ChREBP: A Glucose-activated Transcription Factor Involved in the Development of Metabolic Syndrome

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Abstract. Excess carbohydrate intake leads to fat accumulation and insulin resistance. Glucose and insulin coordinately regulate de novo lipogenesis from glucose in the liver, and insulin activates several transcription factors including SREBP1c and LXR, while those activated by glucose remain unknown. Recently, a carbohydrate response element binding protein (ChREBP), which binds to the carbohydrate response element (ChoRE) in the promoter of rat liver type pyruvate kinase (LPK), has been identified. The target genes of ChREBP are involved in glycolysis, lipogenesis, and gluconeogenesis. Although the regulation of ChREBP remains unknown in detail, the transactivitity of ChREBP is partly regulated by a phosphorylation/dephosphorylation mechanism. During fasting, protein kinase A and AMP-activated protein kinase phosphorylate ChREBP and inactivate its transactivity. During feeding, xylulose-5-phosphate in the hexose monophosphate pathway activates protein phosphatase 2A, which dephosphorylates ChREBP and activates its transactivity. ChREBP controls 50% of hepatic lipogenesis by regulating glycolytic and lipogenic gene expression. In ChREBP−/− mice, liver triglyceride content is decreased and liver glycogen content is increased compared to wild-type mice. These results indicate that ChREBP can regulate metabolic gene expression to convert excess carbohydrate into triglyceride rather than glycogen. Furthermore, complete inhibition of ChREBP in ob/ob mice reduces the effects of the metabolic syndrome such as obesity, fatty liver, and glucose intolerance. Thus, further clarification of the physiological role of ChREBP may be useful in developing treatments for the metabolic syndrome.

Key words: ChoRE, ChREBP, L-PK, Metabolic syndrome

Carbohydrate Response Element Binding Protein (ChREBP)

Increased consumption of high-carbohydrate and high-fat diets (so-called cafeteria diet) is one of the most important risk factors in the development of the metabolic syndrome. Excess carbohydrate is mainly converted to triglyceride in the liver, and excess fat accumulation in the body leads to insulin resistance and metabolic syndrome [1]. When a high-carbohydrate diet is ingested, carbohydrate is converted into triglyceride in the liver by key glycolytic enzymes such as glucokinase and liver-type pyruvate kinase (L-PK) and enzymes of de novo lipogenesis such as acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS) [2]. Both insulin and glucose are potent factors in inducing the transcription of these key enzyme genes (Fig. 1).
Glucose and insulin signals coordinately regulate lipogenesis

In the fed state, glucose and insulin coordinate hepatic lipogenesis by regulating glycolytic and lipogenic gene expression at the transcriptional level. ChREBP and SREBP1c share lipogenic genes and genes related to the hexose monophosphate (HMP) shunt (Fig. 2) [12, 13]. Some groups have reported that hepatic glucokinase is required for the synergistic effects of ChREBP and SREBP1c on glycolytic and lipogenic gene expression (Fig. 2) [14, 15]. Uyeda et al. showed that glucose-activated ChREBP directly binds the ChoRE of the L-PK promoter and activates L-PK gene expression [7]. However, whether SREBP1c physiologically mediates the action of insulin on glucokinase remains controversial. We reconfirmed that the overexpression of dominant active SREBP1c induces glucokinase gene expression in hepatocytes, which we previously confirmed as unpublished data. However, Liang et al. reported that the response of glucokinase to high-carbohydrate diet refeeding is still conserved in SREBP1c knockout mice [13]. In addition, Lynedjian et al. reported that SREBP1c cannot bind to liver-type glucokinase promoter [16], and Pichard et al. reported that SREBP1c knockdown by small interfering RNAs results in impaired induction of the FAS gene in response to glucose and insulin but does not prevent induction of the glucokinase gene [17]. Glucokinase is a key molecule regulating glycolytic flux, and it is important to identify the various transcription factors that mediate the activation of glucokinase gene expression by insulin.
Regulation of ChREBP transcriptional activity

Many glycolytic and lipogenic enzymes are induced by high-carbohydrate feeding and suppressed by fasting and starvation [6]. During starvation, hepatic glycolysis and de novo lipogenesis are suppressed. In contrast, gluconeogenesis, the beta-oxidation of fatty acyl CoA, and ketogenesis are upregulated. This change from anabolism to catabolism is regulated by stress hormone and AMP accumulation. During starvation, concentrations of plasma glucagon and epinephrine are increased. Glucagon and epinephrine increase the intracellular cAMP concentration and activate cAMP-activated protein kinase (PKA). PKA phosphorylates ChREBP, inactivating it [18]. Phosphorylation of ChREBP at Serine residue 196 (Ser196) inactivates nuclear import, and phosphorylation at Threonine residue 666 (Thr666) prevents DNA binding by ChREBP (Fig. 3A). Similarly, intracellular AMP accumulation inhibits ChREBP transactivity by activating AMP-activated protein kinase (AMPK) and phosphorylating ChREBP (Fig. 3A) [19]. In contrast, glucose activates ChREBP transactivity. Glucose is converted to xylulose-5-phosphate (Xu-5-P) in the hexose monophosphate (HMP) shunt, and Xu-5-P activates protein phosphatase 2A delta (PP2A delta) and dephosphorylates ChREBP protein (Fig. 3B) [20]. Xu-5-P-mediated PP2A activation also is seen in the activation of 6-phosphofructo-2, 6-kinase/bisphosphatase [21–23]. Xu-5-P is a key molecule in regulat-
ing not only transcription but also enzyme activity in glycolysis. Thus, ChREBP, by a phosphorylation/dephosphorylation mechanism, would seem to regulate the expression of glycolytic and lipogenic enzyme genes (L-PK, FAS, ACC). Excess glycogen accumulation is due to decreased G6Pase and L-PK enzyme activity. Liver triglyceride content is increased by decreased L-PK and lipogenic enzyme activity. G6P, glucose-6-phosphate; G6Pase, glucose-6-phosphatase; PEP, phosphoenol pyruvate; ChREBP, carbohydrate response element binding protein; L-PK, liver type pyruvate kinase; GK, glucokinase; OAA, oxaloacetate; Tkt, transketolase.

**Fig. 4.** Deficiency of ChREBP induces glycogen accumulation and decreases triglyceride synthesis in the liver. ChREBP regulates target genes of glycolysis (L-PK), gluconeogenesis (G6Pase) and lipogenesis (FAS, ACC). Excess glycogen accumulation is due to decreased G6Pase and L-PK enzyme activity. Liver triglyceride content is increased by decreased L-PK and lipogenic enzyme activity. G6P, glucose-6-phosphate; G6Pase, glucose-6-phosphatase; PEP, phosphoenol pyruvate; ChREBP, carbohydrate response element binding protein; L-PK, liver type pyruvate kinase; GK, glucokinase; OAA, oxaloacetate; Tkt, transketolase.

expression at the transcriptional level [27]. The mouse ChREBP gene promoter contains an LXR response element at about 2.4 kbp, and LXR agonists increase hepatic ChREBP mRNA in wild-type mice but not in LXR-α double knockout mice. Moreover, Saez et al. reported that LXR is activated by glucose and that high-glucose treatment increased ChREBP mRNA two-fold in HepG2 cells [28]. Insulin also regulates the expression and transactivity of the LXR gene [29]. However, despite the hyperinsulinemia and hyperglycemia seen in ob/ob mice, the level of ChREBP mRNA in liver of ob/ob mice is only twice as high as in liver of wild-type mice [30]. These results suggest that ChREBP transactivity is regulated mainly at the post-transcriptional level rather than at the transcriptional level.

**ChREBP knockout mice**

To identify the physiological role of ChREBP in hepatic glucose and lipid metabolism, we established ChREBP knockout mice (ChREBP−/−) [10]. ChREBP−/− mice are viable and appear to have a normal lifespan. These mice show a phenotype with hepatic lipogenesis from glucose 65% lower than in wild-type mice, and adipose tissue weight correspondingly lower. In addition, the mRNAs of many glycolytic and lipogenic enzymes in liver of ChREBP−/− mice are suppressed [10]. Consistent with the in vivo data, our CHIP and EMSA assays show that ChREBP binds directly to ChoREs in the promoters of LPK, ACC, and FAS [31]. These data also indicate that ChREBP directly regulates the expression of glycolytic and lipogenic enzyme genes.

In contrast to decreased lipid content, liver glycogen content is increased and hepatomegaly appears in ChREBP−/− mice [10]. The mechanism by which glycogen content in ChREBP knockout mice is increased is partly understood (Fig. 5). In these mice, most metabolites in the glycolytic pathway, except for pyruvate, are increased. Pyruvate is converted from phosphoenol pyruvate (PEP) by L-PK. In addition, L-PK activity in liver of ChREBP−/− mice was markedly lower and the PEP/pyruvate ratio was higher. G6Pase activity also was decreased in these mice, and the G6P content was increased. Because G6P activates glycogen synthase and stimulates glycogen synthesis in liver, glycogen accumulates in the liver of ChREBP−/−
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mice. These results indicate that ChREBP is a transcription factor that preferentially regulates triglyceride storage (Fig. 5).

Both glucose and insulin are required for full induction of most lipogenic enzyme genes. In addition, the actions of insulin in regard to lipid metabolism are mediated through the transcription factor SREBP1c, and in ChREBP−/− mice, SREBP1 mRNA and protein are unchanged compared with wild-type mice. Furthermore, as with glycolytic genes, SREBP and ChREBP regulate glucokinase and LPK, respectively. These data indicate that ChREBP and SREBP independently regulate de novo lipogenesis.

**Inhibition of ChREBP as a treatment for metabolic syndrome**

Excess fat accumulation in the liver (fatty liver) leads to insulin resistance in the body, and reducing the fat content of the liver readily improves insulin sensitivity [1]. We intercrossed ChREBP−/− mice with ob/ob mice and established ob/ob ChREBP double-cross (ob/ob ChREBP−/−) mice [30]. In ob/ob ChREBP−/− mice, complete inhibition of the ChREBP gene throughout the body ameliorated symptoms of the metabolic syndrome such as obesity, insulin resistance, fatty liver, and glucose intolerance (Fig. 6). Consistently, glycolytic and lipogenic gene expression was normalized in ob/ob ChREBP−/− mice. Postic et al. reported that delivery of adenovirus-bearing ChREBP short hairpin RNA (Ad-shChREBP) into the liver effectively improved the metabolic syndrome in ob/ob mice [32]. Their data suggest that liver-specific inhibition of ChREBP ameliorates both fatty liver and also glucose intolerance. In contrast, liver-specific inhibition of SREBP1c and PPARγ was found to ameliorate fatty liver but not glucose intolerance [33, 34]. Although the source of the phenotypic differences between these liver-specific SREBP1c or PPARγ knockout mice and Ad-shChREBP mice is not known, ChREBP might regulate G6Pase gene expression at the transcriptional level. G6Pase is a key enzyme in the regulation of gluconeogenesis and glucose output in liver, and decreased G6Pase activity in Ad-shChREBP mice can lower the plasma glucose concentration [32]. Comparison of liver-specific ChREBP knockdown mice with liver-specific SREBP1c or PPARγ knockout mice should provide a clearer understanding of the roles of these substances in the metabolic syndrome.
In addition, food intake in ob/ob ChREBP−/− mice was lower than in ob/ob mice, which was not the case in ob/ob mice infected with Ad-shChREBP. In addition, since ChREBP is also expressed in the brain, ChREBP may regulate appetite control, likely in the hypothalamus (Fig. 6).

Marked hepatomegaly and massive glycogen accumulation are thought to be effects of ChREBP inhibition. Although hepatoma was not visible, excess glycogen accumulation could well finally induce liver fibrosis and carcinogenesis. With the aim of applying these results on ChREBP inhibition to the treatment of metabolic syndrome, we are now identifying ChREBP target genes to suggest new drug therapies based on inhibition of ChREBP transactivity.

Activation of PKA and AMPK has been used in the treatment of obesity-related disorders [35, 36]. Exendine-4 (GLP-1 analogue) increases hepatic cAMP content and ameliorates fatty liver by suppressing de novo lipogenesis in ob/ob mice [35]. Metformin also inhibits de novo lipogenesis and ameliorates fatty liver by AMPK in genetically obese mice [37]. In addition, acetate can be taken daily in the form of vinegar; when acetate is converted to acetyl CoA, the AMP/ATP ratio is increased and AMPK is activated [38, 39]. Intake of acetate reduces lipogenesis and improves fatty liver in obese mice and rats. In addition, polyunsaturated fatty acids (PUFA) also can be taken daily in the form of fish oil to improve obesity-related disorders [40, 41]. Since these drugs and foods modulate transactivity not only of SREBP but also of ChREBP, they are promising means of mitigating the metabolic syndrome, but the mechanisms by which they act remain unclear.

Role of ChREBP in other tissues

ChREBP is expressed ubiquitously, but mainly in lipogenic organs such as liver, intestine, and white adipose tissues. Interestingly, ChREBP also is expressed in pancreatic islets [42, 43]. In islets, glucose stimulates insulin secretion and is an important signal for cellular events. Using DNA microarrays, many researchers have identified glucose responsive genes in islets that are common to those in liver [44]. In insulin-producing INS-1 cells, overexpression of ChREBP was found to upregulate LPK, FAS, and ACC1 mRNAs, but the insulin response to glucose in these cells was the same as in control cells [42]. In islets of ChREBP−/− mice, glucose-stimulated insulin secretion was the same as in wild-type mice (unpublished data). ChREBP also regulated lipogenic genes in islets, but only overexpression of ChREBP prevented the accumulation of lipid droplets, unlike overexpression of SREBP1c [45]. These findings suggest the action of an insulin signal in addition to ChREBP activation that is important in the induction of lipogenesis.

ChREBP also is abundantly expressed in adipose tissues. During 3T3-L1 preadipocyte adipogenesis, ChREBP is dramatically induced and the expression of its gene in 3T3L1 cells is modulated by various factors including glucose, free fatty acids, insulin, and the antidiabetic agent troglitazone [46]. However, the expression of ChREBP mRNA in adipose tissue in vivo is barely responsive to changes in nutrient status. Moreover, ChREBP mRNA is induced in the late stage of adipogenesis and ChREBP has little part in this process. Thus, the physiological role of ChREBP in adipose tissue remains unclear.

Conclusion

The liver is an important organ in the maintenance of glucose homeostasis and energy storage. Excess triglyceride in the liver induces fatty liver and eventually insulin resistance. To prevent the metabolic syndrome, it is important to gain understanding of the mechanism by which certain glucose/insulin-regulated transcription factors coordinate hepatic energy metabolism. Among these transcription factors, glucose-activated transcription factor ChREBP regulates the balance between glycogen and triglyceride storage by coordinately regulating glycolytic and lipogenic gene expression. In genetically obese mice, complete deficiency of ChREBP ameliorates glucose intolerance, fatty liver, and obesity, although hepatomegaly and liver glycogen accumulation develop. Thus, the identification of the roles of ChREBP and its target genes in glucose and lipid metabolism should be useful in developing treatments for the metabolic syndrome.

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