Hepatic Gene Regulation by Glucose and Polyunsaturated Fatty Acids: A Role for ChREBP

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ABSTRACT The liver is a major site for carbohydrate metabolism (glycolysis and glycogen synthesis) and triglyceride synthesis (lipogenesis). In the last decade, increasing evidence has emerged to show that nutrients, in particular, glucose and fatty acids, are able to regulate hepatic gene expression in a transcriptional manner. Indeed, although insulin was long thought to be the major regulator of hepatic gene expression, it is now clear that glucose metabolism rather than glucose itself also contributes substantially to the coordinated regulation of carbohydrate and lipid homeostasis in liver. In fact, the recent discovery of the glucose-signaling transcription factor carbohydrate responsive element binding protein (ChREBP) shed some light on the molecular mechanisms by which glycolytic and lipogenic genes are reciprocally regulated by glucose and fatty acids in liver. Here, we will review some of the recent studies that have begun to elucidate the regulation and function of this key transcription factor in liver. Indeed, a better understanding of the mechanisms by which glucose and fatty acids control hepatic gene expression may provide novel insight into the development of new therapeutic strategies for a better management of diseases involving blood glucose and/or disorders of lipid metabolism. J. Nutr. 136: 1145–1149, 2006.

KEY WORDS: • ChREBP • glucose • polyunsaturated fatty acids • transcriptional regulation • glycolytic and lipogenic gene expression

The liver plays a central role in the control of glucose and lipid metabolism. When a high-carbohydrate meal is ingested, several metabolic events aimed at decreasing endogenous glucose production by the liver (glycogenolysis and gluconeogenesis) and increasing glucose uptake (glycolysis) and storage in the form of glycogen in the liver are turned on. In addition, when glucose is delivered into the portal vein in large quantities and once hepatic glycogen concentrations are restored, glucose can be converted in the liver into triglycerides through de novo lipogenesis. In fact, triglycerides represent the principal energy storage fuel in mammals. Enzymes of the lipogenic pathway that are transcriptionally regulated include acetyl-CoA-carboxylase (ACC)², fatty acid synthase (FAS), and stearoyl-CoA desaturase (SCD-1). Absorption of a high-carbohydrate diet is concomitant with increases in the concentrations of substrates such as glucose but also with changes in the concentrations of pancreatic hormones, such as insulin and glucagon. Although it has long been thought that insulin was the main regulator of glycolytic and lipogenic gene transcription, it is now well established that glucose also regulates gene transcription (1). In fact, transcriptional activation of glycolytic and lipogenic genes requires the presence of both insulin and glucose, neither of which is active alone (2,3). A pathway by which insulin can control gene expression was discovered through the study of the transcription factor sterol regulatory element binding protein (SREBP) [for review, see (4)]. In contrast, despite efforts from several laboratories (1,5,6) as we will discuss further, the exact nature of the glucose signaling compound remained largely unknown until recently.

Although storage as triglycerides is the principal energy storage fuel in mammals, excessive accumulation of triglycerides in tissues, including liver, is associated with insulin resistance and enhanced cellular apoptosis (i.e., lipotoxicity) (7). The excessive accumulation of cellular lipids is due to an increased expression of enzymes from the glycolytic and lipogenic pathway combined with the impaired entry of fatty acids into the mitochondrial β-oxidation pathway. Interestingly, a diet that provides 2–5% of energy from (n-3) and (n-6) PUFA leads to a coordinate suppression of glycolytic and lipogenic genes and to an induction of genes involved in fatty acid oxidation (8). This metabolic balance in liver leads to a “partitioning” of fatty acids away from triglyceride synthesis toward fatty acid oxidation. The positive effects of PUFA may in fact delay the onset of insulin resistance and lipotoxicity and in turn improve the metabolic

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2 Abbreviations used: ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; bHLH/LZ, basic/helix-loop-helix/leucine zipper; ChREBP, carbohydrate responsive element binding protein; dmChREBP, double mutant ChREBP; ER, endoplasmic reticulum; FAS, fatty acid synthase; GK, glucokinase; G6P, glucose 6-phosphate; HNF, hepatic nuclear factor; KO, knockout; L-PK, liver-pyruvate kinase; LXR, liver X receptor; MiR, Max-like protein X; PKA, cAMP-dependent protein kinase; PP2A, protein phosphatase 2A; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; SRE, sterol regulatory element; SREBP-1c, SRE binding protein-1c; USF, upstream stimulatory factor; XBP, xylose 5-phosphate.
phosphorylation of ChREBP (25) (Fig. 1). However, recent work by Tsatsos and Towle strongly challenged these concepts (26). First, using an in-gel phosphoprotein stain, the authors showed that although the overall phosphorylation status of ChREBP does increase under cAMP conditions, it does not decrease and/or change when hepatocytes are switched from low to high glucose concentrations (26). These experiments suggest that dephosphorylation is not responsible for the activation of ChREBP. In addition, mutants of ChREBP, in which one or several of the proposed PKA phosphorylation sites are lacking, still retain the ability to respond to high glucose concentrations and to be partially inhibited by cAMP (26). More importantly, preventing PKA-mediated phosphorylation at these residues did not create a glucose-independent active form because these mutants of ChREBP were not active under low glucose concentrations (26). Therefore, it is clear from these studies that mechanisms other than PKA phosphorylation may be required to mediate the glucose-activation of ChREBP.

Another question that must be further addressed is the exact nature of the glucose metabolite responsible for ChREBP activation. Although it is clear that a metabolite of glucose, and not glucose per se, is responsible for this glucose signal, both glucose 6-phosphate (G6P) (1) and X5P (25,27) were proposed as critical metabolites. Several lines of evidence suggested that G6P could be the metabolite acting as a signal on lipogenic gene transcription (3,28). Recent work from our laboratory using mice with a specific deletion of the glucokinase (GK) gene in liver (GK-KO mice) (29) confirmed the fact that G6P production by GK is necessary for the acute induction of ChREBP gene expression (12) and for its nuclear translocation in response to high glucose concentrations (R. Dentin, P. D.
Denechaud, C. Postic, unpublished data). However, this mouse model does not allow us to discriminate the specific role of G6P vs. X5P because the concentrations of these 2 metabolites were found to be decreased in liver of GK-KO mice (12). Over the past years, some experimental evidence favored a possible role for X5P as the signaling compound. This was based mainly on the fact that xylitol, a precursor of X5P, is able to stimulate the transcription of a reporter gene driven by the L-PK promoter in hepatocytes and that X5P was shown to specifically activate PP2A mediated-dephosphorylation of ChREBP (25).

It is interesting to note that Uyeda’s group implicated X5P and PP2A previously in the acute control of glycolysis (30). The authors showed that the X5P-activated PP2A also catalyzes the dephosphorylation of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, the bifunctional enzyme that synthesizes and degrades fructose 2,6 biphosphate, the most potent activator of phosphofructokinase, a key regulatory enzyme of glycolysis. Therefore, according to Uyeda’s model, X5P would be the coordinating metabolite that controls phosphofructokinase acutely and promotes the activation of ChREBP, thereby integrating both short- and long-term-regulation of the glycolytic and lipogenic pathways [see (31) for review]. However, definitive evidence of the involvement of X5P or G6P must be obtained. For example, an approach in which selective overexpression or inhibition of one of the limiting enzymes of the pentose phosphate pathway could help discriminate the effects of these 2 metabolites.

Although additional experiments will be necessary to gain a better understanding of the regulation of the endogenous ChREBP protein by phosphorylation/dephosphorylation as well as to identify the nature of the glucose metabolite involved in ChREBP activation, it is clear from several studies, including ours, that ChREBP is crucial for mediating the transcriptional effect of glucose on both glycolytic and lipogenic genes (12, 32, 33). Studies from our laboratory using ChREBP small interfering RNA (siRNA) in cultured hepatocytes showed that ChREBP is required for the induction of L-PK, FAS, and ACC gene expression in response to glucose (12). These results were also confirmed in vivo using ChREBP gene KO mice (32), which also display reduced glycolysis, lipogenesis, and intolerance to dietary fructose, emphasizing the critical role of ChREBP in glucose and lipid homeostasis (32).

Interestingly, ChREBP does not homodimerize or bind to the ChRE as a homodimer (33, 34). Using the yeast 2-hybrid system, Stoeckman et al. (33) identified a bHLH/LZ protein that interacts with the bHLH/LZ domain of ChREBP, named Mlx (Max-like protein X). Mlx is a member of the Myc/Max/Mad family of transcription factors that can serve as a common interaction partner of a transcription factor network (35). The evidence that Mlx is the functional partner of ChREBP was recently confirmed (34). Through the use of an adenovirus expressing a dominant negative form of Mlx, Ma et al. (34) demonstrated that the inhibition of Mlx interferes directly with the endogenous ChREBP/Mlx complex and abrogates the glucose-response of the ACC reporter gene in primary cultures of hepatocytes. This blunted glucose response can be partially restored, however, when ChREBP is overexpressed. The fact that this rescue occurs only at high glucose concentrations of the recombinant ChREBP adenovirus suggests that sufficient ChREBP must be provided in the cell to titrate out the dominant negative effect of Mlx. According to a model proposed by Towle et al. (36), 2 ChREBP-Mlx heterodimers would bind to the 2 E boxes of the ChoRE to provide a transcriptional complex necessary for glucose regulation. It remains to be determined whether Mlx is also regulated by glucose and more importantly, when and where the association between ChREBP and Mlx occurs. In addition, the fact that ChREBP interacts with Mlx, may suggest that other partners of ChREBP (other transcription factors or cofactors for example) may be required to fully regulate glucose-responsive gene expression in liver.

**Implication of ChREBP in the negative effects of PUFA.**

As mentioned in the introduction, PUFA are potent inhibitors of hepatic glycolysis and de novo lipogenesis, through the inhibition of genes involved in glucose utilization and lipid synthesis, including L-PK, FAS, and ACC. By regulating this pathway, PUFA promote a shift from fatty acid synthesis and storage to oxidation (37). In fact, dietary fat regulates hepatic gene expression by controlling the activity or abundance of several key transcription factors, including peroxisome proliferator-activated receptors (PPARα, β, γ1, and γ2), hepatic nuclear factors (HNF-4α), retinoid X receptor (RXRα), Liver X receptor (LXRα), and SREBP-1c [see (38) for review]. In the past, different studies have implicated SREBP-1c in the negative effects of PUFA. Members of the SREBP family, including SREBP-1c, are bHLH/LZ transcription factors synthesized as inactive precursors bound to the membranes of the endoplasmic reticulum (ER). Each SREBP precursor is organized into 3 domains: an N-terminal domain that contains the transcription factor itself, a central domain of 80 amino acids containing 2 transmembrane sequences separated by 31 amino acids that are in the lumen of the ER, and a COOH-terminal regulatory domain. Upon activation, the ER-anchored SREBP precursor undergoes a sequential 2-step cleavage process to release the N-terminal active domain, designated the nuclear form of SREBP. PUFA inhibit lipogenic gene expression by suppressing SREBP-1c nuclear abundance through several mechanisms, including inhibition of SREBP-1c gene transcription (39), enhancement of proteasomal degradation, and mRNA decay (40–43). Although SREBP-1c may be a major target of PUFA control in liver, the PUFA-mediated suppression of SREBP-1c alone cannot explain the inhibition of the glycolytic L-PK gene by PUFA. Indeed, L-PK expression is not subject to direct SREBP-1c regulation (44) and, more importantly, its promoter does not contain a sterol regulatory element (SRE) binding site (45). Although functional mapping analysis showed that the PUFA response element of the L-PK promoter is co-localized with the ChRE binding site (46, 47), the identity of the transcription factor(s) involved in mediating the negative effects of PUFA on the L-PK gene was uncertain until recently.

Because we previously implicated the transcription factor ChREBP in mediating the positive effect of glucose on the L-PK gene (12), we hypothesized that the suppressive effect of PUFA on this gene could be mediated, at least in part, through a decrease in ChREBP gene expression and/or through a defect in its nuclear translocation (13). Our hypothesis was also supported by the fact that the DNA binding activity of ChREBP was shown previously to be decreased in liver nuclear extracts from rats fed a high-fat diet compared with those fed a high-carbohydrate diet. We demonstrated that PUFA [linoleate (18:2), eicosapentaenoic acid (20:5), and docosahexaenoic acid (22:6)] suppress ChREBP activity by increasing its mRNA decay and by altering ChREBP protein translocation from the cytosol to the nucleus both in primary cultures of hepatocytes and in liver in vivo. The PUFA-mediated alteration in ChREBP translocation is linked to an inhibition of glucose metabolism (i.e., an inhibition of GK activity and a decrease in G6P concentrations) (13) (Fig. 2). Because we also observed a concomitant decrease in the concentrations of X5P under our experimental conditions, once again we could not exclude the possibility that the decrease in X5P was indeed responsible for the alteration in ChREBP translocation. More importantly, our studies demonstrated that overexpression in cultured hepatocytes of a constitutive nuclear ChREBP isofrom, double mutant ChREBP...
FIGURE 2  PUFA inhibit ChREBP nuclear translocation in liver. In the presence of PUFA, the specific inhibition of GK and G6PDH activities (Step 1), 2 key enzymes of glycolysis and of the pentose phosphate pathway, respectively, by decreasing both G6P and X5P concentrations retain ChREBP in the cytosol (Step 2). Under our experimental conditions, AMPK was not involved in this mechanism of inhibition of ChREBP translocation. Although the ability of PUFA to activate AMPK remains controversial, the AMPK-mediated phosphorylation of ChREBP on Ser272 previously described (10) should not be excluded as having potential importance under specific physiological or physiopathologic conditions (Step 3).

(dmChREBP) (33), totally reversed the PUFA inhibition on the L-PK gene and significantly reduced that associated with the FAS gene (13). Together, our results demonstrate that the suppressive effect of PUFA on both glycolytic and lipogenic genes is caused primarily by an alteration of ChREBP activity. Of course, we do not exclude a role for SREBP-1c, especially on the FAS gene. Indeed, the fact that FAS gene expression was not completely restored under dmChREBP overexpression confirmed that SREBP-1c, acting through its SRE binding element, plays a determinant role for the regulation of FAS gene expression. The fact that chromatin immunoprecipitation experiments showed direct binding of SREBP-1c (48) and ChREBP (11) on the promoter region of the FAS gene supports the hypothesis that insulin/glucose stimulation and PUFA suppression are mediated by the coordinated action of ChREBP and SREBP-1c.

It is important to note that the mechanism previously described to explain the inhibitory effect of fatty acids on ChREBP function involved an AMPK-mediated phosphorylation of ChREBP (10) rather than a defect of ChREBP translocation (Fig. 2). However, the model described by Kawaguchi et al. raises some issues (10). Indeed, the authors reported that short-, medium-, and long-chain saturated fatty acids activate AMPK in cultured hepatocytes (10) and that this activation of AMPK was not involved in this mechanism of inhibition of ChREBP activity. Although the ability of PUFA to activate AMPK remains controversial, the AMPK-mediated phosphorylation of ChREBP by the coordinated action of ChREBP and SREBP-1c.

nuclear translocation and that this effect is independent of AMPK, as evidenced by studies performed in AMPK KO mice (13). Indeed, we showed that the ability of PUFA to inhibit ChREBP translocation into the nucleus of hepatocytes was sustained in the absence of either AMPKα1 or α2 subunits in liver (13).

Finally, our studies do not exclude the possibility that PUFA also exert a transcriptional effect on ChREBP gene expression. In the case of SREBP-1c, the mechanisms by which PUFA suppress its gene transcription are beginning to emerge. The SREBP-1 gene contains 2 response elements for the lipogenic transcription factor LXR. The activation of LXR by oxygenated ligands induces the transcription of SREBP-1c (52) and PUFA, by displacing oxygenated from LXR, thus antagonizing the trans-activation of LXR, at least in HEK293 (53). Whether LXR regulates ChREBP gene expression transcriptionally and is involved in mediating the inhibitory effects of PUFA on both SREBP-1c and ChREBP in liver cells remain to be addressed. Analysis of the ChREBP promoter is currently progressing in our laboratory.

Perspectives and future studies. With the discovery of ChREBP, our understanding of the long-term regulation of glucose and lipid metabolism in liver has recently made considerable progress. ChREBP is a major determinant of the reciprocal effects of glucose and PUFA on both glycolytic and lipogenic genes in liver. Although the post-translational regulation of ChREBP by phosphorylation/dephosphorylation may be a more complex process than previously thought, it is clear from recently published studies that ChREBP is the long-sought glucose transcription factor. Indeed, ChREBP is crucial for the glucose-induction of both glycolytic and lipogenic gene expression and plays a determinant role in the control of fatty acid synthesis. Therefore, in a more physiopathologic context, determining its possible involvement in the development of hepatic steatosis and/or insulin resistance will be important in the near future and may help develop some therapeutic targets. Finally, given that ChREBP is also expressed outside of the liver, in tissues that contribute greatly to glucose and lipid homeostasis (i.e., skeletal muscles, white adipose tissue, brain), understanding ChREBP regulation and function in these tissues may be crucial for the regulation of overall energy homeostasis.

LITERATURE CITED

HEPATIC GENE REGULATION BY CHREBP


