Introduction

Glucose is incorporated into the cells via glucose transporter (GLUT), and is initially converted to glucose-6-phosphate by hexokinase (Fig. 1)1). Approximately 1-3% of the glucose-6-phosphate is metabolized via the hexosamine biosynthetic pathway after conversion to fructose-6-phosphate2), although the majority of the incorporated glucose is metabolized by glycolysis and is utilized as an energy source to produce ATP (Fig. 1)1). In the hexosamine biosynthetic pathway, the fructose-6-phosphate is first converted to glucosamine-6-phosphate by glutamine:fructose-6-phosphate amidotransferase (GFAT), the rate-limiting enzyme of the hexosamine biosynthetic pathway; and the glucosamine-6-phosphate is then acetylated by glucosamine-6-phosphate N-acetyltransferase (GNA) to form N-acetylglucosamine (GlcNAc)-6-phosphate, which is isomerized to GlcNAc-1-phosphate and uridinylated by the action of uridine-5′-diphosphate (UDP)-GlcNAc pyrophosphorylase (UAP) (Fig. 1)1). The synthesized UDP-GlcNAc is partially transported into the Golgi apparatus via the UDP-GlcNAc transporter3), and is then utilized as a donor substrate for the N-linked glycosylation (N-glycosylation) and O-linked glycosylation of extracellular and membrane proteins; alternatively, it is also utilized in the cytosol for the O-linked GlcNAc (O-GlcNAc) modification (O-GlcNAcylation) of intracellular proteins (Fig. 1)1).

Recently, it was reported that regular exercise training modulates the hexosamine biosynthetic pathway and O-GlcNAcylation1). However, the relationships among exercise, the hexosamine biosynthetic pathway, and glycosylation as well as their physiological significances still remain unclear. In this short review, therefore, the authors discuss this unknown area focusing mainly on the relationships among exercise, the hexosamine pathway, O-GlcNAcylation, and insulin resistance.

N-glycosylation

Glycosylation, one of the most abundant posttranslational modification reactions, is necessary for protein stability, as well as the localization and trafficking of proteins. Approximately 50% of proteins in mammalian cells are modified with a variety of glycans, which are classified into two major groups, N- and O-glycans5). N-glycans are attached to certain asparagine residues of proteins with the Asn-X-Ser/Thr motif, whereas O-glycans are attached to a subset of serine and threonine residues6). The addition of GlcNAc from UDP-GlcNAc to N- and O-glycans is catalyzed by enzymatic activity of various types of GlcNAc transferase (GnT) localized in the Golgi appa-
The formation of branched structures and resultant elongation and processing of N-glycans by GnT modulate the physiological functions of the glycoproteins, and are associated with a variety of disorders, such as cancer and diabetes mellitus.

Among different types of GnT, GnT-V catalyzes the transfer of a single GlcNAc from UDP-GlcNAc to a α1,3-mannose in N-glycans to form a β1,4 GlcNAc branch (Fig. 2). A loss of GnT-IVα attenuates the half-life of GLUT2 on the pancreatic β-cell surface, and resultant impairment of glucose-stimulated insulin secretion leads to metabolic dysfunctions that are characteristics of type 2 diabetes. Furthermore, chronic high-fat diet feeding of mice reduces the expression and nuclear localization of the transcription factors, forkhead box protein A2 (FOXA2) and hepatocyte nuclear factor 1 homeobox A (HNF1A), resulting in a deficiency of GnT-IVa expression in the pancreatic β-cells, and such a pathogenic process is observed also in human islet cells from patients with type 2 diabetes.

On the contrary, GnT-III catalyzes the transfer of a single GlcNAc from UDP-GlcNAc in a β1,4-linkage to the mannose residue at the base of the trimannosyl core in N-glycans to form a so-called “bisecting GlcNAc” (Fig. 2). The introduction of a bisecting GlcNAc prevents the formation of branched structures in N-glycans, because other GnT are not able to utilize an N-glycan with a bisecting GlcNAc as an acceptor substrate. Actually, the overexpression of GnT-III blocks integrin α3β1-mediated cell migration on laminin 5 in GnT-V transfectant. Conversely, the knockdown of GnT-III increases cell migration as well as β1,6-branched N-glycan levels on integrin αv subunits.

In particular, the intracellular UDP-GlcNAc level modulates N-glycosylation patterns, because GnT have different Km values for UDP-GlcNAc. Among the different types of GnT, GnT-V and GnT-IV have much higher Km values, and their enzymatic activities are enhanced in response to increased intracellular UDP-GlcNAc levels. In fact, β1,6-branched N-glycan levels in Jurkat T-cells are elevated by supplementation of metabolic precursors of the hexosamine biosynthetic pathway, which regulates autoimmunity reactions of T-cells. Thus, glycosylation is affected not only by the levels of expression of a single GnT enzyme, but also by a variety of other factors, including flux via the hexosamine biosynthetic pathway.

**O-GlcNAcylation**

O-GlcNAcylated is a highly reversible, inducible and dynamic post-translational modification, which mediates the addition of GlcNAc from UDP-GlcNAc to serine and/or threonine residues of cytosolic and nuclear proteins. To date, more than 600 proteins have been identified as substrate proteins for O-GlcNAcylated, and are associated with a variety of cellular processes, including cell proliferation, transcription, signal transduction, and metabolism. This modification is reversibly regulated by the enzymatic activity of O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA). OGT catalyzes the transfer of a single GlcNAc from UDP-GlcNAc to a α1,3-mannose in N-glycans to form a β1,4 GlcNAc branch (Fig. 2). A loss of GnT-IVα attenuates the half-life of GLUT2 on the pancreatic β-cell surface, and resultant impairment of glucose-stimulated insulin secretion leads to metabolic dysfunctions that are characteristics of type 2 diabetes.

Furthermore, chronic high-fat diet feeding of mice reduces the expression and nuclear localization of the transcription factors, forkhead box protein A2 (FOXA2) and hepatocyte nuclear factor 1 homeobox A (HNF1A), resulting in a deficiency of GnT-IVa expression in the pancreatic β-cells, and such a pathogenic process is observed also in human islet cells from patients with type 2 diabetes.
transfer of a single GlcNAc from UDP-GlcNAc to serine/threonine residues of substrate proteins\textsuperscript{25,26}, whereas OGA catalyzes the removal of the GlcNAc from such residues\textsuperscript{27,28}. Since the affinity of OGT for substrate proteins is highly sensitive to the intracellular UDP-GlcNAc level\textsuperscript{29}, glucose flux via the hexosamine biosynthetic pathway directly influences O-GlcNAc levels. Indeed, hyperglycemia\textsuperscript{30}, glucosamine infusion\textsuperscript{30}, GLUT overexpression\textsuperscript{31}, and GFAT overexpression\textsuperscript{32}, all of which increase flux via the hexosamine biosynthetic pathway, result in increased intracellular UDP-GlcNAc and O-GlcNAc levels.

It is suggested that excess flux via the hexosamine biosynthetic pathway in adipocytes is one mechanism for the development of insulin resistance. Actually, irreversible inactivation of glutamine-requiring enzymes, including GFAT, by treatment with glutamine analogues improves hyperglycemia\textsuperscript{30}, glucosamine infusion\textsuperscript{30}, GLUT overexpression\textsuperscript{31}, and GFAT overexpression\textsuperscript{32}, all of which increase flux via the hexosamine biosynthetic pathway, result in increased intracellular UDP-GlcNAc and O-GlcNAc levels.

In addition, O-GlcNAcylation catalyzed by the actions of OGT and OGA. OGT catalyzes the transfer of a single GlcNAc from UDP-GlcNAc to serine/threonine residues of substrate proteins\textsuperscript{25,26}, whereas OGA catalyzes the removal of the GlcNAc from such residues\textsuperscript{27,28}. The affinity of OGT for substrate proteins is highly sensitive to the intracellular UDP-GlcNAc level\textsuperscript{29}, GlcNAc, N-acetylglucosamine; GnT, GlcNAc transferase; Asn, asparagine.

levels, indicating that cross-talk between adipocytes and skeletal muscle cells contributes to the development of whole body insulin resistance\textsuperscript{25}. In contrast, transgenic mice overexpressing GLUT1 in skeletal muscle show impaired insulin-stimulated glucose transport activity in skeletal muscle\textsuperscript{37}. The discrepancy in results between GFAT and GLUT1 transgenic mouse models indicates that the hyperglycemia-induced impairment of insulin-stimulated glucose uptake is mediated by increased flux via the hexosamine biosynthetic pathway as well as other mechanisms\textsuperscript{34,38}, such as accumulation of excess glyco-}

Since O-GlcNAcylation is abundantly identified on signaling proteins, including insulin signaling, and antagonizes O-phosphorylation of certain proteins, such as insulin receptor substrate 1 (IRS-1), it is also suggested that accumulation of UDP-GlcNAc, the end product of the hexosamine biosynthetic pathway, mediates the development of insulin resistance partially via elevating O-GlcNAc levels on such signaling proteins\textsuperscript{24}. In fact, transgenic mice overexpressing OGT in adipose tissue and striated muscle show decreased insulin-stimulated whole-body glucose disposal, and hyperinsulinemia and hyperleptinemia\textsuperscript{39}, which are consistent with the phenotypes observed in GFAT transgenic mice\textsuperscript{24,38}. Furthermore, insulin stimulation recruits OGT from the nucleus to the plasma membrane, wherein OGT catalyzes O-GlcNAcylation on some insulin signaling proteins and attenuates its signal transduction by altering their phosphorylation status, and hepatic overexpression of OGT impairs the transcription of insulin-responsive genes and causes the perturbation of insulin-induced inhibition of gluconeo-
On the other hand, pharmacological approaches are carried out to address whether the inhibition of OGA enzymatic activity and resultant elevated O-GlcNAc levels lead to the development of insulin resistance. Competitive inhibition of OGA glycosidase activity using a GlcNAc analogue O-(2-acetamido-2-deoxy-D-glucopyranosylidene)-amino-N-phenylcarbamate (PUG-Nac) results in elevation of global O-GlcNAc levels, and suppresses insulin-stimulated glucose uptake and Akt phosphorylation in 3T3-L1 adipocytes. However, such insulin-desensitizing effects are not observed when 3T3-L1 adipocytes are treated with 1,2-dideoxy-2-propyl-α-D-glucopyranosyl-[2,1-D]-Δ2'-thiazoline (NButGT), a more potent and selective OGA inhibitor, and similar results were also obtained in a rodent model, indicating that non-targeted globally elevated O-GlcNAc levels do not independently cause the development of insulin resistance.

In contrast, several genetic and epidemiological approaches imply that OGA enzymatic activity is associated with the development of insulin resistance. An animal experiment using high-fat diet-fed mice and genetically obese and insulin-resistant db/db mice demonstrates that hepatic overexpression of OGA ameliorates the elevated plasma glucose level and promotes O-GlcNAc removal from the transducer of regulated cyclic adenosine monophosphate response element-binding protein (CREB) 2 (CRTC2), indicating that OGA improves glucose tolerance and insulin sensitivity via the reduction of O-GlcNAc levels on the insulin signaling protein. In addition, a single nucleotide polymorphism (SNP) of the human MGEA5 gene, encoding OGA, is suggested to be a potential risk factor for type 2 diabetes in Mexican Americans.

Exercise, the hexosamine biosynthetic pathway, and glycosylation

Little is known so far how exercise affects the hexosamine biosynthetic pathway and glycosylation, and whether the ameliorating and preventive effects of exercise on a variety of pathophysiological processes, including insulin resistance, are mediated via modulating the metabolic pathway and glycosylation. Nelson et al. conducted the first study to address whether exercise reduces glucose flux via the hexosamine biosynthetic pathway and mediates exercise-stimulated glucose uptake in hindlimb skeletal muscle. They demonstrated that an acute bout of swim exercise with high intensity significantly increases UDP-N-acetylhexosamine (HexNAc) levels post-exercise in hindlimb skeletal muscle in ad libitum fed rats but not in fasted rats. In addition, an in vitro assay using extracts from hindlimb skeletal muscle revealed that this type of exercise does not change the enzymatic activity of GFAT, indicating that the expression level of GFAT is not affected. Thus, it is concluded that the hexosamine biosynthetic pathway is not involved in the exercise-stimulated glucose uptake in skeletal muscle. However, it still remains unclear how exercise affects O-GlcNAc levels on signaling proteins and transcription factors downstream of the insulin receptor, and expression levels of enzymatic activities of OGT and OGA in the skeletal muscle. In addition, it is also unclear whether exercise affects the hexosamine biosynthetic pathway and O-GlcNAcylation in obese and diabetic animals with increased glucose flux via the hexosamine biosynthetic pathway.

Furthermore, it is of critical importance to clarify the effects of different types and doses of exercise on the hexosamine biosynthetic pathway and glycosylation. Actually, a more recent study by Belke demonstrated that regular swim exercise training in mice decreases O-GlcNAc levels on cardiac proteins, including the transcription factor, specificity protein 1 (Sp1), accompanied by reduced expression levels of GFAT2, OGT, and OGA, some of which are shown not to be affected by an acute bout of swim exercise. These results strongly suggest

![Figure 4](https://example.com/figure4.png)

**Fig. 4** Summary of the effects of exercise on the hexosamine biosynthetic pathway and O-GlcNAcylation. An acute bout of swim exercise increases UDP-HexNAc levels post-exercise in hindlimb skeletal muscle in ad libitum fed rats, without changing GFAT expression levels, although it is unclear how the exercise influences O-GlcNAcylation. The effects of an acute swim exercise on the hexosamine biosynthetic pathway and O-GlcNAcylation in adipose tissue and liver are not reported. On the other hand, regular swim exercise training decreases the levels of GFAT, OGT, OGA, and O-GlcNAc in heart in mice. However, the effects of regular swim exercise training on the hexosamine biosynthetic pathway and O-GlcNAcylation in skeletal muscle, adipose tissue, and liver are not reported. GFAT, glutamine:fructose-6-phosphate amidotransferase; UDP, uridine-5'-diphosphate; HexNAc, N-acetylhexosamine; GlcNAc, N-acetylglucosamine; OGT, O-linked GlcNAc transferase; OGA, O-GlcNAcase; O-GlcNAc, O-linked GlcNAc.
that the reduced biosynthetic activity of UDP-GlcNAc and the attenuated enzymatic activity for the O-GlcNAcylation cycle in response to regular exercise training synergistically lead to the decreased O-GlcNAc levels in the heart. Thus, it is interesting to address systematically the effects of regular exercise training on flux via the hexosamine biosynthetic pathway and O-GlcNAc levels in skeletal muscle, adipose tissue, and liver, all of which are important sites to develop insulin resistance. Indeed, increased responsiveness to insulin stimulation via the IRS/phosphoinositide 3-kinase (PI3K)/Akt O-phosphorylation signaling pathway is observed in adipocytes isolated from exercise-stimulated plasma glucose clearance is impaired in endurance exercise-trained rats, and reduced insulin signaling in adipocytes inhibit phosphorylation of Tyr-X-X-Met, a PI3K p85 subunit binding motif, in IRS-1 and results in reduced insulin signaling. On the other hand, treadmill exercise-stimulated plasma glucose clearance is impaired by glucosamine infusion, indicating a possible link between exercise and the hexosamine biosynthetic pathway.

Conclusion

The findings obtained to date on the effects of exercise on the hexosamine biosynthetic pathway and O-GlcNAcylation are summarized in Fig. 4. As illustrated in the figure, there are still a lot of unclear points. It is also interesting to address how exercise modulates other types of glycosylation, including N-glycosylation. The elucidation of these unclear points may lead to further understanding of molecular mechanisms of the ameliorating and preventive effects of exercise on a variety of pathophysiological processes, including insulin resistance.

References

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