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.

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 LXRα 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 PEPC 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 modification in liver 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 LXRαβ/− and LXRαβ/−/− 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 refeed (12h) wild type and LXRαβ/−/− control and STZ-treated mice were subjected to RNA, protein and ChIP analysis and analysed for LXR, ChREBP,

Figure 1. The nutrient sensing hexosamine biosynthetic pathway (HBP) and protein O-GlcNAcylation. The activated sugar substrate UDP-GlcNAc for nucleocyttoplasmic 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 glucose: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. Hyperglycaemia 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.
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 (re-fed 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 re-fed 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 re-fed 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 re-fed 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 re-fed 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 LXRs 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 LXRs by mass spectrometric analysis, and a novel O-GlcNAc site in OGT (unpublished data). Identified O-GlcNAc sites will be mutated and the biological
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 LXRα/β-/- 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. O-GlcNAcylation of nuclear localized ChREBP stabilizes the protein and increases its transcriptional activity on L-PK and lipogenic genes. We now report 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 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. 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. 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 in accordance with the above mentioned study by Ido-Kitamura et al.

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. Interestingly, FXR has been shown to interact with ChREBP and inhibit its transcriptional activity in human hepatocytes. 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. Hepatic LXR expression is upregulated in liver-specific FXR knock out mice, 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, 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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