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The roles of *O*-linked β-*N*-acetylglucosamine in cardiovascular physiology and disease

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Zachara NE. The roles of *O*-linked β-*N*-acetylglucosamine in cardiovascular physiology and disease. *Am J Physiol Heart Circ Physiol* 302: H1905–H1918, 2012. First published January 27, 2012; doi:10.1152/ajpheart.00445.2011.—More than 1,000 proteins of the nucleus, cytoplasm, and mitochondria are dynamically modified by *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc), an essential post-translational modification of metazoans. *O*-GlcNAc, which modifies Ser/Thr residues, is thought to regulate protein function in a manner analogous to protein phosphorylation and, on a subset of proteins, appears to have a reciprocal relationship with phosphorylation. Like phosphorylation, *O*-GlcNAc levels change dynamically in response to numerous signals including hyperglycemia and cellular injury. Recent data suggests that *O*-GlcNAc appears to be a key regulator of the cellular stress response, the augmentation of which is protective in models of acute vascular injury, trauma hemorrhage, and ischemia-reperfusion injury. In contrast to these studies, *O*-GlcNAc has also been implicated in the development of hypertension and type II diabetes, leading to vascular and cardiac dysfunction. Here we summarize the current understanding of the roles of *O*-GlcNAc in the heart and vasculature.

cardioprotection; glycosylation; phosphorylation; signaling; survival

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Introduction

The modification of intracellular proteins by monosaccharides of *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) was first described in 1984 (162). Found on more than 1,000 proteins localized to the nucleus, cytoplasm, and mitochondria, *O*-GlcNAc is a common post-translational modification of metazoans (11, 51, 140, 159, 166, 170, 171, 174) and has recently been found on some prokaryotic proteins (148). *O*-GlcNAc modified proteins, or *O*-GlcNAcylated proteins, fall into diverse functional groups including: histones and other chromatin-associated proteins, transcription factors and RNA polymerase II, ribosomal proteins, proteasomal proteins, cytoskeletal proteins, signaling proteins such as protein kinases and phosphatases, metabolic enzymes, and viral proteins (11, 51, 140, 159, 166, 170, 171, 174).

The following observations support the notion that *O*-GlcNAc is a regulatory post-translational modification analogous to protein phosphorylation: *1*) *O*-GlcNAc is not further extended into more complex oligo- or polysaccharides with the exception of plant nuclear pore proteins (62, 162); *2*) the half-life of the *O*-GlcNAc modification is shorter that that of the protein it modifies (38, 86, 144); *3*) *O*-GlcNAc levels respond dynamically to both extracellular (e.g., insulin) and intracellular (e.g., stress) stimuli (12, 13, 22, 38, 53, 74, 91, 118, 153, 188, 195); *4*) sites of *O*-GlcNAc modification are similar to those used by protein kinases and are identical to those used by kinases on a subset of proteins (57); *5*) *O*-GlcNAc is essential for life in mammals, *arabidopsis* and *drosophila* (45, 59, 131, 146, 149), and the deletion of the *O*-GlcNAc transferase (OGT) is lethal in mice [embryonic day (E) 4.5] cells and some tissues (131, 146); and *6*) numerous studies have implicated *O*-GlcNAc in regulating enzyme activity, DNA binding, protein binding, localization, half-life, and regulating phosphorylation levels either by regulating protein kinases or by blocking amino acids that would otherwise be phosphorylated (58). For a subset of proteins, *O*-GlcNAc can block phosphorylation either by modifying the same Ser/Thr residue that would usually be modified (e.g., C-Myc, Thr 58) (23, 24), or sterically by modifying a nearby Ser/Thr residue (e.g., casein kinase 2, Ser347). Supporting a model in which a protein quickly cycles between *O*-GlcNAc modified and phosphorylated, OGT is found in a complex with protein phosphatase 1β and 1γ (177), and during cytokinesis OGT is found in a complex with OGT, *O*-GlcNAcase (the enzyme that removes *O*-GlcNAc), protein phosphatase 1, and aurora B kinase (152). Notably, on many proteins, such as α-B-crystalline, there is no relationship between phosphorylation and *O*-GlcNAcylation.

Recently, *O*-GlcNAc has been shown to modify many proteins that play key roles regulating the heart and vasculature (Table 1) and has been implicated in the etiology of hypertension and diabetes, as well as regulating the heart's ability to

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Table 1. *O-GlcNAc modified proteins in heart, erythrocytes, and vascular tissue**

Protein and Site	Potential Role of *O*-GlcNAc	Tissue/Organism	Reference
α-Actin	None	Neonatal cardiomyocytes	(70)
Actin (Ser54, Ser157, Ser201, Ser234, Ser325, Ser370)	Unknown, although *O*-GlcNAc levels appear elevated in diabetic models (STZ treated rats and *ob/ob* mice).	Heart	(138)
Akt§	*O*-GlcNAcylation is associated with reduced phosphorylation of Akt and is implicated in reducing angiogenesis.	Aorta	(95, 96, 106)
Ankyrin-1 (Ser288, Ser794, Ser960, Ser1162)	Unknown, but *O*-GlcNAc levels are elevated in patients with diabetes (Ser794 and Ser1162).	Erythrocytes	(169)
Aquaporin-1 (Ser236)	Unknown, but *O*-GlcNAc levels are elevated in patients with diabetes.	Erythrocytes	(169)
Band-3 anion transport protein (Ser162, Ser224, Ser745)	Unknown, but *O*-GlcNAc levels are reduced in patients with diabetes (Ser162 and Ser224).	Erythrocytes	(169)
Carbonic anhydrase (Ser130, Ser218)	Unknown, but *O*-GlcNAc levels are reduced in patients with diabetes (Ser30 and Ser218).	Erythrocytes	(169)
Catalase (Ser114, Ser254)	Unknown, but *O*-GlcNAc levels are elevated in patients with diabetes (Ser254).	Erythrocytes	(169)
Complex II, core 1†	Increased *O*-GlcNAcylation is linked to mitochondrial dysfunction in diabetes.	Neonatal cardiomyocytes	(64)
Complex III, core 2†	Increased *O*-GlcNAcylation is linked to mitochondrial dysfunction in diabetes.	Neonatal cardiomyocytes	(64)
Cox1†	Increased *O*-GlcNAcylation is linked to mitochondrial dysfunction in diabetes.	Neonatal cardiomyocytes	(64)
Desmin	Unknown	Neonatal cardiomyocytes	(70)
eNOS	Enhanced *O*-GlcNAc modification is associated with a reduction in phosphorylation at Ser1177 and a subsequent reduction in NO production. Increased *O*-GlcNAcylation in DOCA-salt treated rats is associated with reduced phosphorylation at Ser1177. This may alter vascular relaxation contributing to hypertension.	Aorta	(95, 119)
Equilibrative nucleoside transporter 1 (Ser63)	Unknown.	Erythrocytes	(169)
Erythrocyte membrane protein band 4.2 (Ser82)	Unknown, but *O*-GlcNAc levels are reduced in patients with diabetes.	Erythrocytes	(169)
Fructose-bisphosphate aldolase	Unknown	Neonatal cardiomyocytes	(70)
Glut1 (Ser465)	Unknown	Erythrocytes	(169)
Glutathione *S*-transferase ω-1 (Ser13)	Unknown, but *O*-GlcNAc levels are elevated in patients with diabetes.	Erythrocytes	(169)
Glyceraldehyde-3-phosphate	Unknown	Neonatal cardiomyocytes	(70)
Hemoglobin subunit-α (Ser4, Ser36, Ser134)	Unknown, but *O*-GlcNAc levels are elevated in patients with diabetes (Ser 4, Ser 36).	Erythrocytes	(169)
Hemoglobin subunit-β (Ser50, Ser73, Thr85)	Unknown, but *O*-GlcNAc levels are elevated in patients with diabetes (Ser50 and Ser73).	Erythrocytes	(169)
HSP27	Unknown	Neonatal cardiomyocytes	(70)
HSP60	Unknown	Neonatal cardiomyocytes	(70)
Malate dehydrogenase	Unknown	Neonatal cardiomyocytes	(70)
Myosin heavy chain 6 (Ser172 Ser179, Ser196, Ser392, Ser622, Ser626, Ser644, Ser645, Ser749, Ser880, Ser1038, Ser1148, Ser1159, Thr1189, Ser1200, Ser1308, Ser1336, Ser1470, Ser1471, Ser1597, Thr1600, Thr1606, Ser1711, Ser1777, Ser19161)	Unknown	Heart	(138)
Myosin regulatory light chain 1 (Thr93, Thr164)	Unknown	Heart	(138)
Myosin regulatory light chain 2 (Ser15)	Unknown	Heart	(138)
NDUFA9∫† (Ser156)	Increased *O*-GlcNAcylation is linked to mitochondrial dysfunction in diabetes.	Neonatal cardiomyocytes	(64)
Peroxiredoxin-2 (Ser112, Thr18)	Unknown, but *O*-GlcNAc levels are reduced in patients with diabetes (Ser112 and Thr18).	Erythrocytes	(169)
Phospholamban† (Ser16, site identified by mutation of Ser residue to alanine)	*O*-GlcNAcylation is associated with reduced phosphorylation, and a subsequent increase in the association of phospholamban with SERCA2a. This association is correlated with decreased cardiac function in diabetic cardiomyopathy.	Neonatal cardiomyocytes	(189)
Proteasome subunit-α type-5 (Ser 198)	Unknown, but *O*-GlcNAc levels are elevated in patients with diabetes.	Erythrocytes	(169)
Protein 4.1 (Ser 491, Ser 792)	Unknown	Erythrocytes	(169)

Continued

Table 1.—*Continued*

Protein and Site	Potential Role of *O*-GlcNAc	Tissue/Organism	Reference
Pyruvate kinase	Unknown	Neonatal cardiomyocytes	(70)
Sp1†	Increased *O*-GlcNAcylation is associated with reduced expression of SERCA2a in models of diabetes.	Neonatal cardiomyocytes	(26)
Spectrin-α chain (Ser844, Ser1250, Ser1737)	Unknown	Erythrocytes	(169)
Spectrin-β chain (Ser671, Ser767, Ser1297, Ser1652, Ser1936)	Unknown	Erythrocytes	(169)
Troponin I (Ser150).	Unknown	Heart	(138)
VDAC§†‡	Decreased *O*-GlcNAcylation correlates with increased mitochondrial permeability.	Neonatal cardiomyocytes	(70, 127)
α-Synuclein (S87)	Unknown, but *O*-GlcNAc levels are elevated in patients with diabetes.	Erythrocytes	(169)

*As more than 1,000 proteins are now known to be *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) modified, we refer readers to the following 2 sites for complete lists of *O*-GlcNAc modified proteins and amino acids: *1*) http://cbsb.lombardi.georgetown.edu/hulab/OGAP.html and *2*) http://www.phosphosite.org/homeAction.do;jsessionid=D89E4F582F1A5BE65C3034BC1883CDFF. ∫*O*-GlcNAc modification sites were mapped by BEMAD. †Identification by immunoprecipitation of the target protein was followed by Western blot with an *O*-GlcNAc specific antibody. §Identification by immunoprecipitation with an *O*-GlcNAc specific antibody or a GlcNAc-specific lectin was followed by detection with a protein specific antibody (this technique can produce false positives). ‡Spots that were reactive with an *O*-GlcNAc specific antibody on by two-dimensional PAGE were identified by mass spectrometry (this technique can produce false positives). STZ, streptozotocin; Cox1, cyclooxygenase 1; eNOS, endothelial nitric oxide (NO) synthase; DOCA, deoxycorticosterone acetate; HSP, heat shock protein; NDUFA9, NADH dehydrogenase [ubiquitin] 1α subcomplex subunit 9, mitochondrial; SERCA2a, cardiac sarcoplasmic reticulum Ca^{2+}-ATPase; VDAC, voltage-dependent anion-selective channel.

respond to both ischemia-reperfusion injury and trauma hemorrhage. Here we will focus on the role of *O*-GlcNAc in regulating physiological processes relevant to the heart and vasculature.

The Biosynthesis of O-GlcNAc

The *O*-GlcNAc cycle is perpetuated by the differential regulation of just two enzymes, the OGT and *O*-GlcNAcase, as well as the levels of the substrate UDP-GlcNAc. How the cell regulates the dynamic modification of more than 1,000 proteins appropriately with just two enzymes has not yet been defined experimentally, though is thought to be regulated by protein-protein interactions of both OGT and *O*-GlcNAcase as well as alternative splicing of both enzymes (58).

The hexosamine biosynthetic pathway. UDP-GlcNAc, the high-energy sugar donor used by OGT is synthesized by the hexosamine biosynthetic pathway (HBP). A small percentage of glucose imported into the cell is converted to UDP-GlcNAc through the HBP (Fig. 1), the rate-limiting step of which is catalyzed by glutamine:fructose-6 phosphatase amidotransferase (GFAT; *Gfpt1*). Notably, glucosamine, glutamine, and *N*-acetylglucosamine all feed into the HBP at different steps altering UDP-GlcNAc concentrations. These and other data have led to the hypothesis that many of the physiological effects of these compounds and hyperglycemia may be mediated and/or exacerbated by OGT and *O*-GlcNAcylation of proteins (56, 61, 143). Importantly, UDP-GlcNAc is also used for the biosynthesis of *N*-linked and *O*-linked glycans in the endoplasmic reticulum (ER)/Golgi, glycosylphosphatidylinositol anchors, and proteoglycans/glucosaminoglycans, and as such, changes to cell physiology attributed to altered UDP-GlcNAc concentrations may be independent of *O*-GlcNAc.

Consistent with the OGT knockout, disrupting flux through the HBP and thus concentrations of UDP-GlcNAc has severe consequences (5, 49). Deletion of glucosamine-phosphate *N*-acetyltransferase 1 (EMeg32; *Gnpnat1*; Fig. 1), which reduces UDP-GlcNAc levels to 5% of normal, is embryonic lethal. However, embryonic fibroblasts isolated from these animals are viable although not adherent (5). More recently, the activity of phosphoglucomutase 3 (*Pgm3*), also known as phosphoacetylglucosamine mutase 1, which catalyzes the penultimate step of the HBP (Fig. 1), has been manipulated genetically (49). Similar to deleting OGT and glucosamine-phosphate *N*-acetyltransferase 1, deletion of *Pgm3* results in embryonic lethality between E3.5 and E6.5, again highlighting the importance of *O*-GlcNAcylation and other glycoconjugates (49). Notably, the authors also generated a number of hypomorphic mutations in *Pgm3*, which allowed them to study the effects of incremental changes in UDP-GlcNAc levels (49). Male mice carrying the hypomorphic mutations are infertile, and structural changes are observed in the testis, kidney, pancreas, and salivary gland and in certain hematopoietic cell lineages (red blood cells, lymphocytes platelets) (49). The latter is not surprising, as *Pgm3* is upregulated by erythropoietin. These mice should be useful tools for studying the role of flux though the HBP and subsequent *O*-GlcNAc addition.

The UDP-GlcNAc: polypeptide OGT (EC 2.4.1.94; GI: 6006036) catalyzes the addition of O-GlcNAc. OGT is a noncanonical glycosyltransferase expressed as a soluble protein in the nucleus, cytoplasm, and mitochondria (84, 104). OGT is characterized by two functional domains, the NH_2-terminal tetratrichopeptide repeat domain (TPR domain) (84, 104) that is structurally similar to importin-α (69) and a COOH-terminal catalytic domain that belongs to the glycogen phosphorylase superfamily (179). Recently, the structures of a bacterial homolog of OGT (27, 116), a truncated human OGT (with only 4.5 of the 11.5 TPR domains) (90), and the TPR domain have been solved (69). Collectively, these data suggest that the TPR domain forms a long helical domain that is capped by the globular catalytic domain. Interestingly, the catalytic domain contains two Rossman-type folds separated by an intervening sequence (90, 179). The catalytic domain appears to have two conformations: an open conformation in which extended peptide substrates can access the narrow active site and a closed conformation (90). These and other data suggest

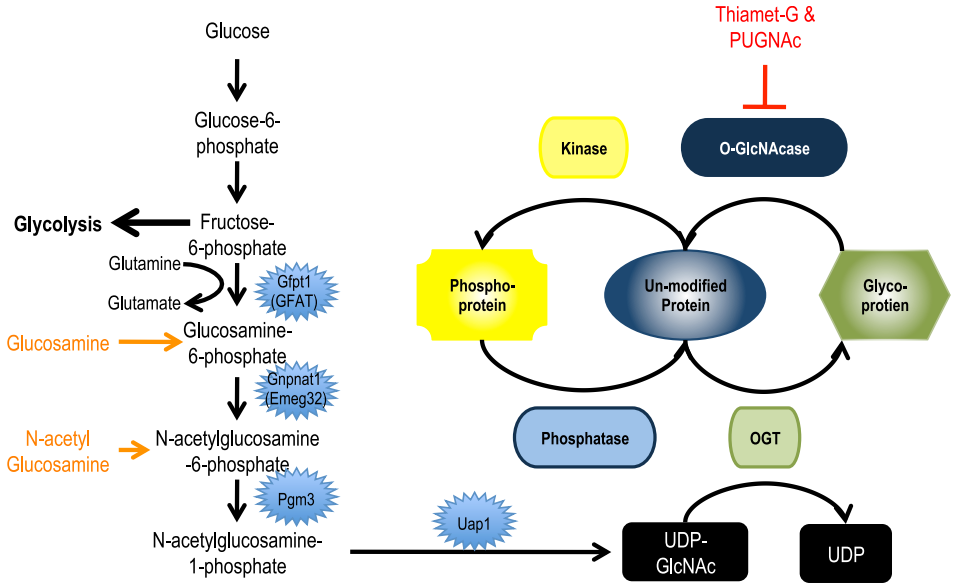

Fig. 1. Metabolism of the *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) modification. A small percentage of glucose imported into the cell is converted through the hexosamine biosynthetic pathway to UDP-GlcNAc. UDP-GlcNAc is the high-energy sugar donor for glycosylation by the *O*-GlcNAc transferase (OGT) and glycosylation in the endoplasmic reticulum and Golgi apparatus. Glucosamine and glutamine (orange) can be used for the synthesis of UDP-GlcNAc. Thiamet-G and *O*-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino-*N*-phenylcarbamate (PUGNAc; red) are commercially available inhibitors of the *O*-GlcNAcase, the enzyme that removes *O*-GlcNAc. Notably, on a subset of proteins, the *O*-GlcNAc modification site is the same as that reported for phosphorylation. Thus, for some proteins, there is a reciprocal relationship between phosphorylation and the *O*-GlcNAc modification. In support of this model, OGT has been found in a complex with protein phosphatase 1α and γ, and during cytokinesis, OGT and *O*-GlcNAcase are found in a complex together and with protein phosphatase 1 and aurora kinase B. Gene names are as follows: *Gfpt1*, glutamine fructose-6-phosphate transaminase 1 (GFAT); *Gnpnat1*, glucosamine-phosphate *N*-acetyltransferase 1; *Pgm3*, phosphoglucomutase 3; *Uap1*, UDP-*N*-acetylglucosamine pyrophosphorylase 1.

that UDP-GlcNAc is bound first, before the peptide gains access to the active site (90).

While there is only one copy of OGT in the genome, on the X-chromosome, several splice variants exist of which three are well characterized (55, 84, 103, 104): *1*) full-length OGT which is the predominant form in the nucleus and cytoplasm (ncOGT), *2*) short OGT, and *3*) mitochondrial OGT. These splice variants, as well as interacting proteins, and the concentrations of UDP-GlcNAc are thought to regulate the substrate specificity of OGT (19, 20). Data that supports these conclusions includes the following observations. First, ncOGT associates with p38 MAPK in response to glucose deprivation. While ncOGT and p38 MAPK do not modify each other, p38 MAPK appears to target ncOGT to its substrates (19). Second, short truncations of the TPR domain reduce the activity of ncOGT toward peptide and protein substrates (85, 105). Third, yeast two-hybrid and pull-down analyses demonstrate that OGT associates with numerous proteins (20, 21). Fourth, ncOGT changes its preferences for peptide substrates in vitro in the presence of different UDP-GlcNAc concentrations (85). These data suggest that at different UDP-GlcNAc concentrations in the cell, the enzyme would glycosylate different subsets of proteins and associate with different proteins providing differential targeting. Thus these changing specificities, as well as protein-protein interactions, may explain why there is no consensus motif for the addition of *O*-GlcNAc.

Walker and coworkers have isolated a number of OGT inhibitors that work well in vitro (50) and in some cells in vivo (10, 127). However, in our hands these inhibitors (5–100 μM, 4–18 h) have not had a significant effect on *O*-GlcNAc levels in U2OS, HeLa, MEF, and Cos-7 cells (N. E. Zachara, unpublished observations). Recently, Vocadlo and coworkers (48) have reported the use of a metabolic inhibitor that appears to work well in cell culture. Researchers interested in detecting the addition of *O*-GlcNAc to their proteins of interest are directed to recent reviews on the subject (196, 197). As both peptides and proteins modified by asparagine-*N*-GlcNAc (170, 171) and asparagine-*N*-$GlcNAc_2$ (67) have been reported in the literature, particular care should be taken to perform appropriate controls sinces many techniques used to detect *O*-GlcNAc will also detect these modifications.

O-GlcNAcase catalyzes the removal of O-GlcNAc (EC 3.2.1.52, GI:1364613, mgea5). *O*-GlcNAcase, sometimes known as hexosaminidase C, is a neutral β-*N*-acetylglucosaminidase that is expressed predominantly in the cytoplasm although it is also found in the mitochondria and nucleus (47, 64, 176). Unlike the lysosomal hexosaminidases, hexosaminidase A and hexosaminidase B, *O*-GlcNAcase is specific for β-*N*-acetylglucosamine and has a neutral pH optimum (pH 4.5–6) (32, 33, 47, 175). There are two major splice variants of *O*-GlcNAcase, a short form (~75 kDa) and a longer form (~130 kDa) (29, 47). Each splice variant contains the NH_2-terminal hexosaminidase domain that is similar to hyaluronidases (32, 33, 47, 175). The COOH-terminal domain has similarity to acetyltransferases and has been suggested to have histone acetyltransferase activity, although the physiological significance of this activity is unknown (160). Supporting a role for *O*-GlcNAc in the development of type II diabetes, mutations and alternative splice variants of *O*-GlcNAcase are associated with the onset of type II diabetes (40, 92). Goto-Kakizaki rats express a 90-kDa isoform, Δ250–345, of *O*-GlcNAcase that appears to lack *O*-GlcNAcase activity (37).

Little is known about the regulation of *O*-GlcNAcase, although it purifies in a complex with other proteins (20, 47, 176), is both phosphorylated and *O*-GlcNAc modified (47,

175), and interestingly is cleaved by caspase-3 during apoptosis (9, 175). *O*-GlcNAcase is efficiently inhibited in vitro and in vivo by a number of compounds (25, 34, 35, 78–80, 89, 145, 147, 157, 191), although only two are commercially available (52, 191): *O*-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino-*N*-phenylcarbamate (PUGNAc) and Thiamet-G.

Several investigators have shown that modulating *O*-GlcNAc levels pharmacologically or genetically does not result in the desired outcome, in part as the cell responds to this intervention by modulating the expression of the endogenous OGT and *O*-GlcNAcase (73, 154). For example, elevating *O*-GlcNAc levels is often accompanied by a suppression of the endogenous OGT and an induction of the endogenous *O*-GlcNAcase, presumably to restore basal levels of *O*-GlcNAc. Conversely, when *O*-GlcNAc levels are lowered, the expression of the *O*-GlcNAcase is lowered and OGT expression is elevated. The mechanism that regulates this internal *O*-GlcNAc rheostat is unknown; however, it appears to be regulated by the levels of *O*-GlcNAc rather than the association of OGT and *O*-GlcNAcase (20). Using an inducible OGT null cell line, Kazemi and coworkers (73) demonstrated that the OGT null is effectively an *O*-GlcNAcase null as well. Notably, if *O*-GlcNAc levels were elevated with an inhibitor of *O*-GlcNAcase at the induction of the knockout, *O*-GlcNAcase expression was stabilized in a dose-dependent manner (73). The mechanism that underlies these observations remains to be elucidated.

The Roles of O-GlcNAc in Regulating the Heart and Cardiovascular System

O-GlcNAc has been implicated in regulating many processes in the heart and vasculature, and these are discussed in detail below. Incongruously, *O*-GlcNAc has been implicated in regulating both the detrimental effects of diabetes as well as survival in models of trauma hemorrhage and ischemia-reperfusion injury. The molecular basis for these paradoxical observations has not yet been defined but may reflect changes in *O*-GlcNAc cycling, altered specificity of the enzymes, or the interaction of *O*-GlcNAc modified proteins with other signaling pathways that are differentially regulated during cellular stress/injury and diabetes.

O-GlcNAc as a regulator of cell survival. *O*-GlcNAc, A NOVEL REGULATOR OF THE CELLULAR STRESS RESPONSE. In 2004, *O*-GlcNAcylation was reported to increase on myriad proteins in response to multiple forms of cellular stress [heat shock, ER Stress (DTT), reductive stress, the hypoxia mimic $CoCl_2$, oxidative stress, ethanolic stress, osmotic stress, genotoxic stress (UVB), arsenite stress, viral infection, translational inhibition (cycloheximide), and potentially ubiquitin stress (inhibition of the proteasome)] (195). Notably, the increase in *O*-GlcNAc levels was post-translational, found in numerous primary and transformed cell lines, and dose dependent (195). Moreover, elevating the levels of *O*-GlcNAc either genetically or pharmacologically promoted cell survival, whereas depressing the levels of *O*-GlcNAc suppressed cell survival (195). Together, these data suggested that *O*-GlcNAc is a novel post-translational modification used to sense and transmit cellular stress signals. In support of this hypothesis, *O*-GlcNAc appears to regulate numerous pathways (Table 2) in a manner consistent with stress tolerance in both in vivo and in vitro models of heat stress (156, 195), hypoxia (125, 127, 128), UV irradiation (102), oxidative stress (70), acute vascular injury (181), trauma hemorrhage (129, 130, 183, 198, 199), and ischemia-reperfusion injury (15, 16, 43, 44, 66, 88, 98–100, 126, 128).

While several pathways have been implicated in promoting cell survival in an *O*-GlcNAc-dependent manner (Table 2), only one of these has been attributed to *O*-GlcNAcylation of a specific protein. Recently, Ku and coworkers (87) demonstrated that *O*-GlcNAcylation of Keratin 18 (Ser30, Ser31, Ser49) was essential for signaling by Akt and that subsequent inactivation of the proapoptotic kinase GSK3β in a model of streptozotocin or FAS/PUGNAc induced apoptosis in the liver and pancreas (87). Another study has highlighted the role of *O*-GlcNAc in the inactivation of GSK3β, although here no defect in Akt signaling was observed (73). In search of additional mechanisms by which *O*-GlcNAc is protective, a number of studies have identified proteins whose *O*-GlcNAcylation status changes in response to stress [Table 3; (159, 194)]. These and other studies suggest that some proteins are modified in response to diverse forms of injuries, whereas other proteins are modified only in response to specific injuries.

The mechanisms that lead to enhanced levels of *O*-GlcNAc in response to different forms of cell stress appear varied, and it is unclear if this depends on the type of injury or the cell/tissue type. There is some evidence that glucose uptake and flux through the HBP are important in ischemia-reperfusion injury and trauma hemorrhage (16, 43, 156). This leads to the attractive hypothesis that one end point of "flight or fight response"-induced hyperglycemia is *O*-GlcNAc (17). Reinforcing this idea, it appears that various forms of stress can activate GFAT, which catalyzes the rate-limiting step in UDP-GlcNAc biosynthesis (36, 137). In response to a number of forms of stress the expression of OGT appears elevated, these include UV irradiation, osmotic stress, and ethanolic stress (195), whereas heat stress appears to increase the activity of OGT increases threefold (195). Finally, as discussed in section *The UDP-GlcNAc: polypeptide OGT (EC 2.4.1.94; GI: 6006036) catalyzes the addition of O-GlcNAc*, in models of nutrient deprivation OGT appears to be targeted to substrates in the absence of increases in UDP-GlcNAc levels by p38 MAPK (19). Little is known about the regulation of *O*-GlcNAcase during stress; however, its expression and activity is not altered by heat stress (195).

O-GlcNAc, A NOVEL ENDOGENOUS CARDIOPROTECTIVE POST-TRANSLATIONAL MODIFICATION. Ischemia-reperfusion injury in the heart is characterized by calcium overload, oxidative stress, ER stress, structural and functional changes to the mitochondria (17), and changes in the *O*-GlcNAcylation status of numerous proteins. In hearts subjected to either ischemia-reperfusion injury (in vivo) or simulated ischemia (ex vivo), *O*-GlcNAc levels appear to drop before increasing during reperfusion (15, 16, 43, 70). This can be mimicked in isolated neonatal cardiomyocytes subjected to hypoxia and reoxygenation, where lower levels of *O*-GlcNAc are observed during hypoxia and then increase above basal levels during reoxygenation (127). Notably, both the acute and delayed models of ischemic preconditioning lead to elevated levels of *O*-GlcNAc. As yet, it is unclear if increased *O*-GlcNAcylation is required for the protective effects of ischemic preconditioning (70).

Table 2. *Pathways implicated in protecting cells and tissues from cellular injury*

Pathway	Role of *O*-GlcNAc	Tissue	Reference
Regulation of reactive oxygen species	Reducing *O*-GlcNAc levels reduces catalase mRNA expression, whereas elevating *O*-GlcNAc levels increased catalase mRNA expression.†	Isolated neonatal cardiomyocytes	(128)
Reduced expression of proinflammatory cytokines	Reduced expression of circulating inflammatory cytokines (TNFα, IL-6, IL-10), probably through regulation of the NF-κB signaling pathway.	Cardiac tissue and isolated neonatal cardiomyocytes, kidney, liver	(129, 130, 183, 198, 199)
Regulation of protein stability	In proteins overexpressing OGT, proteins such as Sp1 appear more heat stable, and this enhances transcription of proteins such as HSPs.	HeLa	(94)
Expression of stress-related genes	OGT and *O*-GlcNAcase have been found on the promoters of numerous stress-related genes. Deletion of OGT renders *Caenorhabditis elegans* sensitive to UV irradiation.	*C. elegans*	(102)
Regulating the expression of HSPs	Regulation of GSK3β, Sp1, and HSF1 have been implicated in regulating the expression of HSPs.	Cos-7 Cells, mouse embryonic fibroblasts, neonatal cardiomyocytes, hepatocytes	(73, 94, 150, 195)
Regulation of GSK3β	*O*-GlcNAc appears to regulate pathways upstream of GSK3β, leading to inactivation by phosphorylation at Ser09.	MEFs, Cos-7 cells	(73, 87)
Mitochondrial membrane potential	Numerous studies have demonstrated the elevated levels of *O*-GlcNAc protect membrane potential and cellular ATP levels.	Isolated neonatal cardiomyocytes	(15, 70, 125, 127, 128)
Reduced calpain activation	May be regulated indirectly, though modulation of intracellular calcium levels	Isolated neonatal cardiomyocytes	(99)
Calcium homeostasis	The mechanisms by which *O*-GlcNAc mediates capacitive calcium entry and calcium overload are unknown, although recently *O*-GlcNAc was shown to modify and regulate the IP_3 receptor.	Isolated neonatal cardiomyocytes	(122, 125, 127, 128, 132, 139)
Regulation of the p38 MAPK pathways	Glucosamine enhances signaling of p38 MAPK in response to agonist stimulation, leading to enhanced phosphorylation of HSP27 and αB-crystallin.	Isolated hearts	(43)
Formation of mPTP	*O*-GlcNAc reduces calcium overload and reactive oxygen species, two triggers known to stimulate pore opening. VDAC, which is thought by some to be a component of the mPTP, is *O*-GlcNAc modified.§	Isolated neonatal cardiomyocytes	(125, 127, 128)

†The molecular mechanism by which this occurs has not been defined. §The importance of VDAC in forming the mitochondrial permeability transition pore (mPTP) is controversial. However, VDAC has been implicated in regulating cell death through other mechanisms including its association with hexokinase 2. OGT, *O*-GlcNAc transferase; GSK3β, glycogen synthase kinase 3β; HSF1, heat shock factor 1; IP_3, inositol 1,4,5-trisphosphate.

Elevating *O*-GlcNAc levels before and after ischemia have been reported to reduce both cell/tissue death in isolated neonatal cardiomyocytes, isolated hearts, and in hearts in vivo by mitigating each of the characteristics of ischemia-reperfusion injury (15, 16, 43, 44, 66, 70, 88, 98–100, 122, 125–128, 132, 198) (Table 2). In addition, elevating *O*-GlcNAc levels prior to injury appears to reduce proinflammatory mediators (TNFα, IL-1, IL-6) in other models of tissue injury (18, 66, 181, 183, 198), suggesting additional pathways by which *O*-GlcNAc promotes survival of myocardial tissue. Glucose, glutamine, and glucosamine have all been shown to be cardioprotective, and several studies have attributed this to changes in *O*-GlcNAcylation (15, 16, 43, 98, 111, 121, 161). Interestingly, the cardioprotective effects of salidroside, a β-glucose analog, have also been attributed to *O*-GlcNAc by a mechanism that is still unclear (180).

O-GlcNAc, HYPERTROPHY, AND THE FAILING HEART. In models of pressure overload-induced hypertrophy, the cellular concentrations of UDP-GlcNAc become elevated, and this is believed to be a consequence of increased expression of GFAT2 (190). Consistent with the idea that hypertrophy is associated with increased flux through the HBP, increased expression of GFAT and elevated levels of *O*-GlcNAcylation are observed in aged Brown-Norway rats, which also demonstrate age-dependent increases in cardiac hypertrophy (41). Moreover, in the failing heart, elevated levels of *N*-acyl-D-glucosamine 2-epimerase, which can contribute to HBP flux, have been reported (83). Together, these data suggest that flux through the HBP alters the physiology of hearts/cardiomyocytes in an *O*-GlcNAc-dependent manner. One consequence of this signaling appears to be a glucose-dependent change in gene expression. Young and coworkers (190) demonstrated in both ex vivo and in vivo

Table 3. *Proteins whose O-GlcNAc modification state changes in response to stress or injury*

Protein and Site	Stress/Injury	Tissue/Organism	Reference
Carm1	Heat stress	Cos-7 cells	(194)
Cytokeratin 8/18	Heat stress	Cos-7 cells	(93)
DNA PK	Heat stress, ER Stress	Cos-7 cells	(194)
Histone H2B	Heat stress	HeLa cells	
IRS-1	Urea (20 mM), probably working through increased ROS	3T3-L1 adipocytes	(31)
Neurofilament H	Glucose deprivation	Neuro-2A	(19)
NF90 and NF110	Heat stress	Cos-7 cells	(194)
Nup153	Heat stress	Cos-7 cells	(194)
P125i (Associates with Sec23)	Heat stress	Cos-7 cells	(194)
Sec24b	Heat stress	Cos-7 cells	(194)
WNK1	Heat stress	Cos-7 cells	(194)

Recently 2 screens have focused on identifying proteins whose *O*-GlcNAcylation status responds to cellular injury (heat stress, hydrogen peroxide, hypoxia, urea, and trauma hemorrhage). Such proteins are listed here only if their *O*-GlcNAcylation status was confirmed either by immunoblot or the *O*-GlcNAc-peptide linkage was confirmed by mass spectrometry. Carm1, coactivator-associated arginine methyltransferase 1; DNA PK, DNA protein kinase; IRS-1, insulin receptor substrate 1; Nup153, nuclear pore protein 153; WNK1, with no lysine kinase 1.

models that glucose (25 mM) metabolism induced a change in the ratio of myosin heavy chain isoforms, increasing the expression of myosin heavy chain-β (fetal). Recently, reducing *O*-GlcNAc levels has been shown to restore hypertrophy signaling in a rodent model of diabetes (112). In contrast to these data, in exercise-induced hypertrophy, reduced levels of *O*-GlcNAc have been observed, suggesting that more research in this field is required before a clear model is delineated (4).

Recently, Watson and colleagues (172) demonstrated a role for *O*-GlcNAc in a model of infarct-induced heart failure (172). In addition to the typical phenotypes associated with heart failure (scaring, elevated heart weight, pulmonary edema, and increased atrial natriuretic peptide), elevated levels of *O*-GlcNAc were observed (172). Consistent with the models of hypertrophy discussed above, elevated expression of GFAT1 was observed, as well as increased expression of OGT and reduced expression of *O*-GlcNAcase. To study the role *O*-GlcNAc in heart failure further, OGT was deleted from hearts using Cre-lox technology and perhaps unexpectedly the significant reduction in *O*-GlcNAc did not result in cardiac dysfunction, hypertrophy, apoptosis, or fibrosis. However, deleting OGT appeared to exacerbate infarct-induced heart failure, resulting in reduced survival of mice, increasing the rate of apoptosis in the remote noninfarcted myocardium, and reducing ventricular function (172).

TRAUMA HEMORRHAGE. Hypovolemia, a result of trauma hemorrhage, is a leading cause of death as a result of multiple organ failure. Enhanced glucose levels have been associated with improved survival in models of trauma hemorrhage, and recent studies have suggested that this is in part due to changes in *O*-GlcNAcylation (18, 129, 130, 183, 198, 199). In vivo trauma hemorrhage suppresses *O*-GlcNAc levels, and simply maintaining *O*-GlcNAc levels with glucosamine treatment improves the function of multiple organs and blood pressure and reduces the expression of the proinflammatory cytokines TNFα and IL-6 (129, 130, 183, 198, 199). Indicating that these affects are mediated by *O*-GlcNAc, these data could be recapitulated by treatment with PUGNAc or by altering the expression of OGT (199). More importantly, treating animals during resuscitation with glucosamine or PUGNAc maintained *O*-GlcNAc levels and elevated survival from 53 (control) to 85 and 86%, respectively (129).

INFLAMMATION. One mechanism by which *O*-GlcNAc is thought to promote survival is by reducing the detrimental effects of prolonged or unregulated inflammation (66, 129, 181, 198), although *O*-GlcNAc has also been associated with hyperglycemia-induced inflammation (184). In numerous models, altering *O*-GlcNAc levels metabolically, pharmacologically, or genetically leads to a depression in the circulating levels of proinflammatory cytokines such as TNFα, IL-10, and IL-6 (66, 129, 181, 198). Moreover, the expression of intracellular adhesion molecule 1 (ICAM-1) is reduced, which should reduce neutrophil infiltration preventing further injury (198). Consistent with this observation, elevated levels of *O*-GlcNAc result in lower activity of cardiac myeloperoxidase in a model of trauma hemorrhage (198). *O*-GlcNAc also appears to regulate the NF-κB signaling pathway, resulting in these observations (198), both directly as NF-κB is *O*-GlcNAc modified (68) and indirectly as the modulation of *O*-GlcNAc levels appears to regulate the phosphorylation of IκB (198). This is probably a general response of cells, as this can be recapitulated in models in which cells are treated with LPS (198).

It is also possible that cells/tissues are less sensitive to inflammatory mediators. In endothelial cells, one outcome of TNFα activation is expression of monocyte chemoattractant protein-1 (MCP1) and ICAM-1 (71). Glucosamine, which was shown to elevate global *O*-GlcNAc levels, repressed the induction of MCP-1 and ICAM-1, suggesting that in human umbilical vein endothelial cells, glucosamine and *O*-GlcNAc are acting to suppress inflammatory signaling. Notably, TNFα acts through p38 MAPK to phosphorylate and activate the p65 subunit of NF-κB, and glucosamine appears to suppress this pathway (71).

Another model in which *O*-GlcNAc appears to reduce inflammation is a model of acute arterial injury, preventing neointima formation (181). Neointima, or thickening of the arterial intima, contributes to the pathology of atherosclerosis, in-stent restenosis, vein bypass graft failure, and transplant vasculopathy (120). Xing and coworkers (181) demonstrated that in response to acute injury of the carotid artery, the levels of *O*-GlcNAc dropped globally (181). Pretreating animals with glucosamine or PUGNAc increased *O*-GlcNAc on a subset of proteins and reduced the expression of a number of chemokines (cytokine-induced neutrophil chemoattractant-2β, MCP-1) and adhesion molecules (p-selectin, VCAM-1) associated with vascular remodeling (181). Consistent with this observation, elevating *O*-GlcNAc levels reduced infiltration of granulocytes

and monocytes into the arterial wall and the appearance of neointima (181).

O-GlcNAc and diabetes. The seminal work of Marshall and coworkers (114) demonstrated that the HBP (HBP; Fig. 1) plays a key role in the development and complications associated with type II diabetes. Here, high levels of glucose were unable to induce insulin resistance if the rate-limiting step of the HBP, GFAT, was inhibited pharmacologically with either 6-diazo-5-oxonorleucine or azaserine [Fig. 1; (114)]. Conversely, overexpression of GFAT exacerbated the effects of hyperglycemia (30, 61). Several laboratories have suggested that this is due to both changes in UDP-GlcNAc levels and thus the *O*-GlcNAc modification (1, 3, 8, 31, 39, 40, 46, 54, 60, 72, 82, 101, 109, 110, 133–136, 155, 167, 169, 185, 187), as well as other metabolites (115, 124) and possibly other forms of protein glycosylation (65, 173). Key data that support a role for *O*-GlcNAc in mediating the complications associated with type II diabetes includes *1*) overexpression of *O*-GlcO-GlcOGT in the muscle and adipose of mice results in insulin resistance and hyperleptinemia, two hallmarks of type II diabetes (117); *2*) deletion of either OGT or *O*-GlcNAcase in *Caenorhabditis elegans* leads to changes in glucose and trehalose metabolism, as well as dauer phenotypes characteristic of disrupted insulin signaling (40, 54); *3*) PUGNAc treatment, an inhibitor of the *O*-GlcNAcase and lysosomal hexosaminidases, leads to insulin resistance in cell culture models and tissue explants (3, 134, 167); *4*) in several models of diabetes *O*-GlcNAc levels are elevated on a subset of proteins (1, 2, 8, 42, 64, 113, 133, 138, 169); *5*) numerous pathways are regulated by NAc, such as insulin signaling via Akt (3, 6, 40, 54, 119, 134–136, 167, 185); and *6*) reducing the levels of *O*-GlcNAc in models of type II diabetes reverses the some of the complications associated with type II diabetes (26, 63, 64, 125). However, recent data suggests that elevating *O*-GlcNAc levels alone may not be sufficient to induce diabetes: *1*) treating 3T3-L1 adipocytes with PUGNAc but not a more specific inhibitor of *O*-GlcNAcase (Thiamet-G) results in insulin resistance (107), *2*) treating mice for long periods of time with Thiamet-G does not alter glucose metabolism (108), and *3*) overexpressing *O*-GlcNAcase does not restore glucose metabolism in 3T3-L1 adipocytes (142). Collectively, these data suggest that elevating *O*-GlcNAc levels alone may not be sufficient for the induction of diabetes but must be associated with some other type of dysregulation [e.g., advanced glycation end-product formation, increased PKC activity, increased flux though the polyol pathway, and increased oxidative stress (7)] or that dysregulation of other pathways impacts the sites and dynamics of the *O*-GlcNAc modification contributing to diabetes. One mechanism by which diabetes may affect the sites of *O*-GlcNAcylation is increased UDP-GlcNAc levels, as discussed in section *The UDP-GlcNAc: polypeptide OGT (EC 2.4.1.94; GI:6006036) catalyzes the addition of O-GlcNAc*. An alternative mechanism may be altered targeting of the OGT, and recently Yao and coworkers (186) have demonstrated one example of this: one side effect of diabetes is increased modification of Arg/Lys residues with methylglyoxal, a highly reactive α-oxoaldehyde. Modification of the corepressor mSin3A with methylglyoxal at Arg925 and Lys938 resulted in recruitment of OGT and subsequent *O*-GlcNAcylation of the transcription factor specificity protein 3. Ultimately, *O*-GlcNAcylation of Sp3 transcription factor leads to an induction of angiopoitien-2, which sensitizes the microvasculature of the kidney to TNFα (186).

O-GlcNAc, DIABETES, AND CARDIOVASCULAR DYSFUNCTION. Several laboratories have clearly demonstrated a role for *O*-GlcNAc in regulating proteins in a manner that would result in cardiac or vascular dysfunction, and these are discussed below. Several studies have determined the levels of *O*-GlcNAc in models of diabetes in cardiovascular tissues *1*) on erythrocyte proteins in patients with type II diabetes (133, 169), *2*) in hearts from Zucker diabetic fatty rats (42), *3*) in hearts from *db/db* mice (112), *4*) in rat hearts treated with streptozotocin (63), and *5*) in hearts from *ob/ob* mice (138). In all cases diabetes results in elevated levels of *O*-GlcNAc on a subset of proteins.

One of the pathophysiologies associated with type II diabetes is the development of cardiomyopathy, in which many aspects of cardiac contractility are impaired. Underlying these observations are changes in cardiac calcium (Ca^{2+}) handling thought to result from decreased sarcoplasmic reticulum (SR) Ca^{2+} uptake, reduced SR Ca^{2+} release, and reduced SR Ca^{2+} content. Like many of the studies discussed in *O-GlcNAc and diabetes*, glucose and glucosamine treatment of cardiomyocytes had been associated with impaired cardiac contractility and increased intracellular calcium. These data led to the hypothesis that *O*-GlcNAcylation of key proteins may lead to cardiac dysfunction in models of type II diabetes (26, 63, 125). Supporting this hypothesis, several studies have demonstrated that the diastolic decay phase of calcium transients is delayed in neonatal cardiomyocytes treated with high concentrations of extracellular glucose (25 mM), glucosamine (8 mM), or PUGNAc (50 μM) (26, 63, 125). Notably, they also demonstrated that overexpressing the *O*-GlcNAcase ablated the effect of high glucose on calcium handling, whereas overexpressing OGT exacerbated the effect (26, 63, 125). Together, these data suggest that nonphysiological elevations in *O*-GlcNAc levels alter calcium handling in neonatal cardiomyocytes but that this was dependent on high-glucose levels, since overexpressing either OGT or *O*-GlcNAcase had no effect on cells maintained in physiological concentrations of glucose (5.5 mM).

Several mechanisms have been reported that would alter calcium handling in the diabetic heart, and these center on cardiac sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a), the Ca^{2+} ATPase that transfers Ca^{2+} from the cytosol of the cell to the lumen of the SR. In one model, changes in the expression of SERCA2a appear to be regulated transcriptionally, and this is attributed to increased *O*-GlcNAcylation of Sp1 and decreased expression of myocytes enhancer factor 2A, two transcription factors known to regulate the expression of SERCA2a (26, 63, 125). *O*-GlcNAc has also been reported to regulate SERCA2a through its association with phospholamban. Yokoe and coworkers (189) showed that phospholamban was *O*-GlcNAcylated at Ser16 and that diabetes led to higher levels of *O*-GlcNAc at this site and lower phosphorylation. They suggest that this ultimately reduces SERCA2a activity through a direct association with phosphorylated-phospholamban (189). In an alternative model, diabetes has been shown to reduce expression of phospholamban, which would result in the same phenotype (63).

High glucose in neonatal cardiomyocytes also alters mitochondrial function, in part by direct modulation of mitochondrial proteins. Recently, Hu and coworkers (63) have shown that elevated levels of *O*-GlcNAc are associated with decreased

function of the mitochondrial electron transport complexes—complex I, III, and IV. Importantly, reducing the levels of *O*-GlcNAc by overexpression of *O*-GlcNAcase reversed the high-glucose phenotype. Consistent with reduced mitochondrial function, lower levels of cellular ATP were observed in cells grown in high levels of glucose (63).

Hyperglycemia, and subsequent upregulation of *O*-GlcNAcylation, has also been linked to the development of atherosclerosis. Federici and coworkers (39) demonstrated that in human coronary artery endothelial cells high glucose and glucosamine elevated the levels of *O*-GlcNAc on a number of key proteins involved in insulin signaling: insulin receptor substrate 1, insulin receptor substrate 2, and the p85 subunit of phosphatidylinositol 3-kinase. Moreover, elevated *O*-GlcNAc levels were associated with decreased insulin signaling downstream of the insulin receptor, in particular through the phosphatidylinositol 3-kinase Akt/PKB signaling pathway. Increased levels of *O*-GlcNAc led to reduced phosphorylation of endothelial nitric oxide synthase (eNOS) at Ser1177; this inhibition of eNOS led to increased activity and expression of matrix metalloproteinase (MMP)-2 and MMP-9 (39). An altered balance between MMPs and inhibitors of MMPs has been implicated in the etiology of atherosclerosis. Consistent with increased MMP activity, plaques from patients with diabetes demonstrated higher levels of *O*-GlcNAcylation than controls (39).

O-GlcNAc and hypertension. Hypertension is characterized by abnormal vascular reactivity, impaired endothelium-dependent relaxation, and enhanced sensitivity to vasoconstrictors. Hypertension is also a major risk factor for cardiovascular disease and is often associated with diabetes. Interestingly, the kinase "with no lysine kinase 1 (WNK1)" has been isolated as being *O*-GlcNAc modified in several studies (77, 159, 194), and *O*-GlcNAcylation increases in response to heat stress (194). While the function of WNK1 *O*-GlcNAcylation has not been studied, mutations in WNK1 and WNK4 lead to familial hypertension (178, 182).

Several studies have demonstrated that elevating *O*-GlcNAc levels induces many of the hallmarks of hypertension, such as increasing reactivity to constrictor stimuli (phenylephrine) and impaired endothelium-dependent vasodilatation (95–97). The mechanisms that underlie these observations appear to be regulated in part by decreased phosphorylation of Akt (Ser473) and eNOS (Ser1177) (95–97). Previously, eNOS was shown to be hyper-*O*-GlcNAcylated in a model of diabetes-induced erectile dysfunction. Notably, *O*-GlcNAcylation of eNOS prevented phosphorylation by Akt at this site and was associated with reduced nitric oxide production (119).

Lima and coworkers (96) demonstrated that hypertensive rats, induced by deoxycorticosterone acetate and salt (DOCA-salt), had elevated levels of *O*-GlcNAc on numerous proteins in both aorta and mesenteric arteries. The effect of hypertension, impaired endothelium-dependent relaxation, and enhanced sensitivity of vasoconstrictors could be recapitulated simply by elevating *O*-GlcNAc levels with an inhibitor of the *O*-GlcNAcase (PUGNAc, Fig. 1). However, PUGNAc and DOCA-salt did not have an additive effect, suggesting that DOCA-salt may be working through *O*-GlcNAc. Again, the effects of hypertension were linked to a reduction in phosphorylation of key signaling molecules eNOS and Akt. Notably, the reduction in eNOS phosphorylation may result from direct competition with *O*-GlcNAc, as Lima and coworkers (96) demonstrated that DOCA induced eNOS *O*-GlcNAcylation.

In subsequent work, Lima and coworkers (97) have demonstrated the endothelin-1 (ET-1) and PUGNAc augment vascular contraction to phenylephrine in vascular smooth muscle cells. Interestingly, ET-1 appears to enhance *O*-GlcNAc levels, suggesting that like DOCA-salt, ET-1 may work via *O*-GlcNAc. Consistent with the hypothesis that ET-1 regulates the RhoA/Rho kinase pathways in an *O*-GlcNAc-dependent manner, ET-1-dependent phosphorylation of protein phosphatase 1 regulatory protein, myosin light chain, and myosin light chain phosphatase target protein 1 could be reversed with an OGT inhibitor or small interfering RNA of OGT (97). Moreover, ET-1 induction of RHO activity was again blocked by small interfering RNA of OGT (97). Together,these data may suggest that *O*-GlcNAc plays a role in regulating vascular reactivity.

Conclusions

O-GlcNAc is a novel post-translational modification that appears to be a key regulator of cell function that is crucial for heart and vascular function. Our understanding of the molecular mechanisms by which *O*-GlcNAc regulates key proteins and signaling events and how this is altered by diseases such as aging, hypertension, and diabetes is in its infancy. One challenge to advancing the field lies in the modification itself, which is challenging to detect and resists genetic/pharmacological manipulation. There have been a number of recent advances in methodologies for detecting *O*-GlcNAc modified proteins (28, 75, 76, 81, 123, 141, 151, 158, 159, 163–166, 170, 171, 192, 193), mapping the sites of addition of *O*-GlcNAc (11, 14, 81, 141, 151, 170), probing the dynamics of *O*-GlcNAcylation (12, 13, 77, 165, 168, 194), and genetic models in which *O*-GlcNAc levels can be manipulated (5, 49, 73, 131, 146, 172). Together, this suite of technologies should allow researchers to answer some of the key remaining questions: *1*) How is the specificity of OGT and *O*-GlcNAcase regulated, and how is this altered during aging or in disease models? *2*) What is the molecular basis of the *O*-GlcNAc paradox, and why is *O*-GlcNAc protective in acute models of injury and destructive in models of sustained injury? and, finally, *3*) What is the function of *O*-GlcNAc on proteins, and how does this network of *O*-GlcNAc modified proteins act to regulate heart and vascular function?

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AUTHOR CONTRIBUTIONS

N.E.Z. prepared figures, drafted manuscript, edited and revised manuscript, and approved final version of manuscript.

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