conclude that low concentrations of GlcN are rapidly taken up by adipocytes, phosphorylated to GlcN-6-P, and then routed through the HBP resulting in a 4–5-fold increase in UDP-GlcNAc levels. At higher levels of extracellular GlcN (>250 μM), we believe that the uptake and the intracellular phosphorylation of GlcN exceed the capacity of the HBP, which leads to a progressive accumulation of GlcN-6-P. The second conclusion is derived by comparing panels D and F in Fig. 2, which show that loss of UDP-glucose is closely correlated with the rise in GlcN-6-P levels. Thus, we believe that depletion of UDP-glucose is mediated by the accumulation of GlcN-6-P and the resulting allosteric activation of GS.

Removal of Extracellular GlcN Decreases Intracellular GlcN-6-P Levels and Increases UDP-glucose Levels—To gain additional insights into the kinetics of hexosamine flux, we pretreated adipocytes for 30 min with insulin and then added 2 mM GlcN. After 1 h, adipocytes were washed three times to remove extracellular GlcN. As shown in Fig. 3A, we found that intracellular GlcN-6-P levels were reduced from 1500 nmol/g to <200 nmol/g after 45 min (t$_{1/2}$ of 31 min). In contrast, UDP-GlcNAc levels actually increased during this time (Fig. 3B). From this observation we conclude that the high intracellular levels of GlcN-6-P were reduced by the continued flux of GlcN-6-P through the HBP. In Fig. 3C we measured UDP-glucose levels as a function of time following GlcN removal. As can be seen, the increase in UDP-glucose levels correlates well with the time-dependent decrease in GlcN-6-P levels (but not with UDP-GlcNAc levels). This finding adds further support for the supposition that GlcN enhances GBS through allosteric regulation of GS rather than O-linked glycosylation.

GlcN Rapidly Lowers UDP-glucose Levels Under Euglycemic Conditions (Presence of 5 mM Glucose)—Several in vitro studies have shown that prolonged GlcN infusion in rats can increase skeletal muscle hexosamine levels and induce whole body insulin resistance (10, 19–23). To reproduce euglycemic-hyperinsulinemic clamp conditions under defined in vivo conditions, we modified the protocol used in Fig. 2 such that all treatment groups contained 5 mM glucose (in addition to GlcN). As can be seen in the time course experiment depicted in Fig. 4A, GlcN-6-P levels were again markedly increased by 5 min (undetectable to about 160 nmol/g) and reached maximal levels by 90 min (t$_{1/2}$ of 12 min). It should be noted that compared with cells treated with GlcN alone (Fig. 2A), total intracellular accumulation was reduced by 50%. This can be explained by the fact that glucose competitively inhibits GlcN uptake through the insulin-responsive GTS. In other words, when a fixed concentration of GlcN was added to insulin-treated cells, glucose uptake was diminished.
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After GlcN washout, recovery of UDP-glucose levels correlates with the time-dependent decrease in GlcN-6-P levels. Insulin-pretreated adipocytes were incubated for 1 h with 2 mM GlcN, washed four times, and then incubated in GlcN-free buffer for the indicated times (wash-out time). Concentrations of GlcN-6-P (A), UDP-GlcNAc (B), and UDP-glucose (C) were measured as described under "Experimental Procedures." Each point represents the mean of duplicate determinations.

As depicted in Fig. 4, D–F, GlcN dose-response curves for all three metabolites were shifted to the right in the presence of 5 mM glucose (compared with cells similarly treated in the absence of glucose as shown in Fig. 2). This rightward shift is most likely due to the reduced uptake of GlcN in the presence of glucose, because glucose has a higher affinity for cell surface glucose transporters and can competitively inhibit GlcN uptake. Although glucose was able to reduce the steady-state levels of GlcN-6-P by half (Fig. 4, A and D), no such reduction was observed when measuring UDP-GlcNAc levels (Fig. 4, B and E). In fact, the combination of glucose and GlcN increased UDP-GlcNAc levels by ~30% above values obtained with maximally effective concentrations of GlcN alone (compare Fig. 4E with Fig. 2E). When the dose-response curves in Fig. 4, D and E are compared, it can be seen that that increases in UDP-GlcNAc levels occur before any measurable changes in GlcN-6-P levels. Specifically, 750 μM GlcN failed to elevate GlcN-6-P,
Adipocytes were treated with 2 mM GlcN for various time periods (time course experiments) or for 4 h with various GlcN concentrations (dose-response experiments). At the end of each treatment time, cells were lysed with perchloric acid, and the neutralized cell extract was deproteinated. Intracellular concentrations of GlcN-6-P (that do not exceed the biosynthetic capacity of the HBP) can have a profound effect on lowering UDP-glucose levels. This makes sense, because insulin stimulates GlcN uptake ~10-fold in isolated adipocytes. It is interesting to note that insulin treatment alone can lower UDP-glucose levels but that it affects neither GlcN-6-P nor UDP-GlcNAc levels. From these insulin-free experiments, we conclude that GlcN is the major variable responsible for the observed metabolite changes and that insulin serves simply to enhance the rate of uptake by its ability to stimulate the GTS. It is also readily apparent from the time course studies that relatively small increases in GlcN-6-P (that do not exceed the biosynthetic capacity of the HBP) can have a profound effect on lowering UDP-glucose levels.

**DISCUSSION**

Hyperglycemia is the hallmark of type 2 diabetes mellitus and contributes to disease pathogenesis by impairing both insulin action and insulin secretion (24–27). Thus, hyperglycemia is not only a consequence of diabetes, it is a pathophysiological factor that can perpetuate and sustain the diabetic state. This detrimental effect of hyperglycemia is generally referred to as “glucose toxicity” (26–28), and for many years the question of how hyperglycemia mediates desensitization at the cellular level remained unanswered. In 1991 we provided an explanation by showing that an excessive flux of glucose through the HBP culminates in the induction of insulin resistance in isolated adipocytes (1). Evidence supporting the hexosamine hypothesis of glucose toxicity included the finding that the induction of insulin resistance requires three components, namely insulin, glucose, and glutamine. When any one of these components is omitted, little or no desensitization is observed (1, 2, 4). This was explained by the fact that the formation of hexosamine products requires a supply of both glucose (in the form of fructose-6-phosphate) and glutamine as a cofactor for glutamine:fructose-6-phosphate amidotransferase in the transfer of an amide group to fructose-6-phosphate. The primary role of insulin in this scheme is to facilitate the uptake of glucose (~10- to 20-fold) by increasing the number of cell surface glucose transporters. Two independent approaches were used to substantiate this hypothesis (1). First, we demonstrated that glucose-induced desensitization could be prevented by glutamine analogs that irreversibly inactivate glutamine-requiring enzymes such as glutamine:fructose-6-phosphate amidotransferase, the first and rate-limiting enzyme of the HBP. Second, we showed that GlcN was >40 times more potent than glucose in inducing insulin resistance by virtue of its ability to undergo internalization though the GTS and directly enter the HBP at the level of GlcN-6-P (the first product of the HBP). Taken together, these studies led to the conclusion that the HBP serves as a glucose sensor (or nutrient sensor) coupled to a complex signal transduction system that plays an integral role in the development of glucose-induced insulin resistance.

With the realization of the glucose-sensing/transductional capabilities of the HBP, several investigators began exploring the potential role of the HBP in regulating glycogen metabolism (29–32). In part, these studies were initiated because hyperglycemia and type 2 diabetes culminate in an impaired ability of insulin to enhance GS activity and stimulate GBS (33, 34). Thus, it was logical to postulate that insulin resistance and defective glycogen metabolism were both causally related to hyperglycemia and enhanced flux through the HBP. Because avenues available to test this hypothesis were limited, several...
investigators used a direct pharmacological approach that entailed the use of GlcN. When these glycogen studies were collectively considered, a conceptual problem emerged in that GlcN treatment yielded results that differed from the defects in glycogen metabolism found under hyperglycemic conditions associated with type 2 diabetes. Moreover, findings among the various studies were not in good agreement. For example, when rats were infused with GlcN during a 6-h euglycemic-hyperinsulinemic clamp, glycogen content in heart and rectus abdominis tissue remained constant (or increased), whereas glycogen synthase activity was diminished in both tissues (18). In contrast, GlcN treatment in vitro was found to abolish insulin stimulation of glycogen synthesis in isolated rat diaphragm but to activate glycogen synthase (29). In fibroblasts, GlcN treatment similarly induced a defect in GBS and increased GS activity (31). From these studies, it is apparent that information derived from the use of GlcN is often conflicting and inconsistent and does not fit into a coherent understanding of GlcN action on glycogen metabolism.

A potential pitfall in the aforementioned studies lies in the use of excessively high concentrations of GlcN for prolonged times. Thus, rather than examining the physiological consequences of increased hexosamine flux, it is possible these investigators were actually assessing the actions of abnormally high intracellular accumulation of GlcN-6-P. Among the first to address this consideration were Virkamaki and Yki-Jarvinen (10), who found that GlcN-6-P levels were increased 500–700-fold in muscle tissue after rats were infused with GlcN for 6 h. These investigators postulated that GlcN-6-P could allosterically regulate GS based on the structural similarities between glucose-6-P (a known allosteric activator of GS) and GlcN-6-P. When the ability of GlcN-6-P to stimulate glycogen synthase activity was directly assessed in vitro using enzyme derived from rectus abdominis and heart muscle, a 21 and 542% increase in enzyme activity was seen.

Although GlcN-6-P was shown to allosterically activate GS, several key questions remained unanswered. For example, could elevated levels of GlcN-6-P stimulate GBS in intact cells and, if so, what is the dose-response relationship between intracellular levels of GlcN-6-P and enhanced GBS? In the current study we addressed these questions and designed experimental protocols with three goals in mind. The first goal was to test the hypothesis that in vivo accumulation of GlcN-6-P (in adipocytes) has a direct effect on GBS. Second, we sought to determine the rapidity of GlcN action, because it should be manifested in minutes if allosteric regulation is involved. Our third goal was to test whether the actions of GlcN on glycogen metabolism were biphasic, with stimulatory effects seen at low concentrations of GlcN and inhibitory actions manifested at high doses. Such a finding could provide a tentative explanation for the conflicting data regarding GlcN action on glycogen metabolism. Our approach was direct in that we sought to elucidate the temporal and dose-dependent relationship between GlcN treatment and the resulting changes in glycogen metabolism (GBS and UDP-glucose levels) and intracellular hexosamine levels (GlcN-6-P and UDP-GlcNac). More specifically, GlcN action was evaluated as a function of treatment time (5 min to 4 h) and GlcN dose (20 μM to 10 mM) by using insulin-treated cells, glucose-treated cells, and control cells (no insulin or glucose treatment).

Based on obtained results, we conclude that GlcN-6-P accumulation in intact adipocytes enhances GBS within minutes, resulting in lower steady-state levels of UDP-glucose (due to rapid precursor utilization). Supporting this idea was the finding that the incorporation of [14C]glucose into glycogen was markedly enhanced over 10 min (2-fold increase; t1/2 of <5 min) in insulin-pretreated adipocytes exposed to 2 mM GlcN. During this time, intracellular levels of UDP-glucose were concomitantly reduced (t1/2 of 1.4 min; >90% decrease). It is unlikely that the rapid and extensive decrease in UDP-glucose levels was due to the glucose-free conditions (with GlcN treatment), because similar results were obtained under euglycemic conditions (the presence of 5 mM glucose). For several reasons, we believe that the stimulation of GBS and the depletion of UDP-glucose are the result of the intracellular accumulation of GlcN-6-P and the allosteric activation of GS. First, we found that GlcN-induced effects on glycogen metabolism correlated well with the early and rapid rise in GlcN-6-P levels (a marked increase by 5 min), which is consistent with the finding that GlcN-6-P is an in vitro allosteric activator of GS. Second, we observed a rapid reduction in GlcN-6-P levels after the removal of GlcN (by washing) and a concomitant restoration of UDP-glucose.

With prolonged GlcN treatment (at 2 mM), we observed a progressive and massive accumulation of GlcN-6-P (>1,400 nmol/g tissue; t1/2 of ~32 min; GlcN ED50 of 620 μM) that correlated with a reduction in GBS. As we reported previously, high GlcN concentrations can overwhelm the biosynthetic capacity of the HBP, resulting in a massive accumulation of GlcN-6-P and the depletion of cellular ATP levels (14). ATP depletion likely results from phosphate sequestration (with the formation of GlcN-6-P) or the increased energy demands of phosphorylation. Thus, the observed reduction in GBS with excessive GlcN treatment may result from a cytotoxic effect rather than a physiological action mediated through the HBP. This finding is significant, because most studies have examined the functional role of the HBP by using high GlcN concentrations and treatment periods from hours to days. The use of such protocols could easily explain the confusion in the literature regarding the role of HBP in glycogen metabolism.

The storage of intracellular glucose as a branched glucose polymer of glycogen is crucial to glucose homeostasis and provides a means to regulate energy balance between meals (35). After eating, elevated blood glucose levels are reduced through a combination of glucose utilization and glucose storage. The liver plays a major role in storage because it can convert glucose to glycogen, which can later be released to maintain constant blood glucose levels. In skeletal muscle, the catabolism of stored glycogen to glucose provides much of the postprandial energy needed for muscle contraction. In both tissues, GS is the rate-limiting enzyme in GBS and is regulated by complex mechanisms, which include rapid allosteric activation by glucose-6-P, inhibition of GS activity by phosphorylation, and possibly the intracellular compartmentalization of GS (11, 35). Interestingly, it has been recently postulated that GS activity may also be regulated by posttranslational modification through the addition of a single GlcNac monosaccharide on serine/threonine residues (36, 37). In other words, defect(s) in glycogen storage observed in hyperglycemic, type 2 diabetics may be mediated by increased flux of glucose into the HBP, enhanced formation of UDP-GlcNac, and rapid O-linked glycosylation of GS (which inhibits enzyme activity). Despite the physiological appeal of this hypothesis, several aspects of the current study are inconsistent with this proposal. For example, we found that increased hexosamine flux resulted in marked stimulation of GBS, not inhibition. Moreover, we found that rapid activation of GS occurred prior to measurable increases in UDP-GlcNac levels. Although additional studies will be required to resolve this apparent discrepancy, one potential explanation is that both mechanisms are operative, with rapid allosteric activation of glycogen synthase by GlcN-6-P followed by a slower inactivation due to O-linked glyco-
and the long term consequences of hyperglycemia and the finding that various clinical parameters are assessed under chronic hyperglycemic conditions. However, it is difficult to separate the initial triggering events of hyperglycemia from the complicated and long term adaptive actions of various cells and tissues. We know that the HBP plays a key role in sensing hyperglycemic conditions, and we postulate that this pathway initiates or triggers the adaptive responses that ultimately lead to the clinical abnormalities of diabetes and the development of diabetic complications. What is required is an understanding of both the short term and long term consequences of hyperglycemia and the finding of new insights into how these changes are interrelated.

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J. Biol. Chem. 2005, 280:11018-11024.
doi: 10.1074/jbc.M413499200 originally published online January 12, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M413499200

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