Review

Role of protein O-linked N-acetyl-glucosamine in mediating cell function and survival in the cardiovascular system

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Received 27 April 2006; received in revised form 14 July 2006; accepted 18 July 2006
Available online 29 July 2006
Time for primary review 21 days

Abstract

There is growing recognition that the O-linked attachment of N-acetyl-glucosamine (O-GlcNAc) on serine and threonine residues of nuclear and cytoplasmic proteins is a highly dynamic post-translational modification that plays a key role in signal transduction pathways. Numerous proteins have been identified as targets of O-GlcNAc modifications including kinases, phosphatases, transcription factors, metabolic enzymes, chaperons, and cytoskeletal proteins. Modulation of O-GlcNAc levels has been shown to modify DNA binding, enzyme activity, protein–protein interactions, the half-life of proteins, and subcellular localization. The level of O-GlcNAc is regulated in part by the metabolism of glucose via the hexosamine biosynthesis pathway (HBP), and the metabolic abnormalities associated with insulin resistance and diabetes, such as hyperglycemia, hyperlipidemia, and hyperinsulinemia, are all associated with increased flux through the HBP and elevated O-GlcNAc levels. Increased HBP flux and O-GlcNAc levels have been implicated in the impaired relaxation of isolated cardiomyocytes, blunted response to angiotensin II and phenylephrine, hyperglycemia-induced cardiomyocyte apoptosis, and endothelial and vascular cell dysfunction. In contrast to these adverse effects, recent studies have also shown that O-GlcNAc levels increase in response to acute stress and that this is associated with increased cell survival. Thus, while the relationship between O-GlcNAc levels and cellular function is complex and not well-understood, it is clear that these pathways play a critical role in the regulation of cell function and survival in the cardiovascular system and may be implicated in the adverse effects of metabolic disease on the heart.

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Keywords: Hexosamine biosynthesis; Protein O-glycosylation; O-GlcNAc transferase; Diabetes

1. Introduction

The association of metabolic diseases with increased risk for heart failure raises the distinct possibility that alterations in myocardial metabolic pathways play a critical role in the development and progression of the disease. However, a causal relationship between metabolic dysfunction and increased risk of heart failure has been hard to establish. Even in animal models of metabolic disease, where there is evidence of specific cardiomyocyte dysfunction, there is no consensus as to the specific metabolic or nutrient signaling pathways that contribute to this dysfunction [1]. The hexosamine biosynthesis pathway (HBP, Fig. 1) has been proposed as a nutrient sensor, and excess glucose flux into the HBP has been implicated in the development of insulin resistance as well as the vascular complications of diabetes [2]. Glucose metabolism via the HBP leads to the formation of uridine 5'-diphosphate-N-acetylglucosamine (UDP-GlcNAc), which is the substrate for the secretory pathway as well as for a distinct O-GlcNAc transferase within the nucleocytoplasmic compartment (OGT, Fig. 1). This OGT catalyzes the formation of a reversible post-translational modification in which GlcNAc is attached via an O-linkage...
to specific serine and threonine residues on numerous nuclear and cytoplasmic proteins [3]. There is growing evidence that the impact of increased HBP flux on cell function is mediated via elevated levels of O-GlcNAc on specific proteins [2], and it is apparent that changes in O-GlcNAc levels can have diverse effects on cell function and survival [3,4]. It is noteworthy that most of the metabolic abnormalities associated with insulin resistance and diabetes, namely hyperglycemia, hyperlipidemia and hyperinsulinemia, are all associated with increased HBP flux and increased O-GlcNAc levels. Consequently, these pathways may play a critical role in the adverse effects of metabolic disease on the heart.

The goal of this review is to summarize our current understanding of the role of the HBP and O-GlcNAc on the regulation of cell function and survival in the cardiovascular system. We begin with a general overview of the HBP and O-GlcNAc (the reader is also referred to several excellent reviews on these topics that provide much more detailed information than can be covered here [2–5]). This will be followed by a discussion of the role of the HBP and O-GlcNAc in mediating the contractile abnormalities associated with diabetes as well as their influence on the regulation of cardiomyocyte hypertrophy and apoptosis. We will also examine the role of these pathways on the development of vascular complications associated with diabetes. As a contrast to the adverse effects of increased HBP and O-GlcNAc, we also discuss recent studies that have demonstrated that these same pathways may be part of an endogenous stress survival response and that acute activation of these pathways is cardioprotective.

2. The hexosamine biosynthesis pathway (HBP)

It has been estimated that in cultured adipocytes the HBP consumes 2–5% of the glucose taken up by cell [6]; however, a direct measure of glucose flux through this pathway has yet to be determined in either the intact heart or in isolated cardiomyocytes. The key regulatory enzyme of the pathway is glutamine: fructose 6-phosphate amidotransferase (GFAT), which converts fructose 6-phosphate to glucosamine 6-phosphate with glutamine as the amine donor [7]. UDP-GlcNAc the end-product of the HBP serves as a substrate for the addition of a single N-acetylglucosamine to serine or threonine residues of both nuclear and cytosolic proteins (O-glycosylation, O-GlcNAc), which as discussed below is increasingly recognized as an important regulatory mechanism involved in signal transduction [2,3,5]. Flux through the HBP can be increased by the addition of exogenous glucosamine (Fig. 1), which enters cells via the glucose transporter system and is phosphorylated to glucosamine-6-phosphate by hexokinase, thereby, bypassing GFAT and elevating UDP-GlcNAc levels.

The role of the HBP in the development of insulin resistance was first described by Marshall et al. in rat adipocytes [6]. Transgenic mice overexpressing GFAT in skeletal muscle and adipose tissue develop insulin resistance and hyperleptinemia [8,9]. In vivo glucosamine infusion also resulted in
skeletal muscle insulin resistance [10–12]. These data support the notion that increased flux through the hexosamine biosynthesis pathway plays an important role in the development of insulin resistance and glucose toxicity. Activation of the HBP has also been associated with glucose-induced transcriptional upregulation of TGFα [13], TGFβ [14], leptin [15], PAI-1 [16] and decreased phosphorylation of Akt and GSK3 [17].

3. Nuclear and cytoplasmic O-glycosylation

O-glycosylation is an ubiquitous post-translational modification present in higher eukaryotes that is increasingly recognized as an important regulatory mechanism involved in signal transduction [2–5]. Unlike other glycosylation events this reaction occurs in the cytosol and the nucleus rather than in the Golgi or the endoplasmic reticulum, and is regulated by two key enzymes: uridine diphospho-N-acetylglucosamine: polypeptide β-N-acetylglucosaminyltransferase (O-GlcNAc transferase; OGT) — catalyzing the O-glycosylation and N-acetylglucosaminidase (O-GlcNAcase) — catalyzing the removal of sugar moiety from the proteins [3,5].

Although, O-glycosylation is a highly dynamic post-translational modification similar to phosphorylation, in contrast to more than 500 kinases regulating phosphorylation [18], there is a single gene encoding OGT [19,20]. A single copy of the OGT gene is located on the X chromosome in humans and mice and OGT gene deletion in mice was embryonically lethal, demonstrating that OGT activity/O-glycosylation is vital for life [21]. The complex interplay between O-GlcNAc and phosphorylation, is reinforced by the observation that OGT forms a complex with protein phosphatase-1 [22]. While O-glycosylation and phosphorylation can be reciprocal on some proteins (e.g., eNOS [23], estrogen receptor-β [24], C-terminal domain of RNA polymerase II [25], c-myc [26]), there can also be multiple phosphorylation/O-glycosylation sites on the same protein [3,5].

In some tissues (e.g., liver, muscle and kidney) OGT is a heterotrimer consisting of two 110 kDa and one 78 kDa subunits [27], whereas in other tissues it exists as a homotrimer of three 110 kDa subunits [19]. The structure of the 110 kDa subunit contains two main domains; an N-terminal domain containing multiple tetratricopeptide repeats (TPR), a catalytic domain in the C-terminus, and a nuclear localization sequence [19,28]. The TPR domains mediate protein–protein interactions and at least 5 TPR domains are required for substrate binding [20]. There is also a mitochondrial form of OGT (mOGT) primarily consisting of 103 kDa subunits, which contains a unique mitochondrial targeting sequence in the N-terminus [5]. Although, mOGT is enzymatically active in vitro, only a few O-glycosylated proteins have been found in mitochondria [5].

Regulation of OGT activity is complex and not yet fully understood. At low micromolar concentrations of UDP-GlcNAc the $K_m$ for OGT was found to be 545 nM, giving a competitive advantage over UDP-GlcNAc transporters [27], which transport UDP-GlcNAc from the cytosol to the Golgi or endoplasmic reticulum. However, others have reported that OGT has three distinct $K_m$ values for UDP-GlcNAc (6, 35, 217 μM) and that multimerization of the enzyme altered the affinity for UDP-GlcNAc [20]. OGT is also known to be a target for both tyrosine phosphorylation and O-glycosylation [3,5]. The effect of these modifications on the function of OGT is not well-defined; however, preliminary studies suggest that tyrosine phosphorylation activates OGT [29]. Wischent et al., demonstrated that OGT directly associates with the O-GlcNAcase [30], the enzyme responsible for removing O-GlcNAc moiety from the Ser/Thr residues of proteins.

O-GlcNAcase (N-acetylglucosidase) is a 106 kDa heterodimeric complex containing a 54 kDa α and a 51 kDa β subunit [31] and the gene is localized to the 10q24 chromosomal location [32]. O-GlcNAcase is distinct from lysosomal hydrolases, including hexosaminidase A and B and is specific for N-acetyl-β-d-glucosaminides, and shows no N-acetyl-β-d-galactosaminidase activity [31–33]. O-GlcNAcase activity and protein is localized primarily in the cytosol (90%), with the remaining 10% found in the nucleus [32]. Toleman et al., recently reported that the active site is found in the N-terminal third of O-GlcNAcase between amino acids 63 and 238 [34]. It has also been shown that O-GlcNAcase has an intrinsic histone acetyl transferase (HAT) activity and a single point mutation abolished HAT activity [35]. Interestingly, there is a caspase-3 cleavage site linking the O-GlcNAcase and HAT regions; however cleavage by caspase-3 does not alter the catalytic activity of O-GlcNAcase [32,35].

Changes in O-GlcNAc levels have been associated with the development of cancers [26,36] and the development of neurodegenerative disorders, such as Alzheimer’s disease [5,37,38]. There is also considerable evidence linking increased O-GlcNAc levels to the development of insulin resistance and diabetic complications [2]. For example, overexpression of OGT in vivo resulted in insulin resistance and hyperleptinaemia [39] and in 3T3-L1 adipocytes increased levels of O-GlcNAc impaired insulin stimulated Akt and GSK3β phosphorylation and cellular insulin resistance [17]. Glucosamine-induced insulin resistance has been associated with increased O-glycosylation of IRS-1 and IRS-2 [12]. Also, increased O-GlcNAc levels in adipocytes decreased insulin stimulated GLUT4 translocation, reduced insulin-stimulated phosphorylation of IRS-1 and Akt and increased O-glycosylation of GLUT4, IRS-1 and Akt2 [40]. Toleman et al. identified O-GlcNAcase splice variants from Goto–Kakizaki rat, a model of type-2 diabetes, which had no O-GlcNAcase activity but the HAT activity was preserved [35]. A correlation between a polymorphism in the O-GlcNAcase gene with Type 2 diabetes has also been reported in Mexican-Americans [41].

Interestingly, the commonly used diabetogenic agents alloxan and streptozotocin (STZ) have been reported to inhibit OGT [42] and O-GlcNAcase respectively [43]. However, whether these mechanisms are responsible for the β-cell toxicity of these agents is uncertain. For example, alloxan has been shown to inhibit the β-cell glucokinase [44]
and may also inhibit other enzymes that recognize uracil moieties. Further, despite reports that STZ is an O-GlcNAc-case inhibitor [43], Macauley et al., [45] found that in vitro STZ had no effect on O-GlcNacase activity suggesting that the increase in O-GlcNac seen with STZ [46] is likely secondary to other toxic effects of this drug.

Thus, there is strong evidence that elevated O-GlcNac levels as a consequence of increased HBP flux combined with changes in expression/activity of OGT and O-GlcNacase may play an important role in the development of insulin resistance and diabetes. There are also an increasing number of studies linking increased O-GlcNac levels to complications associated with diabetes [2,23,47–49]. A list of proteins that have been implicated in the development of diabetic complications and that have been shown to be directly modified by O-GlcNac, or the activity of which has been reported to be altered in response to changes in O-GlcNac, are listed in the Table 1. A comprehensive list of proteins that are modified by O-GlcNac can be found in the review by Love and Hanover [5]. In the following sections we discuss in more detail our current understanding of the influence of O-GlcNac on cell function and survival in the cardiovascular system.

4. Diabetic cardiomyopathy

Diabetic cardiomyopathy is defined as a ventricular dysfunction in the absence of hypertension and/or coronary vascular disease [1]. Diabetes leads to diabetic cardiomyopathy either by affecting function at the cardiomyocyte level [50] or by altering cardiac structure as a result of increased inflammation or fibrosis [51]. Other complications associated with diabetes such as micro- and macrovascular disease and hypertension may also contribute to increased cardiac dysfunction associated with diabetes and may act synergistically with impaired cardiomyocyte function and structural changes. Below we discuss first the contribution of O-GlcNac in mediating adverse effects of diabetes at the cardiomyocyte level, this is followed by consideration of other factors including fibrosis and vascular dysfunction.

4.1. O-GlcNac and abnormal cardiomyocyte function in diabetes

Adult cardiomyocytes isolated as little as 4–6 days following STZ-induced diabetes exhibit slower relaxation and slower rates of Ca2+ transient decay [52] consistent with decreased sarcomplasmic reticulum Ca2+-ATPase (SERCA) activity; however, SERCA expression was unchanged. Incubation of normal adult cardiomyocytes in hyperglycemic media induced similar alterations in excitation–contraction (EC) [53] that were not associated with changes in SERCA, or phosphorylamban expression or phosphorylation [54]. Since, hyperglycemia increases HBP flux and O-GlcNac levels, it is possible that O-GlcNac modification of Ca2+-handling proteins may contribute to hyperglycemia-induced cardiomyocyte dysfunction. This is supported by the

<table>
<thead>
<tr>
<th>Group</th>
<th>Protein</th>
<th>Cell/tissue</th>
<th>Modified by O-GlcNac</th>
<th>Effect of increased O-GlcNac</th>
<th>Ref.</th>
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<td>Transcription factors</td>
<td>Sp1</td>
<td>NRVM*</td>
<td>Yes</td>
<td>Increased activity</td>
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<td>Mef-2</td>
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<td>Reduced expression</td>
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<tr>
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<td>Reduced phosphorylation, impaired insulin signaling</td>
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<td>PI3K</td>
<td>HCAEC</td>
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<td>Decreased activity and phosphorylation</td>
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<tr>
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<td>Yes</td>
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<td>Not known</td>
<td></td>
<td>[95]</td>
<td></td>
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<tr>
<td>PKCα,E</td>
<td>SVG cells*</td>
<td>Not</td>
<td></td>
<td>[96]</td>
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</tbody>
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(continued on next page)
fact that glucosamine, which rapidly increases O-GlcNAc levels, had a similar effect on EC coupling as hyperglycemia [55].

A more direct link between increased O-GlcNAc levels and cardiomyocyte dysfunction was reported by Clark et al., [48], who showed that in neonatal cardiomyocytes increasing O-GlcNAc levels with hyperglycemia, glucosamine or increased OGT expression prolonged Ca\(^{2+}\) transient decays, whereas overexpression of O-GlcNAcase improved Ca\(^{2+}\) transients in cells exposed to hyperglycemia. In contrast to the effects of hyperglycemia in adult cardiomyocytes [54], hyperglycemia significantly decreased SERCA mRNA and protein expression in neonatal cardiomyocytes and this was prevented by increased O-GlcNAcase expression [48]. Increased O-GlcNAc levels on the transcription factor Sp1 were also observed. This may account for the decrease in SERCA expression since the SERCA promoter contains multiple Sp1 binding sites that are required for adequate expression. Sp1 expression levels were unaffected by increased O-GlcNAc levels; however, the expression levels of MEF-2, a transcription factor important for cardiomyocyte function and maturation, was depressed but it was not subject to O-GlcNAc modification itself. Subsequently, Hu et al., [49] showed that in hearts from STZ-induced diabetic mice there was an increase in OGT expression and O-GlcNAc levels, which was accompanied by cardiomyocyte dysfunction. Increased expression of O-GlcNAcase significantly lowered cardiac O-GlcNAc levels and improved function at both the isolated cardiomyocyte and whole heart levels, which was associated with increased SERCA expression relative to diabetic controls.

Pang et al. [56] found that short-term diabetes blunted the inotropic response of the heart to the α-adrenergic agonist phenylephrine and that this effect was partially abrogated by pre-treatment with azaserine, an inhibitor of the HBP [56]. In contrast, a brief pre-treatment of normal hearts with 5 mM glucosamine, sufficient to increase O-GlcNAc levels approximately 3-fold [57] also attenuated the response to phenylephrine [56]; whereas the response to β-adrenergic stimulation was unaltered. Kim et al., [58] demonstrated in C2C12 myoblasts that increased O-GlcNAc levels, blunted the bradykinin mediated increase in intracellular Ca\(^{2+}\), which like phenylephrine is also mediated by PLC activation. Importantly, they showed that PLC-β1 was subject to O-GlcNAc modification and this modification was enhanced by glucosamine, PUGNAc or hyperglycemia. Consequently, they proposed that increased O-GlcNAc levels on PLC-β1 decreased its activity, thereby attenuating the response to bradykinin. Since PLC activation is critical to G-protein coupled signaling, O-GlcNAc induced attenuation of PLC activity could have broad implications for the development of diabetic cardiomyopathy.

4.2. HBP and O-GlcNAc and cardiomyocyte apoptosis and hypertrophy

Another contributing factor to the increased risk of heart failure in patients with diabetes is increased cardiomyocyte apoptosis [59,60]. Incubation of adult cardiomyocytes in hyperglycemic conditions resulted in increased O-GlcNAc levels on the transcriptional regulator p53 leading to increased angiostatin II synthesis and increased apoptosis [61]. They found that an AT\(_1\) receptor antagonist blocked hyperglycemia-induced apoptosis, whereas inhibition of O-glycosylation with BAG (benzyl-2-acetamido-2-deoxy-α-D-galactopyranoside) prevented the O-GlcNAc modification of p53 and blocked the synthesis of Ang II. However, since BAG was originally described as an inhibitor of mucin glycosylation in the Golgi [62], which is not catalyzed by OGT, it lacks specificity. Nevertheless, the link between hyperglycemia, O-GlcNAc and increased angiostatin II synthesis is supported by the fact that in others tissues both glucosamine and hyperglycemia have been shown to increase angiostatinogen gene expression [63].

In addition to inducing apoptosis angiostatin II also stimulates cardiomyocyte growth and hypertrophy, via G\(_s\)-coupled protein complex and activation of PLC. In neonatal cardiomyocytes hyperglycemia attenuated angiostatin II and phenylephrine induced hypertrophy [64]. This was associated with a reduction in agonist-induced increase in cytosolic Ca\(^{2+}\) and the nuclear translocation of the transcription factor, NFAT (nuclear factor of activated T-cells), which plays a critical role in regulating cardiomyocyte hypertrophy. The effects of hyperglycemia were attenuated by inhibiting GFAT, thus demonstrating the involvement of increased HBP flux [64]. Glucosamine also blunted the hypertrophic response to angiostatin II and phenylephrine as well as the increase in cytosolic Ca\(^{2+}\) and NFAT translocation [65]; however, glucosamine did not inhibit Ca\(^{2+}\)-independent hypertrophy induced by constitutive activation of MEK1. It was subsequently demonstrated that the attenuation of angiostatin II induced increase in cytosolic Ca\(^{2+}\) by glucosamine was mediated via increased O-GlcNAc levels [66].

It is also noteworthy that in cell culture studies increased levels of O-GlcNAc interfered with cell growth and division, which was associated with altered cyclin expression [67]. Although cardiomyocytes are generally considered to be terminally differentiated, D-type cyclins are critical regulators of cardiac hypertrophy [68]. Thus, the effect of O-GlcNAc on cyclin expression, raises the possibility that increased cardiomyocyte O-GlcNAc levels could impair cyclin-mediated hypertrophic signaling pathways, thereby contributing to the adverse response to hypertrophic stimuli.
Since hypertrophy is a significant risk factor for heart failure it may not be obvious why an impaired response to hypertrophic stimuli could be detrimental. However, the initial hypertrophic response is typically considered to be a beneficial adaptive response to the increased hemodynamic demand; it is only at a later stage that these changes become maladaptive leading to decompensation and heart failure. Thus, if the initial adaptive hypertrophic response to increased demand is blocked this may accelerate the progression to failure. This could account for the more rapid onset of heart failure seen in diabetic patients following myocardial infarction [69,70] and may also contribute to the poor prognosis of patients with both diabetes and hypertension [71].

4.3. O-GlcNAc and inflammatory response and myocardial fibrosis

Myocardial fibrosis plays an important role in the development of diabetic cardiomyopathy [51]. There is no direct data linking this to increased O-GlcNAc levels; however, in rat mesangial cells increased flux through the HBP lead to NF-κβ promoter activation, and increased expression of inflammatory cytokines which may contribute to glomerulosclerosis [72]. Augmentation of O-GlcNAc levels by glucosamine or PUGNAc administration in human neutrophil leukocytes was shown to enhance agonist-induced chemotaxis and to stimulate chemotaxis in the absence of agonist [73]. These data suggest that elevated O-GlcNAc levels could contribute to the increased inflammation and myocardial fibrosis seen with diabetes.

4.4. HBP and O-GlcNAc and vascular dysfunction

Following balloon-injury there is marked increased expression of GLUT1 and GLUT4 in the neointima [74]. Overexpression of GLUT1 in vascular smooth muscle cells (VSMC) also significantly increased glucose uptake and decreased apoptosis [74], providing a potential link between increased VSMC glucose metabolism and the increased risk of restenosis and atherosclerosis in patients with diabetes. The effects of increased GLUT1 expression were mediated by GSK-3β [74], which has been reported to be a target for OGT [75]. The effect of O-GlcNAc on cell growth and division may also contribute to increased VSMC proliferation seen in diabetes [67].

Nitric oxide is critical for normal endothelial function and in cultured endothelial cells hyperglycemia inhibited eNOS activity and increased O-GlcNAc modification of the enzyme with a reciprocal decrease in phosphorylation [23]; this was blocked by GFAT inhibition suggesting that flux through HBP was a contributing factor. These data provide a possible mechanism underlying impaired endothelial dependent vasodilatation in patients with diabetes [76,77]. Furthermore, in human coronary endothelial cells increased O-GlcNAc levels impaired activation of the metabolic branch of the insulin signaling pathway (i.e. IRS/Pi3K/Akt) but enhanced the mitogenic branch (i.e., ERK1/2 and p38) [47]. Phosphorylation of eNOS by Akt was also attenuated by both hyperglycemia and glucosamine; hyperglycemia also increased expression of MMP-2 and MMP-9 and decreased TIMP-3 and this was prevented by inhibition of the HBP. Since MMPs and TIMPs play a critical role in the development of macrovascular disease [78], the inhibition of eNOS by O-GlcNAc may contribute to the increased incidence of atherosclerosis in diabetic patients. Consistent with this, carotid plaques from patients with Type-2 diabetes showed increased levels of endothelial O-GlcNAc compared to patients without diabetes and a more than 6-fold increase in O-GlcNAc in the cap plaque [47].

Increased expression of the plasminogen activator inhibitor-1 (PAI-1) has been implicated in increased cardiovascular disease seen with diabetes [79]. Du et al., [16] demonstrated that hyperglycemia-induced expression of PAI-1 in endothelial cells was associated with increased O-GlcNAc levels on Sp1 and that when the Sp1 binding sites on the PAI-1 promoter were mutated hyperglycemia had no effect on PAI-1 expression. A more direct link between O-GlcNAc and regulation of PAI-1 expression was subsequently provided by Goldberg et al., in glomerular mesangial cells [80]. Hyperglycemia and increased HBP flux also increases TGF-β1 expression [16,81,82], which may also be mediated by Sp1. TGF-β1 is a potent stimulator for the synthesis of proteoglycans and extracellular matrix proteins and has been implicated in mediating vascular smooth muscle cells and fibroblast proliferation [83].

5. HBP and O-GlcNAc and cell survival

Most of our understanding of the effects of HBP and O-GlcNAc on cellular function is in the context of nutrient excess and the adverse effects of insulin resistance and diabetes. However, the fact that OGT gene deletion is embryonically lethal demonstrates that O-GlcNAc is required for normal cell function [21]. Furthermore, a variety of stress stimuli increased the levels of protein O-GlcNAc in mammalian cells, suggesting that acute activation of the pathways leading to O-GlcNAc formation was an endogenous stress response [84]. Importantly, decreasing OGT expression blunted this response and decreased cell survival in response to stress, while increased levels of O-GlcNAc with PUGNAc protected cells against the same stress [84]. Others reported that inhibition of GFAT decreased O-GlcNAc levels and reduced cell survival following heat stress [85]. In the isolated perfused heart, glucosamine increased cardiac O-GlcNAc levels 3-fold and protected the heart against injury resulting from the calcium paradox and ischemia/reperfusion [57]. Glucosamine cardioprotection could be mediated via mechanisms independent of its effect on O-GlcNAc; however, alloxan an inhibitor of OGT, blocked the protection associated with glucosamine and prevented the increase in O-GlcNAc levels [57]. Furthermore, we have also shown...
in isolated cardiomyocytes that both glucosamine and PUGNAc have a similar protective effect against hypoxia and reoxygenation stress [86]. Glucosamine treatment also improved cardiac function in vivo in a rat model of trauma-hemorrhage and this was associated with increased cardiac O-GlcNAc levels compared to untreated controls [87].

While the specific mechanisms underlying the cardioprotection associated with increased O-GlcNAc levels remain to be determined there is evidence to suggest that this could be mediated at least in part via increased Hsp70 expression [84]. However, in the isolated heart protection was conferred following only 15 min treatment with glucosamine suggesting [57] that de novo protein synthesis is not required for protection. The fact that elevated O-GlcNAc levels attenuate increases in cytosolic Ca$^{2+}$ suggests that protection could also be due to the inhibition of calcium influx into the cell [66,86]. Increased levels of O-GlcNAc also result in the inhibition of the proteasome [88] and this could also contribute to increased cell survival.

One explanation for the potential contradiction between the beneficial effects of increased O-GlcNAcylation and the adverse effects described earlier could be that the protection is observed in response to acute increases in O-GlcNAc levels, whereas adverse effects are seen in response to chronic activation. For example, target proteins may be different in response to a short-term increase in O-GlcNAc relative to a sustained increase in flux through OGT. However, to date there is no information regarding the temporal relationship between increased OGT flux and target protein specificity. An alternative explanation is the concept of allostatics, which suggests that the initial biological response to an acute stress is the activation of processes that are protective and improve survival; however, as the allostatic load increases and the stress becomes more frequent or continuous, activation of the same pathways results in the development of pathophysiology [89]. Thus, if increased O-GlcNAcylation is an acute survival response, the development of pathophysiology may be a consequence of chronic activation as seen in metabolic disease. Furthermore, as described by Zachara and Hart [4] an excessive elevation of O-GlcNAc may induce apoptosis; consequently, stress induced increase in O-GlcNAc on top of an already elevated level of O-GlcNAc may lead to cell death.

6. Conclusions

Although diabetes is clearly an important risk factor for the development of cardiac dysfunction and contractile failure, there continues to be a lack of consensus as to whether this is due to primary defects at the cardiomyocyte level or is secondary to hypertension and increased vascular disease [1]. However, as discussed above experimental studies have shown that diabetes leads to impaired cardiomyocyte function and increased cardiomyocyte apoptosis both of which appear to be mediated by increased O-GlcNAc levels on nucleocyttoplasmic proteins. Furthermore, elevated O-GlcNAc levels impair hypertrophic signaling pathways and also alter cyclin expression both of which could contribute to adverse responses to increased hemodynamic demand such as hypertension. In addition, there is evidence to suggest that increased O-GlcNAc levels may be associated with increased vascular cell proliferation, endothelial and vascular cell function as well as increased fibrosis and inflammation. Thus, chronically elevated levels of O-GlcNAc may represent a common mechanism underlying the adverse effects of diabetes on cardiomyocyte, vascular and endothelial cell function, thereby contributing to both primary and secondary factors associated with diabetic cardiomyopathy. Consequently, strategies targeted towards a reduction of O-GlcNAc levels in cardiac and vascular tissues could represent novel therapeutic approaches for the treatment of diabetic patients with cardiovascular disease. However, such approaches may be complicated by the fact that low levels of O-GlcNAc may also have adverse effects [4].

It is important to note that despite the accretion of experimental data demonstrating the role of O-GlcNAc in mediating the effects of diabetes on the cardiovascular system there is as yet little clinical data to link increased O-GlcNAc levels to cardiovascular complication in diabetic patients. However, what limited data are available [47] are consistent with experimental studies. Clearly, further studies into the regulation of cytosolic and nuclear O-glycosylation in the heart, under both normal and pathophysiological conditions, as well as in experimental and clinical settings, are essential to better understand the role of this post-translational modification in the regulation of the cardiovascular system.

Acknowledgements

This work was supported in part by research grants from NIH HL067464 and HL079364 (JCC); HL076165 (RBM) and SCCOR grant HL077100.

References


