Lipoproteins and cardiovascular reactivity

Laurence G. Howes, Denise Abbott & Nora E. Straznicky[1]

Department of Clinical Pharmacology, St George Hospital, UNSW, Kogarah, NSW, Australia 2217 and [1]Department of Clinical Pharmacology, Austin and Repatriation Medical Centre, Heidelberg, Victoria, Australia 3084

The observation that relatively short periods of cholesterol lowering therapy can reduce the incidence of coronary artery disease events has prompted interest in the short term effects of lipoproteins on cardiovascular responsiveness. Numerous studies in animals and humans have demonstrated that oxidized LDL-cholesterol can impair endothelial dependent vasodilation in coronary arteries and peripheral resistance vessels. Reduction of plasma LDL-cholesterol levels in hypercholesterolaemic patients improves nitric oxide mediated vasodilator responses in the coronary and peripheral circulation. LDL-cholesterol also potentiates responses to vasoconstrictors such as noradrenaline and endothelin-1 in the absence of endothelium, possibly by enhancing calcium influx into vascular smooth muscle cells. Pharmacological reduction of plasma LDL-cholesterol levels has been shown to reduce blood pressure responses to intravenous infusions of pressor hormones and to stress. However, the relative contribution of changes in endothelial dependent vasodilation and vasoconstrictor or inotropic responses remains to be established. Short term changes in LDL-cholesterol produce changes in cardiovascular responsiveness that may influence the development of ischaemic events.

Keywords: cholesterol, HDL, LDL, VLDL, endothelium, hypercholesterolaemia, vascular reactivity

Introduction

Epidemiological and clinical studies have provided overwhelming evidence that high plasma concentrations of total cholesterol and low-density lipoprotein-cholesterol (LDL-C) are strong predictors of coronary heart disease [1, 2]. This is due to the development of atherosclerosis which results from the infiltration of blood vessel walls by cholesterol rich lipoproteins. During this process alterations in vascular reactivity have been observed, including impaired endothelial dependent vasodilation and an increased responsiveness to contractile agonists. There has been considerable interest in these short term effects of lipoproteins on vascular function in recent years, partly because of the observation that coronary artery disease events are reduced following relatively short (1–2 years) periods of cholesterol lowering therapy.

The first study to show a short-term effect of drug induced LDL-C lowering on coronary events used the HMG-CoA reductase inhibitor pravastatin and studied patients at high risk of coronary events (serum total cholesterol levels from 5.2 to 7.8 mmol l^{-1} plus two additional atherosclerotic risk factors [3]). This study in 1062 patients demonstrated a significant reduction in cardiovascular events in patients receiving pravastatin that was apparent after as little as 20 to 30 days of therapy and statistically significant after 26 weeks. This was followed by two further studies of HMG-CoA reductase inhibitor therapy following myocardial infarction—the 4S study (simvastatin) [4] and the CARE study (pravastatin) [5] which demonstrated early reductions in mortality and recurrent coronary events. These effects became apparent after approximately 1–2 years of therapy. A similar result was obtained in a primary prevention study in males using pravastatin [6] and in patients with atheromatous carotid disease treated with pravastatin [7]. An earlier study of the bile acid binding resin, cholestyramine, in the prevention of first coronary events in males produced a 19% reduction in the risk of coronary heart disease, death or non-fatal myocardial infarction that became apparent after 3–4 years [8]. However, this trial produced a reduction in LDL-C of only 12.6% compared with placebo whereas the reduction in the trials that have used HMG-CoA reductase inhibitors has been in the range of 26–38%. Thus differences in the apparent time taken to influence coronary events between these trials may be more a reflection of the degree of LDL-C reduction rather than the pharmacological agent used. In the only study of fibrate therapy (which predominantly lowers very low-density lipoprotein (VLDL) levels and increases high-density lipoprotein (HDL) levels) of sufficient size to assess the relationship between duration of therapy and reduction in ischaemic events a reduction in cardiovascular endpoints was apparent between 1 and 2 years after continuous therapy [9].

Plasma cholesterol reduction with statin therapy has also been shown to reduce the incidence and severity of symptomatic angina [10] and to reduce the frequency of ischaemic episodes on Holter monitoring [11]. Lovastatin therapy has been reported to reduce blood pressure (BP) responses to mental arithmetic stress in hypercholesterol-

Correspondence: Professor L. G. Howes, Department of Clinical Pharmacology, St George Hospital, Kogarah, NSW, Australia 2217.

aemic subjects [12], while simvastatin reduces BP and forearm vascular responses to cold pressor stress in hypercholesterolaemic hypertensives [13]. In patients with coronary artery disease, cholesterol reduction with fluvastatin therapy increases myocardial perfusion both at rest and during exercise [14]. These findings suggest that haemodynamic changes induced by reductions in plasma cholesterol levels may contribute to a reduction in coronary artery disease events, in addition to the longer term effects of retarding the progression of atheroma. The mechanisms responsible for these alterations in vascular reactivity are not yet fully understood. This article reviews the current knowledge concerning the relationship between plasma lipoproteins and cardiovascular reactivity.

Effects of LDL-cholesterol on vasodilator mechanisms

In recent years most attention in the area of lipoprotein induced changes in vascular reactivity has been focused on the effects of LDL-C on endothelial dependent (nitric oxide (NO) mediated) vasodilator responses, particularly in coronary arteries. The important finding that has emerged is that endothelial dysfunction associated with hypercholesterolaemia is rapidly reversible by serum cholesterol level reduction or by antioxidant therapy [15, 16]. Animal studies have shown that hypercholesterolaemia produces endothelial dysfunction and impaired endothelial dependent relaxation before the appearance of atheroma. These animal studies have previously been reviewed [16–18].

Several studies have investigated the effects of hypercholesterolaemia on vascular function in non-atherosclerotic human arteries. Endothelial dependent vasodilation in response to acetylcholine (ACh) [19, 20] and methacholine [21, 22] is significantly impaired in forearm resistance vessels of hypercholesterolaemic patients compared with controls. The same finding has been demonstrated in coronary arteries of hypercholesterolaemic patients with no angiographic evidence of atherosclerosis [23, 24]. Preliminary investigations suggest that sex may be an important determinant of endothelial function in human vasculature. Chowienczyk *et al.* [25] found impaired forearm blood flow responses to ACh in hypercholesterolaemic men but not in premenopausal hypercholesterolaemic women.

A number of animal studies have shown that oxidation of LDL-C is a critically important step in the impairment of endothelial dependent vasodilation by LDL-C [26]. Various mechanisms have been proposed to explain this finding. Galle *et al.* [26] showed that NO released from cultured endothelial cells is inactivated by oxidized LDL (ox-LDL). In another study, stimulation of smooth muscle guanylate cyclase (cyclic GMP) by NO was diminished in a dose dependent manner by ox-LDL [27]. An important feature of ox-LDL is its high content of amphiphiles such as lysolipids, lipid peroxides and oxygenated sterols [28]. Treatment of ox-LDL with albumin, which binds lysophospholipids, has been shown to attenuate its inhibitory effect on endothelial dependent relaxation [28–30]. These and other studies suggest that lysolipids such as lysolecithin and lysophosphatidylcholine in ox-LDL may participate in the apparent neutralization of NO by ox-LDL [31]. In addition, there is evidence that LDL-C increases the production of superoxide anion by endothelial cells [32], resulting in an enhanced rate of inactivation of NO. The formation of vascular NO is enhanced in experimental hypercholesterolaemia [33]. Thus the impaired NO mediated vasodilation associated with hypercholesterolaemia appears to be the result of a diminished effectiveness of NO rather than reduced formation. The reduced effectiveness probably results from the sequestration of NO by ox-LDL, increased inactivation of NO by increased superoxide formation and impaired c-GMP formation in response to NO. The reduced activity of NO in the presence of ox-LDL can be overcome by the administration of L-arginine (the precursor of NO) [11, 15, 34, 35] or tetrahydrobiopterin, an essential cofactor of NO synthase [34].

The effects of hypercholesterolaemia on endothelial dependent vasodilation has generated considerable interest as a possible factor influencing coronary artery spasm and coronary perfusion in hypercholesterolaemic patients either with or without coronary artery disease. In humans, 6 months of cholesterol lowering therapy using diet and cholestyramine has been shown to restore normal vasodilator responses to ACh in coronary arteries of hypercholesterolaemic patients (without angiographic evidence of coronary artery disease) who previously exhibited vasoconstrictor responses to ACh [24]. Egashira [36] showed that the reduction of serum cholesterol with pravastatin improved NO dependent coronary vascular responses in hypercholesterolaemic patients. Anderson *et al.* [15] found that antioxidant therapy and plasma LDL reduction with lovastatin had an additive effect on improving coronary artery responses to ACh in patients with coronary artery disease. Improvement in NO mediated vasodilation following cholesterol lowering therapy in humans does not appear to be limited to the coronary vasculature. Plasma cholesterol reduction with simvastatin has been shown to increase the forearm blood flow response to ACh in hypercholesterolaemia [37].

While ACh stimulated (NO mediated) vasodilation in forearm resistance vessels has been shown to be reduced in patients with hypercholesterolaemia, vascular resistance in the basal (unstimulated) state does not differ from controls [20, 37]. Inhibition of NO synthesis by the continuous intra-brachial artery infusion of L-NMMA (an NO synthase inhibitor) has been shown to increase forearm vascular resistance to the same extent in hypercholesterolaemic and normal subjects [20, 38], while abolishing the NO mediated vasodilator responses to ACh in the normal subjects. Similarly, reduction of LDL-C levels by simvastatin therapy in hypercholesterolaemic subjects improves the vasodilator response to ACh without altering resting forearm vascular resistance [37, 39]. Inhibition of NO synthase by L-NMMA abolishes the beneficial effect of simvastatin on vasodilator responses to ACh in hypercholesterolaemics, but increases resting forearm vascular resistance to the same extent as prior to simvastatin therapy [37]. These findings suggest that hypercholesterolaemia does not significantly influence basal NO mediated vasodilator function in the peripheral circulation, but that the alterations in vasodilator responses to ACh that occur in response to changes in plasma LDL-C levels are due to changes in NO mediated responses. However, these findings may not be completely applicable to the coronary circulation where LDL-C reduction with

statin therapy has been shown to reduce basal coronary vascular resistance [14].

Studies by Goode & Heagerty [40] have suggested that endothelial independent relaxation is also impaired in hypercholesterolaemics, although to a lesser extent than endothelial dependent vasodilation. To distinguish abnormalities in endothelial function from abnormalities in vascular smooth muscle several studies have examined the vasodilator response to directly acting smooth muscle relaxants sodium nitroprusside, nitroglycerin or papaverine. Most human [19, 20, 23] studies have reported preservation of endothelial independent relaxation in vasculature exposed to hypercholesterolaemia. However, Creager *et al.* [21] found that forearm blood flow responses to nitroprusside were attenuated in hypercholesterolaemic subjects compared with controls, and Goode & Heagerty [40] reported similar results from isolated resistance vessel preparations from human biopsies of hypercholesterolaemic subjects.

Effects of LDL on vasoconstrictor mechanisms

In addition to effects on vasodilator function there is evidence from animal studies that lipoproteins may enhance vascular responsiveness to vasoconstrictors by mechanisms that are not related to impaired NO mediated vasodilation. However, the effects of hypercholesterolaemia on vasoconstrictor responses in animals appears to depend upon the species and the blood vessel studied as well as the choice of vasoconstrictor. In general, hypercholesterolaemia increases vascular responsiveness to noradrenaline (NA) in small resistance vessels, while decreasing NA responses in large vessels such as the aorta. Vasoconstrictor responses to serotonin in large vessels are often enhanced by hypercholesterolaemia [41–44]. Galle & Bassenge [26] have extensively studied the effects of LDL-C and ox-LDL on the vascular responsiveness of isolated perfused rabbit femoral arteries, and concluded that while these lipoproteins both inactivate NO, vasoconstrictor responses to NA in the presence of LDL-C and ox-LDL are *greater* in arteries denuded of endothelium. These findings suggest that endothelial dependent vasodilator mechanisms protect against LDL-C enhanced responses to NA, despite inactivation of NO by LDL-C. Oxidation of LDL-C appears to be an important factor in the potentiation of these vasoconstrictor effects on LDL-C in animals [26].

The mechanisms underlying the endothelial independent enhancement of vasoconstrictor responses to ox-LDL are uncertain. In cultured arterial smooth muscle cells, cholesterol enrichment of the plasma membrane increases unstimulated calcium influx and cytosolic calcium levels [45]. As calcium channel blockers abolish this effect on calcium uptake, it has been hypothesized that an increase in the cholesterol content of the cell membrane induces alterations in the conformation or position of calcium channel proteins, thereby exposing or activating previously 'silent' ion channels [45]. Thus increased vascular responsiveness to vasoconstrictors associated with hypercholesterolaemia may be mediated by changes in the cholesterol content of the cell membrane of vascular smooth muscle which result in enhanced calcium influx. It is also possible that similar events occur in cardiac myocytes, leading to a potentiation of contractile responses [46]. The notion of a generalized increase in membrane calcium permeability in vascular smooth muscle cells and cardiac myocytes after cholesterol exposure is supported by some [47–51] but not other studies [41, 52].

Elevated LDL-C levels may also increase vasoconstrictor responses by increasing endothelin release. Hypercholesterolaemic patients have been reported to have elevated plasma endothelin-1 levels compared with controls [35]. Ox-LDL has been shown to induce the expression and release of endothelin from human vascular endothelial cells [53], and endothelin has been shown to potentiate vasoconstrictor responses to NA [54]. However it is uncertain to what extent increased endothelin release results from impaired NO action, as NO is known to suppress endothelin release [55].

A previous study reported that short-term alterations in plasma LDL-C with pravastatin were accompanied by a significant reduction in BP responses to intravenous infusions of both NA and angiotensin II (AII) [56]. Changes in systolic BP responses to AII between pravastatin and placebo therapy correlated significantly with changes in LDL-C suggesting that pravastatin therapy is accompanied by beneficial effects on cardiovascular reactivity which are most likely a consequence of the decrease in circulating LDL-C levels. Baroreceptor responsiveness (measured as the slope of change in heart period versus change in BP) did not change between the two treatments. Resting BPs did not differ between pravastatin and placebo therapy, a result which has been verified in larger clinical trials of similar duration [57, 58]. A recent study reported that short term (2 week) reduction of LDL-C levels by simvastatin reduced forearm vascular responses to intra brachial arterial NA in hypercholesterolaemic men [59]. Similar results were obtained both prior to and during the infusion of L-NMMA in a dose previously shown to inhibit NO synthase. The results suggest that the reduction in vascular responsiveness to NA during simvastatin therapy is not a result of increased NO responsiveness.

Effects of other lipoproteins on vasodilator and vasoconstrictor responses

The effects of lipoproteins other than LDL-C (VLDL, lipoprotein (a) and HDL) on vascular responsiveness in humans are uncertain, and the effects of fibrate therapy (which lowers VLDL and increases HDL-cholesterol) on vascular responses in patients with mixed hyperlipidaemia has not been investigated. However, three studies of coronary artery responses to infusions of ACh during angiography in humans have reported that high levels of HDL protect against the abnormal contractile responses to ACh [44, 60, 61]. Zeiher *et al.* [61] demonstrated that elevated HDL levels were associated with a smaller coronary artery response to cold pressor testing in patients with hypercholesterolaemia. HDL has been shown to reverse the inhibition of endothelial dependent vasodilator responses to ACh by LDL-C in isolated strips of rabbit aorta [62]. NO dependent vasodilator responses in the forearm circulation of patients with non-insulin dependent diabetes have been reported to correlate inversely with serum triglyceride levels and positively with HDL-cholesterol levels [63]. These data

suggest that high HDL levels may protect against the impaired NO mediated vasodilator responses that accompany elevated LDL-C levels. Oxidized lipoprotein (a) has been shown to inhibit endothelial dependent vasodilation of rabbit renal arteries in a more potent manner than ox-LDL [64].

Bezafibrate (a fibrate) treatment of rats fed a lipid raising diet has been reported to prevent the development of increased tail artery vasoconstrictor responses to NA [47] (rat lipoproteins are predominately of the VLDL type). These effects were observed in the absence of endothelium, indicating that they were not mediated via changes in NO metabolism [47].

Conclusions

Short term changes in LDL-C levels, and probably in the extent of oxidation of LDL-C, can modify both vasodilator responses and vasoconstrictor responses. The effects on vasodilator responsiveness appear to be principally due to an impaired action of NO, despite increased NO production, that probably results from sequestration of NO by ox-LDL, enhanced NO inactivation due to ox-LDL stimulated superoxide formation, and impaired NO mediated c-GMP formation. The mechanism underlying the enhancement of vasoconstrictor responses by ox-LDL is uncertain. Increased endothelin production may play a role, but a significant component of the increased vasoconstrictor responsiveness in animals appears to be independent of the endothelium. The enhanced vasoconstrictor responsiveness dose not appear to be specific for NA and may be due to ox-LDL stimulation of calcium uptake in vascular and cardiac muscle cells.

The pathogenetic significance of lipoprotein-induced changes in vascular reactivity is of particular interest. In addition to predisposing to coronary artery spasm, enhanced cardiovascular responses to stress may precipitate ischaemic events by increasing cardiac work and oxygen consumption.

All of the studies on the effects of plasma lipid modifications on vascular responsiveness in man performed so far have used drugs which lower LDL-C. The effects of fibrates (which predominantly lower VLDL triglyceride and cholesterol and increase HDL-cholesterol) on cardiovascular responsiveness in patients with mixed hyperlipidaemia is unknown.

Further work is needed to characterize the mechanisms by which lipoproteins influence vascular reactivity and the significance of these changes in the precipitation of ischaemic events. The relevance of these mechanisms in high risk patients such as the elderly and non-insulin dependent diabetics may be of particular importance in developing treatment strategies for hypercholesterolaemia and in setting targets for lipid lowering therapy.

References

1 Castelli WP, Garrison RJ, Wilson PWF, Abbott RD, Kalousidan S, Kannel WB. The Framingham Study. *JAMA* 1986; **256**: 2835–2838.

2 Hunninghake D. LDL-cholesterol as a determinant of coronary heart disease. *Clin Ther* 1990; **129**: 370–375.

3 The Pravastatin Multinational Study Group for Cardiac Risk Patients. Effects of pravastatin in patients with serum total cholesterol levels from 5.2 to 7.8 mmol/liter (200 to 300 mg/dl) plus two additional atherosclerotic risk factors. *Am J Cardiol* 1993; **72**: 1031–1037.

4 The Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994; **344**: 1383–1389.

5 Sacks FM, Pfeffer MA, Moye LA, *et al.* The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. *N Engl J Med* 1996; **335**: 1001–1009.

6 Shepherd J, Cobbe S, Ford I, *et al.* Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N Engl J Med* 1995; **333**: 1301–1307.

7 Crouse JR, Byington RP, Bond MG, *et al.* Pravastatin, lipids, and atherosclerosis in the carotid arteries (PLAC-II). *Am J Cardiol* 1995; **75**: 455–459.

8 Lipid Research Clinics Program. The lipid research clinics coronary primary prevention trial results. *JAMA* 1984; **251**: 351–364.

9 Frick MH, Elo O, Haapa K, *et al.* Helsinki Heart Study: Primary-prevention trial with gemfibrozil in middle-aged men with dyslipidemia. *N Engl J Med* 1987; **317**: 1237–1245.

10 Kjekshus J, Pedersen TR, Pyorala K, Olsson AG. Effect of simvastatin on ischemic signs and symptoms in the Scandinavian Simvastatin Survival Study (4S) (abstract). *J Am Coll Cardiol* 1997; **29** (2 Suppl A): 75A.

11 Andrews TC, Raby K, Barry J, *et al.* Effect of cholesterol reduction on myocardial ischemia in patients with coronary disease. *Circulation* 1997; **95**: 324–328.

12 Sung B, Izzo J, Orlowski W, Bateson M, Wilson M. HMG-CoA reductase inhibitor exaggerated blood pressure response to mental stress in normotensives with hypercholesterolaemia (abstract). *Circulation* 1994; **90**: 1565.

13 Nazzaro P, Manzari M, Merlo M, *et al.* Simvastatin reduces the vasoconstrictive stress response and the vascular damage in hypercholesterolemic hypertensives. In *International Society of Hypertension*, Glasgow, 1996: S96.

14 Eichstadt HW, Eskotter H, Hoffmann I, Amthauer HW, Weidinger G. Improvement of myocardial perfusion by short-term fluvastatin therapy in coronary artery disease. *Am J Cardiol* 1995; **76**: 122A–125A.

15 Anderson TJ, Meredith IT, Yeung AC, Frei B, Selwyn AP, Ganz P. The effect of cholesterol-lowering and antioxidant therapy on endothelium-dependent coronary vasomotion. *N Engl J Med* 1995; **332**: 488–493.

16 Cox DA, Cohen ML. Effects of oxidised low density lipoproteins on vascular contraction and relaxation: clinical implications in atherosclerosis. *Pharmacol Rev* 1996; **48**: 3–19.

17 Noll G, Luscher TF. Influence of lipoproteins on endothelial function. *Thrombosis Res* 1994; **74** (Suppl 1): S45–S54.

18 Howes LG, Krum H. Plasma lipoproteins, cardiovascular reactivity and the sympathetic nervous system. *J Autonom Pharmacol* 1989; **9**: 253–262.

19 Chowienczyk PJ, Watts GF, Cockcroft JR, Ritter JM. Impaired endothelium-dependent vasodilation of forearm resistance vessels in hypercholesterolaemia. *Lancet* 1992; **340**: 1430–1432.

20 Casino P, Kilcoyne C, Quyyumi A, Hoeg J, Panza J. The role of nitric oxide in endothelium-dependent vasodilation of hypercholesterolaemic patients. *Circulation* 1993; **88**: 2541–2547.

21 Creager MA, Cooke JP, Mendelsohn ME, *et al.* Impaired vasodilation of forearm resistance vessels in hypercholesterolaemic subjects. *J Clin Invest* 1990; **8**: 228–234.

22 Creager M, Gallagher S, Girerd X, Coleman S, Dzau V,

Cooke J. L-Arginine improves endothelium-dependent vasodilation in hypercholesterolemic humans. *J Clin Invest* 1992; **90**: 1248–1253.

23 Drexler H, Zeiher A, Meinzer K, Just H. Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolaemic patients by L-arginine. *Lancet* 1991; **338**: 1546–1550.

24 Leung W, Lau C, Wong C. Beneficial effects of cholesterol lowering therapy on coronary endothelium-dependent relaxation in hypercholesterolaemic patients. *Lancet* 1993; **341**: 1496–1500.

25 Chowienczyk PJ, Watts GF, Cockcroft JR, Brett SE, Ritter JM. Sex differences in endothelial function in normal and hypercholesterolaemic subjects. *Lancet* 1994; **344**: 305–306.

26 Galle J, Bassenge E. Effects of native and oxidised low-density lipoproteins on endothelium-independent vasomotion. *Basic Res Cardiol* 1991; **86**(S2): 127–142.

27 Schmidt K, Graier WF, Kostner GM, Mayer B, Bohme E, Kukovetz WR. Stimulation of soluble guanylate cyclase by endothelium-derived relaxing factor is antagonized by oxidized low-density lipoprotein and lysolecithin on soluble guanylyl cyclase. *J Cardiovasc Pharmacol* 1991; **17** (Suppl 3): S83–S88.

28 Kugiyama K, Kerns SA, Morrisett JD, Roberts R, Henry PD. Impairment of endothelium-dependant arterial relaxation by lysolecithin in modified low-density lipoproteins. *Nature* 1990; **344**: 160–162.

29 Plane F, Kerr P, Bruckdorfer KR, Jacobs M. Inhibition of endothelium-dependent relaxation by oxidized low-density lipoproteins. *Biochem Soc Trans* 1990; **18**: 1177–1178.

30 Mangin EL, Kugiyama K, Nguy JH, Kerns SA, Henry PD. Effects of lysolipids and oxidatively modified low density lipoprotein on endothelium-dependant relaxation of rabbit aorta. *Circ Res* 1993; **72**: 161–166.

31 Yokoyama M, Hirata K, Miyake R, Akita H, Ishikawa Y, Fukazaki H. Lysophosphatidylcholine: essential role in the inhibition of endothelium-depandant vasorelaxation by oxidised low density lipoprotein. *Biochem Biophys Res Commun* 1990; **168**: 301–308.

32 Ohara Y, Peterson TE, Harrison DG. Hypercholesterolemia increases endothelial superoxide anion production. *J Clin Invest* 1993; **91**: 2546–2551.

33 Minor RL Jr, Myers PR, Guerra R Jr, Bates JN, Harrison DG. Diet-induced atherosclerosis increases the release of nitrogen oxides from rabbit aorta. *J Clin Invest* 1990; **86**: 2109–2116.

34 Stroes E, Kastelein J, Cosentino F, *et al.* Tetrahydrobiopterin restores endothelial function in hypercholesterolemia. *J Clin Invest* 1997; **99**: 41–46.

35 Haak T, Marz W, Jungmann E, *et al.* Elevated endothelin levels in patients with hyperlipoproteinemia. *Clin Investig* 1994; **72**: 580–584.

36 Egashira K, Hirooka Y, Kai H, *et al.* Reduction in serum cholesterol with pravastatin improves endothelium-dependent coronary vasomotion in patients with hypercholesterolemia. *Circulation* 1994; **89**: 2519–2524.

37 Stroes E, Koomans H, de Bruin T, Rabelink T. Vascular function in the forearm of hypercholesterolaemic patients off and on lipid-lowering medication. *Lancet* 1995; **346**: 467–471.

38 Munzel T, Hayoz D, Hornig B. Increased vascular sensitivity to nitroglycerin in patients with hypercholesterolemia and peripheral endothelial dysfunction. *Deutsche Medizinische Wochenschrift* 1994; **119**(31–32): 1065–1070.

39 Chin J, Dart A. Therapeutic restoration of endothelial function in hypercholesterolaemic subjects: effect of fish oils. *Clin Exp Pharmacol Physiol* 1994; **21**: 749–755.

40 Goode GK, Heagerty AM. *In vitro* responses of human peripheral small arteries in hypercholesterolemia and effects of therapy. *Circulation* 1995; **91**: 2898–2903.

41 Heistad D, Armstrong M, Piegors D, Mark A. Augmented responses to vasoconstrictor stimuli in hypercholesterolaemic and atherosclerotic monkeys. *Circ Res* 1984; **54**: 711–718.

42 Bomzon L, Kew M, Rosendorff C. Renovascular hypersensitivity to noradrenaline in dietary induced hypercholesterolaemia in baboons. *Clin Exp Pharmacol Physiol* 1978; **5**: 181–185.

43 Bloom D, McCalden TA, Rosendorff C. The effects of hypercholesterolaemic plasma on vascular sensitivity to noradrenaline. *Br J Pharmacol* 1975; **54**: 421–427.

44 Galle J, Ochslen M, Schollmeyer P, Christoph W. Oxidised lipoproteins inhibit endothelium-dependent vasodilation. Effects of pressure and high-density lipoprotein. *Hypertension* 1994; **23**: 556–564.

45 Gleason MM, Medow MS, Tulenko TN. Excess membrane cholesterol alters calcium movements, cytosolic calcium levels, and membrane fluidity in arterial smooth muscle. *Circ Res* 1991; **69**: 216–227.

46 Kuwahara M, Hirose H, Doi K, Sugano S. Effect of hyperlipidemia on cardiovascular responses to adrenergic stimulation in piglets. *J Vet Med Sci* 1992; **54**: 637–642.

47 Trzeciak H, Boguslaw O, Kozlowski A, Chocilowska D, Bara A. Contractility of the tail artery in rats treated with bezafibrate and fed atherogenic diet. *J Cardiovasc Pharmacol* 1993; **22**: 761–767.

48 Thorin E, Hamilton C, Pruncizak A, Reid J. Change in endothelial cell membrane properties after chronic exposure to oxidised low density lipoproteins. *J Hypertens* 1994; **12** (S3): S180.

49 Orlov S, Resink T, Bernhardt J, Ferracin F, Buhler F. Vascular smooth muscle cell calcium fluxes. Regulation by angiotensin II and lipoproteins. *Hypertension* 1993; **21**: 195–203.

50 Liu K, Pierce G. The effects of low density lipoproteins on calcium transients in isolated rabbit cardiomyocytes. *J Biol Chem* 1993; **268**: 3767–3775.

51 Yokoyama M, Henry PD. Sensitization of isolated canine coronary arteries to calcium ions after exposure to cholesterol. *Circ Res* 1979; **45**: 479–486.

52 Broderick R, Bialecki R, Tulenko TN. Cholesterol induced changes in rabbit arterial smooth muscle sensitivity to adrenergic stimulation. *Am J Physiol* 1989; **257**: H170–H178.

53 Boulanger CM, Tanner FC, Bea M-L, Hahn AW, Werner A, Luscher TF. Oxidised low density lipoproteins induce mRNA expression and release of endothelin from human and porcine endothelium. *Circ Res* 1992; **70**: 1191–1197.

54 Yang Z, Richard V, von Segesser L, *et al.* Threshold concentrations of endothelin-1 potentiate contractions to norepinephrine and serotonin in human arteries. *Circulation* 1990; **82**: 188–195.

55 Cao WB, Zeng ZP, Zhu YJ, Luo WC, Cai BQ. Inhibition of nitric oxide synthesis increases the secretion of endothelin-1 in vivo and in cultured endothelial cells. *Clin Med J* 1994; **107**: 822–826.

56 Straznicky N, Louis W, Lam W, Howes LG. Pravastatin reduces cardiovascular reactivity to noradrenalin and angiotensin II in hypercholesterolaemic hypertensives. *Am J Cardiol* 1995; **75**: 582–586.

57 O'Callaghan C, Krum H, Conway E, *et al.* Short term effects of pravastatin on blood pressure in hypercholesterolaemic hypertensive patients. *Blood Pressure* 1994; **3**: 404–406.

58 O'Callaghan C, Krum H, Conway E, *et al.* Efficiency of

pravastatin in combination with captopril in hypertensive patients. *Med J Aust* 1995; **162**: 206–208.
59 Abbott D, Daher A, Manwaring P, Howes LG. Simvastatin reduces forearm vascular responsiveness to norepinephrine (letter). *Atherosclerosis* 1997; (in press).
60 Kuhn F, Mohler E, Satler L, Reagan K, Lu D, Rackley C. Effects of high-density lipoprotein on acetylcholine-induced coronary vasoreactivity. *Am J Cardiol* 1991; **68**: 1425–1430.
61 Zeiher AM, Schachinger V, Hohnloser SH, Saurbier B, Just H. Coronary atherosclerotic wall thickening and vascular reactivity in humans. *Circulation* 1994; **89**: 2525–2532.
62 Matsuda Y, Hirata K, Inoue N, *et al*. High density lipoprotein reverses inhibitory effect of oxidised low density lipoprotein on endothelium-dependent arterial relaxation. *Circ Res* 1993; **72**: 1103–1109.
63 Watts GF, O'Brien SF, Silvester W, Millar JA. Impaired endothelium-dependent and independent dilatation of forearm resistance arteries in men with diet-treated non-insulin-dependent diabetes: role of dyslipidaemia. *Clin Sci* 1996; **91**: 567–573.
64 Galle J, Bengen J, Schollmeyer P, Wanner C. Impairment of endothelium-dependent dilation in rabbit renal arteries by oxidized lipoprotein (a). *Circulation* 1995; **92**: 1582–1589.

(*Received 23 October 1996, accepted 1 May 1997*)

