Review

O-GlcNAcylation: The Underestimated Emerging Regulators of Skeletal Muscle Physiology

Yang Liu †, Ya-Jie Hu †, Wen-Xuan Fan, Xin Quan, Bin Xu * and Shi-Ze Li *

National Experimental Teaching Demonstration Center of Animal Medicine Foundation, College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing 163319, China;
lymissive@163.com (Y.L.); huyajiess@163.com (Y.-J.H.); 15945687925@163.com (W.-X.F.); 13359612081@163.com (X.Q.)
* Correspondence: xubin@byau.edu.cn (B.X.); byndlsz@163.com (S.-Z.L.)
† These authors contributed equally to this work.

Abstract: O-GlcNAcylation is a highly dynamic, reversible and atypical glycosylation that regulates the activity, biological function, stability, sublocation and interaction of target proteins. O-GlcNAcylation receives and coordinates different signal inputs as an intracellular integrator similar to the nutrient sensor and stress receptor, which target multiple substrates with spatio-temporal analysis specifically to maintain cellular homeostasis and normal physiological functions. Our review gives a brief description of O-GlcNAcylation and its only two processing enzymes and HBP flux, which will help to better understand its physiological characteristics of sensing nutrition and environmental cues. This nutritional and stress-sensitive properties of O-GlcNAcylation allow it to participate in the precise regulation of skeletal muscle metabolism. This review discusses the mechanism of O-GlcNAcylation to alleviate metabolic disorders and the controversy about the insulin resistance of skeletal muscle. The level of global O-GlcNAcylation is precisely controlled and maintained in the “optimal zone”, and its abnormal changes is a potential factor in the pathogenesis of cancer, neurodegeneration, diabetes and diabetic complications. Although the essential role of O-GlcNAcylation in skeletal muscle physiology has been widely studied and recognized, it still is underestimated and overlooked. This review highlights the latest progress and potential mechanisms of O-GlcNAcylation in the regulation of skeletal muscle contraction and structural properties.

Keywords: O-GlcNAc; skeletal muscle; metabolism; insulin resistance; sarcomere contraction and structure

1. Introduction

The proteome is constantly changing and finding harmony with the needs of the organism and its cells, and various PTMs play a unique physiological function in these processes [1–3]. Glycosylation, the most extensive and diverse forms of PTMs in eukaryotic cells, contains different types of glycosylation pathways, involves complex metabolic networks and greatly amplifies the proteome by producing the multiple protein forms to instruct a myriad of functions [4,5]. O-GlcNAcylation is a dynamic, reversible and atypical glycosylation [6]. As its full name O-linked β-N-acetylglucosamine modification indicates, it involves the binding of a single GlcNAc to the serine and threonine residues of the target protein via a β-configuration O-glycosidic bond [7]. The precise dynamic homeostasis of O-GlcNAcylation is accurately completed only by OGT and OGA [8]. UDPGlcNAc is the only donor substrate of O-GlcNAcylation, which comes from HBP and responds to carbohydrate metabolism, fatty acid metabolism, protein metabolism and nucleotide metabolism [9,10]. It is worth noting that O-GlcNAcylation is also unique from other classical glycosylation in other aspects [11], and the enzymatic mechanism, amino acid residue sequence and conformation involved in O-GlcNAcylation are shown in Table S1.
O-GlcNAcylation exists in almost all organisms and is extremely conserved in filamentous fungi, worms, insects, plants and humans [12]. O-GlcNAcylation is also found in all major human organs, even in saliva and urine [13]. O-GlcNAcylation is abundant in the brain, liver, pancreas, skeletal muscle, adipose tissue and other organs and tissues, and plays an essential regulatory role in their physiology and pathology [7,14]. The species distribution of O-GlcNAcylation and its tissues distribution in Homo sapiens are illustrated in Figure 1. O-GlcNAcylation is present in almost all cellular compartments, such as the nucleus, cytoplasm, cytomembrane and mitochondria [15,16]. The distribution characteristics of O-GlcNAcylation also mean that almost all functions of proteins in regulating various cellular processes are covered [17]. O-GlcNAcylated proteins are grouped by protein function as shown in Figure 2. In the past 40 years since O-GlcNAcylation was first discovered, the O-GlcNAcylation of protein has been deeply understood and fruitful results have been obtained. Nearly 5000 human proteins and more than 7000 O-GlcNAcylated sites have been identified in thousands of related research studies [13,18]. O-GlcNAcylation affects the activity, stability, sublocation and biological function of target proteins. The abundance and cycle time scale of O-GlcNAcylation are very similar to that of phosphorylation [19]. Indeed, O-GlcNAcylation has surprisingly extensive crosstalk and forms a yin–yang relationship with phosphorylation, as do acetylation, ubiquitination and other PTMs [20,21]. Crosstalk between O-GlcNAcylation and these PTMs is shown in Box 1. O-GlcNAcylation receives and integrates metabolic signal pathway inputs from different partners to perceive external environmental disturbance, and ultimately induces adaptive molecular and physiological responses by targeting multiple substrates with the time-space specificity [22,23]. This physiological property makes O-GlcNAcylation extremely sensitive to nutrient availability and environmental changes and to become a nutrient sensor and stress receptor, thus participating in many biological processes [24]. For instance, O-GlcNAcylation plays a cytoprotective role under adverse conditions such as cold stress and oxidative stress [25–27]. O-GlcNAcylation helps to ensure the normal physiological function of cells by regulating a series of cellular processes, such as signal transduction [28,29], transcription [19,30], translation [31,32], autophagy [33,34], apoptosis [35], immune response [36–38], inflammation [39], chromatin remodeling [40] and metabolic reprogramming [41]. The destruction of the homeostasis of O-GlcNAcylation is closely related to the pathogenesis and progression of multiple diseases [42], such as multiple cancers [43–45], X-linked intellectual disability [46], neurodegenerative diseases [47,48], obesity [49], cardiovascular diseases [50,51], aging [52], diabetes and diabetes’ complications [53,54].

**Box 1.** Extensive crosstalk between O-GlcNAcylation and other PTMs.

More than 400 different types of PTMs individually or mutually regulate almost all aspects of protein function. Most proteins are heavily modified and the combination of various PTMs on a certain protein has explosively expanded its functional range. These PTMs often overlap the same domain on a certain protein and response to various cellular physiological states, and recruit other proteins to bind or regulate their activity. Therefore, the PTM code hypothesis has been proposed. This holds that the combination of multiple PTMs on a given protein produces a dynamic and specific “code” that is “read” and “translated” by the cell to drive complex biological outcomes. The corresponding enzymes involved in PTMs edit the code as editors and erasers to directly regulate protein function or indirectly regulate protein complex formation and signal transduction. In the past few years, innovations in PTMs detection methods have led to a rapid increase in proteomic data. However, the complexity of PTMs and the superficial understanding of thire biological function have hindered the development of this hypothesis. The O-GlcNAcylation plays a unique and important role in the PTM code due to its high sensitivity to cellular environmental and metabolic cues and extensive interactions with other PTMs. This will briefly discuss the crosstalk between O-GlcNAcylation and some common PTMs. The emphasis is on the interaction between O-GlcNAcylation and phosphorylation, and the interaction of O-GlcNAcylation with other PTMs is less well studied.
1. Phosphorylation

The intermodulation of O-GlcNAcylation and phosphorylation has attracted the most attention. O-GlcNAcylation occurs at Ser and Thr residues, which are also potential phosphorylation sites. However, phosphorylation at Tyr residues and O-GlcNAcylation also interact, even this regulation is even more efficient. There are currently four accepted forms of intermodulation between them. That is, the competitive modification at the same site, the alternating modification at different sites, the respective modification at different sites in adjacent regions, and the site-dependent alternating or simultaneous modification. O-GlcNAcylation and phosphorylation at the same or adjacent sites that do not occur simultaneously show negative interactions in a similar “on-off” pattern. Some proteins are simultaneously O-GlcNAcylated and phosphorylated at different sites. In this situation, O-GlcNAcylation often strongly inhibits phosphorylation in specific motifs that have been identified, and vice versa. However, there are also a few examples of O-GlcNAcylation enhanced phosphorylation and their physiological function. Thus, it can be seen that competition and dependence coexist between O-GlcNAcylation and phosphorylation.

In addition to the above way that O-GlcNAcylation directly acts on phosphorylated proteins, O-GlcNAcylation also interacts with phosphorylation by modifying phosphorylation related kinases. OGT and OGA form complexes with kinase and phosphatase. More than 100 kinases have been shown to contain identified O-GlcNAcylation sites, emphasizing the role of O-GlcNAcylation in directly regulating kinase function. A specific suitable example of complex regulation between kinase and O-GlcNAcylation is CaMKIV. CaMKIV contained at least five O-GlcNAcylated sites. CaMKIV is activated by the phosphorylation at Thr200 and the displacement of PP2A. During the activation process, the increase of interaction between CaMKIV and OGA during activation led to the rapid decrease of O-GlcNAcylation level. Inactivated CaMKIV recruited OGT to restore O-GlcNAcylation. CaMKIV also activate OGT by promoting the phosphorylation of OGT. AMPK is another interesting example and is regulated by its own O-GlcNAcylation. AMPK phosphorylates GFAT at Ser243, resulting in decreased GFAT activity to reduce O-GlcNAcylation levels. AMPK phosphorylates OGT at Thr144, which leads to the changes in cell localization and substrate specificity of OGT. O-GlcNAcylation of PTP1B at Ser104, Ser201 and Ser386 increases its enzyme activity, but other phosphatase studies were few.

On balance, O-GlcNAcylation and phosphorylation form a highly dynamic balance and cooperate to complete a variety of complex biological processes, which is widely known as the “Yin-Yang” hypothesis. However, cross dialogue between O-GlcNAcylation and phosphorylation are complex at present. Therefore, it is still impossible to predict in advance how they will interact on a single protein.

2. Acetylation

O-GlcNAcylation of Rela/p65 at Thr305 and Thr315 promotes its acetylation at Lys310, which is necessary for the full transcriptional activity of Rela/p65. Meanwhile, HDAC4 has been shown to be modified by O-GlcNAcylation at Ser342, further demonstrating the direct interaction between O-GlcNAcylation and acetylation. Crosstalk between O-GlcNAcylation and acetylation is also involved in epigenetic regulation. For example, ManNAc induces switching from the inactive state by Ogt-Sirt1 to the active state by Oga, p300, and CBP at the Hcrt gene locus. The histone acetyltransferase domain of OGA also shows that they have an intrinsic reciprocal relationship. SIN3A interacts directly with OGT, which is a histone deacetylase and transcription corepressor. In addition, OGT and OGA are also acetylated, but the effect on their function needs to be further confirmed.

3. Ubiquitylation

O-GlcNAcylation of target proteins prevents their ubiquitination to enhances their own stability. The potential mechanism may be the crosstalk between O-GlcNAcylated protein and phosphorylation to indirectly regulate ubiquitination or directly regulate ubiquitination by recruiting deubiquitylases. O-GlcNAcylation of histone H2B at Ser112 promotes its K120 monoubiquitination to transcriptional activation by using GlcNAc as an anchor of ubiquitin ligase. OGT and OGA are ubiquitinated, but the detailed impact and mechanism need to be further clarified.

4. Methylation

O-GlcNAcylation of histone methyltransferase enhancer of EZH2 at Ser75 enhances its stability to promote the formation of H3K27me3, which helps to regulate gene expression related to skeletal muscle insulin sensitivity, tumor inhibition and neuronal memory formation. TET promotes DNA demethylation by hydroxylating 5mC. It was found that OGT interacted with TET protein to regulate transcription. This interaction provides a link between DNA modification, methylation and O-GlcNAcylation. The effect of this interaction on gene expression varies depending on TET family members and the environment.
Figure 1. Species distribution of O-GlcNAcylation and its tissues’ distribution in Homo sapiens. The universality and conservation of O-GlcNAcylation is self-evident in filamentous fungi, worms, insects, plants and humans. There are more reports on the O-GlcNAcylation in human, mouse or rat, fruit fly and Caenorhabditis elegans species. However, O-GlcNAcylation has not been identified in yeast, and its similar role may be replaced by the O-mannosylation of nucleocytoplasmic proteins in yeast. O-GlcNAcylation was found to occur in almost all major organs of Homo sapiens. This suggests that O-GlcNAcylation is essential for the survival of metazoans and is the root cause of the lethality of OGT and OGA knockout. Tissue distribution analysis emphasizes the strong characteristics of O-GlcNAcylation in the brain and liver of Homo sapiens. Some organs or tissues with less distribution of O-GlcNAcylation may be limited by their own characteristics of less proteins.
Figure 2. Classification, cellular distribution and physiological process of O-GlcNAcylated proteins. Studies on O-GlcNAcylation have been flourishing since its discovery. To date, a total of 5072 O-GlcNAcylated proteins have been identified by various techniques, and 1803 of these proteins contain 7002 different O-GlcNAcylation sites. However, such a large number may still be only a portion of the abundant dynamically modified proteins within the cellular compartments. The universality and conservation of O-GlcNAcylation is self-evident in filamentous fungi, worms, insects, plants and humans. There are more reports on the O-GlcNAcylation in human, mouse or rat, fruit fly and Caenorhabditis elegans species. However, O-GlcNAcylation has not been identified in yeast, and its similar role may be replaced by the O-mannosylation of nucleocytoplasmic proteins in yeast. These O-GlcNAcylated proteins occur in almost all cellular compartments. O-GlcNAcylated proteins are mainly located in the nuclear and cytoplasmic compartments of all metazoans and their infected viruses. Therefore, O-GlcNAcylation is considered to be one of the most abundant PTMs in the nucleocytoplasmic compartment. Secondly, some mitochondrial proteins are also O-GlcNAcylated. In addition, cytosolic domains of membrane proteins are also O-GlcNAcylated, as well as proteins involved in autophagy and proteasomal degradation of proteins, chaperone proteins, vesicle proteins and numerous cytosolic proteins and enzymes. Meanwhile, the distribution characteristics of O-GlcNAcylation also mean that almost all functions of proteins in regulating various cellular processes are covered. In other words, all functional classes of proteins are affected by O-GlcNAcylation, and
these O-GlcNAcylated proteins are distributed according to protein function grouping, as shown above. Some of the largest classes of proteins include those in regulating metabolism, transcription and translation as well as structural proteins. Therefore, O-GlcNAcylation is involved in many cellular processes and pathology, including signal transduction, transcription, translation, chromatin remodeling, protein sublocation and stability, mitochondrial function and cell survival, etc.

Many studies have found that fast and slow skeletal muscles have different characteristics of O-GlcNAcylation during rest, exercise or muscle atrophy [55–58]. Recent data also show that O-GlcNAcylation regulates energy metabolism [59], mediates insulin resistance [59,60] and participates in different physiological processes of skeletal muscle [61,62]. Meanwhile, O-GlcNAcylation plays a potential role in many diseases related to skeletal muscle defects, such as neuromuscular diseases and myotrophy [63–65]. Moreover, a great quantity of O-GlcNAcylated contractile and structural proteins were identified in sarcomeres, such as actin and myosin [66,67]. O-GlcNAcylation is a physiological mediator of skeletal muscle, such as contractile and structural properties, and myocardial and smooth muscle are no exception [68–71]. However, the effect of O-GlcNAcylation on skeletal muscle is still underestimated. Therefore, this review mainly explores the role and potential physiological mechanism of OGT in mediating skeletal muscle metabolism, regulating skeletal muscle contraction and maintaining the basic structure of skeletal muscle.

2. Dynamic O-GlcNAcylation Cycle and Hexosamine Biosynthesis Pathway

2.1. OGT and OGA Are the Only Antagonistic Enzymes for Precisely Regulating the O-GlcNAcylation Cycle in Space-Time Specificity

Unlike the complex regulation of other PTMs, the O-GlcNAcylation cycle of thousands of proteins is regulated by the synergistic action of OGT and OGA alone [72]. OGT transfers GlcNAc from UDP-GlcNAc to the hydroxyl groups in the threonine and serine residues of the target protein [73]. In contrast, OGA hydrolyzes GlcNAc from the O-GlcNAcylated protein [74]. The biological properties of OGT and OGA allow for the addition and removal of reversible GlcNAc multiple times quickly during the protein lifetime to produce high kinetics of O-GlcNAcylation. OGT and OGA recognition and prompt mechanisms have always been the focus of OGT research. However, Ogt knockout is embryo lethal, which hinders the understanding of the precise biological functions of OGT and OGA [75]. This research was followed by the rapid development of highly effective and specific OGT and OGA inhibitors [76]. In addition, techniques and tools, such as purification and identification of O-GlcNAcylation, have greatly improved over the past few decades [77]. Here, we summarize the tools, tactics and objectives commonly used in O-GlcNAcylation research in Table S2, hoping to provide a basis for innovative solutions to overcome new challenges.

OGT has been found to exist in many organisms with high homology. Here, three subtypes of human OGT are taken as an example to demonstrate its gene and protein structure characteristics. OGT has only three isoforms: ncOGT, mimOGT and sOGT [78]. The genomic structure of human OGT is shown in Figure 3A. Ogt is a single gene residing on the chromosome X (Xq13.1) [79]. X inactivation regulates Ogt transcription in female mammals, and Xq13.1 is a region involved in Parkinson’s pathology [80]. Ogt contains 23 exons with alternatively spliced variants [81]. Exon 1 and promoter 1 produce ncOGT. Promoter 2 and the alternative start codon in exon 5 produce mOGT. The alternative start codon in exon 10 generates sOGT. All subtypes of OGT are composed of the amino-terminal TPR domain, the same central linker domain and carboxyl-terminal catalytic domain [82]. All subtypes of OGT are shown in Figure 3B. The three subtypes of OGT contain different numbers of TPRs: 13.5 TPRs in nOGT, 9.5 TPRs in mOGT and 2.5 TPRs in sOGT [83]. Each TPR unit is comprised of 34 amino acid motifs. These TPR units are α-helical and clustered to form a repeating antiparallel helix-turn-helix super spiral [84]. TPRs acts like a “gatekeeper” to identify and interact with the target substrates by contacting their side chains [85]. The mutation of conserved aspartate residues in TPRs has resulted in significant changes in the selectivity, preference and O-GlcNAcylated rate of OGT for target substrates [86]. The
influence of TPRs on substrate preference may also be the potential reason for their different cellular localization: mOGT exists in the mitochondria, while ncOGT and sOGT exist in the cytoplasm and nucleus, respectively [87]. In addition, TPRs also have a certain effect on the activity of OGT, and this effect can be exerted only by partial TPRs [88]. TPRs deleting the front 3 or 6 units do not affect the glycosylation of substrates by OGT; however, TPRs deleting the front 9 or 11 units will deactivate OGT [83,89]. OGT belongs to the GT41 gene family of GT-B glycosyltransferase superfamily [90]. Therefore, the carboxyl-terminal catalytic region in OGT has high homology, structural characteristics and catalytic activity of GT-B glycosyltransferases superfamily. Consistent with the structural characteristics of GT-B, OGT contains two similar Rossmann folds separated by a deep fissure, which are called CDI and CD II, respectively, forming the catalytic core of OGT without the assistance of divalent metal ions [91]. CD I mainly consists of a UDP identification pocket and some catalytic groups composed of acidic amino acids that stabilize the pyrophosphate bond through the synergistic action of divalent cations [92]. CD II is a lectin-like domain that can be used to recognize and bind the glycoside of UDP-GlcNAc due to its strong affinity for carbohydrates caused by its own abundant trimer structure [93]. However, an Int-D exists in OGT and packs against the carboxyl-terminal catalytic lobe, which is distinct from the structural characteristics of GT-B superfamily [94]. It is preliminarily inferred that Int-D may interact with negatively charged membranes or nucleic acids based on the structural characteristics that contain an unusually large number of surface-exposed basic residues [82]. Meanwhile, PPO is located at the carboxyl terminal of OGT and strongly interacts with PIP3, which promotes the recruitment of OGT to the membrane under insulin induction for catalyzing the dynamic O-GlcNAcylation of the insulin signaling pathway [95]. Targeting special localization of this interaction led to the alteration in the phosphorylation of pivotal insulin signal molecules and weakening of the insulin signal transduction [96]. This suggests the indispensable role of OGT in diabetic pathology. In addition, there is a central flexible linker domain composed of about 120 amino acids [97]. The linker domain seems to exist only in metazoans without high conservatism and its function is unknown, which is the root cause of the difficulty in crystallization of OGT in higher metazoans [98]. The activity and substrate recognition of OGT are also regulated by phosphorylation [99]. For example, OGT is phosphorylated by GSK3β on Ser3/4, increasing in its own activity [100]. IRS phosphorylates OGT on Tyr976 with a similar effect [7]. OGT is phosphorylated by AMPK on Thr454, which changes its substrate-binding targets and subcellular localization [101]. The mutation of the phosphorylation site of OGT on Thr12 and Ser36 significantly changed the substrate binding of more than 500 proteins [102]. OGT is phosphorylated by CHK1 on Ser20, which changes its stabilization and required for cytokinesis [103]. OGT is also phosphorylated by CaMKII on Ser20, which increases its activity [104]. In addition, OGT is also O-GlcNAcylated by itself in Ser3/4 and Thr1045, and their role is unknown [7]. There are multiple acetylation sites in OGT [105]. The presence of these acetylation sites in the catalytic domain suggests that they may modulate OGT activity [18]. The advanced structure of OGT and the sites modified by various PTMs are shown in Figure 3C.
Figure 3. Genomic and proteomic structures of various subtypes of human OGT and OGA. (A) Human Ogt gene mapping and structure. (B) Primary protein structure of three subforms of human OGT. Subtype. (C) Advanced structures of human OGT in 3D and its PTMs. The advanced structure of OGA is displayed in cartoon and surface form with 5M7R in the protein data bank by PyMOL Molecular Graphics System, v2.5.2 (Schrödinger, LLC, New York, NY, USA). The various post-translational modification sites of OGT are present. These predicted modification sites are derived from the PhosphoSitePlus database (https://www.phosphosite.org, accessed on 10 March 2022). (D) Human Oga gene mapping and structure. (E) Primary protein structure of three subforms of human OGA. Subtype. (F) Advanced structures of human OGA in 3D and its PTMs.
Oga is highly conserved in eukaryotic species, especially in mammals, but absent in prokaryotes and yeast [106]. Oga is mapped to chromosome 10 (10q24.32) as a single gene copy [107]. It is selectively spliced to produce ncOGA and sOGA, which are different at different carboxyl terminals [87]. Gene and protein structures of OGA are shown in Figure 3D,E. Cell fractionation analysis showed that ncOGA was mainly located in the cytoplasm, while the sOGA subtype existed in the nucleus [108]. ncOGA contains the amino-terminal catalytic domain and the central stalk domain and the carboxyl-terminal pseudo-HAT domain linked through two highly disordered (or low complexity) regions [109]. The amino-terminal catalytic domain of OGA is the GlcNAc hydrolysis domain with sequence homology to GH84 [110]. The stalk domain is a hinged region containing multiple alpha helices [111]. It is not conserved between species, which makes it a flexible region that facilitates the folding of the entire protein [112]. Although it has been reported that the HAT-like domain of ncOGA in mice has histone acetyltransferase activity in vitro, it has not been supported by more studies in vivo for lacking the critical residues for the binding of acetyl-coenzyme A [113]. However, the HAT-like domain is evolutionarily conserved, indicating that the pseudo-HAT domain may play an important role in the deglycosylation-associated functions [114]. sOGA lacks the HAT-like domain but contains 15 unique amino acid residues at the carboxyl terminal [115]. Interestingly, it has been reported that sOGA has higher hydrolytic activity in vitro. OGA preferentially removes GlcNAc from some sites, indicating that it has an equal cooperative relationship with OGT in regulating the replacement of O-GlcNAcylation [116]. The active form of OGA appears as homodimer [117]. OGA forms a homodimer in the form of arm to arm, in which the glycoside hydrolase domain of each monomer is covered by the stalk domain of another monomer, thus forming a potential substrate-binding cleft comprising conserved hydrophobic residues [97]. The glycopeptide of the O-GlcNAcylated protein is tightly bound in the substrate-binding cleft through the abundant GlcNAc contacts of the catalytic pocket in OGA, which involves the peptide side chain and the backbone interactions with cleft surface residues [118]. Meanwhile, OGA recognizes the specific characteristics of substrate peptides and hydrolyzes GlcNAc from a wide range of peptide sequences [119]. In addition, some specific residues on OGA contribute to its interaction with different peptide substrates, which means the differential regulation of O-GlcNAcylation on various proteins [120]. OGA is also affected by PTMs such as phosphorylation and O-GlcNAcylation [121]. There are abundant phosphorylation and ubiquitination sites in the domains of glycoside hydrolase and the HAT-like domain [7], but the effect of these modifications at corresponding sites on OGA activity remains to be further determined. The advanced structure of OGA and the sites modified by various PTMs are shown in Figure 3F. The O-GlcNAcylation of OGA at Ser405 is located in the central highly disordered region, suggesting a role in the regulation of OGA-OGT interactions because this is the binding region of OGA-OGT [122]. OGA is also SUMOylated at Lys358 and acetylated at Lys599, respectively [7,123].

2.2. Nutrient Availability Drives Global O-GlcNAcylation through HBP

Extracellular glucose is transported into the intracellular via GLUT-4 [124]. Only 2~3% of the intracellular glucose enters the HBP, while most of the remaining intracellular glucose enters the glycolysis, pentose phosphate pathway (PPP), glycogen synthesis and even polyol pathways, respectively [125]. Therefore, the O-GlcNAcylation cycle is strictly controlled by the flow of glucose through the HBP [126]. Initially, in a study, intracellular glucose was phosphorylated to Glc-6-P by HK, and then Glc-6-P was further isomerized to Fru-6-P by GPI [127]. Subsequently, 3~5% of Fru-6-P was added with an amino group from glutamine to synthesize GlcN-6-P and glutamate by GFAT, while the other 95% of Fru-6-P was used for glycolysis [128]. The enzymatic reaction is the rate-limiting step of HBP, and GFAT is also the key rate-limiting enzyme of HBP [129]. The activity of GFAT is still regulated by multiple pathways [130]. Firstly, the activity of GFAT is regulated by substrate availability, which is positively activated by the concentration of glucose and glutamine, and the negative feedback is inhibited by the concentration of UDP-GlcNAc.
and GlcN-6-P [131]. The activity of GFAT is also closely related to some PTMs. The Ser\textsuperscript{243} of GFAT is phosphorylated and its activity is reduced by AMPK, mTORC2 and CaMKII, and a similar effect is also caused by 2-Deoxy-D-glucose [132,133]. PKA also promotes the phosphorylation of GFAT at Ser\textsuperscript{205}/235 [134]. Succinylation of GFAT at Lys\textsuperscript{529}, acetylation of GFAT at Lys\textsuperscript{114, 547, 650} and multiple ubiquitination Lys sites of GFAT are predicted by PhosphoSitePlus® v6.6.0.2 (https://www.phosphosite.org, accessed on 10 March 2022). Meanwhile, it has been reported that specificity protein 1, activating transcription factor 4 and X-box-binding protein 1 regulate GFAT at the transcriptional level [135,136]. Glutamine is necessary for this enzymatic reaction, but this restriction can be bypassed by glucosamine as an extended supplement [137]. Therefore, incubating cells with glucosamine or high concentration glucose or glutamine can bypass the rate-limiting step catalyzed by GFAT, thereby increasing global O-GlcNAcylation. GNA converts GlcNAc-6-P using acetyl-CoA [138]. Then, GlcNAC-6-P is catalytically translocated to GlcNAC-1-P by AGM [139]. It is worth noting that the only difference of HBP in prokaryotes is that GlcN-6-P is isomerized to GlcN-1-P and then GlcN-1-P is acetylated to form GlcNAC-1-P [140]. The HBP process in eukaryotes is as shown above. Finally, UTP is then utilized by UAP to convert GlcNAC-1-P into UDP-GlcNAc and release iPPi [141]. The HBP process involves the participation of glucose, glutamine, uridine, acetyl-CoA and ATP [29]. Therefore, UDP-GlcNAc, as the end-product of HBP, integrates the metabolisms of carbohydrates, amino acids, fats and nucleotides [142]. UDP-GlcNAc is a unique donor of O-GlcNAcylation, which provides GlcNAc, which is necessary and irreplaceable for O-GlcNAcylation [143]. GlcNAc provided by UDP-GlcNAc is used and transferred by OGT to the oxygen atom of the hydroxyl group of serine or threonine residues of the target protein [54]. On the contrary, the GlcNAc moiety is removed from O-GlcNAcylated proteins by OGA [144]. These hydrolyzed GlcNAc or other free GlcNAc obtained by lysosomal or nutrient degradation are converted to GlcNAc-6-P through N-Acetylglucosamine kinase (NAGK) and then used again for the synthesis of UDP-GlcNAc [145]. Therefore, GlcNAc can also bypass the rate-limiting step of HBP and GFAT, which is also effective for salvage pathways such as glucosamine and glutamine [146]. In addition, UDP-GlcNAc is also used as a substrate for the synthesis of proteoglycans, hyaluronic acid, glycolipids, GPI anchor, N-glycosylation and other O-glycosylation [147]. The activated UDP-GlcNAc is utilized by concentration-sensitive enzymes in the nucleus, cytoplasm and membrane to glycosylate the substrate or generate glucose conjugates [147]. UDP-GlcNAc is actively transported by nucleotide sugar transporters to cellular organelles, such as the ER and Golgi apparatus [148]. The differences in UDP-GlcNA permeability and relative cell volume of these organelles complicate the estimation of the cytoplasmic and nuclear concentrations of UDP-GlcNAc [149]. The relative abundance of O-GlcNAcylation is roughly negatively correlated with the more complex glycans [150]. These characteristics make UDP-GlcNAc and its derivatives extremely sensitive to the variations in cellular nutrients, so that the dynamic O-GlcNAcylation can be used as a reporter of the functional status of multiple pathways and regarded as a metabolic sensor [24]. Meanwhile, the mutual conversion and complex relationship of the intermediate products in the HBP, polyol pathway, PPP, glycogen, glycolysis and TCA cycle intermediates greatly enlarge the nutritional sensitivity of O-GlcNAcylation [151] and also suggest the potential mechanism of O-GlcNAcylation’s negative feedback regulation of these glucose metabolism branches. Indeed, O-GlcNAcylation is involved in multiple modes of metabolic regulation. Almost all the enzymes involved in glycolysis were identified to have been modified by O-GlcNAcylation [152]. The O-GlcNAcylated enzymes exist in every step of glycolysis, including GLUT4, HK, GPI, PFK, FBA, GAPDH, PGK, PDM, ENO, PK and PDC [59,153–155]. Glycogen synthesis is also regulated by O-GlcNAcylated GSK3β, and PPP activity is affected by O-GlcNAcylated G6PD [156,157]. In addition, increased HBP flux and O-GlcNAcylation also promotes fatty acid oxidation in the heart and adipose tissue [158]. The O-GlcNAylation of several transcription factors, such as PGC1α, FoxO3, NF-κB and CREB, also indirectly participates in transcriptional regulation of metabolism [159–161]. Although only briefly shown in Figure 4, it is worth
noting that almost all enzymes in the TCA cycle are also modified by O-GlcNAcylation, such as AH, IDH, KGD, SL, SDH, MDH and the several subunits of respiratory chain complexes [162,163]. CS and FH may be potentially O-GlcNAcylated, but there is still a lack of supporting evidence [152].

**Figure 4.** Nutrient availability drives the dynamic cycle of protein O-GlcNAcylation via the hexosamine biosynthesis pathway. The GlcNAc provided by UDP-GlcNAc is necessary and unique for O-GlcNAcylation, and the only source of UDP-GlcNAc is HBP. Changes in nutritional availability, such as carbohydrates, lipids, amino acids, nucleotides and ATP, fluctuate HBP flux. Therefore, UDP-GlcNAc and HBP link carbohydrate metabolism, lipid metabolism, amino acid metabolism and nucleotide metabolism. These physiological characteristics make OGT extremely sensitive to nutrient fluctuations.
3. O-GlcNAcylation, Energy Metabolism and Insulin Sensitivity in Skeletal Muscle

Skeletal muscle is a repository of nutrients, enabling it to serve as the consumer and producer of energy during exercise, stress and starvation [164]. The energy requirements of skeletal muscle are enormous, and its energy expenditure increases 300 times from the base state to the full contraction state [165,166]. This directly affects glucose homeostasis in skeletal muscle, where CaMKII or GLUT4 is activated to increase glucose uptake [167]. In fact, skeletal muscle processes more than 80% of insulin to stimulate glucose uptake and is considered as one of the most critical insulin-sensitive tissues [168]. The metabolic flexibility in skeletal muscle ensures an adequate supply of energy for its work [169]. O-GlcNAcylation as a cellular nutrient sensor plays a key role in glucose metabolism during this physiological process [170]. In addition, skeletal muscle is the main target organ of insulin, and nutrient-driven O-GlcNAcylation is a key regulator of insulin signaling in skeletal muscle [62]. O-GlcNAcylation is considered to be critical in the dysregulation of the insulin signaling cascade and the molecular mechanism of insulin resistance [171].

3.1. O-GlcNAcylation Is the Key Regulator of Glucose Metabolism in Skeletal Muscle

Glucose uptake and disposal are the most important limiting factors in fuel metabolism and energy homeostasis of skeletal muscle [172]. Ample data show that O-GlcNAcylation regulates early glucose metabolism [159]. GLUT4 and its transport vesicle proteins in skeletal muscle and adipose tissue are modified and directly regulated by O-GlcNAcylation [173]. O-GlcNAcylation plays an essential role in maintaining glucose uptake by altering GLUT4 translocation, blocking GLUT4 phosphorylation, regulating GLUT4 downstream signal transduction or directly regulating vesicle proteins [174]. Hypoxia inducible factor 1α, a key transcriptional regulator of GLUT1, is modified by O-GlcNAcylation, which indirectly regulates and increases GLUT1 transcription and glucose uptake [175,176]. In addition, HK is a major regulator of cellular glucose uptake, and O-GlcNAcylated hexokinase IV or glucokinase in vivo/vitro positively regulates its expression, which is of positive significance in regulating glucose flux [177]. Similar changes have occurred in GPI [178]. The increase of global O-GlcNAcylation may further promote glucose into HBP and UDP-GlcNAc synthesis through these ways. The contribution of O-GlcNAcylation to glucose uptake reverses metabolic disorder, stress and cell death in skeletal muscle [27]. The increase of glucose uptake has been related to the increase of glycolytic flux and enzyme activity. It is important to note that almost all enzymes involved in the glycolysis pathway are O-GlcNAcylated, which modulates their expression or activity to participate in the regulation of glycolysis in skeletal muscle [152]. The O-GlcNAcylation of PFK at the Ser252 inhibits its activity and oligomerization, which redirects glucose flux into the PPP and reduces glycolytic flux [155]. PGK is a critical metabolic enzyme and catalyzes the production of the first ATP in the glycolysis pathway [179]. PGK is O-GlcNAcylated at the site of Thr255 [153]. The O-GlcNAcylation of PGK enhances its activity to promote glycolysis and translocation to mitochondria to inhibit the TCA cycle [180]. PFKFB3 is O-GlcNAcylated at Ser172 and competes with phosphorylation under metabolic stress [181]. PK is one of the main rate-limiting enzymes in glycolysis, and one of its subtypes is PKM2 [182]. The O-GlcNAcylation of PKM2 occurs at Thr405 and Ser406, which destroys its testamer stability, reduces its activity and causes its nuclear translocation [154,183]. These changes lead to the Warburg effect characterized by increased glucose consumption and lactate production [184]. The product pyruvate enters the anaerobic or aerobic pathway. The downstream LDH and PDC are also modified by O-GlcNAcylation [185]. These data suggest that O-GlcNAcylation plays a key regulatory role in the utilization of the glycolysis end-product. Note that almost all metabolic enzymes of the mitochondrial TCA cycle are also O-GlcNAcylated [186]. Meanwhile, O-GlcNAcylation modifies various mitochondrial proteins to change the morphology, function and quality of mitochondria [187,188]. For example, O-GlcNAcylation of PGC1α is beneficial for maintaining mitochondrial biogenesis and metabolic reprogramming [189]. However, the mechanism of O-GlcNAcylation regulation on mitochondria affecting energy metabolism homeostasis and muscle fiber type switching in skeletal mus-
cle is extremely complex, so it will not be discussed in detail here. Not all of these data are from skeletal muscle tissues or cell lines, and the exact role of O-GlcNAcylation in the regulation of energy metabolism in skeletal muscle remains to be further clarified [190]. Indeed, there is a complex relationship between myofibrils and the metabolic enzymes not limited to what we discussed above. For example, FBA is localized to the Z-line in association with α-actinin within the metabolon [191]. The interaction between FBA and downstream metabolic enzymes of glycolysis occurs in the thin filaments in the same pattern [192]. These specific interactions between glycolytic metabolon and the contractile apparatus may ensure a very efficient and dynamic localized production of ATP for myosin ATPase and actomyosin interactions resulting in force development [193]. In addition, the latest research found that the fluctuation of the global O-GlcNAcylation level also leads to the regulation of protein–protein interactions in multiple protein complexes [194]. Glycogen decomposition is conducive to maintaining the level of global O-GlcNAcylation. GSK3β is modified by O-GlcNAcylated, which may compete with the phosphorylation at Ser9 to inhibit its activity. The PPI required to produce UDPG is also O-GlcNAcylated, and the specific mechanism needs to be further confirmed [195]. These results suggest that O-GlcNAcylation is a regulator of glycogen synthesis. G6PD catalyzes the first speed-limiting step of PPP and is considered as the pacemaker of PPP and the main regulation point of NADPH production [196]. O-GlcNAcylation of G6PD at the site of Ser84 activates its activity and diverts glucose flow to PPP [197]. The absence of O-GlcNAcylation increases the secretion of IL-15 in skeletal muscle, which serves as a myokines regulating systemic oxidative metabolism [198]. Similarly, the secretion of myogenic IL-6 is indirectly regulated by O-GlcNAcylated p65 to maintain energy homeostasis in skeletal muscle [199]. In addition, creatine kinase is O-GlcNAcylated [200]. Creatine shuttle transmits information between ATP site consumption and mitochondria, so O-GlcNAcylation regulates this process.

3.2. O-GlcNAcylation-Mediated Insulin Sensitivity in Skeletal Muscle

HBP has long been known to be involved in glucose-induced insulin resistance. It has been widely recognized that the chronic high flow of HBP represents one of the mechanisms of insulin resistance caused by hyperglycemia [201]. Mice overexpressing GLUT1 in skeletal muscle have insulin resistance and are accompanied by medium- and long-term increased glucose flow and increased UDP-GlcNAc concentration in muscle [202]. Continuous exposure to high glucose or glucosamine can lead to impaired insulin stimulated GLUT4 translocation, resulting in subsequent reduced insulin-stimulated glucose uptake in muscle cells [168]. High glucose or glucosamine leads to the increase of HBP flux and UDP-GlcNAc level, in which glucosamine is more effective [203]. It is worth noting that these changes are related to the level of UDP-GlcNAc in the GLUT4-containing vesicles of the skeletal muscle under the condition of diabetes [204]. These data indicate that there is a strong correlation between HBP flux and UDP-GlcNAc level in skeletal muscle and insulin resistance and diabetes pathology. The catalytic activity of OGT is highly sensitive to HBP flux and UDP-GlcNAc concentration [6]. Therefore, elevated global O-GlcNAcylation levels in skeletal muscle induce insulin resistance [205]. Increasing UDP-GlcNAc enhanced many O-GlcNAcylated muscle proteins on bone through the co-infusion of insulin and glucosamine in a study [206]. Therefore, another possible mechanism of HBP induced insulin resistance is that the increase of O-GlcNAcylation of insulin signal-related proteins antagonizes their phosphorylation. PIP3 recruits OGT to the cytomembrane through the strong interaction with the PPO domain of OGT under diabetes or another insulin-insensitive state [95]. Meanwhile, OGT is phosphorylated at certain tyrosine residues and enhances its activity through the insulin-stimulated insulin receptor [207]. Subsequently, OGT catalyzes the dynamic O-GlcNAcylation of IRS, PDK1, AKT, FoxO1 and other insulin signal molecules [31]. The O-GlcNAcylation of IRS-1 at Ser1101 and IRS-2 at Ser144 inhibits their phosphorylation at the same site, resulting in the attenuation of the insulin signal [208]. The O-GlcNAcylation of AKT at the Thr308 and Thr312 inhibits its phosphorylation at Thr308 through disrupting the interaction between AKT and PDK1 [209,210]. Therefore, excessive nutrients, such as
glucose and fatty acids, lead to an abnormal increase in global O-GlcNAcylation, which reduces insulin signal transduction efficiency, produces insulin resistance and forms a vicious circle and glucose toxicity [211].

4. O-GlcNAcylation Is an Emerging Mediator of Contractile and Structural Properties in Skeletal Muscle

4.1. O-GlcNAcylation Is an Essential Regulator of Contractile Properties in Skeletal Muscle

To date, many O-GlcNAcylated contractile proteins and contractile-related regulatory proteins in sarcomere have been identified [67]. These key contractile proteins include actin, myosin, MLC and MHC, tropomyosin and troponin, etc. [55,66,212]. In view of this, the physiological role of O-GlcNAcylation on skeletal muscle contractile activity has been concerned. Many data emphasize that O-GlcNAcylation mediates calcium activation properties to regulate the contractile activity of skeletal muscle. Increased O-GlcNAcylation of MHC, α-tropomyosin and α-sarcomeric actin in myocardium of diabetic mice resulted in the decrease of sarcomere calcium sensitivity [213]. Reversible reductions in calcium affinity and sensitivity of muscular fibers occur when exposed to GlcNAc, and O-GlcNAcylation of some critical contractile proteins increased, such as MHC, MLC and actin [214]. Moreover, similar results appeared in skinned fibers and cardiac trabeculae. Phosphorylation of Tn I at the Ser23 and Ser24 by PKA improves the calcium sensitivity of cardiomyocytes and alter of myofilament properties [215]. Interestingly, Tn I, Tn T and Tn C of the troponin complex are O-GlcNAcylated in skeletal muscle and myocardial tissue [216]. The O-GlcNAcylation levels of Tn I in fiber cells exposed to GlcNAc or OGA inhibitors increased, and the calcium activation properties reduced [217]. However, this treatment did not affect the phosphorylation levels of Tn I at Ser23/24 [218]. One of the possible mechanisms is that the GlcNAc part destroys the protein–protein interaction [212]. For example, weak electrostatic force maintains the interaction between tropomyosin and actin and O-GlcNAcylation just change specific electrostatic charges [219]. Four O-GlcNAcylated sites were found on myosin in skeletal muscle. These sites are located in the marginal of the carboxy terminal, which is closely related to the polymerization and the interaction of MHC [220]. Further evidence is that one of the sites is associated with a hereditary myosin myopathy because its mutation destroys the polymerization of myosin to myomesin, M protein and titin [67]. Unfortunately, although these specific O-GlcNAcylation changes are associated with calcium sensitivity, they have not been appreciated and studied, and precise O-GlcNAcylated site identification is lacking.

Many reports have shown that O-GlcNAcylation may play an equally crucial role in skeletal muscle physiology as phosphorylation [65,67,221,222]. Another possible mechanism is that O-GlcNAcylation plays its biological function by interacting with phosphorylation, as shown in Box ???. For example, the potential antagonism between the O-GlcNAcylation at Ser190 and the phosphorylation at Ser208 of Tn T plays a therapeutic role in the course of ischemic heart failure [223]. It must be recognized that MLC2 is the most suitable example to explain this mechanism. The elevated sarcoplasmic Ca2+ is bound to four divalent metal-binding sites on calmodulin to form the “Ca4+—calmodulin” complex [224]. The complex then interacts with the inactive catalytic subunit of myosin light chain kinase (MLCK) to form an active holoenzyme complex, namely “Ca4+—calmodulinMLCK” [225]. The “Ca4+—calmodulin—MLCK” complex phosphorylates the slow fiber subtype MLC2 at the Ser14 [226]. Meanwhile, MLC2 non-covalently surrounds the neck region of myosin and provides it additional and powerful mechanical support [227]. This process is Ca2+/calmodulin dependent, and the fast fiber subtype MLC2 is phosphorylated at Ser15 [228]. This effect is eliminated by the recognition and dephosphorylation of MLC2 by MYPT and PP1 [229,230]. The phosphorylation of MLC2 is an pivotal regulation to enhance the contractile capacity of sarcomeres by increasing calcium sensitivity, although it is not necessary for contraction [231]. These data also show that MLC2 phosphorylation causes changes in the structure of thick filaments and increase the number of cross bridges and their attachments because calcium sensitivity is directly proportional to the ATPase.
activity of actomyosin [232]. It is further explained that the electrostatic repulsion between the negative charge of phosphate added on MLC and MHC causes the position of myosin head to move, thus promoting the formation of cross bridges [233]. MLC2 phosphorylation is slower than contraction in terms of kinetics, which is regarded as a biochemical memory [234]. This helps to fight muscle fatigue by enhancing muscle mechanical function during prolonged or repeated activities [231]. MLC2 was found to be O-GlcNAcylated in the myocardium and skeletal muscle. O-GlcNAcylation of MLC2 in myocardial tissue occurred at the site of Ser15. It is worth noting that the only phosphorylation site overlaps with the only O-GlcNAcylation site on MLC2. This suggests that the close potential interaction between O-GlcNAcylation and phosphorylation of MLC2 is in the calcium activation properties of sarcomere. This interaction has been proved to be mutually exclusive, and the dynamics of this interaction vary according to the pattern of skeletal muscle activity [235]. The precise regulation of phosphorylation and O-GlcNAcylation on MLC2 involves a multienzyme cluster. This multienzyme cluster contains MLCK/MYPT2/PP1 and OGT/OGA, which are involved in the phosphorylation and O-GlcNAcylation of MLC2 [226]. This multienzyme cluster is preferentially located at the Z disk of the sarcomere and responds to the physiological signals of skeletal muscle to strengthen the interaction between the enzymes contained in itself [236]. For example, the partial recombination of this multienzyme clusters in skeletal muscle dysfunction, such as increased co-localization between MLCK and OGA. The location of OGT and OGA in the diabetic heart is redistributed in the sarcomere [237]. This is due to enhanced OGA activity and increased the interaction with α-actin, tropomyosin and MLC1, while OGT was the opposite. This results in the removal of abnormal O-GlcNAcylation to restore myofilaments to Ca2+ response [238]. Nevertheless, the exact effect and mechanism of O-GlcNAcylation on MLC2 need to be further confirmed. One credible assumption is that O-GlcNAcylation causes steric hindrance between MLC2 and MHC due to its stokes radius being many times larger than phosphate [19].

In general, O-GlcNAcylation is considered as a new mechanism to regulate the contractile properties of skeletal muscle by modifying critical contractile proteins to regulate the interaction between other proteins or with itself, and phosphorylation to mediate calcium activation. In addition, the regulatory effect of O-GlcNAcylation on the contractile activity of skeletal muscle is also related to its effect on sarcomere structure [239], which is discussed in the next section.

4.2. O-GlcNAcylation Is an Emerging Maintainer of the Structural Properties in Skeletal Muscle

Skeletal muscle is an extremely complex and organized machine with a unique stripe morphological characteristic [240]. This is based on the precise assembly and regular arrangement of sarcomeres with the combination of myofibrillar proteins and structural proteins [241]. Not only the actin and myosin discussed above, but also many critical myofibrin proteins involved in sarcomere structure are modified by O-GlcNAcylation, including actinin, desmin, titin, ZASP, filamin C, myomesin, myopalladin, plectin, BAG3, etc. [217,242]. Meanwhile, a variety of pivotal structural proteins form a complex and ordered “sarcomere cytoskeleton”, and their interactions are essential for maintaining the basic structure of sarcomere and performing physiological functions of sarcomere such as contraction [243]. Moreover, the nodes of multiple dynamic interactions between these various proteins are in the Z disk, M line and I band [244]. Most of the above pivotal structural proteins involved in sarcomere structure are modified by O-GlcNAcylation, including as cytokeratin, laminin, spectrin, α/β-crystallin, integrin, vinculin, etc. [245–248]. First of all, the increased overall O-GlcNAcylation level in C2C12 myotubes resulted in an increase in the width of A band and M line in a study, while the width of I band and sarcomere length decreased [247]. These changes in the morphometric parameters of sarcomere caused by the fluctuation of O-GlcNAcylation level are the most powerful evidence to support that O-GlcNAcylation is an essential modulator of sarcomere structure. Phosphorylation is known to significantly regulate protein–protein interactions on sarcomeres, such as titin-myomesin and ZASP-myotilin interactions [249]. Hence, the dynamic balance between O-GlcNAcylation and phospho-
rlylation may affect sarcomere structure. For example, the Z disk is the most concerned node of both O-GlcNAcylation and phosphorylation [249]. Desmin is capable of multiple PTMs, such as O-GlcNAcylation, phosphorylation and ubiquitination. It is recognized that phosphorylation regulates the polymerization of desmin [250]. O-GlcNAcylation also regulates the polymerization of desmin, just as it regulates the polymerization of tubulin and cytokeratin filaments 8/18 [251–253]. α/β-crystallin serve as molecular chaperones to facilitate the localization, aggregation, and assembly of desmin [254]. O-GlcNAcylation of α/β-crystallin at Thr170 and Thr182 regulates its localization and its interaction with desmin, respectively [255,256]. The multiple O-GlcNAcylation sites of mouse titin are located on the kelch-12 domain, and the absence of titin leads to the change of muscle structure and the decrease of muscle performance [249,257]. These O-GlcNAcylation sites are located in the key regions of sarcomere assembly and myosin polymerization and its interaction with MyBP-C and MHC [258,259]. Interestingly, O-GlcNAcylation sites of MHC have also been found. These sites are adjacent to its domain of polymerization and interaction with myosin and titin [220]. O-GlcNAcylation of MHC is located at Ser1708, and it is further considered to be involved in Laing early-onset distal myopathy due to its proximity to mutant Leu1706 residue. Meanwhile, O-GlcNAcylation sites in the PxxP domain of BAG3 and the plakin domain repeat B5 of plectin have been identified, which are related to the interaction with SH3-containing protein and intermediate filament proteins, respectively [249]. This evidence suggests that O-GlcNAcylation occurs in specific domains of certain structural proteins to regulate interactions other proteins for maintaining sarcomere structure and function. This view was confirmed by a recent study that O-GlcNAcylated milkin binds to FHL2 to anchor mitochondria to F-actin [221,260]. The main targets and pathways of O-GlcNAcylation in skeletal muscle physiology are shown in Figure 5.

![Image of Figure 5](image-url)

**Figure 5.** The major targets and pathways shown to be altered by O-GlcNAcylation in in the skeletal muscle physiology. Thousands of O-GlcNAcylated proteins have been identified in skeletal muscle cells. These O-GlcNAcylated proteins are classified into contractile proteins, sarcolemma proteins, structural
proteins and cytoskeletal proteins, as well as mitochondrial proteins, metabolic enzymes, transcription factors and signal proteins. Therefore, the effects of O-GlcNAcylation on various physiological processes of skeletal muscle may be realized from the following four aspects: (1) its regulation of carbohydrate metabolism with sensing nutritional availability; (2) its maintenance of structural protein synthesis/degradation balance; (3) its improvement of sarcomere contractile activity by modulating the calcium activation properties; (4) its promotion of adaptation and protection under exercise and certain adverse circumstances.

5. Conclusions and Perspectives

In the past few decades, the molecular mechanism and biological function of O-GlcNAcylation have been thoroughly studied. However, the physiological mechanisms by which OGT and OGA accurately identify thousands of substrates and dynamically maintain their O-GlcNAcylation homeostasis remain to be further clarified. One of the current obstacles is that it is difficult to obtain OGT and OGA crystals in higher organisms. Another obstacle is the identification of precise sites of O-GlcNAcylation. This will be an urgent difficulty to overcome and a research node worth exploring. Meanwhile, the research of efficient and specific inhibitors of OGT and OGA has always been a hotspot. The solution of these difficulties will depend on innovative biotechnology and strategies in the future. For example, the calculation and prediction of O-GlcNAcylation site of proteins is an interesting research direction.

O-GlcNAcylation-mediated glucose homeostasis and the insulin sensitivity of skeletal muscle endow the plasticity of metabolic properties to adapt to nutritional availability and physiological clues. However, changes in the fine characterization of global O-GlcNAcylation of skeletal muscle are extremely complex, depending on muscle fiber type, abandonment, rest and exercise patterns such as type and intensity. However, we have not discussed these factors in this review due to the limited space. Further study on the metabolic regulation difference of O-GlcNAcylation in basal and exercise states is helpful to explore and understand how O-GlcNAcylation responds to and coordinates various molecular signals.

The role of abnormal O-GlcNAcylation levels in the pathophysiology of various cancers, neurodegeneration, obesity, diabetes and its complications were also highlighted. However, these research studies have only explored the tip of the iceberg of the pathological role of O-GlcNAcylation, which needs to be studied further. There has been a lot of evidence to support the essential role of O-GlcNAcylation in skeletal muscle physiology and pathology, but these studies have been underestimated and ignored. The abnormality of global O-GlcNAcylation level is one of the pathogenic factors of skeletal muscle atrophy [226,261]. Destruction of OGA activity mediates high levels of O-GlcNAcylation, resulting in muscle atrophy [262]. One possible underlying mechanism is that O-GlcNAcylation appears to negatively regulate myogenesis. Increased global O-GlcNAcylation was shown to inhibit the terminal differentiation program of myogenesis in skeletal muscle [263]. The O-GlcNAcylation of MeF2D on its DNA-binding and transactivation domain, which is reduced by myogenic stimulation, inhibits its recruitment to the myogenin promoter [264]. Another possible mechanism is that O-GlcNAcylation involves the regulation of some catabolic and anabolic pathways, leading to atrophy, such as increased Murf-1 expression [261]. Proteostasis of all components of the cytoskeletal framework is necessary for the structure and function of skeletal muscle [265]. Due to autophagy and proteasome degradation pathways, skeletal muscle effectively recycles damaged or aged organelles and accumulated protein aggregates and breaks down proteins to meet the body’s energy needs [266]. With the continuous understanding of the extensive effects of O-GlcNAcylation on cell function, the benefits of O-GlcNAcylation in mediating autophagy, apoptosis and proteasome have been recognized [47]. In addition, the fine characterization changes of O-GlcNAcylation in muscle fibers in muscular dystrophy, myositis and rhabdomyolysis
have been researched [64]. This adverse effect occurs in myocardium and smooth muscle, rather than being confined to skeletal muscle, such as cardiovascular diseases and vasculopathy caused by diabetes [70,267]. However, these potential pathogenic mechanisms are extremely complex, and we did not discuss them in this review due to limited space. Further research on the role of O-GlcNAcylation in the pathology of these skeletal muscle diseases will provide us with a variety of new therapeutic targets in the future. In particular, the further studies of O-GlcNAcylation mediated skeletal muscle autophagy and its physiological mechanism in muscle mass and atrophy and systemic energy metabolism will help to find emerging signaling pathways.

What is describe in this review is only a superficial and less in-depth understanding of the role of O-GlcNAcylation in skeletal muscle physiology. We believe that the exploration of O-GlcNAcylation-mediated differential regulation of skeletal muscle energy metabolism under various exercise modes and the discovery of the potential mechanism of O-GlcNAcylation in various skeletal muscle pathologies will be a vigorous research hotspot and direction in the future. Over time, these studies will provide new valuable insights in the fields for skeletal muscle diseases and exercise rehabilitation.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/cells11111789/s1, Table S1: Types and process of protein glycosylation, Table S2: Common tools for studying, identification, and detection of O-GlcNAcylation.

**Author Contributions:** Y.L. wrote the manuscript and produced these charts. Y.-J.H. conducted a more extensive and detailed literature search. W.-X.F. and X.Q. corrected the language of the manuscript for publication. B.X. and S.-Z.L. checked and corrected the manuscript for publication. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was funded by General Project of National Natural Science Foundation of China (31972637), Key Project of Heilongjiang Natural Science Foundation (ZD2019C004 and YQ2021C027), Heilongjiang Bayi Agricultural University for San Heng San Zong (ZRCQC202003), Graduate Innovative Research Project of Heilongjiang Bayi Agricultural University (YJSCX2021-Z01).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

**Abbreviations**

1,3BPG 1,3-bisphosphoglycerate  
2PG 2-phosphoglycerate  
3PG 3-phosphoglycerate  
5mC 5-methylcytosine  
5S-GlcNAc 5-thio-N-acetylglucosamine  
5S-GlcNHex 2-deoxy-2-N-hexanamide-5-thio-d-glucopyranoside  
6PGD 6-phosphogluconate dehydrogenase  
6-PGL 6-phosphogluconolactone  
AGM phosphoacetylglucosamine mutase  
AH aconitate hydratase  
AK adenylate kinase  
AMPK AMP-activated protein kinase  
Ara L-arabinose  
BAG benzyl-2-acetamido-2-deoxy-D-galactopyranoside or GalNAc-α-O-benzyl for short  
BAG3 BCL2-related athanogene 3  
BIN1 bridging integrator 1  
BZX 4-methoxyphenyl 6-acetyl-2-oxo-2,3-dihydro-1,3-benzoazole-3-carboxylate;  
CaMKIIδ calcium-calmodulin-dependent protein kinase 2 delta  
CaMKIV calcium-dependent protein kinase type IV
| Abbreviation | Full Name |
|--------------|-----------|
| CARP         | cardiac ankyrin repeat protein |
| Cav α1/β2    | L-type Ca2+ channel subunit alpha 1 and beta 2 |
| CBP          | CREB-binding protein |
| CD I         | catalytic domain I |
| CD II        | catalytic domain II |
| CK           | creatine kinase |
| COLGALT      | collagen O-Gal transferase |
| CREB         | cyclic AMP-response element-binding protein |
| CS           | citrate synthase |
| DB           | dystrobrevin |
| Dia          | DiActrideoxyhexose |
| DOGT         | GlcNAc transferase |
| Dol-P-Man    | dolichol phosphate mannose |
| Dol-P-Oligo  | dolichylpyrophosphate Glc₃Man₉GlcNAc₂ |
| DON          | 6-Diazo-5-oxo-L-norleucine |
| DYSF         | dysterlin |
| E4P          | erythrose-4-phosphate |
| EGF          | epidermal growth factor-like repeat |
| ENO          | beta-enolase |
| ER           | endoplasmic reticulum |
| EthNP        | ethanolamine phosphate |
| EZH2         | enhancer of zeste homolog 2 |
| FBA          | fructose-1,6-bisphosphate aldolase |
| FBP          | fructose-1,6-bisphosphate |
| FH           | fumarate |
| FHL2         | four and a half LIM domains protein 2 |
| Fru-6-P      | fructose-6-phosphate |
| Fuc          | L-fucose |
| FucNAc       | N-Acetyl-fucosamine |
| G6PD         | glucose-6-phosphate dehydrogenase |
| Gal          | D-galactose |
| GALE         | UDP-galactose-4-epimerase |
| GalNAc       | N-acetyl-D-galactosamine |
| GALNT        | polypeptide GalNAc transferase |
| GALP         | glyceraldehyde-3-phosphate; |
| GAPDH        | glyceraldehyde phosphate dehydrogenase |
| GBE          | Glycogen-branching enzyme |
| GDP-Fuc      | GDP-fucose |
| GDP-Man      | GDP-Mannosse |
| GFAT         | glutamine Fru-6-P aminotransferase |
| GH94         | glycoside hydrolase family 94 |
| Glc          | D-glucose; |
| Glc-6-P      | glucose-6-phosphate |
| GlcN-6-P     | glucosamine-6-phosphate |
| GlcNAc       | N-acetylglucosamine |
| GlcNAc       | N-acetyl-D-glucosamine; |
| GlcNAc-1-P   | N-acetylglucosamine-1-phosphate |
| GlcNAc-6-P   | N-acetylglucosamine-6-phosphate |
| GN           | glycogenin |
| GNA          | glucosamine-6-phosphate N-acetyltransferase 1 |
| GNE          | UDP-GlcNAc 2-epimerase/ManNAc kinase |
| GPI          | Glc-6-P isomerase |
| GPI          | glycosylphosphatidylinositol |
| GS           | glycogen synthase |
| GYG          | glycogenin |
| HAT          | histone acetyltransferase |
| HBP          | hexosamine biosynthesis pathway |
| HDAC4        | histone deacetylase 4 |
| Acronym  | Description                                      |
|----------|--------------------------------------------------|
| HK       | hexokinase                                       |
| Hyl      | hydroxylysine                                    |
| IDH      | isocitrate dehydrogenase                          |
| Int-D    | intervention domain                              |
| I-T-C    | troponin complex (Tn I, Tn T, Tn C)              |
| KGD      | ketoglutarate dehydrogenase                      |
| LA       | lactate                                          |
| LDH      | lactate dehydrogenase                            |
| Man      | Mannose                                          |
| ManNAc   | N-acetylmannosamine                               |
| MDH      | malate dehydrogenase                             |
| Mef2 C/D | myocyte-specific enhancer factor 2 C/D           |
| MHC      | myosin heavy chain                               |
| MLC2     | myosin light chain 2                             |
| MLP      | muscle LIM protein                               |
| mOGT     | mitochondrial OGT                                 |
| MyBP-C   | Myosin-binding protein C                         |
| MYPT1    | myosin phosphatase target subunit 1              |
| NButGT   | 1,2-dideoxy-2′-propyl-alpha-D-glucopyranoso-[2,1-D]-Delta 2′-thiazoline |
| ncOGA    | nucleocytoplasmic OGA                            |
| ncOGT    | nucleocytoplasmic OGT                            |
| OGA      | O-GlcNAcase                                      |
| OGT      | O-GlcNAc transferase                              |
| OST      | oligosaccharyltransferase                        |
| PA       | pyruvate                                         |
| PDC      | pyruvate dehydrogenase complex                   |
| PEP      | phosphoenolpyruvate                              |
| PFK      | phosphofructokinase                              |
| PGK      | phosphoglycerate kinase                          |
| PGM      | phosphoglucomutase                               |
| PIP3     | phosphatidylinositol (3,4,5)—triphosphate        |
| PK       | pyruvate kinase                                  |
| PKA      | cAMP-dependent protein kinase                    |
| POFUT    | protein O-fucosyltransferase                     |
| POGLUT   | protein O-Glc transferase                        |
| POMT     | protein O-Man transferase                        |
| PP1      | protein phosphatase 1                            |
| PP2A     | protein phosphatase 2α                            |
| PPi      | inorganic phosphate                              |
| PPO      | Phosphoinositide-binding domain                   |
| Pse      | pseudaminic                                      |
| PTase    | phosphoglucosyltransferase                       |
| PTM      | post-translational modification                  |
| PUGNAc   | O-(2-acetamido-2-deoxy-D-glucopyranosylidene) amino N-phenyl carbamate |
| PY19L    | dpy-19 like C-Man transferase                    |
| R5P      | ribose-5-phosphate                               |
| RER      | rough endoplasmic reticulum                      |
| Rha      | L-rhamnose                                       |
| RPE      | Ru5P epimerase                                   |
| RPI      | Ru5P isomerase                                   |
| Ru5P     | ribulose-5-phosphate                             |
| S1P      | sphingosine-1-phosphate                          |
| S7P      | sedoheptulose-7-phosphate                        |
| SC       | satellite cell                                   |
| SDH      | succinate dehydrogenase                          |
| SIN3A    | SIN3 transcription regulator family member A     |
SIRT1 sirtuin 1  
SL succinyl-CoA ligase  
sOGA short OGA  
sOGT short OGT  
STZ streptozotocin  
TALDO transaldolase  
TET ten-eleven translocation protein  
Thiamet G O-(2-acetamido-2-deoxy-D-glucopyranoseylidene)  
TKT transketolase  
TM tropomyosin  
TMOD tropomodulin  
TPR tetratripeptide repeat domain  
TRIM32 tripartite motif protein  
TT04 3-[2-(1-adamantyl)ethyl]-2-(4-chlorophenyl)imino-4-oxo-1,3-thiazine-6-carboxylic acid  
UAP UDP-N-acetylglucosamine pyrophosphorylase  
UDP-5S-GlcNAc uridine diphospho-5-thio-N-acetylglucosamine or Ac4-5SGlcNAc  
UDPG UDP-Glucose  
UDP-Gal UDP-galactose  
UDP-GalNAc UDP-N-acetylgalactosamine  
UDP-GlcNAc UDP-N-acetylglucosamine  
UDP-xyl UDP-xylene  
UP UDP-Glucose pyrophosphorylase  
UTP uridine triphosphate  
Xu5P xylulose-5-phosphate  
Xyl D-xylose  
XYLT protein O-Xyl transferase  
ZASP Z-band alternatively spliced PDZ-motif protein  
α/β-DG α/β-dystroglycan  
α-GlcNAc 2-acetamido-2-deoxy-1-S-(4-methylbenzenesulfonyl)-1-thio-α-D-glu  
Thiosulfonate copyranose

References
1. Conibear, A.C. Deciphering protein post-translational modifications using chemical biology tools. Nat. Rev. Chem. 2020, 4, 674–695. [CrossRef]  
2. Liu, J.; Qian, C.; Cao, X. Post-Translational Modification Control of Innate Immunity. Immunity 2016, 45, 15–30. [CrossRef] [PubMed]  
3. Aebersold, R.; Agar, J.N.; Amster, I.J.; Baker, M.S.; Bertozzi, C.R.; Boja, E.S.; Costello, C.E.; Cravatt, B.F.; Fenselau, C.; Garcia, B.A.; et al. How many human proteoforms are there? Nat. Chem. Biol. 2018, 14, 206–214. [CrossRef] [PubMed]  
4. Schjoldager, K.T.; Narimatsu, Y.; Joshi, H.J.; Clausen, H. Global view of human protein glycosylation pathways and functions. Nat. Rev. Mol. Cell Biol. 2020, 21, 729–749. [CrossRef]  
5. Reily, C.; Stewart, T.J.; Renfrow, M.B.; Novak, J. Glycosylation in health and disease. Nat. Rev. Nephrol. 2019, 15, 346–366. [CrossRef]  
6. Yang, X.; Qian, A.K. Protein O-GlcNAcylation: Emerging mechanisms and functions. Nat. Rev. Mol. Cell Biol. 2017, 18, 452–465. [CrossRef]  
7. Chatham, J.C.; Zhang, J.; Wende, A.R. Role of O-Linked N-Acetylglucosamine Protein Modification in Cellular (Patho)Physiology. Physiol. Rev. 2021, 101, 427–493. [CrossRef]  
8. Hu, C.W.; Worth, M.; Li, H.; Jiang, J. Chemical and Biochemical Strategies To Explore the Substrate Recognition of O-GlcNAc-Cycling Enzymes. Chembiochem 2019, 20, 312–318. [CrossRef]  
9. Sharma, N.S.; Saluja, A.K.; Banerjee, S. “Nutrient-sensing” and self-renewal: O-GlcNAc in a new role. J. Bioenerg. Biomembr. 2018, 50, 205–211. [CrossRef] [PubMed]  
10. Sun, C.; Shang, J.; Yao, Y.; Yin, X.; Liu, M.; Liu, H.; Zhou, Y. O-GlcNAcylation: A bridge between glucose and cell differentiation. J. Cell Mol. Med. 2016, 20, 769–781. [CrossRef]  
11. Spiro, R.G. Protein glycosylation: Nature, distribution, enzymatic formation, and disease implications of glycopeptide bonds. Glycobiology 2002, 12, 43r–56r. [CrossRef] [PubMed]  
12. Itkonen, H.M.; Loda, M.; Mills, I.G. O-GlcNAc Transferase—An Auxiliary Factor or a Full-blown Oncogene? Mol Cancer Res. 2021, 19, 555–564. [CrossRef] [PubMed]
13. Wu, L.; Berenst, R.R.; Massman, L.; Danner, L.; Malard, F.; Vora, J.; Kharsay, R.; Olivier-Van Stichelen, S. The human O-GlcNAc database and meta-analysis. *Sci. Data* 2021, 8, 25. [CrossRef]

14. Morino, K.; Maegawa, H. Role of O-linked N-acetylglucosamine in the homeostasis of metabolic organs, and its potential links with diabetes and its complications. *J. Diabetes Investig.* 2021, 12, 130–136. [CrossRef][PubMed]

15. Eustice, M.; Bond, M.R.; Hanover, J.A. O-GlcNAc cycling and the regulation of nucleocytoplasmic dynamics. *Biochem. Soc. Trans.* 2017, 45, 427–436. [CrossRef][PubMed]

16. Sakaidani, Y.; Nomura, T.; Matsuura, A.; Ito, M.; Suzuki, E.; Murakami, K.; Nadano, D.; Matsuda, T.; Furukawa, K.; Okajima, T. O-linked-N-acetylgalactosamine on extracellular protein domains mediates epithelial cell-matrix interactions. *Nat. Commun.* 2011, 2, 583. [CrossRef]

17. Li, Y.; Xie, M.; Men, L.; Du, J. O-GlcNAcylation in immunity and inflammation: An intricate system (Review). *Int. J. Mol. Med.* 2019, 44, 363–374. [CrossRef]

18. Malard, F.; Wu, L. O-GlcNAcylation and Akt pathway regulates glucose metabolism in Drosophila melanogaster. *Cell. Mol. Life Sci.* 2015, 72, 3173–3183. [CrossRef] [PubMed]

19. Hart, G.W.; Slawson, C.; Ramirez-Corra, G.; Lagerlof, O. Cross talk between O-GlcNAcylation and phosphorylation: Roles in signaling, transcription, and chronic disease. *Annu. Rev. Biochem.* 2011, 80, 825–858. [CrossRef]

20. Yuan, A.; Tang, X.; Li, J. Centrosomes: O-GlcNAc Deacetylase DoUs Apart. *Front. Endocrinol.* 2020, 11, 621888. [CrossRef]

21. Cui, Z.; Scruggs, S.B.; Gilda, J.E.; Ping, P.; Gomes, A.V. Regulation of cardiac proteasomes by ubiquitination, SUMOylation, and beyond. *J. Mol. Cell. Cardiol.* 2014, 71, 32–42. [CrossRef][PubMed]

22. Ong, Q.; Han, W.; Yang, X. O-GlcNAc as an Integrator of Signaling Pathways. *Front. Endocrinol.* 2018, 9, 599. [CrossRef][PubMed]

23. Zhu, Y.; Hart, G.W. Targeting O-GlcNAcylaton to develop novel therapeutics. *Mol. Aspects Med.* 2021, 79, 100885. [CrossRef][PubMed]

24. Liu, Y.; Yao, R.Z.; Lian, S.; Liu, P.; Hu, Y.J.; Shi, H.Z.; Lv, H.M.; Yang, Y.Y.; Xu, B.; Li, S.Z. O-GlcNAcylation: The “stress and nutrition receptor” in cell stress response. *Cell Stress Chaperones* 2021, 26, 297–309. [CrossRef][PubMed]

25. Chen, P.H.; Chi, J.T.; Boyce, M. Functional crosstalk among oxidative stress and O-GlcNAc signaling pathways. *Glycobiochemistry* 2018, 28, 556–564. [CrossRef]

26. Han, C.; Gu, Y.; Shan, H.; Mi, W.; Sun, J.; Shi, M.; Zhang, X.; Lu, X.; Han, F.; Gong, Q.; et al. O-GlcNAcylation of SIRT1 enhances its deacetylase activity and promotes cytoprotection under stress. *Nat. Commun.* 2017, 8, 1491. [CrossRef]

27. Liu, Y.; Xu, B.; Hu, Y.; Liu, P.; Lian, S.; Lv, H.; Yang, Y.; Ji, H.; Yang, H.; Liu, J.; et al. O-GlcNAcylation of Akt pathway regulates glucose metabolism and reduces apoptosis in liver of piglets with acute cold stress. *Cryobiology* 2021, 100, 125–132. [CrossRef]

28. Ma, Z.; Vosseller, K. Cancer metabolism and elevated O-GlcNAc in oncogenic signaling. *J. Biol. Chem.* 2014, 289, 34457–34465. [CrossRef]

29. Hart, G.W. Nutrient regulation of transcription and translation. *J. Biol. Chem.* 2019, 294, 2211–2231. [CrossRef]

30. Qian, K.; Wang, S.; Fu, M.; Zhou, J.; Singh, J.P.; Li, M.D.; Yang, X.; Zhang, K.; Wu, J.; Nie, Y.; et al. Transcriptional regulation of O-GlcNAc homeostasis is disrupted in pancreatic cancer. *J. Biol. Chem.* 2018, 293, 13989–14000. [CrossRef]

31. Schwein, P.A.; Woo, C.M. The O-GlcNAc Modification on Kinases. *ACS Chem. Biol.* 2020, 15, 602–617. [CrossRef][PubMed]

32. Li, X.; Zhu, Q.; Shi, X.; Cheng, Y.; Li, X.; Xu, H.; Duan, X.; Hsieh-Wilson, L.C.; Chu, J.; Pelletier, J.; et al. O-GlcNAcylation of core components of the translation initiation machinery regulates protein synthesis. *Proc. Natl. Acad. Sci. USA* 2019, 116, 7857–7866. [CrossRef][PubMed]

33. Guo, B.; Liang, Q.; Li, L.; Hu, Z.; Wu, F.; Zhang, P.; Ma, Y.; Zhao, B.; Kovacs, A.L.; Zhang, Z.; et al. O-GlcNAc-modification of SNAP-29 regulates autophagosome maturation. *Nat. Cell Biol.* 2014, 16, 1215–1226. [CrossRef][PubMed]

34. Park, S.; Lee, Y.; Pak, J.W.; Kim, H.; Choi, H.; Kim, J.W.; Roth, J.; Cho, J.W. O-GlcNAc modification is essential for the regulation of autophagy in Drosophila melanogaster. *Cell. Mol. Life Sci.* 2015, 72, 3173–3183. [CrossRef][PubMed]

35. Li, X.; Gong, W.; Wang, H.; Li, T.; Attiri, K.S.; Lewis, R.E.; Kalil, A.C.; Bhinderwala, F.; Powers, R.; Yin, G.; et al. O-GlcNAc Transferase Suppresses Inflammation and Necrosis by Targeting Receptor-Interacting Serine/Threonine-Protein Kinase 3. *Immunity* 2019, 50, 576–590.e6. [CrossRef]

36. Chang, Y.H.; Weng, C.L.; Lin, K.I. O-GlcNAcylation and its role in the immune system. *J. Biomed. Sci.* 2020, 27, 57. [CrossRef]

37. Machacek, M.; Slawson, C.; Fields, P.E. O-GlcNAc: A novel regulator of immunometabolism. *J. Bioenerg. Biomembr.* 2018, 50, 223–229. [CrossRef]

38. Dong, H.; Liu, Z.; Wen, H. Protein O-GlcNAcylation Regulates Innate Immune Cell Function. *Front. Immunol.* 2020, 11, 437–448. [CrossRef][PubMed]

39. Singh, J.P.; Zhang, K.; Wu, J.; Yang, X. O-GlcNAc signaling in cancer metabolism and epigenetics. *Cancer Lett.* 2015, 356, 244–250. [CrossRef][PubMed]
44. Parker, M.P.; Peterson, K.R.; Slawson, C. O-GlcNAcylation and O-GlcNAc Cycling Regulate Gene Transcription: Emerging Roles in Cancer. Cancers 2021, 13, 1666. [CrossRef] [PubMed]
45. Rao, X.; Duan, X.; Mao, W.; Li, X.; Li, Z.; Li, Q.; Zheng, Z.; Xu, H.; Chen, M.; Wang, J.G.; et al. O-GlcNAcylation of G6PD promotes the pentose phosphate pathway and tumor growth. Nat. Commun. 2015, 6, 9468. [CrossRef]
46. Vaidyanathan, K.; Niranjan, T.; Selvan, N.; Teo, C.F.; May, M.; Patel, S.; Weatherly, B.; Skinner, C.; Opitz, J.; Carey, J. Identification and characterization of a missense mutation in the O-linked β-N-acetylgalactosamine (O-GlcNAc) transferase gene that segregates with X-linked intellectual disability. J. Biol. Chem. 2017, 292, 8948–8963. [CrossRef]
47. Akam, I.; Olivier-Van Stichel, S.; Bond, M.R.; Hanover, J.A. Nutrient-driven O-GlcNAc in proteostasis and neurodegeneration. J. Neurochem. 2018, 144, 7–34. [CrossRef]
48. Hwang, H.; Rhim, H. Functional significance of O-GlcNAc modification in regulating neuronal properties. Pharmacol. Res. 2018, 129, 295–307. [CrossRef]
49. Yang, Y.; Fu, M.; Li, M.D.; Zhang, K.; Zhang, B.; Wang, S.; Liu, Y.; Ni, W.; Ong, Q.; Mi, J.; et al. O-GlcNAc transferase inhibits visceral fat lipolysis and promotes diet-induced obesity. Nat. Commun. 2020, 11, 181. [CrossRef]
50. Chatham, J.C.; Young, M.E.; Zhang, J. Reprint of: Role of O-linked N-acetylgalactosamine (O-GlcNAc) modification of proteins in diabetic cardiovascular complications. Curr. Opin. Pharmacol. 2020, 54, 209–220. [CrossRef] [PubMed]
51. Issad, T.; Masson, E.; Pagesy, P. O-GlcNAc modification, insulin signaling and diabetic complications. Diabetes Metab. 2010, 36 6 Pt 1, 423–435. [CrossRef]
52. Banerjee, P.S.; Lagerlöf, O.; Hart, G.W. New insights: A role for O-GlcNAc in chronic diseases of aging. Crit. Rev. Biochem. Mol. Biol. 2016, 51, 1–15. [CrossRef] [PubMed]
53. Peterson, S.B.; Hart, G.W. New insights: A role for O-GlcNAc in diabetic complications. Crit. Rev. Biochem. Mol. Biol. 2016, 51, 150–161. [CrossRef] [PubMed]
54. Gurel, Z.; Sheibani, N. O-Linked β-N-acetylgalactosamine (O-GlcNAc) modification: A new pathway to decode pathogenesis of diabetic retinopathy. Clin. Sci. 2018, 132, 185–198. [CrossRef] [PubMed]
55. Stevens, L.; Bastide, B.; Hedou, J.; Cieniewski-Bernard, C.; Montel, V.; Cochron, L.; Dupont, E.; Mounier, Y. Potential regulation of human muscle plasticity by MiLC2 post-translational modifications during bed rest and countermeasures. Arch. Biochem. Biophys. 2013, 540, 125–132. [CrossRef] [PubMed]
56. Nagy, T.; Káta, E.; Fisi, V.; Takács, T.T.; Stréda, A.; Wittmann, I.; Miseta, A. Protein O-GlcNAc Modification Increases in White Blood Cells After a Single Bout of Physical Exercise. Front. Immunol. 2018, 9, 970. [CrossRef]
57. Petermel, T.T.; Marsh, S.A.; Strobel, N.A.; Matsumoto, A.; Briskey, D.; Dalbo, V.J.; Tucker, P.S.; Coombes, J.S. Glutathione depletion and acute exercise increase O-GlcNAc protein modification in rat skeletal muscle. Mol. Cell. Biochem. 2015, 400, 265–275. [CrossRef]
58. Murata, K.; Morino, K.; Ida, S.; Ohashi, N.; Lemecha, M.; Park, S.Y.; Ishikado, A.; Kume, S.; Choi, C.S.; Sekine, O.; et al. Lack of O-GlcNAcylation enhances exercise-dependent glucose utilization potentially through AMP-activated protein kinase activation in skeletal muscle. Biochem. Biophys. Res. Commun. 2018, 495, 2098–2104. [CrossRef]
59. Lambert, M.; Bastide, B.; Cieniewski-Bernard, C. Involvement of O-GlcNAcylation in the Skeletal Muscle Physiology and Physiopathology: Focus on Muscle Metabolism. Front. Endocrinol. 2018, 9, 578. [CrossRef]
60. Wang, X.; Feng, Z.; Wang, X.; Yang, L.; Han, S.; Cao, K.; Xu, J.; Zhao, L.; Zhang, Y.; Liu, J. O-GlcNAcase deficiency suppresses skeletal myogenesis and insulin sensitivity in mice through the modulation of mitochondrial homeostasis. Diabetologia 2016, 59, 1287–1296. [CrossRef]
61. Pedowitz, N.J.; Bhat, A.R.; Darabedian, N.; Pratt, M.R. MYPT1 O-GlcNAc modification regulates sphinogosine-1-phosphate mediated contraction. Nat. Chem. Biol. 2021, 17, 169–177. [CrossRef] [PubMed]
62. Shi, H.; Munk, A.; Nielsen, T.S.; Daugtry, M.R.; Larsson, L.; Li, S.; Høyer, K.F.; Geisler, H.W.; Sulek, K.; Kjøbsted, R.; et al. Skeletal muscle O-GlcNAc transferase is important for muscle energy homeostasis and whole-body insulin sensitivity. Mol. Metab. 2018, 11, 160–177. [CrossRef] [PubMed]
63. D’Souza, D.M.; Al-Sajee, D.; Hawke, T.J. Diabetic myopathy: Impact of diabetes mellitus on skeletal muscle progenitor cells. Front. Physiol. 2013, 4, 379. [CrossRef] [PubMed]
64. Nakamura, S.; Nakano, S.; Nishii, M.; Kaneko, S.; Kusaka, H. Localization of O-GlcNAc-modified proteins in neuromuscular diseases. Med. Mol. Morphol. 2012, 45, 86–90. [CrossRef] [PubMed]
65. Cieniewski-Bernard, C.; Lambert, M.; DuPont, E.; Montel, V.; Stevens, L.; Bastide, B. O-GlcNAcylation, contractile protein modifications and calcium affinity in skeletal muscle. Front. Physiol. 2014, 5, 421. [CrossRef]
66. Akimoto, Y.; Yan, K.; Miura, Y.; Tsumoto, H.; Toda, T.; Fukutomi, T.; Sugahara, D.; Kudo, A.; Brai, T.; Chiba, Y.; et al. O-GlcNAcylation and phosphorylation of β-actin Ser(199) in diabetic nephropathy. Am. J. Physiol. Renal. Physiol. 2019, 317, F1359–F1374. [CrossRef]
67. Lambert, M.; Claeyssens, C.; Bastide, B.; Cieniewski-Bernard, C. O-GlcNAcylation as a regulator of the functional and structural properties of the sarcomere in skeletal muscle: An update review. Acta Physiol. 2020, 228, e13301. [CrossRef]
68. Ng, Y.H.; Okolo, C.A.; Erickson, J.R.; Baldi, J.C.; Jones, P.P. Protein O-GlcNAcylation in the heart. Acta Physiol. 2021, 233, e13696. [CrossRef]
69. Ngoh, G.A.; Facundo, H.T.; Zafir, A.; Jones, S.P. O-GlcNAc signaling in the cardiovascular system. Circ. Res. 2010, 107, 171–185. [CrossRef]
126. Hanover, J.A.; Krause, M.W.; Love, D.C. The hexosamine signaling pathway: O-GlcNAc cycling in feast or famine. Biochim. Biophys. Acta 2010, 1800, 80–95. [CrossRef]

127. Adeva-Andany, M.M.; Pérez-Felpeto, N.; Fernández-Fernández, C.; Donapetrey-García, C.; Pazos-García, C. Liver glucose metabolism in humans. Biophis. Biophys. Acta 2016, 36, e00416. [CrossRef]

128. Filhoulaud, G.; Guillemain, G.; Scharffmann, R. The hexosamine biosynthesis pathway is essential for pancreatic beta cell development. J. Biol. Chem. 2009, 284, 24583–24594. [CrossRef]

129. Love, D.C.; Hanover, J.A. The hexosamine signaling pathway: Deciphering the “O-GlcNAc code”. Sci. STKE 2005, 2005, re13. [CrossRef]

130. Zibrova, D.; Vandermoere, F.; Göransson, O.; Peggie, M.; Mariño, K.V.; Knierim, A.; Spengler, K.; Weigert, C.; Viollet, B.; Morrice, N.A.; et al. GFAT1 phosphorylation by AMPK promotes VEGF-induced angiogenesis. Biochem. J. 2017, 474, 983–1001. [CrossRef]

131. Ruegenberg, S.; Horn, M.; Pichilo, C.; Allmeroth, K.; Baumann, U.; Denzel, M.S. Loss of GFAT-1 feedback regulation activates the hexosamine pathway that modulates protein homeostasis. Nat. Commun. 2020, 11, 687. [CrossRef] [PubMed]

132. Leto, D.; Saltiel, A.R. Regulation of glucose transport by insulin: Traffic control of GLUT4. Nat. Rev. Mol. Cell Biol. 2012, 13, 383–396. [CrossRef] [PubMed]

133. Eguchi, S.; Oshiro, N.; Miyamoto, T.; Yoshino, K.; Okamoto, S.; Ono, T.; Kikkawa, U.; Yonezawa, K. AMP-activated protein kinase phosphorylates glutamine: Fructose-6-phosphate amidotransferase 1 at Ser243 to modulate its enzymatic activity. Genes Cells 2009, 14, 179–189. [CrossRef] [PubMed]

134. Ruegenberg, S.; Mayr, F.; Atanassov, I.; Baumann, U.; Denzel, M.S. Protein kinase A controls the hexosamine pathway by tuning the feedback inhibition of GFAT-1. Nat. Commun. 2021, 12, 2176. [CrossRef] [PubMed]

135. Al-Mukh, H.; Baudoin, L.; Bouaboud, A.; Sanchez-Salgado, J.L.; Maraña, N.; Khair, M.; Pagesy, P.; Bismuth, G.; Niedergang, F.; Issad, T. Lipopolysaccharide Induces GFAT2 Expression to Promote O-Linked β-N-Acetylgalcosaminylatation and Attenuate Inflammation in Macrophages. J. Immunol. 2020, 205, 2499–2510. [CrossRef] [PubMed]

136. Horn, M.; Denzel, S.I.; Srinivasan, B.; Allmeroth, K.; Schiffer, I.; Breuer, P.; Antebi, A.; Denzel, M.S. Hexosamine Pathway Activation Improves Protein Homeostasis through the Integrated Stress Response. iScience 2020, 23, 100887. [CrossRef]

137. Denzel, M.S.; Antebi, A. Hexosamine pathway and (ER) protein quality control. Curr. Opin. Cell Biol. 2015, 33, 14–18. [CrossRef]

138. Zachara, N.E.; Hart, G.W. 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. [CrossRef]

139. Biwi, J.; Biot, C.; Guerrardel, Y.; Vercoutter-Edouart, A.S.; Lefebvre, T. The Many Ways by Which O-GlcNAcylated Metabolites Enhance Protein Quality Control and Prolong Life. Biochim. Biophys. Acta 2020, 1800, 80–95. [CrossRef]

140. Lam, C.; Low, J.Y.; Tran, P.T.; Wang, H. The hexosamine signaling pathway: O-GlcNAc cycling in feast or famine. Biochim. Biophys. Acta 2015, 1800, 241–248. [CrossRef] [PubMed]

141. Olivier-Van Stichelen, S.; Hanover, J.A. You are what you eat: O-linked N-acetylglucosamine in disease, development and epigenetics. Curr. Opin. Clin. Nutr. Metab. Care 2008, 11, 339–345. [CrossRef] [PubMed]

142. Coussement, P.; Bauwens, D.; Peters, G.; Maertens, J.; De Mey, M. Mapping and refactoring pathway control through metabolic pathway metabolites enhance protein quality control and prolong life. Biochim. Biophys. Acta 2014, 1840, 1467–1476. [CrossRef] [PubMed]

143. Vincenz, L.; Hartl, F.U. Sugarcoating ER Stress. J. Bioenerg. Biomembr. 2013, 45, 719–733. [CrossRef] [PubMed]

144. Saha, A.; Bello, D.; Fernández-Tejada, A. Advances in chemical probing of protein O-GlcNAc glycosylation: Structural role and molecular mechanisms. Chem. Soc. Rev. 2020, 50, 10451–10485. [CrossRef]

145. Akella, N.M.; Ciraku, L.; Reginato, M.J. Fueling the fire: Emerging role of the hexosamine biosynthetic pathway in cancer. BMC Biol. 2019, 17, 52. [CrossRef] [PubMed]

146. Chatham, J.C.; Nöt, L.G.; Fülpö, N.; Marchase, R.B. Hexosamine biosynthesis and protein O-glycosylation: The first line of defense against stress, ischemia, and trauma. Shock 2008, 29, 431–440. [CrossRef] [PubMed]

147. Vasconcelos-Dos-Santos, A.; Oliveira, I.A.; Lucena, M.C.; Mantuano, N.R.; Whelan, S.A.; Dias, W.B.; Todeschini, A.R. Biosynthetic Machinery Involved in aberrant Glycosylation: Promising Targets for Developing of Drugs Against Cancer. Front. Oncol. 2015, 5, 138. [CrossRef]

148. Denzel, M.S.; Storm, N.J.; Gutschmidt, A.; Baddi, R.; Hinze, Y.; Jarosch, E.; Sommer, T.; Hoppe, T.; Antebi, A. Hexosamine pathway metabolites enhance protein quality control and prolong life. Cell 2014, 156, 1167–1178. [CrossRef] [PubMed]

149. Groves, J.A.; Lee, A.; Yildirim, G.; Zachara, N.E. Dynamic O-GlcNAcylation and its roles in the cellular stress response and homeostasis. Cell Stress Chaperones 2013, 18, 535–558. [CrossRef] [PubMed]

150. Bolanle, I.O.; Palmer, T.M. Targeting Protein O-GlcNAcylation, a Link between Type 2 Diabetes Mellitus and Inflammatory Disease. Cells 2022, 11, 705. [CrossRef]

151. Ma, Z.; Vosseller, K. O-GlcNAc in cancer biology. Amino Acids 2013, 45, 719–733. [CrossRef] [PubMed]

152. Bacigaluppa, Z.A.; Bhadiara, C.H.; Reginato, M.J. O-GlcNAcylation: Key regulator of glycolytic pathways. J. Bioenerg. Biomembr. 2018, 50, 189–198. [CrossRef] [PubMed]

153. Nie, H.; Jia, H.; Fan, J.; Shi, X.; Cheng, Y.; Cang, X.; Zheng, Z.; Duan, X.; Yi, W. O-GlcNAcylation of PGK1 coordinates glycolysis and TCA cycle to promote tumor growth. Nat. Commun. 2020, 11, 36. [CrossRef] [PubMed]

154. Singh, J.P.; Qian, K.; Lee, J.S.; Zhou, J.; Han, X.; Zhang, B.; Ong, Q.; Ni, W.; Jiang, M.; Ruan, H.B.; et al. O-GlcNAcase targets pyruvate kinase M2 to regulate tumor growth. Oncogene 2020, 39, 560–573. [CrossRef]
155. Yi, W.; Clark, P.M.; Mason, D.E.; Keenan, M.C.; Hill, C.; Goddard, W.A.; Peters, E.C.; Driggers, E.M.; Hsieh-Wilson, L.C. Phosphofructokinase 1 glycosylation regulates cell growth and metabolism. *Science* 2012, 337, 975–980. [CrossRef]

156. Maury, J.J.; Ng, D.; Bi, X.; Bardor, M.; Choo, A.B. Multiple reaction monitoring mass spectrometry for the discovery and quantification of O-GlcNAc-modified proteins. *Anal. Chem.* 2014, 86, 395–402. [CrossRef]

157. Ou, W.; Liang, Y.; Qin, Y.; Wu, W.; Xie, M.; Zhang, Y.; Zhang, Y.; Ji, L.; Yu, H.; Li, T. Hypoxic acclimation improves cardiac redox homeostasis and protects heart against ischaemia-reperfusion injury through upregulation of O-GlcNAcylation. *Redox Biol.* 2021, 43, 101994. [CrossRef]

158. Baldini, S.F.; Lefebvre, T. O-GlcNAcylation and the Metabolic Shift in High-Proliferating Cells: All the Evidence Suggests that Sugars Dictate the Flux of Lipid Biogenesis in Tumor Processes. *Front. Oncol.* 2016, 6, 6. [CrossRef]

159. Benhamed, F.; Filhoulaud, G.; Caron, S.; Lefebvre, P.; Staels, B.; Pistic, C. O-GlcNAcylation Links ChREBP and FXR to Glucose-Sensing. *Front. Endocrinol.* 2014, 5, 230. [CrossRef]

160. Lim, K.; Yoon, B.H.; Ha, C.H. O-Linked N-acetylgalcosaminylation of Sp1 interferes with Sp1 activation of glycolytic genes. *Biochem. Biophys. Res. Commun.* 2015, 468, 349–353. [CrossRef]

161. Kuo, M.; Zilberfarb, V.; Gangneux, N.; Christeff, N.; Issad, T. O-glycosylation of FoxO1 increases its transcriptional activity towards the glucose 6-phosphatase gene. *FEBS Lett.* 2008, 582, 829–834. [CrossRef] [PubMed]

162. Reggiori, F.; Gabius, H.J.; Aureli, M.; Römer, W.; Sonnino, S.; Eskelinen, E.L. Glycans in autophagy, endocytosis and lysosomal functions. *Glycoconj J.* 2021, 38, 625–647. [CrossRef] [PubMed]

163. Cao, W.; Cao, J.; Huang, J.; Yao, J.; Yan, G.; Xu, H.; Yang, P. Discovery and confirmation of O-GlcNAcylated proteins in rat liver mitochondria by combination of mass spectrometry and immunological methods. *PLoS ONE* 2013, 8, e76399. [CrossRef] [PubMed]

164. Deshmukh, A.S. Insulin-stimulated glucose uptake in healthy and insulin-resistant skeletal muscle. *Horm. Mol. Biol. Clin. Investig.* 2016, 26, 13–24. [CrossRef] [PubMed]

165. Jørgensen, S.B.; Richter, E.A.; Wojtaszewski, J.F. Role of AMPK in skeletal muscle metabolic regulation and adaptation in relation to exercise. *J. Physiol.* 2006, 574, 17–31. [CrossRef] [PubMed]

166. Hargreaves, M.; Spriet, L.L. Skeletal muscle energy metabolism during exercise. *Nat. Metab.* 2020, 2, 817–828. [CrossRef] [PubMed]

167. Ojuka, E.O.; Goyaram, V.; Smith, J.A. The role of CaMKII in regulating GLUT4 expression in skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 2012, 303, E322–E331. [CrossRef]

168. Merz, K.E.; Thurmond, D.C. Role of Skeletal Muscle in Insulin Resistance and Glucose Uptake. *Cell Metab.* 2002, 5, 162–184. [CrossRef]

169. Hortemo, K.H.; Lunde, P.K.; Anonsen, J.H.; Kvaløy, H.; Munkvik, M.; Rehn, T.A.; Sjaastad, I.; Lunde, I.G.; Aronsen, J.M.; Sejersted, O.M. Exercise training increases protein O-GlcNAcylation in rat skeletal muscle. *Physiol. Rep.* 2016, 4, e12896. [CrossRef]

170. Teo, C.F.; Wollast-On Hayden, E.E.; Wells, L. Hexosamine flux, the O-GlcNAc modification, and the development of insulin resistance in adipocytes. *Mol. Cell. Endocrinol.* 2010, 318, 44–53. [CrossRef] [PubMed]

171. Santos, J.M.; Ribeiro, S.B.; Gaya, A.R.; Appel, H.J.; Duarte, J.A. Skeletal muscle pathways of contraction-enhanced glucose uptake. *Int. J. Sports Med.* 2008, 29, 785–794. [CrossRef]

172. Jaldin-Fincati, J.R.; Pavarotti, M.; Frendo-Cumbo, S.; Bilan, P.J.; Klip, A. Update on GLUT4 Vesicle Traffic: A Cornerstone of Insulin Action. *Trends. Endocrinol. Metab.* 2017, 28, 597–611. [CrossRef] [PubMed]

173. Guo, X.; Sun, W.; Luo, G.; Wu, L.; Xu, G.; Hou, D.; Hou, Y.; Guo, X.; Mu, X.; Qin, L.; et al. Panax notoginseng saponins alleviate skeletal muscle insulin resistance by regulating the IRS1-PI3K-AKT signaling pathway and GLUT4 expression. *FEBS Open Bio.* 2019, 9, 1008–1019. [CrossRef] [PubMed]

174. Buse, M.G.; Robinson, K.A.; Marshall, B.A.; Hresko, R.C.; Mueckler, M.M. Enhanced O-GlcNAc protein modification is associated with insulin resistance in GLUT1-overexpressing muscles. *Am. J. Physiol. Endocrinol. Metab.* 2002, 283, E241–E250. [CrossRef] [PubMed]

175. Ferrer, C.M.; Lynch, T.P.; Sodi, V.L.; Falcone, J.N.; Schwab, L.P.; Peacock, D.L.; Vocadlo, D.J.; Seagroves, T.N.; Regnato, M.J. O-GlcNAcylation regulates cancer metabolism and survival stress signaling via regulation of the HIF-1 pathway. *Mol. Cell* 2014, 54, 820–831. [CrossRef] [PubMed]

176. Baldini, S.F.; Steenackers, A.; Olivier-Van Stichelen, S.; Mir, A.M.; Mortuaires, M.; Lefebvre, T.; Guinez, C. Glucokinase expression is regulated by glucose through O-GlcNAc glycosylation. *Biochem. Biophys. Res. Commun.* 2016, 478, 942–948. [CrossRef]

177. Dong, X.; Li, Y.; Chang, P.; Tang, H.; Hess, K.R.; Abbruzzese, J.L.; Li, D. Glucose metabolism gene variants modulate the risk of pancreatic cancer. *Cancer Prev. Res.* 2011, 4, 758–766. [CrossRef]

178. Qian, X.; Li, X.; Shi, Z.; Xia, Y.; Cai, Q.; Xu, D.; Tan, L.; Du, L.; Zheng, Y.; Zhao, D.; et al. PTEN Suppresses Glycolysis by Dephosphorylating and Inhibiting Autophosphorylated PGK1. *Mol. Cell.* 2019, 76, 516–527.e7. [CrossRef]

179. Li, X.; Jiang, Y.; Meisenhelder, J.; Yang, W.; Hawke, D.H.; Zheng, Y.; Xia, Y.; Aldape, K.; He, J.; Hunter, T.; et al. Mitochondria-Translocated PGK1 Functions as a Protein Kinase to Coordinate Glycolysis and the TCA Cycle in Tumorigenesis. *Mol. Cell* 2016, 61, 705–719. [CrossRef]

180. Lei, Y.; Chen, T.; Li, Y.; Shang, M.; Zhang, Y.; Jin, Y.; Yu, Q.; Guo, F.; Wang, T. O-GlcNAcylation of PKFB3 is required for tumor cell proliferation under hypoxia. *Oncogenesis* 2020, 9, 21. [CrossRef] [PubMed]
182. Gao, X.; Wang, H.; Yang, J.J.; Liu, X.; Liu, Z.R. Pyruvate kinase M2 regulates gene transcription by acting as a protein kinase. *Mol. Cell* **2012**, *45*, 598–609. [CrossRef] [PubMed]

183. Chaiyawat, P.; Chokchaicharnakit, D.; Lirdprapamongkol, K.; Srisomsap, C.; Svasti, J.; Champattanachai, V. Alteration of O-GlcNAcylation affects serine phosphorylation and regulates gene expression and activity of pyruvate kinase M2 in colorectal cancer cells. *Onco. Rep.* **2015**, *34*, 1933–1942. [CrossRef] [PubMed]

184. jóźwiak, P.; Forma, E.; Bryś, M.; Krzesiak, A. O-GlcNAcylation and Metabolic Reprograming in Cancer. *Front. Endocrinol.* **2014**, *5*, 145.

185. Woolbright, B.L.; Rajendran, G.; Harris, R.A.; Taylor, J.A. 3rd. Metabolic Flexibility in Cancer: Targeting the Pyruvate Dehydrogenase Kinase Pyruvate Dehydrogenase Axis. *Mol. Cancer* **2019**, *18*, 1673–1681. [CrossRef]

186. Ande, S.R.; Padilla-Meier, G.P.; Mishra, S. Mutually exclusive acetylation and ubiquitylation among enzymes involved in glucose metabolism. *Adipocyte* **2013**, *2*, 256–261. [CrossRef]

187. Tan, E.P.; Villar, M.T.; Lezi, E.; Lu, J.; Selfridge, J.E.; Artigues, A.; Swerdlow, R.H.; Lawson, C. Altering β-N-acetylgalcosamine cycling disrupts mitochondrial function. *J. Biol. Chem.* **2014**, *289*, 14719–14730. [CrossRef]

188. jóźwiak, P.; Ciesielski, P.; Zakrzewski, P.K.; Kozal, K.; Oracz, J.; Budryn, G.; Żyżelewicz, D.; Flament, S.; Vercoutter-Edouart, A.S.; Bray, F.; et al. Mitochondrial O-GlcNAc Transferase Interacts with and Modifies Many Proteins and Its Up-Regulation Affects Mitochondrial Function and Cellular Energy Homeostasis. *Cancers* **2021**, *13*, 2956. [CrossRef]

189. Ohashi, N.; Morino, K.; Ida, S.; Sekine, O.; Lemecha, M.; Kume, S.; Park, S.Y.; Choi, C.S.; Ugi, S.; Maegawa, H. Pivotal Role of O-GlcNAc Modification in Cold-Induced Thermogenesis by Brown Adipose Tissue Through Mitochondrial Biogenesis. *Diabetes* **2017**, *66*, 2351–2362. [CrossRef] [PubMed]

190. Liu, Y.; Chen, Q.; Zhang, N.; Zhang, K.; Tou, T.; Cao, Y.; Liu, Y.; Li, K.; Hao, X.; Xie, X.; et al. Proteomic profiling and genome-wide mapping of O-GlcNAc chematin-associated proteins reveal an O-GlcNAc-regulated genotoxic stress response. *Nat. Commun.* **2020**, *11*, 5898. [CrossRef]

191. Gizał, A.; Duda, P.; Wisniewski, J.; Rakus, D. Fructose-1,6-bisphosphatase: From a glucose metabolism enzyme to multifaceted regulator of a cell fate. *Adv. Biol. Regul.* **2019**, *72*, 41–50. [CrossRef] [PubMed]

192. Rakus, D.; Mamczur, P.; Gizał, A.; Dus, D.; Dzugaj, A. Colocalization of muscle FBPase and muscle aldolase on both sides of the Z-line. *Biochem. Biophys. Res. Commun.* **2021**, *311*, 294–299. [CrossRef] [PubMed]

193. Gizał, A.; Maciaszczyk, E.; Dzugaj, A.; Eschrich, K.; Rakus, D. Evolutionary conserved N-terminal region of human muscle fructose 1,6-bisphosphatase regulates its activity and the interaction with aldolase. *Proteins* **2008**, *72*, 209–216. [CrossRef] [PubMed]

194. Issad, T. O-GlcNAc glycosylation and regulation of cell signaling. *Med. Sci.* **2019**, *7*, 1451–1460. [CrossRef] [PubMed]

195. Martinez, M.; Renuse, S.; Kreimer, S.; O’Meally, R.; Natov, P.; Madugundu, A.K.; Nirujogi, R.S.; Tahir, R.; Cole, R.; Pandey, A. Roles of the hexosamine biosynthetic pathway and pentose phosphate pathway in bile acid-induced cancer development. *Mol. Cell. Proteom.* **2018**, *17*, 2351–2362. [CrossRef] [PubMed]

196. Rakus, D.; Mamczur, P.; Gizał, A.; Dus, D.; Dzugaj, A. Colocalization of muscle FBPase and muscle aldolase on both sides of the Z-line. *Biochem. Biophys. Res. Commun.* **2021**, *311*, 294–299. [CrossRef] [PubMed]

197. Gizał, A.; Maciaszczyk, E.; Dzugaj, A.; Eschrich, K.; Rakus, D. Evolutionary conserved N-terminal region of human muscle fructose 1,6-bisphosphatase regulates its activity and the interaction with aldolase. *Proteins* **2008**, *72*, 209–216. [CrossRef] [PubMed]

198. Ansari, S.A.; Emerald, B.S. The Role of Insulin Resistance and Protein O-GlcNAcylation in Neurodegeneration. *Front. Neurosci.* **2019**, *13*, 473. [CrossRef] [PubMed]
207. Kebede, M.; Ferdaoussi, M.; Mancini, A.; Alquier, T.; Kulkarni, R.N.; Walker, M.D.; Poitout, V. Glucose activates free fatty acid receptor 1 gene transcription via phosphatidylinositol-3-kinase-dependent O-GlcNAcylation of pancreas-duodenum homebox-1. *Proc. Natl. Acad. Sci. USA* 2012, 109, 2376–2381. [CrossRef]

208. Kaleem, A.; Javed, S.; Rehman, N.; Abdullah, R.; Iqtedar, M.; Aftab, M.N.; Haq, I.U. Phosphorylated and O-GlcNAc Modified IRS-1 (Ser1101) and -2 (Ser1149) Contribute to Human Diabetes Type II. *Protein Pept. Lett.* 2021, 28, 333–339. [CrossRef]

209. Wang, S.; Huang, X.; Sun, D.; Xin, X.; Fan, Q.; Peng, S.; Liang, Z.; Luo, C.; Yang, Y.; Jiang, H.; et al. Extensive crosstalk between O-GlcNAc modification and phosphorylation regulates Akt signaling. *PLoS ONE* 2012, 7, e37427. [CrossRef] [PubMed]

210. Copeland, R.J.; Bullen, J.W.; Hart, G.W. Cross-talk between GlcNAcylation and phosphorylation: Roles in insulin resistance and glucose toxicity. *Am. J. Physiol. Endocrinol. Metab.* 2008, 295, E17–E28. [CrossRef] [PubMed]

211. Ramirez-Correa, G.A.; Jin, W.; Wang, Z.; Zhong, X.; Gao, W.D.; Dias, W.B.; Vecoli, C.; Hart, G.W.; Murphy, A.M. O-linked GlcNAc modification of cardiac myofilament proteins: A novel regulator of myocardial contractile function. *Circ. Res.* 2008, 103, 1354–1358. [CrossRef] [PubMed]

212. Ramirez-Correa, G.A.; Ma, J.; Slawson, C.; Zeidan, Q.; Lugo-Fagundo, N.S.; Xu, M.; Shen, X.; Gao, W.D.; Caceres, V.; Chakir, K.; et al. Removal of Abnormal Myofilament O-GlcNAcylation Restores Ca2+ Sensitivity in Diabetic Cardiac Muscle. *Diabetes* 2015, 64, 3573–3587. [CrossRef] [PubMed]

213. Hedou, J.; Cieniewski-Bernard, C.; Leroy, Y.; Michalski, J.C.; Mounier, Y.; Bastide, B. O-linked N-acetylglucosaminylation is involved in the Ca2+ activation properties of rat skeletal muscle. *J. Biol. Chem.* 2007, 282, 10360–10369. [CrossRef]

214. Metzger, J.M.; Westfall, M.V. Covalent and noncovalent modification of thin filament action: The essential role of troponin in cardiac muscle regulation. *Circ. Res.* 2004, 94, 146–158. [CrossRef]

215. Salhi, H.E.; Hassel, N.C.; Siddiqui, J.K.; Brundage, E.A.; Ziolo, M.T.; Janssen, P.M.; Davis, J.P.; Biesiadecki, B.J. Myofilament Calcium Sensitivity: Mechanistic Insight into Tnl Ser-23/24 and Ser-150 Phosphorylation Integration. *Front. Physiol.* 2016, 7, 567. [CrossRef]

216. Ramirez-Correa, G.A.; Jin, W.; Wang, Z.; Zhong, X.; Gao, W.D.; Dias, W.B.; Vecoli, C.; Hart, G.W.; Murphy, A.M. O-linked GlcNAc modification of cardiac myofilament proteins: A novel regulator of myocardial contractile function. *Circ. Res.* 2008, 103, 1354–1358. [CrossRef] [PubMed]

217. Cieniewski-Bernard, C.; Montel, V.; Berthoin, S.; Bastide, B. Increasing O-GlcNAcylation level on organ culture of soleus modulates the calcium activation parameters of muscle fibers. *PLoS ONE* 2012, 7, e48218. [CrossRef]

218. Bayliss, C.R.; Jacques, A.M.; Leung, M.C.; Ward, D.G.; Redwood, C.S.; Gallon, C.E.; Copeland, O.; McKenna, W.J.; Dos Remedios, C.; Marston, S.B.; et al. Myofibrillar Ca2+ sensitivity is uncoupled from troponin I phosphorylation in hypertrophic obstructive cardiomyopathy due to abnormal troponin T. *Cardiovasc. Res.* 2013, 97, 500–508. [CrossRef]

219. Manstein, D.J.; Meiring, J.C.M.; Hardeman, E.C.; Gunning, P.W. Actin-tropomyosin distribution in non-muscle cells. *J. Muscle Res. Cell Motil.* 2020, 41, 11–22. [CrossRef]

220. Hédou, J.; Bastide, B.; Page, A.; Michalski, J.C.; Morelle, W. Mapping of O-linked beta-N-acetylglucosamine modification sites in key contractile proteins of rat skeletal muscle. *Proteomics* 2009, 9, 2139–2148. [CrossRef]

221. Basu, H.; Pekkurnaz, G.; Falk, J.; Wei, W.; Chin, M.; Steen, J.; Schwarz, T.L. FHL2 anchors mitochondria to actin and adapts mitochondrial dynamics to glucose supply. *J. Cell Biol.* 2021, 220, e201912077. [CrossRef] [PubMed]

222. Ryder, J.W.; Lau, K.S.; Kamm, K.E.; Stull, J.T. Enhanced skeletal muscle contraction with myosin light chain phosphorylation by a multienzymatic and sarcomeric complex. *Arch. Biochem. Biophys.* 2021, 650, 1–15. [CrossRef]

223. Butkinaree, C.; Park, K.; Hart, G.W. O-Linked beta-N-acetylglucosamine (O-GlcNAc): Extensive crosstalk with phosphorylation to regulate signaling and transcription in response to nutrients and stress. *Biochem. Biophys. Acta* 2010, 1800, 96–106. [CrossRef] [PubMed]

224. Dubois-Deruy, J.; Belliard, A.; Mulder, P.; Bouvet, M.; Smet-Nocca, C.; Janel, S.; Lafont, F.; Beseme, O.; Amouyel, P.; Richard, V.; et al. Interplay between troponin T phosphorylation and O-N-acetylglucosaminylolation in ischaemic heart failure. *Cardiovasc. Res.* 2009, 85, 37211–37214. [CrossRef] [PubMed]

225. Kubista, M.; de Koning, S.; van der Goes, Y.J.; van der Velden, J.; M?= Claus, J.; de Vos, L.; Bruls, T.; de Vries, R. Phospho-GlcNAc modulation of slow MLC2 during soleus atrophy through a multienzymatic and sarcomeric complex. *Pflugers Arch.* 2014, 466, 2139–2151. [CrossRef]

226. Ryder, J.W.; Lau, K.S.; Kamm, K.E.; Stull, J.T. Enhanced skeletal muscle contraction with myosin light chain phosphorylation by a multienzymatic-sensing kinase. *J. Biol. Chem.* 2007, 282, 20447–20454. [CrossRef]

227. Jiang, Y.; Wang, Y.; Wang, T.; Hawke, D.H.; Zheng, Y.; Li, X.; Zhou, Q.; Majumder, S.; Bi, E.; Liu, D.X.; et al. PKM2 phosphorylates MLC2 and regulates cytokinesis of tumour cells. *Nat. Commun.* 2014, 5, 5566. [CrossRef]

228. Hartshorne, D.J.; Ito, M.; Erdödi, F. Role of protein phosphatase type 1 in contractile functions: Myosin phosphatase. *J. Biol. Chem.* 2004, 279, 37211–37214. [CrossRef]

229. Kiss, A.; Erdödi, F.; Lontay, B. Myosin phosphatase: Unexpected functions of a long-known enzyme. *Biochim. Biophys. Acta Mol. Cell Res.* 2019, 1866, 2–15. [CrossRef]

230. Stull, J.T.; Kamm, K.E.; Vandenboom, R. Myosin light chain kinase and the role of myosin light chain phosphorylation in skeletal muscle. *Arch. Biochem. Biophys.* 2011, 510, 120–128. [CrossRef]

231. Agarwal, P.; Zaidel-Bar, R. Principles of Actomyosin Regulation In Vivo. *Trends Cell Biol.* 2019, 29, 150–163. [CrossRef] [PubMed]
233. Li, L.; Li, Y.; Lin, J.; Jiang, J.; He, M.; Sun, D.; Zhao, Z.; Shen, Y.; Xue, A. Phosphorylated Myosin Light Chain 2 (p-MLC2) as a Molecular Marker of Antemortem Coronary Artery Spasm. Med Sci. Monit. 2016, 22, 3316–3327. [CrossRef] [PubMed]

234. Kanaya, N.; Gable, B.; Murray, P.A.; Damron, D.S. Propofol increases phosphorylation of troponin I and myosin light chain 2 via protein kinase C activation in cardiomyocytes. Anesthesiology 2003, 98, 1363–1371. [CrossRef] [PubMed]

235. Hortemo, K.H.; Aronsen, J.M.; Lunde, I.G.; Sjaastad, I.; Lunde, P.K.; Sejersted, O.M. Exhausting treadmill running causes dephosphorylation of sMLC2 and reduced level of myofilament MLCK2 in slow twitch rat soleus muscle. Physiol. Rep. 2015, 3, e12285. [CrossRef]

236. Cai, L.X.; Tanada, Y.; Bello, G.D.; Fleming, J.C.; Alkassis, F.F.; Ladd, T.; Golde, T.; Koh, J.; Chen, S.; Kasahara, H. Cardiac MLC2 kinase is localized to the Z-disc and interacts with α-actinin2. Sci. Rep. 2019, 9, 12580. [CrossRef]

237. Wang, L.; Geist, J.; Grogan, A.; Hu, L.R.; Kontrogianni-Konstantopoulos, A. Thick Filament Protein Network, Functions, and Disease Association. Compr. Physiol. 2018, 8, 631–709.

238. Wende, A.R. Unsticking the Broken Diabetic Heart: O-GlcNAcylation and Calcium Sensitivity. Diabetes 2015, 64, 3339–3341. [CrossRef]

239. Rassier, D.E. Sarcomere mechanics in striated muscles: From molecules to sarcomeres to cells. Am. J. Physiol. Cell Physiol. 2017, 313, C134–C145. [CrossRef]

240. Galitska-Rakocz, A.; Engel, P.; Xu, C.; Jung, H.; Tobacman, L.S.; Lehman, W. Structural basis for the regulation of sarcomere organization in vertebrate skeletal muscle. Cell 2021, 184, 2135–2150.e13. [CrossRef] [PubMed]

241. Khaitlina, S.Y. Tropomyosin as a Regulator of Actin Dynamics. J. Mol. Biol. 2008, 379, 929–935. [CrossRef]

242. Leung, M.C.; Hitchen, P.G.; Ward, D.G.; Marston, S.B. Z-band alternatively spliced PDZ motif protein (ZASP) is thiazolines at the time of reperfusion is cardioprotective in an O-GlcNAc-dependent manner. J. Mol. Biol. 2015, 349, 101–111. [CrossRef] [PubMed]

243. Henderson, C.A.; Gomez, C.G.; Novak, S.M.; Mi-Mi, L.; Gregorio, C.C. Overview of the Muscle Cytoskeleton. Compr. Physiol. 2017, 7, 891–944.

244. Wang, Z.; Grange, M.; Wagner, T.; Kho, A.L.; Gautel, M.; Raunser, S. The molecular basis for sarcomere organization in vertebrate skeletal muscle. J. Cell Biochem. 2021, 122, 1092–1100. [CrossRef]

245. Ahmad, I.; Hoessli, D.C.; Walker-Nasir, E.; Choudhary, M.I.; Rafik, S.M.; Shakoori, A.R. Phosphorylation and glycosylation interplay: Protein modifications at hydroxy amino acids and prediction of signaling functions of the human beta3 integrin family. J. Cell Biochem. 2006, 99, 706–718. [CrossRef] [PubMed]

246. Laczy, B.; Marsh, S.A.; Brocks, C.A.; Wittmann, I.; Chatham, J.C. Inhibition of O-GlcNAcase in perfused rat hearts by NAG-thiazolines at the time of reperfusion is cardioprotective in an O-GlcNAc-dependent manner. Am. J. Physiol. Heart Circ. Physiol. 2010, 299, H1715–H1727. [CrossRef] [PubMed]

247. Lambert, M.; Richard, E.; Duban-Deweer, S.; Krzewinski, F.; Deracinois, B.; Dupont, E.; Bastide, B.; Cieniewski-Bernard, C. O-GlcNAcylation is a key modulator of skeletal muscle sarcomeric morphometry associated to modulation of protein-protein interactions. Biochim. Biophys. Acta 2016, 1860, 2017–2030. [CrossRef]

248. von Nandelstadh, P.; Ismail, M.; Gardin, C.; Suila, H.; Zara, I.; Carpen, O.; Faulkner, G. A A class III PDZ binding motif in the myotillin and FATZ families binds enigma family proteins: A common link for Z-disc myopathies. Mol. Cell. Biol. 2009, 29, 822–834. [CrossRef] [PubMed]

249. Lambert, M.; Bocquier, D.; Bocquier, D.; Deracinois, B.; Dupont, E.; Bastide, B.; Cieniewski-Bernard, C. O-GlcNAcylation site mapping by (azide-alkyne) click chemistry and mass spectrometry following intensive fractionation of skeletal muscle cells proteins. J. Proteome. 2018, 186, 83–97. [CrossRef] [PubMed]

250. Hnia, K.; Rampsacher, C.; Vernot, J.; Laporte, J. Desmin in muscle and associated diseases: Beyond the structural function. Cell Tissue Res. 2015, 360, 591–608. [CrossRef]

251. Srikanth, B.; Vaidya, M.M.; Kalraiya, R.D. O-GlcNAcylation determines the solubility, filament organization, and stability of keratins 8 and 18. J. Biol. Chem. 2010, 285, 34062–34071. [CrossRef] [PubMed]

252. Ise, H.; Kobayashi, S.; Goto, M.; Sato, T.; Kawakubo, M.; Takahashi, M.; Ikeda, U.; Akaike, T. Vimentin and desmin possess GlcNAc-binding lectin-like properties on cell surfaces. Glycobiology 2010, 20, 843–864. [CrossRef] [PubMed]

253. Ji, S.; Kang, J.G.; Park, S.Y.; Lee, J.; Cho, J.W. O-GlcNAcylation of tubulin inhibits its polymerization. Amino Acids 2011, 40, 809–818. [CrossRef] [PubMed]

254. Lin, B.L.; Song, T.; Sadayappan, S. Myofilaments: Movers and Rulers of the Sarcomere. Compr. Physiol. 2017, 7, 675–692. [PubMed]

255. Ma, J.; Wang, W.H.; Li, Z.; Shabanowitz, J.; Hunt, D.F.; Hart, G.W. O-GlcNAc Site Mapping by Using a Combination of Chemoenzymatic Labeling, Copper-Free Click Chemistry, Reductive Cleavage, and Electron-Transfer Dissociation Mass Spectrometry. Anal. Chem. 2019, 91, 2620–2625. [CrossRef]

256. Krishnamoorthy, V.; Donofrio, A.J.; Martin, J.L. O-GlcNAcylation of aβ-crystallin regulates its stress-induced translocation and cytoprotection. Mol. Cell. Biochem. 2013, 379, 59–68. [CrossRef]

257. Sziklai, D.; Salaj, I.; Papp, Z.; Kellermayer, D.; Mártonfalvi, Z.; Pires, R.H.; Kellermayer, M.S.Z. Nanosurgical Manipulation of Titin and Its M-Complex. Nanomaterials 2022, 12, 178. [CrossRef]

258. Kontrogianni-Konstantopoulos, A.; Ackermann, M.A.; Bowman, A.L.; Yap, S.V.; Bloch, R.J. Muscle giants: Molecular scaffolds in sarcomerogenesis. Physiol. Rev. 2009, 89, 1217–1267. [CrossRef]
259. Li, Y.; Lang, P.; Linke, W.A. Titin stiffness modifies the force-generating region of muscle sarcomeres. Sci. Rep. 2016, 6, 24492. [CrossRef]

260. Canault, M.; Tellier, E.; Bonardo, B.; Mas, E.; Aumailley, M.; Juhan-Vague, I.; Nalbone, G.; Peiretti, F. FHL2 interacts with both ADAM-17 and the cytoskeleton and regulates ADAM-17 localization and activity. J. Cell. Physiol. 2006, 206, 363–372. [CrossRef]

261. Massaccesi, L.; Goi, G.; Tringali, C.; Barassi, A.; Venerando, B.; Papini, N. Dexamethasone-Induced Skeletal Muscle Atrophy Increases O-GlcNAcylation in C2C12 Cells. J. Cell. Biochem. 2016, 117, 1833–1842. [CrossRef] [PubMed]

262. Huang, P.; Ho, S.R.; Wang, K.; Roessler, B.C.; Zhang, F.; Hu, Y.; Bowe, D.B.; Kudlow, J.E.; Paterson, A.J. Muscle-specific overexpression of NCOATGK, splice variant of O-GlcNAcase, induces skeletal muscle atrophy. Am. J. Physiol. Cell Physiol. 2011, 300, C456–C465. [CrossRef] [PubMed]

263. Ogawa, M.; Mizofuchi, H.; Kobayashi, Y.; Tsuzuki, G.; Yamamoto, M.; Wada, S.; Kamemura, K. ermal differentiation program of skeletal myogenesis is negatively regulated by O-GlcNAc glycosylation. Biochim. Biophys. Acta 2012, 1820, 24–32. [CrossRef] [PubMed]

264. Ogawa, M.; Sakakibara, Y.; Kamemura, K. Requirement of decreased O-GlcNAc glycosylation of Mef2D for its recruitment to the myogenin promoter. Biochem. Biophys. Res. Commun. 2013, 433, 558–562. [CrossRef]

265. Joumaa, V.; Bertrand, F.; Liu, S.; Poscente, S.; Herzog, W. Does partial titin degradation affect sarcomere length nonuniformities and force in active and passive myofibrils? Am. J. Physiol. Cell Physiol. 2018, 315, C310–C318. [CrossRef]

266. Neel, B.A.; Lin, Y.; Pessin, J.E. Skeletal muscle autophagy: A new metabolic regulator. Trends Endocrinol. Metab. 2013, 24, 635–643. [CrossRef]

267. Byon, C.H.; Kim, S.W. Regulatory Effects of O-GlcNAcylation in Vascular Smooth Muscle Cells on Diabetic Vasculopathy. J. Lipid. Atheroscler. 2020, 9, 243–254. [CrossRef]