Chapter 12
Non-Alcoholic Fatty Liver Disease and the Metabolic Syndrome

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is a constellation of diseases ranging from benign hepatic steatosis to fibrosing nonalcoholic steatohepatitis (NASH) [1, 2]. The incidence of NAFLD is growing worldwide in parallel to the epidemic rise of obesity and metabolic syndrome [3]. It is currently estimated to be at ~20–25% in the general population, and at ~75–90% in the morbidly obese population; ~3–5% of patients with NAFLD will develop the more progressive form, NASH [4, 5] (Fig. 1). With NASH progressing to cirrhosis and/or hepatocellular carcinoma [6], the disease is projected to become the leading liver disease and cause of liver transplantation due to cirrhosis in western countries [7]. In 1980, Ludwig et al. [8] first described NASH in a small pool of patients with no history of alcoholism. Histologically, these patients exhibited liver macrosteatosis with inflammatory infiltrates, Mallory bodies, fibrosis, and cirrhosis.

The pathogenesis of NASH is not fully elucidated, but a "multiple-hit" hypothesis, previously referred to as the "two-hit hypothesis" has gained traction in recent years [9]. According to this hypothesis, hepatic steatosis develops initially (first hit) (Fig. 2), and predisposes to lipid peroxidation and inflammation, leading to hepatitis, hepatocyte loss by apoptosis, fibrosis and ultimately, cirrhosis. With liver steatosis being mechanistically linked to insulin action, it is natural to associate NAFLD with insulin resistance (Fig. 3). However, this notion remains somehow controversial, largely because of the limited availability of animal models that replicate the human disease. This review will discuss novel mechanisms linking NASH pathogenesis to the Carcino-Embryonic Antigen-related Cell Adhesion Molecule 1 (CEACAM1), a protein that regulates insulin sensitivity by mediating hepatic insulin clearance [10, 11] (Fig. 4), and a negative acute effect of insulin on fatty acid synthase (FAS) activity [12]. CEACAM1 also exerts an anti-inflammatory effect [13].

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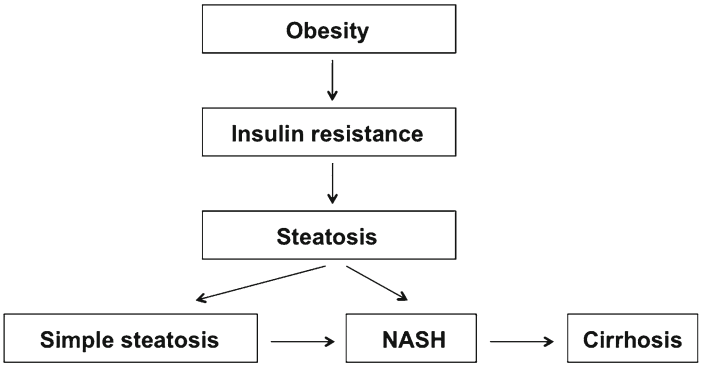

Fig. 1 Natural history of Non-alcoholic fatty liver disease (NAFLD). Obesity is associated with insulin resistance and hepatic steatosis. A subset of patients (~5%) develop nonalcoholic steatohepatitis (NASH), which may progress to cirrhosis

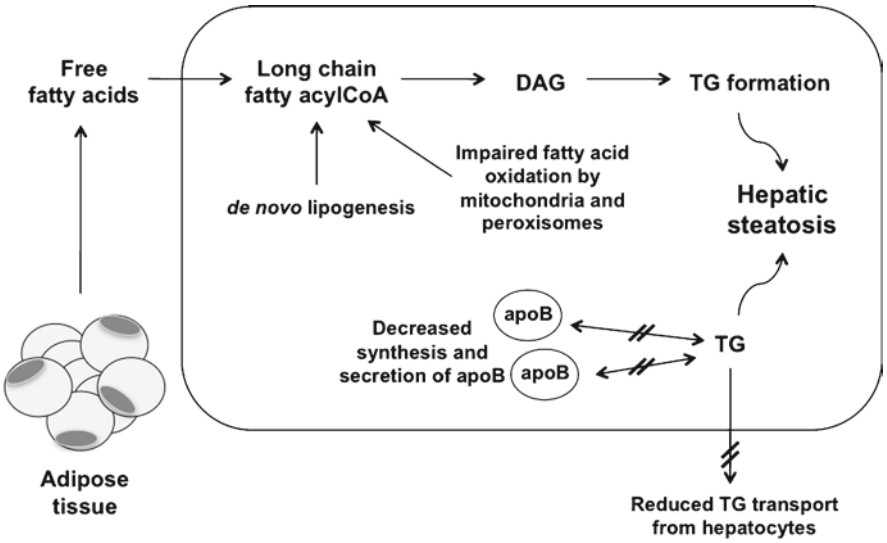

Fig. 2 Pathogenesis of hepatic steatosis. In obese individuals, insulin resistance in adipose tissue increases lipolysis, leading to enhanced hepatic fatty acid influx, generation of LCFACoAs, and triglyceride formation. De novo hepatic lipogenesis is also increased in obesity. On the other hand, hepatic fatty acid oxidation is attenuated in obesity. The result is a net accumulation of hepatic triglycerides, i.e., steatosis

Fig. 4 (continued) binding phosphorylates the receptor at many sites, including Y960 and Y1316 of the β-subunit of the IR. Phosphorylation of Y1316 regulates phosphorylation of CEACAM1 on Y488, causing CEACAM1 binding to an intracellular molecule (X1), which then interacts with phosphorylated Y960 in IR. CEACAM1, through Y513 and/or other proteins targets insulin for degradation

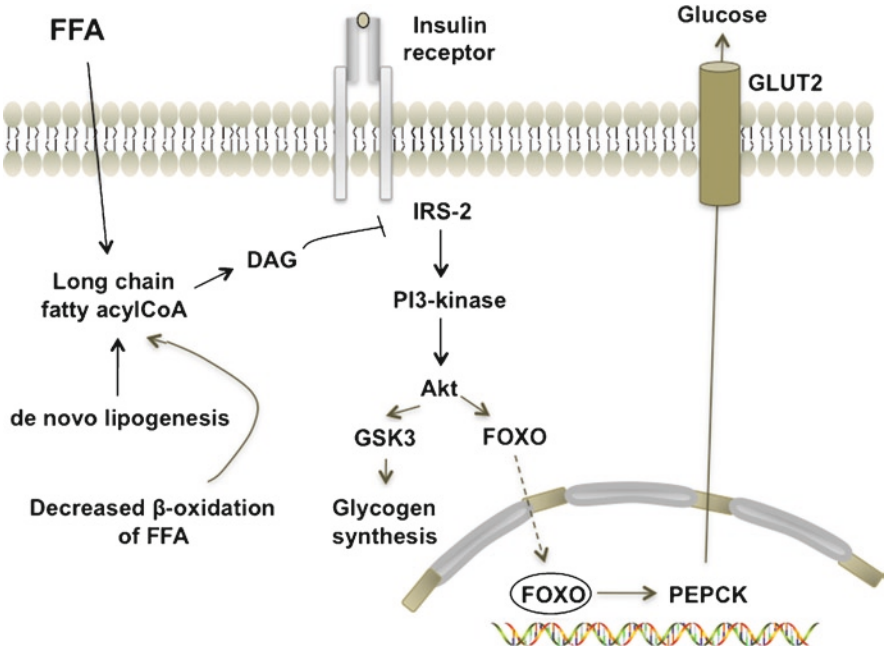

Fig. 3 Hepatic insulin resistance in NAFLD. Diacylglycerol (DAG) content is increased as a result of free fatty acids (FFA) influx from circulation or increased de novo lipogenesis. DAGs activate protein kinase C (PKC), which inhibits the insulin signaling cascade

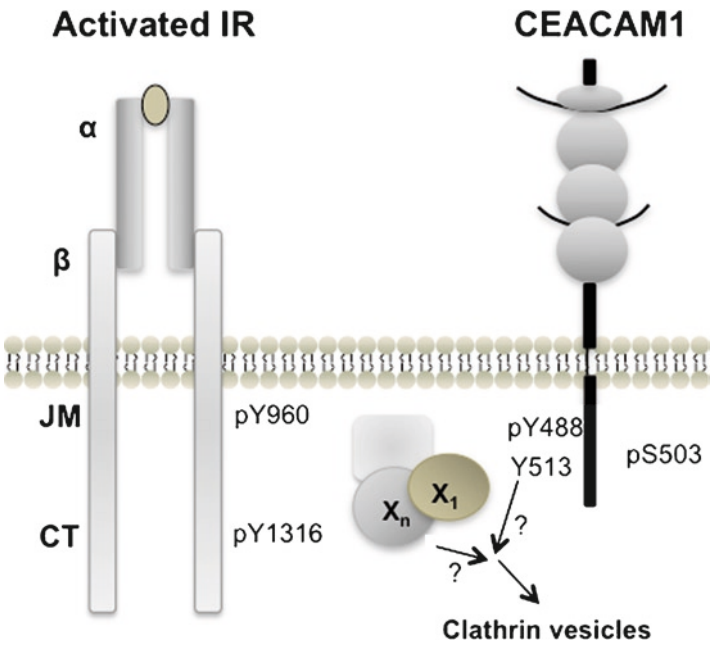

Fig. 4 Model of insulin endocytosis mediated through Carcino-Embryonic Antigen-related Cell Adhesion Molecule (CEACAM). Activation of tyrosine kinase of the insulin receptor (IR) by insulin

NAFLD and the Metabolic Syndrome

As pointed out by Marra et al. [14], NASH can be viewed as the liver manifestation of the metabolic syndrome. Abundant epidemiological evidence indicates that the two conditions are often associated, and that treating the metabolic syndrome improves liver function. The metabolic syndrome is also known as insulin resistance and is characterized clinically by increased body fat content, dyslipidemia, increased blood pressure, and by biochemical features of reduced insulin action, such as fasting hyperinsulinemia, impaired glucose tolerance, and reduced glucose disposal during glucose clamps. It is therefore all the more surprising that no clear-cut molecular mechanism linking hepatic insulin resistance to NAFLD has been identified [15]. This could be due to the fact that insulin affects virtually all the pathways implicated in the pathogenesis of NAFLD, including lipid and glucose metabolism, cellular turnover and survival, production of inflammatory cytokines, and fibrogenesis. The main stumbling block appears to be that insulin affects these processes in different ways: for example, hepatocyte survival is decreased, consistent with resistance to the pro-survival actions of insulin, but lipogenesis is increased, consistent with sensitivity to insulin's lipogenic actions [16–19]. Reconciling these disparate findings is challenging and this could cast doubt about a positive relationship between insulin resistance and NAFLD.

The Intertwined Paths of Insulin and Lipid Metabolism in the Pathogenesis of Hepatic Steatosis

Insulin action is tightly regulated by insulin and fat metabolism in liver. By promoting insulin clearance [10, 11] and mediating a decrease in FAS activity [12], CEACAM1 is well positioned to act as a unifying mechanism for the regulation of insulin action and lipid metabolism in liver.

Insulin resistance is a key factor in the etiology of metabolic diseases, and is commonly associated with hyperinsulinemia. Considerable evidence in humans supports the view that impaired hepatic insulin extraction causes chronic hyperinsulinemia in obesity [20, 21]. Hyperinsulinemia, caused by impaired insulin clearance, worsens insulin resistance by downregulating insulin receptors and escalating de novo lipogenesis, by virtue of activating the nuclear sterol regulatory element-binding protein 1c (SREBP-1c), a master transcriptional regulator of lipogenic enzymes, including FAS [22].

Studies on the role of CEACAM1 in insulin clearance provide more convincing evidence that hyperinsulinemia causes insulin resistance [10]. CEACAM1, a transmembrane glycoprotein in liver, but not muscle or adipose tissue [23], undergoes phosphorylation on tyrosine (Tyr^{488}) by the insulin receptor tyrosine kinase [24]. This requires an intact serine (Ser^{503}) residue. Whereas other substrates of the insulin receptor

mediate insulin action by taking part in the insulin signaling pathways, CEACAM1 regulates insulin action by promoting insulin extraction via endocytotic vesicular insulin uptake and degradation. This finding is buttressed by impaired hepatic insulin clearance and resulting hyperinsulinemia in mice expressing a liver-specific dominant-negative, phosphorylation-defective S503A CEACAM1 mutant (L-SACC1), null mutant mice (*Cc1*$^{-/-}$) [10, 11], and mice with activated SH-containing phosphatase-1 (SHP-1), which dephosphorylates CEACAM1 [25]. Hyperinsulinemic clamp studies reveal that hyperinsulinemia causes secondary insulin resistance in these mice [11, 25, 26]. Ceacam1 mutant mice also develop liver steatosis, resulting from the lipogenic effect of chronic hyperinsulinemia. This increases triglyceride output and redistribution to white adipose tissue, as reflected by increased visceral obesity.

The phenotype of Ceacam1 mutant mice demonstrates a connection between insulin clearance and insulin action in lipid metabolism [27]. It reveals that impaired insulin clearance causes hyperinsulinemia and subsequently, hepatic insulin resistance and increased hepatic de novo fatty acid synthesis. Based on the normal physiology of insulin action, one would predict that insulin resistance would not be associated with increased hepatic triglyceride content [18, 19], as demonstrated by unaltered triglyceride synthesis in the liver-specific insulin receptor knockout mouse (LIRKO) [28]. However, unlike LIRKO, Ceacam1 mutant mice maintain a certain level of insulin receptor signaling. This may explain the peculiar admixture of insulin sensitivity (increased lipogenesis) and resistance (altered glucose homeostasis), observed in these mice. This mixed insulin sensitivity-insulin resistance is the defining feature of NASH [17], and for this reason the Ceacam1 mutant model could be used as a useful tool to investigate the relationship between insulin resistance and hepatic steatosis.

FAS, a key enzyme in the de novo synthesis of fatty acids, is highly expressed in liver and to a lower extent, in white visceral adipose tissue [29]. In contrast to the long-term positive effect of insulin on FAS transcription, we have presented evidence that insulin acutely decreases FAS activity in liver, but not in adipose tissue [12]. The decrease in hepatic FAS activity depends on the ability of insulin to induce CEACAM1 phosphorylation, internalization as part of the insulin endocytosis complex and binding to FAS. We propose that insulin acutely decreases FAS activity to limit lipogenesis and protect the liver against higher levels of insulin in the portal circulation [30]. The negative effect of insulin on FAS activity is abolished in chronic hyperinsulinemia, in light of reduced insulin signaling and CEACAM1 phosphorylation. Together with increase FAS levels as a consequence of activating SREBP-1c by elevated insulin levels, which may result from altered insulin removal in liver, this leads to increased de novo lipogenesis and accumulation of fatty acids in microsomal compartments.

In addition to fatty acid synthesis, CEACAM1 exerts a downregulatory effect on the de novo synthesis of cholesterol [31] and the accumulation of free cholesterol in mitochondria, as suggested by reduced Niemann Pick type C1 (NPC-1) in the liver of L-SACC1 mice [32]. With the latter being an important determinant of glutathione (GSH) level and progression to steatohepatitis [33], this suggests that loss-of-CEACAM1 is at the crossroads of altered insulin metabolism and action and hepatic steatosis, and progression to steatohepatitis.

Pathogenesis of NASH

NAFLD is a multi-faceted disease. The mechanisms underlying the pathogenesis of the progressive form-NASH are not well delineated. The genetic and environmental factors underlying the disease and the progression of fibrosing steatohepatitis have not been fully identified. The "multiple-hit" hypothesis has only slightly promoted our understanding of the pathogenesis of the disease and its progression. Several factors contribute to this limitation. In addition to the mixed insulin sensitivity-insulin resistance in metabolic diseases, the paucity of animal models that replicate adequately all features of the human disease [34, 35] has not helped resolve the issue concerning the relationship between insulin resistance and hepatic steatosis. Few models develop some of the clinical manifestation of the disease, in particular its progressive NASH form [36, 37]. The most common method to induce fibrosing steatohepatitis is the use of a methionine-choline deficient diet in animals. However, this diet does not cause insulin resistance, and NASH patients do not develop methionine or choline deficiency. Mice with liver-specific null deletion of the inositol-phosphatase Pten exhibit severe steatosis while maintaining insulin sensitivity [38]. Transgenic mice with adipose tissue-specific expression of SREBP-1c display marked steatosis and histological changes similar to NASH [39], but they also develop severe insulin resistance. With much dissimilarity with human NASH, these models fail to adequately probe the role of insulin resistance in the disease process.

In contrast, Ceacam1 mutant mice which manifest insulin resistance resulting from hyperinsulinemia, display features of benign NAFLD when fed a regular chow diet, and of the most progressive form -NASH when fed a high-fat diet [32]. Mechanistically, this progression implicates a rise in TNFα secretion from activated resident macrophages and presentation to the increased pool of intrahepatic CD4+ T cells [40]. In addition to infiltrated adipokines from the white adipose tissue, excessive lipid accumulation in the hepatocyte activates liver macrophages [40, 41]. Progression to steatohepatitis involves a Th1 cytokine response, which is characterized by increased release of cytokines from intrahepatic CD4+ T cells [42–44]. With CEACAM1 mediating an anti-inflammatory effect in T cell [13], which depends on SHP-1 activation and on the phosphorylation of immunoreceptor tyrosine-based inhibition motifs (ITIM) within the cytoplasmic domain [45], inactivating Ceacam1 in hepatocytes would limit the CEACAM1-dependent inhibitory responses in T lymphocytes and lead to a robust inflammatory response to cytokines. Thus, progression to steatohepatitis in Ceacam1 mutant mice results from increased accumulation of free cholesterol and hence, reduction in the GSH-defense system against elevated levels of cytotoxic TNFα, which by activating T cells, can lead to a robust inflammatory response especially in light of the loss of the anti-inflammatory function of CEACAM1.

The Ceacam1 mutant mice provide evidence that CEACAM1 protects against NASH in a cell-autonomous fashion through its actions in the hepatocyte, which include prevention of metabolic (insulin resistance and increased triglyceride synthesis) and inflammatory abnormalities (Th1 cytokine response). With its

shared role in all these processes, loss-of-CEACAM1 function provides a unifying underlying mechanism of progressive NAFLD, which results from the culmination of multiple interconnected abnormalities in insulin action, lipid metabolism, and inflammatory response.

Conclusions

NAFLD is becoming an epidemic in parallel to obesity and metabolic syndrome. It is predicted that the incidence of the disease would be higher if better diagnostic means are developed. Identifying the mechanisms linking insulin action with hepatocyte response to oxidative stress, regulation of lipid synthesis, and inflammatory function, can be exploited for diagnosis and treatment of NASH. It can also lead to the development of strategies to prevent the progression from steatosis to steatohepatitis and cirrhosis.

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