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REVIEW

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 transactivity 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

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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). Insulin signaling regulates transcription of these glycolytic and lipogenic enzymes by activation of SREBP1c

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Abbreviations: Carbohydrate Response Element Binding Protein (ChREBP), Carbohydrate Response Element (ChoRE), Liver type pyruvate kinase (LPK), Fatty Acid synthase (FAS), Acetyl CoA Carboxylase (ACC), Malic Enzyme (ME), Hexose Monophosphate Shunt (HMP Shunt), Malate-Pyruvate Shunt (MP Shunt), Glucokinase (GCK), glucose 6 phosphatase (G6Pase), Glucose 6 phosphate (G6P), AMP activated protein kinase (AMPK), Xylulose-5-phosphate (Xu-5-P), Protein phosphatase 2A delta (PP2Adelta), cAMP-activated protein kinase (PKA), Phosphoenol Pyruvate (PEP)

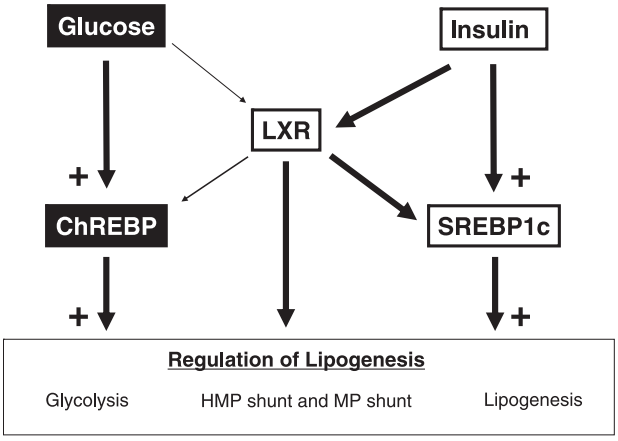

Fig. 1. Glucose and insulin activate transcription factors and regulate *de novo* lipogenesis in liver.
Glucose activates ChREBP and insulin activates SREBP1c and LXR. LXR, liver protein X receptor; SREBP1c, sterol regulatory element binding protein 1c; ChREBP, carbohydrate response element binding protein; HMP, hexose monophosphate; MP, malate-pyruvate shunt.

and liver X receptor (LXR), respectively [3, 4]. In contrast, the mechanism by which glucose signaling activates the expression of these enzymes is poorly understood. For example, transcription of Spot14 (S14) in liver is induced by a carbohydrate diet. Towle *et al.* reported that the carbohydrate response element (ChoRE) of the S14 gene consists of two E-box-like consensus sequences (CAYGNGN5CNCRTG) [5]. Like S14, many glucose response genes (such as LPK, FAS, and ACC) contain a similar ChoRE in their promoter regions [2]. This suggests that a common transcription factor binds to the conserved ChoRE, and many researchers have addressed this problem over a long period [6]. Uyeda *et al.* successfully purified a transcription factor that binds to the rat L-PK ChoRE [7]. This transcription factor is in the same region as the Williams-Beuren syndrome critical region 14 (WBSCR14) protein, which is now renamed the carbohydrate response element binding protein (ChREBP) [7, 8]. Williams-Beuren syndrome is a neurodevelopmental disorder affecting several systems, and is caused by a heterozygous deletion in chromosomal region 7q11.23 in human. WBSCR14/ChREBP is expressed as a 4.2 kb transcript, and the WBSCR14/ChREBP locus encompasses 33 kb of genomic DNA with 17 exons [9]. The distribution of ChREBP mRNA is ubiquitous, but it is most abundant in lipogenic organs such as liver, brown and white adipose tissues, small intestine, kidney, and muscle [7, 10]. ChREBP is a member of the basic helix-loop-helix/leucine zipper (bHLH/ZIP) family of transcription factors with Mr = 94,600, and forms heterodimers with the bHLHZip protein Mlx to bind the ChRoE [8, 11]. Target genes of ChREBP are involved in glycolysis (L-PK), the NADPH supply system (glucose-6-phosphate dehydrogenase, transketolase, malic enzyme, etc.), gluconeogenesis (G6Pase), and lipogenesis (ACC, FAS) [10, 12].

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, Iynedjian *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.

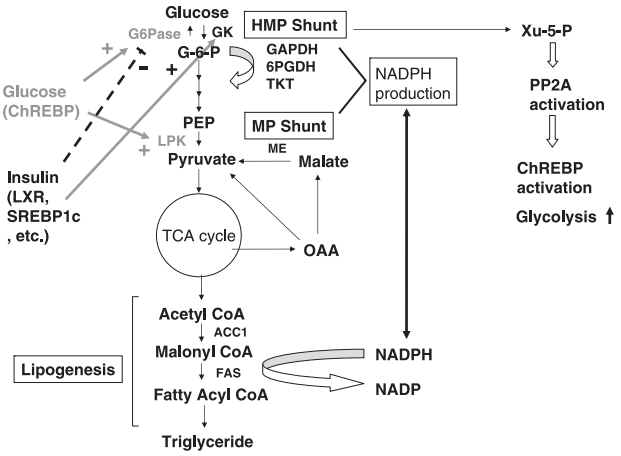

Fig. 2. ChREBP and SREBP-1c regulate different steps in glycolysis and gluconeogenesis.
ChREBP and SREBP share the regulation of lipogenesis and the hexose monophosphate (HMP) and malate-pyruvate (MP) shunts (black and yellow). Glucose (blue) and insulin (red) activate LPK and GK respectively. Glucose also activates G6Pase but insulin inhibits it. G6P, glucose-6-phosphate; GK, glucokinase; G6Pase, glucose-6-phosphatase; PEP, phosphoenol pyruvate; Xu-5-P, xylulose-5-phosphate; ChREBP, carbohydrate response element binding protein; LXR, liver protein X receptor; SREBP1c, sterol regulatory element binding protein 1c; ME, malic enzyme; G6PDH, glucose-6-phosphate dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; LPK, liver-type pyruvate kinase; OAA, oxaloacetate; Tkt, transketolase; FAS, fatty acid synthase; ACC1, acetyl CoA carboxylase.

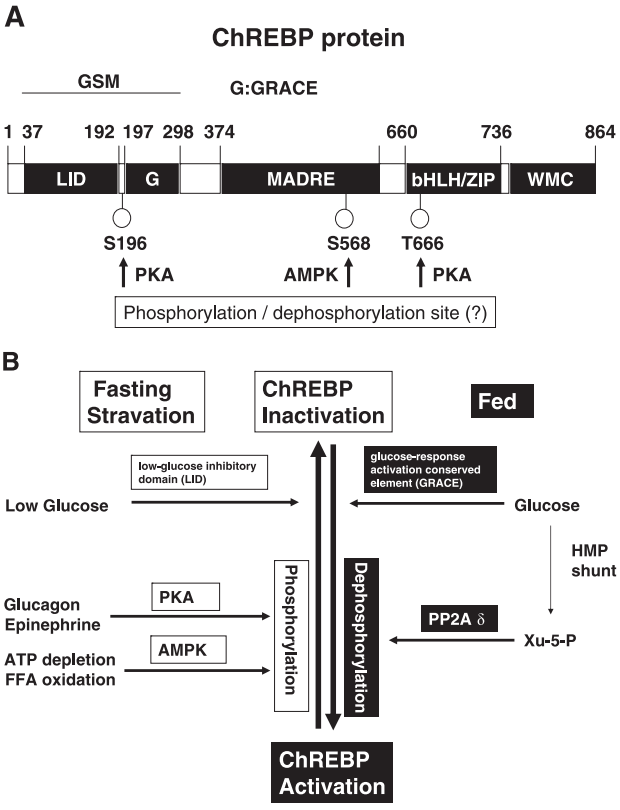

Fig. 3. ChREBP regulation by nutritional state.
(A) ChREBP protein structure. Ser196, Ser568, and Thr666 are putative phosphorylation sites. MADRE, middle activation domain as in RelB; bHLHZip, basic helix-loop-helix leucine zipper domain; WMC, WBSCR14-Mlx C-tail homologous domain; GRACE, glucose response activation conserved element; LID, low-glucose inhibitory domain; GSM, glucose sensing module.
(B) Nutritional conditions determine ChREBP transactivity. PKA, protein kinase A; AMPK, AMP-activated protein kinase; PP2A, protein phosphatase 2A; FFA, free fatty acid; Xu-5-P, xylulose-5-phosphate; HMP, hexose monophosphate; ChREBP, carbohydrate response element binding protein.

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 (PP2Adelta) 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-

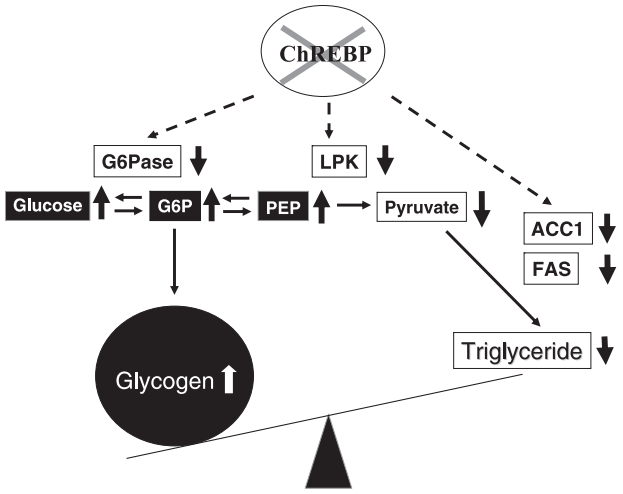

Fig. 4. Deficiency of ChREBP induces glycogen accumulation and decreases triglyceride synthesis in the liver.
ChREBP regulates target genes of glycolysis (L-PK), gluconeogenesis (G6Pase) and lipogenesis (FAS, ACC). Excess glycogen accumulation is due to decreased G6Pase and L-PK enzyme activity. Liver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

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, S14, etc.). However, some groups have reported evidence against such a phosphorylation/dephosphorylation mechanism. Despite lacking phosphorylation sites by PKA, a S196A/T666A mutant of ChREBP retains glucose responsiveness and cAMP-dependent inhibition of ACC promoter transactivity [24]. Nevertheless, the ChREBP protein contains a glucose-sensing module that mediates its glucose responsiveness (Fig. 3A and 3B) [25], so it is clear that PKA and AMPK inhibit ChREBP transactivity and that PP2A activates it. However, the mechanism by which glucose activates the transactivity of ChREBP remains unknown (Fig. 4).

At the transcriptional level, ChREBP also is regulated loosely in several conditions. The level of ChREBP mRNA in liver in the fed state is the same as or twice as high as the level during fasting [26]. Repa *et al.* reported that LXR directly regulates ChREBP gene 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$^{-/-}$

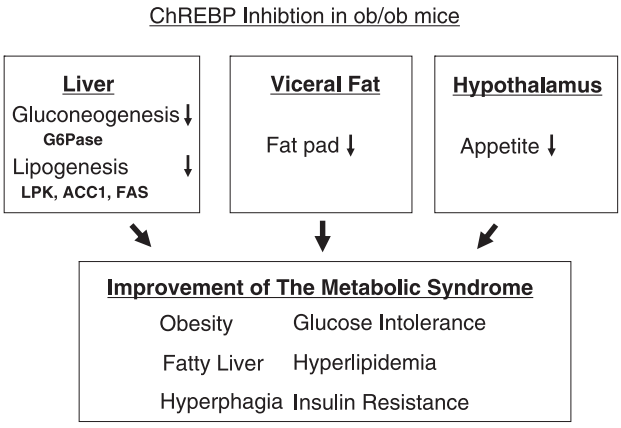

Fig. 5. Deletion of the ChREBP gene improves the metabolic syndrome in ob/ob mice.
In these mice, glycolysis and lipogenesis are increased. Paradoxically, G6Pase activity and gluconeogenesis are increased. When the complete ChREBP gene is deleted, glycolytic genes (LPK) and lipogenic genes (FAS and ACCI) are decreased. Appetite also is decreased in ob/ob ChREBP$^{-/-}$ mice. G6Pase, glucose 6 phosphatase; ChREBP, carbohydrate response element binding protein; LPK, liver-type pyruvate kinase; FAS, fatty acid synthase; ACC1, acetyl CoA carboxylase.

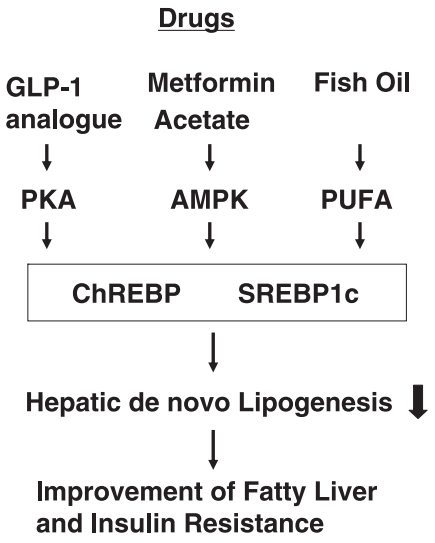

Fig. 6. ChREBP and SREBP1c are potential targets for the treatment of the metabolic syndrome.
PKA, protein kinase A; AMPK, AMP-activated protein kinase; PP2A, protein phosphatase 2A; FFA, free fatty acid; Xu-5-P, xylulose-5-phosphate; HMP, hexose monophosphate; ChREBP, carbohydrate response element binding protein; PUFA, polyunsaturated fatty acids.

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 additon, 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]. Exedine-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 its *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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