The role of the lipogenic pathway in the development of hepatic steatosis

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Abstract

Non-alcoholic fatty liver disease (NAFLD) represents a wide spectrum of diseases, ranging from simple fatty liver (hepatic steatosis) through steatosis with inflammation and necrosis to cirrhosis. NAFLD, which is strongly associated with obesity, insulin resistance and type 2 diabetes, is now well recognized as being part of the metabolic syndrome. The metabolic pathways leading to the development of hepatic steatosis are multiple, including enhanced non-esterified fatty acid release from adipose tissue (lipolysis), increased de novo fatty acids (lipogenesis) and decreased β-oxidation. Recently, several mouse models have helped to clarify the molecular mechanisms leading to the development of hepatic steatosis in the pathogenesis of NAFLD. This review describes the models that have provided evidence implicating lipogenesis in the development and/or prevention of hepatic steatosis.

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Résumé

Rôle de la lipogenèse dans le développement de la stéatose hépatique

Les maladies métaboliques du foie représentent plusieurs syndromes qui vont de la simple stéatose hépatique à la stéatose hépatique inflammatoire (stéatohépatite) pouvant évoluer vers la nécrose et même la cirrhose. La stéatose hépatique est très fortement associée à l’obésité, la résistance à l’insuline et le diabète de type 2. Les voies métaboliques, qui peuvent conduire au stockage excessif de lipides dans le foie (principalement des triglycerides), sont multiples et peuvent être liées à une augmentation exacerbée de la lipolyse adipocytaire, une synthèse accrue de la synthèse de novo des acides gras par la voie de la lipogenèse ainsi qu’à une réduction conjointe de la β-oxydation des acides gras. Au cours des dernières années, des modèles animaux ont permis une meilleure compréhension des mécanismes moléculaires impliqués dans le développement de la stéatose hépatique. Cette revue présente et discute certains des modèles qui ont permis de révéler l’importance de la voie de la lipogenèse dans l’apparition et/ou la prévention de la stéatose hépatique.

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is an increasingly common health concern that is considered to be a component of the metabolic syndrome. Excessive accumulation of triglycerides (TG) in hepatocytes is the hallmark of NAFLD. The spectrum of NAFLD can range from simple fatty liver (hepatic steatosis), with a benign prognosis, to the potentially progressive form of non-alcoholic steatohepatitis (NASH), which can lead to fibrosis and cirrhosis, resulting in increased morbidity and mortality. All features of the metabolic syndrome, including obesity, type 2 diabetes, arterial hypertension and hyperlipidemia (elevated TG levels), are associated with NAFLD/NASH [1,2]. The diagnosis of NAFLD is based clinically on high transaminase levels, a high body mass index (BMI), and ultrasound evidence of fat and features of the metabolic syndrome. Liver biopsies are, however, necessary to determine the presence of NASH and to assess the degree of fibrosis [3]. There is currently no generally accepted treatment.
for NAFLD. To date, the only effective treatments of NAFLD are lifestyle changes (diet, weight reduction and exercise). As NAFLD seems to be caused and worsened by insulin resistance, the most promising agents are drugs that restore insulin sensitivity such as thiazolidinediones (TZDs), a class of oral antidiabetic drugs that improves insulin sensitivity by acting as a selective agonist of the nuclear peroxisome proliferator-activated receptor PPAR-γ. They can reduce hepatic and peripheral insulin resistance, decrease hepatic steatosis and attenuate the inflammatory response [4-6]. TZDs exert insulin-sensitizing actions directly on adipocytes (increase number and differentiation, stimulate glucose uptake) and indirectly via decreased lipolysis and altered release of adipokines. TZDs decrease the secretion of anti-insulin adipokines (TNF-α and resistin), and increase the secretion of insulin-like adipokine (adiponectin) by adipocytes [7]. However, although effective in the treatment of hepatic steatosis, the limitations of TZDs in NAFLD patients are weight gain and increased body adiposity.

2. Metabolic pathways leading to the development of hepatic steatosis

Excessive accumulation of fat in hepatocytes is the earliest response to and the most common feature of NAFLD. However, the origin of the fat (mainly TG) that accumulates is complex and only partially understood. The potential sources of fat contributing to hepatic steatosis include: (i) dietary fatty acids [mainly through the uptake of intestine-derived chylomicron (CM) remnants]; (ii) increased lipolysis of peripheral fats stored in white adipose tissue that flow to the liver as plasma non-esterified fatty acids (NEFA); and (iii) fatty acids newly made within the liver through de novo lipogenesis. After the esterification step (converting fatty acids into TG), TG can then be stored as lipid droplets within hepatocytes or secreted into the blood as very low-density lipoproteins (VLDL), but they can also be hydrolyzed and the fatty acids channeled towards the β-oxidation pathway. Therefore, excessive fat accumulation in the liver can occur as a result of increased fat delivery, increased fat synthesis, reduced fat oxidation and/or reduced fat export in the form of VLDL.

Strong evidence demonstrates that, in NAFLD patients, insulin does not suppress lipolysis to the same extent that it does in healthy individuals [8]. Because insulin has a potent suppressive effect on hormone-sensitive lipase (HSL) [8], studies have examined whether resistance of HSL to insulin in insulin-resistant states is the predominant defect accounting for the increased flux of NEFA from adipose tissue. Studies have revealed that HSL-knockout mice show increased hepatic sensitivity due to reduced plasma NEFA and hepatic TG concentrations [9,10]. Thus, these studies suggest that restricted lipolysis could help to prevent a ‘spillover’ of fat from adipose tissue to the liver and so prevent hepatic steatosis and/or insulin resistance. Using a multiple stable isotope approach, Donnelly et al. [11] estimated that, while 60% of TG accumulated in the liver of NAFLD patients originates from NEFA, a little over 10% comes from the diet and almost 30% from de novo lipogenesis. This study underscores the contribution of de novo fat synthesis to the pathology of NAFLD.

3. Targeting the lipogenic pathway to prevent hepatic steatosis in mice

De novo fat synthesis (lipogenesis) is the metabolic pathway leading to the conversion of an excess of carbohydrates into fatty acids, which are ultimately esterified with glycerol-3-phosphate to form TG. The activity of the lipogenic pathway is strongly dependent upon nutritional conditions, and it is now clearly established that lipogenic enzyme transcription requires both insulin and glucose to be fully induced [12]. Conditions associated with high rates of lipogenesis, such as a low-fat/high-carbohydrate (LF/HC) diet, hyperglycemia and hyperinsulinemia, are associated with a shift in cellular metabolism from lipid oxidation to TG esterification, thereby increasing the availability of liver TG. The enzymes involved in the synthesis of TG in liver include: (i) glucokinase (GK) [13] and L-pyruvate kinase (L-PK) [14] for glycolysis; (ii) ATP citrate lyase [15], acetyl-CoA carboxylase (ACC) [16] and fatty acid synthase (FAS) [17] for lipogenesis, and long-chain elongase (Elovl6; LCE) [18] and stearoyl-CoA desaturase 1 (SCD1) [19], catalyzing fatty acid elongation and desaturation steps; and (iii) mitochondrial glycerol-3-phosphate acyltransferase (GPAT) and diacylglycerol acyltransferase (DGAT) for TG synthesis [20] (Fig. 1).
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 nature of the glucose-signaling compound was recently identified as the transcription factor ChREBP (carbohydrate responsive element-binding protein). Glucose activates ChREBP by stimulating its gene expression and mediating its post-translational modification(s). ChREBP is required for the induction of L-PK, which is exclusively dependent on glucose. Induction of lipogenic genes, such as ACC, FAS, SCD-1, is under the combined actions of ChREBP and SREBP-1c. Transcription factor SREBP-1c also mediates the effect of insulin on GPAT, although the direct action of ChREBP on GPAT gene expression has not been established. As the nuclear receptor LXR is required for insulin action on SREBP-1c expression, insulin must, in some manner, stimulate the production of an endogenous sterol ligand of LXR (oxysterols). ChREBP is also a direct target of LXR when activated by pharmacological agonists such as T0-901317, but LXR is unable to activate ChREBP expression in response to glucose (Adapted from Robichon et al. [54]).

Although rodent models of hepatic steatosis and/or insulin resistance do not always perfectly reproduce the human pathology of NAFDL, the use of transgenic, knockout and knockdown mouse models has helped, over the years, to achieve a better understanding of the molecular determinants of NAFDL [21]. Key enzymes of fatty acid synthesis/desaturation/elongation/esterification such as ACC, SCD1, Elovl6, GPAT and DGAT [22-28] have been shown, when knocked down, to reverse many of the metabolic defects associated with hepatic steatosis and/or insulin resistance, indicating that decreased TG synthesis in liver is a potential and interesting target for the treatment of NAFDL. Among them, SCD1 has emerged as a particularly interesting target for the reversal of hepatic steatosis and insulin resistance [29]. SCD1 is a delta-9 fatty acid desaturase that converts saturated fatty acids (SFA) into monounsaturated fatty acids (MUFA), particularly oleate (C18: 1n-9) and palmitoleate (C16: 1n-7). MUFA are major components of membrane phospholipids, TG and cholesterol esters. SCD1-deficient mice [23] or mice treated with SCD1 antisense nucleotides [24] are protected against diet-induced obesity and insulin resistance when fed a high-carbohydrate/high-fat (HC/HF) diet. The protective effect of SCD1 deficiency is attributed to these mice to a combined decrease in lipogenic rates and activation of the β-oxidation pathway, underlying the metabolic link between these two pathways. Indeed, elevated malonyl-CoA concentrations, the metabolic product of lipogenic ACC, inhibit carnitine palmitoyltransferase 1 (CPT-1), the rate-limiting enzyme of β-oxidation, and regulate the transfer of long-chain acyl-CoAs (LCCoAs) from the cytosol into the mitochondria, thereby resulting in a shift from an oxidative to a reesterification pathway [30]. However, it is not clear how SCD1 deficiency affects and/or regulates lipogenic rates in liver. Liver-specific knockout of SCD1 (LKO mice) also protects against diet-induced obesity and hepatic steatosis [31]. Under both short- and long-term conditions, LKO mice exhibit reduced rates of fatty acid synthesis in liver and decreased expression of key genes of the lipogenic pathway (namely, ACC and FAS). Interestingly, hepatic SCD1 deficiency reduces the nuclear content of two key factors—carbohydrate responsive element-binding protein (ChREBP) and sterol regulatory element-binding protein (SREBP-1c) [31]—involved in the transcriptional control of lipogenic gene expression in response to glucose and insulin, respectively, as discussed below (Fig. 1). However, once again, the mechanism by which SCD1 affects the maturation and/or translocation of these two transcription factors is not clear, but could be linked to MUFA concentrations in hepatocytes. Clearly, a better knowledge of the function and/or regulation of the transcription factors involved in the activity of lipogenic enzymes may, in the future, help in the development of potential therapeutic approaches.

4. Transcriptional control of fat synthesis via SREBP-1c, LXR and ChREBP

Lipogenic gene expression is coordinately controlled by key transcriptional regulators: SREBP-1c in response to insulin; and ChREBP in response to glucose [12,32]. Liver X receptors (LXRs) are ligand-activated transcription factors that belong to the nuclear hormone-receptor superfamily [33]. LXRs play a key role in cholesterol and bile acid metabolism, but are also important regulators of the lipogenic pathway, as LXRs are essential for transcriptional control of SREBP-1c by insulin [34-36]. Direct targets of LXR include FAS and SCD1 [27,37]. ChREBP is regulated by glucose at the transcriptional level [38] and was also recently identified as a direct target of LXRs [39,40]. ChREBP is particularly important for the induction of liver pyruvate kinase (L-PK), which is exclusively dependent on glucose [41]. Induction of lipogenic genes (ACC, FAS, SCD1) is under the concerted action of ChREBP, SREBP-1c and LXRs in response to nutritional signals [12,21,36] (Fig. 1).
So far, the relative importance of these transcriptional factors in controlling the synthesis of fat in response to glucose and insulin signals has been difficult to ascertain because they act either independently and/or synergistically to regulate their target genes. We have recently demonstrated that liver-specific inhibition of ChREBP by decreasing the rate of hepatic lipogenesis improved hepatic steatosis and insulin resistance in obese ob/ob mice [42]. These results suggest that ChREBP is a potential therapeutic target and, therefore, accurate knowledge of the mechanisms involved in regulating its expression and activation is crucial for the development of pharmacological approaches in the treatment of metabolic diseases. The mechanism responsible for ChREBP activation at the post-translational level involves an increase in intracellular glucose metabolism [43]. At low glucose concentrations, ChREBP is an inactive phosphorylated cytosolic protein whereas, at high glucose concentrations, ChREBP undergoes dephosphorylation (on Ser-196), and is translocated into the nucleus to activate its target genes [44]. Because this mechanism has only recently been demonstrated with the endogenous protein, the regulation of ChREBP by phosphorylation/dephosphorylation was controversial [45,46]. However, the use of a phospho-specific antibody that we developed provided, for the first time, a direct correlation between the modulation of Ser-196 phosphorylation and intracellular localization of the endogenous ChREBP protein in liver [40].

5. Is hepatic steatosis always associated with insulin resistance?

As already mentioned in the introduction, the excess accumulation of TG in hepatocytes is the hallmark of NAFLD, which is strongly associated with insulin resistance [2,47]. However, despite the correlation between fatty liver and insulin resistance, it remains unclear whether or not insulin resistance causes the excess accumulation of TG in liver, or whether or not the increase in TG itself or of metabolite intermediates plays a causal role in the development of hepatic or systemic insulin resistance. Recent studies have favored the hypothesis that the accumulation of intrahepatic lipids precedes the state of insulin resistance, although others have shown that hepatic TG per se are not toxic and may, in fact, protect the liver from lipotoxicity by buffering the accumulation of fatty acids [48,49]; this suggests that hepatic steatosis is not necessarily associated with insulin resistance. Indeed, the overexpression of key enzymes of the esterification pathway (such as DGAT2) [50] or blockade of VLDL secretion [51] show a clear dissociation between marked hepatic steatosis and insulin resistance. Recent studies have also shown that the lipid species (length of the carbon chain and/or the degree of saturation) that accumulate in the steatotic liver may not be equally deleterious for hepatic insulin sensitivity [28,31]. Further experiments are needed to better understand how fatty acid composition influences hepatic insulin sensitivity.

6. Conclusion

NAFLD appears to be one of the most frequent causes of liver dysfunction, and its incidence has increased markedly over the years. While the mechanisms involved in the pathogenesis of NAFLD in humans have not been thoroughly investigated, a recent study has reevaluated the contribution of lipogenesis to the development of hepatic steatosis and revealed that the expression of fatty acid metabolism-related genes, such as ACC and FAS, are indeed increased in NAFLD [52] (Fig. 2). Analyses of the expression of lipogenic transcription factors—namely, ChREBP, SREBP-1c, and LXR—have revealed that expression levels of LXR are four times greater in the liver of NAFLD patients than in that of controls and was significantly correlated with SREBP-1c, but not ChREBP, levels [53]. In our opinion, more information on the ChREBP contribution to NAFLD in needed, and additional studies of ChREBP activity (nuclear protein content/phosphorylation levels) are also required.

![Diagram](https://example.com/diagram.png)

**Fig. 2.** Role of the lipogenic pathway in the development of hepatic steatosis. Non-alcoholic fatty liver disease (NAFLD) is one of the most frequent causes of liver dysfunction, and its incidence has increased markedly over the years. While the mechanisms involved in the pathogenesis of NAFLD in humans have not been thoroughly investigated, enhanced activity of the lipogenic pathway very likely contributes to the development of hepatic steatosis in NAFLD. In response to insulin and glucose, sterol regulatory element-binding protein (SREBP)-1c and carbohydrate responsive element-binding protein (ChREBP) are activated, respectively, and induce the expression of lipogenic genes, including ACC, FAS and SCD1. SREBP-1c and ChREBP are also transactivated by the nuclear receptor LXR that regulates the metabolism of cholesterol and fatty acids. More knowledge of the respective roles of these transcription factors in the pathogenesis of NAFLD is now needed.

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