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Invited Review

Hexosamines, insulin resistance, and the complications of diabetes: current status

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Buse, Maria G. Hexosamines, insulin resistance, and the complications of diabetes: current status. *Am J Physiol Endocrinol Metab* 290: E1–E8, 2006; doi:10.1152/ajpendo.00329.2005.—The hexosamine biosynthesis pathway (HBP) is a relatively minor branch of glycolysis. Fructose 6-phosphate is converted to glucosamine 6-phosphate, catalyzed by the first and rate-limiting enzyme glutamine:fructose-6-phosphate amidotransferase (GFAT). The major end product is UDP-*N*-acetylglucosamine (UDP-GlcNAc). Along with other amino sugars generated by HBP, it provides essential building blocks for glycosyl side chains, of proteins and lipids. UDP-GlcNAc regulates flux through HBP by regulating GFAT activity and is the obligatory substrate of *O*-GlcNAc transferase. The latter is a cytosolic and nuclear enzyme that catalyzes a reversible, posttranslational protein modification, transferring GlcNAc in *O*-linkage (*O*-GlcNAc) to specific serine/threonine residues of proteins. The metabolic effects of increased flux through HBP are thought to be mediated by increasing *O*-GlcNAcylation. Several investigators proposed that HBP functions as a cellular nutrient sensor and plays a role in the development of insulin resistance and the vascular complications of diabetes. Increased flux through HBP is required and sufficient for some of the metabolic effects of sustained, increased glucose flux, which promotes the complications of diabetes, e.g., diminished expression of sarcoplasmic reticulum Ca^{2+}-ATPase in cardiomyocytes and induction of TGF-β and plasminogen activator inhibitor-1 in vascular smooth muscle cells, mesangial cells, and aortic endothelial cells. The mechanism was consistent with enhanced *O*-GlcNAcylation of certain transcription factors. The role of HBP in the development of insulin resistance has been controversial. There are numerous papers showing a correlation between increased flux through HBP and insulin resistance; however, the causal relationship has not been established. More recent experiments in mice overexpressing GFAT in muscle and adipose tissue or exclusively in fat cells suggest that the latter develop in vivo insulin resistance via cross talk between fat cells and muscle. Although the relationship between HBP and insulin resistance may be quite complex, it clearly deserves further study in concert with its role in the complications of diabetes.

hexosamine biosynthesis pathway; *N*-acetylglucosamine; *O*-linked *N*-acetylglucosamine; modification of proteins

INSULIN RESISTANCE IS A HALLMARK of type 2 diabetes, of uncontrolled type 1 diabetes, and of obesity and the metabolic syndrome (91) and is associated with numerous other conditions, such as cystic fibrosis, uremia, septicemia, glucocorticoid excess, polycystic ovary syndrome, etc. Clinically, insulin resistance is defined as the reduced ability of insulin to lower plasma glucose, which reflects in great part impaired insulin-stimulated glucose transport into tissues, which express the insulin-responsive glucose transporter GLUT4 (skeletal and heart muscle and adipocytes). Except for a few rare conditions, the major defect(s) is downstream of insulin's binding to its receptors. Type 2 diabetes is a polygenic disease, and several recent, excellent reviews discuss the insulin receptor signaling cascade and proposed mechanisms of impaired signal transduction in insulin resistance (57, 69, 70, 72). The propensity to insulin resistance is likely genetically determined (79); however, the expression of the phenotype is modulated by various factors, including diet, exercise, and aging.

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Sustained hyperglycemia causes insulin resistance in humans (89) and in animal models (67), which leads to the concept of "glucose toxicity." The latter accounts for the insulin resistance in uncontrolled type 1 diabetes (89). Similarly, sustained elevations of circulatory nonesterified fatty acids (NEFA) also induce insulin resistance ("lipotoxicity"). Thus insulin resistance may represent an adaptive mechanism that may serve to protect cells from the deleterious effects of excessive nutrient flux, such as oxidative stress. This would imply the existence of cellular biochemical sensors, which monitor the flux of nutrients. McGarry (47) first identified malonyl-CoA as a biochemical sensor that regulates the switch from fatty acid to glucose oxidation in the liver. Several laboratories have proposed that flux through the hexosamine synthesis pathway (HSP) may function as a cellular nutrient sensor and play a role in the development of insulin resistance and the vascular complications of diabetes (4, 5, 24, 44, 66). This review addresses the experimental evidence that supports and questions this hypothesis and the proposed mechanisms by which the HSP may exert these effects.

A role for excess glucose flux via HSP in insulin resistance was first proposed by Marshall et al. in 1991 (43) on the basis

of experiments using isolated rat adipocytes. In this system, preexposure of the cells to insulin and high glucose act synergistically to induce resistance of glucose transport to subsequent acute stimulation by insulin. The insulin resistance develops only if a complete amino acid mixture or glutamine is present in the medium during preincubation with high glucose and insulin. The requirement for glutamine suggested the involvement of the HSP (43).

The HSP is a relatively minor branch of the glycolytic pathway, encompassing ~3% of total glucose utilized (43) (Fig. 1). Entry into the HSP is catalyzed by the first and rate-limiting enzyme glutamine:fructose 6-phosphate (F-6-*P*) amidotransferase (GFAT), which converts F-6-*P* and glutamine to glucosamine 6-phosphate (GlcN-6-*P*) and glutamate. Subsequent steps metabolize GlcN-6-*P* to UDP-*N*-acetylglucosamine (UDP-GlcNAc), UDP-*N*-acetylgalactosamine (UDP-GalNAc), and CMP-syalic acid, essential building blocks of the glycosyl side chains of glycoproteins, glycolipids, proteoglycans, and gangliosides. UDP-GlcNAc is of particular interest because *1*) quantitatively it is the major end product of the HSP; *2*) it is an allosteric feedback inhibitor of GFAT, which regulates glucose entry into the pathway; and *3*) it is the obligatory substrate of *O*-GlcNAc transferase (OGT). The latter is a cytosolic and nuclear enzyme that catalyzes a reversible posttranslational protein modification, whereby GlcNAc is transferred in *O*-linkage to specific serine/threonine residues of numerous proteins (37, 42). The sites of *O*-GlcNAc modification (*O*-GlcNAcylation) are often identical or adjacent to known phosphorylation sites, suggesting a regulatory function (14). Functional significance of *O*-GlcNAcylation has been reported for several proteins (85), including the transcription factors Sp1 (16, 26, 65, 78, 86), c-myc (34), cAMP response element-binding protein (40), signal transducer and activator of transcription-5 (21), and pancreatic duodenal homeobox-1 (18), as well as cytosolic and nuclear enzymes, e.g., glycogen synthase (54, 55) and RNA polymerase II (14). Of particular interest in the context of insulin resistance is that insulin receptor substrates (IRS)-1 and -2 (1, 17, 56, 77), and probably also GLUT4 (8), are subject to *O*-GlcNAcylation. Although the *O*-GlcNAc modification of IRS-1 in the references cited was based on immunological methods, an *O*-GlcNAcylation site on IRS-1 was recently identified by mass spectrometry (2). The reversible, *O*-GlcNAc modification of proteins has been suggested by many investigators as a mechanisms by which increased HSP activity could cause insulin resistance and the complications of diabetes.

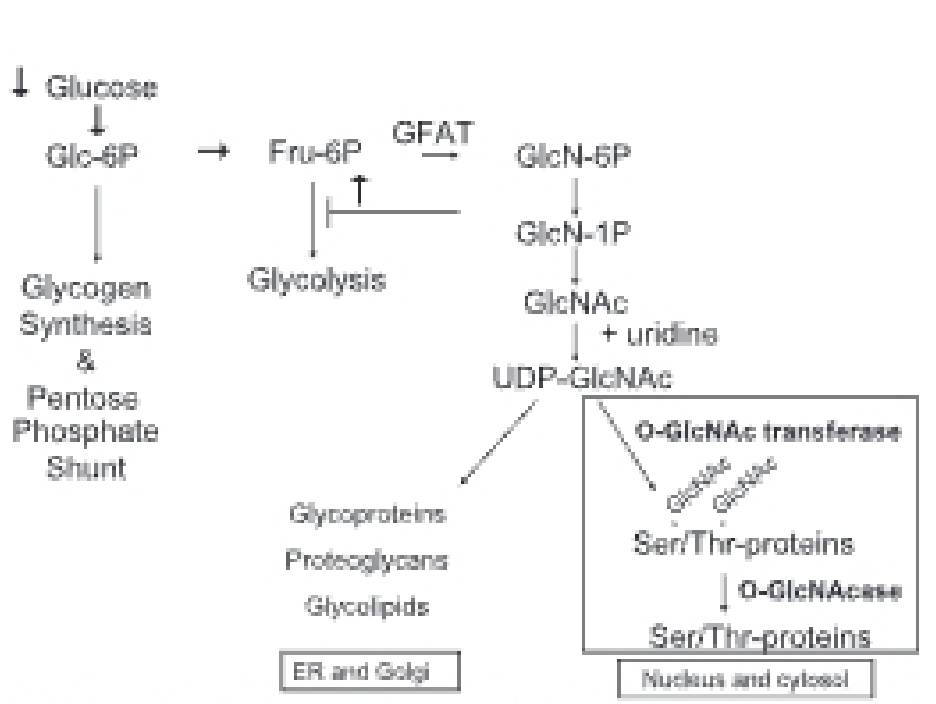

Fig. 1. A simplified schematic representation of the hexosamine biosynthetic pathway. Black arrows indicate that flux through the pathway can be increased by accelerating glucose entry or by inhibiting glycolysis distal to fructose 6-phosphate. GFAT, glucosamine:fructose-6-phosphate amidotransferase; Glc-6P, glucose 6-phosphate (G-6-*P*); Fru-6P, fructose 6-phosphate (F-6-*P*); GlcN, glucosamine; GlcNAc, *N*-acetylglucosamine; ER, endoplasmic reticulum (adapted from Ref. 2).

There is considerable evidence indicating that increased activity of the HSP can cause insulin resistance in cell culture models and in rodents in vivo. In the model mentioned above, where sustained exposure to high glucose in the presence of insulin caused insulin resistance in adipocytes, treatment of the cells with inhibitors of GFAT activity prevented this effect. Furthermore, glucosamine (GlcN), which enters the HSP bypassing GFAT, also caused insulin resistance but at much lower doses than glucose. The effect of GlcN infusions on the development of insulin resistance in rodents undergoing insulin clamp studies has been extensively studied (3, 56, 68). Rossetti et al. reported in 1995 that infusion of GlcN increased the concentrations of UDP-GlcNAc in muscle and markedly decreased insulin-stimulated total body glucose utilization in healthy controls, but not in diabetic rats, which were already insulin resistant (68). Previous studies had demonstrated that in vitro treatment of isolated muscles with GlcN inhibited the insulin response of glucose transport without affecting insulin receptor and GLUT4 expression (62). Sustained hyperglycemia, which causes insulin resistance, also increased UDP-*N*-acetylhexosamine (HexNAc) concentrations in muscles (63). The insulin resistance that develops in rats infused with lipid emulsions is also associated with increased UDP-GlcNAc in muscle, presumably reflecting impaired glycolytic flux distal to F-6-*P*, resulting in increased flux via HSP (28). On the basis of various clamp studies, Hawkins and colleagues (27, 28) proposed that the UDP-GlcNAc concentration in skeletal muscle may modulate the insulin responsiveness of glucose transport. However, this conclusion has been questioned by Choi et al. (12), who found that increasing circulating free fatty acids induces peripheral insulin resistance without concomitant increases in the concentrations of UDP-GlcNAc or UDP-GalNAc in muscle.

Mice overexpressing GLUT1 in muscle exhibit chronically increased muscle glucose flux, increased muscle glycogen, and mild fasting hypoglycemia without significant changes in circulating insulin or glucagon. Insulin fails to stimulate glucose transport in the insulin-resistant muscles in vitro, although GLUT4 expression is unchanged.

Other stimuli that normally stimulate glucose transport, e.g., IGF-I, hypoxia, and contractile activity, are also ineffective in GLUT1-overexpressing mice (22). UDP-HexNAc concentrations and GFAT activity are markedly increased in these muscles (9), as well as the *O*-GlcNAc modification of numerous membrane-associated proteins, which may include GLUT4 and/or proteins associated with GLUT4 (8), suggesting but not proving that the HSP may be involved. Other suggestive correlations include that GFAT activity and UDP-HexNAc concentrations are increased in leptin-deficient, insulin-resistant *ob/ob* mice (7), whereas at the other end of the spectrum UDP-HexNAc concentrations are reduced in muscles of growth hormone-deficient rats (64) and in rats with chronic caloric restriction and enhanced insulin sensitivity (20).