Hypothesis

Hepatic pseudocapillarisation and atherosclerosis in ageing

David G Le Couteur, Robin Fraser, Victoria C Cogger, Allan J McLean

Cardiovascular disease secondary to atherosclerosis is the main cause of death and disability in industrialised countries, and ageing is the foremost risk factor for atherosclerosis. We present a hypothesis linking age-specific structural change in the liver with accepted pathogenic mechanisms leading to atherosclerosis. Ageing in the liver is associated with pseudocapillarisation of the sinusoidal endothelium, which is characterised by thickening of endothelium, basement membrane formation, and defenestration (loss of pores). Fenestrations (pores) normally form a liver sieve that allows passage of chylomicron remnants for subsequent uptake and metabolism by hepatocytes. Ageing is associated with impaired clearance of chylomicron remnants, postprandial hypertriglyceridaemia, and hence, atherosclerosis, which we propose is linked directly to loss of permeability of the liver sieve because of defenestration associated with pseudocapillarisation. Development of methods to maintain fenestrations of sinusoidal endothelium or to facilitate refenestration might be a new therapeutic strategy for management of cardiovascular disease in old people.

Ageing is the main risk factor for atherosclerosis, and cardiovascular disease is the foremost cause of death and disability in industrialised countries.[1,2] The Framingham study attributes vascular risk to ageing, which is a more than two to three times greater predictor of vascular risk than smoking, hypertension, diabetes mellitus, and hypercholesterolaemia.[1] As people get older, the importance of hypertension and smoking as risk factors for ischaemic heart disease decline whereas the effect of age becomes dominant.[1] Risk of cardiovascular disease secondary to atherosclerosis rises exponentially with ageing,[2] and in older people, vascular disease is the main contributor to disability and death.[2]

Despite this close association, there is no generally accepted mechanism linking ageing and atherosclerosis. Atherosclerosis might take many years to develop, and risk factors such as hyperlipidaemia and hypertension are often seen in old people. Even so, the association between ageing and atherosclerosis presents ". . . a conundrum of significant proportions to clinicians and basic scientists alike".[2]

We postulate that age-specific changes in the sinusoidal endothelium of the liver[3] initiate atherosclerosis by well recognised and specific age-related changes in lipid metabolism. All individual links in the mechanistic chain between the ageing liver and cardiovascular disease have been established. What remains to be established is the contribution of this mechanism to vascular disease in old people.

Lancet 2002; **359:** 1612–15

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Ageing and the liver sieve

Ageing in the liver was previously thought to be associated with few important changes, apart from reduction in mass and blood flow.[4] Motivated by the observation that such changes cannot fully explain the age-related impairment of hepatic drug metabolism,[4] we examined structures between the blood vessel and the hepatocyte that could block substrate transfer—ie, the sinusoidal endothelium and space of Disse.

The capillaries within the normal liver are highly specialised. The sinusoidal endothelium is very thin and contains pores, called fenestrations, with diameters of

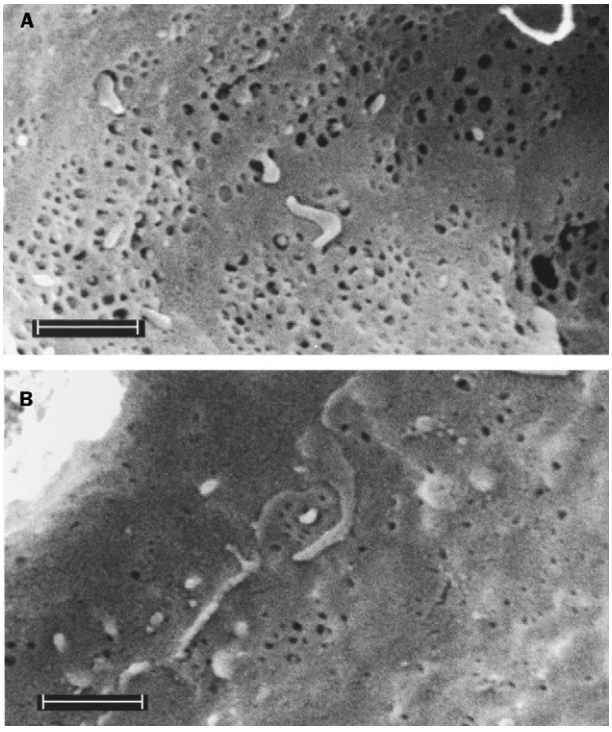

Figure 1: **Scanning electronmicrographs of the sinusoidal endothelium from the liver of (A) an adult rat aged 6 months and (B) an old rat aged 26 months**
There is loss of fenestrations from the sinusoidal endothelium of the old rat liver. Bar=1 µm. Reproduced from reference 3 with permission of W B Saunders.

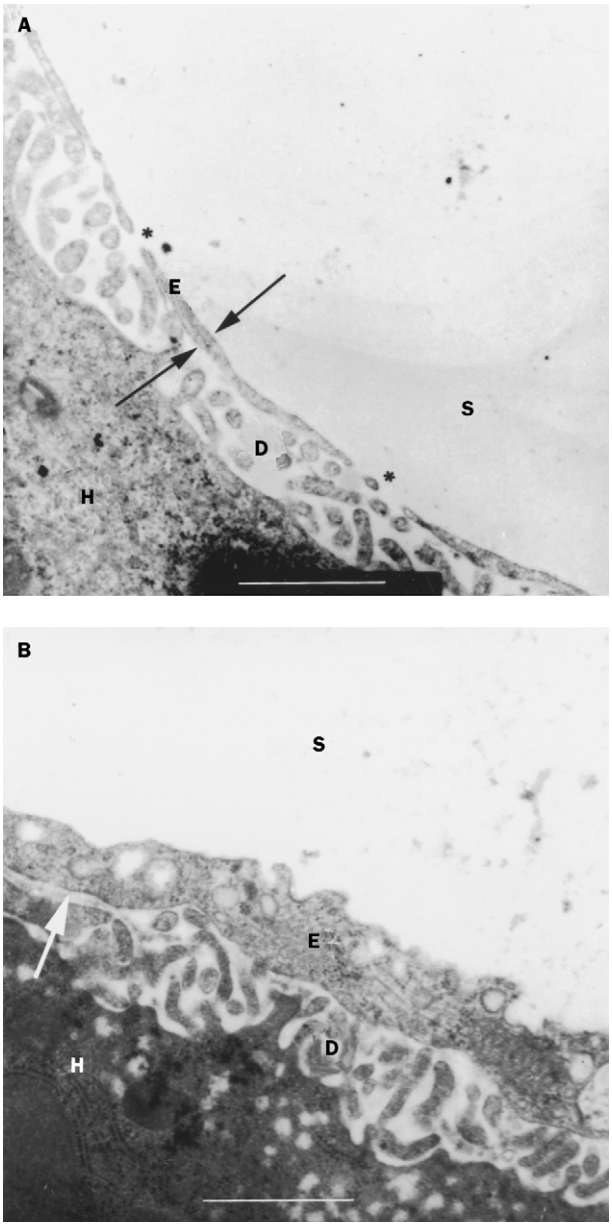

Figure 2: **Transmission electronmicrograph of the liver of (A) an adult rat aged 6 months and (B) an old rat aged 26 months**
The hepatocyte (H) is separated from the sinusoid (S) by the sinusoidal endothelium (E) and space of Disse (D). *Fenestration; black arrows=narrow endothelium; white arrow=basal lamina. Bar=1µm. Reproduced from reference 3 with permission of W B Saunders.

about 100 nm (figures 1 and 2). There is no basal lamina and the space of Disse is essentially free of collagen. The sinusoidal endothelium was termed the liver sieve by Fraser[5] and Wisse,[6] because these structural features facilitate transfer of all but the largest colloids from blood to hepatocytes.

Ageing in the liver is associated with profound changes in the sinusoidal endothelium and space of Disse.[3] By electronmicroscopic analysis, ageing was shown to be associated with a 50% increase in thickness of the endothelium and a 50% reduction in the number of fenestrations in the sinusoidal endothelium (defenestration; figures 1 and 2). Immunohistochemical analysis showed expression of factor VIII-related antigen, collagen IV, and collagen I in the sinusoids of old livers. Ageing was also shown to be associated with deposition of minor amounts of collagen and formation of basement membrane within the space of Disse (figure 2). These factors are seen in capillaries of non-hepatic tissues and cirrhosis but not in healthy young livers.

These changes have been termed pseudocapillarisation because the ageing sinusoidal endothelium loses its porous liver sieve structure and becomes like capillaries seen in other vascular beds.[3] The changes have been recorded in the livers of old rats[3] and in preliminary examination of human livers.[7]

The pathogenesis of these changes with ageing is unclear. The liver receives most of its blood supply via the portal vein, and the sinusoidal endothelium will probably be exposed to high concentrations of gut-derived toxins. Various xenobiotics (alcohol, nicotine, endotoxins)[8] and oxidants[9] can produce striking changes in sinusoidal endothelium.

The liver sieve and lipid metabolism

Chylomicrons are spherical lipoproteins rich in triglycerides that are formed in the intestine from dietary lipids. They are large particles with diameters of 100–1000 nm that are unable to pass through the fenestrations of hepatic sinusoidal endothelium (figure 3).[5,8] Chylomicrons are metabolised to chylomicron remnants by lipoprotein lipase, which is present on the endothelium of systemic capillaries. In rats that have had hepatectomy, chylomicron remnants accumulate in the blood,[10] which indicates the importance of the liver in metabolism of chylomicron remnants. Chylomicron remnants (diameter 30–80 nm) are smaller than chylomicrons and have acquired apolipoprotein E. These particles pass through fenestrations of the sinusoidal endothelium[8,11] and are sequestered within the space of Disse, in which they undergo further metabolism by hepatic lipase. Chylomicron remnants bind to hepatocyte membrane receptors that recognise apolipoprotein E (LDL receptor, LDL receptor-related protein) thus allowing uptake into, and subsequent metabolism by, hepatocytes.[8,11]

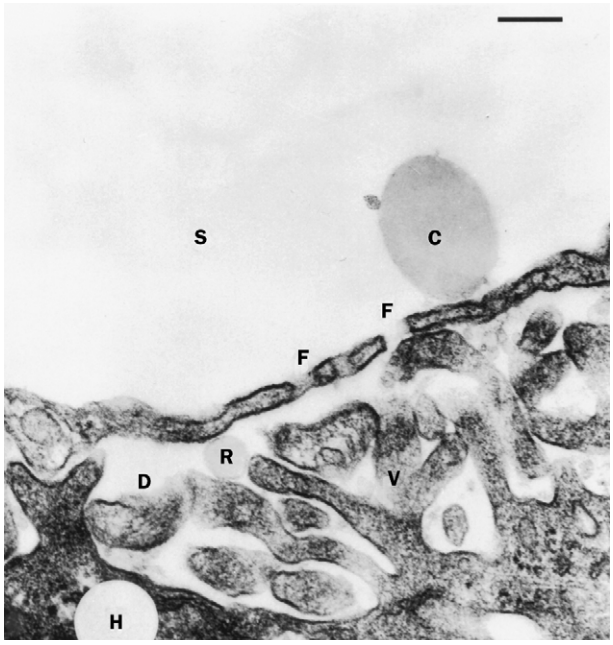

Figure 3: **Transmission electronmicrograph of the liver**
A chylomicron (C) is within the lumen of the sinusoid (S) and a chylomicron remnant (R) is trapped within the space of Disse (D). F=fenestration; V=hepatocyte microvilli; H=hepatocyte. Bar=100 nm. Reproduced from reference 8 with permission of W B Saunders.

The role of fenestrations in steric selection of chylomicrons and chylomicron remnants has been well established in vivo.[8,11] Wisse[12] postulated that fenestrations filter chylomicrons and other lipoproteins according to size. Membranes isolated from hepatocytes cannot differentiate between chylomicrons and chylomicron remnants, whereas in vivo, livers selectively take up chylomicron remnants.[13] Electronmicroscopy has shown that large chylomicrons are present in sinusoidal blood whereas smaller particles (remnants) are seen in the space of Disse (figure 3).[5] The liver differentially traps radiolabelled chylomicrons of different sizes, such that smaller particles are trapped to a greater extent than those larger than 100 nm.[5]

The effect of defenestration on lipid metabolism has also been investigated. Nicotine, endotoxins, and dimethylnitrosamine induce defenestration and are associated with enhanced susceptibility to dietary cholesterol.[8] Likewise, rabbits and chickens, which have reduced fenestrations compared with man and rodents, are vulnerable to dietary cholesterol, resulting in hyperlipidaemia and atherosclerosis.[8] In man, there is correlation between alcohol consumption, defenestration, and hyperlipidaemia, and we have postulated that loss of the liver sieve contributes to alcohol-induced hypertriglyceridaemia.[14]

Defenestration causes impaired clearance of chylomicron remnants after meals because circulating chylomicron remnants produced from dietary fat are unable to enter the space of Disse.[8,14] Because remnants are rich in triglycerides, defenestration is manifested as postprandial hypertriglyceridaemia.

Ageing and postprandial hyperlipidaemia

Ageing is associated with hyperlipidaemia.[2] Abbott and colleagues[15] however, report a reduction in cholesterol concentrations in the very old, perhaps as a result of concomitant disease and preterminal loss of bodyweight.

Postprandial hyperlipidaemia specifically is common in old age.[16–18] The magnitude of postprandial hypertriglyceridaemia after a fat-rich meal correlates with age in man.[16] Fasting triglyceride concentrations are greater in old people, and the rise after a meal is nearly two times greater in old than in young people.[17] Old age is associated with raised postprandial retinyl-ester concentrations (a marker of chylomicron remnants) and two-fold lengthening of residence time of retinyl esters in triglyceride-rich lipoproteins.[18]

Ageing is also associated with reduced hepatic expression of LDL receptor and LDL receptor-related protein, and this reduction in expression has been proposed as one explanation for diminished clearance of chylomicron remnants in old age.[19] However, the effects of any age-related changes in expression of LDL receptor and LDL receptor-related protein—and other lipid-binding substrates in the space of Disse, such as apolipoprotein E and heparan sulphate proteoglycan—will be constrained if chylomicron remnants are unable to enter the space of Disse because of loss of fenestrations.

Diabetes mellitus, a disorder sometimes regarded as premature ageing, is associated with qualitatively similar ultrastructural changes in the liver[20] and with impaired hepatic clearance of chylomicron remnants,[21] and these findings could be relevant.

Postprandial hyperlipidaemia and atherosclerosis

The hypothesis that postprandial hyperlipidaemia is atherogenic was proposed by Moreton,[22] Fraser,[23] and Zilversmit,[24] and is well established after much review.[11]

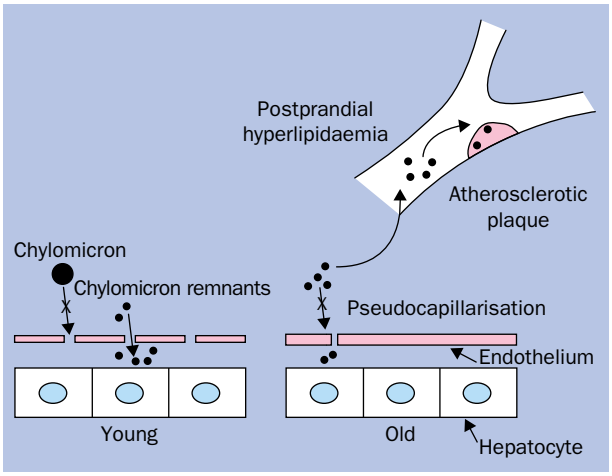

Figure 4: **Hypothesis linking age-related reduction in porosity of hepatic sinusoidal endothelium (pseudocapillarisation) with development of atherosclerosis**

Clinical evidence for the association includes the observation that coronary artery disease is associated with raised postprandial hypertriglyceridaemia independent of fasting LDL and HDL concentrations.[11,25] Furthermore, clearance of chylomicron remnants is reduced substantially in normolipidaemic patients with coronary artery disease.[25] With respect to the mechanism, chylomicron remnants and retinyl esters are taken up by the arterial vessel wall by transcytosis, and have been noted in atherosclerotic plaques.[24,26]

Testing the hypothesis

The hypothesis is simple, and every step in the pathogenic chain has already been validated. The hepatic sinusoidal endothelium contains pores called fenestrations that allow passage of particles of a certain size (chylomicron remnants; figure 4). With old age, the diameter and frequency of fenestrations lessens, with a rise in matrix in the space of Disse, thus leading to diminished porosity of the liver sieve and consequent reduction in clearance of chylomicron remnants. This impaired clearance is linked to atherosclerosis.

Testing the hypothesis will depend on establishment of how much this mechanism contributes to postprandial hyperlipidaemia and atherosclerosis in old age, and whether interventions that modify fenestrations improve chylomicron remnant clearance; three specific examples of intervention follow. First, we could examine the effects of age on clearance and volume of distribution of chylomicron remnants in the livers of animals and systemic clearance in human beings—eg, with new techniques for measurement of chylomicron remnant clearance.[27] Second, we could investigate agents that prevent defenestration or facilitate refenestration (eg, pantethine)[8] in old age. Third, analysis of the effects of age on deposition in atherosclerotic plaques of retinyl esters and remnant-specific apolipoprotein B48 could be done. Development of methods to maintain fenestrations of sinusoidal endothelium or to facilitate refenestration represents a novel therapeutic strategy for management of cardiovascular disease in old people.

Conflict of interest statement
None declared.

Acknowledgments
The study was sponsored by the National Health and Medical Research Council of Australia, University of Sydney SESQUI Grant, Ageing and Alzheimer's Research Foundation, Private Practice Trust Fund of the

Canberra Hospital, Health Research Council of New Zealand, Canterbury Medical Research Foundation, and Lottery Health Board of New Zealand.

References

1 Grundy SM, Pasternak R, Greenland P, Smith S, Fuster V. Assessment of cardiovascular risk by use of multiple-risk-factor assessment equations: a statement for healthcare professionals from the American Heart Association and the American College of Cardiology. *J Am Coll Cardiol* 1999; **34:** 1348–59.

2 Hazzard W. Dyslipoproteinemia in the elderly. *Clin Geriatr Med* 1992; **8:** 89–102.

3 Le Couteur DG, Cogger VC, Markus AM, et al. Pseudocapillarization and associated energy limitation in the aged rat liver. *Hepatology* 2001; **33:** 537–43.

4 Le Couteur DG, McLean AJ. The aging liver: drug clearance and an oxygen diffusion barrier hypothesis. *Clin Pharmacokinet* 1998; **34:** 359–73.

5 Fraser R, Bosanquet AG, Day WA. Filtration of chylomicrons by the liver may influence cholesterol metabolism and atherosclerosis. *Atherosclerosis* 1978; **29:** 113–23.

6 Wisse W, De Zanger RB, Charels K, Van Der Smissen P, McCuskey RS. The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse. *Hepatology* 1985; **5:** 683–92.

7 Cogger VC, McLean AJ, Le Couteur DG. The liver sinusoidal endothelium in ageing. 2nd International Conference on Hepatic and Splanchnic Circulation in Health and Disease, Aug 24–26, 2001, Dunedin, New Zealand.

8 Fraser R, Dobbs BR, Rogers GW. Lipoproteins and the liver sieve: the role of fenestrated sinusoidal endothelium in lipoprotein metabolism, atherosclerosis, and cirrhosis. *Hepatology* 1995; **21:** 863–74.

9 Cogger VC, Mross P, Hosie MJ, Ansselin AD, McLean AJ, Le Couteur DG. The effect of acute oxidative stress on the ultrastructure of the perfused rat liver. *Pharmacol Toxicol* 2001; **89:** 306–11.

10 Redgrave TG. Formation of cholesteryl ester-rich particulate lipid during metabolism of chylomicrons. *J Clin Invest* 1970; **49:** 465–71.

11 Yu KC, Cooper AD. Postprandial lipoproteins and atherosclerosis. *Frontiers Biosci* 2001; **6:** 332–54.

12 Wisse E. An electron microscopic study of the fenestrated endothelial lining of rat liver sinusoids. *J Ultrastruct Res* 1970; **31:** 125–50.

13 Floren CH. Binding of apolipoprotein E-rich remnant lipoproteins to human liver membranes. *Scand J Gastroenterol* 1984; **19:** 473–79.

14 Clark SA, Cook HB, Oxner RB, Angus HB, George PM, Fraser R. Defenestration of hepatic sinusoids as a cause of hyperlipoproteinaemia in alcoholics. *Lancet* 1988; **2:** 1225–27.

15 Abbott RD, Yano K, Hakin AA, et al. Changes in total and high-density lipoprotein cholesterol over 10- and 20-year periods (The Honolulu Heart Program). *Am J Cardiol* 1998; **82:** 172–78.

16 Cohn JS, McNamara JR, Cohn SD, Ordovas JM, Schaefer EJ. Postprandial plasma lipoprotein changes in human subjects of different ages. *J Lipid Res* 1988; **29:** 469–79.

17 Cassader M, Gambino R, Rulu G, Marena S, Bodoni P, Pagano G. Postprandial triglyceride-rich lipoprotein changes in elderly and young subjects. *Aging Clin Exp Res* 1996; **8:** 421–28.

18 Krasinski SD, Cohn JS, Schaefer EJ, Russell RM. Postprandial plasma retinyl ester response is greater in older subjects compared with younger subjects. Evidence for delayed plasma clearance of intestinal lipoproteins. *J Clin Invest* 1990; **85:** 883–92.

19 Field PA, Gibbons GF. Decreased hepatic expression of the low-density lipoprotein (LDL) receptor and LDL receptor-related protein in aging rats is associated with delayed clearance of chylomicrons from the circulation. *Metabolism* 2000; **49:** 492–98.

20 Latry P, Bioulac-Sage P, Echinard E, et al. Perisinusoidal fibrosis and basement membrane-like material in the livers of diabetic patients. *Hum Pathol* 1987; **18:** 775–80.

21 Phillips C, Murugasu G, Owens D, Collins P, Johnson A, Tomkin GH. Improved metabolic control reduces the number of postprandial apolipoprotein B-48-containing particles in type 2 diabetes. *Atherosclerosis* 2000; **148:** 283–91.

22 Moreton JR. Atherosclerosis and dietary hyperlipemia. *Science* 1947; **106:** 190–91.

23 Fraser R. The role of dietary triglycerides in cholesterol metabolism. *Atherosclerosis* 1974; **19:** 327–36.

24 Zilversmit DB. Atherogenesis: a postprandial phenomenon. *Circulation* 1979; **60:** 473–85.

25 Weintraub MS, Grosskopf I, Rassin T, et al. Clearance of chylomicron remnants in normolipidaemic patients with coronary heart disease: case control study over three years. *BMJ* 1996; **312:** 935–39.

26 Rapp JH, Lespine A, Hamilton RL, et al. Triglyceride-rich lipoproteins isolated by selected affinity anti-apolipoprotein B immunosorption from human atherosclerotic plaque. *Arterioscler Thromb* 1994; **14:** 1767–74.

27 Watts GF, Barrett PH, Marais AD, et al. Chylomicron remnant metabolism in familial hypercholesterolaemia studied with a stable isotope breath test. *Atherosclerosis* 2001; **157:** 519–23.