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O-GlcNAc and the Cardiovascular System

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Abstract

The cardiovascular system is capable of robust changes in response to physiologic and pathologic stimuli through intricate signaling mechanisms. The area of metabolism has witnessed a veritable renaissance in the cardiovascular system. In particular, the post-translational β -O-linkage of N-acetylglucosamine (O-GlcNAc) to cellular proteins represents one such signaling pathway that has been implicated in the pathophysiology of cardiovascular disease. This highly dynamic protein modification may induce functional changes in proteins and regulate key cellular processes including translation, transcription, and cell death. In addition, its potential interplay with phosphorylation provides an additional layer of complexity to post-translational regulation. The hexosamine biosynthetic pathway generally requires glucose to form the nucleotide sugar, UDP-GlcNAc. Accordingly, O-GlcNAcylation may be altered in response to nutrient availability and cellular stress. Recent literature supports O-GlcNAcylation as an autoprotective response in models of acute stress (hypoxia, ischemia, oxidative stress). Models of sustained stress, such as pressure overload hypertrophy, and infarct-induced heart failure, may also require protein O-GlcNAcylation as a partial compensatory mechanism. Yet, in models of Type II diabetes, O-GlcNAcylation has been implicated in the subsequent development of vascular, and even cardiac, dysfunction. This review will address this apparent paradox and discuss the potential mechanisms of O-GlcNAc-mediated cardioprotection and cardiovascular dysfunction. This discussion will also address potential targets for pharmacologic interventions and the unique considerations related to such targets.

Keywords

Hexosamine biosynthetic pathway; Ischemia-reperfusion injury; Heart failure; Hypertrophy; Mitochondria

Accessory Pathways of Glucose Utilization

Glucose functions not only as a ubiquitous source of energy but also confers significant capacity for inter/intracellular signaling. Upon entering a cell, glucose is phosphorylated to glucose-6-phosphate. During glycolysis it is further metabolized to fructose-6-phosphate

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permitting entry into a host of accessory pathways of glucose metabolism; one such pathway is the Hexosamine Biosynthetic Pathway (HBP). The HBP culminates in the formation of a high-energy glycoside precursor (UDP-GlcNAc) for the posttranslational modification of nuclear and cytoplasmic proteins. This resulting β -O-linkage of N-acetylglucosamine (O-GlcNAc) to proteins has been identified in altering expression, translation, and function of the target proteins. Recently O-GlcNAc has emerged as key player in the primary pathophysiology of many cardiovascular diseases. This review focuses on the role of O-GlcNAc in the cardiovascular system and examines the therapeutic potential for pharmacological management of this signaling mechanism.

Hexosamine Biosynthetic Pathway

Approximately 5% of intracellular glucose enters the HBP. The four enzymatic reactions of the HBP convert fructose-6-phosphate to uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc), the monosaccharide donor for the O-GlcNAc modification. The first reaction is the rate-limiting conversion of fructose-6-phosphate to glucosamine-6-phosphate by L-glutamine: fructose-6-phosphate amidotransferase (GFAT) with the concomitant conversion of Gln to Glu^{1, 2}. The second reaction is the conversion of glucosamine-6-phosphate to N-acetylglucosamine-6-phosphate through glucosamine-6-phosphate acetyl-transferase (Emeg32; Gnpnat1) using acetyl-CoA. The penultimate reaction converts N-acetylglucosamine-6-P to N-acetylglucosamine-1-P with phosphoglucomutase 3 (Pgm3). Interestingly, deletion of either Emeg32³ or Pgm3⁴ is embryonic lethal. Finally, pyrophosphorylase catalyzes the conjugation of a uridine nucleotide to form UDP-GlcNAc, which serves as the monosaccharide donor for O-GlcNAcylation. As shown in Figure 1, nutrient-derived glucose, glutamine, acetyl-CoA, and glucosamine all feed into the HBP at different points linking it with amino acid metabolism, lipolysis/lipogenesis, and glucose oxidation. Because the production of UDP-GlcNAc requires nutrients derived from other metabolic pathways, O-GlcNAcylation may serve as a nutrient or metabolic sensor^{5–11}.

Identifying O-GlcNAc modification of proteins has been a major challenge in this field. Thus, techniques¹² to identify O-GlcNAcylation continue to rapidly evolve. The O-GlcNAc modification was first identified on lymphocytes in 1984 using radiolabeled galactose tagging¹³. As the O-GlcNAc field has grown and branched towards diverse biological processes, the impetus to chemically monitor O-GlcNAc cycling has increased. The combination of conventional antibody staining and fluorophore labeling remain preferred techniques for O-GlcNAc detection. Although the CTD clone is probably the most commonly used antibody, some have challenged its fidelity¹⁴. Recently, several antibodies were developed to expand our ability to detect O-GlcNAcylation¹⁵. To detect site-specific modifications, investigators must rely on more labor-intensive methods and expensive, highly specialized mass spectrometry instrumentation^{16–22}. Unfortunately, its small size, lack of charge, labile nature, and its similarity to other small sugars, makes O-GlcNAc particularly challenging to unequivocally identify.

Regulation of O-GlcNAc

GFAT

Flux through the HBP can be altered based on the availability of nutrients and activity of enzymes. The availability of glucose to shunt through the HBP is paramount. Further progression through the HBP requires sources of glutamine, glucosamine and acetyl-CoA. The first and rate-limiting enzyme of the HBP is GFAT², which is highly conserved and exists in two different isoforms, GFAT1 and GFAT2²³. GFAT1 is expressed in the pancreas, placenta, testes, and skeletal muscle^{24, 25}. GFAT2 is expressed in the heart and the central nervous system. It can be regulated transcriptionally, post-transcriptionally, and through

HBP-feedback inhibition by UDP-GlcNAc^{1, 26–29}. GFAT uses glutamine as a substrate for the formation of glucosamine-6-phosphate³⁰. GFAT antagonists, such as O-diazoacetyl-L-serine (azaserine) and 6-diazo-5-oxonorleucine (DON), irreversibly inhibit GFAT, resulting in reduced flow of glucose through the HBP; however, a primary issue with such analogs of glutamine is that they are general amidotransferase inhibitors. The rate-limiting nature of GFAT can be bypassed by exogenous or naturally occurring glucosamine. Given the conceptual difficulty in targeting GFAT via a glutamine analog approach and the fact that the ultimate product, UDP-GlcNAc, is essential for traditional complex glycosylation as well as other sugar nucleotide synthesis, GFAT seems to be a poor therapeutic candidate to specifically reduce O-GlcNAcylation.

OGT

Ultimately, two highly conserved enzymes, O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), directly regulate O-GlcNAcylation of proteins. OGT catalyzes the addition of a single GlcNAc moiety to serine/threonine residues on proteins^{31–34}. Interestingly, in addition to glycosylation OGT functions in a proteolytic capacity. It has been highlighted to O-Glycosylate and catalyze the proteolysis of cell cycle regulator HCF-1^{35, 36}. OGT is expressed ubiquitously but is found to be most abundant in glucose-sensing cells of the pancreas, and, the brain. The predominant isoform of OGT is nucleocytoplasmic in localization and is coded by a single X-linked gene^{31, 32, 37}. Alternate splicing of the message produces three isoforms of OGT: nucleocytoplasmic OGT (ncOGT), mitochondrial OGT (mOGT), and short OGT (sOGT)^{31, 32}.

The mechanism of substrate specificity of OGT has yet to be determined; however, recent advances in the study of its structural and kinetic properties may yield important insights. OGT consists of an N-terminal domain with tetratricopeptide repeats (TPR) and an intervening domain flanked by two glycotransferase catalytic domains^{38, 39}. The ubiquitous, 34 amino acid TPR motif produces a structure with two antiparallel α -helices^{40, 41}. Multiple tandem arrays of TPRs generate a right-handed helical structure with potential to mediate protein-protein interactions. The different isoforms of OGT vary only in the number of TPR regions. As determined by X-ray crystallography, the N-terminal region of human OGT has a canonical superhelical fold typified by TPRs and a groove⁴². Further studies with bacterial OGT demonstrated that the TPRs and the catalytic domain are juxtaposed such that the superhelical groove extends into the active site^{43, 44}. Through binding with the continuous groove, protein substrates may influence substrate specificity in the catalytic center⁴⁵. This notion was consistent with *in vitro* studies that identified the role of TPR in modifying proteins⁴⁶.

The interactions between OGT and protein substrates require the presence of UDP-GlcNAc. Kinetic studies by the Walker group indicated that OGT employs an ordered bi-bi kinetic mechanism where UDP-GlcNAc binds first followed by the substrate⁴⁷. The interaction between UDP-GlcNAc and OGT induces a conformational change between TPR 12 and 13 that is hypothesized to allow protein substrate entry into the catalytic domain active site. Post-translational modifications involving tyrosine kinases, nitrosylation of cysteine residues, and O-GlcNAc modification may also regulate OGT activity^{31, 48}. Yet, unlike most kinases, there is no known consensus sequence for OGT. Thus, the splice variants themselves, interacting proteins, and potentially the concentrations of UDP-GlcNAc may largely regulate substrate selection^{49, 50}.

The Walker laboratory also identified, through a high-throughput screen, a number of potential OGT inhibitors⁵¹. Two compounds, TT04 and TT40, characterized with an oxobenzo[d]oxazole core were found to have irreversible action through modifying the catalytic base at the active site⁵²; however, these compounds demonstrated low water

solubility, which limited their application⁵³. TT04 has been successfully used in the Jones lab as an OGT inhibitor^{54, 55}. Recent work by Vocadlo led to the development of an O-GlcNAc substrate analog named 5-thioglucoamine (SSGlcNAc)⁵⁶. The acetylated SSGlcNAc readily crosses the cell membrane due to its hydrophobic nature, becomes converted to UDP-5sGlcNAc and ultimately binds to the active site of OGT competitively inhibiting its function. Preliminary cell culture treatment with the inhibitor resulted in marked reduction in overall O-GlcNAcylation and did not affect cell viability. Genetic deletion and translational silencing techniques have also been employed to reduce activity of OGT. Neonatal cardiomyocytes from loxP-flanked OGT mice were infected with adenoviral Cre recombinase (to knockout OGT) or transfected with short interfering RNA directed against OGT; both approaches decreased global O-GlcNAcylation and sensitized the cardiomyocytes to post-hypoxic death⁵⁴. Accordingly, there are several biological methods to suppress OGT activity; however, the efficacy of such traditional pharmacologic inhibitors requires further validation and may require the development of new compounds.

O-GlcNAcase

O-GlcNAcase (OGA) catalyzes the removal of the O-GlcNAc modification from proteins³⁸. It primarily resides in the cytoplasm but can be found in nuclei and, potentially, mitochondria^{38, 57, 58}. The structure of OGA consists of two main domains: an N-terminal domain with glycoside hydrolase activity and a C-terminal histone acetyltransferase (HAT) domain. These domains flank a region containing a caspase-3 cleavage site⁵⁹. There are two confirmed splice variants of OGA. The full-length protein variant is predominately found in the cytosol whereas the shorter variant, which lacks the C-terminal domain, resides in the nucleus⁶⁰. The shorter form of OGA also lacks apparent HAT activity.

Human OGA employs a two-step catalytic mechanism and the transient formation of a bicyclic oxazoline intermediate. The highly conserved active site has two adjacent aspartate residues^{45, 61}. They are proposed to play a key role in the cleavage of O-GlcNAc from protein substrates. The first aspartate residue acts as a base to attack the 2-acetamido group of the anomeric carbon. The second aspartate acts as an acid to permit the departure of the leaving group.

Accessibility to the sugar moiety and restricted conformational freedom of the modified protein dictate OGA substrate specificity⁴⁵. Recent work with the mutagenesis of b-N-acetylglucosaminidase from *Oceanicola granulosus*, an enzyme homologous (37%) to human OGA in sequence and function, revealed that peptide binding on the surface of human OGA is important for its function⁶². Further studies with the *Clostridium perfringens* OGA homologue demonstrated the importance of conformational restraint when deletion of hydrogen bonding within glycopeptides resulted in decreased affinity for human OGA⁶³. These studies extend the hypothesis that accessibility to the sugar is important and that conformational change in protein substrates serves to impair OGA processing⁴⁵.

Several OGA inhibitors have been developed to study the biological roles of O-GlcNAc. O-(2-acetamido-2-deoxy-D-glucopyranoseylidene)amino N-phenyl carbamate (PUGNAc), GlcNAcstatin, and Thiamet G are three inhibitors found to effectively limit OGA activity⁵³. PUGNAc acts as a transition state analog but is unstable in aqueous solutions^{64, 65}. GlcNAcstatins, following a similar structure to PUGNAc, were developed to have higher selectivity to human OGA⁶⁶. These proved to penetrate the cell and increase global O-GlcNAcylation 2–3 fold but at the cost of low solubility in aqueous solutions⁶⁷. Yuzwa et al. mimicked electrostatic interactions that occur once the bicyclic oxazoline intermediate forms during the reaction mechanism of OGA with another inhibitor called Thiamet G^{53, 68}. Currently, it is the most potent inhibitor of OGA and is exceptionally stable in aqueous solutions⁶⁸.

In addition to traditional pharmacologic approaches, Ngoh et al. demonstrated successful inhibition of OGA through RNA interference. Neonatal rat cardiac myocytes (NRCMs) transfected with short interfering RNA directed against OGA resulted in significantly augmented O-GlcNAc levels when compared to addition of a scrambled non-silencing control sequence. Primed with increased O-GlcNAcylation, these myocytes were less susceptible to post-hypoxic cell death⁶⁹. Compared to OGT, OGA inhibitors are generally more reliable and better characterized.

O-GlcNAcylation and Phosphorylation

Because it shares some similarities with phosphorylation, O-GlcNAcylation, and its impact on signaling, can become exceptionally complicated. Both O-GlcNAcylation and phosphorylation modifications occur on serine and threonine residues, are dynamically added and removed, and alter function of proteins⁷⁰. Although hundreds of genes regulate phosphorylation, only two known genes encode enzymes for the addition and removal of O-GlcNAc in mammals. Interestingly, a gene with functional relationship to OGT, EGF repeat-specific O-GlcNAc transferase (Eogt), was identified in flies and later in mammals. Eogt O-GlcNAcylates EGF-repeats, may be sequestered in the ER, and, unlike OGT, may be restricted to a specific consensus sequence^{71, 72}. Part of the complexity arises within sites of occupancy for O-GlcNAc and O-phosphate. Site mapping studies have revealed evidence for at least three types of interplay between these signaling mechanisms⁷³. The first is competitive site occupancy where O-GlcNAc and O-phosphate compete for the same threonine or serine moiety at the same site^{74–77}. The second is alternative occupancy at adjacent sites. Here, occupancy of O-GlcNAc and O-phosphate at adjacent sites can influence the turnover or function of proteins^{78, 79}. A third type of interaction involves O-GlcNAc and O-phosphate at the same sites, on adjacent sites, or on distant sites^{80, 81}. Although O-GlcNAc and phosphorylation are often described as having a Yin-Yang relationship⁸², it is difficult to imagine such a relationship as universal and obligatory.

O-GlcNAc Acts as an Alarm or Stress Signal

The O-GlcNAc post-translational modification may sense and trigger a pro-adaptive response to cellular stressors. Some of the earliest evidence to support such a contention involved the association between hyperglycemia and O-GlcNAcylation and/or OGT^{83–86}. Although this can be retrospectively viewed as ‘stress’ studies, the first *bona fide* investigation of O-GlcNAc as a stress signal came in 2004⁸⁷. Zachara et al. found that O-GlcNAcylation increased in response to various forms of stress, including heat, oxidative, osmotic, ultraviolet light, and others⁸⁷. O-GlcNAcylation increased in a roughly dose-dependent manner in response to these stressors in varying cell lines. More importantly, augmenting O-GlcNAc levels promoted cell survival, whereas depressing O-GlcNAc levels reduced survival⁸⁷. Such insights offered the clarion call for new investigations into a previously unrecognized role for O-GlcNAcylation in cell survival.

The Role of O-GlcNAc in the Cardiovascular System

Acute Cardioprotection

The O-GlcNAc modification confers protection to subsequent lethal insults, which has been reviewed extensively^{8–10, 70, 88–92}, elsewhere. Briefly, in response to myocardial ischemia-reperfusion injury – which is characterized by calcium overload, oxidative stress, and ER stress – global levels of O-GlcNAcylation are augmented^{54, 93–98}. Temporal changes in O-GlcNAcylation have been observed *in vitro* and *in vivo* studies of cardiomyocyte survival. In *in vitro* studies, O-GlcNAc levels drop during hypoxia then increase during reoxygenation in isolated, perfused hearts subjected to simulated ischemia-reperfusion^{96–99}; this same effect

occurs during hypoxia-reoxygenation of NRCMs⁵⁴. Interestingly, *in vivo* myocardial ischemia-reperfusion studies demonstrate similar findings⁹⁹. The elevation in O-GlcNAc before and after ischemia functions to reduce cell death^{96–99}, and, antecedent elevation of O-GlcNAcylation promotes myocardial tissue viability in other models of tissue injury^{100, 101}. Regulation of the HBP and enzymatic regulators of O-GlcNAcylation may provide potential targets for therapeutic intervention in cardiovascular disease.

Hypertrophy and the Failing Heart

Nowhere has the rebirth of metabolic investigation in the cardiovascular system been more evident than in heart failure. Metabolic changes during heart failure result in changes in substrate utilization and the reversion to a fetal metabolic profile. The heart suppresses fatty acid oxidation and augments its reliance on carbohydrate oxidation^{102, 103}. Increased flux of glucose enters the HBP, could result in protein O-GlcNAcylation, and play an important role in cardiomyocyte hypertrophy. In models of pressure-overload hypertrophy, UDP-GlcNAc concentrations are greatly increased¹⁰⁴. Accordingly, levels of O-GlcNAc and GFAT increased in Brown-Norway rats as their hearts with age-related cardiac hypertrophy¹⁰⁵. This observation suggests that HBP and/or O-GlcNAc may be involved in the development of hypertrophy.

Cardiac myocytes isolated from db/db diabetic and non-diabetic mice demonstrated altered hypertrophic signaling response to changes in protein O-GlcNAcylation¹⁰⁶. To directly identify the role of O-GlcNAcylation in hypertrophy, Facundo et al.¹⁰⁷ determined that NFAT activation during cardiomyocyte hypertrophy required O-GlcNAcylation. O-GlcNAc-dependent activation of NFAT resulted in hypertrophy whereas depressing O-GlcNAcylation blunted cardiomyocyte NFAT activation and hypertrophy¹⁰⁷. Because others showed NFAT could be a target of O-GlcNAc in other model systems¹⁰⁸, and, that NFAT is phosphorylated when excluded from the nucleus (i.e. not activated¹⁰⁹), it is tempting to speculate that cardiomyocyte hypertrophy is driven by the direct O-GlcNAcylation of NFAT.

Watson et al.¹¹⁰ reported increased O-GlcNAcylation and OGT expression during heart failure in mice. In this infarct-induced heart failure model, OGT expression was elevated and OGA expression was reduced. Cardiac-specific deletion of OGT and consequent reduction in cardiac O-GlcNAcylation significantly exacerbated infarct-induced heart failure¹¹⁰. Specifically, survival was somewhat reduced, ventricular dysfunction was significantly depressed, and apoptosis was augmented. The results demonstrate that the pro-adaptive increase in O-GlcNAc is required in the failing heart. Interestingly, cardiomyocyte OGT deletion did not result in any acute signs of heart failure in otherwise naïve (non-infarcted) mice. The question remains whether constitutive (early in life) or long-term (beyond four weeks) deletion of cardiomyocyte OGT results in myocardial pathology.

Stem Cells

Recent success with cardiac stem cell (CSC) therapy in the post-infarcted heart stimulated questions regarding the basic biology of CSCs, specifically involving O-GlcNAcylation. O-GlcNAcylation may partially regulate pluripotency and reprogramming of embryonic stem cells^{111, 112}. In addition, O-GlcNAc serves to regulate cell survival in development^{113, 114}. Zafir et al. directly examined the role of O-GlcNAc in cardiac stem cells by demonstrating a time-dependent increase in protein O-GlcNAcylation in CSCs following hypoxia-reoxygenation. Furthermore, pharmacological intervention or gene deletion-mediated reduction in O-GlcNAcylation in CSCs sensitized them to post-hypoxic injury⁵⁵. In contrast, augmenting O-GlcNAc levels enhanced CSC survival. Thus, induction of O-GlcNAcylation in CSCs prior to *in vivo* transfer to infarcted myocardium may provide an adjuvant

therapeutic benefit by promoting cell survival, which represents a current pursuit of this laboratory.

Diabetes

Diabetes mellitus, characterized by elevated blood glucose levels, represents a primary, independent risk factor for cardiovascular disease. Given the simple association of hyperglycemia with diabetes and elevated O-GlcNAcylation, it is not surprising that protein O-GlcNAcylation has been linked to diabetes. Indeed, several reports indicate elevated O-GlcNAcylation in diabetic models^{58, 83, 84, 115–118}. Although some may question this relationship in individual tissues or cell types – particularly insulin sensitive tissues – evidence supports a general relationship between diabetes and O-GlcNAcylation. Interesting proof-of-concept studies demonstrated a relationship between the HBP and insulin resistance *in vitro*. Specifically, overexpression of GFAT1 in rat fibroblasts leads to decreased insulin sensitivity¹¹⁹, and, transgenic overexpression of GFAT resulted in insulin resistance¹²⁰. Marshall et al.² rescued hyperglycemia-induced insulin resistance through GFAT inhibition. Similarly, overexpression of OGT in muscle and adipose tissue leads to insulin resistance and hyperleptinemia¹²¹, while inhibiting O-GlcNAcase with PUGNAc (to increase O-GlcNAcylation) leads to insulin resistance in cell culture^{122–124}. Such findings support a role for the HBP and O-GlcNAcylation in promoting, or at least participating in the pathogenesis of insulin resistance and diabetes.

However the nonselective nature of PUGNAc has led to concern over the veracity of this correlation¹²⁵. Treatment with PUGNAc, in addition to increasing O-GlcNAcylation, has been found to induce proteins involved in ubiquitin-proteasome degradation and insulin signaling pathways¹²⁶. Specifically, PUGNAc appears to increase ubiquitination of proteins and decrease not only Akt phosphorylation but also the protein itself¹²⁶. Similarly, recent studies from the Buse lab^{127–129} contend that elevating O-GlcNAc alone may be insufficient to induce diabetes. The Vocadlo group used a more selective OGA inhibitor than PUGNAc (i.e. NButGT) to determine a correlation between insulin resistance and O-GlcNAcylation. NButGT rapidly increased O-GlcNAcylation without the added effect of causing insulin resistance in 3T3-L1 adipocytes^{130–132}. Neither overexpression of OGA nor knockout of OGT appeared to be required for insulin resistance¹³³; however, Dentin et al. showed overexpression of OGA in a diabetic mouse model rescues glucose tolerance and insulin sensitivity¹³⁴. Taken together, these results suggest that global O-GlcNAcylation may not necessarily cause insulin resistance on its own. Thus, the role of the HBP and O-GlcNAc in diabetes may not be as clearly cause-and-effect as initially thought.

O-GlcNAc Mediates Diabetic Cardiac Dysfunction?

Similar to the basic cell studies tying O-GlcNAc to diabetic pathogenesis, several groups have suggested an association between increased O-GlcNAcylation and cardiac dysfunction. At a reductionist level, cells grown in hyperglycemic conditions demonstrated reduced ATP production as a potential result of altered mitochondrial proteins¹³⁵. Increased O-GlcNAcylation, secondary to hyperglycemia, may lead to depressed function of mitochondrial electron transport complexes I, III, and IV in NRCMs¹³⁵; overexpression of O-GlcNAcase attenuated this effect. Nevertheless, it still remains unclear whether all of the necessary components (i.e. OGT, UDP-GlcNAc, appropriate binding partners) to produce O-GlcNAcylation are present in the mitochondria, and, although such studies are exciting, further work is required to fully understand how O-GlcNAc may regulate mitochondrial function.

Not only may O-GlcNAc alter basic aspects of mitochondrial function, it may also affect cardiac contractile function through altering calcium handling. Treating cardiomyocytes

with high concentrations of extracellular glucose, glucosamine, or PUGNAc resulted in delayed calcium transients and overexpression of OGT exacerbated this effect, whereas OGA overexpression ablated it^{69, 135, 136}. The interaction between phospholamban and SERCA2a may also be modified by O-GlcNAcylation. O-GlcNAcylation at Ser16 on phospholamban is increased in the diabetic condition¹³⁷. This led to a reduction in phosphorylation of phospholamban and a decreased association with SERCA2a thereby decreasing the pump's activity. Such studies suggest the increased O-GlcNAcylation during hyperglycemia/diabetes may be part of the cause of the pathology, either at the mitochondrial level and/or the level of calcium handling. Yet, certain fundamentals remain unclear such as how cardiomyocytes, which (according to conventional wisdom) normally require insulin for most glucose uptake, might exhibit increased glucose uptake during diabetes, at least to a level sufficient to drive flux, and consequently O-GlcNAcylation, through the HBP. On an even larger scale, some investigators contend that myocardial insulin resistance serves as a pro-adaptive, defensive response against nutrient excess¹³⁸.

O-GlcNAc Modulates Vascular Activity

Sustained alteration of cardiovascular reactivity can result in hypertension, a primary risk factor for cardiovascular disease. Deranged vascular activity, endothelial reactivity, and hypersensitivity to vasoconstrictors are all hallmarks of hypertension and vascular dysfunction. Interestingly, the development of these hallmark characteristics appears to coincide with augmented O-GlcNAcylation. Lima et al. described increased O-GlcNAcylation in the vasculature of hypertensive rats¹³⁹. Vascular function studies demonstrated impaired endothelium-dependent relaxation and enhanced sensitivity to vasoconstrictors. Augmenting O-GlcNAcylation with PUGNAc was sufficient to recapitulate these vascular effects in normoglycemic conditions, indicating a role for O-GlcNAc in the development of hypertension.

Furthermore, Lima et al. correlated a link between O-GlcNAcylation and endothelin-1 (ET-1), a major player in the development of vascular dysfunction¹⁴⁰. ET-1 is responsible for inducing vasoconstriction and triggering a host of transcription factors that lead to inflammation, oxidative stress, and eventually tissue death¹⁴¹. The authors found that protein O-GlcNAcylation increases following incubation with ET-1 and alters vascular reactivity, and, when OGT is inhibited in the presence of ET-1, the effect of ET-1 on O-GlcNAc and vascular reactivity is abrogated. Moreover, blocking the ET_A receptor with atrasentan, resulted in decreased O-GlcNAcylation. ET-1 treatment depresses OGA expression and activity, which could result in the observed increase in O-GlcNAcylation. Thus, a complex interplay exists between ET-1 and the induction of O-GlcNAcylation.

Conversely, data from the Oparil lab supports a role of O-GlcNAc in preventing vascular dysfunction. First, they demonstrated that the induction of O-GlcNAcylation inhibits acute inflammatory responses to endoluminal injury¹⁴². Further studies showed that pretreatment of aortic rings with Thiamet G and glucosamine lead to increased global O-GlcNAcylation and prevented TNF α induced hypo-contractility and endothelial dysfunction¹⁴³. This inhibition of TNF α 's effects may be the result of O-GlcNAcylation of the NF κ B p65¹⁴⁴. As in the myocardium, the vasculature may host divergent responses to O-GlcNAcylation, which may largely relate to duration of the stimulus.

Exercise

Because O-GlcNAc is a fuel or metabolic sensor, several groups have interrogated the potential role of O-GlcNAcylation in exercise. Recently, Bennet et al. studied the effects of exercise on O-GlcNAcylation in diabetic mice¹⁴⁵. They found that exercise treatment of diabetic mice resulted in improved rate of relaxation (Tau) and a reduction in O-

GlcNAcylation. Also, Belke showed decreased protein O-GlcNAcylation and expression of OGT in mice with exercise-induced (i.e. physiologic) hypertrophy¹⁴⁶. In addition, the de-O-GlcNAcylation of OGT resulted in the dissociation from the repressor element 1 silencing transcription factor (REST) complex and the initiation of physiological hypertrophic signaling in the exercised mice¹⁴⁷. So, how do the aforementioned diabetic results relate to the exercise studies? It is not entirely clear; however, hypertrophic signaling following decreased O-GlcNAcylation may indicate a potential for other mediators of pathological cardiac dysfunction in diabetes. In essence, increased O-GlcNAcylation may not be the requisite factor for cardiac dysfunction but rather a perpetuating factor stemming from the initial insult of the diabetic condition.

Conclusion

O-GlcNAcylation plays a vital role in cardiac and vascular function, especially during disease. Although O-GlcNAcylation uniformly promotes cardiomyocyte survival in the context of acute cell injury, the role of O-GlcNAc in the vasculature or in the chronically failing/diabetic heart is less clear-cut. So, is any of this clinically relevant? In the case of acute cardioprotection, therapeutic augmentation of O-GlcNAc levels is a clinically feasible option. One such strategy would be to limit de-O-GlcNAcylation through pharmacological inhibition of OGA. Another method would be to prime cardiomyocytes through genetically enhancing O-GlcNAcylation. Either approach could be used in cases such as heart transplant or coronary artery bypass grafting – both of which involve predictable stress/ischemic events in the heart.

The implementation of targeted O-GlcNAcylation, though difficult to imagine at this point, may provide a therapeutic benefit to the cardiovascular system prior to or following acute cardiovascular injury. These strategies may provide protection during ischemic events in the heart resulting in improved survival and cardiac function. In the meantime, laboratory efforts should be directed toward understanding which proteins are modified and what such modification does to the target proteins to influence cardiovascular cell fate and function. Curiously, we have virtually no understanding of the transcriptional and post-transcriptional regulation of the enzymes regulating O-GlcNAcylation. Such basic insights, along with the identification of more reliable inhibitors of OGT, remain as major limitations in the field.

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Abbreviations

HBP	Hexosamine Biosynthetic Pathway
O-GlcNAc	β-O-linkage of N-acetylglucosamine
GFAT, L-glutamine	fructose-6-phosphate amidotransferase
UDP-GlcNAc	uridine diphosphate-N-acetylglucosamine
OGT	O-GlcNAc transferase

OGA	O-GlcNAcase
NRCM	Neonatal rat cardiac myocytes
DON	6-diazo-5-oxonorleucine
TPR	tetratricopeptide repeats
PUGNAc	O-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino N-phenyl carbamate
Eogt	EGF repeat-specific O-GlcNAc transferase

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Figure 1.

Depiction of glucose entry into a cell and, subsequently, the hexosamine biosynthetic pathway (HBP). The four reactions and enzymes of the HBP are indicated along with corresponding inhibitors (red). The formation of UDP-GlcNAc, the donor for the O-GlcNAc modification, is the final product of the HBP. O-GlcNAc can be added to protein substrates via OGT. Conversely, OGA functions to remove this moiety. Inhibitors are highlighted in red and juxtaposed with their targets.

Table 1

Summary of the literature showing the specific impact of increasing or decreasing O-GlcNAc on different models of disease or injury.

Cell Type	Stimulus/Model	Effect	Reference
Neonatal rat cardiomyocytes	Hyperglycemia	Impaired activity of complex I, III, and IV.	58
Neonatal rat cardiomyocytes	Hyperglycemia and ischemia reperfusion injury	Increased cell viability, decreased apoptosis and necrosis	97, 98
Rat whole hearts	Glucosamine perfusion in streptozotocin-induced diabetic rats	Increased recovery from ischemia and calcium paradox	94
Rat whole hearts	Glucosamine perfusion in ischemia/reperfusion	Attenuated ischemic contracture and improved functional recovery	96
Neonatal rat cardiomyocytes	Glucosamine perfusion in ischemia/reperfusion	Increased cell viability, decreased apoptosis and necrosis	97, 98
Adult rat cardiomyocytes	Glucosamine treatment following trauma-hemorrhage	Improvement in cardiac function	100
Skeletal muscle	GFAT transgenic overexpression	Insulin resistance	119, 120
Cardiac stem cells	Inhibition of OGA via Thiamet under hypoxia/reoxygenation	Enhanced cell survival	55
3t3 cells, Skeletal muscle	Inhibition of OGA via PUGNAc	Insulin resistance	122–124, 126
Neonatal rat cardiomyocytes	Inhibition of OGA via PUGNAc under hypoxia/reoxygenation	Attenuated post-hypoxic injury	69
Neonatal rat cardiomyocytes	Inhibition of OGA via PUGNAc under ischemia/reperfusion	Increased cell viability, decreased apoptosis and necrosis	97, 98
Neonatal rat cardiomyocytes	Inhibition of OGA via PUGNAc prior to ER stress	Attenuated ER-stress induced cell death	93
Neonatal rat cardiomyocytes	Adenoviral overexpression of OGT	Increased post-hypoxic cellular viability	54
Neonatal rat cardiomyocytes	Adenoviral overexpression of OGT prior to ER stress	Attenuated ER-stress induced cell death	93
Neonatal rat cardiomyocytes	Gene deletion of OGA via RNAi under hypoxia/reoxygenation	Decreased post-hypoxic injury	69
Rat whole hearts	Ischemic preconditioning prior to ischemia/reperfusion	Reduced infarct size	95
Adult mouse cardiomyocytes	Angiotensin II/Phenylephrine	Hypertrophy	106, 107
DOCA rats	Hypertension	Impaired vasodilatory activity	139
Neonatal rat cardiomyocytes	GFAT inhibition via azaserine	Decreased post-hypoxic cellular viability	98
Rat whole hearts	GFAT inhibition via azaserine in hearts treated with streptozotocin	Increased sensitivity to calcium paradox	93
Neonatal rat cardiomyocytes	Inhibition of OGT by TT04	Exacerbated post-hypoxic injury	54
Cardiac stem cells	Inhibition of OGT by TT04 under hypoxia/reoxygenation	Decreased post-hypoxic cellular viability	55
Neonatal rat cardiomyocytes	Adenoviral overexpression of OGA under hypoxia/reoxygenation	Augmented post-hypoxic death and calcium overload	69
Neonatal rat cardiomyocytes	Adenoviral overexpression of OGA prior to ER stress	Exacerbated brefeldin A-induced cell death	93
Neonatal rat cardiomyocytes	Gene deletion of OGT with RNAi	Enhanced sensitivity to post-hypoxic death	54
Adult rat cardiomyocytes	Gene deletion of OGT with RNAi	Increased I κ B- α phosphorylation	100

O-GlcNAc

Cell Type	Stimulus/Model	Effect	Reference
cmOGT KO	Gene deletion of OGT prior to Myocardial Infarction	Exacerbated cardiac dysfunction	110
Streptozotocin-induced diabetic mice	Exercise	Decreased OGT and GFAT mRNA, O-GlcNAcylation, hypertrophy	146, 147
293 Cells	siRNA against OGT	Decreased site-specific proteolysis of HCF-1	35, 36