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RESEARCH HIGHLIGHT

Liver X receptors connect nuclear O-GlcNAc signaling to hepatic glucose utilization and lipogenesis

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Insulin is a central regulator of glycolysis and *de novo* lipogenesis in the liver. However, hepatic glucose metabolism has been shown to activate the transcription of glycolytic and lipogenic enzymes independently of insulin. The nuclear liver X receptors LXRα and LXRβ play a major role in glucose and lipid metabolism, regulating transcription of glycolytic and lipogenic enzymes in liver, which is believed to be mediated by oxysterol ligand activation and insulin signaling. The majority of hepatic glucose-responsive genes are regulated by carbohydrate response element-binding protein (ChREBP), a transcriptional regulator that requires glucose metabolism via the hexosamine biosynthetic pathway and O-GlcNAc transferase (OGT)-mediated O-GlcNAc modification for full activation. We have previously shown that also LXRs are targets for O-GlcNAc modification in response to glucose and refeeding, promoting lipogenic gene expression. We recently addressed the relative roles of insulin, glucose and LXR in regulating hepatic glycolytic and lipogenic gene expression *in vivo* by subjecting untreated control and streptozotocin (STZ)-treated LXRα/β$^{+/+}$ and LXRα/β$^{-/-}$ mice to a fasting-refeeding regime. STZ was used to destroy pancreatic β-cells and insulin production. We found that under hyperglycemic and hypoinsulinemic conditions, LXRs maintained their ability to upregulate the expression of glycolytic and lipogenic enzymes, including glucokinase (GK), sterol regulatory element-binding protein (SREBP-1c), ChREBPα and the newly identified shorter isoform ChREBPβ. ChREBPα expression became dependent on LXR under hyperglycemic and hypoinsulinemic conditions, which was mediated, at least in part, by OGT signaling. Moreover, we found that LXR and OGT interacted and co-localized in the nucleus in Huh7 cells and that loss of LXRs profoundly reduced nuclear O-GlcNAc signaling, ChREBP O-GlcNAcylation and activity *in vivo*. We propose that LXR regulation of nuclear O-GlcNAc signaling and ChREBP O-GlcNAcylation is part of a mechanism linking hepatic glucose utilization with lipid synthesis.

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The liver plays a central role in maintaining whole-body metabolism in response to nutritional cues. Following a meal rich in carbohydrates, the liver converts excess glucose into fatty acids through *de novo* lipogenesis, a process that mainly is regulated at the transcriptional level through upregulation of glycolytic and lipogenic enzymes including glucose kinase (GK), liver pyruvate kinase (L-PK), acetyl-CoA carboxylase (ACC) and fatty-acid synthase (FAS) [1]. Glucose not only serves as an energy source and substrate for lipogenesis, but is also acting in concert with insulin as a signaling molecule in the regulation of glycolytic and lipogenic gene expression in response to feeding. Once taken up by hepatocytes, glucose is converted to glucose-6-phosphate (G6P) by GK, which is obligatory for glycogen storage, glycolysis and metabolism through the nutrient and glucose sensing hexosamine biosynthetic pathway (HBP). The end product of HBP is the

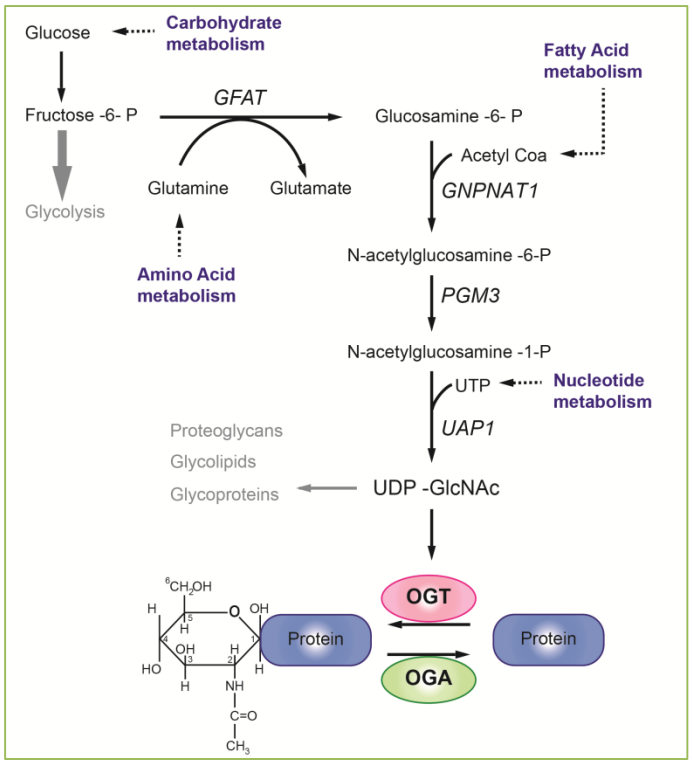

Figure 1. The nutrient sensing hexosamine biosynthetic pathway (HBP) and protein O-GlcNAcylation. The activated sugar substrate UDP-GlcNAc for nucleocytoplasmic O-GlcNAc transferase (OGT) is synthesized via the HBP that is sensitive to different metabolic pathways, particularly glucose and glutamine metabolism via the rate limiting enzyme glutamine:fructose-6-phosphate amidotransferase (GFAT). OGT catalyzes the reversible addition of N-acetylglucosamine (GlcNAc) to hydroxyl groups of Ser and Thr (O-GlcNAc) while OGA (O-GlcNAcase) hydrolyzes the sugar. The sites of O-GlcNAc modification can be the same or near sites for phosphorylation. Nucleocytoplasmic OGT is distinct from UDP-GlcNAc regulated glycosyltransferases in the secretory pathway generating proteoglycans, glycolipids and glycoproteins.

high-energy substrate UDP-N-acetylglucosamine (UDP-GlcNAc), which is used as a building block for glycosylation in the endoplasmic reticulum (ER) and Golgi apparatus and as a substrate for the enzyme O-GlcNAc transferase (OGT), that dynamically adds GlcNAc in O-glycosidic linkage to serine and threonine residues on nuclear, mitochondrial and cytoplasmic targets proteins (protein O-GlcNAcylation) [2, 3] (Figure 1). As the activity of OGT is increased with UDP-GlcNAc concentration, OGT can be viewed as a nutritional sensor that regulates the activity, stability and localization of proteins. Hyperglycemia increases glucose flux into the HBP and transgenic mice overexpressing OGT show a diabetic phenotype due to insulin resistance [4] and hepatic steatosis [5]. We and others have shown that high glucose levels activate the glycolytic and lipogenic transcriptional regulators LXRα and LXRβ and carbohydrate-responsive element-binding protein (ChREBP) via OGT-mediated O-GlcNAc modification of these proteins [5-7]. Together with sterol regulatory element-binding protein 1c (SREBP-1c), these factors cooperate in regulating glycolytic and lipogenic gene expression in response to feeding, where LXRs and ChREBP are essential for expression of SREBP-1c and L-PK expression, respectively [1, 8, 9].

LXRs are classically known as cholesterol sensing nuclear receptors via ligand activation by oxygenated derivatives of cholesterol (oxysterols) [10]. Activation of LXRs with synthetic ligands (GW3965 and T0901317) have been shown to improve whole-body glucose uptake and homeostasis through upregulation of the insulin-responsive glucose transporter (Glut4) in adipose tissue and downregulation of the gluconeogenic enzymes PEPCK and G6Pase in liver [11-13]. Also, LXR ligand activation induces the expression of GK in liver, which promotes hepatic glucose flux and thus enhances glucose utilization [11]. GK activity as measured by phosphorylation of glucose to G6P and GK expression are also reduced in LXR deficient mice, supporting that LXR dependent regulation of GK is important under physiological conditions, possibly through a direct regulation of LXR binding to the identified LXR response element in the GK promoter [7, 14-16]. Moreover, and as mentioned above, LXRs are central regulators of fatty acid synthesis in response to ligand activation and insulin signaling [17, 18]. The mechanisms of insulin activation of LXRs are currently not known, but may involve increased expression of LXR, phosphorylation of LXR and/or synthesis of endogenous ligand(s). Another possible mechanism of insulin activation of LXR-mediated lipogenesis is its ability to inhibit the gluconeogenic transcription factor Forkhead box protein 1 (Foxo1) via phosphorylation and sequestration in the cytoplasm. As Foxo1 has been shown to inhibit LXR-mediated transactivation of the SREBP-1c promoter [19, 20], the insulin signal would relieve LXR and cooperating transcription factors from transrepression by Foxo1 allowing for full activation of SREBP-1c transcription [21]. Finally, as insulin plays a permissive role in determining the flux rate through glycolysis and glucose metabolic pathways, insulin is necessary for proper glucose utilization and O-GlcNAc signaling to LXR and other glucose-regulated proteins, including ChREBP.

We recently addressed the role of glucose, insulin and LXR in hepatic lipogenesis by subjecting control and streptozotocin (STZ)-treated LXRα/β$^{+/+}$ and LXRα/β$^{-/-}$ mice to a fasting-refeeding regime [7]. The mice were fed a diet containing 64 % carbohydrates (45 % starch and 4 % sugars), 31.5 % protein, 4.5 % fat, and no cholesterol. Liver lysates from fasted (24h) and refed (12h) wild type and LXRα/β$^{-/-}$ control and STZ-treated mice were subjected to RNA, protein and ChIP analysis and analysed for LXR, ChREBP,

SREBP-1c, GK, L-PK and downstream lipogenic target gene expression. Our results demonstrated that LXRs maintain their ability to induce expression of glycolytic and lipogenic enzymes including GK, SREBP-1c and ChREBP under hyperglycemic conditions independently of postprandial insulin levels (STZ-treatment). However, fold-inductions of GK mRNA expression in response to feeding were reduced to 30-fold in control LXRα/β$^{-/-}$ mice as compared to 50-fold in control wild type mice, suggestive of maintained GK-expression by other transcriptional regulators than LXR and SREBP-1c in control LXRα/β$^{-/-}$ mice, possibly PPARγ [22]. Under hyperglycemic conditions lacking the postprandial insulin signal (refed STZ-treated mice), hepatic GK expression was upregulated 10-fold and the response was completely abolished in STZ-treated LXRα/β$^{-/-}$ mice, suggestive of insulin independent transactivation of the GK promoter via LXRs and SREBP-1c. Thus, regulation of glycolysis and lipogenesis in response to a diet devoid of cholesterol under hyperglycemic conditions seem to require LXR and SREBP-1c in the absence of postprandial insulin signaling.

Despite lower GK mRNA and protein expression in control and STZ-treated refed LXRα/β$^{-/-}$ mice, cytoplasmic protein O-GlcNAc levels were maintained as compared to wild type mice suggestive of sustained metabolism via the HBP and formation of UDP-GlcNAc in response to feeding in LXRα/β$^{-/-}$ mice. Interestingly, nuclear protein O-GlcNAc levels were reduced in control and STZ-treated refed LXRα/β$^{-/-}$ mice, suggestive of reduced nuclear OGT activity as nuclear OGT levels were similar in wild type and LXRα/β$^{-/-}$ mice liver. Accordingly, nuclear (but not cytosolic) O-GlcNAc levels were elevated in human liver hepatoma Huh7 cells ectopically expressing LXRα or LXRβ (unpublished observations from our laboratory). OGT activity is dependent on UDP-GlcNAc substrate availability, post-translational modifications and protein interactions [2]. Apparently, the nucleus is largely permeable to UDP-GlcNAc [23], making it unlikely that LXR affect UDP-GlcNAc transport to the nucleus. However, as the perinuclear space between the outer and inner nuclear membrane is contiguous with the ER lumen, uptake of UDP-GlcNAc across the ER membrane (for N-linked GlcNAc glycosylation) may impact transport to the nuclear compartment. However, unpublished qPCR results from our laboratory, show no significant down-regulation of the ER-localized UDP-GlcNAc transporter SLC35B4 [24] in livers from LXRα/β$^{-/-}$ mice. Interestingly, we observed nuclear protein expression of glutamine: fructose-6-phosphate amidotransferase-1 (GFAT1), the rate limiting enzyme in the HBP, suggestive of nuclear synthesis of UDP-GlcNAc. The GFAT1 isoform was only modestly expressed in the cytoplasm with a stronger expression of the GFAT2 isoform, which was not expressed in the nucleus. Although GFAT1/2 protein expression was not changed by knocking out LXRs, LXR deficiency may affect their activities. GFAT1 and GFAT2 are both phosphorylated by PKA negatively affecting GFAT1 and positively affecting GFAT2 activity [25, 26]. This may suggest that cytosolic and nuclear UDP-GlcNAc pools are regulated differently during fasting and feeding and that the fraction of UDP-GlcNAc that is utilized for O-GlcNAc modification during refeeding is increased towards the cytoplasmic pool in LXRα/β$^{-/-}$ mice. In hepatocytes, cytosolic O-GlcNAc is involved in negative feedback regulation of insulin signaling [27], suggesting a role for LXRs in cross-talk between glucose utilization and insulin signaling via regulation of the HBP. Experiments are currently underway in our laboratory to elucidate UDP-GlcNAc nucleocytoplasmic levels in fasted and refed wild type and LXRα/β$^{-/-}$ mice livers.

A second alternative to how LXR impact nuclear OGT activity is through direct binding of LXR with OGT. Previous studies have shown that OGT subcellular localization, activity and target gene specificity are determined by the availability of various interacting protein substrates and regulatory protein partners [28, 29]. In our recent study we observed that LXR and OGT colocalize in the nucleus in EGFP-LXR and Cherry-OGT overexpressing Huh7 cells [7]. Both *in vitro* in Huh7 cells and *in vivo* in wild type refed liver, OGT and LXR were co-immunoprecipitated and GST-pulldown experiments in GST-LXR and His-OGT co-expressing E.Coli cells, were evident of a direct interaction between OGT and LXRα and LXRβ. Current peptide array analysis in our laboratory, further support a direct interaction between OGT and LXRs. Our fluorescence imaging revealed a uniform distribution of LXR and OGT in the nucleus outside of the rRNA synthesizing nucleolus. In some cells, we observed a strong colocalization in small confined subdomains. Previous studies have shown that O-GlcNAc is found highly concentrated at nuclear pores and subdomains within the nucleus [30] and OGT is known as an important coregulator of nutrient-responsive gene transcription [31-33]. We reported nuclear colocalization of LXR and OGT in Huh7 cells cultivated at both low and high glucose concentrations and apparently independently of synthetic LXR ligand, suggesting that LXRs interact constitutively with OGT via their N-terminal ligand-independent domain. However, the activities of complexed LXR and OGT are most likely dynamically regulated by posttranslational modifications and coregulators in response to hormonal and nutrient signals [28]. In agreement with this hypothesis, we have identified two O-GlcNAc sites in the N-terminal part of *in vitro* O-GlcNAcylated LXRα by mass spectrometric analysis, and a novel O-GlcNAc site in OGT (unpublished data). Identified O-GlcNAc sites will be mutated and the biological

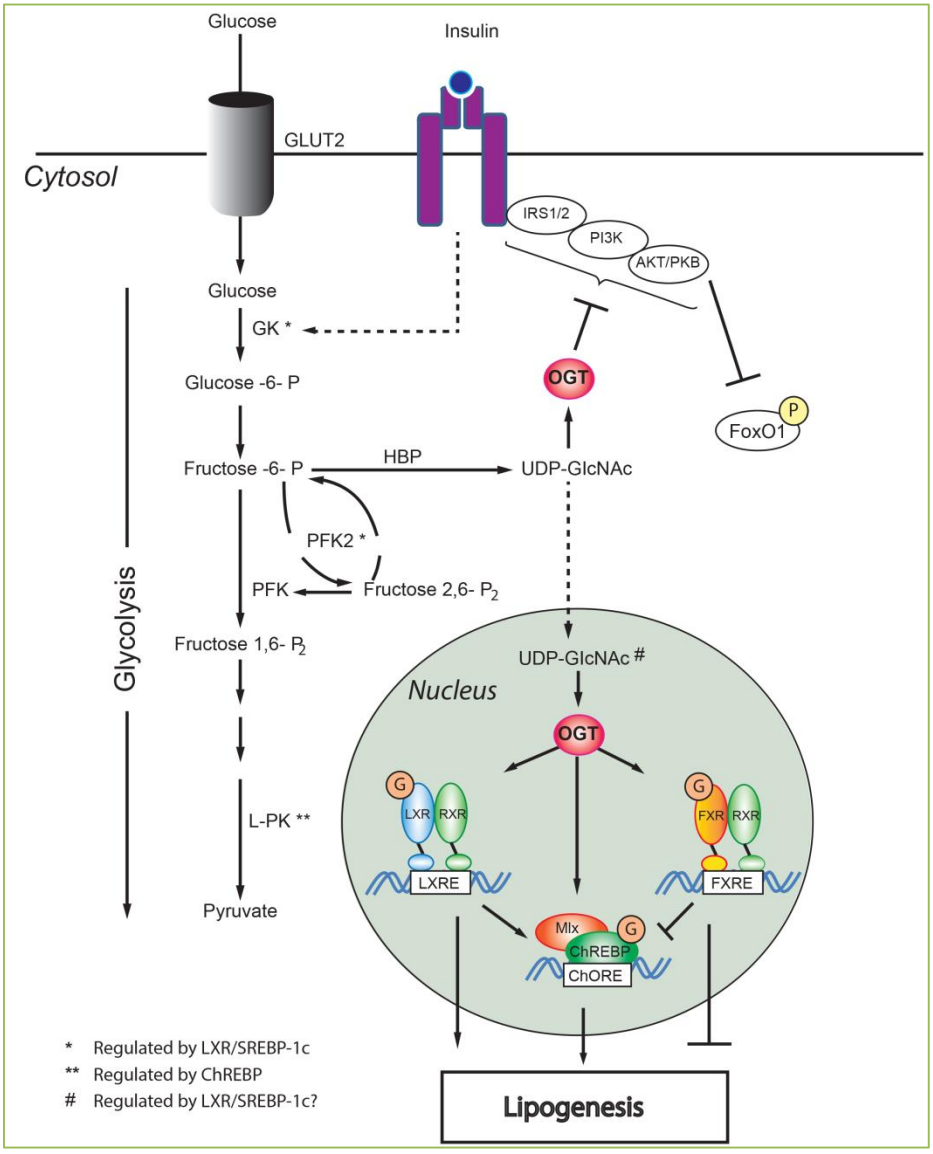

Figure 2. The role of LXR and cooperating transcription factors in glucose-O-GlcNAc and insulin regulated hepatic lipogenesis. Hepatic *de novo* lipogenesis is dependent upon glucose both as a fuel where insulin plays a permissive role, as well as a signaling component via HBP/OGT and O-GlcNAcylation of transcription factors LXR and ChREBP. LXR is essential for optimal glycolysis and nuclear O-GlcNAc signaling via regulation of GK expression and possibly also nuclear UDP-GlcNAc availability and/or OGT activity. ChREBP expression is dependent on LXR under hyperglycemic and hypoinsulinemic conditions and optimal ChREBP O-GlcNAcylation and activity is dependent on LXR under physiological insulin sensitive conditions. FXR has also been shown to be O-GlcNAc modified in response feeding and high glucose levels, but its role in lipogenesis is currently not known. However, in response to bile acids, FXR is known as acting antagonistic to LXR and ChREBP, supressing lipogenesis. Under physiological insulin sensitive conditions, Foxo1 is retained in the cytoplasm, preventing it from inhibiting LXR and ChREBP activity. Paradoxically, under hyperglycemic insulin resistant conditions, Foxo1 is O-GlcNAc modified and promotes hepatic gluconeogenesis. The role of O-GlcNAc modified Foxo1 in hepatic lipogenesis, is currently not known.

consequences of the O-GlcNAc modifications and impact on OGT interaction, LXR and OGT activity will be investigated.

One consequence of reduced nuclear O-GlcNAc signaling in response to refeeding as observed in our LXR$\alpha/\beta^{-/-}$ mice is altered transcription mediated by O-GlcNAc targeted transcription factors. As mentioned above, regulation of hepatic glucose and lipid homeostasis is tightly regulated at the transcriptional level by several transcription factors including LXR, ChREBP, Foxo1 and the bile acid responsive

farnesoid X receptor (FXR), all of which have been shown to be O-GlcNAc modified in response to glucose and feeding [5, 6, 34, 35]. O-GlcNAcylation of nuclear localized ChREBP stabilizes the protein and increases its transcriptional activity on L-PK and lipogenic genes [5]. We now report [7] a link between LXR activity and glucose sensing via O-GlcNAc to ChREBP expression and activation. Under hyperglycemic conditions lacking the postprandial insulin signal (refed STZ-treated mice), hepatic ChREBPα mRNA and protein expression became dependent on LXR and *in vitro* transfection studies suggest a role for LXR and OGT in high glucose regulation of the ChREBPα promoter. Moreover, O-GlcNAcylation of ChREBP and the potential of ChREBPα to induce its target genes L-PK and the shorter glucose-regulated ChREBPβ isoform [36] were significantly lower in LXRα/β$^{-/-}$ refed mice. Intriguingly, the ChREBP protein level was not reduced in refed LXR deficient mice compared to wild type mice, suggesting that ChREBPα activity is dependent on LXR and O-GlcNAc signaling under physiological insulin-dependent conditions. Our observations are in accordance with observations in Foxo1 knockout liver and Foxo1 overexpressing primary mouse hepatocytes, in which ChREBP O-GlcNAcylation, and recruitment to the L-PK promoter were increased and decreased, respectively [37]. Under physiological conditions, insulin retains Foxo1 in the cytoplasmic compartment, keeping it from inhibiting nuclear LXR and ChREBP. Under insulin resistant and hyperglycemic conditions however, nuclear Foxo1 is O-GlcNAc modified and paradoxically potentiating gluconeogenic gene transcription and hepatic glucose output [34]. The role of O-GlcNAc modified Foxo1 on lipogenic gene expression is currently not known, but hepatic Foxo1 ablation has been shown to result in a slight increase in expression of LXR and ChREBP targets genes FAS and L-PK [38] in accordance with the above mentioned study by Ido-Kitamura *et al* [37].

Recently, FXR was shown to be O-GlcNAc modified in response to high glucose and refeeding, increasing FXR target gene expression and decreasing bile acid content [35]. Interestingly, FXR has been shown to interact with ChREBP and inhibit its transcriptional activity in human hepatocytes [39]. The relative roles of bile acid ligand activation and O-GlcNAc modification of FXR in this inhibition and whether it involves inhibition of ChREBP O-GlcNAc modification are currently not known. LXR and FXR act antagonistically in regulating expression of lipogenic SREBP-1c and cytochrome P450 7α-hydroxylase (CYP7A1), the rate limiting enzyme in cholesterol conversion into bile acid, where LXR upregulate and FXR downregulate expression of these proteins [40]. Hepatic LXR expression is upregulated in liver-specific FXR knock out mice [41], and CYP7A1 expression were strongly downregulated in our LXRα/β$^{-/-}$ refed control and STZ-treated mice, suggesting increased FXR activity in mice lacking LXRs. These data support the yin/yang relationship between these nuclear receptors in bile acid and lipid synthesis and suggests a potential role for LXR and FXR in regulating ChREBP activity in a reciprocal manner. The relative roles of ligand activation and glucose/O-GlcNAc signaling in this scenario, and whether LXR/FXR regulation of ChREBP involves spatiotemporal regulation via dynamic interactions with OGT and coregulator proteins on ChREBP-targeted promoters, is an intriguing subject for future investigations. Interestingly, bile acid activation of FXR has previously been shown to regulate glucose homeostasis and inhibit Foxo1 activity [42-44], suggesting a link between LXR, FXR and Foxo1 signaling in regulation of ChREBP activity (Figure 2).

In conclusion, our studies reveal LXRs as essential for nuclear O-GlcNAc signaling, and place LXRs at the focal point of hepatic glucose utilization and lipid metabolism upstream of ChREBP. Overall, our data suggest that feeding-responses to LXR involve cross-talk between insulin, O-GlcNAc and oxysterol signaling and that there is a tight interplay between LXRs and ChREBP in regulating hepatic lipogenic gene transcription in response to feeding and hyperglycemia. The relative roles of LXRs and ChREBP and cooperating transcription factors in regulating glucose and lipid homeostasis in response to feeding under physiological and pathophysiological conditions, are clearly dependent on glucose and insulin signaling involving posttranslational modifications, availability of nuclear receptor ligands, and spatiotemporal coregulator interactions. As aberrant O-GlcNAc signaling during nutrient stress and diabetes leads to excessive glucose production and lipid accumulation in the liver, studies including mutated O-GlcNAc residues in Foxo1, FXR, LXR and ChREBP, will provide a better understanding of the relevance of coordinated O-GlcNAcylation of these proteins under physiological and pathophysiological conditions.

Conflicting interests

The authors have declared that no competing interests exist.

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References

1. Strable MS, Ntambi JM. Genetic control of de novo lipogenesis: role in diet-induced obesity. Crit Rev Biochem Mol Biol 2010; 45:199-214.

2. Hart GW, Slawson C, Ramirez-Correa G, Lagerlof O. Cross talk between O-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease. Annu Rev Biochem 2011; 80:825-858.

3. Ruan HB, Singh JP, Li MD, Wu J, Yang X. Cracking the O-GlcNAc code in metabolism. Trends Endocrinol Metab 2013; 24:301-309.

4. McClain DA, Lubas WA, Cooksey RC, Hazel M, Parker GJ, Love DC, *et al*. Altered glycan-dependent signaling induces insulin resistance and hyperleptinemia. Proc Natl Acad Sci U S A 2002; 99:10695-10699.

5. Guinez C, Filhoulaud G, Rayah-Benhamed F, Marmier S, Dubuquoy C, Dentin R, *et al*. O-GlcNAcylation increases ChREBP protein content and transcriptional activity in the liver. Diabetes 2011; 60:1399-1413.

6. Anthonisen EH, Berven L, Holm S, Nygard M, Nebb HI, Gronning-Wang LM. The nuclear receptor LXR is O-GlcNAc modified in response to glucose. J Biol Chem 2009; 285:1607-1615

7. Bindesboll C, Fan Q, Norgaard RC, MacPherson L, Ruan HB, Wu J, *et al*. Liver X receptor regulates hepatic nuclear O-GlcNAc signaling and carbohydrate responsive element-binding protein activity. J Lipid Res 2015; 56:771-785.

8. Grønning-Wang LM, Bindesbøll C, Nebb HI. The Role of Liver X Receptor in Hepatic de novo Lipogenesis and Cross-Talk with Insulin and Glucose Signaling. Lipid Metabolism. InTech 2013.

9. Filhoulaud G, Guilmeau S, Dentin R, Girard J, Postic C. Novel insights into ChREBP regulation and function. Trends Endocrinol Metab 2013; 24:257-268.

10. Janowski BA, Grogan MJ, Jones SA, Wisely GB, Kliewer SA, Corey EJ, *et al*. Structural requirements of ligands for the oxysterol liver X receptors LXRalpha and LXRbeta. Proc Natl Acad Sci U S A 1999; 96:266-271.

11. Laffitte BA, Chao LC, Li J, Walczak R, Hummasti S, Joseph SB, *et al*. Activation of liver X receptor improves glucose tolerance through coordinate regulation of glucose metabolism in liver and adipose tissue. Proc Natl Acad Sci U S A 2003; 100:5419-5424.

12. Cao G, Liang Y, Broderick CL, Oldham BA, Beyer TP, Schmidt RJ, *et al*. Antidiabetic action of a liver x receptor agonist mediated by inhibition of hepatic gluconeogenesis. J Biol Chem 2003; 278:1131-1136.

13. Dalen KT, Ulven SM, Bamberg K, Gustafsson JA, Nebb HI. Expression of the insulin-responsive glucose transporter GLUT4 in adipocytes is dependent on liver X receptor alpha. J Biol Chem 2003; 278:48283-48291.

14. Kalaany NY, Gauthier KC, Zavacki AM, Mammen PPA, Kitazume T, Peterson JA, *et al*. LXRs regulate the balance between fat storage and oxidation. Cell Metabolism 2005; 1:231-244.

15. Beaven SW, Matveyenko A, Wroblewski K, Chao L, Wilpitz D, Hsu TW, *et al*. Reciprocal regulation of hepatic and adipose lipogenesis by liver x receptors in obesity and insulin resistance. Cell Metab 2013; 18:106-117.

16. Oosterveer MH, van Dijk TH, Grefhorst A, Bloks VW, Havinga R, Kuipers F, *et al*. Lxralpha deficiency hampers the hepatic adaptive response to fasting in mice. J Biol Chem 2008; 283:25437-25445.

17. Tobin KA, Ulven SM, Schuster GU, Steineger HH, Andresen SM, Gustafsson JA, *et al*. Liver X receptors as insulin-mediating factors in fatty acid and cholesterol biosynthesis. J Biol Chem 2002; 277:10691-10697.

18. Chen G, Liang G, Ou J, Goldstein JL, Brown MS. Central role for liver X receptor in insulin-mediated activation of Srebp-1c transcription and stimulation of fatty acid synthesis in liver. Proc Natl Acad Sci U S A 2004; 101:11245-11250.

19. Kamei Y, Miura S, Suganami T, Akaike F, Kanai S, Sugita S, *et al*. Regulation of SREBP1c gene expression in skeletal muscle: role of retinoid X receptor/liver X receptor and forkhead-O1 transcription factor. Endocrinology 2008; 149:2293-2305.

20. Liu X, Qiao A, Ke Y, Kong X, Liang J, Wang R, *et al*. FoxO1 represses LXRalpha-mediated transcriptional activity of SREBP-1c promoter in HepG2 cells. FEBS Lett 2010; 584:4330-4334.

21. Deng X, Zhang W, I OS, Williams JB, Dong Q, Park EA, *et al*. FoxO1 inhibits sterol regulatory element-binding protein-1c (SREBP-1c) gene expression via transcription factors Sp1 and SREBP-1c. J Biol Chem 2012; 287:20132-20143.

22. Kim TH, Kim H, Park JM, Im SS, Bae JS, Kim MY, *et al*. Interrelationship between liver X receptor alpha, sterol regulatory element-binding protein-1c, peroxisome proliferator-activated receptor gamma, and small heterodimer partner in the transcriptional regulation of glucokinase gene expression in liver. J Biol Chem 2009; 284:15071-15083.

23. Bond MR, Hanover JA. A little sugar goes a long way: the cell biology of O-GlcNAc. J Cell Biol 2015; 208:869-880.

24. Maszczak-Seneczko D, Olczak T, Olczak M. Subcellular localization of UDP-GlcNAc, UDP-Gal and SLC35B4 transporters. Acta Biochim Pol 2011; 58:413-419.

25. Chang Q, Su K, Baker JR, Yang X, Paterson AJ, Kudlow JE. Phosphorylation of human glutamine:fructose-6-phosphate amidotransferase by cAMP-dependent protein kinase at serine 205 blocks the enzyme activity. J Biol Chem 2000; 275:21981-21987.

26. Hu Y, Riesland L, Paterson AJ, Kudlow JE. Phosphorylation of mouse glutamine-fructose-6-phosphate amidotransferase 2 (GFAT2) by cAMP-dependent protein kinase increases the enzyme activity. J Biol Chem 2004; 279:29988-29993.

27. Zhang K, Yin R, Yang X. O-GlcNAc: A Bittersweet Switch in Liver. Front Endocrinol (Lausanne) 2014; 5:221.

28. Nagel AK, Ball LE. O-GlcNAc transferase and O-GlcNAcase: achieving target substrate specificity. Amino Acids 2014; 46:2305-2316.

29. Cheung WD, Sakabe K, Housley MP, Dias WB, Hart GW. O-linked beta-N-acetylglucosaminyltransferase substrate specificity is regulated by myosin phosphatase targeting and other interacting proteins. J Biol Chem 2008; 283:33935-33941.

30. Hanover JA. Glycan-dependent signaling: O-linked N-acetylglucosamine. FASEB J 2001; 15:1865-1876.

31. Bond MR, Hanover JA. O-GlcNAc cycling: a link between metabolism and chronic disease. Annu Rev Nutr 2013; 33:205-229.

32. Lewis BA, Hanover JA. O-GlcNAc and the epigenetic regulation of gene expression. J Biol Chem 2014; 289:34440-34448.

33. Harwood KR, Hanover JA. Nutrient-driven O-GlcNAc cycling -

think globally but act locally. J Cell Sci 2014; 127:1857-1867.

34. Housley MP, Rodgers JT, Udeshi ND, Kelly TJ, Shabanowitz J, Hunt DF, *et al*. O-GlcNAc regulates FoxO activation in response to glucose. J Biol Chem 2008; 283:16283-16292.
35. Berrabah W, Aumercier P, Gheeraert C, Dehondt H, Bouchaert E, Alexandre J, *et al*. Glucose sensing O-GlcNAcylation pathway regulates the nuclear bile acid receptor farnesoid X receptor (FXR). Hepatology 2014; 59:2022-2033.
36. Herman MA, Peroni OD, Villoria J, Schon MR, Abumrad NA, Bluher M, *et al*. A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism. Nature 2012; 484:333-338.
37. Ido-Kitamura Y, Sasaki T, Kobayashi M, Kim HJ, Lee YS, Kikuchi O, *et al*. Hepatic FoxO1 integrates glucose utilization and lipid synthesis through regulation of Chrebp O-glycosylation. PLoS One 2012; 7:e47231.
38. Haeusler RA, Han S, Accili D. Hepatic FoxO1 ablation exacerbates lipid abnormalities during hyperglycemia J Biol Chem. 2010; 285:26861-26868.
39. Caron S, Huaman Samanez C, Dehondt H, Ploton M, Briand O, Lien F, *et al*. Farnesoid X receptor inhibits the transcriptional activity of carbohydrate response element binding protein in human hepatocytes. Mol Cell Biol 2013; 33:2202-2211.
40. Kalaany NY, Mangelsdorf DJ. LXRS and FXR: the yin and yang of cholesterol and fat metabolism. Annu Rev Physiol 2006; 68:159-191.
41. Schmitt J, Kong B, Stieger B, Tschopp O, Schultze SM, Rau M, *et al*. Protective effects of farnesoid X receptor (FXR) on hepatic lipid accumulation are mediated by hepatic FXR and independent of intestinal FGF15 signal. Liver Int 2015; 35:1133-1144.
42. Ma K, Saha PK, Chan L, Moore DD. Farnesoid X receptor is essential for normal glucose homeostasis. J Clin Invest 2006; 116:1102-1109.
43. Yamagata K, Daitoku H, Shimamoto Y, Matsuzaki H, Hirota K, Ishida J, *et al*. Bile acids regulate gluconeogenic gene expression via small heterodimer partner-mediated repression of hepatocyte nuclear factor 4 and Foxo1. J Biol Chem 2004; 279:23158-23165.
44. Shin DJ, Osborne TF. FGF15/FGFR4 integrates growth factor signaling with hepatic bile acid metabolism and insulin action. J Biol Chem 2009; 284:11110-11120.