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REVIEW ARTICLE

Control of lipid metabolism by the dynamic and nutrient-dependent post-translational modification *O*-GlcNAcylation

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Abstract

O-GlcNAcylation is a post-translational modification belonging to the large group of glycosylations. It consists of the modification of cytoplasmic, nuclear, and mitochondrial proteins with a single N-acetylglucosamine residue by *O*-GlcNAc transferase (OGT). Despite its structural simplicity, *O*-GlcNAcylation orchestrates many functions inside the cell. This modification regulates fatty acids synthesis, fat storage, and utilization. The generation of white and brown adipocyte-OGT knock-out mice has highlighted the marked interference of *O*-GlcNAcylation in adiposity and, as a consequence, in metabolic pathologies. OGT is more especially involved in the regulation of lipolysis, and thermogenesis in brown adipose tissue. In addition, *O*-GlcNAcylation directly regulates fatty acid synthase, the main enzyme responsible for fatty acids synthesis, and other lipogenic enzymes and transcription factors. Nevertheless, only a few studies reported connections between *O*-GlcNAcylation and homeostasis of cholesterol or its derivatives. This knowledge gap is surprising due to the crucial importance of cholesterol in structuring animal biological membranes and as a precursor of a wide variety of biological compounds. Here, we review the current literature about this topic and discuss future prospects in the field.

Key points:

- As a PTM, *O*-GlcNAcylation exponentially expands protein functions.
- *O*-GlcNAcylation orchestrates many biological functions in living beings including metabolic fluxes.
- *O*-GlcNAcylation is crucial for fat storage and mobilization, and for fatty acid synthesis but its function in the metabolism of other lipid compounds is less documented.

KEYWORDS
lipids, metabolism, O-GlcNAcylation

POST-TRANSLATIONAL MODIFICATIONS: TO THE RESCUE OF A RELATIVELY MODEST HUMAN PROTEOME

In 1988, James Wyngaarden, heading NIH at that time, coordinated a working group aiming at developing an international consortium focusing on complex genomes and on deciphering the entire human genome. The Human Genome Project started in 1990 under the supervision of the Nobel Prize winner James D. Watson. After claiming that the DNA sequence is 99.9% identical between two individuals, the International Human Genome Sequencing Consortium identified 20,000–25,000 protein-coding genes instead of the 100,000 expected.[1] Since then, the Human Proteome Organization (HUPO) evaluated the number of predicted protein-coding genes to 19,823.[2] This seems very low given the extreme biological complexity of some species such as *Homo sapiens*, for whom hundreds of millions of different biochemical and biological processes are estimated. Fortunately, the proteome can be greatly expanded by alternative splicing of mRNA and by post-translational modifications (PTMs):[3] the expression of a protein-coding gene does not lead to a single product but up to tens of different variants in some cases. A PTM is the result of reversible or irreversible covalent linkage(s) of a chemical group (or even of large peptides), or of the proteolytic cleavage of a protein once it has been translated by the ribosomal machinery.[4] Co-translational modifications (*N*-myristoylation or *N*-glycosylation) can also be considered PTMs. PTMs may occur shortly after protein synthesis is achieved and help in polypeptide folding and stability. According to the final subcellular localization of the protein, PTMs can also play a crucial role in protein fate. As we previously discussed elsewhere,[3] PTMs generate an exponential increase in protein isoforms, each having its own network of interactants; the behavior and function(s) of the protein depending on its partners. Among hundreds of PTMs identified in living beings, a large variety of glycosylations are found (Figure 1).

O-GlcNAcylation, A DECEPTIVE STRUCTURAL SIMPLICITY: THE IRONY OF THE FOREST THAT HIDES THE TREE

Glycosylations form a vast and heterogeneous group of PTMs ranging from archaea to eubacteria and eukaryotes.[5–7] Around 80% of proteins are glycosylated in the diverse subcellular compartments of human cells and 2%–4% of the genome encodes proteins involved in glycosylation processes.[8,9] Therefore, glycosylation is a set of spatially and temporally well-organized PTMs, which is matter and energy consuming. Oligosaccharides, polysaccharides, and glycans are formed by a panel of a dozen monosaccharides the most commonly found in the nature and linked together by nearly 50 different bonds, making these branched structures much more complex than nucleic acids and proteins.[5–9]

In contrast to complex glycosylations that include *N*-glycosylproteins, *O*-glycosylproteins (comprising mucins), proteoglycans (modification of proteins by glycosaminoglycans), and glycolipids (Figure 1), *O*-GlcNAcylation consists of the transfer of a single N-acetylglucosamine moiety through a β-linkage onto serine and threonine residues of proteins.[10,11] *O*-GlcNAcylated proteins are located in the nucleus, the cytosol, and the mitochondria of animal cells.[12,13] A fraction of *O*-GlcNAcylated proteins is resident of the cytoplasmic side of the plasma membrane, more especially in lipid microdomains or rafts.[14]

Under its apparent simplicity, *O*-GlcNAcylation intervenes at many different levels in the regulation of cellular and tissue homeostasis, as well as at the level of a whole organism.[10,15] Unlike many glycosylations, *O*-GlcNAcylation is a highly dynamic modification (Figure 2). A target protein can undergo several cycles of *O*-GlcNAcylation/de-*O*-GlcNAcylation during its lifetime, the time and the occupancy rate of the *O*-GlcNAc moiety being very variable from one protein to another, and even from one *O*-GlcNAc site to another.[16–18] This reversibility is often compared to that of phosphorylation with which *O*-GlcNAcylation can compete.[19] The *O*-GlcNAc transferase (OGT) catalyzes the transfer of the GlcNAc group provided by UDP-GlcNAc,[12,20] the end-product of the hexosamine biosynthetic pathway (HBP),[21] and its counterpart *O*-GlcNAcase (OGA) hydrolyses the GlcNAc moiety[22] (Figure 2). Since HBP is connected to different metabolic pathways and supplied by nutritional fluxes to produce UDP-GlcNAc, it is well appreciated that *O*-GlcNAcylation levels are nutrient dependent.[10,12] *O*-GlcNAcylation interferes not only with phosphorylation but also with other PTMs such as methylation,[23,24] acetylation,[23] and ubiquitination,[24–27] giving sugar a prominent place in offering near-infinite combinations of PTMs to finely tune protein functions. The large variety of *O*-GlcNAcylated proteins[28] makes this glycosylation a regulator of key cellular processes such as maintaining DNA integrity,[29] metabolic fluxes,[30] and cell cycle.[31] A deregulation of *O*-GlcNAcylation homeostasis is actively responsible for the emergence and etiology of metabolic pathologies including hypertension,[32] metabolic syndrome,[33] obesity,[34] and diabetes.[35]

Here, we will focus on current knowledge regarding the interference of *O*-GlcNAcylation process on lipid metabolism and will open up on future perspectives.

O-GlcNAcylation AND LIPID BIOGENESIS

Lipids represent around 15% of the dry weight of the body. Along with carbohydrates, proteins, and nucleic acids, lipids are molecules of

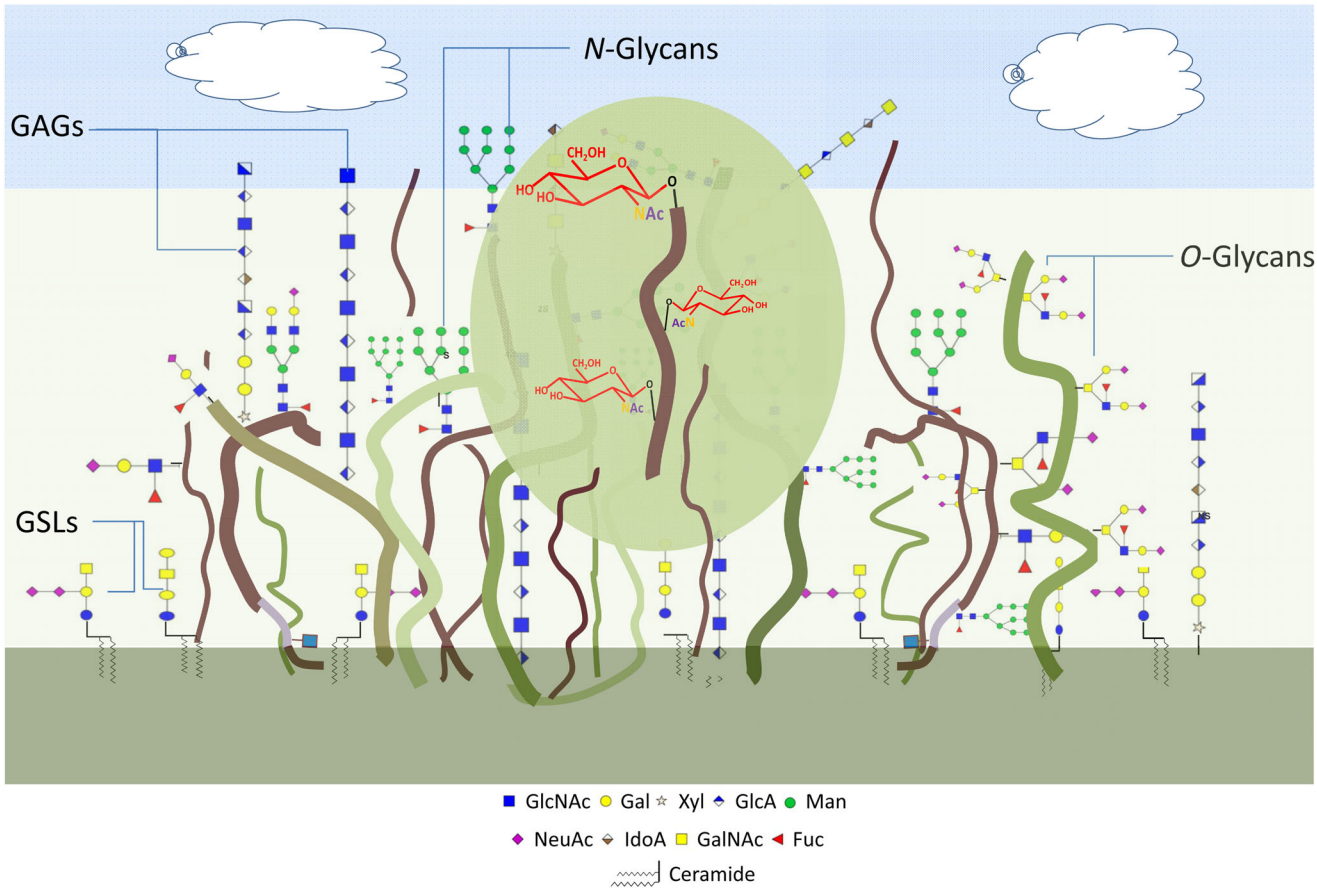

FIGURE 1 *O*-GlcNAcylation, a branch in a forest of complex glycosylations. *O*-GlcNAcylation (in green oval) consists of the modification of proteins by a single residue of N-acetylglucosamine that is neither elongated nor epimerized. In contrast, *N*-glycans, *O*-glycans, GAGs, and GSLs are much more complex since they are built with a wide variety of monosaccharides linked by different types of glycosidic bonds. Abbreviations: Fuc, fucose; GAGS, glycosaminoglycans; Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine; GSL, glycosphingolipid; IdoA, iduronic acid; Man, mannose; NeuAc, neuraminic acid; Xyl, xylose

major biological interest.[36] Yet, lipids suffer from a bad press, mainly on their role in energy storage in the adipose tissue, that is commonly associated with weight gain, obesity, and other related pathologies.[37] However, lipids are vital in many ways.

Lipids are major membrane components (phospholipids, glycosphingolipids, cholesterol).[36] But they endorse many other types of role in the homeostasis of living organisms: they are signaling molecules, including secondary messengers (phosphatidylinositol-3,4,5-triphosphate [PIP3], diacylglycerol [DAG]) or prostaglandins,[38] vitamin precursors or transporters of fat-soluble vitamins,[39] they can endow hormonal functions (ecdysone in arthropods, or estrogens, androgens, glucocorticoids, mineralocorticoids in mammals),[40] are used as co-/post-translational modifications (acylation, prenylation, cholesteroylation),[41] are carriers of odoriferous activity in plants (monoterpenes in essential oils),[42] of sexual pheromone like bombykol,[43] are crucial players in digestion (bile acids and salts),[44] and can endorse many other roles in the homeostasis of living organisms (Figure 2).

Fat diet and *O*-GlcNAcylation

It has been shown in various tissues (human stomach, liver and skeletal muscle; mouse intestine and adipose; rat skeletal muscle and heart) that hyperlipidemia interferes with the *O*-GlcNAcylation content as extensively discussed in a recent paper[45] (Figure 3). Obesity and high-fat diet increase *O*-GlcNAcylation in numerous mice tissues with the exception of hippocampus, pancreatic islets, and macrophages. Also, long-chain fatty acids enhance the level of *O*-GlcNAcylation in various *ex vivo* models (e.g., rat L6 myotubes, immortalized cell lines, human gastric-derived cell lines) but decrease it in the human neuroblastoma cell line SH-SY5Y[45] (Figure 3).

Reciprocally, the use of adipocyte-specific OGT knock-out mice strengthened the marked interference of *O*-GlcNAcylation in adiposity and thus metabolic pathologies (for review, see [46]) (Figure 4). White adipose tissue (WAT) conditional OGT KO mice led to the conclusion that this glycosyltransferase enables a dialog between adipocytes and brain;[47] this axis drives hyperphagia and obesity when animals were

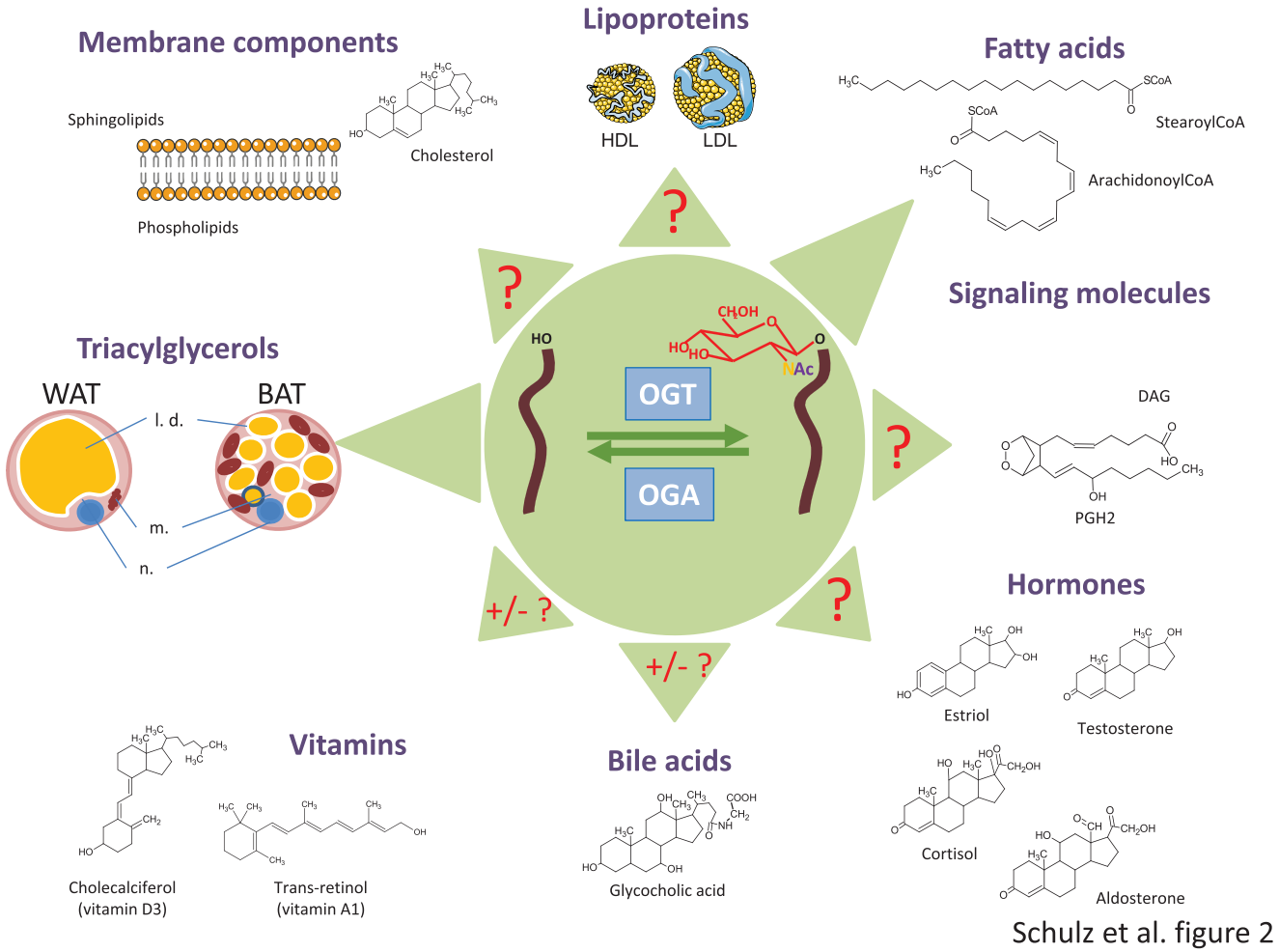

FIGURE 2 Proven and putative regulation of lipid metabolism by *O*-GlcNAcylation. Lipids belong to a large family of biological compounds that are as diverse in terms of structure as they are in function. Currently, *O*-GlcNAcylation dynamics has been shown to control fat storage and utilization, and fatty acids biogenesis. In contrast, data regarding the metabolism of cholesterol, its derivatives, and other kind of lipids are either scattered or non-existent. Examples of structures are given for certain sub-families of lipids. Fatty acids are shown in their activated forms (condensed to coenzyme-A). Abbreviations: BAT, brown adipose tissue; DAG, diacylglycerol; HDL, high-density lipoprotein; l.d, lipid droplets; LDL, low-density lipoprotein; m, mitochondria; n, nucleus; OGA, *O*-GlcNAcase; OGT, *O*-GlcNAc transferase; PGH2, prostaglandin H2; WAT, white adipose tissue

placed on a high-fat diet.[47] The authors proposed that OGT senses fat by a mechanism that remains to be elucidated and activates the lipid desaturase enzyme stearoyl-CoA desaturase (SCD), leading to the accumulation of *N*-arachidonoyl ethanolamine (AEA), an activator of the cannabinoid receptor-1 (CB1) that induces hyperphagia (Figure 4). Another study showed that *O*-GlcNAcylation of perilipin-1 (PLIN1) at Ser492 and Ser517, both sites being targeted by protein kinase A (PKA) to stimulate lipolysis during fasting, blocks the catabolic pathway by preventing access of adipocyte lipases to triacylglycerols[48] (Figure 4). Then, OGT could increase obesity by restricting lipolysis. Analyses of GTEx data revealed that individuals suffering type 2 diabetes harbor a higher OGT/OGA mRNA ratio in subcutaneous and visceral adipose tissue than healthy patients.[48] In a second report on a mouse model of diet-induced obesity, OGT knock-out in macrophages also increased lipolysis in the adipose tissue but induced an accumulation of lipids in liver and muscles[49] (Figure 4). Later, Xiaoyong Yang's group revealed that OGT genetic ablation in the ventromedial hypothalamus led to a reduction in energy expenditure and to mice obesity by reduction of lipolysis through a PKA-hormone-sensitive lipase axis.[50] A similar phenotype was observed in mice knocked-out for OGT in the kidney[51] (Figure 4). Deletion of OGT resulted in a decrease of lipid droplets lipolysis and consequently to a depletion in ATP. According to the authors, it seems that renal lipolysis is driven by an FXR (farnesoid X receptor)-carboxylesterase-1 (CES1) axis since, while FXR is modified and regulated by *O*-GlcNAcylation (see section *Current knowledge on the regulation of cholesterol and derivatives metabolism by O-GlcNAcylation* for details), the direct relationship between FXR and CES1 was not completely elucidated in their hands and thus needs further investigation.[51] In a study that focused on the role of the AgRP (agouti-related peptide) neurons in adipose tissue browning, Ruan and colleagues demonstrated that deletion of OGT in these neurons protected mice from diet-induced obesity[52] (Figure 4). According to this

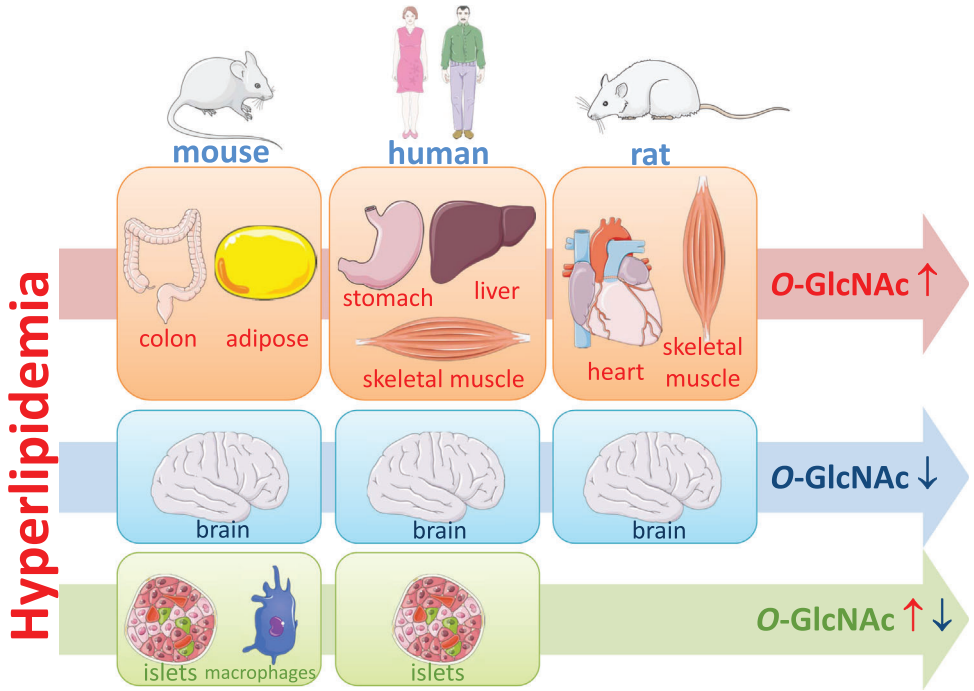

FIGURE 3 Known interference of hyperlipidemia on *O*-GlcNAcylation levels in human and rodents. Numerous studies have revealed that hyperlipidemia interferes with the *O*-GlcNAcylation content in several mice, rat, and human tissues. For full details, refer to the text.

set of studies, it is obvious that OGT and its product, *O*-GlcNAcylation, are critical in fat mass accumulation and in the etiology of obesity.

Of particular interest, an original regulation mode of OGT activity has recently been highlighted in obesity.[53] The authors identified a novel interacting partner of OGT, SELENOV (Selenoprotein V), belonging to the selenoprotein family (Figure 4). Knock-out of SELENOV reduced the activity of OGT, resulting in a concomitant decrease of *O*-GlcNAcylation and phosphorylation of AMP-kinase. A blunted lipolysis and an increased lipogenesis were observed in WAT; a concomitant decrease in lipolysis and thermogenesis was also noticed in brown adipose tissue (BAT). The function of OGT in the regulation of cold-induced thermogenesis in BAT has been previously reported by Ohashi et al.[54] Ablation of *Ogt* in BAT decreases the expression of peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) and impairs mitochondrial functions with a loss in uncoupling protein-1 (UCP-1) content (Figure 4).

Regulation of fatty acid metabolism

The regulation by *O*-GlcNAcylation of fatty acids biosynthesis is one of the best addressed processes in the field of lipid metabolism, the alteration of the PTM cycling resulting in modifications of lipogenesis rate. Fatty acid synthase (FASN), the multi-enzymatic protein that builds fatty acids, utilizes acetyl-CoA, malonyl-CoA, and NADPH,H^+ through a complex cooperation of six different catalytic domains organized into a single polypeptide in animal cells.[55] Using hepatic cell lines and liver tissues from C57Bl6 and ob/ob mice, we demonstrated that FASN interacted with and was modified by OGT.[56] In the same study, we used various conditions of treatment of immortalized human hepatocytes (IHH). This included normal and high concentrations of glucose in conjunction with (or not) insulin and nutrients upregulating the flux of HBP. We first observed as expected that insulin increased the expression level of FASN, mostly through a transcriptional mechanism, and concomitantly *O*-GlcNAcylation content. Of particular interest, the incubation of IHH with fructose, glucosamine, and glutamine, all three suppliers of the HBP increased the expression of FASN and its glycosylation. At last, we inhibited the glutamine:fructose-6-phosphate amidotransferase (GFAT), the rate-limiting enzyme of HBP, and observed a reduction of FASN.[56] In agreement with our data, another group downregulated GFAT-1 and also noticed a reduction in FASN content, formation of lipid droplets, and inhibition of adipogenesis.[57] From the mechanistic point of view, *O*-GlcNAcylation of FASN increased its interaction with the deubiquitinase USP2A, protecting the lipogenic enzyme from proteasomal degradation: its catalytic activity was consequently increased.[56] Accordingly, while it is obvious that insulin plays a major function in lipogenesis, these two reports highlight the importance of HBP in the biosynthesis of lipids.

In addition, expression of FASN fluctuates along the cell cycle, and silencing or inhibiting OGT delays FASN upregulation and slows down cell cycle progression upon serum stimulation of previously serum-starved cells.[58] Transcription of FASN is controlled by several transcription factors: liver X receptor-α (LXR-α), sterol responsive element binding protein (SREBP), and carbohydrate responsive element binding protein-1c (ChREBP). While we observed that OGT blockade impacted FASN expression level independently of SREBP level in HepG2 cells, Sodi and collaborators showed that silencing OGT in breast cancer cells decreased SREBP level and therefore FASN and

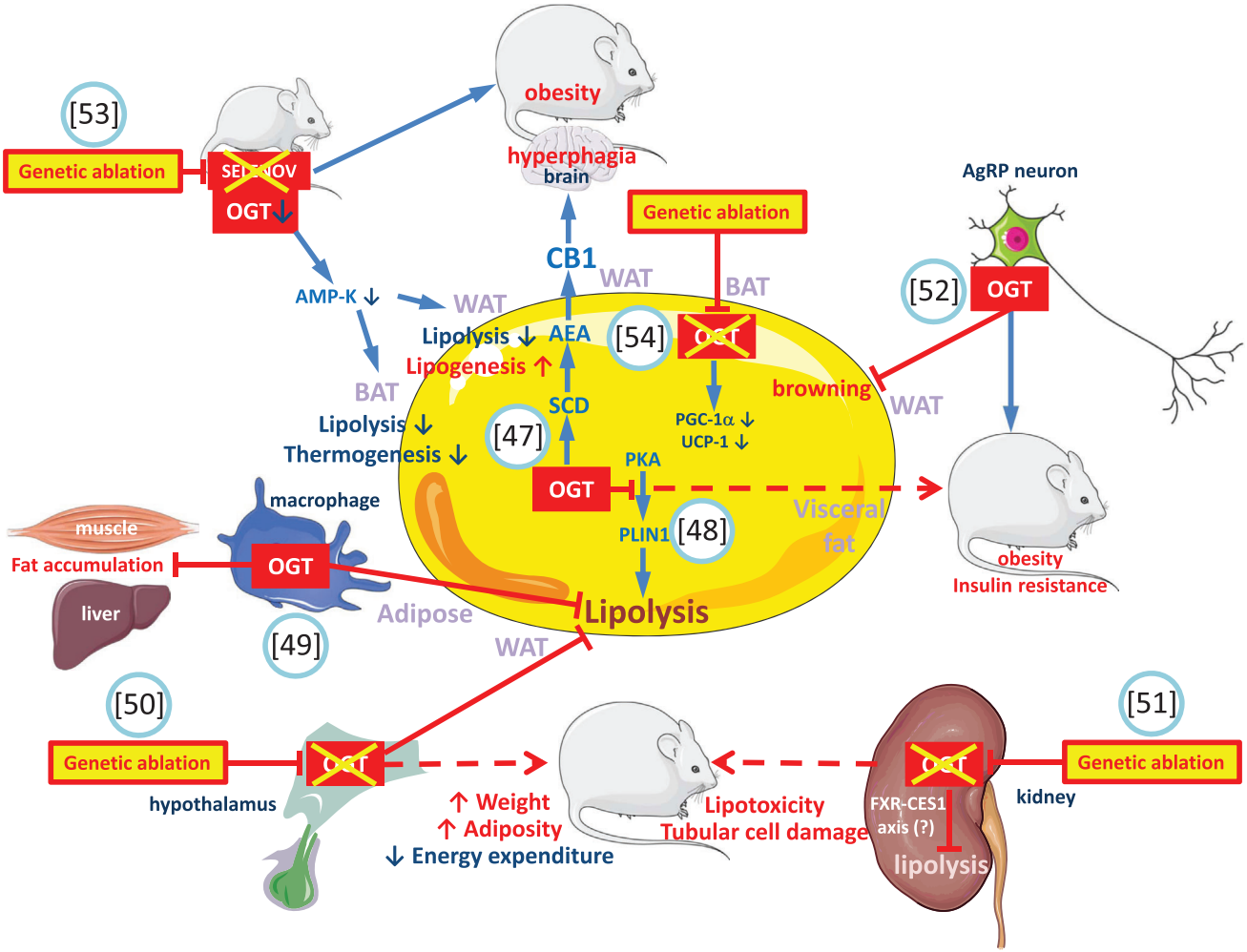

FIGURE 4 Overview of the main studies dealing with OGT activity in lipogenesis and lipolysis in animal models. The use of several adipocyte-specific OGT knock-out mice have strengthened the marked interference of *O*-GlcNAcylation in the adiposity process. The respective reference to each study is indicated in the blue circles. Each type of adipose tissue evoked in these different experiments is indicated in purple. For full details, refer to the text. Abbreviations: AEA, *N*-arachidonoyl ethanolamine; AgRP, agouti-related peptide; AMP-K, AMP-dependent kinase; BAT, brown adipose tissue; CB1, cannabinoid receptor-1; CES1, carboxylesterase-1; FXR, farnesoid X receptor; OGT, *O*-GlcNAc transferase; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1α; PKA, protein kinase A; PLIN1, perilipin-1; SCD, stearoyl-CoA desaturase; SELENOV, selenoprotein V; SREBP, sterol responsive element binding protein; UCP-1, uncoupling protein-1; WAT, white adipose tissue

other lipogenic enzymes content.[59] In addition, OGT controls *srebf1* locus activity by modulating DNA hydroxymethylation through its physical interaction with the circadian nuclear receptor REV-ERBα. Thus, the time-dependent formation of the OGT/REV-ERBα complex favors basal expression of *srebf1* gene in order to prepare the liver to respond to the fasting/fed transition state.[60] We evidenced that FASN level was not only driven by *O*-GlcNAcylation but also depended upon the activity of mammalian/mechanistic target of rapamycin (mTOR).[58] Blocking FASN activity reduced OGT level and mTOR activation, highlighting a feedback-loop regulation between FASN, OGT, and mTOR. We did not give the full explanation about this cross-regulation but can propose the following hypothesis. Upon stimulation with serum, previously deprived cells increase the expression of OGT,[61] and the mTOR pathway is activated. Because FASN is pivotal for cell to proliferate, we can assume that its inhibition blocks both processes. Similarly, acyl-CoA ligase 4 (ACSL4) accelerates hepatocellular carcinoma proliferation by activation of mTOR and glucose transporter 1 (GLUT1) and by elevation of *O*-GlcNAcylation levels.[61] In return, *O*-GlcNAcylation increases the expression of ACSL4, providing another example of cross regulation between this PTM and lipogenesis.[62] Phosphorylation of the regulator of pre-mRNA splicing serine/arginine-rich protein-specific-kinase-2 (SRPK-2) by mTOR/S6K1 is implicated in *de novo* lipogenesis and breast cancer cell growth.[63] SRPK2 is modified by three *O*-GlcNAc moieties close to its second nuclear localization signal (NLS). *O*-GlcNAcylation near this NLS is recognized by importin-α that triggers SRPK2 nuclear translocation. SRPK2 then increases the stability of pre-mRNA encoding lipogenic factors such as FASN, farnesyl-diphosphate farnesyltransferase-1 (FDFT-1), ATP-citrate lyase (ACLY), and mevalonate pyrophosphate decarboxylase (MVD), and promotes breast tumorigenesis.[63]

It is usually accepted that *O*-GlcNAcylation content increases in response to a wide variety of stresses and that this rise plays a

cytoprotective role.[64] Accordingly, among many other proteins, FASN is *O*-GlcNAcylated during cell nutrient starvation. Despite the nutritional deprivation, this turns on fatty acid production that protects cells against the starvation stress.[65] This observation is in line with Zachara's team work.[66] In response to oxidative stress, FASN and OGA physically interact resulting in a reduced OGA activity. These observations can be interpreted as a regulation of enzymes modulating *O*-GlcNAcylation in a spatiotemporal manner. In response to stress, the level of *O*-GlcNAcylation increases via the sequestration and neutralization of OGA activity by FASN.

Recently, it has been found in mesenchymal pancreatic cancer cells that FASN and fatty acid desaturase-2 (FADS2) expression are connected to the *O*-GlcNAcylation status of the transcription factor zinc finger E-box-binding homeobox-1 (ZEB-1) at Ser555.[67] The authors highlighted a mechanism by which glucose contributes to mesenchymal pancreatic cancer cells ferroptosis through an *O*-GlcNAcylation-FASN-FADS2 axis.

Acetyl-CoA carboxylase 1 (ACC1) is another key-enzyme involved in fatty acid synthesis and whose expression is controlled by SREBP1. It has been shown in liver and breast cancer cells that upon glutamine deprivation, the glutamine synthetase (GS) is expressed because of SREBP1 transcriptional activity.[68] Then, *O*-GlcNAcylation of Sp1 leads in a feed-forward loop to the transcriptional expression of SREBP1 that in turn drives the transcriptional expression of GS and ACC1: as a result, lipogenesis is increased and lipid droplets are built.

In an attempt to dissect the effect of *O*-GlcNAcylation on the transcriptional properties of TATA-box binding protein (TBP), Hardivillé and collaborators evidenced that under high glucose concentration or in the presence of thiamet-G, a potent inhibitor of OGA, the fraction of TBP bound on chromatin is increased.[69] Mutation of the *O*-GlcNAc site Thr114 of TBP deeply modifies the expression of genes involved in lipogenesis, including 2,4-didenoyl-CoA reductase-2 (DECR2), acyl-CoA synthetase family member-2 (ACSF2), carnitine palmitoyltransferase-2 (CPT2), and perilipin-2 (PLIN2), that correlates with an increase of lipid droplets in HeLa cells. They further demonstrated that *O*-GlcNAcylation at Thr114 deregulates the interaction of TBP with B-TFIID TATA-box binding protein associated factor-1 (BTAF1), that prevents lipid droplet accumulation into cells. This study showed that *O*-GlcNAcylation of TBP redirects cellular metabolic programming leading to an alteration in cellular storage of lipids.

Current knowledge on the regulation of cholesterol and derivatives metabolism by *O*-GlcNAcylation

Cholesterol belongs to the sterol compound family. Its synthesis requires nearly 30 reactions through the mevalonate pathway (Figure 5). Cholesterol is of crucial importance in mammalian cells. It is a key component of lipid microdomains or rafts indispensable for the dynamic assembly of membrane proteins and lipids[70] (Figure 2). Lipid rafts are involved in essential cellular processes such as endocytosis, exocytosis, cell transduction, and cell signaling, and are platforms of interaction for pathogens and toxins. Cholesterol excess is responsible for various pathologies. A too high concentration of blood cholesterol (hypercholesterolemia) increases the risk of atherosclerotic cardiovascular diseases (heart failure, heart attack, arrhythmia, stroke, heart valve complications, etc.)[71]. Cholesterol also contributes to cancer emergence by increasing the rate of cell proliferation.[72] Therefore, understanding the regulation of cholesterol biosynthesis is of crucial importance to fight these diseases and to complement therapies based on the use of statins. The current knowledge on how *O*-GlcNAcylation interferes with cholesterol metabolism is very limited. Nevertheless, it was reported that exposure of 3T3-L1 adipocytes to high concentrations of insulin increased the concentration of cholesterol at the plasma membrane in an *O*-GlcNAcylation dependent manner.[72] Insulin, through an acceleration of the HBP flux and the *O*-GlcNAcylation of Sp1, activates genes encoding the transcription factor SREBP-1 and the rate-limiting enzyme of cholesterol synthesis 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR)[73] (Figure 5). For note, the authors did not find any changes for SREBP-2 that is known to more heavily regulate the expression of HMGR than SREBP-1. We found that insulin stimulates expression of OGT and its interaction with lipid rafts in the hepatocellular carcinoma cell line HepG2.[14] However silencing OGT did not induce any significant changes in cholesterol content compared to control HepG2 cells.[14] The discrepancies between the two studies may be related to the origin of the cell lines, epithelial for HepG2 cells and adipocyte-type for 3T3-L1 cells.

Importantly, cholesterol is also the precursor of various derivative compounds among which bile acids are considered the major end-products of the catabolism of cholesterol (Figure 5). Bile acids are hydroxylated steroids indispensable to the solubilization and digestion of dietary lipids.[43] They are synthesized in the liver and stocked in the gallbladder. Then, they can be conjugated to the amino-acids glycine and taurine in the gastrointestinal tract to form bile salts. Bile acids also play a role of signaling molecules. They are capable of binding the G protein-coupled bile acid receptor 5 (TGR5), vitamin D receptor (VDR), sphingosine-1-phosphate receptor (S1PR), as well as FXR that in turn controls the enterohepatic cycling of bile acids. FXR-α is highly expressed in the liver and the ileon. It negatively regulates bile acids synthesis by repression of cholesterol 7-α hydroxylase (cytochrome P450 7A1, CYP7A1), the first enzyme involved in bile acids biosynthesis (Figure 5). A study led by a German group showed that the synthesis of bile acids decreased after an oral glucose test tolerance (OGTT) performed on a cohort of individuals, suggesting that excess glucose slows down bile acids pool through FXR.[74] A mechanistic connection was made a few years later by Berrabah and collaborators.[75] FXR is modified and stabilized by *O*-GlcNAcylation which consequently increases its transcriptional activity by modulating its interaction with corepressor complexes (Figure 5). Since HBP activity is dependent on nutrient fluxes, this work links bile acid metabolism and FXR activity to the nutritional status.[75] A correlation has thus been made between *O*-GlcNAcylation of FXR, altered expression of its target genes and hepatic bile acids content. However, the role of *O*-GlcNAcylation on the bile acid biosynthetic pathway is unknown. Measurement of the activity of enzymes involved in the production of hepatic bile acids other than that of CYP7A1A[73] (Figure 5) could be considered according

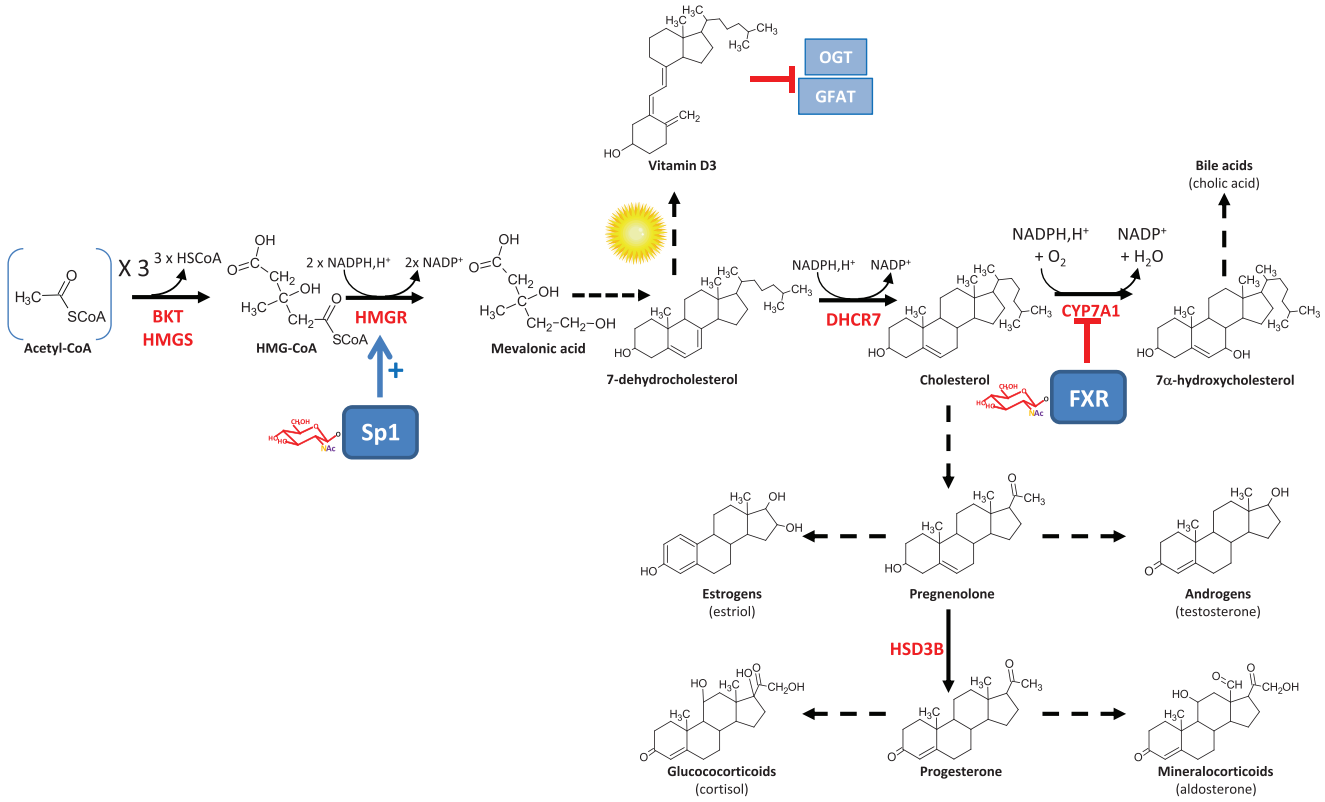

FIGURE 5 Biosynthesis of cholesterol and some of its derivatives. Cholesterol is synthesized in the liver through the mevalonate pathway by using acetyl-CoA and NADPH,H$^+$. 7-Dehydrocholesterol (provided by the Kandutsch–Russell branch of the cholesterol biosynthetic pathway) is a metabolic crossroad and can either provide cholesterol or vitamin D that needs UV-B for synthesis. From cholesterol, bile acids are generated because of the rate-limiting enzyme CYP7A1. Pregnenolone is the precursor of steroids hormones (estrogens and androgens) and progesterone from which mineralocorticoids and corticoids are built. SP1 and FXR when *O*-GlcNAcylated are key-regulators of HMGR and CYP7A1, respectively. Vitamin D3 (cholecalciferol) reduces expression of OGT and GFAT. Abbreviations: BKT, beta-ketothiolase; CYP7A1, cholesterol 7-alpha hydroxylase (cytochrome P450 7A1); DHCR7, 7-dehydrocholesterol reductase; FXR, farnesoid X receptor; GFAT, glutamine:fructose-6-phosphate aminotransferase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; HMGS, 3-hydroxy-3-methylglutaryl-CoA synthase; HSCoA, coenzyme-A; HSD3B, 3β-hydroxysteroid dehydrogenase isomerase ; NADP$^+$/ NADPH,H$^+$, nicotinamide adenine dinucleotide oxidized/reduced forms; OGT, *O*-GlcNAc transferase; SP1, specificity protein 1

to HBP flux, OGT, or OGA activity. A similar investigation on secondary bile acids in the intestine would be also of interest, since the biosynthetic pathways differ from hepatic and intestine bile acids.

CONCLUDING REMARKS AND FUTURE PROSPECTS

We sought to review current knowledge of the control of lipid metabolism by the nutrient-dependent and dynamic modification *O*-GlcNAcylation. Unlike carbohydrates, proteins and nucleic acids for which there is a common structural basis, lipids form a very heterogeneous family making their study much more difficult than any other biological compounds.

Most of the current literature focuses on how OGT drives adipose tissue fat storage and mobilization, and biosynthesis of fatty acids.[46–54] Apart from that, almost everything remains to be done in the field. While few studies were dedicated to bile acids,[74,75] the other cholesterol derivatives among which vitamins of the group D, oxysterols, steroid and sex hormones (cortisol, aldosterone, testosterone, progesterone, estriol, etc.) have received insufficient interest so far (Figure 5). Only one study described a connection between *O*-GlcNAcylation and cholecalciferol (vitamin D3).[76] In a model of type 1 diabetes induced by intraperitoneal injection of streptozotocin in rat, cholecalciferol reduced the expression of OGT and GFAT in parallel with an improvement in hyperglycemia and hypoinsulinemia (Figure 5). However, the regulation of vitamin D3 metabolism by *O*-GlcNAcylation remains fully unexplored (except that the vitamin D receptor, VDR, is *O*-GlcNAcylated[77]). It would be interesting to dissect the subsequent modification occurring on 7-dehydrocholesterol (pre-vitamin D3) in the skin (in response to UV-B) to provide cholecalciferol, then in liver that generates calcidiol (25-hydroxycholecalciferol), and finally kidney to give calcitriol (24,25-di-hydroxyvitamin D3).

The lack of publications dealing with the regulation of cholesterol by OGT/OGA is surprising considering its high relevance in numerous animal physiological functions and implication in metabolic disorders. In its non-esterified (free cholesterol) and esterified forms, this sterol is a major component of lipoproteins (its amount reaches more than 50% of total lipids in low-density lipoproteins [LDL]) (Figure 2). For

obvious public health concerns, it is quite strange that no data were reported in the field of lipoproteins, whose major function is to transport hydrophobic lipids in the hydrophilic blood between intestine, liver, and peripheral tissues. Lipoproteins are complex particles that consist of the non-covalent assembly of lipids (phospholipids, triacylglycerols, and cholesterol) and proteins (generally named apolipoproteins). Investigating the biosynthesis, trafficking and assembly into lipoproteins of apolipoproteins such as apolipoprotein-AI (Apo-AI) and Apo-E for high-density lipoprotein (HDL), Apo-B100 for very low-density lipoprotein (VLDL), intermediary-density lipoprotein (IDL) and LDL, and Apo-B48 for chylomicrons is a scientific field that deserves to be studied. To our knowledge, only one publication deals with *O*-GlcNAcylation of apolipoproteins and relates specifically to Apo-AI.[78] Apo-AI is the major protein component of HDL since it represents nearly 70% of the total protein mass, and it actively participates in the retrograde transport of cholesterol to the liver.[79] It is synthesized and secreted by the liver, and the intestine, in a lipid-poor form of HDL. Because of its detergent-like properties, Apo-AI interacts with and organizes lipids so as to structure nascent HDLs. During its transit in the bloodstream, a transfer of membrane phospholipids and cholesterol from peripheral cells and macrophages to HDLs occurs via the transmembrane protein ATP-binding cassette A1 (ABCA1): discoid nascent HDLs are formed.[80] Free cholesterol is transferred inside the HDL and esterified by lecithin-cholesterol acyltransferase (LCAT) for which Apo-A1 is an activator, HDL becomes spherical and mature. These HDLs are back transported to and uptaken by the liver by the scavenger receptor SR-B1. Cholesterol is metabolized to bile acids and secreted into the bile. It is suggested that after acute myocardial infarction, Apo-AI undergoes changes in its glycosylation pattern and this states that both *N*-glycosylation and *O*-glycosylation and *O*-GlcNAcylation can occur together on the apolipoprotein.[78] These glycosylation modifications may have a relevant prognosis value and a protective role following acute myocardial infarction. While this claim is exciting, it seems unlikely since complex glycosylation and *O*-GlcNAcylation processes follow distinct biosynthesis pathways.[81] Also, the experiments dedicated to proving that Apo-AI is *O*-GlcNAcylated are sorely lacking. Nonetheless, in the endoplasmic *reticulum*, EGF domain-like OGT (EOGT) is the counterpart of the nucleocytoplasmic OGT.[82] It cannot be excluded that Apo-AI is *O*-GlcNAcylated by EOGT in the secretory pathway. It can be assumed that this point will soon receive attention.

It this review, we give some examples of deregulation of the metabolism of lipids in the context of cancer, more specifically in pancreatic and breast cancers. The crucial role of lipids in oncogenesis has been widely characterized but insufficiently in the field of *O*-GlcNAcylation, while metabolic profiling of breast cancer cells depleted of OGT showed marked differences in lipid content.[59] The same group recently reported that the overexpression of OGT in glioblastoma cells resulted in an accumulation of lipids as evidenced by Nile red staining and measurement of total ^{13}C enrichment of palmitic and stearic.[83] Mechanistically, OGT activity favors the phosphorylation of acetyl-CoA synthetase 2 (ACSS2), the enzyme producing acetyl-CoA (the substrate of ACC1 that generates malonyl-CoA for FASN) from acetate, rendering ACSS2 less sensitive to polyubiquitination and as a consequence more stable. The authors describe the involvement of cyclin-dependent kinase-5 (CDK5) as an intermediary component between OGT and ACSS2 and argue that the OGT-CDK5-ACSS2 axis could be an interesting target in the therapy against brain cancer. This hypothesis deserves attention and it would be legitimate to study this same pathway in other types of cancer to find out whether it can be generalized or, on the contrary, specified for glioblastoma alone.

Lastly, the regulation of lipid metabolism by *O*-GlcNAcylation in the brain is an area largely underexploited. The brain is a lipid-rich organ with a wide diversity of lipid structures. It is known that many neuropathologies including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and also glioblastomas as discussed above are associated with perturbations in brain lipid content. In addition, the involvement of *O*-GlcNAcylation cycling disruption in the emergence of neurological diseases is well described.[84,85] Lipidomic analyses would make it possible to gauge the impact of *O*-GlcNAcylation on lipid remodeling in neuronal lines and more particularly animal models; this would be of major interest in potentially opening up new therapeutic perspectives which are lacking in patients suffering from this type of pathology.

In conclusion, this review shows that, apart from lipogenesis and lipolysis, the role of *O*-GlcNAcylation in lipid homeostasis has been not sufficiently investigated despite the pivotal involvement of this post-translational modification in normal and physiopathological lipid metabolisms. Further molecular analyses of the links between *O*-GlcNAcylation and lipids could provide novel future research and treatment directions dedicated to metabolic pathologies and the epidemic increase in overweight and obesity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

The authors confirm that they have followed the ethical policies of the journal.

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