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Nicotine decreases the porosity of the rat liver sieve: a possible mechanism for hypercholesterolaemia

R. Fraser, S.A. Clark, W.A. Day and F.E.M. Murray
Department of Pathology, Christchurch School of Medicine, Christchurch, New Zealand

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Summary. Nicotine was fed to rats for 6 weeks, as a weight adjusted dose equivalent to that of a human being smoking 50 to 100 cigarettes per day. Those rats fed nicotine developed hypercholesterolaemia. Scanning electron microscopy showed the porosity of the hepatic sinusoidal endothelium of nicotine fed animals was about 40% that of control animals. The decline in porosity was found to be due to a reduction in diameter rather than number of fenestrae. We believe that this decreased hepatic sinusoidal porosity may alter cholesterol homeostasis by increasing the circulation time of chylomicron remnants too large to pass through the fenestrae. This phenomenon may be an aetiological factor in the known correlation between cigarette smoking, atherosclerosis, and coronary heart disease in humans.

Keywords: atherosclerosis, cholesterol, chylomicrons, endothelium, lipoproteins, liver, nicotine, sinusoid

The fenestrated endothelium of the hepatic sinusoids, or 'liver sieve', acts as a filter separating large chylomicrons, which are lipoproteins of intestinal origin, from their smaller, triglyceride-depleted, but cholesterol-rich remnants. In this way, it determines which lipoproteins leave the circulation for recognition by specific receptors on the microvilli of the hepatocytes in the space of Disse. This filtration is believed to be one factor influencing the balance between cholesterol from the intestines and that synthesized by the liver (Fraser *et al.* 1978; Naito & Wisse 1978; Wisse *et al.* 1985; Fraser *et al.* 1986*a*, *b*, *c*).

The lower porosity of the sinusoidal endothelium in rabbit and chicken livers compared to that in the rat has been postulated as being implicated in the susceptibility of these two species to experimentally-induced hypercholesterolaemia and atherosclerosis (Wright *et al.* 1983; Fraser *et al* 1986*a*, *b*, *c*). Similarly, the decreased diameter of sinusoidal fenestrae seen in the livers of rats dosed with the hormones adrenaline, noradrenaline, and serotonin is believed to be a factor in stress-related atherogenesis (Wisse *et al.* 1980; Tsukada *et al* 1986). The decline in porosity of the liver sieve in animals over a prolonged period of

Correspondence: Dr Robin Fraser, Department of Pathology, Christchurch School of Medicine, Christchurch, New Zealand.

ethanol ingestion may also be related to the development of alcoholic hyperlipoproteinaemia (Fraser *et al.* 1986*a*).

In a recent report on 'The Prevention of Cardiovascular Disease' (North *et al.* 1986) an advisory committee to the New Zealand Minister of Health concluded that cigarette smokers are twice as likely to develop coronary disease as non-smokers. Smoking has been related to an increase in coronary atherosclerosis in human beings (Auerbach *et al.* 1965), as well as to raised serum lipoprotein levels (Boyle *et al.* 1968; Stefanovitch *et al.* 1969). Hyperlipoproteinaemia has also been noted in rabbits dosed orally with nicotine (Booyse *et al.* 1981).

It is known that rats, which are usually resistant to hyperlipoproteinaemia, have a fairly porous liver sieve compared to species which are susceptible to dietary cholesterol (Fraser *et al.* 1986*c*). A pilot study reported in abstract form has shown that the liver sieve in nicotine-dosed rats decreases in porosity, and that hyperlipoproteinaemia develops (Fraser *et al.* 1987). For this paper, we expanded the pilot experiment to examine changes in the liver sieve in rats, following nicotine ingestion.

Materials and methods

Twenty young, male, Sprague-Dawley rats, weighing 400 ± 55g, were divided randomly into four groups of five animals. Group 1 was fed standard powdered rat pellets and drinking water *ad libitum*; Group 2 received powered pellets to which had been added 1% cholesterol by weight, and drinking water *ad libitum*; Group 3 was given normal powdered rat pellets and drinking water to which had been added 0.005% nicotine, and Group 4 had cholesterol-enriched food and nicotine-enriched drinking water. The rat pellets used in these experiments were composed primarily of vegetable products, but also contained meat, bone meal, and buttermilk powder, to give a fat content of 6%.

The dose of nicotine in the drinking water was adjusted so that the rats drank approximately 5mg/kg body weight per day, or the human equivalent of 50–100 cigarettes per day (Booyse *et al.* 1981; Zimmerman & McGeachie 1985). This dose was derived from nicotine levels in human plasma in which it has been shown that the oral consumption of 2.4 mg nicotine/kg/day yields a level equivalent to that obtained by smoking between one and two packs of cigarettes per day, where an average cigarette contains 2.2 mg of nicotine (Richardson & Morton 1979).

After 5 weeks, the serum cholesterol and triglyceride levels were measured in venous blood from the tail and determined enzymatically (Abbott Laboratories, South Pasadena, CA, USA), and, at 6 weeks, the livers were perfused with glutaraldehyde fixative through the portal vein at a constant physiological pressure of 10 cm water. The livers were then examined by scanning electron microscopy (Wright *et al.* 1983; Fraser *et al.* 1986*c*). Three sinusoids were chosen at random and scanned at a magnification of × 10 000, in order to measure the diameter and frequency of fenestrae. Measurements were made from plates enlarged to a magnification of × 30 000, and about 2000 fenestrae were measured from each group of rats.

Table 1. Comparison of serum cholesterol and triglyceride levels in rats fed nicotine and cholesterol for 5 weeks

Dietary group ($n=5$)	Serum cholesterol (mmol/l) (mean ± s.d.)	Serum triglyceride (mmol/l) (mean ± s.d.)
1 Control	1.45 ± 0.2	0.30 ± 0.09
2 Cholesterol	1.43 ± 0.1	0.25 ± 0.05
3 Nicotine	2.10 ± 0.4*	0.31 ± 0.02
4 Cholesterol and nicotine	2.13 ± 0.4*	0.37 ± 0.07

* Significantly different from control $P<0.05$.

Bonferroni adjusted probabilities from *t*-tests.

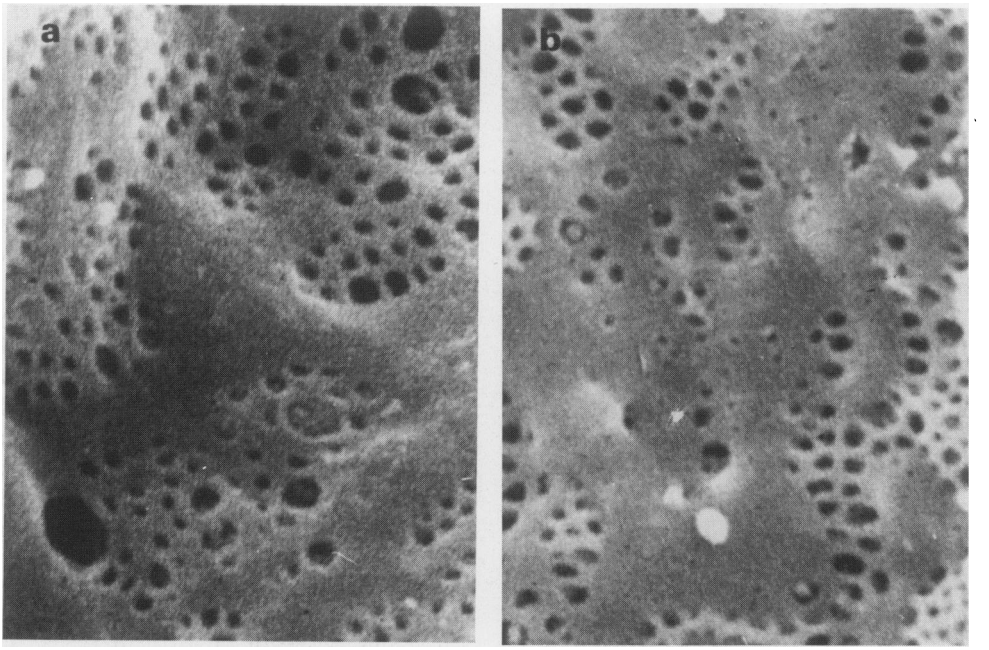

Fig. 1. Scanning Electron Micrograph of the internal surface of the fenestrated liver sinusoids demonstrating the lowered porosity of the endothelium in that from rats fed with nicotine (*b*) compared to controls (*a*). (×22 500).

Table 2. Comparison of sinusoidal porosity in rats fed nicotine and cholesterol for 6 weeks.

Group	Diameter of fenestrae (nm)	Number of fenestrae (per μm^2)	Porosity (per cent area perforated)
1 ($n=3$) (Control)	98±13	20.0±6.3	17.6±6.9
2 ($n=3$) (Cholesterol)	97±22	19.9±10.0	15.2±4.5
3 ($n=4$) (Nicotine)	63±13†	16.7±6.6	6.6±3.5†
4 ($n=4$) (Nicotine and Cholesterol)	68±15†	13.9±3.8	7.0±5.0†

† Significantly different from control $P<0.001$.
Bonferroni adjusted probabilities from *t*-tests.

From these measurements, the sinusoidal porosity was calculated for each rat (Fraser *et al.* 1986*c*). A small proportion of the samples were not suitable for electron microscopy, according to the criterion of blanching and hardening of the liver which indicates successful perfusion-fixation, and these were discarded from the experiment (Table 2).

Results

Rats from Groups 3 and 4, fed cholesterol and nicotine, or nicotine alone, had higher serum cholesterol than the controls, or those fed cholesterol without nicotine (Table 1).

Figure 1 and Table 2 show that the hepatic sinusoids of rats fed nicotine for 6 weeks (Groups 3 and 4) had a porosity of about 40% of that for the control animals (Groups 1 and 2). This was caused mainly by a highly significant decrease in the diameter of the fenestrae. There was no statistical difference in sinusoid porosity between rats which were fed cholesterol and those which received standard pellets.

Discussion

The decline in liver sieve porosity and concomitant increase in serum cholesterol levels following the ingestion of nicotine supports the hypothesis that the sinusoidal endothelium plays a role in the regulation of lipoprotein metabolism. This postulate arose from the observation that sinusoidal fenestrae were of an ideal diameter to separate small from large chylomicrons (Wisse 1970). Experiments demonstrating differential trapping by the liver of a size range of radioactively labelled chylomicrons and their remnants, and the presence of chylomicron-like particles in the space of Disse smaller than those in the sinusoids, support this hypothesis (Fraser *et al.* 1978; Naito & Wisse 1978).

Ethanol was the first drug shown to influence the endothelial fenestrae of the liver sinusoids. Dilation of the fenestrae in rats which had ingested ethanol for 4 weeks was observed by transmission electron microscopy, and has been postulated as a factor in the pathogenesis of steatosis in the alcoholic liver (Fraser *et al.* 1980). Subsequently, scanning electron microscopy of hepatic sinusoids from rats, baboons, and human beings who had ingested ethanol over a prolonged period, showed that, although in some instances the fenestrae dilated, they also became less numerous (Fraser *et al.* 1981; Mak and Lieber 1984; Horn *et al.* 1987). The resulting decrease in porosity has been postulated as a factor in the pathogenesis of the hyperlipoproteinemia associated with alcoholism (Fraser *et al.* 1986*a*).

The liver sieve is also influenced by other drugs, hormones, mechanical factors and species differences, and these have all been implicated in the modification of lipoprotein metabolism and atherogenesis (Fraser *et al.* 1986*a*, *b*, *c*). The liver sieve hinders the apo E receptors of hepatocytes in their recognition of large, intestinally-derived lipoproteins (the chylomicrons and their remnants), and a decrease in porosity might further increase the circulation time of these particles, as well as preventing the inhibition of cholesterol and lipoprotein synthesis in the liver (Florèn 1984; Fraser *et al.* 1986*a*, *b*, *c*).

In this paper, the alkaloid nicotine has been shown to induce hypercholesterolaemia and to markedly decrease the porosity of the liver sieve in rats. Serum cholesterol and triglyceride levels were higher in rats which had ingested nicotine with either a normal or a cholesterol-enriched diet than in controls. The animal fat content of the normal rat pellets used in our laboratory provided a potential source of dietary cholesterol.

The mode of action of nicotine on the hepatic endothelial cell to reduce the diameter of fenestrae is unknown. However it has been shown that endothelial cells have a strongly developed cytoskeleton which, in tissue culture, is acted upon by various vasoactive drugs, ethanol and cytochalasin B (Tsukada *et al.* 1986; Van der Smissen *et al.* 1986).

A report by the US Surgeon General (United States Department of Health and Human Services, 1983) on the consequences of smoking for health concluded that '[Smoking] . . should be considered the most important of the behavior modifiable risk factors for coronary heart disease'. Nicotine has several mechanisms by which to exert its deleterious effect; one is to decrease the porosity of the liver sieve, leading to hypercholesterolaemia. Oral nicotine has been

shown to raise serum cholesterol levels in human beings (Dousset *et al.* 1986), therefore nicotine could be expected to effect the sinusoidal fenestrae whether it reached the blood stream by inhalation or by absorption from the gastrointestinal tract.

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References

AUERBACH O., HAMMOND E.C. & GARFINKEL L. (1965) Smoking in relation to atherosclerosis of the coronary arteries. *New Engl. J. Med.* **273**, 775–779.

BOOYSE F.M., OSIKOWICZ G. & QUARFOOT A.J. (1981) Effects of chronic oral consumption of nicotine on the rabbit aortic endothelium. *Am. J. Pathol.* **102**, 229–238.

BOYLE J.R., MORALES I.B., NICHAMAN M.Z., TALBERT C.R. & WATKINS R.S. (1968) Serum beta lipoproteins and cholesterol in adult men. *Geriatrics.* **23**, 102–111.

DOUSSET J.C., GUTIERRES J.B. & DOUSSET N. (1986) Hypercholesterolaemia after administration of nicotine chewing gum. *Lancet* **ii**, 1393–1394.

FLORÈN C.H. (1984) Binding of Apolipoprotein E-rich remnant lipoproteins to human liver membranes. *Scand. J. Gastroenterol.* **19**, 473–479.

FRASER R., BOSANQUET A.G. & DAY W.A. (1978) Filtration of chylomicrons by the liver may influence cholesterol metabolism and atherosclerosis. *Atherosclerosis* **29**, 113–123.

FRASER R., BOWLER L.M. & DAY W.A. (1980) Damage of rat liver sinusoidal endothelium by ethanol. *Pathology* **12**, 371–376.

FRASER R., DAY W.A. & WRIGHT P.L. (1981) Fatty liver and the sinusoidal cells. In *Festschrift for F.C.Courtice.* Ed. D. Garlick. Sydney University NSW Press. pp. 139–144.

FRASER R., DAY W.A. & FERNANDO N.S. (1986*a*) Review: The liver sinusoidal cells, their role in disorders of the liver, lipoprotein metabolism and atherogenesis. *Pathology.* **18**, 5–11.

FRASER R., DAY, W.A. & FERNANDO N.S. (1986*b*) Atherosclerosis and the Liver Sieve. In *Cells of the Hepatic Sinusoid* Vol 1. Eds A. Kirn, D.L. Knook & E. Wise. Rijswijk, The Netherlands. Kupffer Cell Foundation. pp. 317–322.

FRASER R., HESLOP V.R., MURRAY F.E.M. & DAY W.A. (1986*c*) Ultrastructural studies of the portal transport of fat in chickens. *Br. J. exp. Path.* **67**, 783–791.

FRASER R., MURRAY F.E.M. & DAY W.A. (1987) The effect of nicotine on the endothelial fenestrae of liver sinusoids. *NZ Med. J.* **100**, 357.

HORN T., CHRISTOFFERSEN P. & HENRIKSEN J.H. (1987) Alcoholic liver injury: Defenestration in noncirrhotic livers — A scanning electron microscopic study. *Hepatology* **7**, 77–82.

MAK K.M. & LIEBER C.S. (1984) Alterations in endothelial fenestrations in liver sinusoids of baboons fed alcohol: A scaning electron microscopic study. *Hepatology* **4**, 386–391.

NAITO M. & WISSE E. (1978) Filtration effect of endothelial fenestrations on chylomicron transport in neonatal rat liver sinusoids. *Cell Tissue Res.* **190**, 371–382.

NORTH J.D.K., BEAGLEHOLE R., CHRISTMAS B.W., FRANKISH J.D., HAY D.R., LAUGESON B.M., NEUTZE J.M., NYE E.R., PRIOR I.A.M., SHARPE D.N. & SIMPSON F.O. (1986) *The Prevention of Cardiovascular disease/Advisory Committee to the Minister of Health.* Wellington. Government Printer. 80p.

RICHARDSON D.R. & MORTON R.F. (1979) Differential effects of chronic nicotine and tobacco smoke administration on iliac vascular resistance in the rat (40558). *Proc. Soc. Exp. Biol. Med.* **161**, 386–390.

STEFANOVICH V., GORE I., KAGIYAMA G. & IWANAGA Y. (1969) The effects of nicotine on dietary atherogenesis in rabbits. *Exp. Mol. Pathol.* **11**, 71–81.

TSUKADA N., ODA M., YONEI Y., HONDA K., AKAIWA Y., KIRYU Y. & TSUCHIYA M. (1986) Alterations of the hepatic sinusoidal endothelial fenestrae in response to vasoactive substances in the rat — *in vivo* and *in vitro* studies. In *Cells of the Hepatic Sinusoid.* Vol. 1. Eds A. Kirn, D.L. Knook & E. Wisse. Rijswijk, The Netherlands. Kupffer Cell Foundation. pp. 515–516.

UNITED STATES DEPARTMENT OF HEALTH AND HUMAN SERVICES (1983) *The Health Consequences of Smoking: Cardiovascular Diseases. A report to the Surgeon General.* US Department of Health and Human Services, Public Health Service. DHHS(PHS). pp. 50184–50204.

VAN DER SMISSEN P., VAN BOSSUYT H., CHARELS K.

& Wisse E. (1986) The structure and function of the cytoskeleton in sinusoidal endothelial cells in the rat liver In *Cells of the Hepatic Sinusoid.* Vol. 1. Eds A. Kirn, D.L. Knook & E. Wisse. Rijswijk, The Netherlands. Kupffer Cell Foundation. pp. 517–522.

Wisse E. (1970) An electron microscopic study of the fenestrated endothelial lining of rat liver sinusoids. *J. Ultrastr. Res.* **31**, 125–150.

Wisse E., Van Dierendonck J.H., De Zanger R.B., Fraser R. & McCuskey R.S. (1980) On the role of liver endothelial filter in the transport of particular fat (chylomicrons and their remnants) to parenchymal cells and the influence of certain hormones on the endothelial fenestrae. In *Communications of Liver Cells.* Ed. H. Popper. Lancaster: MTP Press. pp. 195–200.

Wisse E., De Zanger R.B., Charels K., Van der Smissen P. & McCuskey R.S. (1985) The liver sieve: Considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse. *Hepatology* **5**, 683–692.

Wright P.L., Smith K.F., Day W.A. & Fraser R. (1983) Small liver fenestrae may explain the susceptibility of rabbits to atherosclerosis. *Arteriosclerosis* **3**, 344–348.

Zimmerman M. & McGeachie J. (1985) The effect of nicotine on aortic endothelial cell turnover — An autoradiographic study. *Atherosclerosis* **58**, 39–47.