

Multiple Tissue-specific Roles for the *O*-GlcNAc Post-translational Modification in the Induction of and Complications Arising from Type II Diabetes*

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In this minireview, we will highlight work in the last 30 years that has clearly demonstrated that the *O*-GlcNAc modification is nutrient-responsive and plays multiple roles in metabolic regulation of signaling and gene expression. Further, we will examine recent studies that have investigated the impact of *O*-GlcNAc in a variety of glucose- and insulin-responsive tissues and the roles attributed to *O*-GlcNAc in the induction of insulin resistance and glucose toxicity, the hallmarks of type II diabetes mellitus. We will also summarize potential causal roles for the *O*-GlcNAc modification in complications associated with diabetes.

Type II diabetes, also referred to as non-insulin-dependent diabetes mellitus or adult-onset diabetes, is a metabolic disorder in which hyperglycemia and insulin resistance are the hallmarks (1, 2). This disease, which afflicts over 25 million United States citizens, is thought to result from a combination of predisposing genetic traits coupled with chronic low energy expenditure and overnutrition (1–3). Thus, type II diabetes displays traits of both an inherited as well as an acquired disease. Insulin resistance coupled with chronic glucose toxicity are thought to be responsible for the many complications associated with the disease (1, 2). Thus, understanding the mechanisms by which tissues sense and respond to changes in glucose levels and develop resistance to insulin-stimulated signaling is key to developing therapeutics for this multisystem disease of energy metabolism (Fig. 1).

The Hexosamine Biosynthetic Pathway (HBP) as a Glucose Sensor and Modulator of Insulin Signaling

Once glucose enters a cell, it is rapidly converted to glucose 6-phosphate, which can be further utilized in multiple meta-

bolic pathways (Fig. 2A). This intermediate can be utilized for glycogen synthesis, the pentose phosphate pathway, or converted to fructose 6-phosphate. The vast majority of fructose 6-phosphate is converted to fructose 1,6-bisphosphate and thus committed to glycolysis. However, a small percentage (2–5%) is converted to glucosamine 6-phosphate by the rate-limiting enzyme L-glutamine D-fructose 6-phosphate amidotransferase (GFAT)² to commit it to the HBP that generates UDP-GlcNAc (4, 5). Groundbreaking work by multiple laboratories demonstrated that the HBP was a glucose sensor and that decreasing/increasing flux through this path could hamper or induce, respectively, the insulin-resistant state (4, 6–11). For example, in adipocytes, treatment with glucosamine that feeds directly into the HBP, via the generation of glucosamine 6-phosphate via phosphorylation, can abrogate the need for high glucose in the induction of insulin resistance (6). Further, overexpression of GFAT in peripheral insulin-responsive tissues leads to an insulin-resistant phenotype in mice (12). Pathways that utilize UDP-GlcNAc, the end product of the HBP, have been investigated as the mechanism by which the HBP acts as a nutrient sensor and modulator of signaling.

The Regulatory *O*-GlcNAc Post-translational Modification

Although UDP-GlcNAc can be used for the generation of other sugar nucleotides and the synthesis of complex glycans that are free or attached to proteins and lipids, this sugar nucleotide also serves as the donor for the *O*-GlcNAc modification that was originally discovered Torres and Hart (13) in the mid-1980s (Fig. 2B). Unlike classical complex glycosylation, the *O*-GlcNAc modification of protein serine and threonine residues occurs in the nucleus and cytoplasm, and its addition and removal are catalyzed by nucleocytoplasmic enzymes, *O*-GlcNAc transferase (OGT) and neutral β -*N*-acetylglucosaminidase (OGA), respectively (14–18). Several excellent recent reviews have focused on this modification, the thousands of *O*-GlcNAc-modified proteins known, and the cycling enzymes (14, 19–25). Here, we will review the tissue-specific roles for the cycling enzymes and the *O*-GlcNAc modification in energy homeostasis and the type II diabetes mellitus phenotype.

Functions for the *O*-GlcNAc Modification in the Pancreatic β -Cell

The pancreas is a glucose-responsive endocrine tissue that secretes hormones in response to glucose levels (Fig. 1). Dysregulation of the β -cells of the pancreas, which are responsible for the secretion of insulin in response to hyperglycemia, is a major hallmark of the diabetic phenotype (1, 2). The *O*-GlcNAc modification of several proteins, primarily transcription factors, has been implicated in positively regulating the secretion

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² The abbreviations used are: GFAT, L-glutamine:D-fructose-6-phosphate amidotransferase; HBP, hexosamine biosynthetic pathway; *O*-GlcNAc, *O*-linked β -*N*-acetylglucosamine; OGT, *O*-GlcNAc transferase; OGA, neutral β -*N*-acetylglucosaminidase; PPP, pentose phosphate pathway.

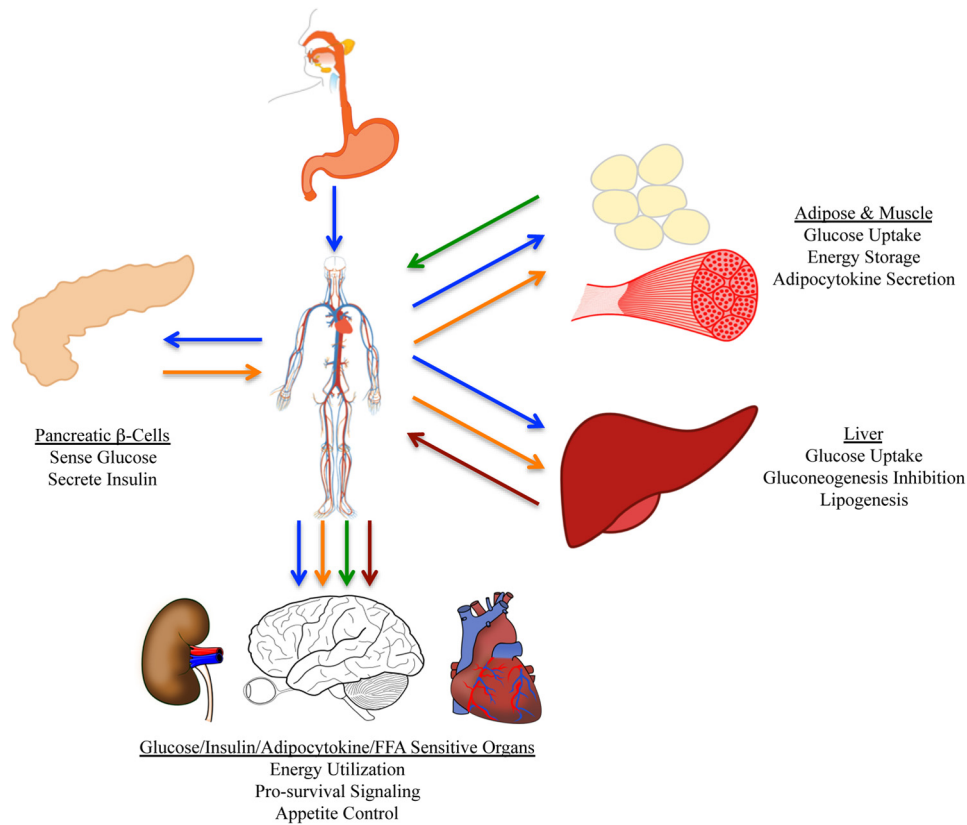


FIGURE 1. **Whole organism energy dynamics and the diabetic phenotype.** Glucose (blue arrows) is absorbed into the bloodstream from the duodenum. Glucose is sensed by the β -cells of the pancreas that respond by secreting insulin into the bloodstream (orange arrows). Insulin triggers adipose and muscle cells to take glucose out of the bloodstream and promotes energy storage. Insulin also modulates adipocytokine secretion (green arrows). In the liver, insulin promotes glucose uptake and inhibition of gluconeogenesis and promotes lipogenesis and FFA secretion (red arrows). Glucose, insulin, adipocytokines, and FFA all act on responsive organs, including the brain, eye, heart, kidney, and circulatory system, to promote/inhibit a variety of processes and responses. In the diabetic phenotype, all of the tissues and organs of the organism become affected due to a disconnect between sugar metabolism and insulin-dependent signal transduction.

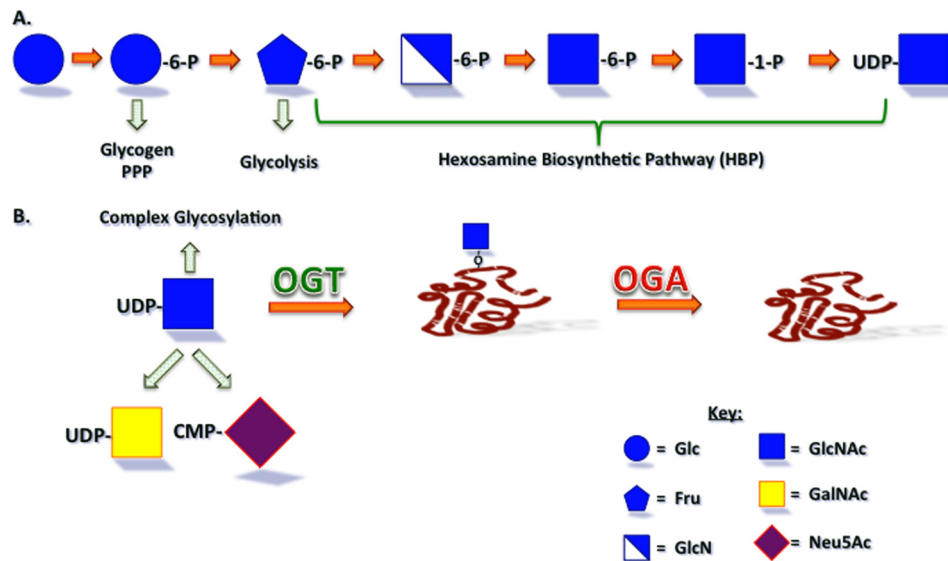


FIGURE 2. **The hexosamine biosynthetic pathway and UDP-GlcNAc utilization.** A, glucose entering a cell can be utilized in glycogen synthesis, the pentose phosphate pathway (PPP), and glycolysis. Approximately 2–5% of entering glucose is shunted into the hexosamine biosynthetic pathway that generates the sugar nucleotide UDP-GlcNAc, the levels of which are proportional to that of the glucose entering the cells and are elevated in diabetic animals and patients. B, UDP-GlcNAc can be utilized for the generation of free, protein-linked, or lipid-linked complex carbohydrates or for the generation of other sugar nucleotides, UDP-GlcNAc and CMP-Neu5Ac, in humans. UDP-GlcNAc also serves as the sugar nucleotide donor for the OGT-catalyzed addition of O-GlcNAc to serine and threonine residues of nuclear and cytoplasmic proteins, which can be removed by the enzymatic action of OGA. Neu5Ac, N-acetylneuraminic acid.

of insulin from the β -cells of the pancreas. In particular, O-GlcNAc modification of NeuroD1, which enhances transcription of the insulin gene and promotes nuclear localization and O-GlcNAc modification of the transcription factor PDX-1, enhances DNA binding (26, 27). However, long-term elevation of O-GlcNAc levels via pharmacological approaches has been associated with β -cell apoptosis, although the mechanism is unclear but may involve the Akt pathway (28, 29). Further studies aimed at uncovering the roles of acute and chronic hyperglycemia that can be assigned to increases in O-GlcNAc levels in the glucose-responsive pancreas are still required.

Functions for the O-GlcNAc Modification in Skeletal Muscle and Adipose Tissue (Insulin-responsive Glucose Uptake and Metabolism)

Skeletal muscle and adipose tissue are responsible for clearing the majority of glucose from the bloodstream in response to insulin (1, 2) (Fig. 1). Insulin promotes energy storage in these tissues as well as the secretion of adipocytokines from adipose tissue (2, 19) (Fig. 1). Pioneering studies using metabolic, pharmacological, and genetic approaches clearly demonstrated an important role for the HBP in peripheral insulin resistance (4, 6–11). These studies were followed up by pharmacological and genetic studies that suggested that elevation in the O-GlcNAc modification leads to adipose and skeletal muscle insulin resistance (30, 31). This work included demonstration that a transgenic mouse overexpressing OGT in skeletal muscle displayed insulin resistance as well as hyperleptinemia (30). Cell culture studies discovered similar insulin-resistant phenotypes upon elevation in O-GlcNAc levels via pharmacological approaches (31–33). Further work uncovered that multiple proteins involved in the insulin signaling pathway were functionally O-GlcNAc-modified (34–38) and that the O-GlcNAc cycling enzymes genetically interacted with components of the insulin-like signaling pathway in the model organism *Caenorhabditis elegans* (39–44). In addition to defects in insulin-dependent glucose uptake and energy storage, the HBP and more specifically the O-GlcNAc modification are also functionally implicated in the secretion of adipocytokines that modulate a number of energy homeostatic functions in the organism, including feeding behavior (30, 33, 45–47) (Fig. 1). For example, Rossetti's group (47) clearly demonstrated that leptin transcription, expression, and secretion were regulated by the HBP, and later, McClain *et al.* (30) demonstrated using genetic approaches that this regulation was directly due to the O-GlcNAc modification. Work by our own group using pharmacological manipulation followed by quantitative mass spectrometry has further determined that multiple mammalian adipocytokines are regulated by levels of the O-GlcNAc modification in adipocytes (45, 46). Thus, multiple groups using a variety of approaches and systems have determined strong links between the O-GlcNAc modification and peripheral insulin resistance. Work by Buse and colleagues (48), who demonstrated that overexpression of OGA did not ameliorate hyperglycemia/chronic insulin-induced insulin resistance in cultured adipocytes, and work by Voadlo and colleagues (49, 50), who demonstrated that a specific inhibitor of OGA did not induce peripheral insulin resistance, clearly show that the field still has much to uncover to

fully understand the role of the O-GlcNAc modification in modulating peripheral tissue insulin responsiveness. In particular, the field needs to acknowledge and address that metabolic treatments (for example, the addition of glucose, glucosamine, GlcNAc, and/or glutamine), pharmacological treatments (inhibition of OGT, OGA, and GFAT via inhibitors with various mechanisms and specificities, for example), and genetic manipulation (O-GlcNAc/HBP enzyme overexpression and knock-down/knock-out) are not necessarily comparable. For example, pharmacological inhibition of OGA potentially elevates O-GlcNAc levels by breaking the dynamic on/off cycle, whereas overexpression of OGT likely elevates O-GlcNAc levels by shifting the equilibrium toward modification.

Functions for the O-GlcNAc Modification in the Liver (Insulin-suppressed Gluconeogenesis)

The liver plays a central role in glucose homeostasis and is insulin-responsive (Fig. 1). In addition to insulin-responsive glucose uptake, insulin also inhibits gluconeogenesis and promotes FFA secretion in the liver (1). Perhaps some of the most compelling studies for the role of O-GlcNAc in the liver and the diabetic phenotype were conducted by the Montminy and Evans groups (51, 52), who showed that overexpression of OGA in the liver rescued circulating glucose levels in a diabetic mouse model and that overexpression of OGT in the liver induced insulin resistance and dyslipidemia in a normal mouse, respectively. Elegant work by Hart and colleagues (53, 54) has demonstrated that expression of key gluconeogenic enzymes is regulated at the level of transcription by the O-GlcNAc modification, and this work has been recently followed up on and extended by Yang's group (55). O-GlcNAc modification of transcription factors is also centrally involved in the regulated expression of lipogenic enzymes (56, 57). Thus, in the liver, metabolic regulation of gene expression via the O-GlcNAc modification appears to be a central theme, and it has been established in multiple systems that the O-GlcNAc modification is a regulator of transcription (58, 59).

Functions for the O-GlcNAc Modification in Organs/Tissues Associated with Diabetic Complications

Multiple tissues and organs are involved in the diabetic phenotype, including the kidney, eye, brain, heart, and vascular system (1, 2) (Fig. 1). Retinopathy and nephropathy that lead to blindness and kidney failure are major complications of diabetes mellitus (60, 61). Insulin plays a pro-survival role in large part by activation of the anti-apoptotic PI3K-Akt pathway (62). Flux through the HBP and elevation in O-GlcNAc modification of proteins have been demonstrated to attenuate this pathway in multiple systems and thus may play a significant role in the induction of apoptosis in these tissues (31, 34, 37, 52, 63). Alternatively or perhaps additively, the O-GlcNAc modification is known to modulate NO production in endothelial cells, promoting macro- and microvascular complications that may lead to organ failure (64, 65). In alignment with the role of O-GlcNAc in vascular regulation, several groups have demonstrated a role for O-GlcNAc in the heart, and recent studies have covered these findings (23, 25, 66–68). Diabetic cardiomyopathy is responsible for a large number of the deaths

associated with diabetes mellitus (69). Recent work has demonstrated that the O-GlcNAc modification of proteins in cardiac tissue is a mechanism for hyperglycemia/insulin-resistant-induced mitochondrial dysfunction, contractile defects, and atherosclerosis as well as perturbations in calcium loading and oxidative stress responses (23, 25, 66–68). Further, OGA protein levels and O-GlcNAc-modified proteins are increased in erythrocytes of pre-diabetic and diabetic patients, paving the way for O-GlcNAc levels being used as a biomarker for early detection and efficacious treatment of diabetes (70, 71). Future studies are needed to uncover the roles for O-GlcNAc in mediating glucose toxicity and the normal physiological roles for this modification in modulating energy utilization and responsiveness to extracellular cues in organs associated with the diabetic phenotype.

Conclusions and Future Directions

As illustrated in Fig. 1, the organism as a whole must be considered when investigating energy homeostasis and its dysregulation in diabetes. This is especially true for the O-GlcNAc modification that has been increasingly implicated as a metabolic sensor that regulates gene expression, *i.e.* an epigenetic mark, of secreted factors. In this review, we have highlighted several such examples, including O-GlcNAc levels regulating insulin secretion in the pancreas, which goes on to regulate multiple processes in other organs, and the O-GlcNAc modification regulating insulin-dependent leptin secretion at the level of transcription in adipose tissue, which then circulates to the brain to regulate eating behavior (Fig. 1). Thus, a major challenge in the O-GlcNAc and diabetes field is elucidating not only direct effects of O-GlcNAc modification on particular proteins in specific cell types, but equally importantly, elucidating indirect effects of changes in O-GlcNAc levels in one tissue having metabolic consequences in another tissue that impact the overall energy dynamics and health of the entire organism.

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