Old Age and the Hepatic Sinusoid

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ABSTRACT

Morphological changes in the hepatic sinusoid with old age are increasingly recognized. These include thickening and defenestration of the liver sinusoidal endothelial cell, sporadic deposition of collagen and basal lamina in the extracellular space of Disse, and increased numbers of fat engorged, nonactivated stellate cells. In addition, there is endothelial upregulation of von Willebrand factor and ICAM-1 with reduced expression of caveolin-1. These changes have been termed age-related pseudocapillarization. The effects of old age on Kupffer cells are inconsistent, but impaired responsiveness is likely. There are functional implications of these aging changes in the hepatic sinusoid. There is reduced sinusoidal perfusion, which will impair the hepatic clearance of highly extracted substrates. Blood clearance of a variety of waste macromolecules takes place in liver sinusoidal endothelial cells (LSECs). Previous studies indicated either that aging had no effect, or reduced the endocytic capacity of LSECs. However, a recent study in mice showed reduced endocytosis in pericentral regions of the liver lobules. Reduced endocytosis may increase systemic exposure to potential harmful waste macromolecules such as advanced glycation end products Loss of fenestrations leads to impaired transfer of lipoproteins from blood to hepatocytes. This provides a mechanism for impaired chylomicron remnant clearance and postprandial hyperlipidemia associated with old age. Given the extensive range of substrates metabolized by the liver, age-related changes in the hepatic sinusoid and microcirculation have important systemic implications for aging and age-related diseases. Anat Rec, 291:672–683, 2008.

Key words: liver; aging; liver sinusoidal endothelial cell; chylomicron remnant; endocytosis; microcirculation; stellate cell; Kupffer cell; caloric restriction

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Since submission, Stacchiotti et al. also confirmed pseudocapillarization in aging mice. (Stacchiotti A, Lavazzza A, Ferroni M, Sberveglieri G, Bianchi R, Rezzani R, Rodella LF. Effects of aluminum sulphate in the mouse liver: similarites to the aging process. Exp Gerontol 43:330–8.)

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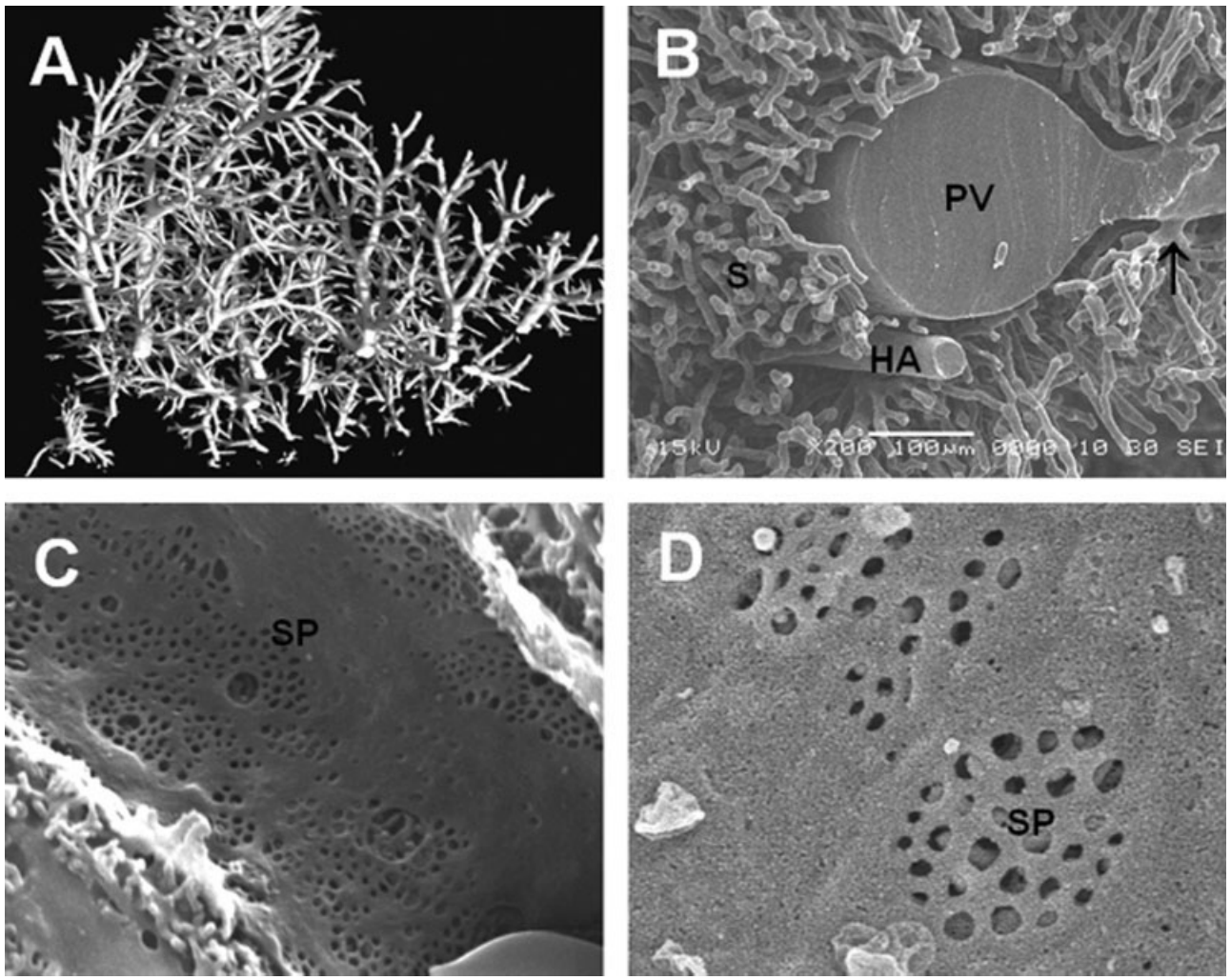

Fig. 1. The hepatic microcirculation and sinusoids. **A:** Three-dimensional reconstruction of vascular cast using micro-computed tomography showing branching of the portal venules and terminal portal venules. **B:** Scanning electron micrograph of a vascular cast showing a branch of the portal vein (PV) and branch of the hepatic artery (HA) with surrounding sinusoidal microvascular network (S). A branch from the PV into the sinusoids is shown (↑). **C:** Scanning electron micrograph showing the luminal surface of the liver sinusoid with fenestrations (approximate diameter 50–100 nm) clustered into sieve plates (SP). **D:** Higher magnification of scanning electron micrograph of a liver sieve plate showing numerous fenestrations.

THE HEPATIC SINUSOID

The liver is highly perfused, receiving approximately one quarter of total blood output from the heart. The liver parenchyma is supplied both by the hepatic artery (25%) and the portal vein (75%), with its rich mixture of gut-derived nutrients, toxins, and microbes. The portal vein branches into portal venules and terminal portal venules, which feed into the hepatic sinusoids. The hepatic arterioles, derived from the hepatic artery, also feed into these sinusoids. Hepatic sinusoids are porous, gossamer-like, cylindrical vessels that are slightly narrower than blood cells (Fig. 1). They connect afferent portal triads to exiting central hepatic venules—a distance of approximately 1 mm. There are approximately one billion sinusoids in the human, forming the rich capillary network of the liver, which permits the vast hepatic blood flow to course intimately between the cords of hepatocytes (McCuskey and Reilly, 1993; Fraser et al., 1995; McCuskey, 2000). This remarkable vascularity facilitates the exchange of substrates between blood and the liver and provides an extensive endothelial surface area for interactions with circulating immune cells and various colloid and soluble macromolecular waste products.

Liver sinusoidal endothelial cells (LSECs) are highly specialized endothelial cells that line the wall of the hepatic sinusoid and separate the sinusoidal blood derived primarily from the portal vein, from hepatocytes. LSECs are very thin and perforated with fenestrations, which are pores approximately 50–150 nm in diameter grouped together in clusters of many fenestrations known as liver sieve plates (Fig. 1). Fenestrations are true discontinuities in the endothelium, lacking either a diaphragm or underlying basal lamina. Approximately 5–10% of the

surface of the LSEC is perforated by fenestrations, and some studies have suggested that there is a zonal gradient with slightly larger fenestrations in the periportal sinusoids and greater porosity being found in pericentral sinusoids (Wisse et al., 1985; McCuskey and Reilly, 1993; Smedsrod et al., 1994; Fraser et al., 1995). LSECs have several important functions that are facilitated at least in part by this unique morphology:

Ultrafiltration

In the 1970s, it was postulated that the fenestrated endothelium acts like a dynamic filter, particularly for lipoproteins, and hence termed "the liver sieve" (Wisse, 1970; Fraser et al., 1978; Naito and Wisse, 1978). It is now recognized that fenestrations permit the passage of a wide range of substrates (plasma and substrates within plasma, plasma proteins including albumin, and smaller lipoproteins) into the extravascular space of Disse (Le Couteur et al., 2005). In addition, blood cells are thought to massage fluid through the fenestrations by virtue of the fact that their diameter is greater than that of a typical sinusoid (Wisse et al., 1985). The thinness of hepatic endothelial cells and the lack of basal lamina and collagen in the space of Disse ensure that any permeability barriers to the diffusion of substrates between blood and hepatocytes are minimized (Le Couteur et al., 2005). Of particular importance is the role of the LSEC and its fenestrations in the hepatic metabolism of lipoproteins (Fraser et al., 1995; Le Couteur et al., 2006). The first stage in the metabolism of dietary fats is the production of chylomicrons. Chylomicrons are triglyceride-rich, spherical lipoproteins formed in the intestine from dietary lipids. They are large particles with diameters of 100–1,000 nm that are unable to pass through the fenestrations of the hepatic sinusoidal endothelium because of their size. Chylomicrons are metabolized to chylomicron remnants by lipoprotein lipase present on the endothelium of systemic capillaries. Chylomicron remnants are smaller particles (30–80 nm) that have acquired apoE. Remnants pass through fenestrations and are sequestered within the space of Disse for receptor-mediated uptake into hepatocytes (Fraser et al., 1995; Yu and Cooper, 2001). There is accumulating evidence for the role of fenestrations in regulating lipoprotein transfer. There is a close correlation between the diameter of fenestrations and chylomicron remnants in the space of Disse and chylomicrons larger than fenestrations have not been seen in the extracellular space (Naito and Wisse, 1978). Membranes isolated from hepatocytes cannot differentiate between chylomicrons and chylomicron remnants, whereas in vivo, livers selectively take up chylomicron remnants (Floren, 1984). Electron microscopy has demonstrated that large chylomicrons are only found in the sinusoidal blood, whereas smaller chylomicron remnants are also observed in the space of Disse. There is differential trapping by the liver of radiolabeled chylomicrons of different sizes such that smaller particles are trapped to a greater extent than those larger than 100 nm (Fraser et al., 1978). Similar results indicating exclusion on the basis of size have been reported for large and small liposomes (Romero et al., 1999) and colloidal gold particles of different diameters (Hardonk et al., 1985). Loss of fenestrations (defenestration) causes impaired clearance of chylomicron remnants after meals and because remnants are still relatively rich in triglycerides, this is manifested as postprandial hypertriglyceridemia (Fraser et al., 1995; Yu and Cooper, 2001). Recently, it has been shown that conditions associated with reduced fenestrations cause impaired lipoprotein uptake and hypertriglyceridaemia: old age (Hilmer et al., 2005); VEGFR (vascular endothelial growth factor receptor) knockout mice (Carpenter et al., 2005); and treatment with poloxamer 407 (Cogger et al., 2006).

Endocytosis

The most striking functional characteristic of LSECs is their very high endocytic activity (Smedsrod et al., 1990). The first evidence for the endocytic activity of the LSEC came when it was reported that the physiological waste product, hyaluronan was avidly and specifically eliminated by LSECs from the circulation of rats and rabbits (Eriksson et al., 1983; Smedsrod et al., 1984). On the basis of this activity, LSECs are often termed scavenger endothelial cells (Seternes et al., 2002). It is now recognized that LSECs play a critical role in the removal of macromolecular waste products from the systemic circulation and probably represent the most active endocytic cells in the body (Smedsrod et al., 1990; Smedsrod, 2004). In particular connective tissue macromolecules (such as hyaluronan, chondroitin sulphate, collagen α-chain, PICP, PINP, and PIIINP) appear to be almost exclusively cleared from the blood circulation by mannose receptor-mediated or scavenger receptor-mediated endocytosis in LSECs (Smedsrod et al., 1990; Smedsrod, 2004; Malovic et al., 2007). In addition, a broad range of other substrates is endocytosed by LSEC including oxidized and acetylated low density lipoproteins (LDLs), advanced glycation end products, immune complexes and microbial CpG motifs (Nagelkerke et al., 1983; Blomhoff et al., 1984; Skogh et al., 1985; Van Berkel et al., 1991; Smedsrod et al., 1997; Martin-Armas et al., 2006).

Immunological

LSECs express many antigens important for interactions with leukocytes and lymphocytes including CD40, CD54, CD80, CD86, MHC class I and II, ICAM-1, PECAM, E-selectin, P-selectin, L-SIGN, and FcγR (Enomoto et al., 2004; Lalor et al., 2006). The liver's lymphocyte population is selectively enriched in natural killer and natural killer T cells, which play critical roles in first line immune defense against invading pathogens, modulation of liver injury and recruitment of circulating lymphocytes (Racanelli and Rehermann, 2006). The LSEC expression of MHC Class II (Knolle and Gerken, 2000) is debated and some authors claim that the LSEC lack this receptor (Katz et al., 2004). LSECs have a possible role in antigen presentation (Limmer et al., 2000) and may be involved in the development of immunotolerance by inducing apoptosis in lymphocytes (Karrar et al., 2007). Fenestrations appear to have a role in mediating interactions between circulating immune cells and hepatocytes. Using a murine transgenic model of autoimmune hepatitis, it was shown that naive T cells interact directly with hepatocytes through fenestrations in the LSEC ("TEHLI" trans-endothelial hepato-

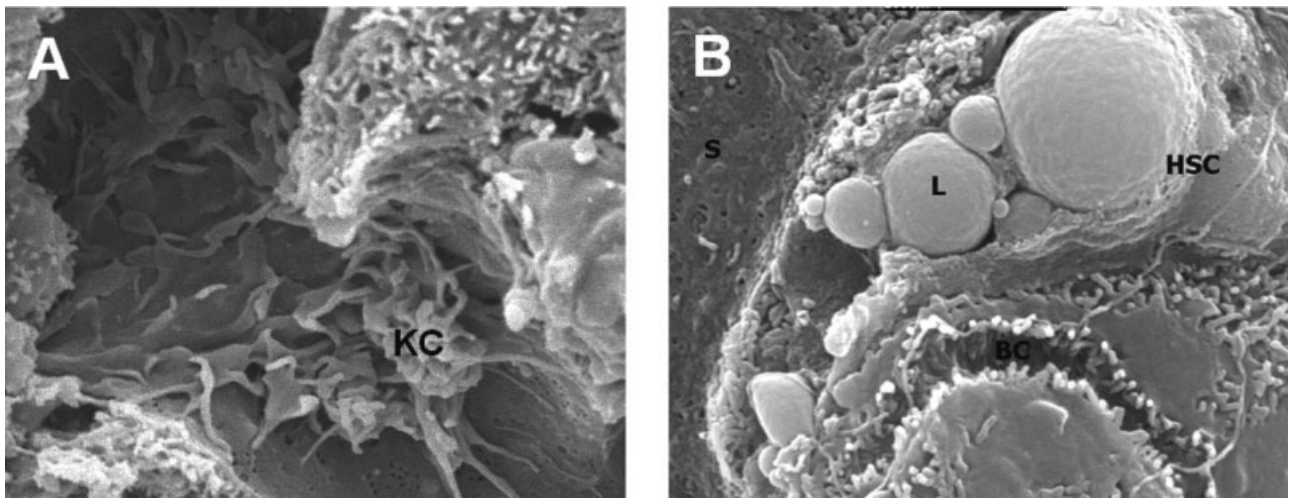

Fig. 2. Scanning electron micrographs of liver. **A:** Kupffer cell lying in a sinusoid. **B:** Stellate cell (HSC) containing lipid droplets (L) and lying between a sinusoid (S) and a bile canaliculus (BC).

cyte lymphocyte interactions), and this appears to be the first step in the development of immunotolerance (Jamieson et al., 2007). It was also found that activated lymphocytes and other leukocytes accessed the liver tissue in hepatitis by means of passage through the fenestrations and conversely that loss of fenestrations almost fully abolished any hepatitis (Warren et al., 2007).

In addition to the LSEC, the hepatic sinusoid contains several other cell types (Fig. 2). Stellate cells lie within the extracellular space of Disse and surround the LSECs with their cytoplasmic extensions. Stellate cells are fat storing cells containing large vitamin A droplets and may have a role in regulating sinusoidal blood flow through contraction or swelling (McCuskey, 2000). After liver injury, stellate cells become activated, characterized by loss of vitamin A-rich lipid droplets, production of extracellular matrix, and expression of α-smooth muscle actin. Kupffer cells (KCs) lie within the sinusoidal lumen and are the body's largest population of resident tissue macrophages. Together with the LSEC, the KCs make up the hepatic reticuloendothelial system with colloids and macromolecular waste substances being cleared mainly by the LSEC, and larger particles being eliminated by phagocytic uptake in KCs (Smedsrod, 2004). Finally, there are resident hepatic T cytotoxic lymphocytes (pit cells) that lie within the sinusoids and identified by their characteristic cytoplasmic inclusions called pits (Wisse et al., 1997).

Until recently, there has been relatively little research reported about the effects of aging on the hepatic sinusoid and microcirculation. In old age, there is a reduction in many hepatic functions such as drug and lipoprotein metabolism (James, 1985; Le Couteur and McLean, 1998; Schmucker, 1998, 2001, 2005). The age-related reduction in the hepatic clearance of medications is as much as 60% particularly for those drugs undergoing phase I and flow limited metabolism (Le Couteur and McLean, 1998; McLean and Le Couteur, 2004). The age-related reduction in the hepatic clearance of chylomicron remnants in humans is approximately 50% but remains unexplained (Krasinski et al., 1990). However, in vitro studies of hepatocyte enzyme function have not shown any consistent aging changes, apart from a reduction in liver ATP content and hepatocyte mitochondrial activity and numbers (Sastre et al., 1996; Le Couteur et al., 2001; Baur et al., 2006). It has been well established that total hepatic blood flow is reduced by 30–50% and liver mass, as a fraction of body weight is likewise reduced in old age by 25–35% (Le Couteur and McLean, 1998), and such changes will impact on liver function (Woodhouse and Wynne, 1988; Le Couteur and McLean, 1998; Schmucker, 1998). Recent studies described below have indicated that there are also substantial changes in the structure and function of the LSEC that are likely to have significant implications for liver function in old age.

OLD AGE AND THE HEPATIC SINUSOID

Pseudocapillarization of the LSEC

Although an early report indicated no major change in the structure of isolated LSECs with age (De Leeuw et al., 1990), it has recently been reported that old age is associated with ultrastructural changes in the hepatic sinusoidal endothelium and space of Disse from intact livers of the rat (Le Couteur et al., 2001; Jamieson et al., 2007), human (McLean et al., 2003), mouse (Warren et al., 2005; Ito et al., 2007), and the nonhuman primate, *Papio hamadryas* (Cogger et al., 2003). These changes were termed "pseudocapillarization," because the aging sinusoidal endothelium had become more like capillaries seen in other nonfenestrated vascular beds (Le Couteur et al., 2001). On electron microscopy, there is an increase of approximately 50% in endothelial thickness and a similar reduction in the porosity and number of fenestrations ("defenestration"; Table 1; Fig. 3). The effect of aging on the diameter of fenestrations was inconsistent; however, there is probably a trend toward a reduction in diameter. These changes were associated with perisinusoidal basal lamina deposition in many old livers and some scattered collagen in the space of Disse.

TABLE 1. Effect of old age on the ultrastructural parameters of the LSEC including endothelial thickness and fenestration porosity

Species	Young	Old	Fractional change with age	Reference
Thickness of LSEC (nm)				
Rat	230 ± 50	320 ± 80	1.39	(Le Couteur et al., 2001)
Human	165 ± 17	289 ± 9	1.75	(McLean et al., 2003)
Baboon	130 ± 8	186 ± 9	1.43	(Cogger et al., 2003)
Mouse	154 ± 4	245 ± 8	1.59	(Warren et al., 2005)
Rat	180 ± 5	211 ± 6	1.17	(Jamieson et al., 2007)
Mouse	230 ± 10	380 ± 20	1.65	(Ito et al., 2007)
Porosity of fenestrations in LSEC on scanning electron microscopy				
Rat	4.1 ± 2.3%	2.5 ± 1.2%	0.61	(Le Couteur et al., 2001)
Baboon	4.2 ± 0.5%	2.4 ± 0.4%	0.57	(Cogger et al., 2003)
Mouse	4.1 ± 2.2%	2.2 ± 3.5%	0.54	(Warren et al., 2005)
Rat	3.4 ± 0.3%	2.4 ± 0.1%	0.71	(Jamieson et al., 2007)
Mouse	130 ± 5 (per 50 μM^2)	63 ± 4 (per 50 μM^2)	0.48	(Ito et al., 2007)
Fenestration count on transmission electron microscopy (per 10 μm)				
Rat	2.7 ± 1.1	0.9 ± 0.8	0.33	(Le Couteur et al., 2001)
Human	7.7 ± 0.7	1.5 ± 0.4	0.19	(McLean et al., 2003)
Baboon	9.4 ± 0.9	5.5 ± 0.7	0.59	(Cogger et al., 2003)
Diameter of fenestrations (nm)				
Rat	73 ± 1	60 ± 1	0.82	(Le Couteur et al., 2001)
Mouse	74 ± 4	58 ± 12	0.78	(Warren et al., 2005)
Baboon	58 ± 1	70 ± 2	1.21	(Cogger et al., 2003)
Rat	68 ± 1	66 ± 2	0.97	(Jamieson et al., 2007)

LSEC = liver sinusoidal endothelial cells.

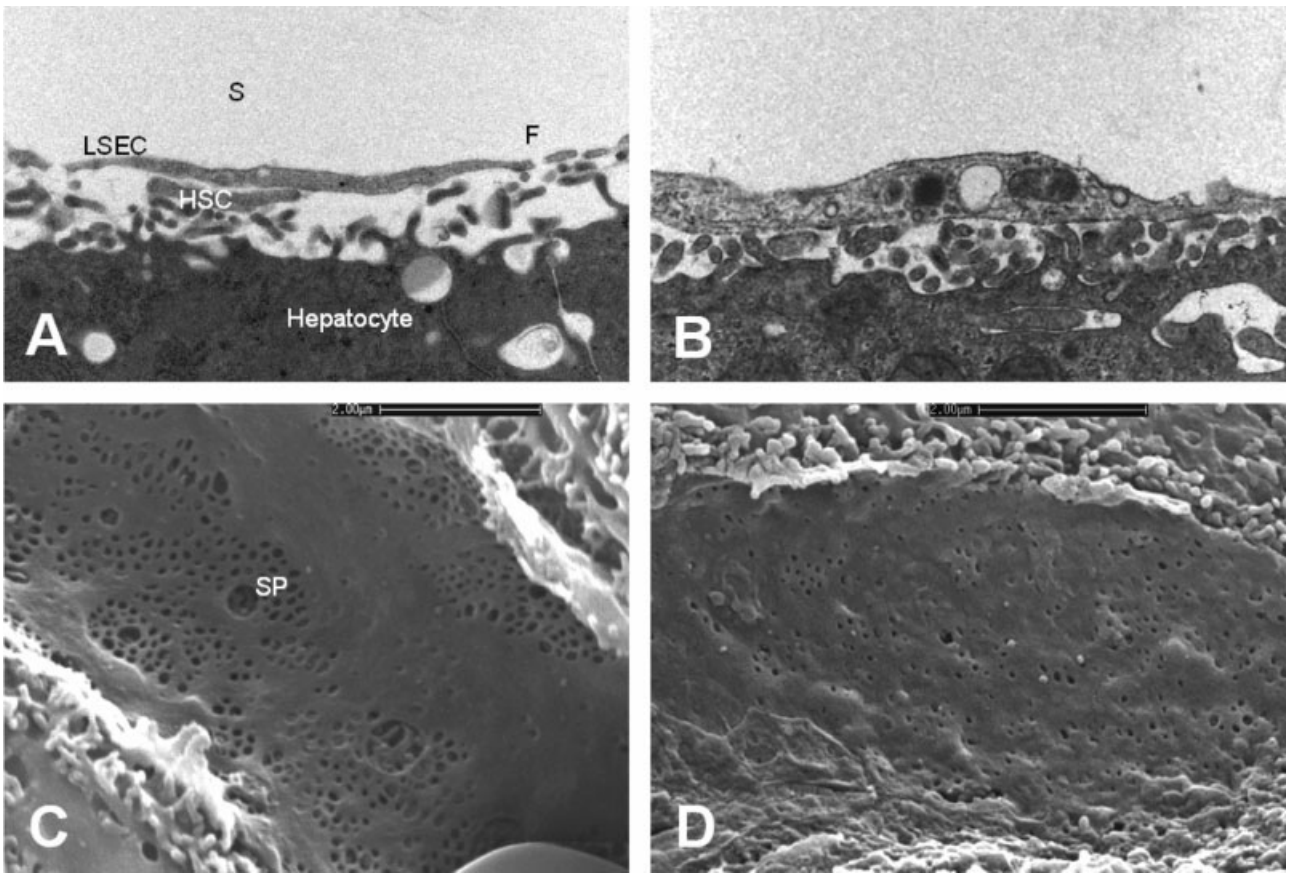

Fig. 3. Transmission and scanning electron micrographs of the liver sinusoidal endothelial cell. **A:** In the young liver, the endothelium (LSEC) is thin and perforated with fenestrations (F). The extension of a stellate cell (HSC) lies beneath the liver sinusoidal endothelial cell. **B:** In old livers, the endothelium is thickened and defenestrated. **C:** Scanning electron micrographs of young liver shows many fenestrations in the endothelial cell clustered into sieve plates (SP). **D:** Scanning electron micrographs shows loss of fenestrations and sieve plate structure in old age.

TABLE 2. Effect of old age on antigen and staining markers of the endothelium, stellate cell and extracellular matrix

Species	Rat	Rat	Rat	Mouse	Mouse	Human	Baboon
Reference	(Le Couteur et al., 2001)	(Jamieson et al., 2007)	(Hilmer et al., 2005)	(Warren et al., 2005)	(Ito et al., 2007)	(McLean et al., 2003)	(Cogger et al., 2003)
Endothelial markers							
vWf	↑	↑	↑	↔		↑	↑
Caveolin-1		↓					
ICAM-1					↑		
Stellate cell activation							
α-SMA							↔
Desmin							↔
Extracellular matrix							
Collagen I	↑					↑	
Collagen IV	↑	↑				↑	↔
Fibronectin							↔
Laminin				↔			↑
Sirius red		↑		↑	↑		↔
Masson's trichrome				↔		↑	↔

vWf = von Willebrand factor; ICAM-1 = intracellular adhesion molecule-1; α-SMA = alpha smooth muscle actin).

The age-related changes in the ultrastructure of the LSECs are associated with altered but inconsistent expression of several antigens and stains used to identify pathology in the hepatic sinusoid. The endothelial marker von Willebrand factor is a glycoprotein mediating attachment of platelets after endothelial injury and is not normally expressed in healthy young liver sinusoids. In most studies, the expression of vWf is increased in old age (Table 2; Fig. 4). In addition, one study showed a reduction in caveolin-1 expression (Jamieson et al., 2007; Fig. 4), and another single report showed increased ICAM-1 expression (Ito et al., 2007). Both findings support the concept of significant aging changes in LSEC biology. There is variable up-regulation of markers of extracellular matrix, mostly collagen IV and Sirius red. These findings, together with the occasional observation of collagen in the space of Disse on electron microscopy, are consistent with the conclusion that there is some patchy perisinusoidal fibrosis in old age. Finally, a single study in baboons found no age-related expression in the markers of stellate cell activation, α-SMA, and desmin, suggesting that stellate cell activation is not present (Cogger et al., 2003).

Old age was associated with some deposition of lipofuscin and multinucleate cells in the hepatic parenchyma; however, there was no other indication of liver disease on the basis of standard pathological definitions and blood liver function tests (Le Couteur et al., 2001; Cogger et al., 2003; McLean et al., 2003; Warren et al., 2005; Ito et al., 2007). However, there is a reduction in ATP levels in the liver in old age from 2.81 ± 0.33 to 2.25 ± 0.26 μmol/g in rats (Le Couteur et al., 2001), and this finding is associated with reduced numbers of mitochondria with increased size and impaired function (Sastre et al., 1996; Baur et al., 2006; Lopez-Lluch et al., 2006).

These findings may bolster very early reports of the effects of aging on the liver. Hinton and Williams noted the occasional occurrence of perisinusoidal fibrosis detected with reticulin staining in aged mice (Hinton and Williams, 1968). Likewise, the presence of slight portal fibrosis in the absence of septa formation, along with other minor changes, were considered to be evidence for "senile hepatopathy" in humans (Findor et al., 1973). Increased lysosomes were noted in LSECs isolated from very old (35 months) rats (Knook and Sleyster, 1976).

To determine whether pseudocapillarization is preventable, the effects of caloric restriction have been investigated. A reduction of caloric intake by approximately 40% increases maximum life expectancy by approximately 40%, and this finding is associated with a delay in the onset of most age-associated disorders and pathology (Everitt et al., 2005). In old caloric restricted rats, endothelial thickness was significantly less (190 ± 7 nm vs. 211 ± 6 nm) and fenestration porosity was significantly greater (3.9 ± 0.3% vs. 2.4 ± 0.1%) than in old ad libitum fed rats. Caloric restriction prevented the age-related decrease in caveolin-1 expression and increase in perisinusoidal collagen IV staining (Jamieson et al., 2007). Given the probable role of caveolin-1 in maintaining fenestral integrity (Yokomori et al., 2001), it is of interest that old age is associated with reduced fenestrations in association with reduced caveolin-1 expression, and that these changes were reversed by caloric restriction. It is possible that old age is associated generally with reduced caveolin-1 expression and the specific effect in the LSEC is defenestration.

Age-related defenestration may have several implications. In particular, the effect of defenestration on the hepatic disposition of lipoproteins such as chylomicron remnants has been reported (Le Couteur et al., 2002; Hilmer et al., 2005; see below). In addition the loss of fenestrations and altered diffusional properties of the aged LSEC and space of Disse might influence the hepatic clearance of medications, hence contributing to adverse drug reactions (Le Couteur et al., 2005). It is also possible that the loss of fenestrations might contribute to autoimmune disease in older people by impeding the interactions between naive T lymphocytes and hepatocytes that are thought to induce immunotolerance (Warren et al., 2006).

In summary, old age is associated with thickening and defenestration of the LSEC and altered expression of some endothelial antigens. This is associated with minor perisinusoidal fibrosis but no other features consistent

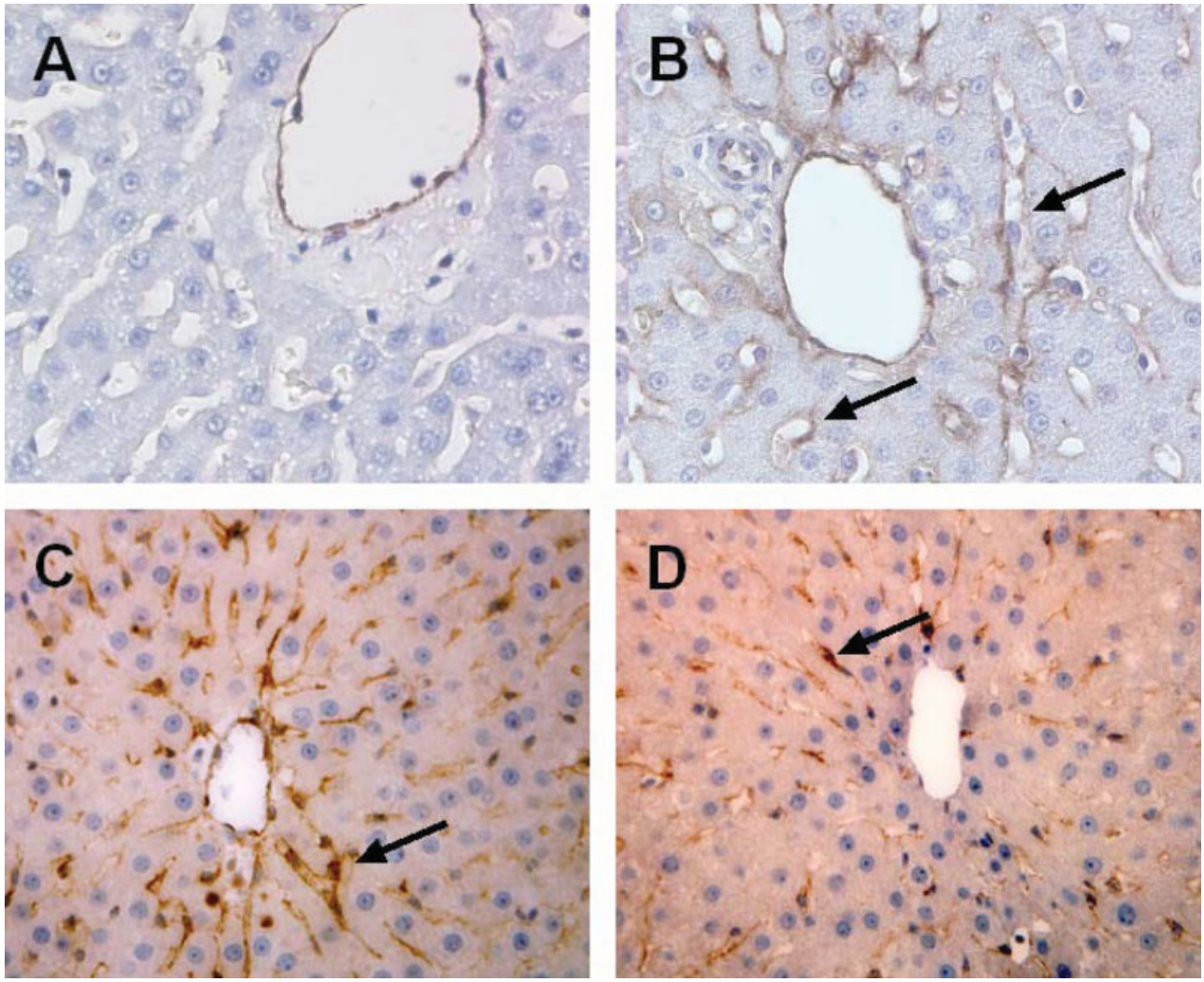

Fig. 4. Immunohistochemical changes in the perisinusoidal region in aging. **A:** In young baboon liver, von Willebrand factor is not expressed along the sinusoids. **B:** In old baboon liver, there is extensive perisinusoidal expression of von Willebrand factor (arrows). **C:** In young rat liver, there is perisinusoidal expression of caveolin-1 (arrows). **D:** In old rat liver, there is reduced perisinusoidal expression of caveolin-1.

with liver disease, thereby indicative of a primary endothelial pathogenesis. At a minimum these changes should be taken into consideration when diagnosing or studying liver disease in older people. However, age-related changes in the LSEC appear to have broader implications for the effects of aging on liver function which may include the metabolic syndrome with its burden of stroke and cardiac disease.

Aging and Hepatic Perfusion

Most studies in humans and animals have shown that total hepatic blood flow whether determined by Doppler or dye dilution is reduced in the order of 30–50% and parallels the age-related reduction in liver mass (Wynne et al., 1989, 1990; Le Couteur and McLean, 1998; Schmucker, 1998, 2001, 2005). Mechanisms for this change remain unclear. The hemodynamic responsiveness of the perfused rat liver does not change (Le Couteur et al., 1992) nor is there any increase in portal pressure in old age (Le Couteur and McLean, 1998).

Using the clearance of colloidal albumin, Brouwer et al calculated that liver perfusion in rats was 1.30 ± 00.13 mL/min/g at 3 months, 1.54 ± 0.19 mL/min/g at 6 months and 1.33 ± 0.28 mL/min/gram of liver at 36 months, suggestive of a reduction in perfusion with in old age (Brouwer et al., 1985). Recently Ito et al. (Ito et al., 2007) used high resolution in vivo microscopy to investigate aging in mouse liver. There was a 14% reduction in the numbers of perfused sinusoids between 0.8 and 27 months of age and a 35% reduction in sinusoidal blood flow. This finding was associated with a marked increase in the perisinusoidal expression of ICAM-1 and an increase in leukocyte adhesion. Slightly narrower sinusoids with thickened LSECs and swollen stellate cells with abundant lipid droplets were also observed. It was concluded that these changes were mechanistically linked to the age-related reduction in

hepatic perfusion and hepatic blood flow (Ito et al., 2007). Similarly, an age-related increase in the number of sinusoidal polymorphs, especially in response to endotoxin has been reported (Durham et al., 1990). On the other hand, Vollmar et al. (2002) used in vivo microscopy to study sinusoidal perfusion in the rat. They reported a minor reduction of sinusoidal density to 87% over life, but concluded that there were no aging changes in sinusoidal perfusion, leukocyte adhesion, or sinusoidal diameter. However, there was a reduction in sinusoidal flow from 9.3 ± 0.7 pL/s at 3 months to 6.8 ± 0.7 pL/s at 24 months. The increased mass of the liver during maturation and in older rats was explained by an increase in lobular size rather than an increase in the number of lobules. The average lobular size changed from 0.188 ± 0.021 mm^2 at 1 month, 0.420 ± 0.066 mm^2 at 3 months, 0.749 ± 0.033 mm^2 at 12 months and 0.655 ± 0.021 mm^2 at 24 months (Vollmar et al., 2002).

Aging and LSEC Endocytosis

The morphological changes in the LSEC in old age might also affect its endocytic function. So far few reports address this issue. In rats, the uptake of colloidal carbon was found to be reduced in old age, and this was attributed to impaired Kupffer cell activity (De Leeuw et al., 1983; Heil et al., 1984; Yamano et al., 2000; Videla et al., 2001). However, colloidal carbon is also taken up by LSEC endocytosis (Fujita et al., 1983); therefore, these results may need to be re-interpreted. In a study by Brouwer et al. (1985), the in vivo LSEC capacity for uptake of colloidal (heat-aggregated) albumin in rat was not influenced by age but other studies have reported a 53% reduction of in vivo LSEC uptake of azoaniline-albumin in 22–24 month rats compared with 6–8 month rats (Caperna and Garvey, 1982), and a 80% reduction of in vivo LSEC uptake of sulfanilate-azo-albumin in 28 month rats compared with 12 month rats (Heil et al., 1984), Interestingly, in the three age groups analyzed by Heil et al. (1984), the endocytosis of sulfanilate-azo-albumin peaked at 12 months of age, whereas the uptake in 28 month rats equaled that of 4 month rats.

Recently, in vivo microscopy was used to detect the LSEC uptake of two fluorescently labeled scavenger receptors ligands, advanced glycation end product (AGE) -modified albumin and formaldehyde-treated albumin in aged mice (Ito et al., 2007). There was suppressed endocytic function in old mice, especially in the pericentral area. It was concluded that such an age-related reduction in this activity will increase the risk of extrahepatic deposition and deleterious effects of circulating waste macromolecules. Of note, advanced AGEs are linked with many age-related diseases such as diabetes mellitus, atherosclerosis, and dementia (Singh et al., 2001).

Aging and Kupffer cells

Two early studies of Kupffer cells in humans noted an increase in their numbers and activity in old age (Schaffner and Popper, 1959; Findor et al., 1973). In rats, an increase in numbers has been reported (from 2.2 ± 0.2 to 5.5 ± 0.6 per HPF) (Hilmer et al., 2007) whereas another study reported a reduction in volume density (from 1.34 ± 0.16% to 1.13 ± 0.23%; Martin et al., 1992). Kupffer cells from old rat livers have less pseudopodia and actin and myosin cytoskeleton (Sun et al., 1998) and an increased number of lysosomes (Knook and Sleyster, 1976).

The effects of age on Kupffer cell endocytic activity are inconsistent. The uptake of colloidal carbon is either reduced by 35% (Videla et al., 2001) or unchanged (Vomel et al., 1981; De Leeuw et al., 1983; Yamano et al., 2000), whereas the uptake of denatured albumin has usually been reported to be reduced, by 23% (but not statistically significant) between 6 and 22 months in rats (Caperna and Garvey, 1982), 58% between 12 and 28 months in rats (Heil et al., 1984) and by 40% between 6 and 36 months in rats (Brouwer et al., 1985). These conclusions may be influenced by any aging effects on LSEC endocytosis of these substrates (Hilmer et al., 2007). The uptake of large microspheres (500–1,100 nm) by Kupffer cells is either largely unchanged (De Leeuw et al., 1983; Vollmar et al., 2002), decreased (Sun et al., 1998), or increased (Hilmer et al., 2007; Ito et al., 2007). Kupffer cell uptake of other substrates such as mitochondria (Martin et al., 1994) and Cu-ceruloplasmin (Vomel et al., 1984) is reduced in old age. The responsiveness of Kupffer cells to stimuli is diminished in old age as determined by carbon-induced oxygen consumption (Videla et al., 2001) and cadmium-induced phagocytosis (Yamano et al., 2000). Despite these inconsistencies, it is likely that the ability of the Kupffer cell to mount an effective immune response will be impaired given the established negative effects of aging on systemic immunity (Weng, 2006). Furthermore, any increase in Kupffer cell number and basal activity is not necessarily unexpected because aging is associated with increased systemic markers of inflammation (Johnson, 2006).

Aging and Stellate Cells

A regularly reported finding has been the presence of fat engorged stellate cells in old age, including mice, rats, and baboons (Durham et al., 1990; Martin et al., 1992; Vollmar et al., 2002; Cogger et al., 2003; Grizzi et al., 2003; Warren et al., 2005; Ito et al., 2007). These are apparent on electron microscopy but also can be identified by their signet ring appearance on light microscopy (Cogger et al., 2003; Warren et al., 2005; Fig. 5). These swollen stellate cells in old age are not activated because stellate cells lose fat droplets during activation. It has also been reported that vitamin A autofluorescence is increased in old age, which is further evidence that stellate cells are not activated (Vollmar et al., 2002). In addition, α-SMA and desmin staining, markers of stellate cell activation, were not increased in the one study where it was reported (Cogger et al., 2003). This is unlike most liver diseases, including hepatic fibrosis, which are characterized by stellate cell activation (Friedman, 2004). Some of the stellate cells are so swollen that they protrude into the sinusoidal lumen and potentially could reduce sinusoidal blood flow.

Implications of Pseudocapillarization on Lipoprotein Disposition

Given the role of fenestrations in the transfer of lipoproteins from blood to the hepatocyte, it is plausible that

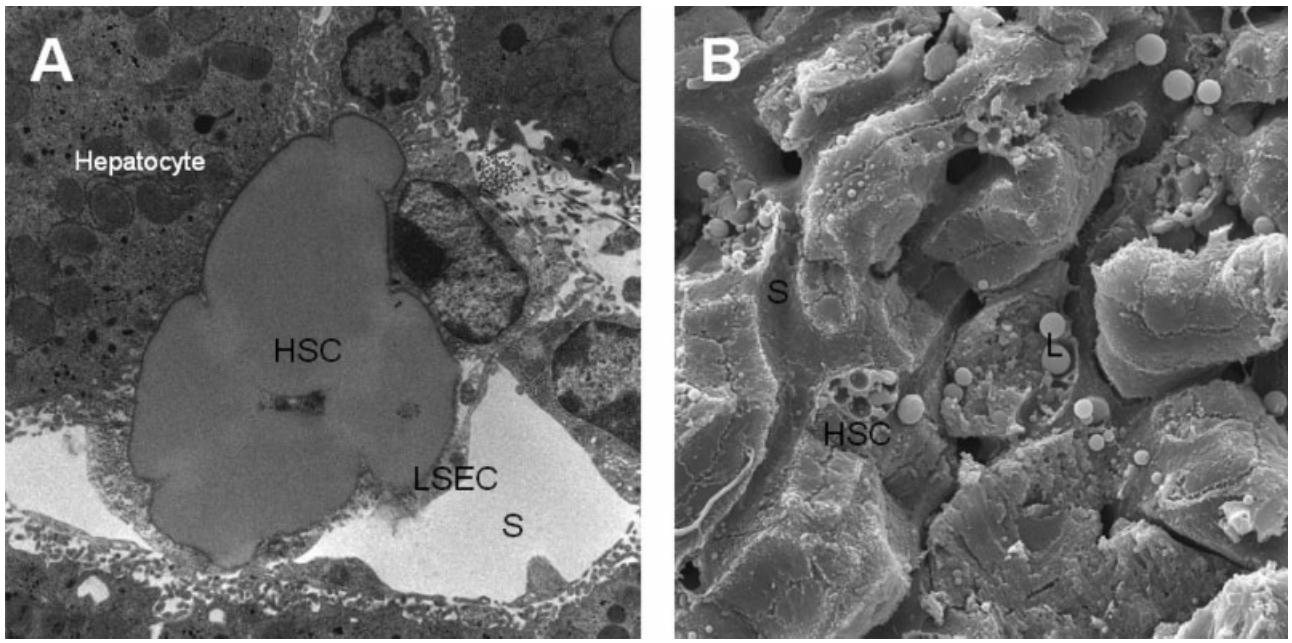

Fig. 5. Electron micrographs of stellate cells from an old mouse. **A:** Transmission electron micrograph showing a fat engorged stellate cell (HSC) protruding into the sinusoid (S). **B:** Scanning electron micrograph showing numerous stellate cells (HSC) engorged with many lipid droplets (L).

age-related defenestration will impair lipoprotein clearance by the liver and contribute to dyslipidemia in older people (Le Couteur et al., 2002). The incidence and prevalence of atherosclerosis increases dramatically with old age, and its clinical manifestations are present in the majority of older people (Lakatta and Levy, 2003). As the population ages, coronary syndromes are occurring primarily in older people rather than in middle-aged patients with standard vascular risk factors such as smoking, fasting hyperlipidemia, and hypertension (Steg et al., 2002). The prevalence of such risk factors does increase in old age, however, their overall impact on susceptibility to cardiovascular disease decreases, as old age itself becomes the predominant influence (Kannal, 2002). The clearance of chylomicron remnants is significantly impaired in older people (Krasinski et al., 1990; Borel et al., 1998), and in people aged 65 years and older, remnant-like lipoprotein cholesterol is strongly associated with the development of coronary artery disease (Simons et al., 2001).

To determine whether age-related defenestration impairs the transfer of lipoproteins across the LSEC and, hence, cause impaired hepatic clearance of chylomicron remnants, the multiple indicator dilution method was used to study lipoprotein disposition in perfused rat livers (Hilmer et al., 2005). In young livers, lipoproteins of average diameter of 53 $\pm$ 8 nm were able to access the entire extracellular space, whereas in old livers, the lipoproteins were confined to the vascular space. These results parallel those seen following the administration of poloxamer 407. This surfactant causes severe hyperlipidemia, marked defenestration and impaired hepatic uptake of lipoproteins (Cogger et al., 2006). The corollary of these results is that modulation of LSEC fenestrations might be a therapeutic target for the treatment of age-related dyslipidemia.

CONCLUSIONS

In the past, the liver was considered to be remarkable for the absence of any major age-related alteration (Popper, 1986). However, recently changes in the hepatic sinusoid with old age have been identified that probably contribute to the substantial age-related changes in liver function. These changes, termed pseudocapillarization, include thickening and defenestration of the liver sinusoidal endothelial cell, sporadic deposition of collagen and basal lamina in the extracellular space of Disse, and fat engorged, nonactivated stellate cells. In addition, there is endothelial up-regulation of von Willebrand factor and ICAM-1 with reduced expression of caveolin-1. Functional implications include reduced sinusoidal perfusion, impaired LSEC endocytosis, and reduced transfer of lipoproteins across the LSEC. Given the extensive range of substrates metabolized by the liver, these age-related changes in the hepatic sinusoid and microcirculation may have important systemic implications for aging and age-related diseases.

The effect of aging on the hepatic sinusoid is a relatively new focus of research, but timely given the aging of the population. Clearly additional studies are required to confirm and clarify the changes of pseudocapillarization and its reversal by caloric restriction. In addition, the implications of any age-related changes in LSEC endocytosis on systemic disease, and of defenestration on immunotolerance are important future areas to pursue. Developing therapeutic agents to modulate fenestrations will provide a definitive answer to the clinical

relevance of age-related changes in the hepatic sinusoid on dyslipidemia.

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