HHS Public Access

Author manuscript

Curr Opin Clin Nutr Metab Care. Author manuscript; available in PMC 2016 July 01.

Published in final edited form as:

Curr Opin Clin Nutr Metab Care. 2015 July ; 18(4): 339–345. doi:10.1097/MCO.0000000000000188.

You are what you Eat: *O*-Linked N-acetylglucosamine in Disease, Development and Epigenetics

Stéphanie OLIVIER-VAN STICHELEN[1] and **John A. HANOVER**[1,*]

[1]National Institute of diabetes and digestive and Kidney diseases, National Institute of Health, Bethesda, MD, 20892, USA

Abstract

Purpose of review—The *O*-linked N-acetylglucosamine (O-GlcNAc) modification is both responsive to nutrient availability and capable of altering intracellular cellular signaling. We summarize data defining a role for *O*-GlcNAcylation in metabolic homeostasis and epigenetic regulation of development in the intrauterine environment.

Recent Findings—*O*-GlcNAc Transferase (OGT) catalyzes nutrient-driven *O*-GlcNAc addition and is subject to random X-inactivation. OGT plays key roles in growth factor signaling, stem cell biology, epigenetics, and possibly imprinting. The *O*-GlcNAcase, which removes *O*-GlcNAc is subject to tight regulation by higher order chromatin structure. *O*-GlcNAc cycling plays an important role in the intrauterine environment where OGT expression is an important biomarker of placental stress.

Summary—Regulation of *O*-GlcNAc cycling by X-inactivation, epigenetic regulation, and nutrient-driven processes make it an ideal candidate for a nutrient-dependent epigenetic regulator of human disease. In addition, *O*-GlcNAc cycling influences chromatin modifiers critical to the regulation and timing of normal development including the polycomb repression complex (PRC2) and the TET proteins mediating DNA methyl cytosine demethylation. The pathway also impacts the Hypothalamic-Pituitary-Adrenal Axis critical to intrauterine programming influencing disease susceptibility in later life.

Keywords

Metabolic syndrome; intrauterine environment; Epigenetics; *O*-GlcNAc; Development

Introduction

Hippocrates, the ‘Father of Medicine’ said, “Let food be thy medicine and medicine be thy food.” Due to recent changes in worldwide nutritional habits, determining the interplay between diet and cell signaling is emerging as a critical area for further research. The challenge is not a new one. Previous work by Conrad Waddington, James Neel, and David Barker has led to the concept that a ‘thrifty phenotype’ emerged during human evolution to

*Corresponding Author: John A. Hanover, NIDDK, NIH, Bethesda MD, 201892-0851; jah@helix.nih.gov.

Conflicts of interest

None

allow the fetus to respond to environmental (primarily nutritional) cues during ontogeny (Reviewed in [1]). Currently, the average American eats around 77 pounds of added sugar every year (e.g., 22 tsp./day), thus, understanding what happens when sugars are metabolized will be of utmost importance for public health. Over the past 30 years, the understanding of *O*-GlcNAc cycling has had a significant impact on scientific research by defining links between sugar metabolism and altered protein function [2]. The process of *O*-GlcNAcylation is defined as the dynamic modification of serine or threonine amino acids by a single residue of N-acetyl glucosamine (GlcNAc) (Figure 1). The GlcNAc substrate is directly provided by metabolism of extracellular glucose through the Hexosamine Biosynthesis Pathway (HBP) [1–3]. This pathway is dependent upon metabolites derived from amino acid metabolism (Glutamine), lipid metabolism (Acetyl-CoA), nucleotide metabolism (UDP) and glucose metabolism (Glucose-6-phosphate) (Figure 1). Consequently, *O*-GlcNAcylation is considered to be a nutrient-sensing 'rheostat' acting posttranslationally upon proteins; an increase in dietary sugar percentage impacts *O*-GlcNAcylated protein pool [4]. Two complementary enzymes regulate this modification: *O*-GlcNAc Transferase (OGT) and *O*-GlcNAcase (OGA), which add and remove the sugar, respectively. By dynamically modifying intracellular proteins, *O*-GlcNAcylation differs substantially from the classical static N- and *O*-glycosylation and represents a new mode of regulation for a signaling pathway [5] and impacts many of the key signaling pathways (insulin, IGF, FGF, mitochondrial sub-complexes) known to be involved in metabolic homeostasis.

To date, almost all functional protein groups are represented among the pool of *O*-GlcNAcylated proteins, from structural proteins to transcription factors, including even the enzymes of *O*-GlcNAc cycling (OGA and OGT) [1]. The consequences of *O*-GlcNAcylation are likely broad, ranging from conformational changes and altered protein-protein interactions, to changes in protein half-life, sub-cellular localization or protein activity. By modification of a large number of proteins, *O*-GlcNAcylation is able to alter transcription, translation and proteasomal degradation. These processes, in turn, are known to regulate complex processes such as the cell cycle and embryonic development [1,6]. *O*-GlcNAcylation has also been implicated in pathologies including neurodegeneration, cardiovascular diseases, type II diabetes and cancers. In these cases, nutrition-related deregulation could impact protein modification and exacerbate disease development [1,6]. Because its high dependence on glucose and other nutrient levels, *O*-GlcNAcylation is poised to regulate biological pathways in a nutrient-dependent manner. Herein, we highlight diet-responsive processes in which *O*-GlcNAcylation is known to be involved (Figure 2). We will also emphasize the importance of this pathway in the intrauterine environment where it may exert trans-generational effects relevant to human health and disease susceptibility.

The Interplay between *O*-GlcNAc and Epigenetics

Epigenetics represents a way to alter the program of development in response to dietary influences. In fact, exposing mice to a high sugar bolus triggers epigenetic changes, which could last as far as 6 days after the initial exposure, and ultimately affect their cardiovascular damage risk [7]. Interestingly, epigenetic changes can also explain how paternal diet may

affect metabolic features of his offspring [8]. Due to its reactivity to glucose flux, *O*-GlcNAcylation is one important potential mediator of diet-induced epigenetic modification.

Since the discovery of *O*-GlcNAc, chromatin has been described as a major *O*-GlcNAc target, suggesting a key role for *O*-GlcNAcylation in the transcriptional regulation of genes (Reviewed in [9*]). *O*-GlcNAc was found to occupy the promoters of a substantial percentage of expressing genes in *C. elegans* [10]. In addition, the *O*-GlcNAc modification was found directly on histones H2A, H2B, H4 and H3 [11,12,13]. Furthermore, OGA possesses a predicted HAT domain is similar to the histone acetyltransferase GCN5, believed to facilitate the association of OGA with histone complexes [14]. Interestingly, OGT is also found associated with epigenetic modulators including the TET proteins [15], the mSin3A-HDACs (Reviewed in [16]) and finally the Polycomb group (PcG) proteins [17]. These recent findings are summarized below:

- The transcriptional corepressor mSin3A physically interacts with OGT and is *O*-GlcNAcylated. OGT-mSin3A interaction is also believed to trigger the repression of specific subsets of regulatory genes, for example, the retinoblastoma tumor suppressor protein (Reviewed in [16]). Under high glucose condition, OGT-mSin3a-HDAC complex is notably targeted and affects proper vascular function [18,19].
- OGT binds to the TET (Ten–Eleven Transcription) family proteins involved in DNA demethylation on CpG islands. OGT modifies TET 1, 2 and 3 proteins [15] but also interacts with them to be directed to chromatin to modify histones [20*]. In addition, a competition between phosphorylation and *O*-GlcNAcylation on TET proteins has recently been demonstrated, and may regulate their functions [21].
- In Drosophila, the PcG gene super sex combs (sxc) encodes the *O*-GlcNAc transferase [22]. In the same organism, major *O*-GlcNAc staining is found on the PcG-binding-sites on polytene chromosomes [23] and a major Polycomb protein (Ph) is *O*-GlcNAcylated for proper functioning [24]. While Drosophila and mammalian are obviously different, a link between PcG protein and OGT was also demonstrated in mammals where PRC2 is needed to maintain normal OGT level in stem cells [17].
- The interplay between PcG gene silencing and *O*-GlcNAcylation becomes more intriguing knowing that the *Oga* gene is localized in the NK cluster, containing three homeobox gene clusters regulated by PcG repression [25, 1, 9*].

Taken together, these observations link *O*-GlcNAcylation to epigenetic phenomena previously shown to play key roles in differentiation and development. *O*-GlcNAc is also intimately involved in one of the most detailed PcG-mediated gene silencing mechanisms: the dosage compensation or X-inactivation in mammals that we detail in the next section.

Dosage of *Ogt* is regulated by imprinted X-inactivation

Dosage compensation mechanisms are necessary in order to balance transcription from the X-chromosome between males and females. Whereas females have two X chromosomes, males have an X and Y chromosome. In mammals, this process for dosage compensation is

called X-inactivation, and is defined by silencing of one of the two female X-chromosomes [26]. During early embryogenesis, X-chromosome inactivation occurs differently for extra-embryonic vs. embryonic tissues. In the extra-embryonic tissues, the paternally inherited X-chromosome (Xp) is silenced (imprinted X-inactivation, iXCI) [26]. Within the inner cell mass (ICM), from which the embryo is derived, cells undergo random X-inactivation (rXCI), e.g. half the cells silence the Xp and the other half silence the maternally inherited X-chromosome (Xm) [26]. Interestingly, the *O*-GlcNAc transferase gene is localized on the X-chromosome close to the X-inactivation center (XIC) (Reviewed in [26]). Because *Ogt* is an X-linked nutrient sensor whose dosage is important for human health and disease, silencing of extra-*Ogt* alleles seems critical for normal female development. Indeed, heterozygous *Ogt* knockout (KO) female is embryonic lethal when the mutant allele is maternally inherited, whereas paternal inheritance is viable (Reviewed in [26]). In fact, in the female placenta, *Ogt* is only expressed by the maternal allele [27]. Consequently, because the Xp is silenced, at least a functional maternal *Ogt* allele seems required in extra-embryonic tissues for proper preimplantation development. Surprisingly though, other studies have shown that *Ogt* does not systematically undergo normal iXCI in extra-embryonic tissues and have therefore defined *Ogt* as an iXCI-escaping gene in mouse trophoblastic stem cells [28–31]. Thus, female placentas sometimes have higher levels of OGT and *O*-GlcNAcylated proteins than male placentas [32**].

To summarize, Ogt seems to have a varied pattern of placental iXCI and, as a consequence, *O*-GlcNAcylation may be a good candidate to link maternal diet to development of the offspring. Indeed, maternal low fat diet (high in carbohydrates) increases *Ogt* expression only in the female mouse placenta [33]. Similarly, as *O*-GlcNAcylation is involved in type-2 diabetes [34], female rodents, who had experienced malnutrition, or defects in glucose homeostasis during perinatal development, had increased incidence of metabolic syndrome that could be explained by sex-specific *O*-GlcNAcylation pattern (Reviewed in [26]). Moreover, maternal high sucrose exposure during pregnancy caused glucose homeostasis defects in female but not male offspring in mice, which may be explain by *O*-GlcNAcylation [35]. While diet during pregnancy does affect on offspring of both genders, female and male mouse preimplantation embryos still have around 600 differentially expressed transcripts that could potentially explain the sex-specific sensitivity to maternal diet [36].

Dosage of *Ogt* is regulated by Random X-inactivation

Compared to iXCI, from which *Ogt* may escape, random X-chromosome inactivation (rXCI) occurring in the embryo itself serves to silence extra-*Ogt* alleles. Due to its location near XIC, *Ogt* seems to be under tight transcriptional control in mouse embryo. Nevertheless, prior to widespread X-inactivation, most X-linked genes are biallelically expressed in mouse embryonic stem cells. However, allelic analysis in these cells indicated that *Ogt* expression is already monoallelic (Reviewed in [26]). This last observation suggests that, due to its close proximity to *Xist* locus in the XIC, *Ogt* can be repressed prior to rXCI in mice. But because many more X-genes escape X-inactivation in humans (15%) than in mice (3%) (Reviewed in [26]), an investigation of *Ogt* gene dosage in human was necessary. Our lab demonstrated that only one copy of *Ogt* gene is also activated in female human fibroblast 37*]. This study also demonstrated that *Ogt* might be reactivated by removal of XCI

repression marks on DNA. As an example, Systemic Lupus Erythematosus (SLE) presents a reactivation of the silenced X-chromosome due to a large demethylation process and diet is thought to be part of disease progression. Interestingly, a reactivation of *Ogt* is observed in SLE and is believed to worsen or even trigger this disease [38*]. In other words, monoallelic inactivation of *Ogt* likely controls OGT protein content in cells, preventing major imbalance in *O*-GlcNAc cycling.

Intrauterine Effects of *O*-GlcNAcylation

Hyperglycemia, due to diabetes or permanent high sugar consumption, makes a pregnancy high risk and can cause many negative effects on the fetus. Blood sugar is the baby's food source and it passes from the mother through the placenta. Ubiquitously present in the developing placenta and embryo, *O*-GlcNAcylation has been proposed to be one of the explanations of how maternal diet may effect development of the offspring [25]. Indeed, hyperglycemia-mediated *O*-GlcNAcylation has an impact as early as blastocyst stage of embryogenesis [39]. Similarly, creating a permanent diet-induced-hyper-*O*-GlcNAcylation by *Oga* KO triggers serious developmental defects [40**]. In these studies, *Oga* null pups exhibit a high incidence of neonatal lethality owing to decreased glycogen stores, a smaller size and a higher fat percentage.

O-GlcNAcylation is essential for proper vertebrate development. In different models (Zebrafish and Xenopus), *O*-GlcNAcylation has also been found critical for early development processes. More importantly, *Ogt* KO causes lethality, with mouse embryos dying around 4.5 days post coitus (blastocyst) [39] (Reviewed in [26]).

O-GlcNAc Impacts Stem Cell Biology and Neurogenesis

O-GlcNAcylation also plays a key role in stem cell biology. Numerous stem cell factors have been shown *O*-GlcNAcylated such as Oct4 [41] or Sox2 [42]. Whereas the role of Sox2 *O*-GlcNAcylation is still unclear, Oct4 interacts with OGT and is modified in order to regulate pluripotency gene networks [43]. Furthermore, elevating *O*-GlcNAcylation, triggers alterations in stem cells differentiation, particularly described in neuronal lineages [44, 45, 46*].

Glucose levels were recently correlated to brain developmental delay in type 1 diabetic patients although the mechanism of this delay was unclear from this analysis [47]. Interestingly, *O*-GlcNAcylation is found especially abundant in brain tissues. Furthermore, disruption of placental *Ogt* affects the Hypothalamus-Pituitary-Adrenal (HPA) axis, critical for proper fetal growth and metabolism, thus linking placental Ogt with neurodevelopmental programming [48**]. Prenatal stress induced by loss of OGT in the placenta also induces hypothalamic mitochondrial dysfunction. In related experiments, decreasing cellular *Oga* also deregulates numerous genes linked to cell proliferation and metabolism, with a particular interest for *Nr3c1*, a gene involved in intrauterine programming of the HPA axis [40**]. As a consequence, the *Oga* null mice show a general perturbation of insulin-glucose homeostasis. A key homeobox protein involved in pituitary development, Otx2, is also *O*-GlcNAcylated but the role of the modification remains unknown [49]. Furthermore, secretion of orexin A and B by the hypothalamic orexin neuron, notably involved in sleep/

wake and feeding behaviors, is regulated by a *O*-GlcNAc cycling at the pre-Orexin *Hrct* locus [50]. Production of Orexin stimulates orexigenic neurons such as the AgRP neurons, which are localized in the hypothalamus and stimulate metabolism. Interestingly, in AgRP neurons, removal of *Ogt* promotes the browning of white adipose tissue and protects mice again diet-induced obesity and insulin resistance [51]. Taken together, these findings highlight the emerging role of *O*-GlcNAcylation as key player in brain-induced metabolic deregulation. *O*-GlcNAcylation is therefore a key regulator important for stem cell biology, neurogenesis and epigenetics and therefore is uniquely poised to integrate nutritional information during early development in the intrauterine environment.

Conclusion

Proper dietary balance has long been known to be critical for the maintenance of human health. Malnutrition, such as rapid increase in glucose or fat consumption, disturbs this equilibrium, with many consequences leading to the progression of human diseases linked to nutrient excess. As a nutrient sensor, *O*-GlcNAcylation is one of the key homeostatic mechanisms responding to dietary imbalance [52]. Such imbalance can occur due to the supplementation of our modern diet with high fructose corn syrup or by an overabundance of protein-rich animal protein and fat. Imbalance can also be introduced by dietary supplementation in such as the widespread use of glucosamine supplements that directly feed into the hexosamine biosynthetic pathway driving the *O*-GlcNAc modification. As demonstrated in this review, *O*-GlcNAcylation may also be altered by epigenetic phenomena including random and incomplete X-inactivation in females [53]. *O*-GlcNAc cycling may now be considered to be a major mediator of diet-responsive signaling and must be considered when studying nutrition-responsive diseases. Diseases linked to over-nutrition including obesity, type-2 diabetes, Alzheimer's disease and cancer have all been linked to aberrant *O*-GlcNAcylation. As we have argued here, the intrauterine environment where embryonic development proceeds is a locus where *O*-GlcNAc may exert its most direct effect on future disease susceptibility for the developing child. Future research is aimed at understanding how the homeostatic *O*-GlcNAc cycling pathway has evolved to buffer the developing fetus from metabolic perturbations at this most critical stage in human development.

Acknowledgments

We would like to thank Drs. Dona Love, Lara Abramowitz, and Michelle Bond for helpful discussions.

Financial support and sponsorship

This work was supported by the Intramural Program of the National Institutes of Diabetes, Digestive, and Kidney Diseases (NIDDK), National Institutes of Health. Dr. Stephanie Olivier-Van Stichelen received support from the Rotary Foundation Ambassadorial Scholarship from the Rotary Foundation.

References and recommended reading

* of special interest

** of outstanding interest

1. Hanover JA, Krause MW, Love DC. Bittersweet memories: linking metabolism to epigenetics through *O*-GlcNAcylation. Nat Rev Mol Cell Biol. 2012; 13:312–21. [PubMed: 22522719]
2. Hart GW. Three décades of research on O-GlcNAcylation- a major nutrient sensor that régulâtes signaling, transcription and cellular metabolism. Front Endocrinol. 2014; 5:183–190.
3. Bond MR, Hanover JA. A Little Sugar goes a Long Way: The Cell Biology of O-GlcNAc. J Cell Biol. 2015; 208:869–880. [PubMed: 25825515]
4. Medford HM, Chatham JC, Marsh SA. Chronic ingestion of a Western diet increases *O*-linked-β-N-acetylglucosamine (*O*-GlcNAc) protein modification in the rat heart. Life Sci. 2012; 90:883–8. [PubMed: 22575823]
5. Ghosh SK, Bond MR, Love DC, Ashwell GG, Krause MW, Hanover JA. Disruption of O-GlcNAc Cycling in C. elegans Perturbs Nucleotide Sugar Pools and Complex Glycans. Front Endocrinol. 2014; 5:197–205.
6. Lefebvre T, Dehennaut V, Guinez C, et al. Dysregulation of the nutrient/stress sensor *O*-GlcNAcylation is involved in the etiology of cardiovascular disorders, type-2 diabetes and Alzheimer's disease. Biochim Biophys Acta. 2010; 1800:67–79. [PubMed: 19732809]
7. Villeneuve LM, Natarajan R. The role of epigenetics in the pathology of diabetic complications. Am J Physiol Renal Physiol. 2010; 299:F14–25. [PubMed: 20462972]
8. Öst A, Lempradl A, Casas E, et al. Paternal diet defines offspring chromatin state and intergenerational obesity. Cell. 2014; 159:1352–64. [PubMed: 25480298]
9*. Lewis BA, Hanover JA. *O*-GlcNAc and the epigenetic regulation of gene expression. J Biol Chem. 2014; 289:34440–8. This Article summarizes current knowledge of *O*-GlcNAc and epigenetic regulation. [PubMed: 25336654]
10. Love DC, Ghosh S, Mondoux MA, et al. Dynamic *O*-GlcNAc cycling at promoters of Caenorhabditis elegans genes regulating longevity, stress, and immunity. Proc Natl Acad Sci USA. 2010; 107:7413–8. [PubMed: 20368426]
11. Sakabe K, Wang Z, Hart GW. Beta-N-acetylglucosamine (*O*-GlcNAc) is part of the histone code. Proc Natl Acad Sci USA. 2010; 107:19915–20. [PubMed: 21045127]
12. Fujiki R, Hashiba W, Sekine H, et al. GlcNAcylation of histone H2B facilitates its monoubiquitination. Nature. 2011; 480:557–60. [PubMed: 22121020]
13. Xu Q, Yang C, Du Y, et al. AMPK regulates histone H2B *O*-GlcNAcylation. Nucleic Acids Res. 2014; 42:5594–604. [PubMed: 24692660]
14. He Y, Roth C, Turkenburg JP, Davies GJ. Three-dimensional structure of a Streptomyces sviceus GNAT acetyltransferase with similarity to the C-terminal domain of the human GH84 *O*-GlcNAcase. Acta Crystallogr D Biol Crystallogr. 2014; 70:186–95. [PubMed: 24419391]
15. Zhang Q, Liu X, Gao W, et al. Differential regulation of the ten-eleven translocation (TET) family of dioxygenases by *O*-linked β-N-acetylglucosamine transferase (OGT). J Biol Chem. 2014; 289:5986–96. [PubMed: 24394411]
16. Harwood KR, Hanover JA. Nutrient-driven *O*-GlcNAc cycling - think globally but act locally. J Cell Sci. 2014; 127:1857–67. [PubMed: 24762810]
17. Myers SA, Panning B, Burlingame AL. Polycomb repressive complex 2 is necessary for the normal site-specific *O*-GlcNAc distribution in mouse embryonic stem cells. Proc Natl Acad Sci US A. 2011; 108:9490–5.
18. Medford HM, Porter K, Marsh SA. Immediate effects of a single exercise bout on protein *O*-GlcNAcylation and chromatin regulation of cardiac hypertrophy. Am J Physiol Heart Circ Physiol. 2013; 305:H114–23. [PubMed: 23624624]
19. Cox EJ, Marsh SA. Exercise and diabetes have opposite effects on the assembly and *O*-GlcNAc modification of the mSin3A/HDAC1/2 complex in the heart. Cardiovasc Diabetol. 2013; 12:101. [PubMed: 23835259]
20*. Ito R, Katsura S, Shimada H, et al. TET3-OGT interaction increases the stability and the presence of OGT in chromatin. Genes Cells. 2014; 19:52–65. This paper demonstrates that the TET3-OGT interactions stabilize OGT on chromatin. [PubMed: 24304661]
21. Bauer C, Göbel K, Nagaraj N, et al. Phosphorylation of TET Proteins Is Regulated via *O*-GlcNAcylation by the *O*-Linked N-Acetylglucosamine Transferase (OGT). J Biol Chem. 2015; 290:4801–12. [PubMed: 25568311]

22. Sinclair DA, Syrzycka M, Macauley MS, et al. Drosophila *O*-GlcNAc transferase (OGT) is encoded by the Polycomb group (PcG) gene, super sex combs (sxc). Proc Natl Acad Sci US A. 2009; 106:13427–32.

23. Gambetta MC, Oktaba K, Müller J. Essential role of the glycosyltransferase sxc/Ogt in polycomb repression. Science. 2009; 325:93–6. [PubMed: 19478141]

24. Gambetta MC, Müller J. *O*-GlcNAcylation prevents aggregation of the Polycomb group repressor polyhomeotic. Dev Cell. 2014; 31:629–39. [PubMed: 25468754]

25. Love DC, Krause MW, Hanover JA. *O*-GlcNAc cycling: emerging roles in development and epigenetics. Semin Cell Dev Biol. 2010; 21:646–54. [PubMed: 20488252]

26. Olivier-Van Stichelen S, Abramowitz LK, Hanover JA. X marks the spot: does it matter that *O*-GlcNAc transferase is an X-linked gene? Biochem Biophys Res Commun. 2014; 453:201–7. [PubMed: 24960196]

27. McGraw S, Oakes CC, Martel J, et al. Loss of DNMT1o disrupts imprinted X chromosome inactivation and accentuates placental defects in females. PLoS Genet. 2013; 9:e1003873. [PubMed: 24278026]

28. Splinter E, de Wit E, Nora EP, et al. The inactive X chromosome adopts a unique three-dimensional conformation that is dependent on Xist RNA. Genes Dev. 2011; 25:1371–83. [PubMed: 21690198]

29. Calabrese JM, Sun W, Song L, et al. Site-specific silencing of regulatory elements as a mechanism of X inactivation. Cell. 2012; 151:951–63. [PubMed: 23178118]

30. Dubois A, Deuve JL, Navarro P, et al. Spontaneous reactivation of clusters of X-linked genes is associated with the plasticity of X-inactivation in mouse trophoblast stem cells. Stem Cells. 2014; 32:377–90. [PubMed: 24115267]

31. Mugford JW, Starmer J, Williams RL, et al. Evidence for local regulatory control of escape from imprinted X chromosome inactivation. Genetics. 2014; 197:715–23. [PubMed: 24653000]

32**. Howerton CL, Morgan CP, Fischer DB, Bale TL. *O*-GlcNAc transferase (OGT) as a placental biomarker of maternal stress and reprogramming of CNS gene transcription in development. Proc Natl Acad Sci U S A. 2013; 110:5169–74. This paper argues that OGT is a biomarker of maternal Stress and reprogramming of gene expression in the intrauterine environment. [PubMed: 23487789]

33. Mao J, Zhang X, Sieli PT, et al. Contrasting effects of different maternal diets on sexually dimorphic gene expression in the murine placenta. Proc Natl Acad Sci US A. 2010; 107:5557–62.

34. Ma J, Hart GW. Protein *O*-GlcNAcylation in diabetes and diabetic complications. Expert Rev Proteomics. 2013; 10:365–80. [PubMed: 23992419]

35. Samuelsson A-MM, Matthews PA, Jansen E, et al. Sucrose feeding in mouse pregnancy leads to hypertension, and sex-linked obesity and insulin resistance in female offspring. Front Physiol. 2013; 4:14. [PubMed: 23423541]

36. Hale M, Xu P, Scardina J, et al. Differential gene expression in male and female rainbow trout embryos prior to the onset of gross morphological differentiation of the gonads. BMC Genomics. 201110.1186/1471-2164-12-404

37*. Olivier-Van Stichelen S, Hanover JA. X-inactivation normalizes *O*-GlcNAc transferase levels and generates an *O*-GlcNAc-depleted Barr body. Front Genet. 2014; 5:256. This work describes the regulation OGT by X-inactivation and its absence in the Barr body resulting from X-inactivation. [PubMed: 25136351]

38*. Hewagama A, Gorelik G, Patel D, et al. Overexpression of X-linked genes in T cells from women with lupus. J Autoimmun. 2013; 41:60–71. This paper shows that OGT is overexpressed in T cells from women with lupus. [PubMed: 23434382]

39. Pantaleon M, Tan HY, Kafer GR, Kaye PL. Toxic effects of hyperglycemia are mediated by the hexosamine signaling pathway and o-linked glycosylation in early mouse embryos. Biol Reprod. 2010; 82:751–8. [PubMed: 20032283]

40**. Keembiyehetty C, Love DC, Harwood KR, et al. Conditional Knock-out Reveals a Requirement for *O*-Linked N-Acetylglucosaminase (*O*-GlcNAcase) in Metabolic Homeostasis. J Biol Chem. 2015; 290:7097–113. This paper demonstrates a role for *O*-GlcNAcase in metabolic homeostasis

and evidence that *O*-GlcNAc cycling impacts the Hypothalamic-Pituitary-Adrenal axis in mice. [PubMed: 25596529]

41. Webster DM, Teo CF, Sun Y, et al. *O*-GlcNAc modifications regulate cell survival and epiboly during zebrafish development. BMC Dev Biol. 2009; 9:28. [PubMed: 19383152]
42. Jang H, Kim TW, Yoon S, et al. *O*-GlcNAc regulates pluripotency and reprogramming by directly acting on core components of the pluripotency network. Cell Stem Cell. 2012; 11:62–74. [PubMed: 22608532]
43. Pardo M, Lang B, Yu L, et al. An expanded Oct4 interaction network: implications for stem cell biology, development, and disease. Cell Stem Cell. 2010; 6:382–95. [PubMed: 20362542]
44. Kim H-SS, Park SY, Choi YR, et al. Excessive *O*-GlcNAcylation of proteins suppresses spontaneous cardiogenesis in ES cells. FEBS Lett. 2009; 583:2474–8. [PubMed: 19591829]
45. Maury JJ, Chan KK, Zheng L, et al. Excess of *O*-linked N-acetylglucosamine modifies human pluripotent stem cell differentiation. Stem Cell Res. 2013; 11:926–37. [PubMed: 23859804]

46*. Speakman CM, Domke TC, Wongpaiboonwattana W, et al. Elevated *O*-GlcNAc levels activate epigenetically repressed genes and delay mouse ESC differentiation without affecting naïve to primed cell transition. Stem Cells. 2014; 32:2605–15. This paper shows that *O*-GlcNAc cycling may impact ESC differentiation. [PubMed: 24898611]

47. Mauras N, Mazaika P, Buckingham B, et al. Longitudinal Assessment of Neuroanatomical and Cognitive Differences in Young Children with Type 1 Diabetes: Association with Hyperglycemia. Diabetes. 201410.2337/db14-1445

48**. Howerton CL, Bale TL. Targeted placental deletion of OGT recapitulates the prenatal stress phenotype including hypothalamic mitochondrial dysfunction. Proc Natl Acad Sci US A. 2014; 111:9639–44. This paper demonstrate that OGT deletion leads to a prenatal stress phenotype including altered hypothalamic function.

49. Ortiz-Meoz RF, Merbl Y, Kirschner MW, Walker S. Microarray discovery of new OGT substrates: the medulloblastoma oncogene OTX2 is *O*-GlcNAcylated. J Am Chem Soc. 2014; 136:4845–8. [PubMed: 24580054]
50. Hayakawa K, Hirosawa M, Tabei Y, et al. Epigenetic switching by the metabolism-sensing factors in the generation of orexin neurons from mouse embryonic stem cells. J Biol Chem. 2013; 288:17099–110. [PubMed: 23625921]
51. Ruan H-BB, Dietrich MO, Liu Z-WW, et al. *O*-GlcNAc transferase enables AgRP neurons to suppress browning of white fat. Cell. 2014; 159:306–17. [PubMed: 25303527]
52. Bond MR, Hanover JA. *O*-GlcNAc cycling: a link between metabolism and chronic disease. Annu Rev Nutr. 2013; 33:205–29. [PubMed: 23642195]
53. Abramowitz LK, Olivier-Van Stichelen S, Hanover JA. Chromosome imbalance as a driver of sex disparity in disease. J Genomics. 2014; 2:77–88. [PubMed: 25031659]

Key Points

- *O*-GlcNAc is a nutrient-responsive posttranslational modification of proteins involved in transcription, translation, protein stability and interaction. It also interacts with numerous epigenetic regulators.
- *O*-GlcNAc Transferase (OGT), which catalyzes *O*-GlcNAc addition, is an X-linked gene subject to strict regulation by X-inactivation in females.
- The human *O*-GlcNAcase (OGA) gene is a susceptibility locus for Type 2 diabetes and obesity and is subject to regulation by higher order chromatin structure.
- Altered *O*-GlcNAc cycling has been linked to chronic human diseases including Alzheimer's disease, Obesity, Diabetes, Systemic Lupus Erythematosus, Cardiovascular disease, and Cancer.
- *O*-GlcNAc metabolism may be particularly important in the intrauterine environment where OGT is a key biomarker for placental stress linked to subsequent disease susceptibility.

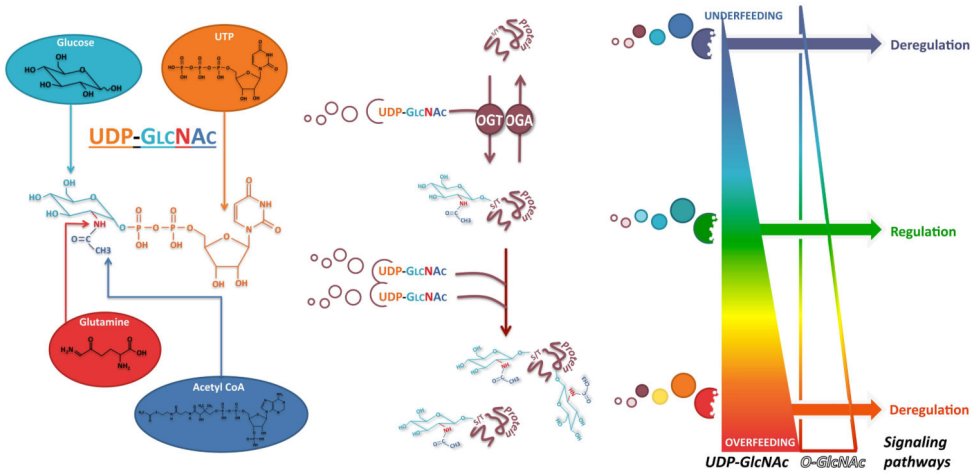

Figure 1. The Hexosamine Signaling Pathway terminating in dynamic *O*-GlcNAc cycling

O-GlcNAc cycling is a nutrient-dependent modification capable of generating a graded signaling output to a multitude of simultaneous targets. Directly supplied by the breakdown of food, 2 to 3% of glucose entering cells is driven to the **H**exosamine **B**iosynthetic **P**athway to generate UDP-GlcNAc using other nutrient derived precursors. The breakdown of foods to produce metabolites is represented by the stylized bubbles. *O*-GlcNAc Transferase (OGT) uses the varying levels of UDP-GlcNAc to *O*-GlcNAcylate serine or threonine residue of diverse intracellular proteins (nuclear, cytoplasmic or mitochondrial). The *O*-GlcNAcase (OGA) hydrolyzes the GlcNAc moiety and creates a dynamic posttranslational protein modification. This, in turn, impacts canonic phosphorylation-dependent signaling cascades, alters protein stability, and may cause direct enzyme activation. Thus, levels of food-derived metabolites are integrated, amplified and transduced by the Hexosamine signaling pathway.

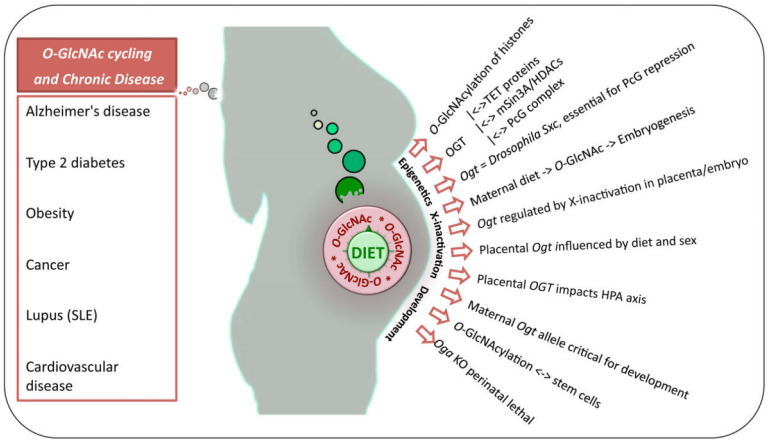

Figure 2. Diet-responsive *O*-GlcNAcylation is linked to the Pathophysiology of Human disease and may influence Disease Susceptibility in the Developing Fetus and influence its Germline

Dietary metabolites act through *O*-GlcNAc cycling to impact epigenetics, X-inactivation and development through *O*-GlcNAcylation. This diagram represents some of the major recent discoveries in these different fields. The breakdown of foods to produce metabolites is represented by the stylized bubbles. O-GlcNAc cycling in response to nutrient flux may impact chronic diseases (panel on left). The epigenetic factors are also particularly important in the intrauterine environment where maternal-fetal communication occurs during embryonic development. Thus, imbalanced *O*-GlcNAcylation is not only associated with disease in adulthood. By acting in the intrauterine environment, Imbalanced *O*-GlcNAc cycling may influence disease susceptibility in subsequent generations. (Abbreviations: SLE- Systemic Lupus Erythematosus, PcG- Polycomb Group, OGT- *O*-GlcNAc Transferase, Sxc-*Super Sex Combs.)*