Role of ChREBP in hepatic steatosis and insulin resistance

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is emerging as the most common chronic liver disease in the Western countries. NAFLD, which describes a large spectrum of liver histopathological features including simple steatosis, non-alcoholic steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma [1] is associated in the vast majority of the cases, with obesity, insulin resistance and type 2 diabetes. Excessive accumulation of triglycerides (TG) is one of the main characteristics of non-alcoholic fatty liver disease and fatty acids utilized for the synthesis of TG in liver are available from the plasma non-esterified fatty acid pool but also from fatty acids newly synthesized through hepatic de novo lipogenesis. Recently, the transcription factor ChREBP (carbohydrate responsive element binding protein) has emerged as a central determinant of lipid synthesis in liver through its transcriptional control of key genes of the lipogenic pathway, including fatty acid synthase and acetyl CoA carboxylase. In this mini-review, we will focus on the importance of ChREBP in the physiopathology of hepatic steatosis and insulin resistance by discussing the physiological and metabolic consequences of ChREBP knockdown in liver of ob/ob mice.

Keywords: ChREBP; LXR; Hepatic steatosis; Insulin resistance; ob/ob Mice

Abstract Non-alcoholic fatty liver disease is tightly associated with insulin resistance, type 2 diabetes and obesity, but the molecular links between hepatic fat accumulation and insulin resistance are not fully identified. Excessive accumulation of triglycerides (TG) is one of the main characteristics of non-alcoholic fatty liver disease and fatty acids utilized for the synthesis of TG in liver are available from the plasma non-esterified fatty acid pool but also from fatty acids newly synthesized through hepatic de novo lipogenesis. Recently, the transcription factor ChREBP (carbohydrate responsive element binding protein) has emerged as a central determinant of lipid synthesis in liver through its transcriptional control of key genes of the lipogenic pathway, including fatty acid synthase and acetyl CoA carboxylase. In this mini-review, we will focus on the importance of ChREBP in the physiopathology of hepatic steatosis and insulin resistance by discussing the physiological and metabolic consequences of ChREBP knockdown in liver of ob/ob mice. © 2007 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.

but they can also be hydrolyzed and the fatty acids channelled towards the β-oxidation. Although the mechanisms involved in the pathogenesis of NAFLD have not been thoroughly investigated in humans, both increased de novo fatty acid synthesis and decreased β-oxidation in the mitochondria have been implicated in the development of the pathology [3,4].

De novo lipogenesis is nutritionally regulated and both glucose and insulin signaling pathways are elicited in response to dietary carbohydrates to synergistically induce glycolytic and lipogenic gene expression. The transcription factor SREBP-1c (sterol regulatory element binding protein-1c) has previously emerged as a major mediator of insulin action on lipogenic genes, such as acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS) [5] (Fig. 1). However, SREBP-1c activity alone is not sufficient to account for the stimulation of glycolytic and lipogenic gene expression in response to carbohydrate since SREBP-1c gene deletion in mice only results in a 50% reduction in fatty acid synthesis [6]. More importantly, L-pyruvate kinase (L-PK), one of the rate-limiting enzyme of glycolysis is exclusively dependent on glucose [7] and is not subjected to SREBP-1c regulation [8]. Until recently, the nature of the glucose-signaling compound was not known, but the recent identification of a glucose-responsive basic/helix–loop–helix/leucine zipper (bHLH/LZ) transcription factor named ChREBP (carbohydrate responsive element binding protein) has shed light on the mechanism whereby glucose affects gene transcription [9,10]. ChREBP is a large protein (864 amino acids and Mr = 94600) that contains several domains including a nuclear localization signal (NLS) near the N-terminus, proline domains, a basic loop–helix–leucine zipper (bHLH/LZ) and a leucine-zipper-like (Zip-like) domain. Glucose activates ChREBP by regulating its entry from the cytosol into the nucleus [11,12] thereby promoting its binding to carbohydrate responsive element (ChoRE) present in the promoter regions of glycolytic and lipogenic genes [13]. Studies by the Towle laboratory have revealed that ChREBP binds as a heterotetramer, together with its functional partner Mlx (Max like protein) on ChoRE elements [14,15].

ChREBP is also regulated by glucose at the transcriptional level [16]. Interestingly, ChREBP was also recently identified as a direct target of liver X receptors (LXRs) [17] (Fig. 1). LXRs are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily [18]. LXRs play a central role in cholesterol and bile acid metabolisms [19] but are also important regulators of the lipogenic pathway [20] through the transcriptional control of SREBP-1c [21], FAS [22] ACC [23] and stearoyl-CoA desaturase 1 (SCD-1) [24], the
The conversion of glucose into fatty acids through de novo lipogenesis is nutritionally regulated and both glucose and insulin signaling pathways are elicited in response to dietary carbohydrates to synergistically induce glycolytic and lipogenic gene expression. The transcription factor SREBP-1c (Sterol Regulatory Element binding Protein) has emerged as a major mediator of insulin action on lipogenic genes, such as fatty acid synthase (FAS) and acetyl CoA carboxylase (ACC) [5]. The mechanism of the glucose-signaling compound was recently identified as being ChREBP (carbohydrate responsive element binding protein). Glucose activates ChREBP by stimulating its gene expression and by regulating its entry from the cytosol into the nucleus thereby promoting its binding to carbohydrate responsive element (ChoRE) present in the promoter regions of its target genes [10]. ChREBP is required for the induction of L-pyruvate kinase (L-PK), which is exclusively dependant on glucose. Induction of FAS and ACC genes is under the combined action of ChREBP and of SREBP-1c in response to glucose and insulin, respectively. ChREBP is also a direct target of LXRs (Liver X receptors) [17], nuclear receptors implicated in the lipogenic pathway, through the direct transcriptional activation of ACC and FAS but also indirectly via SREBP-1c. Indeed, LXRs are central for the insulin-mediated activation of SREBP1-c. Recently, the observation that glucose binds and activates nuclear LXRs placed LXRs at the center masters of the glucose-signaling pathway [27]. Whether LXRs are implicated in the glucose-induction of ChREBP and other glucose-regulated genes needs to be addressed in a physiological context.

We chose to elucidate the implication of ChREBP in the physiopathology of hepatic steatosis in ob/ob mice. Indeed, ob/ob mice have been extensively studied and represent a naturally occurring model of NAFLD. These mice are leptin-deficient due to a mutation in the ob gene, which encodes leptin and therefore prevents its synthesis [32]. Leptin, a satiety hormone synthesized by the white adipose tissue, inhibits feeding behavior and increases energy expenditure by acting on anorexigenic neurons in the ventral median nucleus of the hypothalamus [33]. In the absence of leptin, ob/ob mice are hyperphagic, inactive and become obese. These mice are also insulin resistant and hyperinsulinemic, with resultant hyperglycemia and hyperlipidemia. Most importantly, ob/ob mice develop spontaneously fatty livers due, in part to an exacerbated glycolytic and lipogenic pathway [34]. The up-regulation of the lipogenic pathway in liver is rather puzzling since ob/ob mice are insulin-resistant as evidenced by the decreased in the insulin-mediated phosphorylation of key effectors of the signaling pathway, including insulin substrate 1 (IRS-1) and phosphatidylinositol-3-kinase (PI3K) [35,36]. However, despite a clear state of hepatic insulin resistance, the expression of key genes of the lipogenic pathway, including FAS and SREBP-1c is markedly elevated in livers of ob/ob mice [37]. The mechanisms involved in this up-regulation are not clear especially considering that genes of the gluconeogenic pathway (phosphoenol pyruvate carboxykinase (PEPCK) and glucose 6 phosphatase (G6Pase)), inhibited by insulin under normal conditions, are up-regulated in liver ob/ob mice, in agreement with their state of insulin resistance [38]. The sustained expression of PEPCK and G6Pase causes an enhanced hepatic glucose production and resultant hyperglycemia in ob/ob mice.

We first established a potential molecular link between ChREBP and hepatic steatosis in ob/ob mice by determining that ChREBP gene expression and nuclear protein content were markedly increased in livers of these mice under both fasted and fed conditions [39]. Under fed conditions, we observed a concomitant increase in nuclear ChREBP and SREBP-1c content supporting the fact that these two transcription factors contribute to the high rates of lipogenesis that leads to hepatic steatosis in ob/ob mice. However, under fasted conditions, only ChREBP content was increased compared to ob/+ controls suggesting that ChREBP by itself may be responsible for the increased rates of lipogenesis measured after a 24 h fast in ob/ob mice [39] (Fig. 2). Interestingly, a recent study reports that increased metabolism via GK, the first and rate-limiting enzyme of the glycolytic pathway is sufficient to induce lipogenic gene expression in liver of streptozotocin-
treated rats, independently of the insulin action via SREBP-1c [40]. In fact, in this rat model, the over-expression of GK re-
stores the expression of ChREBP and rescues lipogenic gene
expression. It should be noted however that this study only re-
ported ChREBP and SREBP-1c expression at mRNA levels
when it is well known that these two transcription factors
are also submitted to important post-traductional regulations
[10,41]. Clearly, the respective roles of ChREBP and
SREBP-1c in controlling the lipogenic pathway needs to be
determined. While the mechanism by which ChREBP expression
is increased in liver of olob mice is unknown it could be
directly caused by chronic exposure to hyperglycemia since
glucose metabolism through hepatic GK is required for the
induction of ChREBP in liver [16,40]. The fact that both GK
expression and glucose metabolism are elevated in liver of
ob/ob mice supports this hypothesis. Another point that should
be taken in consideration concerning the elevated expression of
ChREBP in liver of ob/ob mice is that these mice being leptin-
deficient. Since leptin has been shown to decrease SREBP-1c
expression in various mouse models including, leptin-treated
ob/ob mice, IRS-2 knockout mice and in lipoatrophic mice
[42,43] it would be interesting to determine whether leptin
treatment in ob/ob mice is able to significantly decrease ChRE-
BP expression in liver.

3. Liver-specific inhibition of ChREBP in liver of ob/ob mice

To determine if beneficial effects could result from reduced
hepatic expression of ChREBP, adenoviral-based short-hair-
pin (shRNA) technology was used to knockdown ChREBP
expression in livers of ob/ob mice. Adenoviral delivery of
shRNA in vivo has been proven to be successful for studying
the effects of knocking down genes involved in liver metabo-
lism [44–46] without the potential compensatory effects ob-
served in gene knockout mice. Under both short (2 days)
and long term (7 days) conditions, ChREBP was efficiently de-
creased after shRNA treatment. A resultant decrease in lipo-
genic rates was observed thereby significantly decreasing
hepatic fat accumulation in liver as well as free fatty acid con-
centrations [39] (Fig. 3). In agreement with our previous
in vitro studies [16], inhibition of ChREBP markedly affected

![Graph](image)

Fig. 2. Correlation between ChREBP nuclear levels and lipogenic
rates in liver. Under both fasted and 18 h high-carbohydrate-fed
(HCHO) conditions, a strong correlation was observed between
ChREBP nuclear protein content and the high rates of lipogenesis in
liver of both ob/+ and ob/ob mice supporting the hypothesis that
ChREBP contributes to the development of hepatic steatosis in ob/ob
mice. Adapted from Dentin et al. [39].

![Graph](image)

Fig. 3. The metabolic parameters of the ob/ob are improved after ChREBP knockdown in liver. Adenoviral delivery of shRNA against ChREBP was
successful after 7 days in decreasing hepatic fat accumulation in liver of ob/ob mice as well as decreasing plasma lipid parameters including
triglyceride (TG) and free fatty acid (FFA) concentrations. The beneficial effect of ChREBP knockdown was therefore apparent on overall glucose
tolerance and insulin sensitivity with a significant improvement in both hyperglycemia and hyperinsulinemia. Adapted from Dentin et al. [39].
the expression of L-PK, ACC and FAS in liver of ob/ob mice but also reduced the expression of SCD-1 as well as glyceraldehyde 3-phosphate acyltransferase (GPAT), the enzyme that controls the first step of triglyceride synthesis. Both SCD1 [47] and GPAT [46] have been previously linked to hepatic steatosis in ob/ob mice. Therefore, ChREBP by transcriptionally controlling most of the lipogenic program (ACC, FAS, SCD-1), TG synthesis at the level of GPAT and potentially VLDL export (R. Dentin and C. Postic, unpublished observations) appears as a central modulator of fatty acid concentrations in liver. Altogether our results suggest a broader transcriptional role for ChREBP and micro-RNA analysis could help identify potential novel targets.

Hepatic insulin resistance has been associated with steatosis in both rodents [48] and humans [49]. This resistance could result from the contribution of adipose tissue through increased flux of free fatty acids (FFA) to the liver or by secretion of numerous adipocytokines, which may affect hepatic insulin action. Although the association between hepatic insulin resistance and TG accumulation in liver is clear, the causative role for hepatic insulin resistance in the accumulation of TG has not clearly established. Nevertheless, by markedly preventing TG accumulation in liver of ob/ob mice, ChREBP knockdown significantly restored insulin sensitivity in liver as evidenced by the restoration of protein kinase B (Akt), and Foxo1 phosphorylation by insulin [39]. The transcription factor Foxo1 plays a pivotal role in the control of gluconeogenesis by transcriptionally regulating the expression of PEPCK and G6Pase in liver [50–52]. Insulin-mediated Akt phosphorylation of Foxo1 leads to its nuclear exclusion, ubiquitination and degradation [53]. The subsequent decrease in nuclear Foxo1 decreases expression levels of PEPCK and G6Pase, thereby decreasing gluconeogenic rates and reducing blood glucose. In agreement with these studies, ChREBP knockdown, by rescuing Foxo1 phosphorylation by insulin, led to an efficient inhibition of PEPCK and G6Pase, associated with a subsequent normalization of blood glucose levels in shChREBP RNA treated-ob/ob mice [39] (Fig. 4).

Interestingly, insulin sensitivity was not only restored in liver but also in skeletal muscles and adipose tissue, in which we also observed a significant improvement in Akt phosphorylation in response to the insulin bolus [39]. As a result, glycogen content was restored to control levels in skeletal muscles from shChREBP RNA treated-ob/ob mice. It is important to note that ChREBP knockdown was only targeted to liver of ob/ob mice and that the metabolic improvements observed on both skeletal muscle and adipose tissue were indirect, probably due to the fact that hyperlipidemia (FFA and plasma TG concentrations) were markedly reduced in these mice [39]. The beneficial effect of ChREBP knockdown was therefore apparent on overall glucose tolerance and insulin sensitivity with a significant improvement in hyperlipidemia, hyperglycemia, and hyperinsulinemia (Fig. 3). Altogether, our results show that modulating the expression of a single gene in liver of ob/ob mice significantly lowered circulating lipid and blood glucose concentrations inducing a beneficial interaction between liver, skeletal muscle and adipose tissue. Liver plays a key role in the control of energy balance [54] and affecting gene expression in liver was previously demonstrated to lead to extra-hepatic consequences. For example, enhancing glycolysis at the level of GK in a mouse model of obesity causes a significant increase in energy expenditure in skeletal muscle, through

Fig. 4. Summary of ChREBP knockdown in liver. Consequently to adenovirus-mediated inhibition of ChREBP in liver of ob/ob mice, lipogenesis and triglyceride (TG) synthesis are decreased. As a result, the restored inhibition of genes from the gluconeogenic pathway (G6Pase and PEPCK) by insulin leads to the improvement of blood glucose levels. Correction of hepatic steatosis also leads to decreased levels of plasma TG and non-esterified fatty acids (NEFA). As a consequence, insulin sensitivity is restored in skeletal muscles and glycogen synthesis is enhanced, therefore contributing to the decrease in blood glucose concentrations observed. The overall phenotype is a significant improvement in hyperglycemia, hyperinsulinemia and hyperlipidemia. Adapted from Dentin et al. [39].
effects secondary to lowering plasma insulin and insulin levels [55].

4. Conclusion and perspectives

Clearly, a deficiency in ChREBP ameliorates the phenotype of ob/ob mice and suggests that an inhibition of ChREBP could be a benefit for the treatment of hepatic steatosis and other related diseases (Fig. 4). However the limitation of our studies is the use of ob/ob mice and a model of diet-induced obesity in C57BL/6J mice would be necessary to address the role of ChREBP in hepatic steatosis in the context of normal leptin signaling. Nevertheless, ChREBP appears as a potential therapeutic target and therefore an accurate knowledge of the mechanisms involved in regulating its expression and activation is crucial for the development of pharmacological approaches for the treatment of metabolic diseases. The mechanism responsible for ChREBP activation is thought to involve the dephosphorylation of Serine residue 196 (Ser-196), a target of protein kinase A (PKA), localized near the nuclear localization signal. At low glucose concentrations ChREBP is an inactive phosphorylated cytosolic protein while at high glucose concentrations, ChREBP undergoes dephosphorylation (on Ser-196), and is translocated into the nucleus to activate its target genes. Since it was not clearly demonstrated on the endogenous protein, the regulation of ChREBP by phosphorylation/dephosphorylation remains controversial [56,57]. Future studies should help in the future to clarify the mechanisms involved in regulating ChREBP expression, nucleo-cytoplasmic shuttling and its post-transductional modifications. These studies will be helpful for the development of potential therapeutic approaches for the treatment of diseases characterized by dysregulations of glucose and/or lipid metabolism.

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