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REVIEW

Insulin resistance in development and progression of nonalcoholic fatty liver disease

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Abstract

Although insulin resistance (IR) is strongly associated with nonalcoholic fatty liver disease (NAFLD), the association of IR and NAFLD is not universal and correlation between IR and severity of NAFLD is still controversial. In this review, we summarize recent evidence that partially dissociates insulin resistance from NAFLD. It has also been reported that single-nucleotide polymorphisms in the diacylglycerol acyltransferase gene, rather than IR, account for the variability in liver fat content. Polymorphisms of the patatin-like phospholipase 3 gene have also been reported to be associated with NAFLD without metabolic syndrome, which suggests that genetic conditions that promote the development of fatty changes in the liver may occur independently of IR. Moreover, environmental factors such as nutrition and physical activity as well as small intestinal bacterial overgrowth have been linked to the pathogenesis of NAFLD, although some of the data are conflicting. Therefore, findings from both genetically engineered animal models and humans with genetic conditions, as well as recent studies that have explored the role of environmental factors, have confirmed the view that NAFLD is a polygenic disease process caused by both genetic and environmental factors. Therefore, IR is not the sole predictor of the pathogenesis of NAFLD.

Key words: Nonalcoholic fatty liver disease; Insulin resistance; Metabolic syndrome; Diabetes; Nonalcoholic steatohepatitis

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Core tip: Insulin resistance is considered as the major contributor for the development and progression of nonalcoholic fatty liver disease (NAFLD). However, recent evidence that has shown that non-obese individuals from developing countries are also affected by NAFLD, thus the conventional paradigm of NAFLD as the "hepatic manifestation of metabolic syndrome" has become outdated. Recent studies have highlighted novel pathophysiological mechanisms for the development and progression of NAFLD. Insulin resistance contributes to the disease process, but it is evident that environmental and genetic factors also contribute for development of necroinflammation and subsequent

progression to fibrosis. This review provides a summary of current knowledge of the pathogenesis of NAFLD and discusses factors that dissociate insulin resistance from NAFLD.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is an emerging public health problem[1] due to increasing prevalence in developed and developing countries. NAFLD is the second leading cause of chronic liver diseases after hepatitis C in Western countries and affects individuals of all age groups[2]. NAFLD includes a wide spectrum of conditions that range from a simple steatosis to nonalcoholic steatohepatitis (NASH) which may further progress to cirrhosis and its complications, in absence of alcohol consumption; or a low daily consumption of alcohol (< 30 g/d for men, < 20 g/d for women)[3-5]. NAFLD has been linked to insulin resistance (IR) and other components of metabolic syndrome such as diabetes mellitus, central abdominal obesity and dyslipidemia[6]. Patients with NAFLD are at an increased risk for all-cause mortality, including liver-related deaths and non-liver-related deaths such as death due to cardiovascular disease and diabetes[7].

Recent evidence that has shown that non-obese individuals from developing countries are also affected by NAFLD; thus, the conventional paradigm of NAFLD as the "hepatic manifestation of metabolic syndrome" has become outdated[8]. Recent studies have highlighted novel pathophysiological mechanisms in the development and progression of NAFLD. IR contributes to the disease process, but it is evident that environmental and genetic factors also have the contribution in the development of necroinflammation and subsequent fibrosis. The dogma of a sequential progression of simple steatosis to NASH to cirrhosis in NAFLD is currently under scrutiny.

The pathogenesis of NAFLD is now conceptualized as a complex and multifaceted process that requires further understanding. This review provides a summary of our current understanding of these processes, particularly the evidence that IR is not the lone predictor for NAFLD, but rather, the disease is multifactorial and may be caused by the involvement of genetic and environmental factors.

RESEARCH

We searched MEDLINE, EMBASE, and PubMed using the MeSH terms "insulin resistance", "nonalcoholic fatty liver disease", and "nonalcoholic steatohepatitis". The reference lists of the articles selected for inclusion were also reviewed for additional relevant papers. The search was limited to studies that were reported in the English language and that were published between 1995 and March 2015. Articles that are specifically related to the epidemiology, diagnosis and current treatment strategies for NAFLD and NASH are summarized.

Burden of NAFLD

The reported prevalence of NAFLD from Western countries is 20%-30%, from Asian countries is approximately 15%[9-11]. In normal-weight individuals without any known metabolic risk factors, the prevalence of NAFLD is reported to be approximately 16%. However, the prevalence is much higher among high-risk groups such as diabetics (60%), patients with hyperlipidemia (90%) and obese patients undergoing bariatric surgery (91%)[9-13]. Only 20% of patients under the age of 20 have NAFLD, but among patients aged 60 and above, the prevalence is more than 40%[14]. This findings further strengthened in another study where older age is identified as an independent risk factor for disease progression from simple steatosis to NASH and for the development of fibrosis and cirrhosis[15]. Hamabe *et al*[16] showed that smoking is an independent risk factor for NAFLD. A few studies have also reported ethnic variation in the prevalence of NAFLD, but these reports present contrasting data[17,18]. The risk of mortality is higher in NASH and advanced fibrosis compared with simple steatosis[19]. The progression to advanced fibrosis has been shown to be associated with the patient's age and the degree of inflammation[20]. In a long-term longitudanal study of 129 patients with NAFLD, Ekstedt *et al*[19] explored that mortality was not increased in patients with simple steatosis but was increased in NASH patient. Although the mortality was primarily due to cardiovascular disease, liver-related deaths were more common in patients with NASH-related cirrhosis[21].

Pathogenesis

Traditional concept: The two-hit hypothesis: Day *et al*[22] first proposed the current concept of the "two-hit hypothesis in NAFLD" in 1998 (Figure 1). The first hit is primarily as a result of IR, increased dietary intake and enhanced hepatic lipogenesis there is accumulation of free fatty acids (FFAs) and triglycerides (TGs) in hepatocytes[22]. The second hits is a combination of oxidative stress, lipid peroxidation, mitochondrial dysfunction and the release of inflammatory mediators, which leads to progressive liver injury which constitute steatohepatitis and fibrosis[22]. The activation of proinflammatory pathways and toll-like receptors merge at the junction of two main intracellular signaling pathways known as nuclear factor-κB (NF-κB) and c-Jun N-terminal kinase (JNK)[23,24]. NF-κB activation has been reported in NASH and can lead to increased transcription of many proinflammatory genes, whereas JNK activation causes IR *via* the direct phosphorylation and degradation of insulin receptor substrate 1 (IRS1); this in turn reduces the intracellular signaling pathway activity downstream of the insulin receptor[23]. Lipid peroxidation can promote

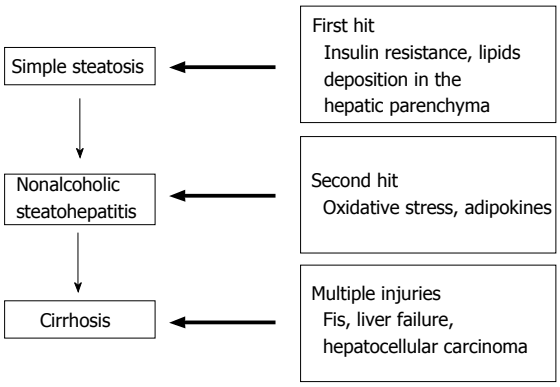

Figure 1 Two-hit hypothesis of nonalcoholic fatty liver disease (traditional view).

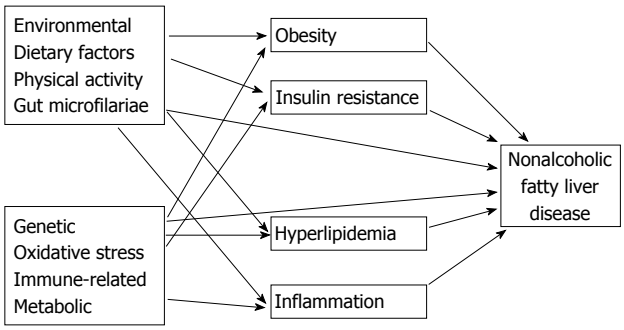

Figure 2 Interplay among environmental and genetic factors in the development of nonalcoholic fatty liver disease.

the proliferation of stellate cells, which contributes to fibrogenesis[25]. Reactive oxygen species induce the release of cytokines from hepatocytes, which leads to the initiation of various immune-mediated mechanisms that contribute to further liver cell injury. The combination of hyperinsulinemia, hepatic iron and lipid peroxidation induces oxidative stress[17], which can cause mitochondrial dysfunction in NASH and can contribute to TG accumulation and eventually to cell necrosis[11].

Multiple-hit pathogenesis

Accumulations of knowledge in recent years have challenged the traditional "two-hit" pathogenesis. Knowledge of interaction between insulin resistance, adipokines, adipose tissue inflammation and other less recognized pathogenic factors has been argued that multiple hits from adipose tissue and the gut occur at the same time and promote liver inflammation (Figure 2). This process suggests that cellular inflammation and insulin resistance occur concurrently[26,27]. Progression of NAFLD to NASH is explained by subsequent "two-hit" theory. In the "multiple-hit" model[28,29] hepatic steatosis may represent an epiphenomenon of several distinct injurious mechanisms including IR rather than a true "first hit"[30]. Hyperinsulinemia, results in increased hepatic *de novo* lipogenesis and increased adipose tissue lipolysis; leads to an increased efflux of free fatty acids to the liver[31,32]. After the initial development of steatosis, the liver becomes extremely vulnerable. Multipe series of pathogenic and injurious factors including oxidative damage, activation of transforming growth factor-beta pathway, dysregulation of multiple adipokines and apoptpsis and activation of hepatic stellate cell may lead to hepatocyte injury and finally to the progression from simple steatosis to NASH and fibrosis[33]. So multiple factors ineract in the complicated ways for development and progression of steatosis, NASH and fibrosis[14,34,35].

Distinct-hit hypothesis

A more recent model has proposed that the development of simple steatosis and NASH follows distinct pathways. The activation of these pathways is a complex process and is not only the result of a simple hepatic insult. Many other factors promote the activation of the pathways that lead to the development of steatosis and NASH[34]. The most important factors include genetic factors, the activation the hedgehog pathway and hepatic progenitor cells[36].

Role of IR in NAFLD

Studies have demonstrated that NAFLD is associated with higher IR compared with controls, even after the exclusion of overweight and obese subjects, and that IR increases with increasing degrees of steatosis[37-40]. IR in NAFLD is predominantly peripheral and occurs in the skeletal muscle and adipose tissue. Peripheral IR in the skeletal muscle causes reduced glucose uptake, which leads to hyperglycemia. In adipose tissue, IR impairs the anti-lipolytic action of insulin, which leads to an increased release of FFA. Elevated plasma concentrations of insulin, glucose, and fatty acids then impair the β-oxidation of fatty acids by negative feedback and promote the uptake of hepatic fatty acids and triglycerides, *de novo* lipid synthesis (*via* SREBP) (sterol-regulatory element-binding protein) and the expression of C/enhancer-binding protein (CCAAT/EBP). Insulin resistance also increases the amount of intra-hepatocytic fatty acids *via* an increase in glycolysis and a decrease in apolipoprotein B-100, which blocks the export of VLDL. The development of IR in NAFLD is most likely related to the imbalance between pro-insulin (adiponectin) and anti-insulin (TNFα) cytokines, specially, those secreted by adipose tissue. Alterations in several molecules, including FFAs, TNFα, membrane glycoprotein PC-1, and leptin, interfere with the insulin signaling pathway. FFAs are both the result and cause of IR. Excess FFAs cause hepatic IR *via* the down regulation of IRS1 signaling and by the activation of the inhibitor kappa B kinase (IKK-B)/NF-κB pathway. Patients with NAFLD have increased insulin resistance not only in muscle but also in liver and adipose tissue[41], and this reduced insulin sensitivity plays a major role in the pathogenesis of NAFLD. This IR, increases peripheral lipolysis in adipose tissue that leads to increase in the delivery of FFAs to the liver and *de novo* lipogenesis[17,35]. In addition, lipid overload in pancreatic-B cells leads to dysregulated insulin secretion and changes in the expression of peroxisome proliferator-activated receptor(PPAR)-α, glucokinase, the glucose

transporter-2, pre-pro-insulin and pancreatic duodenal homeobox-1, which can lead to IR as a result of FFA-induced B-cell apoptosis[12]. It has been suggested that IR in the liver is sufficient to produce dyslipidemia and increase the risk of atherosclerosis[42]. However, current evidences are not sufficient to demonstrate a consistent association between any particular type of adipokine and the histological severity of NAFLD[43].

IR IS THE CAUSE OR A CONSEQUENCE?

Although the development and progression of NAFLD is strongly associted with metabolic syndrome and IR, several studies have evidenced that all obese and diabetic dividuals dont have NAFLD. There are also evideces that NAFLD can occur in nonobese, as well as persons without metabolic syndrome[44]. Therefore, it could be hypothesized that factors other than IR could be the determinant of the development and severity of NAFLD. Familial lustering[45,46] and in the ethnic variation in the prevalence of NAFLD strengthen the initial concept[17]. Single-nucleotide polymorphisms in the adiponectin, interleukin-6, *TNFα* and *apoE* genes has been studied[47-49]. Multiethnic genome-wide association study with NAFLD revealed that the patatin-like phospholipase domain containing protein 3 (also known as adiponutrin) gene is strongly associated with hepatic TG content[50]. Allelic variants of the patatin-like phospholipase domain containing protein 3 (*PNPLA3*) genes have been found to be correlated with amounts of hepatic fat in Hispanics and African-Americans, and to be associted with prevalence of NAFLD. *PNPLA3* has also been independently identified in a separate population-based genome-wide study that influences the alanine aminotransferase (ALT) livel[51]. Environmental factors like; sedentary life styles, excess food intake, constituents of food and intestinal bacterial overgrowth have evidences to contribute in the pathogenesis of NAFLD. Obesity resulting from excess food intake and lack of exercise has been proven to contribute to the progression of fibrosis in patients with NAFLD[19]. An increased consumption of meat, soft drinks, saturated fat and cholesterol and a low consumption of fish and polyunsaturated fat (PUFA) were found to be associated with NAFLD[52-55]. Dietary supplementation with PUFA has been demonstrated in randomized control trial to be benifical in regression of fatty liver and reduction of ALT compared to dietery advice alone[56,57]. On the other hand highcarbohydrate and lowfat diets are associated with more progressive disease[58,59]. Conversely, studies in mice[60] and non-human primates[61], exposure to a maternal high-fat diet associated with development and progression of NAFLD in the offspring. Small intestinal bacterial overgrowth increases gut permeability, which leads to portal endotoxemia and increased numbers of circulating inflammatory cytokines, both of which have crucial role in the progression of NAFLD to NASH[62]. Several studies have reported an association between small intestinal bacterial overgrowth and the progression of NAFLD[63-65]. Dietary supplimentatation of probiotics and treatment with antibiotics resulted in benificial effects in NAFLD, which has furter sterngthen the concept[65].

FROM SIMPLE STEATOSIS TO NASH

Linear progression vs different entity

Although simple steatosis and NASH are currently classified as two histological subtypes of NAFLD, the two conditions are likely distinct from both a histological and a pathophysiological standpoint[34]. The American Association for the Study of Liver Diseases has recently suggested the classification of patients within the NAFLD spectrum into two main categories: NASH and "not steatohepatitis, with steatosis" ("simple steatosis")[66]. Differentiation is on histological variation where NASH is defined by the findings of lobular inflammation, portal inflammation, cellular ballooning, and fibrosis. In contrast, "not steatohepatitis, with steatosis" is characterized by simple fat infiltration with minimal/no inflammation[66]. NASH is a progressive disease, may progress to cirrhosis upto 9%-20% over a period of 5-10 years[67-69]. Vernon *et al*[9] explored that, only NASH is progressive and associated with the development of cirrhosis and hepatocellular carcinoma. In contrast, "simple steatosis" tends to be stable over time[69]. Though there is recent study of progression of steatosis to NASH and also there is progression to fibrosis[70], In agreement with these findings, Musso *et al*[71] in a meta-analysis concluded that a minority of patients with pure fatty liver will progress to NASH and only NASH seems to be associated with an increased risk of progressive liver disease[71]. Along these lines, a community based study of NAFLD outcomes has shown that no patients with simple steatosis died during a 7.6-year follow-up, whereas 35% of patients with NASH died during[69]. All these results established that NASH and "not steatohepatitis, with steatosis" are two distinct entities rather than a real progression of histological changes that can progress over time. For this reason, simple steatosis and NASH should be considered as a separate disease entity that develops along a distinct pathogenic pathway with multiple hits. The conceptualisation of these pathophysiological mechanisms would not only improve our biological understanding of NAFLD but may also allow clinicians to interven the patogenesis more accurately in future.

TREATMENT OF NAFLD

Considering that IR is a primary factor in the pathogenesis of NAFLD, several insulin sensitizers have been used in different settings. Table 1 summarizes a few of these trials. According to these trials, none of these drugs was effective, and thus further studies are warranted to identify their role. Notably, metformin was shown to improve liver injury, but this medication, which is typically used in the treatment of type 2 diabetes, could not prevent fibrosis in patients with steatosis[72]. Additionally, glitazones, which are PPARγ agonists, were found to be efficient in the management

Table 1 Insulin-sensitizing agents and anti-diabetic drug trials for halting nonalcoholic fatty liver disease progression

Insulin-sensitizing agent	Results of the study	Relevance to NAFLD	Ref.
Metformin	Improvements in liver histology and ALT levels in 30% of patients with NASH	Appears to be beneficial for NAFLD patients but not for non-obese patients with early-stage NAFLD	Loomba *et al*[72]
Pioglitazone	Improvement in the biochemical and histological features of NASH	Could be used as a treatment for NAFLD	Promrat *et al*[73]
Pioglitazone	Improvement in insulin resistance but not in hepatic fibrosis and ALT levels	Not adapted to treat NAFLD	Sanyal *et al*[74]

NASH: Nonalcoholic steatohepatitis; NAFLD: Nonalcoholic fatty liver disease; ATL: Alanine aminotransferase.

of NAFLD *via* a notable decrease in liver fibrosis[73]. In contrast, another study revealed that pioglitazone does not promote beneficial effects with respect to liver fibrosis, but it diminished inflammation and steatosis[74]. Therefore, further studies are required to elucidate these contradictory results. Additionally, salsalate, a potential anti-diabetic drug that is currently under development, has been shown to improve glycemia in diabetic patients through a downregulation of the proinflammatory IKKβ/NFκB pathway[75]. Additionally, this agent likely improves NAFLD through an induction of adiponectin[76].

CONCLUSION

Hepatic steatosis is recognized to be the consequence of a complex interplay among diet, environment and liver and adipose tissues, although a comprehensive understanding of pathogenesis of NAFLD has not yet been complete. Therefore, NAFLD is currently perceived as multifactorial pathogenic disease with both genetic and environmental factors. Genome-wide association studies have identified specific genetic associations that are involved in NAFLD. From a therapeutic point of view, pathogenic-based interventions aimed at the reversal of NAFLD are likely to be a rational approach to the prevention and treatment of hepatic IR, metabolic syndrome and related complications. Further studies are required to explore the relationship among adiponutrin mutations, steatosis and IR. A better understanding of the different factors involved in the pathophysiology of NAFLD will open the opportunity to intervene its progression in future.

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