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Minireview

Role of ChREBP in hepatic steatosis and insulin resistance

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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 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

1. Introduction

Non-alcoholic 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 the one main characteristics of hepatic steatosis, a pathological pattern that is now considered as a component of the metabolic syndrome [2]. Fatty acids utilized for the synthesis of TG in liver are available from the plasma non-esterified fatty acid pool (NEFA) but also from fatty acids newly synthesized through hepatic de novo lipogenesis. Triglycerides can then be stored as lipid droplets within the hepatocytes or secreted into the blood as VLDL 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 dependant 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, polyproline domains, a basic loop–helix–leucine-zipper (b/HLH/Zip) 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

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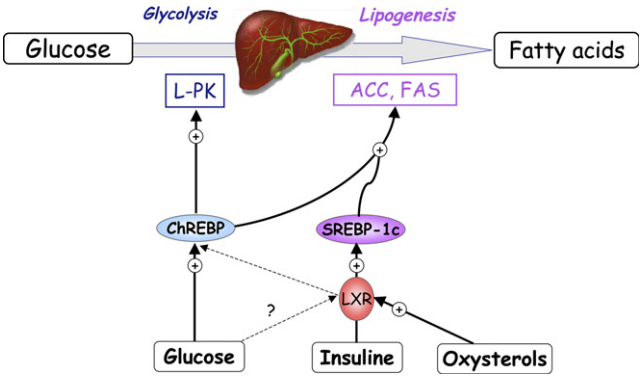

Fig. 1. Transcriptional control of glycolysis and lipogenesis. The conversion of glucose into fatty acids through de novo lipogenesis is nutritionally regulated and both glucose and insulin signaling pathways are elicited in response to dietary carbohydrates to synergistically induce glycolytic and lipogenic 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 nature 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.

enzyme required for the biosynthesis of monounsaturated fats, palmitoleate and oleate from saturated fatty acids [25]. In fact, LXRs are central for the insulin-meditated induction of SREBP-1c [21,26] (Fig. 1). Known ligands of LXRs are oxysterols but, interestingly, glucose was also recently shown to activate LXRs and to induce their target genes, including ChREBP (Fig. 1) [27]. This study directly implicated LXRs as master regulators of the glucose-signaling pathway and challenged the role of previously recognized *glucose-sensors* such as glucokinase (GK), the first enzyme of the glycolytic pathway. Indeed, we have previously shown that hepatic GK is acting as a *glucose-sensor* in liver and is required for the expression of ChREBP as well as the subsequent induction of glycolytic and lipogenic genes. However, as raised by Wilson and Lazar [28] several concerns aroused from the study of Mitro et al. [27] including the fact that both D-glucose and L-glucose (which is inactive in most biological reaction including GK activity) were found to activate LXRs [29] and that the experiments were performed in HepG2 cells, an hepatoma cell line that respond poorly to glucose. Clearly, studies performed in a physiological context will be necessary to help understand the physiological relevance of LXRs as *glucose-sensors* in liver (Fig. 1).

In contrast, the role of ChREBP as a central mediator of the effect of glucose on glycolytic and lipogenic genes is now clearly established. ChREBP is required for the induction of L-PK [11,16] and acts in synergy with SREBP-1c to induce FAS and ACC to glucose and insulin, respectively [30] (Fig. 1). Furthermore, ChREBP silencing in hepatocytes [16] and in mice [31] not only leads to the lack of induction of L-PK, FAS and ACC genes in response to glucose but also causes a significant reduction in lipid synthesis. This mini-review will focus on the recent emergence of ChREBP in the control of the lipogenic pathway and address its role in the physiopathology of hepatic steatosis and insulin resistance in the *ob/ob* mouse model.

2. Use of the genetically obese *ob/ob* mice for the study of hepatic steatosis and insulin resistance

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 susbrate 1 (IRS-1) and phosphatidyl-inositol-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-