Hepatic Microcirculation in Fatty Liver Disease

GEOFF C. FARRELL,[1]* N.C. TEOH,[1] AND R.S. MCCUSKEY[2]

[1]Gastroenterology and Hepatology Unit, and Australian National University Medical School, The Canberra Hospital, Garran, Australia

[2]Department of Cell Biology and Anatomy, College of Medicine, University of Arizona, Tucson, Arizona

ABSTRACT

Nonalcoholic fatty liver disease (NAFLD), the most common cause of steatosis, is associated with visceral obesity and insulin resistance. With more severe risk factors (obesity, type 2 diabetes [T2D], metabolic syndrome), steatosis may be complicated by hepatocellular injury and liver inflammation (steatohepatitis or NASH). NASH can lead to perisinusoidal fibrosis and cirrhosis. Fat-laden hepatocytes are swollen, and in steatohepatitis, further swelling occurs due to hydropic change (ballooning) of hepatocytes to cause sinusoidal distortion, as visualized by in vivo microscopy, reducing intrasinusoidal volume and microvascular blood flow. Involvement of other cell types (sinusoidal endothelial cells, Kupffer cells, stellate cells) and recruitment of inflammatory cells and platelets lead to dysregulation of microvascular blood flow. In animal models, the net effect of such changes is a marked reduction of sinusoidal space (~50% of control), and a decrease in the number of normally perfused sinusoids. Such microvascular damage could accentuate further liver injury and disease progression in NASH. The fatty liver is also exquisitely sensitive to ischemia-reperfusion injury, at least partly due to the propensity of unsaturated fatty acids to undergo lipid peroxidation in the face of reactive oxygen species (ROS). This has important clinical consequences, particularly limiting the use of fatty donor livers for transplantation. In this review, we discuss available data about the effects of steatosis and steatohepatitis on the hepatic microvascular structure and sinusoidal blood flow, highlighting areas for future investigation. Anat Rec, 291:684–692, 2008.

Key words: fatty liver; microcirculation; steatosis; liver transplantation

PATHOLOGICAL DEFINITIONS AND CAUSES OF FATTY LIVER DISEASE

Steatosis is the presence of fat droplets within hepatocytes. Hepatic fat storage is an important physiological function, but abnormal partitioning of fat into the liver produces the most common form of liver pathology. There are numerous causes of steatosis, but, in general, these may be summarized as viral, toxic, genetic, and metabolic causes (Table 1), or various combinations of these etiologies (Chitturi and Farrell, 2001). For example, steatosis is present in approximately 40% of liver biopsies from patients with hepatitis C virus (HCV) infection (Powell et al., 2005); while the significance may vary according to HCV genotype and the presence of metabolic risk factors (Hui et al., 2002, 2003; Powell et al., 2005; Matos et al., 2006), steatosis and associated insulin resistance may accelerate progression to cirrhosis. Fatty liver is also an almost invariable part of liver pathology in alcoholic liver disease. However, the most common type of fatty liver disease in contemporary

*Correspondence to: Geoff C. Farrell, The Canberra Hospital and ANU Medical School, Garran, ACT 2604 Australia. Fax: 02-6244-3092. E-mail: geoff.farrell@act.gov.au

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TABLE 1. Common causes of hepatic steatosis[a]

Etiology	Comments – known effects of microvascular changes
Alcohol	Microvascular changes contribute to portal hypertension and liver injury (see reviews by Reynaert and Geerts in this issue of the Journal)
Hepatitis C	Steatosis contributes to fibrotic progression of liver disease and increased risk of cirrhosis
Total parenteral nutrition	Micronutrient deficiency and high carbohydrate load
Drugs	Tamoxifen, amiodarone, high-dose corticosteroids, nucleoside analogues used in anti-retroviral infections – mitochondrial toxicity
Profound weight loss	Eating disorders, older forms of bariatric surgery, such as jejuno-ilial by-pass surgery
Environmental hepatotoxins	Dimethylformamide, toxic oil syndrome
Genetic metabolic disorders	Wilson's disease and other copper-associated liver diseases of childhood; type 1 glycogen storage disease, abeta-lipoproteinaemia; Alström syndrome
Causes of 'secondary' insulin resistance	Lipodystrophy, polycystic ovarian syndrome, hypopituitarism
Causes of primary insulin resistance (NAFLD/NASH)	Central obesity, obesity, glucose intolerance/type 2 diabetes, dyslipidemia, metabolic syndrome

[a]Reviewed in more detail in Chitturi and Farrell (2001) and Larter and Farrell (2006).

European, American (North and South), and Asian populations is nonalcoholic fatty liver disease (NAFLD), a metabolic disorder now understood to be closely related to central adiposity, insulin resistance, and the metabolic syndrome (Larter and Farrell, 2006; Chitturi et al., 2007). The diagnosis of NAFLD is permissible when fatty liver disease (FLD) occurs without other causes, but particularly when metabolic risk factors are present; it is excluded by alcohol intake of more than two standard drinks per day (20 g ethanol) in men, or one standard drink per day (10 g) in women (Farrell et al., 2007; Chitturi et al., 2007). Unless specifically indicated, all subsequent discussion on fatty liver disease will refer to this entity or experimental models that simulate it.

From the point of view of anatomical pathology, steatosis takes one of two morphological forms: (1) fat stored in multiple small vesicles, that is, microvesicular steatosis; here, the hepatocyte nucleus remains centrally located, and (2) fat stored in a single large vesicle or lipid droplet, that is, macrovesicular steatosis. Macrovesicular steatosis is the predominant morphology in NAFLD, but mixed forms of micro- and macrovesicular steatosis often exist in varying proportions. A noteworthy feature of macrovesicular steatosis is that the lipid droplet tends to displace the hepatocyte nucleus to the periphery of the cell, with the usually abundant organelles (e.g., mitochondria, smooth endoplasmic reticulum) present in an attenuated rim of cytoplasm around this lipid vesicle. This gives the hepatocyte an appearance that resembles mature adipocytes, and in some forms of experimental fatty liver disease there are also molecular markers of such "adipocytic transformation" (Arsov et al., 2006a; Larter CZ, unpublished results).

NAFLD is now conceptualised to embrace a continuum of liver pathology, from simple steatosis (in which the only change is fat accumulation in hepatocytes), through steatohepatitis to cirrhosis. Nonalcoholic steatohepatitis (NASH) is a progressive lesion in which steatosis is complicated by hepatocellular injury, evident as ballooning degeneration and Mallory hyaline, lobular inflammation, and fibrosis. The inflammatory infiltrate contains some polymorphonuclear cells as well as mononuclear cells. Fibrosis is typically perisinusoidal in distribution, often worse in acinar zone 3 (pericentral), which is similar to that found in alcoholic liver disease (Hall and Kirsch, 2005; Kleiner et al., 2005). A variant pattern in which periportal fibrosis is more pronounced is more common in children with NAFLD (reviewed in Hall and Kirsch, 2005). In severe cases, NASH may progress to cirrhosis and possibly hepatocellular carcinoma. The reason why a minority of cases exhibits such progression is unclear, although one suggestion is that vascular changes occurring in more advanced stages of steatohepatitis may lead to subsequent ischemic injury with progressive loss of hepatic parenchyma (Wanless and Shiota, 2004).

CLINICAL SIGNIFICANCE OF FATTY LIVER

NAFLD is the result of overnutrition caused by the combined effects of an energy-dense diet (caloric intake in excess of energy needs) and physical underactivity. Together, these factors cause central (or visceral) obesity and insulin resistance. Central obesity is the factor most strongly associated with steatosis, and it has been proposed that steatosis may cause hepatic insulin resistance, rather than being its inevitable consequence (reviewed in Larter and Farrell, 2006). The more severe are the risk factors [obesity, glucose intolerance, or type 2 diabetes (T2D), metabolic syndrome], the more likely is steatosis to be complicated by steatohepatitis and cirrhosis (Larter and Farrell, 2006). Clearly, for the disorder to be "nonalcoholic" FLD, significant levels of alcohol consumption must be excluded, as mentioned above.

The increasing prevalence of NAFLD/NASH in most societies of the world, conspicuously including much of Asia and South America as well as North America, Australia/New Zealand, and Europe (Larter and Farrell, 2006; Chitturi et al., 2007), parallels that of the obesity and T2D pandemics. Furthermore, more severe forms of NAFLD are inextricably linked to metabolic syndrome, which is present in ~85% of cases of NASH and which becomes manifest after diagnosis of NAFLD at a rate at least 10-fold greater than the general population (Hamaguchi et al., 2005; Fan et al., 2005; Chitturi and Farrell, 2007). Cirrhosis was the third most common cause of death in one North American community study of people with NAFLD (Adams et al., 2005) and is among the most common causes of death in patients with T2D (Brun et al., 2000). Thus, while only a small proportion

of persons with NAFLD are likely to develop cirrhosis or its complications, perhaps 10–25% (Larter and Farrell, 2006), the very high prevalence of fatty liver (15–45% in various regions and population subgroups) means that this disorder will contribute significantly to an increased burden of ill-health in the future. Furthermore, because steatosis on its own is benign, the factors that mediate progression to steatohepatitis and cirrhosis are of considerable interest for their potential therapeutic relevance.

FATTY LIVER IMPAIRS OUTCOMES OF LIVER TRANSPLANTATION

Between 15% and 50% of livers from cadaveric brain-dead adult and child donors for liver transplantation exhibit significant fatty liver disease, as defined by more than 30% of hepatocytes showing steatosis (Todo et al., 1989; Strasberg et al., 1994; Trevisani et al., 1996; Selzner and Clavien, 2001; Alwayn and Porte, 2007). The body mass index is a reliable predictor of the presence of steatosis in a liver transplant donor (Rinella et al., 2001; Hwang et al., 2004). Several clinical and experimental studies have demonstrated that primary graft nonfunction resulting in liver failure is more frequent when such grafts are used for transplantation (Todo et al., 1989; Mor et al., 1992; Strasberg et al., 1994; Trevisani et al., 1996; Cameron et al., 2000; Selzner and Clavien, 2001; Cheng et al., 2001; Imber et al., 2002; Verran et al., 2003; Busuttil and Tanaka, 2003; Soejima et al., 2003; Alwayn and Porte, 2007). There appears to be a relationship between the extent of fatty change in the liver and primary graft nonfunction (Adam et al., 1992; Perez-Daga et al., 2006). One large study assessed 390 frozen-section liver biopsies and found that primary graft nonfunction occurred in 13% of donor livers showing more than 30% steatosis, compared with 2.5% of nonsteatotic grafts (Adam et al., 1992). Furthermore, the more extensive the steatosis, the poorer was graft survival. Ploeg et al., (1993) described rates of primary graft nonfunction up to 80% in severely steatotic livers, but graft failure was also significant (30%) in moderately steatotic livers.

The outcome of these studies has led to the recommendation that liver biopsy is mandatory before flush out and cold storage of donor livers, and that grafts with severe steatosis (>60%) should be discarded. Those with moderate change (30–60% steatosis) should be evaluated in conjunction with other criteria that influence the urgency of transplantation, such as health status of the recipient and availability of other donor livers (Todo et al., 1989; Adam et al., 1992; Ploeg et al., 1993; Strasberg et al., 1994; Trevisani et al., 1996; Selzner and Clavien, 2001; Verran et al., 2003; Alwayn and Porte, 2007). A more recent analysis of 300 liver transplants confirmed that the degree of liver graft steatosis is an important determinant of ischemia–reperfusion injury and that this is what correlated with a higher rate of postoperative complications (such as renal failure requiring hemodialysis) and higher 90-day mortality (Perez-Daga et al., 2006).

The importance of steatosis on graft survival has also been investigated in living-related donor livers (Hayashi et al., 1999; Soejima et al., 2003). Hayashi et al. (1999) showed that early graft function was similar in mild and moderate steatosis, but severe steatosis (>60%) was significantly associated with graft dysfunction and poor outcome. Primary graft nonfunction was not described in this study; one likely explanation is that shorter graft ischemia times are involved in living-related transplantation compared with the cadaveric (brain-dead) donor setting. On the other hand, with living-donor liver transplantation another implication is that there may be an increased risk to the donor, as well as to the recipient of a "marginal liver" (in this case, marginality determined by presence of steatosis). A recent retrospective review of Japanese living (right-lobe) liver donors found that 18% had some fatty change, and 3 of 300 (1%) donors had evidence of NASH (Yamamoto et al., 2007). Importantly, one of the three donors with NASH developed liver failure after hepatic resection and died; the cause was attributed to the increased operative risk to the donor's residual liver, related to underlying NASH and marginal "hepatic reserve."

Because the success of liver transplantation depends on the viability of the graft, objective and uniform assessment of the degree of steatosis in donor livers is critical. In most liver transplant centers, current approaches to quantify and grade hepatic steatosis arise from studies performed in the 1990s (Todo et al., 1989; Adam et al., 1992; Ploeg et al., 1993; Strasberg et al., 1994; Trevisani et al., 1996; Selzner and Clavien, 2001; Verran et al., 2003; Alwayn and Porte, 2007), where fatty change is classified as mild if visualized in 30% of hepatocytes, moderate if 30–60% of cells are involved, or severe (>60%). However, such changes can vary from center to center, with different tissue processing and staining techniques. Consensus in the assessment of hepatic fat in donor grafts is therefore needed in clinical practice to achieve better outcomes. The reasons for the increased susceptibility of fatty liver to injury during hepatic surgery (in the case of living donors) and during liver transplantation have been the subject of several investigations (Selzner et al., 2000; Selzner and Clavien, 2001); that focusing on the hepatic microvasculature is discussed in the later section.

EXPERIMENTAL MODELS OF FATTY LIVER DISEASE

There are numerous rodent models of fatty liver disease, based on nutritional, genetic, toxic, and viral factors; these have been reviewed elsewhere (Koteish and Diehl, 2001; Farrell, 2005). In general, animals fed diets enriched in lipogenic nutrients, particularly simple sugars such as fructose or sucrose and/or saturated fats, develop steatosis rather than steatohepatitis. In older animals, some evidence of hepatocellular injury, focal lobular inflammation, and even early pericellular fibrosis may be observed, particularly during prolonged intake of a lipogenic diet. Other commonly used fatty liver disease models are animals lacking genes that affect appetite regulation, or which predispose to diabetes. Examples include the *fa*/*fa* (or Zucker) rat, and the *db*/*db* mice, both of which lack functional leptin receptors and are thereby predisposed to hyperphagic obesity and T2D, and *ob*/*ob* mice which lack leptin itself. There is not yet complete agreement on the extent to which liver disease in these models resembles NASH, as opposed to simple steatosis. However, the extent of steatosis is often

impressive, with hepatic triglyceride content increased at least fivefold above control lean mice. Experiments on ischemia–reperfusion and organ preservation (for transplantation) in these rodent models have provided solid evidence that fatty liver predisposes to ischemia–reperfusion injury and graft failure of affected livers (Selzner and Clavien, 2001), as indicated in the previous section.

Hepatic microcirculatory blood flow has been studied more extensively in two other nutritional models of steatohepatitis. The methionine and choline deficient (MCD) dietary model has been most used to establish molecular pathways of inflammatory recruitment and liver injury in steatohepatitis (Leclercq et al., 2000, 2004; Ip et al., 2004; McCuskey et al., 2004; de la Peña et al., 2005, 2007; Schattenberg et al., 2005, 2006), and in ischemia–reperfusion injury (Baskin-Bey et al., 2005). The MCD model captures the full pathological spectrum of NASH, including hepatocyte ballooning, extensive lobular inflammation, and pericellular fibrosis, but animals lose weight and are insulin sensitive, rather than obese and insulin resistant. On the basis of certain biochemical changes in fatty acid turnover and serum leptin levels (these are low), Maher and her colleagues have suggested that the MCD model may be more similar to the fatty liver disease which occurs with lipodystrophy than NAFLD/NASH (Rizki et al., 2006), which is strongly associated with insulin resistance and metabolic syndrome. By contrast, two newer models have the pathophysiological settings of NASH, together with the appropriate spectrum of liver pathology. These are the intragastric overfeeding model of Tsukamoto and colleagues (Deng et al., 2005) and the "Fat Aussie" mouse model (Arsov et al., 2006b). Fat Aussie arose as a spontaneous mutation (*foz*) of the murine homologue of the Alström gene (*Alms1*). When fed commercial rodent chow, *foz*/*foz* mice develop obesity, insulin resistance, T2D, and hypoadiponectinemia; livers show extensive steatosis, mostly microvesicular, even at 3 months. When fed a high-fat diet, weight gain and the metabolic changes are accentuated, hypoadiponectinemia is more profound and livers show severe steatohepatitis with fibrosis (Arsov et al., 2006a). The highly reproducible phenotype of liver disease and metabolic syndrome (with T2D) in these obese mice, together with the facility to compare simple steatosis with steatohepatitis, makes this a highly suitable model to examine microvascular changes in fatty liver disease.

MICROVASCULAR ALTERATIONS IN FATTY LIVER DISEASE

Substantial changes in blood flow are present in fatty liver disease, as reviewed most recently in 2003 (Ijaz et al., 2003). Using Doppler flowmetry, Seifalian et al. demonstrated reduced sinusoidal perfusion in fatty human liver donors compared with healthy livers (Seifalian et al., 1998). Analogous studies in rabbits with dietary-induced steatosis of graded severity confirmed that this reduction in perfusion correlated with the severity of fat accumulation in parenchymal cells (Seifalian et al., 1999). The severity of steatosis had a greater effect on the microcirculation than on total hepatic blood flow.

Further experimental studies have used in vivo microscopic methods, light and electron microscopy, and laser Doppler flowmetry to evaluate alterations of the hepatic microvascular in fatty livers of genetically obese Zucker (*fa*/*fa*) rats (Sato et al., 1986; Sun et al., 2001, 2003), *ob*/*ob* mice (Hasegawa et al., 2007), and *foz*/*foz* mice (Teoh et al., unpublished results), as well as in mice and rats with dietary-induced steatosis and steatohepatitis (Teramoto et al., 1993; Caraceni et al., 1999; Hakamada et al., 1997; McCuskey et al., 2004; McCuskey and DeLeve, unpublished results). These studies have all demonstrated that the reductions in sinusoidal perfusion arise initially from the effects of enlarged hepatic parenchymal cells, swollen with accumulated lipid, which widen the parenchymal cell plates, narrow, and distort the lumens of sinusoids so as to reduce the intrasinusoidal volume (Ohara, 1989), as well as altering the architecture of the sinusoidal network (Fig. 1). These changes are illustrated from our recent (unpublished) studies in *foz*/*foz* mice (Fig. 2). As a result of the structural alterations around them, the sinusoids become inefficient conduits of blood with resulting impairment of tissue perfusion, evidenced by the significant reductions in the numbers of perfused sinusoids per microscopic field (Sato et al., 1986; Sun et al., 2001; McCuskey et al., 2004; Hasegawa et al., 2007; Teoh et al., unpublished results).

In more severe fatty liver disease, simple steatosis may progress to steatohepatitis, and then to fibrosing steatohepatitis with the initiation of capillarization of the sinusoids. This change is hallmarked structurally by a progressive loss of fenestrae in the sinusoidal endothelial cells (SEC), concomitant with development of a basal lamina and deposition of collagen in the space of Disse (Fig. 3; McCuskey et al., 2004; McCuskey and DeLeve, unpublished results). There may be accompanying adhesion of leukocytes to the sinusoidal endothelium, followed by leukocyte infiltration into the hepatic parenchyma to form inflammatory foci (McCuskey et al., 2004). The deposition of collagen in the space of Disse accentuates the narrowing and distortion of the sinusoidal lumen, further restricting microvascular blood flow. This is exacerbated by leukocytes either mechanically trapped in the narrowed sinusoids or adhering to the endothelium as a result of activation of a hepatic microvascular inflammatory response.

It seems likely that the microvascular changes in steatosis are secondary to lipid accumulation and oxidative stress in hepatic parenchymal cells because, unlike changes with ischaemia-reperfusion injury (Teoh et al., 2007) and endotoxemia (McCuskey et al., 1995), there is no evidence of swollen SEC, which is an early marker of SEC injury (McCuskey et al., 1995; McCuskey, 2002). There is also a paucity of inflammation, as indicated by adhesion of leukocytes to the SEC, until significant parenchymal changes have already occurred (see below; McCuskey et al., 2004).

CAUSES AND CONSEQUENCES OF INEFFECTIVE SINUSOIDAL PERFUSION

During the development of fibrosing steatohepatitis induced by feeding mice a lipogenic MCD diet, dysfunctional (or effectively obliterated) sinusoids increased significantly from 13% at 3 weeks of dietary feeding to 19% at 5 weeks (McCuskey et al., 2004). Between 5 and 8 weeks, the number of leukocytes adhering to the sinusoidal lining increased substantially, consistent with the

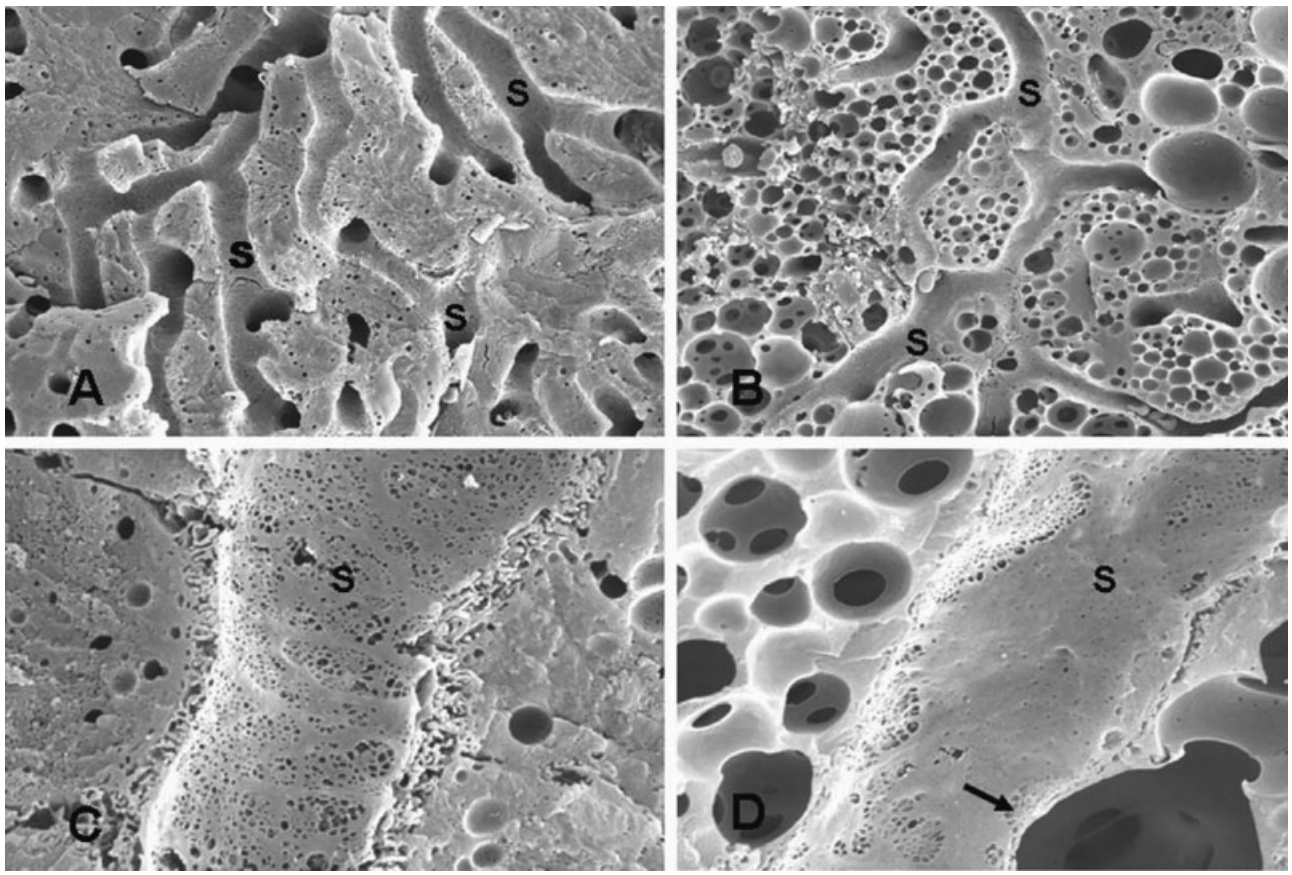

Fig. 1. **A–D:** Scanning electron micrographs of livers from lean mice fed a normal chow diet (A,C) compared with obese mice fed a high fat, high carbohydrate diet (B,D) for 12 months. Note the sinusoids and their pattern distorted by the enlarged, fat-laden parenchymal cells in B versus A and the loss of fenestrae in D versus C. The arrow in D points to the compression of the sinusoid caused by a ballooning hepatocyte containing a large fat droplet. A and B, ×1,000 original magnification; C and D, ×8,000 original magnification.

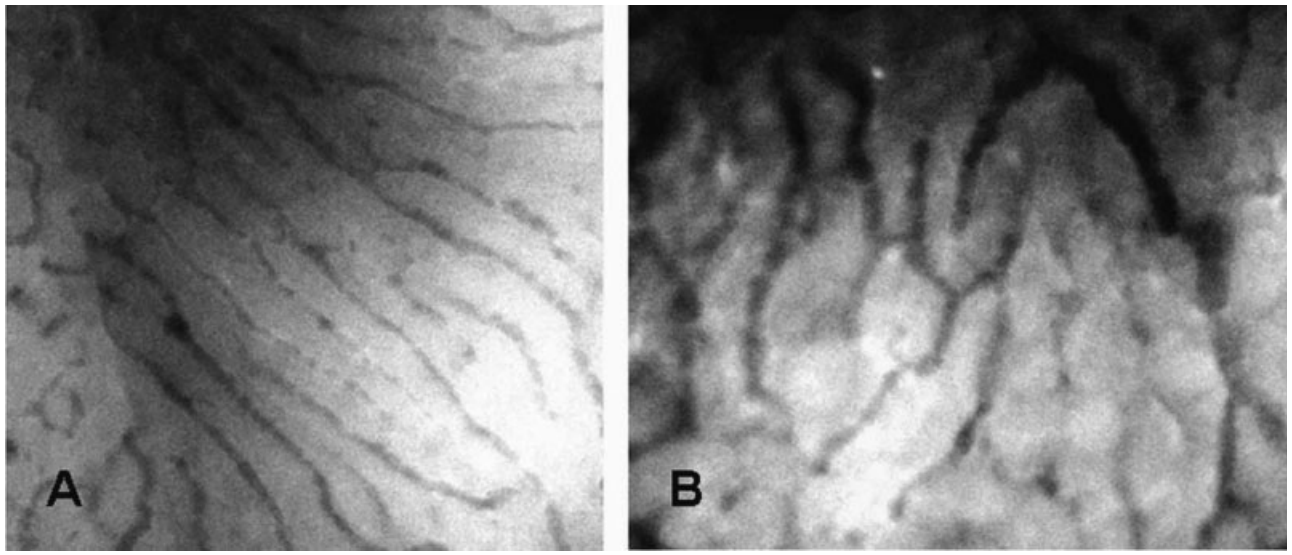

Fig. 2. **A,B:** In vivo microscopic images of the hepatic microcirculation in obese *foz/foz* mouse (B) and lean wild-type (A; ×200 original magnification). Note the distorted sinusoidal network and vessels of varying diameter in the obese *foz/foz* liver (B; ×400 original magnification) compared with the more regular pattern and diameter of sinusoids in the lean wild-type (A).

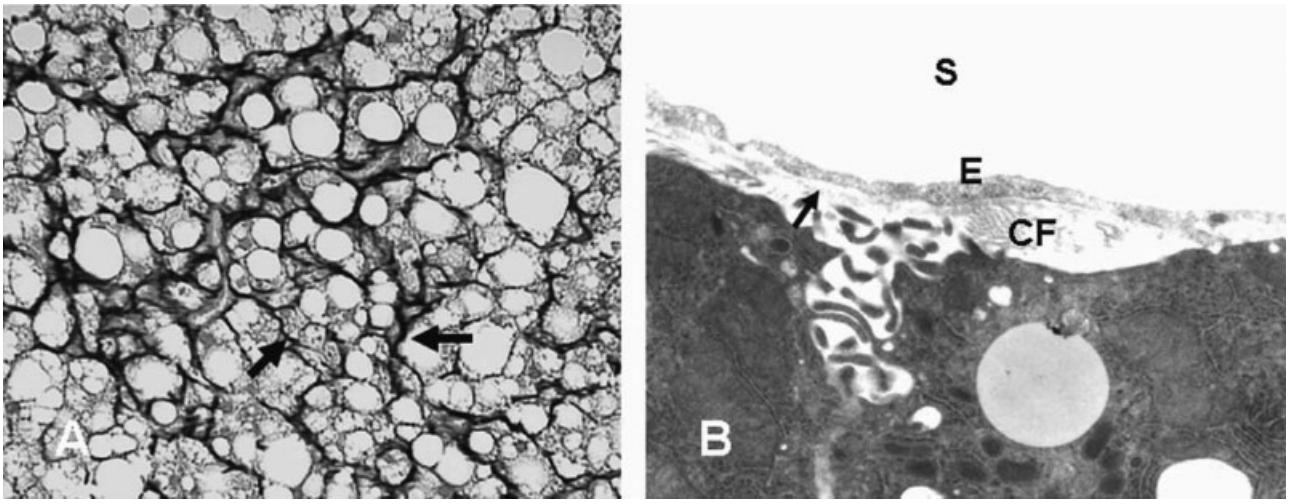

Fig. 3. Perisinusoidal and pericellular deposition of collagen in obese mice fed a high fat, high carbohydrate diet for 12 months. **A:** Arrows point to collagen fibers stained with Sirius Red (×20 objective). **B:** Transmission electron micrograph illustrating collagen deposition (CF) and a subendothelial basal lamina (arrow) in the space of Disse. S, sinusoidal lumen; E. sinusoidal endothelial cell, ×6,000 original magnification.

appearances on light microscopy of a parenchymal mixed cellular inflammation (McCuskey et al., 2004). Such adherence of leukocytes to endothelial cells is characteristic of a hepatic microvascular inflammatory response (McCuskey et al., 1995; McCuskey, 2002; Teoh et al., 2007). The hepatic expression of the intercellular adhesion molecules, ICAM-1 and VCAM also are increased in MCD diet-induced steatohepatitis (Ip et al., 2004; de la Peña et al., 2005). Such changes have been attributed to up-regulation of NF-kappaB, in turn is activated in the liver by the presence of severe oxidative stress (Leclercq et al., 2004; de la Peña et al., 2005). The demonstration that this secondary inflammatory response is focused, at least partially, on the microvasculature of the liver indicates one possible mechanism whereby metabolic oxidative stress attracts an inflammatory response with the potential to perpetuate liver injury by means of changes in liver perfusion. Analogous microvascular changes have been demonstrated that affect tissue oxygen concentration in the centrilobular region of livers of obese Zucker rats (Sun et al., 2003).

The impaired hepatic microcirculation during fatty liver disease has been implicated in the decreased tolerance of the fatty liver to ischemia-reperfusion injury (Teramoto et al., 1993; Hakamada et al., 1997; Sun et al., 2001; Hasegawa et al., 2007), as well as increased sensitivity to drugs and toxins (Yang et al., 1997; Ito et al., 2006).

MACROPHAGE ACTIVITY

Concomitant with the alterations in sinusoidal blood flow observed in several models of fatty liver disease, significant changes occur in the phagocytic activity of hepatic macrophages (McCuskey et al., 1995, 2004; Yang et al., 1997). Because these cells in their activated state are known to release reactive oxygen species (ROS) and nitroradicals, as well as cytokines and vasoactive prostanoids, they are also likely to contribute importantly to parenchymal injury, the microvascular inflammatory response, and activation of the adjacent stellate cells with resultant loss of lipid droplets and increased production of collagen.

Further studies are clearly indicated to elucidate the extent to which products released by Kupffer cells and SECs could mediate hemodynamic changes in the liver of steatohepatitis, especially **since** both increased and decreased activity of these resident tissue macrophages has been reported. For example, during the first several weeks, mice on the MCD diet, or rats on a choline-deficient diet exhibited significant increases in Kupffer cell phagocytic activity (McCuskey et al., 1995, 2004). By eight weeks, however, phagocytic activity was diminished in mice on the MCD diet (McCuskey et al., 2004); In other recent studies in the MCD model, NADPH oxidase, the rate-limiting step in macrophage ROS production, was shown to be dispensable for generation of hepatic oxidative stress and lesion development in steatohepatitis (de la Peña et al., 2007). The reduced activity of hepatic macrophages late in the evolution of steatohepatitis is consistent with the report of diminished phagocytic activity in the livers of obese Zucker rats (Yang et al., 1997). Whether this leads to spill-over of gut-derived endotoxin into the systemic circulation and stimulation of increased cytokine production in peripheral tissues, as suggested for the Zucker rat (Yang et al., 1997), is unknown.

ROLE OF STELLATE CELLS

Activation of stellate cells may also play a role in modifying hepatic microvascular dynamics. Such activation increases stellate cell contractility, and these cells are thought to play a role in the regulation of sinusoidal caliber and hepatic microvascular blood flow (Bauer et al., 1995; Zhang et al., 1994, 1993). It is possible that impairment of blood flow, together with deposition of collagen and other extracellular matrix proteins in the

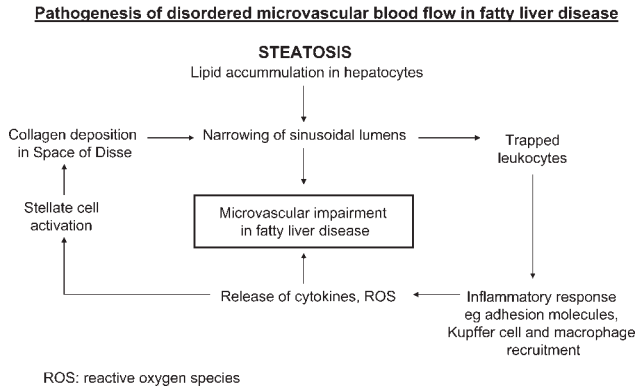

Fig. 4. Pathogenesis of disordered microvascular blood flow in fatty liver disease.

space of Disse, contributes to the continuing liver injury that is a feature of all forms of steatohepatitis. In particular, impaired oxygenation of centrilobular hepatocytes, or ROS generated during spontaneous fluctuations in blood flow that cause ischemia followed by reperfusion would explain, in part, the predominate distribution of liver injury and fibrosis in the centrilobular region (acinar zone 3). It should be noted that defenestration of the sinusoidal endothelium, the formation of a subendothelial basal lamina, and the deposition of collagen in the space of Disse (Fig. 3) reported in dietary models of fatty liver disease (McCuskey et al., 2004; McCuskey and DeLeve, unpublished results) are consistent with the initiation of "capillarization" of the sinusoidal wall that is reported to occur during fibrosis and cirrhosis.

SUMMARY AND CONCLUSIONS

Fatty liver is the most prevalent cause of liver disease, being particularly related to obesity, diabetes and metabolic syndrome (NAFLD). Steatosis contributes to mortality after liver surgery and acute graft failure during liver transplantation because of the increased susceptibility of fatty livers to oxidative forms of liver injury. Hepatocellular injury in fatty liver disease, and especially with the development of fibrosing steatohepatitis, appears to be associated with, and could result from a combination of impaired sinusoidal blood flow due to lipid accumulation in parenchymal cells and collagen deposition in the space of Disse causing narrowing of sinusoid lumens. The resultant changes to the hepatic microcirculation are exacerbated by leukocytes, either mechanically trapped in the narrowed sinusoids or adhering as a result of activation of an hepatic microvascular inflammatory response. The latter may involve activation of Kupffer cells and other hepatic macrophages with the release of proinflammatory cytokines, as well as free radicals. The way in which these changes interact to impair microvascular blood flow are conceptualized in Figure 4. Together, they contribute to the hepatic inflammatory response in steatohepatitis, stimulate stellate cells, and exacerbate the oxidative stress in the liver which progressively increases during the course of fatty liver disease. In addition, these microcirculatory and cellular changes in the fatty liver sensitize the organ the effects of ischemia–reperfusion injury, toxins, and drugs leading to an exacerbated response to liver damage. Further studies are required to identify which steps in this proposed pathogenic chain of events are reversible, ultimately leading to the development of therapeutic interventions that can improve safety and clinical outcomes of liver surgery and donor organ preservation for liver transplantation.

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