Published in final edited form as:
Curr Signal Transduct Ther. 2010 January ; 5(1): 49–59.

Protein O-GlcNAcylation: A critical regulator of the cellular response to stress

John C. Chatham[1,2,*] and **Richard B. Marchase**[2]

[1]Department of Medicine, Division of Cardiovascular Disease, Center for Free Radical Biology, Center for Aging and Clinical Nutrition Research Center, University of Alabama at Birmingham, Birmingham, AL.

[2]Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL.

Abstract

The post-translational modification of serine and threonine residues of nuclear and cytoplasmic proteins by the O-linked attachment of the monosaccharide ß-N-acetyl-glucosamine (O-GlcNAc) is a highly dynamic and ubiquitous protein modification that plays a critical role in regulating numerous biological processes. Much of our understanding of the mechanisms underlying the role of O-GlcNAc on cellular function has been in the context of chronic disease processes. However, there is increasing evidence that O-GlcNAc levels are increased in response to stress and that acute augmentation of this response is cytoprotective, at least in the short term. Conversely, a reduction in O-GlcNAc levels appears to be associated with decreased cell survival in response to an acute stress. Here we summarize our current understanding of protein O-GlcNAcylation on the cellular response to stress and in mediating cellular protective mechanisms focusing primarily on the cardiovascular system as an example. We consider the potential link between O-GlcNAcylation and cardiomyocyte calcium homeostasis and explore the parallels between O-GlcNAc signaling and redox signaling. We also discuss the apparent paradox between the reported adverse effects of increased O-GlcNAcylation with its recently reported role in mediating cell survival mechanisms.

Keywords

Hexosamine biosynthesis; protein O-glycosylation; capacitative calcium entry (CCE); oxidative stress; heart; ischemia

Introduction

The post-translational modification (PTM) of proteins is a common mechanism for regulating protein function, location and turnover. Protein phosphorylation is probably the most widely studied form of PTM; however there are many other PTMs, including acylation, ubiquitylation, methylation, acetylation, thiolation, nitration, and glycosylation [1]. The PTM of serine and threonine residues of nuclear and cytoplasmic proteins by O-linked ß-N-acetylglucosamine (O-GlcNAc), first identified in 1984 [2], is increasing recognized as a highly dynamic PTM that contributes to the regulation of numerous biological processes, including nuclear transport, transcription and translation, cytoskeletal organization, signal transduction, proteasomal function and cell survival [3-14]. The

[*]**Corresponding Author:** John C. Chatham, University of Alabama at Birmingham, Department of Medicine, 703 19th Street South, ZRB 302, Birmingham, AL 35294-0007. Telephone: (205) 934-0240; Fax: (205) 996-6960; jchatham@uab.edu.

majority of our understanding of the contribution of altered O-GlcNAcylation to pathophysiology is in the context of diseases such as aging [15-17], cancer [18-20], neurodegenerative disorders [6, 10, 21, 22] and diabetes and diabetic complications [23, 24]. Glucose metabolism via the hexosamine biosynthesis pathway (HBP) is essential for the synthesis of O-GlcNAc and there is considerable evidence supporting a role for sustained increases of O-GlcNAc in contributing to glucose toxicity and insulin resistance [23, 24].

O-GlcNAc modified proteins have been found in all metazoans studied thus far [25] and O-GlcNAc transferase (OGT), which catalyzes the final step in O-GlcNAc synthesis, has been identified in plants, human, rat, mouse, and the nematode Caenorhabditis elegans [26]. Given that the genes for OGT are highly conserved [27] and that OGT gene deletion is embryonically lethal [28], O-GlcNAc and the pathways involved in regulating O-GlcNAc levels must provide some evolutionary advantage to cells and organisms. Substantive evidence of this was first reported in 2004, when O-GlcNAc levels were shown to increase in response to a diverse array of stress stimuli, and inhibition of this response resulted in reduced cell survival [29]. Conversely, augmentation of O-GlcNAc levels conferred increased tolerance to stress. Subsequently, there have been a number of studies that have examined the role of this pathway in mediating the cell survival response particularly in the setting of the cardiovascular system. Thus, 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. In addition we will explore the apparent paradox between the beneficial pro-survival aspects of increased O-GlcNAc levels and the adverse effects associated with pathophysiological conditions such as diabetes.

Stress, the hexosamine biosynthesis pathway and O-GlcNAcylation

Cells sense stress primarily by two pathways: 1) damage to macromolecules and 2) changes in cellular redox potential, including an increase in reactive oxygen species (ROS) [30]. ROS frequently modify protein function by oxidation of susceptible cysteine residues [31]; within the glucose metabolic pathway, the activity of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), is particularly sensitive to inhibition by ROS and it has been proposed to play a critical role as a sensor of intracellular oxidative stress [32]. GAPDH plays a regulatory role for the entry of glucose into glycolysis and oxidative phosphorylation; thus, its inhibition results in glucose units being channeled into other pathways, such as the pentose phosphate pathway and, of particular relevance here the HBP. Another common stress response is activation of glucose metabolizing pathways; at the cellular level this is reflected in increased glycogenolysis in response to an ischemic or hypoxic stress. At the organismal level this is seen as stress-induced hyperglycemic state, which occurs rapidly in response to severe injury including surgical trauma, hemorrhage, shock, sepsis and burns. In both cases this increase in glucose utilization is typically thought to be a valuable survival response, either by rapidly increasing available fuel for energy production or by facilitating mobilization of interstitial fluid reserves by increasing osmolarity [33-39]. However, the increased intracellular availability of glucose also provides additional substrate for the HBP, and if combined with ROS-mediated inhibition of GAPDH [40], would result in a further increase in flux through the HBP.

The HBP is estimated to consumes ~2-5% of the total glucose entering the cell [41]. It should be noted however, that this is based on a single study in cultured adipocytes, and a quantitative assessment of glucose flux through this pathway has yet to be determined in any intact organ system. The enzyme glutamine-fructose-6-phosphate transaminase (EC 2.6.1.16), also know as L-glutamine-D-fructose 6-phosphate amidotransferase (GFAT), regulates the entry of glucose into the HBP and converts fructose 6-phosphate to glucosamine 6-phosphate with glutamine as the amine donor [42]. Uridine-diphosphate-N-acetylglucosamine (UDP-GlcNAc) is the primary end product of the HBP and is a substrate

for multiple glycosylation reactions resulting in the synthesis of glycoproteins, glycolipids and proteoglycans. UDP-GlcNAc is also the essential sugar nucleotide donor for the synthesis of O-GlcNAc modified proteins catalyzed by a unique glycosyl transferase - UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase (OGT, O-GlcNAc transferase) [43, 44]. OGT is distinct from all other protein glycosyltransferases because it is exists in the nucleocytoplasmic compartment, not the secretory pathway, and it catalyzes the formation of a reversible attachment of GlcNAc via an O-linkage to specific serine and threonine residues on numerous nuclear and cytoplasmic protein [2, 45, 46].

Our understanding of the regulation of OGT activity is complex and incomplete; however, its activity is reported to be sensitive to changes in UDP-GlcNAc concentration [47]. Thus, a stress-induced increase in glucose metabolism via the HBP will lead to an increase in UDP-GlcNAc levels, which in turn will increase the flux through OGT and lead to an elevation of O-GlcNAc levels on nuclear and cytoplasmic proteins [48]. Hypoxic stress has also been shown to increase GFAT expression [49], which would potentially further increase glucose entry into the HBP. However, it should be noted that our understanding of the acute regulation of GFAT activity is remarkably limited. There two isoforms - GFAT1 and GFAT2 – and both are subject to phosphorylation by cAMP-dependent protein kinase. However, phosphorylation of GFAT1 decreases its activity [50], whereas phosphorylation of GFAT2 leads to increased activity [51]; the importance of the differential effects of phosphorylation on GFAT activity is unclear. Some studies indicate that GFAT expression and HBP flux in the heart are subject to regulation in response to chronic stress [17, 52]. Acute ischemic stress also increases UDP-GlcNAc [48, 53]; however, the relative importance of increased substrate availability compared to increased GFAT activity to overall HBP flux is unknown.

In addition to the HBP, GFAT, UDP-GlcNAc and OGT, the level of protein OGlcNAcylation is also regulated by a ß-N-acetylglucosaminidase (O-GlcNAcase; also known as meningioma expressed antigen 5 (mgea5) and nuclear cytoplasmic O-GlcNAcase and acetyltransferase (NCOAT)), which catalyzes the removal of the sugar moiety from the proteins [54, 55]. Like OGT, O-GlcNAcase is also highly conserved in mammals, and is expressed in all tissue types examined with a similar tissue distribution to OGT [55]. O-GlcNAcase activity is specific for N-acetyl-ß-D-glucosaminides and unlike lysosomal hexosaminidases has a pH optimum near neutral [54, 55]. The catalytic domain of O-GlcNAcase is in the N-terminus [56], while the C-terminus has been reported in vitro to have a histone acetyl-transferase activity [57]. Acute inhibition of O-GlcNAcase leads to relatively rapid increases in overall O-GlcNAc levels, which are associated with increased tolerance to stress; however, little is known about effects of ischemic or oxidative stress on O-GlcNAcase activity. It has been shown that O-GlcNAcase contains a caspase-3 cleavage site, and thus it may be regulated in response to apoptosis [58]; however, this may have other regulatory roles such as stabilization, localization, targeting, since enzyme activity is unaffected by cleavage [57-59].

Thus, while O-GlcNAcylation is essential for cell survival and activation of pathways leading to O-GlcNAc synthesis are associated with increased tolerance to stress, the mechanism(s) involved in regulating O-GlcNAc cycling have not been well defined. Furthermore, our knowledge regarding the regulation of GFAT, OGT and O-GlcNAcase activities under either normal physiological conditions or in response to stress remain surprisingly limited.

Hexosamine biosynthesis, O-GlcNAcylation and cell survival

Even though our understanding of the regulatory mechanisms involved in mediating O-GlcNAc levels is limited, there is a relatively extensive literature implicating increased O-

GlcNAcylation as a mediator of the effects of a range of chronic disease states including insulin resistance, diabetes; cancer and neurodegenerative disorders, including Alzheimer's disease [6, 10, 18, 19, 21, 57, 60-70]. However, in 2004 it was demonstrated for the first time that activation of pathways leading to increased O-GlcNAc levels appeared to be an endogenous stress response that was linked to cell survival [29]. Since OGT catalytic activity is highly sensitive to changes in UDP-GlcNAc concentrations [47], an acute increase in UDP-GlcNAc concentrations should be an effective means for increasing O-GlcNAc levels. One approach to acutely increase UDP-GlcNAc levels is the addition of exogenous glucosamine, which is readily transported into cells via the glucose transporter system and subsequently phosphorylated to glucosamine-6-phosphate by hexokinase. Thus, glucosamine bypasses GFAT, which regulates glucose entry into the HBP, resulting in a rapid increase in UDP-GlcNAc levels [71].

Therefore, we postulated that acute pre-treatment of the isolated perfused heart with glucosamine, would lead to increased O-GlcNAc levels and that this would protect the heart against ischemic injury. Perfusion of an isolated rat heart with 5mM glucosamine resulted in a marked increase in O-GlcNAc levels in the heart within 10 min [72], and glucosamine treated hearts exhibited significantly improved functional recovery following ischemia reperfusion compared to untreated hearts [72]. Alloxan, which inhibits OGT [73], both prevented the increase in O-GlcNAc and blocked the protection afforded by glucosamine treatment [72]. While alloxan has significant limitations as an inhibitor of OGT [74], at the time of these studies no other inhibitors were available. Nevertheless, this was the first report that supported the notion that acutely activating the pathways leading to O-GlcNAc synthesis could be an effective cardioprotective strategy. At the cellular level the tolerance of cardiomyocytes to hypoxia and reoxygenation was also improved by increasing O-GlcNAc levels with glucosamine or by increasing glucose concentration [75]. Importantly, the protection observed with high glucose was prevented by inhibiting GFAT, as was the associated increase in O-GlcNAc, demonstrating the role of the HBP in mediating this response. Interestingly, in untreated cardiomyocytes, there was a marked increase in O-GlcNAc early during the reoxygenation period, which was abolished in the absence of glucose [75]. This lends further support to the importance of glucose metabolism via the HBP in mediating the stress induced increase in O-GlcNAc.

Another commonly used approach for increasing O-GlcNAc levels independent of the HBP is to prevent removal of O-GlcNAc from proteins by inhibiting O-GlcNAcase. PUGNAc [O-(2-acetamido-2-deoxy-d-glucopyranosylidene)amino-N-phenylcarbamate] is an O-GlcNAc analog that is a competitive inhibitor of O-GlcNAcase and has been widely used to increase O-GlcNAc levels [76, 77]. PUGNAc treatment had a similar effect on improving cardiomyocyte viability and reducing necrosis as glucosamine treatment [75]. These cardiomyocyte studies confirmed the findings from the isolated heart, demonstrating that increasing O-GlcNAc levels before the ischemic or hypoxic stress enhanced the tolerance to that stress. However, cardioprotective treatment strategies that have been shown to be effective when used prior to ischemia often prove to be ineffective when used only during reperfusion [78]. Therefore, we examined the effectiveness of both glucosamine and PUGNAc in mediating functional recovery when they were administered only during reperfusion [79]. Following 20min of zero-flow ischemia hearts were reperfused for 60 min with no additional treatment or with the addition of glucosamine or PUGNAc for the first 20 min of reperfusion. Both glucosamine and PUGNAc significantly improved functional recovery and decreased tissue injury as indicated by lower cardiac troponin I (cTnI) release during reperfusion [79]. Of note there was a significant correlation between increased O-GlcNAc levels and lower cTnI release further supporting a link between O-GlcNAcylation and improved tolerance to ischemia reperfusion injury [79]. Furthermore the fact that both glucosamine and PUGNAc, which increase O-GlcNAc levels by entirely different

mechanisms, provided similar protection against ischemic/hypoxic injury, lends credence to the notion that the increase in O-GlcNAcylation plays a causative role in mediating the protective response.

Jones et al. demonstrated that administration of PUGNAc in vivo significantly reduced infarct size following ischemia/reperfusion in mice [80]. This indicated that the protection associated with augmentation of O-GlcNAc levels was not limited to the in vitro models described above, but was also effective in more clinically relevant in vivo model of myocardial infarction. Interestingly they also reported that ischemic preconditioning, a widely studied and powerful protective strategy, was also a potent stimulus for increasing O-GlcNAcylation. They also demonstrated that PUGNAc and ischemic preconditioning reduced infarct size to a similar extent [80]. These observations raise the intriguing possibility that activation of O-GlcNAc formation may be one mechanism underlying ischemic preconditioning; however, this hypothesis has yet to tested.

Further evidence that in vivo augmentation of O-GlcNAc levels afforded protection was provided by Yang et al. [81], who demonstrated that administration of glucosamine during resuscitation following trauma-hemorrhage improved cardiac output 2-fold compared to vehicle-treated controls. They also found that glucosamine treatment improved perfusion of various organs systems, including kidney and brain, and attenuated the trauma-hemorrhage-induced increase in serum levels of the inflammatory cytokines IL-6 and TNF-α. Similarly, PUGNAc administration following trauma-hemorrhage also improved cardiac output, decreased total peripheral resistance and increased perfusion of critical organs systems compared to vehicle treated controls [82]. Similar to glucosamine, PUGNAc also attenuated the increase in plasma IL-6 and TNFα levels. Recent studies have demonstrated that both PUGNAc and glucosamine significantly increased 24 hr survival rates in a model of severe trauma hemorrhage and resuscitation. This was associated with decreased inflammatory cytokines and reduced liver injury, particularly in the PUGNAc treated group [83]. Both glucosamine and PUGNAc treatment significantly increased O-GlcNAc levels in multiple tissues compared to the untreated trauma-hemorrhage groups. Surprisingly, however, trauma-hemorrhage and resuscitation resulted in a marked loss of overall O-GlcNAcylation compared to sham surgery control group and treatment with glucosamine or PUGNAc appeared to prevent this loss of O-GlcNAcylation rather than increase levels above normal [82]. The trauma-hemorrhage and resuscitation induced loss of O-GlcNAcylation was present even 24hrs after resuscitation [83]. It is not known whether this reduction in overall O-GlcNAc levels is a result of decreased synthesis due to decreased flux through either GFAT or OGT or is a consequence of increased O-GlcNAcase activity resulting in increased degradation. These results highlight the need for a better understanding of mechanisms underlying the normal regulation of O-GlcNAc turnover and how stressors such as ischemia and reperfusion, hypoxia and reoxygenation or hemorrhagic shock and resuscitation affect the regulation of O-GlcNAc turnover.

The fact that both glucosamine and PUGNAc have similar protective effects at the cellular, isolated organ and whole animal level provide strong support for the fundamental concept that this protection is mediated via increased O-GlcNAcylation. However, glucosamine can modulate a number of pathways other than O-GlcNAc such as ganglioside [84] or cell surface *N*-glycan [85] synthesis and PUGNAc also inhibits other ß-hexosaminidases [86-88], which may alter processing of glycoconjugates in addition to O-GlcNAc. To provide a more definitive cause and effect relationship we used gain and loss of function studies in neonatal cardiomyocytes. We found that both glucosamine and OGT overexpression significantly increased *O*-GlcNAc levels and decreased hypoxia/reoxygenation injury to a similar extent [89]. Both interventions also attenuated the loss of mitochondrial membrane potential and the loss of mitochondrial cytochrome C induced by

acute oxidative stress [89]. Conversely, decreased OGT expression attenuated the ischemia-induced increase in O-GlcNAc, and this was associated with increased necrosis, greater loss of cytochrome C and increased apoptosis following ischemia/reperfusion [89]. Others have also demonstrated that increasing or decreasing OGT levels increased or decreased cardiomyocyte survival respectively [90]. Lowering O-GlcNAc levels by overexpressing O-GlcNAcase also enhanced the sensitivity of cardiomyocytes to hypoxia/reoxygenation [91]. Taken together these studies demonstrate that strategies that augment O-GlcNAc levels are clearly pro-survival, whereas those that decrease O-GlcNAc levels reduce cell survival. In addition, it should be noted that a number of studies have reported potentially adverse effects of glucosamine including growth inhibition, cell cycle arrest and apoptosis, which may be related to effects other than increased O-GlcNAc levels [84, 92, 93]. However, in the setting of acute stress in cardiomyocytes we have typically found that glucosamine and OGT overexpression have remarkably similar protective effects [89, 94], suggesting that under such conditions the effect of glucosamine is mediated primarily via increased flux through OGT.

Although PUGNAc has been widely used pharmacological tool for increase O-GlcNAc levels via its inhibition of O-GlcNAcase. However, while PUGNAc, an O-GlcNAc analog. which is a potent competitive inhibitor of O-GlcNAcase, is not a selective O-GlcNAcase inhibitor (K_I=0.046 μM) as it also inhibits other glycoside hydrolases such as the lysosomal ß-hexosamindases (K_I=0.036 μM) and α-N-acetylglucosaminidases (K_I=6 μM) [88]. Such off target effects has the potential to complicate the interpretation of results obtained with PUGANc However, Macauley and coworkers [88], have recently developed novel O-GlcNAcase inhibitors, analogous derivatives of the parent NAG-thiazoline (1,2 dideoxy-2'-methyl-α-D-glycopyranoso-[2,1-d]-Δ2'-thiazoline), which have been shown to be good transition state mimics for the substrate-assisted catalytic mechanism used by O-GlcNAcase and equally effective but more selective inhibitors of eukaryotic O-GlcNAcase over the functionally related lysosomal ß-hexosamindases than PUGNAc. For example, NAG-Bt (1,2 dideoxy-2'-propyl-α-D-glycopyranoso-[2,1-d]-Δ2'-thiazoline) was found to be a potent O-GlcNAcase inhibitor (K_I=0.23 μM) and highly selective over the ß-hexosamindases (K_I=340 μM) with a ~1500-fold greater specificity than PUGNAc [88]. More recently van Aalten and colleagues have described other O-GlcNAcase inhibitors, "GlcNAcstatins", which they have reported to be effective in the nanomolar range [95, 96]. The availability of such highly specific and effective O-GlcNAcase inhibitors will be especially valuable tools for improving our understanding of the role of O-GlcNAcylation in mediating cell function. On the other hand, while the more specific NAG-thiazolidine O-GlcNAcase inhibitor, NAG-Bt has been reported to have different effects of cell function than PUGNAc [97], in our studies we have found that PUGNAc and NAG-Bt to have similar protective effects against ischemia/reperfusion injury in both cardiomyocytes [75, 89] and the perfused heart [79, 98].

Mechanisms associated with O-GlcNAc mediated cell survival

A number of putative mechanisms have been proposed to account for the increased tolerance to stress and improved survival associated with increasing O-GlcNAcylation. Increased transcription of heat shock protein (HSP) 40 and HSP70 were associated with elevated O-GlcNAc levels, and HSP70 is known to be target for O-GlcNAc modification [99, 100]. Although we found no change in HSP70 expression in cardiomyocytes following glucosamine treatment [75], PUGNAc treatment resulted in augmented HSP70 levels in response to oxidative stress [80]. On the other hand Sohn et al., [101] found that O-GlcNAc mediated protection was not dependent on changes in HSP110, HSP90, HSP70 or HSP27 expression levels. The activity of the transcription factor Sp1 also increases when modified by O-GlcNAc [40, 102] and this may contribute to the increased synthesis of heat shock proteins [103] as well as pro-survival Bcl-2 family members [104].

However, in the isolated perfused heart, cardioprotection is conferred after a very brief (5 min) exposure to glucosamine [48, 72], suggesting that O-GlcNAc-related protection does not necessarily depends on de novo protein synthesis. Activation of O-GlcNAc formation attenuates the mPTP opening, a critical step in the initiation of apoptosis and cell death [80, 89, 90, 105]. Glucosamine, OGT overexpression and O-GlcNAcase inhibition all attenuate H_2O_2-induced loss of mitochondrial membrane potential and cyctochrome *c* release [89]. We found that this was associated with increased mitochondrial Bcl-2 levels; Bcl-2 inhibits the mitochondrial permeability transition pore (mPTP) opening possibly by direct interaction with VDAC (voltage dependant anion channel), one of the putative components of mPTP [106, 107]. Interestingly, cardiac mitochondria isolated from PUGNAc-treated mice and also OGT overexpressing neonatal rat cardiomyocytes are resistant to the Ca^{2+}-induced mPTP formation, whereas inhibition of OGT increased sensitivity to Ca^{2+}-induced mitochondrial swelling [80, 90, 105]. One of the O-GlcNAc modified proteins identified as a potential O-GlcNAc target is VDAC, and it has been hypothesized this modification of VDAC may preserve the mitochondrial integrity by interfering with mPTP formation [80].

Glucosamine-induced increase in O-GlcNAc levels also attenuated the ischemia-induced increase in p38 mitogen activated protein kinase (MAPK) phosphorylation, decreased ischemic contracture and reduced incidence of reperfusion-induced arrhythmias [48]. Paradoxically, at the end of reperfusion phosphorylated p38 levels were increased in response to glucosamine treatment. While p38 activation is frequently considered to be pro-apoptotic, it can also lead to activation of pro-survival pathways through downstream effectors, such as αB-crystallin and HSP27, both of which have been shown to play a role in ischemic protection [108-112] and are also targets for O-GlcNAc modification [10, 113, 114]. Preliminary studies have also shown that ischemia/reperfusion alters the level of O-GlcNAc modification of glycogen phosphorylase b, mitochondrial aconitase 2 and the cytoskeletal protein vinculin [53]. It remains to be determined whether changes in O-GlcNAcylation of these proteins alter the functional response to ischemia/reperfusion injury. Increased levels of O-GlcNAc have also been reported to inhibit protein degradation [5, 14], most likely due to inhibition of the proteasome [115], and this could contribute to enhanced cell survival.

Inflammation also plays an important role in the evolution of tissue injury in vivo, and a number of studies have demonstrated that acute increases in O-GlcNAc attenuate the inflammatory response induced by tissue injury and stress [81-83, 116-118]. Glucosamine and PUGNAc both decreased serum levels of the inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-6 following trauma-hemorrhage and resuscitation [81, 82]. Glucosamine treatment also significantly reduced the trauma-hemorrhage-induced increase in Inter-Cellular Adhesion Molecule (ICAM)-1 expression, IκB-α (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha) phosphorylation, Nuclear factor-kB (NF-κB) expression and NF-κB DNA binding activity in the heart [116], suggesting that elevated O-GlcNAc levels may contribute to the down-regulation of the NF-κB pathway. Glucosamine-induced increase in O-GlcNAc levels in rat aortic smooth muscle cells was shown to inhibit tumor necrosis factor TNF-α stimulated chemokine and adhesion molecule (ICAM-1, vascular cell adhesion molecule (VCAM)-1) expression, IκB-α phosphorylation and NF-κB activation [117]. In isolated cardiomyocytes, glucosamine and OGT overexpression increased O-GlcNAc levels, attenuated lipopolysaccharide (LPS)-induced TNF-α and ICAM-1 expression and decreased IκB-α phosphorylation and nuclear NF-κB levels [119]. Conversely, knockdown of OGT decreased O-GlcNAc levels and enhanced the LPS-induced increase in IκB-α phosphorylation [119]. Glucosamine also increased O-GlcNAc levels in macrophages and attenuated NF-κB activation and iNOS expression[119]. Together, these results demonstrate that modulation of cellular O-GlcNAc signaling events alters NF-κB activation, indicating therefore that attenuation of NF-κB

signaling may be a contributing factor to the protection associated with increased protein O-GlcNAc levels in vivo.

Thus, it is becoming increasingly evident that the protection associated with activation O-GlcNAcylation is multifactorial. Initial acute protection will likely involve transcriptionally independent mechanisms resulting from direct O-GlcNAc modification of proteins leading to changes in protein-protein interactions, modulation of stress-activated kinase pathways, activation of pro-survival pathways or inhibition of pro-apoptotic pathways. Longer-term cellular protection may be facilitated via increased transcription of pro-survival factors such as HSPs mediated in part by increased O-GlcNAcylation of transcription factors such as Sp1. The O-GlcNAc mediated attenuation of NF-κB signaling, indicates that augmentation of O-GlcNAc levels in vivo, may exert a protective effect not only at the level of the heart and other parenchymal tissues but also by attenuation of inflammatory responses in circulating mediators such as macrophages.

O-GlcNAcylation and calcium homeostasis

Maintenance of calcium homeostasis is critical for both normal cell signaling as well as for cell survival. When the concentration of cytoplasmic free Ca^{2+} ($[Ca^{2+}]_i$) is excessive, Ca^{2+} overload occurs and cell death rapidly ensue. Thus, primary adaptations that likely evolved in response to stress are mechanisms designed to suppress Ca^{2+} overload. There are two general mechanisms that regulate cellular calcium homeostasis: voltage gated and non-voltage gated calcium entry. Voltage gated calcium channels are characteristic of excitable cells such as cardiomyocytes where they are activated by membrane depolarization, which leads to an increase in $[Ca^{2+}]_i$ which in the case of cardiomyocytes is responsible for muscle contraction. Non-voltage gated calcium channels are more commonly associated with non-excitable cells and are typically associated with agonist induced increase in $[Ca^{2+}]_I$ leading to the activation of a wide range of calcium-mediated signaling pathways. One such non-voltage gated calcium entry pathway is capacitative calcium entry (CCE), which refers to a Ca^{2+} influx pathway mediated by store-operated calcium channels (SOCs). Typically CCE is activated when endoplasmic (ER) or sarcoplasmic reticulum (SR) Ca^{2+} stores are depleted, usually by agonists leading to the activation of phospholipase C (PLC) and the generation of inositol-1,4,5-trisphosphate (IP_3) [120].

CCE characterizes all non-excitable cells except erythrocytes [121], and has been shown to co-exist with the voltage-gated Ca^{2+} channels in smooth [122] and skeletal muscle cells [123-127]. Although the presence of CCE in the heart is under explored, there has been a gradual accretion of data from us [128-130] and more recently other investigators [131, 132] clearly demonstrating the presence of CCE in cardiomyocytes. The development of links between the HBP and CCE has also evolved over the last decade, beginning with studies of CCE in response to IP_3-generating agonists in excitable cells. In 1995, Rivera et al. [133] were among the first groups to describe a CCE pathway in smooth muscle cells. Of particular relevance, in that study a marked diminution of CCE following exposure to hyperglycemia was observed; however, the specific metabolic pathway responsible was not described. Subsequently, Vemuri and Marchase [134] showed that glucosamine inhibited CCE in the J774 macrophage cell line without affecting ATP levels. As the primary pathway of glucosamine metabolism is via the HBP, this study provided the first evidence that CCE may be regulated by HBP activity and thus by extension O-GlcNAc levels.

The first report that CCE was an important attribute in mediating cardiomyocyte function was from Hunton et al. in 2002 [128]. They showed that neonatal cardiomyocytes exhibited an increase in $[Ca^{2+}]_i$ that was dependent on endoplasmic reticulum (ER) store depletion, which was blocked by agents known to inhibit CCE in other cells, such as SKF96365 but not by inhibitors of voltage-gated channels. Treatment of neonatal cardiomyocytes with IP_3

generating agonists, such as phenylephrine and angiotensin II, lead to a release of calcium from the SR and also caused $[Ca^{2+}]_i$ to increase via a CCE-like pathway. In addition, CCE inhibitors also negated the nuclear localization of nuclear factor of activated T-cells (NFAT), which occurs in response to both phenylephrine and angiotensin II. NFAT is a transcription factor critical in regulating cardiomyocyte hypertrophy; thus, CCE inhibitors also blunted the hypertrophic response of phenylephrine and angiotensin II. Of particular relevance to this discussion, all of these responses to CCE activation were rapidly and reversibly inhibited by both glucosamine [128] and hyperglycemia [130]; importantly, the inhibition of CCE-mediated responses by hyperglycemia was blocked by inhibition of the HBP [130].

Nagy et al. subsequently demonstrated that the effects of glucosamine on CCE in cardiomyocytes were a consequence of increased O-GlcNAc levels and that the O-GlcNAcase inhibitor PUGNAc mimicked the effects of glucosamine and attenuated CCE [135]. Subsequent investigations also demonstrated that CCE was not only present in adult cardiomyocytes, but also that it was inhibited by glucosamine [129]. Additional studies in the isolated perfused heart suggested that CCE contributed to the inotropic effects of phenylephrine and angiotensin II, which were also attenuate by glucosamine as well as an independent inhibitor of CCE, SKF96365 [136]. Taken together these studies demonstrated not only a role for CCE in mediating cardiomyocyte function, but also for the regulation of CCE by the HBP and O-GlcNAcylation. While there are multiple potential calcium entry mechanisms, which could contribute to calcium overload, the fact that increased O-GlcNAcylation specifically attenuates CCE led us to postulate that CCE may be an important mediator of stress-induced Ca^{2+} overload. This is supported by our observations that activation of the HBP and increased O-GlcNAc levels not only attenuates CCE in cardiomyocytes but also protects the isolated perfused heart against Ca^{2+} overload induced by the calcium paradox [72], and blunts ischemia/reperfusion-induced activation of Ca^{2+}-mediated proteases[79]. This is further supported by reports demonstrating that in arterial myocytes hypoxia leads to store operated depletion and activates CCE [137-139]. Thus, one mechanism of protection associated with increased O-GlcNAc levels could be attenuation of the calcium overload mediated via an inhibition of CCE.

A major limitation underlying mechanistic studies into the regulation of CCE has been the lack of channel proteins responsible for mediating this process; however, there is a growing consensus that stromal interacting molecule (STIM)1 and Orai1 are essential for activation of CCE [127, 140, 141]. STIM1 contains an EF hand Ca^{2+}-binding domain that serves as the sensor of Ca^{2+} within the ER or SR. When the intra-ER Ca^{2+} level drops, rapid clustering and translocation to ER near the plasma membrane takes place, where it interacts with of the components of the CCE channel including Orai1 leading to opening of the channel. In support of our notion that this process may be regulated by O-GlcNAc, STIM1 contains a proline-serine rich domain in its cytoplasmic tail that is not only appears to be a prime site for phosphorylation but also shares characteristics with the prime acceptor site recognized by OGT for the addition of O-GlcNAc [25]. Significantly, deletion of this same serine-proline rich region prevented the ability of STIM1 to activate Ca^{2+} influx [141]. It is also of note that in preliminary experiments we have found for the first time that both STIM1 and Orai1 proteins are present in cardiomyocytes (Chatham and Marchase, unpublished data). Thus we hypothesize that an increase in cellular O-GlcNAc levels leads to increased O-GlcNAcylation of the cytoplasmic domain of STIM1, thereby preventing the necessary clustering of STIM1 and blocking the interaction with Orai 1. This prevents the formation of channels required for CCE resulting in the diminution of calcium influx seen in response to IP_3-generating agonists as well as in response to stresses such as ischemia or the calcium paradox that otherwise results in calcium overload and cell damage.

Interaction between redox and O-GlcNAc signaling

It is well established that numerous stress stimuli lead to an increase in intracellular levels of reactive oxygen species (ROS) primarily as a result of increased ROS production from mitochondria [142]. Furthermore, it has been shown that inhibition of glyceraldehyde phosphate dehydrogenase (GAPDH) by mitochondrial superoxide was an important factor leading to the increase in O-GlcNAcylation associated with hyperglycemia [40, 65]. It is also noteworthy that in the original study where O-GlcNAcylation was shown to mediate cell survival [29], the stresses used to increase O-GlcNAc levels, such as ultraviolet (UV) light cobalt chloride, ethanol, and heat shock, are all associated with increased ROS production. We also found that there was a rapid, but transient increase in O-GlcNAc levels following reoxygenation of hypoxic cardiomyocytes, which was glucose dependent [75]. Further, recent studies have shown that augmentation of O-GlcNAc levels in cardiomyocytes attenuates hydrogen peroxide-induced loss of mitochondrial membrane potential [80, 89, 90] and that this may be due to O-GlcNAcylation of mitochondrial proteins such as VDAC [80, 90]. Taken together, these studies suggest that ROS and mitochondria may contribute to the regulation of O-GlcNAc synthesis, which could in turn modulate the response to oxidative stress. It is unclear however, whether, in response to acute stress the increase in O-GlcNAc levels is an indirect consequence of ROS mediated inhibition of GAPDH [40, 65], or whether the activities of GFAT, OGT, and O-GlcNAcase may directly modified in response to ROS.

Another example of the convergence of redox and O-GlcNAc signaling is in the modulation of nitric oxide (NO). NO is widely recognized as central reactive species involved in redox signaling, and nitric oxide synthase (NOS) is subject to both phosphorylation and O-GlcNAcylation. Endothelial NOS (eNOS) is activated by Akt-mediated phosphorylation, thereby increasing NO production. This Akt phosphorylation site (Ser1177) is also subject to O-GlcNAcylation, leading to lower activity and decreased NO production [65]. Thus, changes in O-GlcNAc levels can influence eNOS activity both via direct O-GlcNAc modification as well as indirectly via O-GlcNAcylation of its upstream kinase Akt. This also leads to the notion that the interaction of redox and O-GlcNAc mediating signaling may contribute to the regulation of an entire signaling pathway. For example, insulin signaling is regulated by NO the level of which, as noted above, can be modulated by changes O-GlcNAcylation. In addition a number of components of the insulin signaling pathway, such as IR-ß, IRS1/2, PI3K, Akt, and GSK3ß are all potential targets for O-GlcNAcylation, resulting in reduced activity and decreased insulin response [143].

The recent report demonstrating O-GlcNAcylation of the transcription factor FoxO1 [144], which functions as a nutrient and stress sensor, is yet another example of the intersection between redox and O-GlcNAc signaling. One of the consequence of FoxO1 activation is increased transcription of oxidative stress response enzymes such as catalase and MnSOD [144]. Thus, an ROS induced increase in O-GlcNAc formation, could lead to O-GlcNAc-induced activation of FoxO1 thereby increasing the levels of endogenous antioxidant proteins. Taken together these studies support the notion of a role of O-GlcNAc in modulating redox signaling as well as for ROS mediated regulation of O-GlcNAc signaling.

The paradox of bad versus good O-GlcNAcylation

The majority of literature regarding the pathophysiological role of O-GlcNAcylation is as a mediating factor in various chronic disease states, including cancer, neurodegenerative diseases and diabetes [6, 10, 15-24]. These adverse effects of increased O-GlcNAcylation would appear to be at odds with the recent literature demonstrating a cytoprotective effect. One possible explanation for the apparent paradox between the adverse and beneficial effects of increased O-GlcNAcylation 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, a short-term increase in overall O-GlcNAcylation may result in different target proteins being modified compared to those that are O-GlcNAcylated in response 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. It is also possible that in chronic pathophysiological conditions, there are changes in the activity or expression levels of GFAT, OGT and O-GlcNAcase, which result in an impaired or blunted response to otherwise normal physiological or stress stimuli.

Another explanation could be provided by the concept of allostasis, 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 [145]. 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, a stress induced increase in O-GlcNAc on top of an already elevated level of O-GlcNAc may lead to cell death.

Alternatively, it is possible that the effects of O-GlcNAc modification of the same pathway could contribute to both the adverse and beneficial effects of increased O-GlcNAc synthesis. As discussed above, there is considerable evidence supporting a role for O-GlcNAc in regulating Ca^{2+} entry into the cell via CCE, specifically, for elevated O-GlcNAc levels to inhibit the activation of CCE. CCE is known to play a critical role in regulating numerous Ca^{2+} mediated cell signaling pathways, particularly in non-excitable cells, but also more recently in excitable cells including cardiomyocytes [128, 130]. Sustained inhibition of these Ca^{2+} mediated cell signaling pathways, as might occur in the setting of a chronic increase in O-GlcNAc, could blunt the response to normal physiological stimuli, which would likely contribute to impaired cellular function. However, our data also suggest that O-GlcNAc mediated inhibition of CCE also attenuates Ca^{2+} overload, which plays an important role in protection associated with ischemia/reperfusion injury. Of note, we have shown that hearts from diabetic animals were resistant to Ca^{2+}-overload [72] and also exhibited a blunted response to a-adrenergic stimulation[136]; both of which were reversed by acute inhibition of the HBP. However, in the same studies, we also showed that acute activation of the HBP with glucosamine also protected against Ca^{2+} overload and blunted α-adrenergic stimulation [72, 136]. Taken together these observations suggest that both the acute protection and adverse effects associated with increased O-GlcNAcylation may be mediated, at least in part, by inhibition of the same Ca^{2+} entry pathways.

Conclusion

It took approximately 20 years from the initial identification of O-GlcNAc modified proteins [2] until its beneficial role in mediating cell survival was clearly recognized [4]. It is becoming increasingly evident that O-GlcNAc modification of proteins is a metabolically modulated signaling pathway that regulates cell function and plays a particularly critical role in mediating the response of cells to stress. There is now an emerging consensus, at least in the cardiovascular system, that acute increases in O-GlcNAc synthesis or inhibition of O-GlcNAc degradation affords remarkable cardioprotection against oxidative stress including ischemia/reperfusion; however, the specific mechanisms underlying the protection associated with increased O-GlcNAc levels remains elusive. It should emphasized that it remains to be determined whether the protection against oxidative stress and ischemia/reperfusion injury, associated with short-term increases in O-GlcNAcylation is broadly applicable or whether it is specific to the heart.

In some biological systems, such as the heart, O-GlcNAc levels respond rapidly (i.e. within minutes) to activation of the HBP or inhibition of O-GlcNAcase [72, 79]. This suggests that the O-GlcNAc "on/off" cycle is a highly dynamic process that likely contributes to acute regulation of cell function in response to both physiological and pathophysiological stimuli. However, our knowledge regarding the regulation of O-GlcNAc turnover is remarkably limited. The increase in O-GlcNAc that occurs in response to stress such as ischemia could be due to a substrate driven increase in flux through the HBP, increased GFAT activity, or both. Alternatively, given the presence of such an active O-GlcNAc cycle, ischemia could indirectly or directly modulate the O-GlcNAc enzymes, such that activation of OGT and/or inhibition of O-GlcNAcase could lead to a rapid increase in O-GlcNAc levels independent of changes in the HBP.

At the cellular level gain and loss of function studies further demonstrate that OGT and O-GlcNAcase play critical role in regulating the response to acute oxidative stress [89-91]. At the transcriptional level, protection appears to be a result, at least in part of increased heat shock proteins synthesis [29]. On the other hand, the fact that protection can be afforded with very brief pre-treatment protocols, strongly suggests that transcriptionally independent mechanisms also play an important role. Potential mechanisms include activation of pro-survival kinase pathways [48], increased O-GlcNAcylation of small heat shock proteins or preventing loss of mitochondrial membrane potential [80, 89]. However, we also propose that O-GlcNAc mediated inhibition of CCE attenuates Ca^{2+} overload, may represent a key mechanism underlying the acute protective response associated with increased O-GlcNAcylation. The recent identification of key proteins responsible for mediating CCE, namely STIM1 and Orai1, provide a foundation for testing this hypothesis.

The potential interaction between O-GlcNAc signaling, ROS and mitochondrial function raises the possibility that O-GlcNAc signaling contribute to intracellular redox sensing and conversely that ROS may be involved in regulating cellular O-GlcNAc levels. Regardless, protein O-GlcNAcylation is clearly an integral component of complex signaling networks responsible for the regulation of cellular response to both physiological and pathophysiological stimuli, thereby playing a critical role in mediating cell survival. However, despite our extensive and growing knowledge regarding the influence of O-GlcNAc signaling on cellular function, our knowledge of the factors involved in regulating O-GlcNAc turnover remain substantially under developed. Thus, as we continue to evolve our understanding of role of O-GlcNAcylation in mediating the response to stress, additional efforts are required to better elucidate the mechanisms involved in regulating the activity and transcription of the proteins responsible for controlling O-GlcNAc synthesis and degradation.

Acknowledgments

This work was supported in part by a research grants from NIH HL067464 and HL079364 (JCC) and HL076175 (RBM). We would also like to thank our graduate students, post-doctoral fellows, technical staff who have worked in our laboratories and contributed to the studies described here. We also want to acknowledge the contributions of our colleagues at UAB and elsewhere who have helped develop some of the ideas and concepts presented here.

References

1. Jensen ON. Interpreting the protein language using proteomics. Nat Rev Mol Cell Biol. 2006; 7:391–403. [PubMed: 16723975]
2. Torres CR, Hart GW. Topography and polypeptide distribution of terminal N-acetylglucosamine residues on the surfaces of intact lymphocytes. Evidence for O-linked GlcNAc. J Biol Chem. 1984; 259:3308–17. [PubMed: 6421821]

3. Slawson C, Housley MP, Hart GW. O-GlcNAc cycling: How a single sugar post-translational modification is changing the Way We think about signaling networks. J Cell Biochem. 2006; 97:71–83. [PubMed: 16237703]
4. Zachara NE, Hart GW. O-GlcNAc a sensor of cellular state: the role of nucleocytoplasmic glycosylation in modulating cellular function in response to nutrition and stress. Biochim Biophys Acta. 2004; 1673:13–28. [PubMed: 15238246]
5. Zachara NE, Hart GW. Cell signaling, the essential role of O-GlcNAc! Biochim Biophys Acta. 2006; 1761:599–617. [PubMed: 16781888]
6. Love DC, Hanover JA. The hexosamine signaling pathway: deciphering the “O-GlcNAc code”. Sci STKE. 2005; 2005:re13. [PubMed: 16317114]
7. Hart GW. Dynamic O-linked glycosylation of nuclear and cytoskeletal proteins. Annu Rev Biochem. 1997; 66:315–35. [PubMed: 9242909]
8. Dehennaut V, Lefebvre T, Sellier C, et al. O-linked N-acetylglucosaminyltransferase inhibition prevents G2/M transition in Xenopus laevis oocytes. J Biol Chem. 2007; 282:12527–36. [PubMed: 17329255]
9. Guinez C, Morelle W, Michalski JC, Lefebvre T. O-GlcNAc glycosylation: a signal for the nuclear transport of cytosolic proteins? Int J Biochem Cell Biol. 2005; 37:765–74. [PubMed: 15694836]
10. Wells L, Whalen SA, Hart GW. O-GlcNAc: a regulatory post-translational modification. Biochem Biophys Res Commun. 2003; 302:435–41. [PubMed: 12615051]
11. Liu K, Paterson AJ, Zhang F, et al. Accumulation of protein O-GlcNAc modification inhibits proteasomes in the brain and coincides with neuronal apoptosis in brain areas with high O-GlcNAc metabolism. J Neurochem. 2004; 89:1044–55. [PubMed: 15140202]
12. Comer FI, Hart GW. O-GlcNAc and the control of gene expression. Biochim Biophys Acta. 1999; 1473:161–71. [PubMed: 10580136]
13. Zachara NE, Hart GW. O-GlcNAc modification: a nutritional sensor that modulates proteasome function. Trends Cell Biol. 2004; 14:218–21. [PubMed: 15130576]
14. Han I, Kudlow JE. Reduced O glycosylation of Sp1 is associated with increased proteasome susceptibility. Mol Cell Biol. 1997; 17:2550–8. [PubMed: 9111324]
15. Fulop N, Mason MM, Dutta K, et al. The impact of Type-2 diabetes and aging on cardiomyocyte function and O-Linked N-acetylglucosamine levels in the heart. Am J Physiol Cell Physiol. 2007; 292:C1370–8. [PubMed: 17135297]
16. Rex-Mathes M, Werner S, Strutas D, et al. O-GlcNAc expression in developing and ageing mouse brain. Biochimie. 2001; 83:583–90. [PubMed: 11522386]
17. Fulop N, Feng W, Xing D, et al. Aging leads to increased levels of protein O-linked N-acetylglucosamine in heart, aorta, brain and skeletal muscle in Brown-Norway rats. Biogerontology. 2008; 9:139–51. [PubMed: 18185980]
18. Chou TY, Hart GW, Dang CV. c-Myc is glycosylated at threonine 58, a known phosphorylation site and a mutational hot spot in lymphomas. J Biol Chem. 1995; 270:18961–5. [PubMed: 7642555]
19. Shaw P, Freeman J, Bovey R, Iggo R. Regulation of specific DNA binding by p53: evidence for a role for O-glycosylation and charged residues at the carboxy-terminus. Oncogene. 1996; 12:921–30. [PubMed: 8632915]
20. Donadio AC, Lobo C, Tosina M, et al. Antisense glutaminase inhibition modifies the O-GlcNAc pattern and flux through the hexosamine pathway in breast cancer cells. J Cell Biochem. 2008; 103:800–11. [PubMed: 17614351]
21. Hanover JA. Glycan-dependent signaling: O-linked N-acetylglucosamine. FASEB J. 2001; 15:1865–1876. [PubMed: 11532966]
22. Dias WB, Hart GW. O-GlcNAc modification in diabetes and Alzheimer's disease. Mol Biosyst. 2007; 3:766–72. [PubMed: 17940659]
23. Buse MG. Hexosamines, insulin resistance, and the complications of diabetes: current status. Am J Physiol Endocrinol Metab. 2006; 290:E1–E8. [PubMed: 16339923]
24. Copeland RJ, Bullen JW, Hart GW. Cross-talk between GlcNAcylation and phosphorylation: roles in insulin resistance and glucose toxicity. Am J Physiol Endocrinol Metab. 2008; 295:E17–28. [PubMed: 18445751]

25. Hart GW, Housley MP, Slawson C. Cycling of O-linked beta-N-acetylglucosamine on nucleocytoplasmic proteins. Nature. 2007; 446:1017–22. [PubMed: 17460662]
26. Hanover JA, Forsythe ME, Hennessey PT, et al. A Caenorhabditis elegans model of insulin resistance: altered macronutrient storage and dauer formation in an OGT-1 knockout. Proc Natl Acad Sci U S A. 2005; 102:11266–71. [PubMed: 16051707]
27. Lubas WA, Frank DW, Krause M, Hanover JA. O-Linked GlcNAc transferase is a conserved nucleocytoplasmic protein containing tetratricopeptide repeats. J Biol Chem. 1997; 272:9316–24. [PubMed: 9083068]
28. Shafi R, Iyer SP, Ellies LG, et al. The O-GlcNAc transferase gene resides on the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny. Proc Natl Acad Sci U S A. 2000; 97:5735–9. [PubMed: 10801981]
29. Zachara NE, O'Donnell N, Cheung WD, Mercer JJ, Marth JD, Hart GW. Dynamic O-GlcNAc modification of nucleocytoplasmic proteins in response to stress. A survival response of mammalian cells. J Biol Chem. 2004; 279:30133–30142. [PubMed: 15138254]
30. Kultz D. Molecular and evolutionary basis of the cellular stress response. Annu Rev Physiol. 2005; 67:225–57. [PubMed: 15709958]
31. Adler V, Yin Z, Tew KD, Ronai Z. Role of redox potential and reactive oxygen species in stress signaling. Oncogene. 1999; 18:6104–11. [PubMed: 10557101]
32. Chuang DM, Hough C, Senatorov VV. Glyceraldehyde-3-phosphate dehydrogenase, apoptosis, and neurodegenerative diseases. Annu Rev Pharmacol Toxicol. 2005; 45:269–90. [PubMed: 15822178]
33. Jarhult J. Osmotic fluid transfer from tissue to blood during hemorrhagic hypotension. Acta Physiol Scand. 1973; 89:213–26. [PubMed: 4765041]
34. Stone JP, Schutzer SF, McCoy S, Drucker WR. Contribution of glucose to the hyperosmolality of prolonged hypovolemia. Am Surg. 1977; 43:1–5. [PubMed: 831606]
35. Menguy R, Masters YF. Influence of hyperglycemia on survival after hemorrhagic shock. Adv Shock Res. 1978; 1:43–54. [PubMed: 262089]
36. Friedman SG, Pearce FJ, Drucker WR. The role of blood glucose in defense of plasma volume during hemorrhage. J Trauma. 1982; 22:86–91. [PubMed: 7062368]
37. Ware J, Ljungqvist O, Norberg KA, Nylander G. Osmolar changes in haemorrhage: the effects of an altered nutritional status. Acta Chir Scand. 1982; 148:641–6. [PubMed: 7170899]
38. Ljungqvist O, Boija PO, Ware J. The effect of hyperosmolar infusions on survival after hemorrhage. Acta Chir Scand. 1989; 155:433–8. [PubMed: 2596253]
39. Mizock BA. Alterations in fuel metabolism in critical illness: hyperglycaemia. Best Pract Res Clin Endocrinol Metab. 2001; 15:533–51. [PubMed: 11800522]
40. Du XL, Edelstein D, Rossetti L, et al. Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. Proc Natl Acad Sci U S A. 2000; 97:12222–12226. [PubMed: 11050244]
41. Marshall S, Bacote V, Traxinger RR. Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system. Role of hexosamine biosynthesis in the induction of insulin resistance. J Biol Chem. 1991; 266:4706–4712. [PubMed: 2002019]
42. Kornfeld R. Studies on L-glutamine D-fructose 6-phosphate amidotransferase. I. Feedback inhibition by uridine diphosphate-N-acetylglucosamine. J Biol Chem. 1967; 242:3135–41. [PubMed: 4961641]
43. Haltiwanger RS, Blomberg MA, Hart GW. Glycosylation of nuclear and cytoplasmic proteins. Purification and characterization of a uridine diphospho-N-acetylglucosamine:polypeptide beta-N-acetylglucosaminyltransferase. J Biol Chem. 1992; 267:9005–13. [PubMed: 1533623]
44. Haltiwanger RS, Holt GD, Hart GW. Enzymatic addition of O-GlcNAc to nuclear and cytoplasmic proteins. Identification of a uridine diphospho-N-acetylglucosamine:peptide beta-N-acetylglucosaminyltransferase. J Biol Chem. 1990; 265:2563–8. [PubMed: 2137449]
45. Holt GD, Hart GW. The subcellular distribution of terminal N-acetylglucosamine moieties. Localization of a novel protein-saccharide linkage, O-linked GlcNAc. J Biol Chem. 1986; 261:8049–57. [PubMed: 3086323]

46. Lehle L, Strahl S, Tanner W. Protein glycosylation, conserved from yeast to man: a model organism helps elucidate congenital human diseases. Angew Chem Int Ed Engl. 2006; 45:6802–18. [PubMed: 17024709]
47. Kreppel LK, Hart GW. Regulation of a cytosolic and nuclear O-GlcNAc transferase. Role of the tetratricopeptide repeats. J Biol Chem. 1999; 274:32015–32022. [PubMed: 10542233]
48. Fulop N, Zhang Z, Marchase RB, Chatham JC. Glucosamine cardioprotection in perfused rat heart associated with increased O-Linked N-acetylglucosamine protein modification and altered p38 activation. Am J Physiol Heart Circ Physiol. 2007; 292:H2227–36. [PubMed: 17208994]
49. Manzari B, Kudlow JE, Fardin P, et al. Induction of macrophage glutamine: fructose-6-phosphate amidotransferase expression by hypoxia and by picolinic acid. Int J Immunopathol Pharmacol. 2007; 20:47–58. [PubMed: 17346427]
50. Chang Q, Su K, Baker JR, Yang X, Paterson AJ, Kudlow JE. Phosphorylation of human glutamine:fructose-6-phosphate amidotransferase by cAMP-dependent protein kinase at serine 205 blocks the enzyme activity. J Biol Chem. 2000; 275:21981–7. [PubMed: 10806197]
51. Hu Y, Riesland L, Paterson AJ, Kudlow JE. Phosphorylation of mouse glutaminefructose-6-phosphate amidotransferase 2 (GFAT2) by cAMP-dependent protein kinase increases the enzyme activity. J Biol Chem. 2004; 279:29988–93. [PubMed: 15133036]
52. Young ME, Yan J, Razeghi P, et al. Proposed regulation of gene expression by glucose in rodent heart. Gene Reg Systems Biol. 2007; 1:251–262.
53. Laczy B, Wilson L, Brocks CA, Marchase RB, Chatham JC. Effect of ischemia-reperfusion on O-GlcNAcylation of specific proteins in isolated rat hearts. Faseb J. 2008; 22:750.
54. Dong DL, Hart GW. Purification and characterization of an O-GlcNAc selective N-acetyl-beta-D-glucosaminidase from rat spleen cytosol. J Biol Chem. 1994; 269:19321–30. [PubMed: 8034696]
55. Gao Y, Wells L, Comer FI, Parker GJ, Hart GW. Dynamic O-glycosylation of nuclear and cytosolic proteins: cloning and characterization of a neutral, cytosolic beta-N-acetylglucosaminidase from human brain. J Biol Chem. 2001; 276:9838–9845. [PubMed: 11148210]
56. Toleman C, Paterson AJ, Kudlow JE. Location and characterization of the O-GlcNAcase active site. Biochim Biophys Acta. 2006; 1760:829–39. [PubMed: 16517082]
57. Toleman C, Paterson AJ, Whisenhunt TR, Kudlow JE. Characterization of the histone acetyltransferase (HAT) domain of a bifunctional protein with activable O-GlcNAcase and HAT activities. J Biol Chem. 2004; 279:53665–73. [PubMed: 15485860]
58. Butkinaree C, Cheung WD, Park S, Park K, Barber M, Hart GW. Characterization of beta-N-acetylglucosaminidase cleavage by caspase-3 during apoptosis. J Biol Chem. 2008; 283:23557–66. [PubMed: 18586680]
59. Wells L, Gao Y, Mahoney JA, et al. Dynamic O-glycosylation of nuclear and cytosolic proteins: further characterization of the nucleocytoplasmic beta-N-acetylglucosaminidase, O-GlcNAcase. J Biol Chem. 2002; 277:1755–1761. [PubMed: 11788610]
60. McClain DA, Lubas WA, Cooksey RC, et al. Altered glycan-dependent signaling induces insulin resistance and hyperleptinemia. Proc Natl Acad Sci U S A. 2002; 99:10695–9. [PubMed: 12136128]
61. Vosseller K, Wells L, Lane MD, Hart GW. Elevated nucleocytoplasmic glycosylation by O-GlcNAc results in insulin resistance associated with defects in Akt activation in 3T3-L1 adipocytes. Proc Natl Acad Sci U S A. 2002; 99:5313–5318. [PubMed: 11959983]
62. Patti ME, Virkamaki A, Landaker EJ, Kahn CR, Yki-Jarvinen H. Activation of the hexosamine pathway by glucosamine in vivo induces insulin resistance of early postreceptor insulin signaling events in skeletal muscle. Diabetes. 1999; 48:1562–71. [PubMed: 10426374]
63. Park SY, Ryu J, Lee W. O-GlcNAc modification on IRS-1 and Akt2 by PUGNAc inhibits their phosphorylation and induces insulin resistance in rat primary adipocytes. Exp Mol Med. 2005; 37:220–9. [PubMed: 16000877]
64. Lehman DM, Fu DJ, Freeman AB, et al. A single nucleotide polymorphism in MGEA5 encoding O-GlcNAc-selective N-acetyl-beta-D glucosaminidase is associated with type 2 diabetes in Mexican Americans. Diabetes. 2005; 54:1214–21. [PubMed: 15793264]

65. Du XL, Edelstein D, Dimmeler S, Ju Q, Sui C, Brownlee M. Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. J Clin Invest. 2001; 108:1341–1348. [PubMed: 11696579]
66. Federici M, Menghini R, Mauriello A, et al. Insulin-dependent activation of endothelial nitric oxide synthase is impaired by O-linked glycosylation modification of signaling proteins in human coronary endothelial cells. Circulation. 2002; 106:466–72. [PubMed: 12135947]
67. Akimoto Y, Kawakami H, Yamamoto K, Munetomo E, Hida T, Hirano H. Elevated expression of O-GlcNAc-modified proteins and O-GlcNAc transferase in corneas of diabetic Goto-Kakizaki rats. Invest Ophthalmol Vis Sci. 2003; 44:3802–9. [PubMed: 12939295]
68. Clark RJ, McDonough PM, Swanson E, et al. Diabetes and the accompanying hyperglycemia impairs cardiomyocyte cycling through increased nuclear O-GlcNAcylation. J. Biol. Chem. 2003; 278:44230–44237. [PubMed: 12941958]
69. Akimoto Y, Hart GW, Hirano H, Kawakami H. O-GlcNAc modification of nucleocytoplasmic proteins and diabetes. Med Mol Morphol. 2005; 38:84–91. [PubMed: 15944815]
70. Hu Y, Belke D, Suarez J, et al. Adenovirus-mediated overexpression of O-GlcNAcase improves contractile function in the diabetic heart. Circ Res. 2005; 96:1006–1013. [PubMed: 15817886]
71. Marshall S, Nadeau O, Yamasaki K. Dynamic actions of glucose and glucosamine on hexosamine biosynthesis in isolated adipocytes: differential effects on glucosamine 6-phosphate, UDP-N-acetylglucosamine, and ATP levels. J Biol Chem. 2004; 279:35313–9. [PubMed: 15199059]
72. Liu J, Pang Y, Chang T, Bounelis P, Chatham JC, Marchase RB. Increased hexosamine biosynthesis and protein *O*-GlcNAc levels associated with myocardial protection against calcium paradox and ischemia. J. Mol. Cell Cardiol. 2006; 40:303–312. [PubMed: 16337959]
73. Konrad RJ, Zhang F, Hale JE, Knierman MD, Becker GW, Kudlow JE. Alloxan is an inhibitor of the enzyme O-linked N-acetylglucosamine transferase. Biochem Biophys Res Commun. 2002; 293:207–212. [PubMed: 12054585]
74. Tiedge M, Richter T, Lenzen S. Importance of cysteine residues for the stability and catalytic activity of human pancreatic beta cell glucokinase. Arch Biochem Biophys. 2000; 375:251–60. [PubMed: 10700381]
75. Champattanachai V, Marchase RB, Chatham JC. Glucosamine protects neonatal cardiomyocytes from ischemia-reperfusion injury via increased protein-associated O-GlcNAc. Am J Physiol Cell Physiol. 2007; 292:C178–87. [PubMed: 16899550]
76. Haltiwanger RS, Grove K, Philipsberg GA. Modulation of O-linked N-acetylglucosamine levels on nuclear and cytoplasmic proteins in vivo using the peptide O-GlcNAc-beta-N-acetylglucosaminidase inhibitor O-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino-N-phenylcarbamate. J Biol Chem. 1998; 273:3611–7. [PubMed: 9452489]
77. Shanmugasundaram B, Debowski AW, Dennis RJ, Davies GJ, Vocadlo DJ, Vasella A. Inhibition of O-GlcNAcase by a gluco-configured nagstatin and a PUGNAc-imidazole hybrid inhibitor. Chem Commun (Camb). 2006:4372–4. [PubMed: 17057847]
78. Bolli R, Becker L, Gross G, Mentzer R Jr. Balshaw D, Lathrop DA. Myocardial protection at a crossroads: the need for translation into clinical therapy. Circ Res. 2004; 95:125–34. [PubMed: 15271864]
79. Liu J, Marchase RB, Chatham JC. Increased O-GlcNAc levels during reperfusion lead to improved functional recovery and reduced calpain proteolysis. Am J Physiol Heart Circ Physiol. 2007; 293:H1391–9. [PubMed: 17573462]
80. Jones SP, Zachara NE, Ngoh GA, et al. Cardioprotection by N-acetylglucosamine linkage to cellular proteins. Circulation. 2008; 117:1172–82. [PubMed: 18285568]
81. Yang S, Zou LY, Bounelis P, Chaudry I, Chatham JC, Marchase RB. Glucosamine administration during resuscitation improves organ function after trauma hemorrhage. Shock. 2006; 25:600–607. [PubMed: 16721268]
82. Zou LY, Yang S, Chaudry IH, Marchase RB, Chatham JC. PUGNAc administration during resuscitation improves organ function following trauma-hemorrhage. Shock. 2007; 27:402–408. [PubMed: 17414423]

83. Not LG, Brocks CA, Marchase RB, Chatham JC. O-GlcNAc agonist treatment improves survival, reduces inflammation and organ damage 24 hours after trauma-hemorrhage in rats. Faseb J. 2008; 22:1227.
84. Masson E, Wiernsperger N, Lagarde M, El Bawab S. Glucosamine induces cell-cycle arrest and hypertrophy of mesangial cells: implication of gangliosides. Biochem J. 2005; 388:537–44. [PubMed: 15654767]
85. Stanley P. A method to the madness of N-glycan complexity? Cell. 2007; 129:27–9. [PubMed: 17418781]
86. Horsch M, Hoesch L, Vasella A, Rast DM. N-acetylglucosaminono-1,5-lactone oxime and the corresponding (phenylcarbamoyl)oxime. Novel and potent inhibitors of beta-N-acetylglucosaminidase. Eur J Biochem. 1991; 197:815–8. [PubMed: 2029909]
87. Perreira M, Kim EJ, Thomas CJ, Hanover JA. Inhibition of O-GlcNAcase by PUGNAc is dependent upon the oxime stereochemistry. Bioorg Med Chem. 2006; 14:837–46. [PubMed: 16214344]
88. Macauley MS, Whitworth GE, Debowski AW, Chin D, Vocadlo DJ. O-GlcNAcase uses substrate-assisted catalysis: kinetic analysis and development of highly selective mechanism-inspired inhibitors. J Biol Chem. 2005; 280:25313–22. [PubMed: 15795231]
89. Champattanachai V, Marchase RB, Chatham JC. Glucosamine protects neonatal cardiomyocytes from ischemia-reperfusion injury via increased protein O-GlcNAc and increased mitochondrial Bcl-2. Am J Physiol Cell Physiol. 2008; 294:C1509–20. [PubMed: 18367586]
90. Ngoh GA, Watson LJ, Facundo HT, Dillmann W, Jones SP. Non-canonical glycosyltransferase modulates post-hypoxic cardiac myocyte death and mitochondrial permeability transition. J Mol Cell Cardiol. 2008; 45:313–25. [PubMed: 18539296]
91. Ngoh GA, Facundo HT, Hamid T, Dillmann W, Zachara NE, Jones SP. Unique hexosaminidase reduces metabolic survival signal and sensitizes cardiac myocytes to hypoxia/reoxygenation injury. Circ Res. 2009; 104:41–9. [PubMed: 19023128]
92. Singh LP, Cheng DW, Kowluru R, Levi E, Jiang Y. Hexosamine induction of oxidative stress, hypertrophy and laminin expression in renal mesangial cells: effect of the anti-oxidant alpha-lipoic acid. Cell Biochem Funct. 2007; 25:537–50. [PubMed: 16892452]
93. Masson E, Lagarde M, Wiernsperger N, El Bawab S. Hyperglycemia and glucosamine-induced mesangial cell cycle arrest and hypertrophy: Common or independent mechanisms? IUBMB Life. 2006; 58:381–8. [PubMed: 16801212]
94. Zou L, Yang S, Champattanachai V, et al. Glucosamine improves cardiac function following trauma-hemorrhage by increased protein O-GlcNAcylation and attenuation of NF-{kappa}B signaling. Am J Physiol Heart Circ Physiol. 2009; 296:H515–23. [PubMed: 19098112]
95. Dorfmueller HC, Borodkin VS, Schimpl M, van Aalten DM. GlcNAcstatins are nanomolar inhibitors of human O-GlcNAcase inducing cellular hyper-O-GlcNAcylation. Biochem J. 2009; 420:221–7. [PubMed: 19275764]
96. Dorfmueller HC, Borodkin VS, Schimpl M, Shepherd SM, Shpiro NA, van Aalten DM. GlcNAcstatin: a picomolar, selective O-GlcNAcase inhibitor that modulates intracellular O-glcNAcylation levels. J Am Chem Soc. 2006; 128:16484–5. [PubMed: 17177381]
97. Macauley MS, Bubb AK, Martinez-Fleites C, Davies GJ, Vocadlo DJ. Elevation of global O-GlcNAc levels in 3T3-L1 adipocytes by selective inhibition of O-GlcNAcase does not induce insulin resistance. J Biol Chem. 2008; 283:34687–95. [PubMed: 18842583]
98. Laczy B, Marsh SA, Brocks CA, Marchase RB, Chatham JC. Inhibition of O-GlcNAcase in perfused rat hearts by NAG-thiazolines at the time of reperfusion is cardioprotective in an O-GlcNAc dependent manner. FASEB J. 2009; 23:793.14.
99. Wells L, Vosseller K, Cole RN, Cronshaw JM, Matunis MJ, Hart GW. Mapping sites of O-GlcNAc modification using affinity tags for serine and threonine post-translational modifications. Mol Cell Proteomics. 2002; 1:791–804. [PubMed: 12438562]
100. Walgren JL, Vincent TS, Schey KL, Buse MG. High glucose and insulin promote O-GlcNAc modification of proteins, including alpha-tubulin. Am J Physiol Endocrinol Metab. 2003; 284:E424–E434. [PubMed: 12397027]

101. Sohn KC, Lee KY, Park JE, Do SI. OGT functions as a catalytic chaperone under heat stress response: a unique defense role of OGT in hyperthermia. Biochem Biophys Res Commun. 2004; 322:1045–1051. [PubMed: 15336570]
102. Majumdar G, Wright J, Markowitz P, Martinez-Hernandez A, Raghow R, Solomon SS. Insulin stimulates and diabetes inhibits O-linked N-acetylglucosamine transferase and O-glycosylation of Sp1. Diabetes. 2004; 53:3184–92. [PubMed: 15561949]
103. Lim KH, Chang HI. O-linked N-acetylglucosamine suppresses thermal aggregation of Sp1. FEBS Lett. 2006; 580:4645–52. [PubMed: 16879824]
104. Dong L, Wang W, Wang F, et al. Mechanisms of transcriptional activation of bcl-2 gene expression by 17beta-estradiol in breast cancer cells. J Biol Chem. 1999; 274:32099–107. [PubMed: 10542244]
105. Ngoh GA, Jones SP. O-GlcNAc signaling attenuates mitochondrial membrane permeability transition in cell death. Faseb J. 2008; 22:1130.
106. Tsujimoto Y, Shimizu S. VDAC regulation by the Bcl-2 family of proteins. Cell Death Differ. 2000; 7:1174–81. [PubMed: 11175254]
107. Tsujimoto Y, Shimizu S. Role of the mitochondrial membrane permeability transition in cell death. Apoptosis. 2007; 12:835–40. [PubMed: 17136322]
108. Efthymiou CA, Mocanu MM, de Belleroche J, Wells DJ, Latchmann DS, Yellon DM. Heat shock protein 27 protects the heart against myocardial infarction. Basic Res Cardiol. 2004; 99:392–4. [PubMed: 15309411]
109. Hollander JM, Martin JL, Belke DD, et al. Overexpression of wild-type heat shock protein 27 and a nonphosphorylatable heat shock protein 27 mutant protects against ischemia/reperfusion injury in a transgenic mouse model. Circulation. 2004; 110:3544–52. [PubMed: 15569832]
110. Li G, Ali IS, Currie RW. Insulin-induced myocardial protection in isolated ischemic rat hearts requires p38 MAPK phosphorylation of Hsp27. Am J Physiol Heart Circ Physiol. 2008; 294:H74–87. [PubMed: 17906111]
111. Ray PS, Martin JL, Swanson EA, Otani H, Dillmann WH, Das DK. Transgene overexpression of alphaB crystallin confers simultaneous protection against cardiomyocyte apoptosis and necrosis during myocardial ischemia and reperfusion. Faseb J. 2001; 15:393–402. [PubMed: 11156955]
112. Martindale JJ, Wall JA, Martinez-Longoria DM, et al. Overexpression of mitogen-activated protein kinase kinase 6 in the heart improves functional recovery from ischemia in vitro and protects against myocardial infarction in vivo. J Biol Chem. 2005; 280:669–76. [PubMed: 15492008]
113. Roquemore EP, Chevrier MR, Cotter RJ, Hart GW. Dynamic O-GlcNAcylation of the small heat shock protein alpha B-crystallin. Biochemistry. 1996; 35:3578–86. [PubMed: 8639509]
114. Whelan SA, Hart GW. Proteomic approaches to analyze the dynamic relationships between nucleocytoplasmic protein glycosylation and phosphorylation. Circ Res. 2003; 93:1047–1058. [PubMed: 14645135]
115. Zhang F, Su K, Yang X, Bowe DB, Paterson AJ, Kudlow JE. O-GlcNAc modification is an endogenous inhibitor of the proteasome. Cell. 2003; 115:715–725. [PubMed: 14675536]
116. Zou LY, Yang S, Chaudry IH, Marchase RB, Chatham JC. The protective effect of glucosamine on cardiac function following trauma hemorrhage: Downregulation of cardiac NF-B signaling. FASEB J. 2007; 21:914.
117. Xing D, Feng W, Chen YF, Chatham JC, Oparil S. Glucosamine inhibits TNF-alpha-induced expression of inflammatory mediators in rat aortic smooth muscle cells through inhibition of NF-kappaB activation. Faseb J. 2008; 22:744.
118. Xing D, Feng W, Not LG, et al. Increased protein O-GlcNAc modification inhibits inflammatory and neointimal responses to acute endoluminal arterial injury. Am J Physiol Heart Circ Physiol. 2008; 295:H335–42. [PubMed: 18469144]
119. Zou LY, Champattanachai V, Marchase RB, Chatham JC. In isolated cardiomyocytes NF-kappaB activation is modulated by alterations in protein O-GlcNAc levels. Faseb J. 2008; 22:1227.
120. Broad LM, Braun FJ, Lievremont JP, Bird GS, Kurosaki T, Putney JW Jr. Role of the phospholipase C-inositol 1,4,5-trisphosphate pathway in calcium release-activated calcium

current (Icrac) and capacitative calcium entry. J. Biol. Chem. 2001; 276:15945–15952. [PubMed: 11278938]

121. Putney JW Jr. Broad LM, Braun FJ, Lievremont JP, Bird GS. Mechanisms of capacitative calcium entry. J Cell Sci. 2001; 114:2223–9. [PubMed: 11493662]
122. Trepakova ES, Csutora P, Hunton DL, Marchase RB, Cohen RA, Bolotina VM. Calcium influx factor directly activates store-operated cation channels in vascular smooth muscle cells. J. Biol. Chem. 2000; 275:26158–26163. [PubMed: 10851243]
123. Kurebayashi N, Ogawa Y. Depletion of Ca2+ in the sarcoplasmic reticulum stimulates Ca2+ entry into mouse skeletal muscle fibres. J. Physiol. (London). 2001; 533:185–199. [PubMed: 11351027]
124. Lyfenko AD, Dirksen RT. Differential dependence of store-operated and excitation-coupled Ca2+ entry in skeletal muscle on STIM1 and Orai1. J Physiol. 2008; 586:4815–24. [PubMed: 18772199]
125. Zhao X, Weisleder N, Thornton A, et al. Compromised store-operated Ca2+ entry in aged skeletal muscle. Aging Cell. 2008; 7:561–8. [PubMed: 18505477]
126. Shin DM, Muallem S. Skeletal muscle dressed in SOCs. Nat Cell Biol. 2008; 10:639–41. [PubMed: 18521070]
127. Stiber J, Hawkins A, Zhang ZS, et al. STIM1 signalling controls store-operated calcium entry required for development and contractile function in skeletal muscle. Nat Cell Biol. 2008; 10:688–97. [PubMed: 18488020]
128. Hunton DL, Lucchesi PA, Pang Y, Cheng X, Dell'Italia LJ, Marchase RB. Capacitative calcium entry contributes to nuclear factor of activated T-cells nuclear translocation and hypertrophy in cardiomyocytes. J Biol Chem. 2002; 277:14266–14273. [PubMed: 11827959]
129. Hunton DL, Zou LY, Pang Y, Marchase RB. Adult Rat Cardiomyocytes Exhibit Capacitative Calcium Entry. Am J Physiol Heart Circ Physiol. 2004; 286:H1124–H1132. [PubMed: 14630640]
130. Pang Y, Hunton DL, Bounelis P, Marchase RB. Hyperglycemia inhibits capacitative calcium entry and hypertrophy in neonatal cardiomyocytes. Diabetes. 2002; 51:3461–3467. [PubMed: 12453900]
131. Ohba T, Watanabe H, Murakami M, et al. Upregulation of TRPC1 in the development of cardiac hypertrophy. J Mol Cell Cardiol. 2007; 42:498–507. [PubMed: 17174323]
132. Nakayama H, Wilkin BJ, Bodi I, Molkentin JD. Calcineurin-dependent cardiomyopathy is activated by TRPC in the adult mouse heart. Faseb J. 2006; 20:1660–70. [PubMed: 16873889]
133. Rivera AA, White CR, Guest LL, Elton TS, Marchase RB. Hyperglycemia alters cytoplasmic Ca2+ responses to capacitative Ca2+ influx in rat aortic smooth muscle cells. Am J Physiol. 1995; 269:C1482–8. [PubMed: 8572177]
134. Vemuri S, Marchase RB. The inhibition of capacitative calcium entry due to ATP depletion but not due to glucosamine is reversed by staurosporine. J. Biol. Chem. 1999; 274:20165–20170. [PubMed: 10400631]
135. Nagy T, Champattanachai V, Marchase RB, Chatham JC. Glucosamine inhibits angiotensis II induced cytoplasmic Ca2+ elevation in neonatal cardiomyocytes via protein-associated O-GlcNAc. Am J Physiol Cell Physiol. 2006; 290:C57–65. [PubMed: 16107505]
136. Pang Y, Bounelis P, Chatham JC, Marchase RB. The hexosamine pathway is responsible for the inhibition by diabetes of phenylephrine-induced inotropy. Diabetes. 2004; 53:1074–1081. [PubMed: 15047624]
137. Ng LC, Wilson SM, Hume JR. Mobilization of sarcoplasmic reticulum stores by hypoxia leads to consequent activation of capacitative Ca2+ entry in isolated canine pulmonary arterial smooth muscle cells. J Physiol. 2005; 563:409–19. [PubMed: 15613369]
138. Ng LC, Kyle BD, Lennox AR, Shen XM, Hatton WJ, Hume JR. Cell culture alters Ca2+ entry pathways activated by store-depletion or hypoxia in canine pulmonary arterial smooth muscle cells. Am J Physiol Cell Physiol. 2008; 294:C313–23. [PubMed: 17977940]
139. Becker S, Knock GA, Snetkov V, Ward JP, Aaronson PI. Role of capacitative Ca2+ entry but not Na+/Ca2+ exchange in hypoxic pulmonary vasoconstriction in rat intrapulmonary arteries. Novartis Found Symp. 2006; 272:259–68. discussion 268-79. [PubMed: 16686440]

140. Calloway N, Vig M, Kinet JP, Holowka D, Baird B. Molecular clustering of STIM1 with Orai1/CRACM1 at the plasma membrane depends dynamically on depletion of Ca2+ stores and on electrostatic interactions. Mol Biol Cell. 2009; 20:389–99. [PubMed: 18987344]

141. Varnai P, Hunyady L, Balla T. STIM and Orai: the long-awaited constituents of store-operated calcium entry. Trends Pharmacol Sci. 2009; 30:118–28. [PubMed: 19187978]

142. Gutierrez J, Ballinger SW, Darley-Usmar VM, Landar A. Free radicals, mitochondria, and oxidized lipids: the emerging role in signal transduction in vascular cells. Circ Res. 2006; 99:924–32. [PubMed: 17068300]

143. Fulop N, Marchase RB, Chatham JC. Role of protein O-linked N-acetyl-glucosamine in mediating cell function and survival in the cardiovascular system. Cardiovasc Res. 2007; 73:288–97. [PubMed: 16970929]

144. Housley MP, Rodgers JT, Udeshi ND, et al. O-GlcNAc regulates FoxO activation in response to glucose. J Biol Chem. 2008; 283:16283–92. [PubMed: 18420577]

145. McEwen BS. Protective and damaging effects of stress mediators. N Engl J Med. 1998; 338:171–9. [PubMed: 9428819]