Review

Lipids and the endothelium

Anthony M. Dart*, Jaye P.F. Chin-Dusting

Alfred Baker Medical Unit, Alfred Hospital & Baker Medical Research Institute, Melbourne, Australia

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Abstract

The normal endothelium is characterised by the production of a number of molecules which affect the contractile state of adjacent myocytes and the behavior of formed elements within the blood stream, and by the absence of cell surface adhesion molecules. In addition, endothelial cells are important modulators of coagulation and fibrinolysis. Whilst effects of lipids have been documented on many of these endothelial processes, there is particularly strong evidence for effects on the vasodilatation mediated by endothelium derived nitric oxide and on the interaction between leukocytes and the endothelial surface. Both LDL cholesterol and triglyceride rich lipoproteins impair endothelium dependent vasodilatation. The effects of LDL cholesterol are primarily evident for lipoprotein particles that have been oxidised with evidence for effects of specific constituents of oxidised LDL, such as lysophosphatidylcholine (LPC). LDL effects have been demonstrated at numerous sites of the nitric oxide signaling pathway including receptor-G protein coupling, nitric oxide synthase and NO bioactivity, with evidence for enhanced superoxide formation and the consequent production of the less potent dilator peroxynitrite. The effects of lipids on endothelium dependent vasodilatation can be reversed not only by reducing the level of elevated lipids levels but also by provision of the NOS substrate, L-arginine and by the provision of antioxidants, although the mechanism for these effects are not fully elucidated. The adhesion of leukocytes to the endothelial surface is stimulated by low density and triglyceride rich lipoproteins. As with endothelium dependent vasodilatation, the effects of LDL cholesterol are primarily evident for low-density lipoprotein particles that have been oxidised, and many of the effects of oxidised LDL can be mimicked by LPC. HDL can overcome pro-adhesive effects of oxidised LDL. The effects of LDL on leukocyte adhesion are secondary to the expression of adhesion molecules on the luminal surfaces of endothelial cells. In addition to the likely deleterious effects of lipids on endothelium-mediated vasodilatation and leukocyte-endothelial cell interaction, lipids have been shown to affect a number of other endothelial processes and function. Thus, oxidised LDL affects endothelial ET1 and PGH1 release. Although effects have been shown on endothelial cell growth and apoptosis and on endothelial processes related to thrombosis and fibrinolysis, these effects have been less extensively studied than endothelial dependent vasodilatation and leukocyte-endothelial cell interaction. Many of the effects of elevated or modified low density and TG rich lipoproteins on endothelial cells and endothelial cell processes could be expected to contribute to the development of atherosclerosis and therefore, to the association between lipids and atherosclerotic, particularly coronary, vascular disease. However, the extent to which "endothelial dysfunction" accounts for the known relationships between serum lipid concentrations and CHD is yet to be established. © 1999 Elsevier Science B.V. All rights reserved.

1. Introduction

The endothelium consists of a single layer of cells separating intra-vascular contents from all subjacent tissues. It fulfills a number of physiological functions including the provision of a non-thrombogenic surface, the control of the passage of solutes and macromolecules and, more recently recognised, the production of a number of molecules which modulate the contraction of adjacent smooth muscle cells and cardiac myocytes and the functions of formed elements within the blood. It is important to recognise that the endothelium is also able to respond to pathophysiological circumstances, such as infection and trauma of neighbouring tissue, by changes in these processes to favour, for example, haemostasis and the recruitment of leukocytes.

Endothelial cells are continually exposed to circulating lipids and, in some circumstances, to lipids that have accumulated in sub-endothelial regions. The relation be-
tween lipoprotein classes and the development of atherosclerosis is well established and has given impetus to studies of interaction between lipoproteins and endothelial cells. This is particularly relevant because change in endothelial function can be observed in the absence or before the development of overt atheroma, suggesting that it may be of relevance during the earliest stages of disease development. Many of these changes replicate “appropriate” response of the endothelium to injury, including a shift in the balance between endothelium mediated vasodilatation and vasoconstriction and the expression on endothelial cells of molecules promoting leukocyte adherence and migration.

In considering the interaction between lipids and the endothelium, a large number of issues are relevant. These include consideration of the relevant lipid(s) or lipoprotein(s), whether their actions are exerted luminally or abluminally, which endothelial cell processes are affected, whether lipid induced changes in endothelial cell behaviour are themselves modified by other factors such as changes in shear stress, whether intracellular targets involve modifications of enzyme action and whether there are changes in message transcription or translation. Furthermore lipoproteins are themselves modified by endothelial cell products raising the possibility of positive and negative feedback.

It is beyond the scope of this review to give a comprehensive account of all these matters and a number of previous reviews may be consulted for further details. These include reviews on the physiology and pathophysiology of nitric oxide [1–7] as well as reviews dealing specifically with effects of shear stress [8], adhesion molecules and platelets [9], lipoprotein oxidation [10–12] and reactive oxygen species [13,14]. In addition there are reviews on the function and activity of NO synthase [15,16], and others related to atherosclerosis, lipids and vascular function [7,17–20]. The current review is aimed at updating knowledge on the relation between lipids and the endothelium.

2. Lipids and nitric oxide dependent vasodilatation

2.1. Endothelial NO production and bioactivity

As indicated in the introduction, one of the more recently recognised functions of the endothelium is modulation of the degree of contraction of subjacent vascular smooth muscle cells. A number of established and putative autacoids are responsible for this modulating role and these include an “endothelium derived relaxing factor”, believed to be nitric oxide (NO). NO is produced from the guanidino terminal of L-arginine by the enzyme NO synthase with citrulline as the other reaction product. NO has a biological half-life of seconds, is able to diffuse both luminally and abluminally to exert its effects which are in large part due to the stimulation of guanylate cyclase with the production of increased amounts of cyclic GMP. In vascular smooth muscle cells the increase in cyclic GMP produces a fall in intracellular calcium with a consequent reduction in contractility. NOS exists in a number of isoforms with the isoform NOSIII (ecNOS) being constitutively active in endothelial cells. NOSII (iNOS) can be induced in endothelial cells in response to various cytokines [21–23].

All NOS isoforms require the cofactors iron protoporphyrin IX (heme), flavin mononucleotide (FMN) and tetrahydrobiopterin (BH4). NOS contains an oxidase and a reductase domain. Upon calcium/calmodulin binding NOS receives electrons from NADPH at the reductase domain, which are then shuttled by the flavin moiety to the oxidised domain where they are used for reducing molecular oxygen which is subsequently the source for oxidation of arginine. ecNOS synthase membrane targeting is dependent on myristolation [24], but this is permanent, and does not explain the synthase trafficking seen for example, with bradykinin which causes translocation of NOS from the membrane to the cytosol [25]. Absence of myristolation is however associated with reduced arginine to citrulline conversion [24]. Of particular relevance to modulation by lipids is the recent evidence of calveolae targeting of ecNOS and recognition that activity is modified by its interaction with the calveolae associated protein, calveolin. This is of particular importance given the location of lipoprotein receptors within calveolae. In the absence of calcium/calmodulin, ecNOS is localised within calveolae and inhibited by calveolin. An increase in calcium/calmodulin concentration removes the inhibition of calveolin on the NO synthase complex [26].

In addition to the generation of NO, NO synthase is also a source of the superoxide radical O$_2^-$ . Such superoxide production is favoured under conditions of relative deficiency of BH4 (in relation to flavin). Using recombinant bovine ecNOS, superoxide generation can be demonstrated by chemiluminescence that is inhibited by BH4 and diphenyleneiodonium, an inhibitor of the flavin moiety [27]. BH4, however, also inhibits superoxide production by xanthine oxidase suggesting a direct anti oxidant effect. Superoxide and NO combine, optimally in an equimolar basis, to generate peroxynitrite ($\text{ONOO}^-$) [28]. This ion, although toxic in higher concentration, appears to be vasodilatory, but much less potent and with a shorter half-life than NO [29], so that the generation of superoxide diminishes the vasodilatory and other effects of endothelium derived NO.

3. Nitric oxide dependent vasodilatation

Endothelium dependent vasodilatation has been shown to be impaired by elevated cholesterol in studies in
humans, in animals fed a high cholesterol diet and in a variety of arterial preparations studied in vitro.

3.1. Human studies

A number of studies in man have shown impairment in endothelium mediated vasodilatation in response to elevated total and particularly LDL cholesterol [30–36]. They have involved studies in peripheral conduit vessels, resistance vessels and specifically coronary arteries but importantly, are not restricted to sites of atheroma. Responses can be normalised rapidly by a variety of strategies that lower LDL cholesterol and improvements have been reported within one month of lipid lowering drug therapy or immediately following LDL apheresis [36–39]. However a detrimental effect of cholesterol is evident even down to low levels [40] and effects of cholesterol are not always maintained in multivariate analysis [41].

Endothelial function is also affected by a number of other lipid parameters. Flow mediated dilatation (FMD), a largely endothelium dependent process, was depressed in normal subjects after a fat meal [42] and following an intralipid infusion [43]. A reduced response to acetylcholine but not to sodium nitroprusside (SNP) has recently been demonstrated in patients with high levels of triglyceride but normal plasma LDL levels [44]. Furthermore, fish oils, which are known to lower triglyceride rich lipoproteins, enhance forearm blood flow dilator responses to acetylcholine but not SNP in diabetic and hypercholesteremic subjects [36,45] as well as coronary vasodilator responses to acetylcholine in heart transplant recipients [46]. In the patients with hypercholesterolaemia, the effects of fish oil were observed without any change in plasma or LDL levels [36]. The forearm blood flow increase to serotonin is impaired in patients with familial combined hyperlipidaemia and improved with lipid lowering therapy. Impairment is related to the IDL level and improvement to the reduction in IDL in these subjects who have a normal LDL [47]. Coronary artery responses to acetylcholine and L-NMMA were related to chylomicron remnants rather than to other lipoprotein fractions [48]. Increasing fatty acid concentration, either by infusing intralipid or somatostatin, has also been reported to blunt methacholine, but not SNP blood flow responses in the leg [49]. Elevated Lp(a) affects basal, but not acetylcholine mediated, production of NO [50] in forearm peripheral vessels, but does enhance acetylcholine and cold pressor coronary constrictor responses in patients with normal angiographic appearances [51], an association persisting with multivariate analysis. Furthermore, FMD in the femoral artery of children is negatively correlated with total cholesterol and Lp(a) but not with other fractions although the response to GTN was also depressed in this study [31]. A low HDL in the presence of a low LDL level, however, seems to have no effect on FMD [52].

In addition to restitution of function by cholesterol lowering, L-arginine, the precursor to nitric oxide, also appears to be effective. L-, but not D-arginine [53], augments dilator responses to methacholine in hypercholesterolaemic but not normal subjects studied by forearm plethysmography [53] and responses to acetylcholine studied by quantitative coronary angiography [54]. In adults with coronary artery disease, three days of L-arginine improves FMD [55]. On the other hand, improvement with L-arginine has been seen in the forearm responses of normal but not hypercholesterolaemic subjects [56]. An effect of supplementary L-arginine is puzzling in view of the apparently superfluous concentration of intracellular arginine available as a substrate for NO synthase. A number of alternative mechanisms have been postulated including the presence of endogenous inhibitors such as asymmetric dimethylarginine (ADMA), whose effects could be overcome by additional arginine. However, although plasma levels of ADMA are elevated in hypercholesterolaemic subjects [57], they are not likely to be high enough to be effective as opposed, for example, to chronic renal failure [58]. Arginine also stimulates insulin release that is vasodilatory and thus may contribute to the effects of exogenously administered arginine [59]. This is especially pertinent given that the vasodilatory effects of insulin are reduced by hypercholesterolaemia [60]. An additional possibility is that arginine is compartmentalised, rather than homogeneously distributed, within the cell.

As will be discussed in greater detail below there is convincing evidence that the state of oxidation of lipid particles is a major determinant of their effects on the endothelium. Whilst this has not been directly addressed in man and there is little evidence for an appreciable quantity of LDL oxidation products within the vascular compartment of man, there is evidence regarding the effects of antioxidants and related entities. In patients with coronary disease FMD was improved following the use of L-2-oxothiazolidine-4-carboxylic acid (OTC) which increases intracellular glutathione by providing cysteine as a substrate showing the importance of water soluble redox agents [61]. FMD in normal subjects was depressed after a fat meal but this effect was prevented if vitamin C and E were given immediately prior [42]. However, in a plethysmographic forearm study in hypercholesterolaemic subjects the consumption of mixed antioxidants (beta carotene–vitamin C–vitamin E) for one month, whilst protecting LDL against oxidation, had no effect on the impaired acetylcholine dilatory response [62]. Infused BH4 has transient effects in hypercholesterolaemic subjects in whom it improves the impaired constrictor response to L-NMMA and the impaired dilation response to serotonin but has no effects in control subjects [63].

A number of studies on ex vivo human material are also available. Studies on arterioles in skin biopsies from hypercholesterolaemic subjects while concurring in the observation of impaired endothelial responses [64,65], reported differing effects on SNP responses, in that these
were impaired in one [65] but not the other [64] study. L-arginine had an effect in one [65] but not the other [64]. In human saphenous veins, acetylcholine mediated dilatation is impaired by oxidised LDL but this was not prevented by L-arginine pretreatment [66]. The impaired endothelial response in internal mammary arteries has been shown to be proportional to the pre-operative LDL level [67]. An interesting feature of these studies is that they show endothelial impairment in tissues that have been removed from the influence of circulating lipoproteins and presumably reflect the result of intracellular or sub intimal lipid accumulation during life.

3.2. Animal feeding studies

Animal feeding experiments have also documented impaired endothelium dependent relaxation [68–78] that may be evident earlier to pulse and shear flow than to pharmacologically induced NO release [73]. The early endothelial response to increased flow appears to be associated with pertussis toxin insensitive G protein activation [79–81], which may be relevant to evidence for a preferential effect of lipoproteins on Gi linked receptors (see below). Interestingly an increase rather than decrease in total NO production is seen with cholesterol feeding [70]. This apparent paradox is probably explained by increased superoxide production. Endothelial production of superoxide has been observed in cholesterol fed rabbits [82], with inhibition by allopurinol suggesting involvement of xanthine oxidase [83]. As already discussed superoxide reacts rapidly with NO to form peroxynitrite, a much less potent vasodilator. Superoxide generation can lead to the production of a number of other products which may also be relevant, although the highly reactive OH radical seems not likely to be involved because of its extremely short diffusion distance. Animal experiments have also provided valuable evidence regarding the effects of antioxidants, L-arginine and other “protective” strategies. The impairment in acetylcholine relaxation due to cholesterol feeding can be improved by one week’s intramuscular polyethylene glycol superoxide dismutase (SOD) [75], five days of injected SOD liposomes [77], the hypcholes- terolaemic antioxidant agent probucol [74] or by dietary supplementation with vitamin E [69,71] or vitamin E and beta carotene [72]. In the latter report, the benefit was not however related to change in the resistance of LDL to oxidation [72]. Supplementary vitamin E prevents the reduction in acetylcholine coronary dilator responses seen in hypercholesterolaemic dogs, although there are also effects on SNP dilatation [68]. The effect of acetylcholine in reducing the height of the dicrotic notch, indicative of an effect on large artery properties, is reduced in cholest- erol fed rabbits and restored by concomitant vitamin E supplementation [84]. Effects of cholesterol feeding in mice on endothelium mediated dilatation were only evident if the diets were concomitantly low in vitamin E and selenium [85]. Impaired dilator responses to acetylcholine in cholesterol fed rabbits can also be improved by L. arginine [76,86] but not D arginine, with no effect of L arginine in normal rabbits [86].

3.3. Summary

Both clinical and animal feeding experiments indicate that elevated plasma lipid levels are associated with impairment of the vasodilatation occurring in response to endothelial NO production. Reduced NO bioavailability, at least partly attributable to concomitant superoxide production, contributes to these effects. Impairment in NO dependent vasodilatation is evident for triglyceride rich lipoproteins as well as for LDL. Normalization of responses can be achieved not only by reduction of elevated lipid concentrations but also by supplementation with the NOS substrate L-arginine and by antioxidants, however the mechanisms for these effects are not fully understood. Direct evidence for the importance of oxidised lipoprotein species in man in contributing to endothelial dysfunction is also lacking.

4. Mechanisms for the effects of lipids on endothelium mediated dilatation

The demonstration of impaired endothelium dependent vasodilatation in in vitro arterial segments exposed to lipoprotein fractions allows a more controlled examination of the relevant constituents. Whilst effects on endothelium mediated vasodilatation have been seen with apparently unmodified LDL more marked effects are evident once this has been modified by oxidation. The literature in regard to oxidation is complicated by lack of definitive and comparable measures of oxidation, but generally two extents of oxidation can be recognised. In the first, so-called minimally modified (mm), the lipoprotein is still recognised as a ligand by the LDL receptor. A number of techniques have been used to generate mm-LDL and include cold storage, UV radiation, use of low concentrations of transition metals and the use of soybean lipoxygenase and phospholipase. Such modified LDL usually contains a low level of thiobarbituric acid reactive substances (TBARS), a measure of lipid peroxidation. More extensive modification results in a particle that is not recognised by the LDL receptor and is usually achieved by incubation with higher concentrations of transition metal ions, most commonly copper. Lipoproteins can also be oxidised by contact with a number of different cell types including endothelial cells. An influence of haemodynamic factors on the interaction between lipids and endothelium mediated vasodilatation seems plausible but has been little studied. In one study of rabbit renal arteries exposed to native or oxidised LDL at different perfusion pressures, inhibition of endothelium dependent dilatation was seen at 100 but not at 30 mmHg.
perfusion pressure possibly due to difference in the degree of lipid infiltration of the vessel wall [87].

The particular components of modified LDL responsible for the endothelial effects have also been extensively studied with strong evidence in favour of a role of lysophosphatidylcholine (LPC). This is known to be present in oxidised LDL and can mimic many of the effects of oxidised LDL on endothelial function. Direct evidence for the role of LPC in man is, however, lacking. Effects of a number of other oxidised species as well as of non-LDL related lipoproteins have also been reported. In addition to determination of the relevant lipid species there has been considerable study of the site of action with evidence for effects on receptor mediated G protein coupling, a number of intracellular signal transduction pathways, effects on NO synthase as well as effects post NO production. There is also recent evidence for an effect of LPC on the uptake of arginine into endothelial cells [88].

4.1. Active lipid components

4.1.1. Lipoprotein receptors

The interaction between extracellular lipids and endothelial cells may operate through specific cell surface receptors or, for some molecules, through direct interchange with plasma membranes. In addition to the classical LDL (B/E) receptor a number of other receptors have been shown to be expressed on endothelial cells although they may not be universally present throughout the endothelium and little appears to be known about their location on luminal vis a vis abluminal surfaces. A recently described receptor likely to be of importance to the interplay between lipids and the endothelium is a receptor for oxidised LDL. This receptor recognises oxidised but not acetylated LDL. It is a member of the C-type lectin family [89]. The expression of the oxidised LDL receptor (LOX-1) is increased when cells are incubated with oxidised but not native LDL [90] and is up regulated by shear stress [91]. The demonstration of a specific oxidised LDL receptor, which has been found in human endothelium [89], is important because many of the effects of LDL are seen for the oxidised but not the native lipoprotein (see below). It must also be noted, however, that although oxidised lipids exist in atheromatous tissue [92], their demonstration within the vascular compartment is controversial. The low density lipoprotein receptor protein/α2 macroglobulin receptor has been found in rat endothelial cells although human cells contain mRNA but not protein [93,94]. Microvascular endothelial cells contain class B scavenger receptors (CD 36 and SR-B1) which recognise both acetylated and oxidised LDL, and like the LDL receptor, are located within calveolae [95]. The VLDL receptor has also been demonstrated in endothelium [96]. In addition to receptors, lipoprotein lipase may also be relevant for the interaction between lipids and endothelium.

4.1.2. Oxidised LDL

Copper oxidised LDL attenuates acetylcholine induced relaxation in rat aortic rings [97]. SHT induced relaxation in pig coronary arteries [98], and bradykinin induced IP$_3$ increase in bovine aortic endothelial cells [99]. The degree of impairment is related to the extent of oxidation [100] and to the LPC content rather than the level of TBARS [101]. Effects of LPC on endothelium function have been reported in several studies [102–106]. It has been shown to inhibit acetylcholine responses in rabbit aortic rings [103], serotonin, UK 14304 and thrombin responses in pig coronary arteries [104,107] and bradykinin induced PI hydrolysis in bovine aortic endothelial cells [108]. Endothelium relaxation to acetylcholine, ATP and A23187 is inhibited in rabbit arterial strips by LPC [106] where it also prevents the acetylcholine induced rise in calcium in endothelial cells [109]. LPC also reduces NO release directly measured by differential pulse amperometry from histamine stimulated human umbilical vein endothelial cells (HUVECS) [110]. Whilst LPC is similar to oxidised LDL in effects on acetylcholine induced relaxation, at concentrations above 30–50 μmol/l it becomes toxic due to micelle formation [103]. Other oxidative constituents of LDL reported to affect the l-arginine-nitric oxide pathway include 13-hydroperoxoctadecadienoate (13HPODE) which increases eNOS message levels with increase in protein and activity in bovine aortic endothelial cells [111] and 7-ketocholesterol and 7β-hydroxycholesterol which decrease NO release to histamine [112]. Neither 5-or 6-α-epoxy cholesterol, nor 19-hydroxycholesterol were effective [112]. In contrast to the effects of oxidised LDL, acetylcholine mediated vasodilatation in rat aorta is not impaired by acetylated LDL [100].

4.1.3. Other Apo B containing lipoproteins

In keeping with the in vivo human studies showing effects of lipoproteins other than LDL, dilator responses to endothelium dependent agonists were impaired in rat aortic rings exposed to VLDL (as well as LDL and HDL) and there was a strong correlation with the lipoprotein phospholipid content [113]. Although responses to acetylcholine, substance P and the calcium ionophore A23187 were reduced in rabbit aortic strips by incubation with remnant lipoproteins (produced following absorption to B 100 and A1 antibodies) native VLDL was without effect [114]. Endothelin 3 stimulated NO release, however, is suppressed by triglyceride rich lipoproteins isolated from humans following a fat from each meal [115].

4.1.4. HDL and apo A1

In subcutaneous arterioles obtained from hypercholesterolaemic subjects, the best predictor of endothelium dependent dilation response was the in vivo level of
apoprotein A1 [64]. While separate incubation with HDL and LDL both depressed endothelium dependent dilatation in rat aortic rings, this effect was no longer apparent with co-incubation of both [113]. Pre-incubation with HDL prevents the effects of copper oxidised LDL or LPC in diminishing acetylcholine induced endothelium dependent dilatation in rabbit aortic strips. This may be related to the ability of HDL to reduce incorporation of LPC into endothelial cells while promoting its release into the medium [103].

4.1.5. Fatty acids

Eicosapentaenoic acid (EPA) ethylester causes an increase in intracellular calcium and increased production of nitrogen oxides, as measured by the Griess reaction, in HUVECS [116]. Oleic acid reduces NO activity in bovine pulmonary artery endothelial cells [117].

4.2. Receptors/G proteins

A number of studies have found effects of lipids restricted to particular agonists suggesting that certain receptors or receptor coupled processes are more sensitive to the effects of lipids. In pig coronary arteries copper oxidised LDL inhibits 5HT and thrombin induced dilatation without affecting responses to bradykinin, A23187 or SIN-1. The effect persists with pertussis toxin [104]. Acetylsalicylic acid but not substance P induced relaxation is attenuated in arteries from human buttock skin of hypercholesterolaemic subjects [64]. mm-LDL appears to exert effects particularly on G, although without changing the amount of G proteins present [118,119]. LPC has no effect on bradykinin or ADP relaxation in the circumflex coronary artery of pigs but does reduce the effects of serotonin and UK14304. In a bioassay system LPC inhibited 5HT but not bradykinin mediated relaxation suggesting an effect predominantly on G, [120]. However other studies have found effects on a wide range of agonists including bradykinin and have also found effects of native as well as oxidised LDL and LPC [108,121]. It may be that the different effects observed are concentration dependent and/or dependent on the level of oxidation of the lipid particle and/or the conditions of the experiment such as temperature or pH. For example, at lower concentrations (0.02 mg protein/ml), LDL appears to be selective, only inhibiting responses to acetylcholine in rat aortic rings whereas at higher concentration (0.2 mg protein/ml) inhibition was also observed to histamine and the receptor-independent A23187 [113].

4.3. Effects on intracellular calcium

Native, mildly and fully oxidised LDL have all been reported to increase endothelial calcium concentration [122–127] as has acetylated LDL [122]. Negative studies have also been reported for native LDL [127]. LPC causes a biphasic increase in intracellular calcium in endothelial cells [108]. However, it inhibits the rise in endothelial intracellular calcium produced by acetylcholine in mice [128] and rabbits [109] and also the thrombin and histamine induced rise in calcium in HUVECS [129].

4.4. Effects on NOS protein levels and message

A number of different responses to native LDL have been observed. n-LDL has been found to have no effect on ecNOS message [130,131], to produce a slight increase in NOS message [132] or, in high concentration in human saphenous vein endothelial cells to reduce NO synthase protein and message but to increase total NO release (measured by the Griess reaction) [133]. Mild and moderately copper oxidised LDL decreases ecNOS protein and mRNA in human saphenous vein endothelial cells [134] and ecNOS mRNA is reduced by oxidised LDL in bovine aortic endothelial cells [131]. Effects of oxidised LDL may be both dose and time dependent. Low concentrations of oxidised LDL up regulate and high concentrations down regulate ecNOS mRNA in bovine aortic endothelial cells [130] and in another study oxidised LDL had a biphasic effect on transcription with an initial fall followed by an increase [132]. LPC has been consistently shown to upregulate ecNOS [130,135,136], apparently related to an increase in protein phosphatase activity leading to increased binding of the transcription factor Sp-1 [135], necessary for the activation of NOSIII transcription [137]. Phosphatidylycholine, LPC and phosphatidylethanolamine all increase conversion of L-arginine to citrulline in purified NOS from bovine aortic endothelial cells [138].

4.5. Lipid effects on NO and direct vasodilators

A number of studies have shown an inhibitory effect of oxidised LDL on cyclic GMP or relaxation responses to SNP and GTN [121,139,140]. In addition, responses to SNP as well as to acetylcholine are depressed in arterioles obtained from human buttock biopsies of hypercholesterolaemic subjects [65]. Interestingly, enhanced responses to acetylcholine, A23187 and SNP have been reported in rabbit carotid arteries perfused with liposomes composed of free cholesterol and phospholipid in a 2:1 molar ratio [141].

There is evidence that both normal and oxidised LDL inactivate released NO [142] and whilst highly copper oxidised LDL shows no effect on arginine to citrulline conversion by purified NO synthase complex incubated with arginine, it does reduce subsequent production of cyclic GMP from purified guanylate cyclase. Bioactivity but not production of NO from bovine aortic endothelial cells is reduced by both normal and oxidised LDL [143]. Oxidised LDL impairs the cyclic GMP response in fibroblasts to authentic NO as well as to the effects of
bradykinin or A23187 induced stimulation of bovine aortic endothelial cells [144].

As previously discussed, reduction in bioactivity of NO with maintained or even increased production can be explained by increased superoxide production. Sufficient superoxide is produced in the adventitia of rat aorta to inactivate derived NO [145] and there is evidence in rabbit [146] and rat [145] aorta that NADPH oxidase, and not xanthine oxidase or mitochondrial NADH dehydrogynase, acts as a source of superoxide.

4.6. Summary

Endothelial cells express a number of lipoprotein receptors in addition to the classical LDL (B/E) receptor including a receptor for oxidised LDL and the VLDL receptor. There is considerable evidence favouring a role for oxidised rather than native LDL and LPC mimics many molecules expressed on endothelial cells. The initial receptors in addition to the classical LDL (B/E) receptor leukocyte rolling with subsequent adherence and finally migration. The rolling and adherent phases result from specific interactions between leukocytes and adhesion molecules expressed on endothelial cells. The initial rolling represents an interaction between leukocytes and Selectins with subsequent adherence occurring through the involvement of ICAM and VCAM. Endothelial cells do not normally express these adhesion molecules on their luminal surfaces. However, in response to hypercholesterolaemia, increases in rolling/adhesion and the expression of the relevant adhesion molecules can be demonstrated [156–158]. As with endothelium dependent vasodilatation, a role for oxidised LDL has been clearly established with LPC being a primary candidate [110,159–162]. In contrast to the pro-adhesive effects of elevated LDL, HDL and apo A1 have been shown to reduce the expression of these adhesion molecules when they are stimulated [163] suggesting an alternative or additional explanation for the inverse relation between HDL and clinical coronary heart disease. There is also evidence of an effect of NO on these processes. InHUVECS and human dermal cells TNFα induces adhesion molecules expression. With the use of DETA-NO as a source of NO the TNFα induces cell surface expression of VCAM and ICAM is reduced but there is no effect on E Selectin. The action of TNFα on the transcription factor NFκB is also reduced in the presence of NO and opposite effects are obtained with LNMMA [164].

P Selectin, which is stored in the Weibel-Palade bodies of endothelial cells becomes surface expressed in the atheromatous segments of human arteries but not in normal arteries [165]. The extra-cellular domains of adhesion molecules can be found free in the circulation and elevated levels have been demonstrated in subjects with hyperlipidaemia and coronary heart disease. In humans, circulating ICAM and P Selectin are increased in patients with CHD [166] and soluble ICAM, VCAM and Selectin in patients with hypertriglyceridaemia [167]. Interestingly, while six weeks of n-3 fatty acid supplementation (which decreases plasma triglyceride levels) increases Selectin levels, both Selectin and ICAM levels fall with a further six months treatment [167].

5. Lipids and other endothelium derived vasoactive autocoids

In addition to NO, the endothelium produces a number of other vasoactive substances including prostacyclin, endothelin and endothelium derived hyperpolarising factor. Endothelial cell or copper oxidised LDL suppresses ET1 secretion from pulmonary artery endothelial cells and a similar effect is evident for LPC [147]. While this is also true inHUVECS for heavily copper oxidised LDL, there is no effect of native or minimally oxidised LDL [148]. HDL and apolipoprotein A1, on the other hand, increase endothelin 1 synthesis due to a PKC dependent effect on translation in bovine aortic endothelial cells [149].

Oxidised LDL produces an increase and then a subsequent fall in PGI2 release [150] and native LDL an increase in release [124] from bovine aortic endothelial cells, whilst HDL induces PGI2 generation inHUVECS [151]. The decreased platelet reactivity induced by endothelial cells, due to PGI2, is inhibited by EPA and to lesser extent docosahexaenoic acid (DHA) [152] through an effect on PGH synthase rather then PGI2 synthase [153]. LPC increases release of arachidonic acid and causes increased transcription of COX 2 mRNA with no effect on COX 1 inHUVECS [154,155].

6. Lipid effects on leukocyte-endothelial cell interactions

In addition to changes in endothelium dependent vasodilatation, lipids have also been studied in relation to leukocyte-endothelial interactions. Leukocytes adherence to and penetration of the endothelium are normal responses to tissue injury and can be induced by a number of cytokines such as TNFα and IL-1. The processes start with leukocyte rolling with subsequent adherence and finally migration. The rolling and adherent phases result from specific interactions between leukocytes and adhesion molecules expressed on endothelial cells. The initial rolling represents an interaction between leukocytes and Selectins with subsequent adherence occurring through the involvement of ICAM and VCAM. Endothelial cells do not normally express these adhesion molecules on their luminal surfaces. However, in response to hypercholesterolaemia, increases in rolling/adhesion and the expression of the relevant adhesion molecules can be demonstrated [156–158]. As with endothelium dependent vasodilatation, a role for oxidised LDL has been clearly established with LPC being a primary candidate [110,159–162]. In contrast to the pro-adhesive effects of elevated LDL, HDL and apo A1 have been shown to reduce the expression of these adhesion molecules when they are stimulated [163] suggesting an alternative or additional explanation for the inverse relation between HDL and clinical coronary heart disease. There is also evidence of an effect of NO on these processes. InHUVECS and human dermal cells TNFα induces adhesion molecules expression. With the use of DETA-NO as a source of NO the TNFα induced cell surface expression of VCAM and ICAM is reduced but there is no effect on E Selectin. The action of TNFα on the transcription factor NFκB is also reduced in the presence of NO and opposite effects are obtained with LNMMA [164].

P Selectin, which is stored in the Weibel-Palade bodies of endothelial cells becomes surface expressed in the atheromatous segments of human arteries but not in normal arteries [165]. The extra-cellular domains of adhesion molecules can be found free in the circulation and elevated levels have been demonstrated in subjects with hyperlipidaemia and coronary heart disease. In humans, circulating ICAM and P Selectin are increased in patients with CHD [166] and soluble ICAM, VCAM and Selectin in patients with hypertriglyceridaemia [167]. Interestingly, while six weeks of n-3 fatty acid supplementation (which decreases plasma triglyceride levels) increases Selectin levels, both Selectin and ICAM levels fall with a further six months treatment [167].
6.1. Animal feeding studies

Cholesterol feeding in rabbits increases aortic VCAM staining [168] particularly over areas containing foam cells [169] whilst rabbits bred to have a high or low atherogenic response to cholesterol feeding show differences in their VCAM staining proportional to the atherogenic response [170]. In cholesterol fed rabbits leukocyte rolling and adherence is observed in mesenteric venules together with surface expression of P Selectin, ICAM and VCAM [156]. The increased monocyte adhesion and accumulation seen in cholesterol fed rabbits can be reduced by dietary supplementation with L-arginine [157] without necessarily affecting LDL oxidation or superoxide formation [157]. Cholesterol feeding in rats also results in increased rolling endothelial cells and an increase in VCAM, but not E Selectin, in rabbit aortic endothelial cells, HUVECS and human iliac arterial endothelial cells, HUVECS and human iliac arterial endothelial cells and an increase in VCAM, but not E Selectin, in rabbit aortic endothelial cells and HUVECS [179]. In addition to LPC, 13HPODE causes similar proadhesive effects of LDL are more pronounced for the oxidised than the native lipoprotein and LPC is able to mimic many of the effects of oxidised LDL. TG rich lipoproteins also have a proadhesive effect, whilst HDL and apo A1 inhibits the cytokine induced expression of adhesion molecules. Lipoprotein effects on message transcription and cell signaling pathways have been reported, as has interaction with NO. Elevated levels of soluble adhesion molecules have been found in hyperlipidaemia and coronary heart disease although their prognostic significance is not yet established. In view of the focal and regional nature of atherosclerosis, studies on regional variation and interaction with haemodynamics should be rewarding.

6.2. Acute cell experiments

Native LDL causes an increase in VCAM message in a number of endothelial cells [123,171,172] although there may be no detectable increase in protein [171,172]. Increase in E Selectin [123] and ICAM message [171] have also been reported. These effects are achieved through the B/E (LDL) receptor [123] and are associated with binding of the transcription factors AP1 and GATA but not NFkB [172]. While the latter finding was substantiated in a separate study, native LDL had no effect on monocyte adherence [159]. In human endothelial cells mm-LDL increases mRNA for the monocyte chemotactic protein MCP-1 [173] and P Selectin [160] along with increased intracellular P Selectin protein but no P Selectin surface expression, whereas oxidised LDL did increase P Selectin surface expression [160]. Increased expression of granulocyte-macrophage colony stimulating factor (CSF) and granulocyte-CSF is also observed [174]. In rabbit aortic endothelial cells mm-LDL increased monocyte adherence with activation of NFkB [159]. Effects of mm-LDL are consistent with a cyclic AMP rather than a PKC dependent mechanism [159,160]. LDL oxidized by copper or incubation with endothelial cells increases the endothelial adhesion of monocytoid cells in HUVECS [161], which is prevented by pretreatment with HDL [163]. In human arterial endothelial cells and HUVECS, oxidised LDL modifies the effect of TNFα on VCAM but not E Selectin expression. VCAM expression is controlled by the redox sensitive activation of NFkB like transcription factor that differs from the expression for ICAM and E Selectin [175]. In pig coronary arteries both copper oxidised LDL and LPC increase the adhesion of polymorphonuclear leukocytes and increase the expression of ICAM and these effects are prevented by PKC inhibition [162]. Cell adhesion is also affected by triglyceride rich lipoproteins. Thus monocyte adhesion to bovine aortic endothelial cells is increased if the endothelium is preincubated with βVLDL from cholesterol fed rabbits or VLDL from fat fed monkeys [176]. Interestingly, the IL-1β stimulation induced increase in ICAM, VCAM and E Selectin mRNA in HUVECS is attenuated by the n-3 fatty acids, EPA and DHA [177]. LPC causes increased leukocyte adherence and rolling in rat mesenteric vessels and these effects are attenuated by the use of anti P Selectin antibodies and NO donors [110]. LPC also causes an increase in P Selectin and ICAM 1 expression in these vessels [110], an increase in P Selectin expression in cat coronary endothelium which is sensitive to PKC inhibition [178], an increase in ICAM in rabbit aorta endothelial cells, HUVECS and human iliac arterial endothelial cells and an increase in VCAM, but not E Selectin, in rabbit aortic endothelial cells and HUVECS [179]. In addition to LPC, 13HPODE causes similar findings to oxidised LDL in modifying the effect of TNFα on VCAM expression [175]. There is no effect of acetylated LDL on cell adhesion [162].

6.3. Summary

Leukocyte adherence to endothelial cells requires the surface expression of a number of adhesion molecules. In addition to stimulation by a number of cytokines, both cell adhesion and the expression of adhesion molecules are affected by lipoproteins. As with vasodilatation, the proadhesive effects of LDL are more pronounced for the oxidised than the native lipoprotein and LPC is able to mimic many of the effects of oxidised LDL. TG rich lipoproteins also have a proadhesive effect, whilst HDL and apo A1 inhibits the cytokine induced expression of adhesion molecules. Lipoprotein effects on message transcription and cell signaling pathways have been reported, as has interaction with NO. Elevated levels of soluble adhesion molecules have been found in hyperlipidaemia and coronary heart disease although their prognostic significance is not yet established. In view of the focal and regional nature of atherosclerosis, studies on regional variation and interaction with haemodynamics should be rewarding.

7. Thrombosis and fibrinolysis

Although the endothelium is the source for a number of components important for both thrombosis and fibrinolysis, there is only rather limited information regarding the influence of lipids on these endothelial processes. A number of studies have shown an increase by mm-LDL, but not native LDL, in the expression of tissue factor (TF) by endothelial cells, due to effects on transcription [180,181]. Oxidised LDL produced by irradiation induces thrombomodulin mRNA, thrombomodulin antigen and activity in HUVECS [182]. LPC, at 50 μmol/l, reduces
tissue factor pathway inhibitor (TFPI) messenger RNA and TFPI in HUVECS [183]. Copper oxidised but not native LDL reduces tPA and increases PAI-1 release from human macro-vascular but not micro-vascular endothelial cells [184]. VLDL activates the PAI-1 promoter in HUVECS [185] and stimulates PAI-1 secretion from HUVECS with a more potent effect if from hypertriglyceridaemic subjects. These effects are 75% blocked by antibodies to the B/E (LDL) receptor [186]. The Hind III polymorphism in PAI-1 is associated with differences in activity [187] and effects of VLDL in increasing PAI-1 transcription are more marked in the Hind III polymorphism 2-2 than in other genotypes [188]. In clinical studies the free form of TFPI is reduced in hyperlipidaemic subjects [189,190] whilst TFPI associated with VLDL and LDL is increased [190]. Elevated cholesterol is associated with prolonged euglobulin lysis time suggesting a change in the balance between the release of plasminogen activators, such as tPA, and inhibitors [191]. The same study also noted LDL particle size to be a determinant of plasma von Willebrand factor (vWF) levels. In the ARIC study circulating vWF levels were correlated with plasma triglyceride and HDL but not total cholesterol [192]. In the EURODIAB study vWF levels were correlated with plasma cholesterol and triglyceride in men, but not women [193].

8. Endothelial cell apoptosis and growth

Incubation of bovine aortic endothelial cells with mildly oxidised LDL produced by 2 μmol/l of copper or irradiation, produces a slow rise in calcium prior to cell death [126] whilst iron oxidised LDL causes an increase in cell growth but also more rapid senescence in HUVECS [194]. Rabbit aortic endothelial cells incubated with copper oxidised LDL show a marked calcium uptake and inhibition of tyrosine phosphatase activity [127]. Copper oxidised LDL stimulates tyrosine phosphorylation of epidermal growth factor receptor [195]. Incubation of HUVECS with oxidised LDL but not native LDL leads to endothelial cell apoptosis. This is associated with an increase in CPP-32 like protease activity. Apoptosis can be prevented by vitamins C and E or N-acetylcysteine [196]. Oxysterols rather than LPC appear to be responsible for these effects of oxidised LDL with activation of acid sphingomyelinase and caspases leading to ceramide accumulation [197].

9. Conclusion and clinical implications

There is substantial evidence that many endothelial cell processes are sensitive to the presence of lipids. Although effects have been demonstrated for a number of lipoprotein classes and other lipid moieties, a prominent and consistent effect of oxidised LDL has been noted in relation to several key processes (Fig. 1). These include the production (and action) of a range of vasoactive molecules including NO, endothelin and prostacyclin as well as the surface expression of adhesion molecules. LDL is clearly established as a major factor in the development of atherosclerosis raising the possibility that the endothelial perturbations documented in this review may underlie or contribute to the association between LDL and atherosclerosis. Endothelial cell processes are also influenced by triglyceride rich lipoproteins and HDL (Fig. 1). The inverse association between HDL cholesterol concentrations and atherosclerosis is also established clinically and there is increasing evidence for a pro-atherosclerotic effect of at least some triglyceride enriched lipoprotein particles, again raising the possibility that effects on endothelial cells may contribute to these associations.

There are multiple potential mechanisms by which ‘endothelial dysfunction’ could contribute to the development of atherosclerosis and its clinical sequelae. These include shifting the balance from a vasodilatory to a vasoconstrictory action, favouring recruitment of leucocytes, loss of endothelial protection against inappropriate thrombosis and failure of fibrinolysis. Attempts to more directly prove a causal sequence between ‘endothelial dysfunction’ and clinical outcomes have to date focused on relatively late stages in disease progression with attempts to influence the occurrence of myocardial ischaemia in patients with established coronary heart disease. Whilst cholesterol reduction did reduce the number of episodes of myocardial ischaemia in one study, another failed to show benefit despite documented improvement in vasodilator endothelial function [198,199]. Although, as has been discussed, soluble adhesion molecule concentrations are elevated in patients with hyperlipidaemia and coronary heart disease it is not clear whether the elevation is a consequence of the atherosclerotic process or is indeed an indication that adhesion molecule expression is an early and pivotal factor in disease development.

As illustrated in Fig. 1, perturbations in endothelial cell functions arise secondary to a large number of underlying mechanisms, some of which may have clinical application. Beneficial effects on outcomes by cholesterol reduction or limiting LDL oxidation, whilst of enormous importance to clinical practice, cannot be taken as evidence for a role of ‘endothelial dysfunction’ in the development of atherosclerotic disease since they may be operating by additional mechanisms such as plaque stabilisation. Antioxidant therapy and agents designed to scavenge free radicals, whilst potentially acting through endothelial processes, may also be operating primarily through other mechanisms. A number of the sites of action shown in the figure do however offer potential for more selective and informative interventions. These include supplementation with L-arginine, gene delivery systems to alter endothelial NOS expression and interventions to alter the balance between NO and superoxide production. Further elucidation of the pathways leading to adhesion molecule expression may
Fig. 1. Endothelial cell receptors exist for native (n) and oxidised (ox)-LDL and for VLDL. The lipoprotein receptor related protein/α2 macroglobulin receptor and class B scavenger receptors (not shown) have also been identified in some endothelial preparations. Some lipid moieties, such as LPC, may act by receptor independent mechanisms. n-LDL, ox-LDL and LPC affect basal (1) and stimulated (2) intracellular calcium concentration. (a) ox-LDL suppresses ET-1 secretion (3) whilst HDL increases endothelin synthesis (4). PGI2 is positively affected by n-LDL and HDL whilst ox-LDL exerts a biphasic effect (5). (b) Both LDL, particularly ox-LDL, and triglyceride rich lipoproteins (TGRL; 6) interfere with NO mediated vasodilatation. Co-incubation with HDL diminishes these effects (7). Actions have been demonstrated at the level of l-arginine uptake (8), receptor-G protein coupling (9), NOS protein and transcription (10), as well as alteration in relative production of NO and O2•− with production of the less effective vasodilator, peroxynitrite (ONOO−; 11). Effects on released NO (12) and directly acting NO donors have also been found. (c) LDL, particularly when oxidised, and TGRL increase leucocyte rolling and adhesion (13) with evidence for increased surface expression of adhesion molecules (14). NO involvement has been shown in relation to cytokine induced adhesion molecule expression (15). In addition to the interactions shown, lipid effects have also been documented in relation to thrombosis, fibrinolysis and endothelial cell growth and apoptosis (see text).

also yield potential sites where specific interventions may provide definitive evidence to establish the role of ‘endothelial dysfunction’ in disease initiation and development. Whilst clinical trials of cholesterol (primarily LDL) reduction have yielded impressive results, the reductions in mortality and CHD events have been in the order of 30% implying considerable scope for additional benefit from other interventions, perhaps including those more specifically aimed at the endothelium.

References


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