REVIEW

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Non-parenchymal hepatic cell lipotoxicity and the coordinated progression of non-alcoholic fatty liver disease and atherosclerosis

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Purpose of review
Non-alcoholic fatty liver disease (NAFLD) appears to be independently associated with the development of atherosclerosis. The biological mechanisms underlying this association are complex, and likely involve liver-resident cell types other than hepatocytes. Thus, we review recent evidence that non-parenchymal hepatic cell responses to lipid excess contribute to the pathogenesis of both NAFLD and atherosclerosis.

Recent findings
Significant independent associations between NAFLD and atherosclerosis have been identified through cross-sectional studies and meta-analyses. Mechanistic studies in cell cultures and in rodent models suggest that liver-resident macrophages, activated hepatic stellate cells (HSC) and liver sinusoidal endothelial cells (LSEC) mount lipotoxic responses under NAFLD conditions which can contribute to the progression of both NAFLD and atherosclerosis.

Summary
Non-parenchymal hepatic cell types exhibit some similarity in their responses to lipid excess, and in their pathogenic mechanisms, which likely contribute to the coordinated progression of NAFLD and atherosclerosis. In response to lipotoxic conditions, macrophages, Kupffer cells and HSC initiate robust inflammatory responses, whereas LSEC generate excess reactive oxygen species (ROS). The extent to which inflammatory cytokines and ROS produced by non-parenchymal cells contribute to the progression of both NAFLD and atherosclerosis warrants further investigation.

Keywords
fatty acid, inflammation, macrophage, reactive oxygen species, sinusoidal endothelial cell, stellate cell

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) and atherosclerosis are well recognized as comorbid conditions, particularly in individuals with metabolic syndrome. Accumulating clinical evidence suggests that NAFLD, in fact, contributes to the development of atherosclerosis. A recent meta-analysis of 26 studies (total of 85 395 participants) determined a significant independent association between NAFLD and subclinical atherosclerosis, identified by carotid artery intima-media thickness, arterial stiffness, coronary artery calcification and endothelial dysfunction [1▪▪]. Further independent association between NAFLD and noncalcified coronary artery plaques was provided through a cross-sectional study of 5121 individuals with no prior history of coronary artery disease [2▪▪]. The biological mechanisms responsible for this association are complex, involving hepatic insulin resistance, altered hepatocyte lipoprotein metabolism and dyslipidemia, and chronic hepatocyte inflammation – all in response to hepatic exposure to high concentrations of fatty acids. Adding to this complexity is the likelihood that liver-resident cell types other than hepatocytes, including stellate cells, macrophages and sinusoidal endothelial cells, which are similarly exposed to

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KEY POINTS

- Clinical studies have identified significant independent associations between NAFLD and early atherosclerosis.
- Liver steatosis exposes non-parenchymal cell types, including macrophages, HSC and LSEC to chronic lipid excess.
- The lipotoxic response in macrophages and HSC involves generation of proinflammatory factors, whereas in LSEC it involves generation of ROS.
- Further investigation is required to determine the extent to which non-parenchymal cell-derived inflammatory cytokines and ROS contribute to concomitant NAFLD and atherosclerosis progression.

excess lipid during NAFLD, also contribute to plaque development. Here, we discuss recent evidence implicating non-parenchymal hepatic cell responses to lipid excess in the progression of NAFLD and in the concomitant creation of a proatherogenic environment.

HEPATIC MACROPHAGES AND KUPFFER CELLS

NAFLD progression is partly a consequence of lipotoxicity, which we define as fatty acid-induced cell stress. This process occurs when fatty acid uptake (particularly saturated species) and *de novo* synthesis exceed the ability of cells to oxidize, export or store them safely as triglycerides. The conditions and processes involved in hepatocyte lipotoxicity are quite well understood, and have been reviewed extensively in recent years [3–5]. Hepatocyte fatty acid excess causes endoplasmic reticulum and oxidative stress, triggering response pathways that lead to impaired insulin signaling, inflammation and apoptosis, which promote disease progression from benign steatosis to nonalcoholic steatohepatitis (NASH).

Upon injury, hepatocytes release the chemokine CCL2 into the circulation, which elicits the recruitment of monocytes to the liver through activation of CC chemokine receptor 2 (CCR2). The contribution of this axis to NAFLD pathogenesis is supported by the finding that NAFLD patients exhibited increased hepatic and serum CCL2 concentrations, the latter of which was associated with increased severity of hepatic inflammation [6▪]. Furthermore, in mouse models of diet-induced steatohepatitis, pharmacological inhibition of CCR2 decreased hepatic accumulation of monocytes [6▪] and monocyte-derived macrophages [7]. Lipotoxic hepatocytes have also been shown to release extracellular vesicles containing the macrophage chemokine CXCL10, in a JNK-dependent and mixed lineage kinase 3 (MLK3)-dependent manner, thereby inducing macrophage chemotaxis [8]. Moreover, pharmacological inhibition of MLK3 reduced macrophage chemotaxis *in vitro,* and decreased serum CXCL10 and hepatic macrophage infiltration in mice with diet-induced steatohepatitis [9]. Another mechanism implicated in the development of steatohepatitis is hepatocyte pyroptosis, a form of programmed necrosis. Specifically, the pyroptosis protein Gasdermin D (GSDMD) was observed to be elevated in livers of NAFLD and NASH patients. In mice with diet-induced steatohepatitis, genetic ablation of GSDMD decreased hepatic interleukin-1β (IL-1β), tumor necrosis factor α (TNFα) and CCL2, as well as hepatic infiltration of macrophages [10]. Together, these findings denote hepatocellular injury as a key stimulus for the recruitment of inflammatory cells in the progression of NAFLD to steatohepatitis.

Upon recruitment to the liver, myeloid cells infiltrate hepatic tissue through cell–cell adhesion to liver sinusoidal endothelial cells (LSEC). In obese mice, LSEC showed increased expression of cell adhesion molecules, such as VCAM1 and ICAM1, and monocytes extracted from these mice exhibited increased adhesion to LSEC *in vitro* [11]. This suggests that lipid excess increases cell–cell adhesion, which promotes hepatic infiltration of myeloid cells. Within the fat-laden liver, monocyte-derived macrophages and Kupffer cells are exposed to various lipid species, which can have distinct effects on macrophage phenotype. Kupffer cells exposed to palmitate *in vitro* were polarized to a proinflammatory M1 phenotype, characterized by increased *TNFα* and *IL-6* expression, whereas exposure to polyunsaturated fatty acids elicited an anti-inflammatory M2 profile, shown by elevated expression of *MRC2* and *IL-10* [12]. Together with the knowledge that NASH patients exhibit increased hepatic palmitate content [13], these findings suggest that accumulation of palmitate is critical to hepatic inflammation. Consistent with this, exposure of macrophages to excess palmitate *in vitro* caused intracellular accumulation of palmitate crystals, resulting in lysosomal dysfunction, and subsequent NLRP3 inflammasome activation and IL-1β release [14]. Moreover, hepatic macrophages exposed to cholesterol crystals alone [15▪] or derived from lipid-laden hepatocytes [16] exhibited NLRP3 activation and IL-1β secretion. The involvement of the NLRP3 inflammasome in liver inflammation has been further corroborated by evidence that inhibition of NLRP3 in genetically and diet-induced obese mice with steatohepatitis reduced plasma IL-1β,

CCL2 and IL-6, and reversed hepatic inflammation and fibrosis [15▪].

In lipotoxic conditions characteristic of NAFLD, hepatic macrophages secrete various proinflammatory cytokines, such as IL-1β, IL-6 and TNFα, all of which may enter the circulation and have been directly implicated in the progression of atherosclerosis [17]. However, there may be additional mechanisms through which hepatic inflammatory cells contribute to atherogenesis. Exposure of hepatocytes to TNFα was reported to induce the expression of PCSK9 [18]. Given that PCSK9 is a regulator of hepatic LDL clearance and plasma LDL-C levels, this suggests that hepatic TNFα may disrupt LDL metabolism and contribute to the elevated LDL-C observed in atherosclerosis. A recent study also showed that TNFα increased apoB secretion in mouse hepatocytes *in vitro* and promoted hepatic VLDL secretion in mice [19], indicating a link between hepatic TNFα and elevated plasma triglycerides. Moreover, Kupffer cells were identified as the predominant source of plasma CETP, a protein which mediates the exchange of cholesteryl esters for triglycerides between lipoproteins and is associated with dyslipidemia [20]. In obesity-associated NAFLD, hepatic Kupffer cell content and plasma CETP are increased, concomitant with atherogenic dyslipidemia characterized by small dense LDL, elevated triglycerides and reduced HDL [20]. These findings suggest that activation of liver macrophages in NAFLD may alter lipoprotein metabolism and secretion to promote increased plasma LDL-C and triglycerides, and reduced HDL-C, contributing to an atherogenic lipid profile during NAFLD progression.

HEPATIC STELLATE CELLS

Unlike hepatocyte lipotoxicity, our knowledge of hepatic stellate cell (HSC) responses to fatty acid excess is limited, despite the activation and proliferation of these cells in advanced NAFLD, their chronic exposure to lipid excess and their known contribution to fibrosis. We recently determined the sensitivity of human primary activated HSC to high concentrations of saturated and unsaturated fatty acids. Exposure to either high palmitate or high oleate alone induced cell stress, but through different mechanisms. Palmitate stimulated transient expression of the endoplasmic reticulum stress-induced apoptotic factor, *CHOP*, whereas oleate decreased *CHOP* expression and increased expression of *TXNIP* [21]. TXNIP (thioredoxin-interacting protein) can be induced by activation of either PERK or IRE1 during endoplasmic reticulum stress, and can activate the inflammasome under these conditions [3]. Additional evidence from human and rat HSC lines suggests that palmitate, possibly through the generation of the metabolite dihydroceramide [22], promotes HSC activation and fibrotic activity through XBP-1-mediated induction of autophagy [23], and inflammasome-mediated hedgehog signaling [24].

Accumulating evidence of a robust inflammatory response in HSC upon exposure to high fatty acids supports the possibility that these cells could generate inflammatory cytokines that enter the circulation. In direct support of this, Shoji *et al.* [25] demonstrated that plasma IL-34, derived from liver fibroblasts, is dramatically increased in patients with NAFLD which has progressed to fibrosis. IL-34 has recently been identified as a significant predictor of cardiovascular mortality, and is proposed to contribute to atherosclerosis progression by promoting the release of other proinflammatory cytokines including IL-1β, IL-6 and TNF-α [26]. Similarly, CCL5 and CCL20, both potent chemokines, are increased in serum from individuals with NAFLD/NASH, and originate from both hepatocytes and activated HSC [27,28]. Most recently, circulating IL-6 was independently associated with subclinical atherosclerosis in an NAFLD subgroup of the Multi-Ethnic Study of Atherosclerosis (MESA) cohort [29▪▪]. In fact, IL-6 concentrations stratified NAFLD patients according to their coronary plaque burden. Although this study did not further subdivide NAFLD patients according to severity of liver disease, IL-6 production occurs in a variety of cell types within the liver, including fibroblasts [30], raising the possibility that increased hepatic IL-6 production directly impacts plaque development. Further work is warranted to determine whether activated HSC-derived proinflammatory cytokines are key mediators of NAFLD-related atherosclerosis.

LIVER SINUSOIDAL ENDOTHELIAL CELLS

LSEC are the most abundant non-parenchymal cells in the liver. Similar to HSC, little is known of the contributions of LSEC lipotoxicity to disease progression, despite the chronic exposure of these cells to excess lipid in NAFLD. LSEC are highly specialized and unique from vascular endothelial cells as they lack a basement membrane and have a multitude of fenestrae that regulate transport of macromolecules, including lipids and lipoproteins, across the sinusoid. Under normal conditions, LSEC maintain homeostatic regulation over hepatic vascular tone, primarily through the production of nitric oxide. An important cross-talk also occurs between LSEC and HSC, which serves to maintain HSC quiescence

under homeostatic conditions [31]. However, in the presence of hepatocyte lipotoxicity and injury, LSEC lose their regulatory functions, rapidly become dysfunctional and undergo LSEC capillarization, a process characterized by the development of a basement membrane and loss of fenestrations. This is then followed by angiogenesis [31–33]. In light of recent findings that LSEC dysfunction precedes hepatic inflammation and fibrosis, it is important to recognize that communication between LSEC and other hepatic cell types likely plays a significant role in NAFLD progression.

Accumulation of fatty acids and cholesterol within hepatocytes causes hepatocyte ballooning, which leads to sinusoidal compression, increased intrahepatic vascular resistance (IHVR) and increased shear stress, thereby acting as a mechanical stressor on LSEC to promote dysfunction and capillarization [34]. LSEC dysfunction, elicited by continuous vascular stress, is mainly characterized

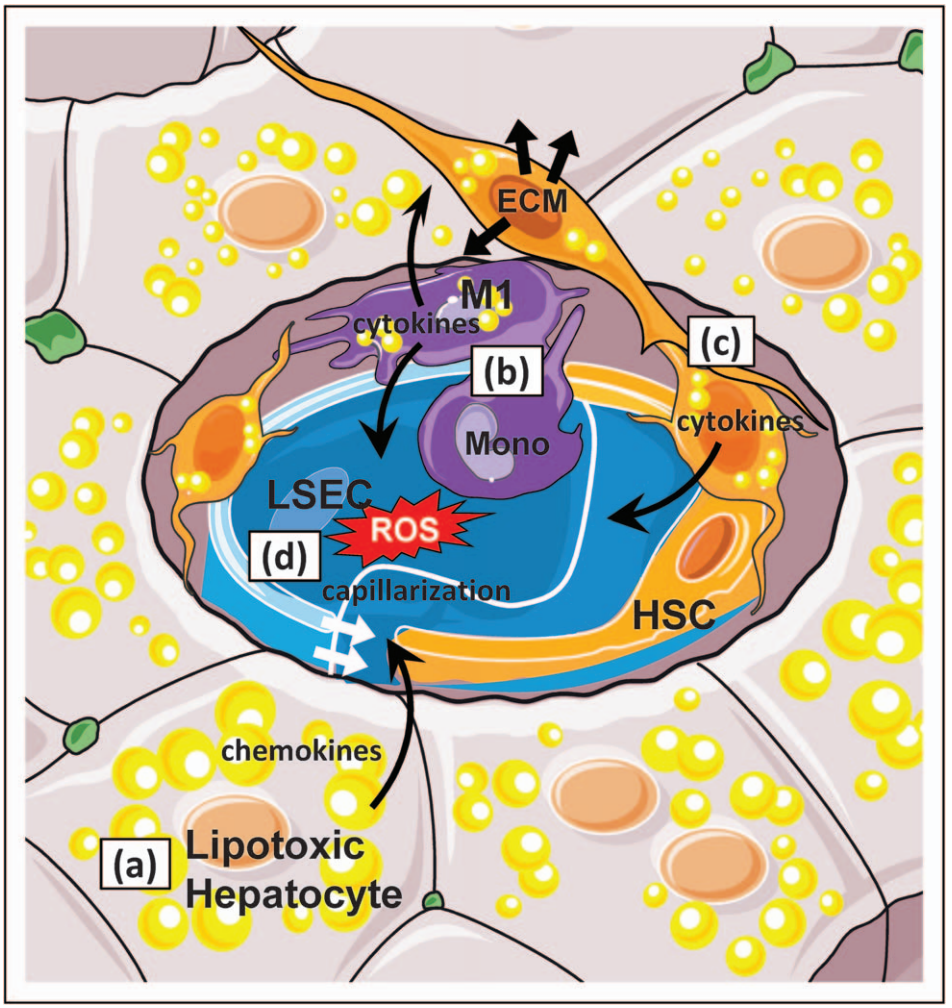

FIGURE 1. Non-parenchymal cell responses to lipid excess during NAFLD. (a) Lipotoxic hepatocytes initiate inflammatory cascades which can include the release of chemokines, such as CCL2 and CXCL10. (b) In response to chemokines, circulating monocytes (purple) infiltrate hepatic tissue through adhesion to LSEC. Upon exposure to high fatty acids, monocyte-derived macrophages and Kupffer cells adopt a proinflammatory M1 phenotype, characterized by increased production of cytokines, such as TNFα, IL-6 and IL-1β. These cytokines can enter systemic circulation via the sinusoids. (c) HSC (orange) become activated and begin to migrate, proliferate, produce ECM components and secrete proinflammatory cytokines, such as IL-34, CCL5 and CCL20, which can also enter systemic circulation via the sinusoids. (d) In the presence of lipotoxic hepatocytes and high fatty acids, LSEC (blue) undergo capillarization and excessive generation of ROS. Some LSEC-derived ROS may enter the circulation as a result of immediate proximity to the sinusoid. Yellow spheres, cytosolic lipid droplets; black arrows, secretion; white arrows, process of LSEC capillarization; green structures, bile canaliculi; ECM, extracellular matrix; HSC, hepatic stellate cell; LSEC, liver sinusoidal endothelial cell; M1, M1 macrophage; Mono, monocyte; NAFLD, non-alcoholic fatty liver disease; ROS, reactive oxygen species.

by decreased nitric oxide bioavailability either through impaired eNOS production, or through its reaction with superoxides [32]. It has also been postulated that impaired LSEC nitric oxide production may be due to eNOS phosphorylation induced by insulin resistance, resulting from hepatocyte lipotoxicity [35]; however, the specific mechanism underlying this effect remains controversial [32].

LSEC oxidative stress, as a consequence of LSEC lipotoxicity, may be an important driver of NAFLD progression. Saturated fatty acids, such as palmitate, have been shown to activate toll-like receptor-4 (TLR4) in LSEC [36]. In this setting, TLR4 activation induces NOX1 and subsequent generation of superoxide, which reacts with nitric oxide to form peroxynitrate, thereby reducing nitric oxide bioavailability [37▪]. Oxidative stress can also modulate LSEC cyclooxygenase (COX) activity and downstream prostanoid production to elicit vasoconstriction, which further increases IHVR and thus exacerbates LSEC stress in a vicious cycle [38]. Moreover, COX activation within LSEC can directly contribute to the generation of superoxides, which further depletes nitric oxide bioavailability [39]. As discussed in the next paragraph, LSEC-derived reactive oxygen species (ROS) can enter the circulation, possibly contributing to a proatherogenic environment, in addition to promoting NAFLD progression.

Activated HSC are pivotal drivers of hepatic fibrosis, but are increasingly recognized for their role in promoting LSEC capillarization and angiogenesis leading to ROS generation. An interesting series of feed-forward loops involving HSC activation and LSEC appear to drive NAFLD progression. HSC activation induces LSEC capillarization via hedgehog signaling [40], and has also been shown to induce LSEC vascular endothelial growth factor expression in a hedgehog-dependent manner to further promote hepatic angiogenesis [41]. Increased LSEC angiogenesis downstream of HSC activation can exacerbate hepatic fibrosis, which further increases IHVR and vascular stress, leading to further stimulation of LSEC ROS production [42,43]. The putative link between LSEC ROS generation and NAFLD progression is supported by the finding that serum reactive oxygen metabolites are significantly higher in patients with advanced NAFLD/NASH [44]. This observation is in line with the concept that superoxides generated by LSEC can enter systemic circulation. Remarkably, serum markers of oxidative stress and increased carotid artery intima-media thickness have been independently associated with NASH [45]. This raises the intriguing possibility that LSEC oxidative stress and superoxide generation are also mediators of atherosclerosis development during NAFLD.

CONCLUSION

Our targeted review suggests that non-parenchymal hepatic cell types mount some similar responses to lipid excess, and share some common pathogenic mechanisms that likely contribute to the coordinated progression of both NAFLD and atherosclerosis (Fig. 1). In particular, monocyte-derived macrophages, Kupffer cells and HSC initiate robust inflammatory responses upon exposure to high saturated fatty acids, whereas the LSEC lipotoxic response involves ROS generation. Consistent with this, inflammatory cytokines produced by macrophages, Kupffer cells and HSC have been implicated in the progression of NAFLD and atherosclerosis, whereas LSEC-derived ROS may independently contribute to the progression of both diseases. Further investigation will be required to determine the extent to which non-parenchymal cell-derived inflammatory cytokines and ROS play a role in both diseases, and whether liver-targeted therapies for NAFLD can modulate the disease promoting behavior of these cell types.

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Conflicts of interest

There are no conflicts of interest.

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- ▪ of special interest
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