Non-specific inhibition by human lipoproteins of endothelium dependent relaxation in rat aorta may be attributed to lipoprotein phospholipids

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Abstract

Objective: In vitro incubation of low-density lipoprotein (LDL) is reported to attenuate endothelium dependent relaxation mediated by acetylcholine (ACh) while not affecting endothelium-independent relaxation. This study was designed to examine the effects of other lipid-carrying lipoproteins as well as to study their effects on responses mediated by endothelium dependent agonists other than ACh. Methods: The effects of human LDL, very-low-density lipoprotein (VLDL) and high density lipoprotein (HDL) on endothelium-dependent relaxation by ACh, histamine and the calcium ionophore, A23187, and endothelium-independent relaxation by sodium nitroprusside (SNP) were investigated in rat isolated aortic rings. The effects of combined LDL and HDL incubation on responses mediated by ACh were also examined. Control experiments included experiments examining the effects of bovine serum albumin on responses mediated by ACh. Thiobarbituric-acid-reactive substances (TBARS) measured before and after organ bath incubation indicated little oxidation of the lipoproteins used. Results: Maximal responses to ACh were inhibited by LDL, VLDL and HDL 0.02 and 0.2 mg protein/ml, to histamine by LDL (0.2 mg protein/ml), VLDL (0.02 and 0.2 mg protein/ml) and HDL (0.02 and 0.2 mg protein/ml) and to A23187 by LDL (0.2 mg protein/ml), VLDL (0.2 mg protein/ml) and HDL (0.02 and 0.2 mg protein/ml). A small but significant correlation was observed between the level of inhibition of the endothelium-dependent responses and lipoprotein phospholipid concentration in the organ bath but not between the level of inhibition and cholesterol (free and esterified) or triglyceride concentrations. Responses to SNP were unaffected by LDL, VLDL and HDL. Combined incubation of tissues with LDL (0.2 mg protein/ml) and HDL (0.2 mg protein/ml) significantly increased maximal responses to ACh (pre-lipoproteins 81.8 ± 5.7 vs plus-LDL/HDL 100 ± 0.0; P < 0.05). Bovine serum albumin had no effect on the maximal responses to ACh. Conclusions: We conclude that inhibition by human lipoproteins of endothelium-dependent agonists occurs with LDL, HDL and VLDL and suggest that this may be due to the phospholipid content of each lipoprotein. However, combined incubation of HDL with LDL negates this effect and an increased maximal response to ACh is reported. © 1997 Elsevier Science B.V.

Keywords: Vascular reactivity; Nitric oxide; Human, lipoproteins; Phospholipids; Aorta; Rat

1. Introduction

The physical disruption of endothelial structure and function due to the formation of an atherosclerotic lesion results in, amongst other factors, a decreased vascular response to agents that stimulate the release of nitric oxide from the endothelium [1]. Endothelial dysfunction occurring before the formation of structural disruption [2] is less well-defined and has led to widespread interest in the role that lipoproteins may play. It is now clear that responses to endothelium-dependent vasodilators such as acetylcholine (ACh), but not direct smooth muscle dilators such as sodium nitroprusside (SNP), are impaired in forearm resistance vessels of hypercholesterolaemic patients, even though these vessels are not prone to atherosclerosis [3,4]. It has been suggested that oxidation of low density lipoproteins (LDL) may play an important role. While in vitro incubation of rabbit aortic rings with non-oxidised LDL has been shown to have no effect on endothelium-dependent relaxation [5,6], copper-oxidised LDL completely
abolished relaxation responses mediated by ACh and reduced maximum relaxation to the calcium ionophore, A23187, by approximately 50% [7].

There is ample evidence that it is the lipid component of LDL that diminishes vascular response to endothelium derived nitric oxide [8–10] and further that lysophosphatidylcholine, a substance produced when native LDL are oxidised [11], is responsible. It remains arguable, however, as to the effect of other lipid-carrying lipoproteins such as high-density lipoprotein (HDL) and very-low-density lipoprotein (VLDL). While unmodified HDL have been demonstrated to antagonise the vascular effects of oxidised LDL [10], oxidised HDL have also been shown to potently inhibit the effects of nitric oxide in their own right [9]. This non-specific inhibitory effect of lipoproteins appears to also extend to unmodified HDL and VLDL obtained from rabbit plasma [8]. It is not known whether this is also true of human lipoproteins.

The physiological significance of copper-oxidised lipoproteins is debatable. In the current paper we describe our studies on the effects of unmodified human lipoproteins, LDL, HDL and VLDL, on endothelium-dependent relaxations of the rat isolated aortic ring.

2. Methods

2.1. Preparation of lipoproteins

Approximately 150 ml of blood was taken from normal healthy volunteers. Individual lipoprotein fractions were obtained using discontinuous density gradient ultracentrifugation on a Beckman vertical rotor (70 Ti) with a Beckman ultracentrifuge; the VLDL fraction was obtained at 1.006 ± 0.001 g/ml, LDL at 1.063 ± 0.001 g/ml and HDL at 1.21 ± 0.001 g/ml. To concentrate the fraction, VLDL was re-spun at 1.006 ± 0.001 g/ml for a further 18–20 h. The isolated fractions were dialysed for a minimum of 18 h in saline containing EDTA 1 mM. All buffers contained EDTA 1 mM. Free and esterified cholesterol, phospholipids and triglycerides were measured using enzymatic colorimetric methods on a Cobas-Bio centrisanlyser (Roche Diagnostics, Tegimenta, Switzerland). Apo A1 and apo B levels were measured using immunoturbidimetric methods on a Cobas-Bio centrisanlyser. The protein content of each lipoprotein fraction was determined by a simplified protein assay method of Lowry [12]. All lipoprotein fractions were filtered through a 0.45 μm milllex-HV filter unit (Millipore MA 01730; Bedford). Fractions were stored for a maximum of 10 days at 4°C.

The lipid peroxide content of the lipoprotein samples obtained was monitored fluorometrically as thiobarbituric-acid-reactive substances (TBARS) [13]; 300 ml of sample was vortexed with 600 ml of TBARS reagent (15% trichloroacetic acid, 0.375% thiobarbituric acid (TBA) and 0.25N HCl/100 ml). The samples were centrifuged at 10,000 r.p.m. for 10 min and the supernatant was measured for TBARS using a Cobas-Bio centrisanlyser. This was repeated on samples which had been incubated in the organ bath experiments.

2.2. Isolation of rat thoracic aorta

Male Sprague-Dawley rats (250 ± 50 g) were gassed with 80% CO₂ and 20% O₂ and then killed by exsanguination. The thoracic aorta was excised and placed in a Krebs modified solution (composition in mmol/L: NaCl 119, HCl 4.7, MgSO₄·7H₂O 1.17, NaHCO₃ 25, KH₂PO₄ 1.18, CaCl₂ 2.5, glucose 11 and EDTA 0.03) kept ice-cold and then trimmed free of fat and connective tissue; 3-mm-wide rings were cut and mounted on 2 parallel stainless steel hooks in a water-jacketed, 5 ml organ bath containing Krebs solution maintained at 37°C and gassed with carbogen (95% O₂, 5% CO₂). Rings were set at a resting tension of 5 g, pre-determined in preliminary experiments to approximate an internal circumference equal to 0.9 × L₁₀₀, where L₁₀₀ denotes the internal circumference at the level of passive stretch equivalent to a transmural pressure of 100 mmHg. The lower hook was attached to a plastic (PVC) support leg which was further attached to a micrometer for re-adjustment of initial tension. The upper hook was attached to a force transducer (FT03 force transducer; Grass Scientific Instruments, Mitutoyo, Japan) for isometric recordings. Changes in isometric force were amplified (Quadbrige amplifier, Scientific Concepts Inc., Victoria, Australia) and recorded on a MacLab data acquisition system (MacLab SE, Apple Computer Inc, Cupertino, CA) connected to an Apple Macintosh SE. Aortic ring preparations were allowed to equilibrate for 30 min at the initial stretch tension of 5 g. At the end of the equilibration period, rings were re-stretched to 5 g and allowed to equilibrate for a further 30 min.

The use of laboratory animals was approved by the Animal Ethics Committee, Baker Medical Research Institute which adheres to National Health and Medical Research Council of Australia guidelines.

2.3. Organ bath experimental protocol

All rings were contracted with a submaximal dose of noradrenaline (NA; 0.1–1 mM). When a steady level of active force was obtained, cumulative concentration–relaxation responses to the endothelium-dependent relaxant agonists, ACh (1 nM–30 μM) and histamine (1 nM–30 μM), the calcium ionophore, A23187 (1 pM–3 nM) and the endothelium-independent vasodilator, SNP (1 nM–30 μM), were obtained. Only one relaxing agonist was tested on any one aortic ring from any one rat.

Any one of the 3 lipoprotein fractions, VLDL, LDL or HDL, was then added to the organ bath to give a final concentration of either 0.02 or 0.2 mg protein/ml. Lipoproteins obtained from only one individual were tested at any one time. Tissues were left to incubate in the
lipoprotein-rich solution for 60 min. In the continued presence of the lipoprotein, cumulative concentration–relaxation curves were repeated with the same agonist as used prior to addition of lipoprotein. At the end of this second curve, rings were thoroughly washed (replacement of fresh Krebs’ solution 3–4 times organ bath volume) every 10–15 min for 60 min. Cumulative concentration–relaxation curves were then repeated for the second time. In addition to these single lipoprotein experiments, the combined effects of HDL (0.2 mg protein/ml) and LDL (0.2 mg protein/ml) were also examined. As before, concentration–relaxation response curves to ACh on noradrenaline pre-constricted rat aortic rings were obtained before and in the continued presence, following a 60 min incubation, of LDL and HDL.

Two series of control experiments were performed. The first were time-control experiments, where relaxation responses to ACh were obtained and repeated at the same time points as the experiments described above, but in the absence of any lipoprotein. The second studied the specificity of the effects of the lipoproteins. Thus, relaxation–response curves to ACh were performed initially and then repeated after a 60 min incubation with bovine serum albumin (0.2 mg protein/ml).

2.4 Data analysis

Individual concentration response curves for each agonist were fitted to a logistic equation of the form:

$$E = MA^P / (A^P \times K^P)$$

where $E$ is the response, $M$ is the maximum response, $A$ is the concentration eliciting $E$, $K$ is the concentration eliciting 50% of the maximum response (i.e., EC$_{50}$) and $P$ is the slope parameter [14]. The location of the EC$_{50}$ value provided a measure of drug potency.

Data are presented as mean ± standard deviation (s.d.) unless otherwise stated. Results were analysed by analysis of variance (ANOVA) followed by Student’s unpaired t-test using Sigmastat Statistical Software (Jandel Scientific, San Rafael, CA) which inherently analyses data for normality prior to performing parametric analysis. Correlations between % inhibition of maximal relaxation of endothelium-dependent agonists by the lipoproteins and calculated organ bath concentrations of cholesterol (free, esterified and total), triglycerides and phospholipids were performed using simple regression analysis (Sigmastat, Jandel Scientific, San Rafael, CA). Percent inhibition of maximal relaxation by lipoproteins is defined as the difference in the maximal response obtained to the endothelium-dependent agonist used (ACh, histamine, A23187) in the presence, compared with that obtained in the absence, of each lipoprotein (LDL, VLDL, HDL). Calculated organ bath concentrations of each lipoprotein component (free and esterified cholesterol, triglyceride and phospholipid) were grouped into deciles of concentrations and matched against the relevant mean % inhibition. Statistical significance was set at $P < 0.05$.

2.5 Drugs and chemicals

Norepinephrine bitartrate salt (Sigma, USA), ACh chloride (Sigma, USA), (2-[4-imidazolyl]ethyl)amine dihydrochloride (Sigma, USA) and sodium nitroferricyanide (Sigma USA) were all dissolved and diluted in Krebs’ solution. Calcium ionophore, A23187 (Sigma, USA), was made up in ethanol as a 2 mM stock and diluted in Krebs’ solution. Boehringer Mannheim kit numbers for analysis of lipoprotein composition were as follows: free cholesterol 310328, total cholesterol 1442350, triglycerides 450032, phospholipids 691844, apo A1 1378686 and apo B 1378694.

3 Results

3.1 Lipoprotein content

The mean (± standard error of the mean) percentage composition (free and esterified cholesterol, triglycerides, phospholipids and protein) of LDL, VLDL and HDL are shown in Fig. 1. As expected, the higher the density of the lipoproteins, the higher the proportion of protein, and the lower the triglyceride concentrations. The levels of malonaldehyde (MDA) reflected the minimally oxidised state of the lipoproteins (Fig. 1). These levels were not significantly altered by incubation in the organ baths (data not shown).

3.2 Organ bath experiments

3.2.1 Control experiments: time controls and bovine serum albumin

Time control experiments where concentration–response curves were obtained to ACh at the same time periods (i.e., before and after 1 h), but without any lipoprotein intervention, revealed little effect of time on maximal responses to ACh (mean ± s.d.: Curve 1, Time 0, 89.0 ± 11.0% vs Curve 2, Time 60 min, 75.6 ± 27.1%; $P > 0.05$). Mean maximum relaxation responses to ACh were not affected after incubation with albumin at 0.2 mg protein/ml (mean ± s.d.: pre-albumin 68.5 ± 8.1% vs plus-albumin 80.8 ± 14.0%, $P < 0.05$). Albumin had no effect on negative log EC$_{50}$ values of ACh (mean ± s.d. pre-albumin 6.77 ± 0.37 vs plus-albumin 6.29 ± 0.26).

3.2.2 LDL

Fig. 2a represents the mean concentration–response curve to ACh obtained in the absence and presence of LDL (0.02 mg protein/ml). Table 1 details the mean maximal responses and potency of all the agonists used in the absence and presence of LDL. Maximal relaxation to
Fig. 1. Mean (±s.e.m.) percentage composition (free and esterified cholesterol, triglycerides, phospholipids and protein) of LDL, VLDL and HDL.

ACh was significantly attenuated in the presence of LDL (0.02 mg protein/ml). The EC$_{50}$ (negative log of the concentration required to produce 50% of the maximum response) of ACh shifted significantly to the right following incubation with LDL. Although maximal relaxation responses to histamine and A23187 were also blunted by LDL (0.02 mg protein/ml), this was not significant at $P<0.05$.

Maximal relaxation responses and EC$_{50}$ values to ACh were significantly attenuated in the presence of LDL (0.02 mg protein/ml) (see Table 1). Maximal relaxation responses to histamine and A23187 were also significantly attenuated. There was no significant shift in the dose–response curves to these agonists. Neither maximal relaxation responses nor potency to SNP were significantly affected by LDL (0.02 or 0.2 mg protein/ml).

3.2.3. VLDL

Mean concentration–response curves to ACh in the absence and presence of VLDL (0.02 mg protein/ml) are shown in Fig. 2b. Table 2 details the mean maximal responses and potency of all the agonists used in the absence and presence of VLDL. Maximal relaxation mediated by ACh and histamine, but not by A23187, was significantly attenuated in the presence VLDL at 0.02 mg protein/ml. Similarly, EC$_{50}$ values to ACh and histamine, but not to A23187 were significantly decreased in the presence of VLDL 0.02 mg protein/ml. On the other hand, maximal relaxation mediated by the 3 endothelium-dependent relaxing agonists used (ACh, histamine and
Table 1
Effect of LDL (0.02 mg protein/ml and 0.2 mg protein/ml) on mean % maximal relaxation response ± standard deviation (s.d.) and mean potency (neg. log M EC$_{50}$) ± s.d. to acetylcholine, histamine, A23187 and sodium nitroprusside.

<table>
<thead>
<tr>
<th>Protein</th>
<th>0.02 mg protein/ml</th>
<th></th>
<th>0.2 mg protein/ml</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>% maximal relaxation</td>
<td>EC$_{50}$ (neg. log M)</td>
<td>% maximal relaxation</td>
<td>EC$_{50}$ (neg. log M)</td>
</tr>
<tr>
<td></td>
<td>Pre-LDL a</td>
<td>Plus-LDL b</td>
<td>Pre-LDL</td>
<td>Plus-LDL</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>94.50 ± 7.3</td>
<td>63.40 ± 32.2</td>
<td>6.86 ± 0.7</td>
<td>5.64 ± 0.8</td>
</tr>
<tr>
<td>Histamine</td>
<td>86.25 ± 13.8</td>
<td>59.63 ± 40</td>
<td>7.23 ± 1.5</td>
<td>6.58 ± 2.4</td>
</tr>
<tr>
<td>A23187</td>
<td>93.13 ± 10.3</td>
<td>67.50 ± 3.1</td>
<td>8.67 ± 2.1</td>
<td>8.40 ± 1.8</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>98.63 ± 1.9</td>
<td>98.50 ± 2.5</td>
<td>7.95 ± 0.7</td>
<td>7.53 ± 0.3</td>
</tr>
</tbody>
</table>

| Protein       | 95.5 ± 5.8        | 57.66 ± 34.0  | 7.42 ± 1.0        | 5.25 ± 1.0   |
|               | 90.50 ± 9.6       | 25.25 ± 30.7  | 4.57 ± 1.3        | 6.01 ± 0.7   |
| A23187        | 93.1 ± 7.3        | 44.75 ± 41.0  | 9.99 ± 1.1        | 7.19 ± 2.0   |
| Sodium nitroprusside | 96.75 ± 6.5  | 91.88 ± 19.5 | 7.39 ± 0.6        | 7.04 ± 0.9   |

a Pre-LDL: obtained from concentration–response curves constructed before incubation with LDL.
b Plus-LDL: obtained from concentration–response curves constructed following, and in the continued presence of, 1 h incubation, with LDL.

Table 2
Effect of VLDL (0.02 mg protein/ml and 0.2 mg protein/ml) on mean % maximal relaxation response ± standard deviation (s.d.) and mean potency (neg. log M EC$_{50}$) ± s.d. to acetylcholine, histamine, A23187 and sodium nitroprusside.

<table>
<thead>
<tr>
<th>Protein</th>
<th>0.02 mg protein/ml</th>
<th></th>
<th>0.2 mg protein/ml</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% maximal relaxation</td>
<td>EC$_{50}$ (neg. log M)</td>
<td>% maximal relaxation</td>
<td>EC$_{50}$ (neg. log M)</td>
</tr>
<tr>
<td></td>
<td>Pre-VLDL a</td>
<td>Plus-VLDL b</td>
<td>Pre-VLDL</td>
<td>Plus-VLDL</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>97.38 ± 5.0</td>
<td>56.00 ± 28.0</td>
<td>6.75 ± 0.6</td>
<td>6.36 ± 1.0</td>
</tr>
<tr>
<td>Histamine</td>
<td>90.13 ± 7.1</td>
<td>62.25 ± 28.7</td>
<td>6.48 ± 0.9</td>
<td>4.89 ± 0.6</td>
</tr>
<tr>
<td>A23187</td>
<td>85.63 ± 6.9</td>
<td>61.38 ± 35.5</td>
<td>6.99 ± 0.4</td>
<td>6.76 ± 0.5</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>99.25 ± 2.1</td>
<td>96.13 ± 5.0</td>
<td>7.02 ± 0.9</td>
<td>7.02 ± 1.0</td>
</tr>
</tbody>
</table>

| Protein       | 91.13 ± 7.6        | 29.13 ± 36.3  | 7.46 ± 1.2        | 6.90 ± 0.4   |
|               | 83.75 ± 8.9        | 47.75 ± 32.4  | 5.67 ± 1.5        | 5.90 ± 0.7   |
| A23187        | 92.25 ± 8.6        | 55.50 ± 39.5  | 8.39 ± 1.4        | 8.19 ± 0.7   |
| Sodium nitroprusside | 97.00 ± 4.6  | 95.75 ± 9.7   | 7.40 ± 0.3        | 7.47 ± 0.5   |

a Pre-VLDL: obtained from concentration–response curves constructed before incubation with VLDL.
b Plus-VLDL: obtained from concentration–response curves constructed following, and in the continued presence of, 1 h incubation with VLDL.

Table 3
Effect of HDL (0.02 and 0.2 mg protein/ml) on mean % maximal relaxation response ± standard deviation (s.d.) and mean potency (neg. log M EC$_{50}$) ± s.d. to acetylcholine, histamine, A23187 and sodium nitroprusside.

<table>
<thead>
<tr>
<th>Protein</th>
<th>0.02 mg protein/ml</th>
<th></th>
<th>0.2 mg protein/ml</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% maximal relaxation</td>
<td>neg. log EC$_{50}$</td>
<td>% maximal relaxation</td>
<td>EC$_{50}$ (neg. log M)</td>
</tr>
<tr>
<td></td>
<td>Pre-HDL a</td>
<td>Plus-HDL b</td>
<td>Pre-HDL</td>
<td>Plus-HDL</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>96.75 ± 3.9</td>
<td>53.00 ± 34.4</td>
<td>6.97 ± 1.1</td>
<td>5.93 ± 0.8</td>
</tr>
<tr>
<td>Histamine</td>
<td>92.38 ± 10.3</td>
<td>43.88 ± 46.2</td>
<td>6.13 ± 1.3</td>
<td>6.44 ± 2.0</td>
</tr>
<tr>
<td>A23187</td>
<td>91.00 ± 3.3</td>
<td>61.88 ± 33.8</td>
<td>7.31 ± 1.5</td>
<td>7.56 ± 1.5</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>98.75 ± 3.5</td>
<td>95.75 ± 7.54</td>
<td>6.93 ± 0.30</td>
<td>7.53 ± 0.3</td>
</tr>
</tbody>
</table>

| Protein       | 95.13 ± 5.2        | 53.63 ± 39.0  | 7.11 ± 0.8        | 7.5 ± 0.9    |
|               | 89.75 ± 12.6       | 28.00 ± 37.0  | 6.60 ± 1.6        | 5.58 ± 2.8   |
| A23187        | 91.63 ± 4.0        | 61.88 ± 36.0  | 9.10 ± 1.1        | 9.67 ± 1.1   |
| Sodium nitroprusside | 99.00 ± 2.1  | 96.13 ± 8.8  | 7.75 ± 0.4        | 7.53 ± 0.3   |

a Pre-HDL: obtained from concentration–response curves constructed before incubation with HDL.
b Plus-HDL: obtained from concentration–response curves constructed following, and in the continued presence of 1 h incubation, with HDL.

n = 8 per set of experiments.

A23187) were all significantly attenuated by VLDL 0.2 mg protein/ml. The EC$_{50}$ value for histamine was significantly attenuated following incubation with VLDL 0.2 mg protein/ml. EC$_{50}$ values to A23187 were unaltered. Mean EC$_{50}$ values for ACh could not be calculated from fitted curves because of the very dampened response to ACh following VLDL incubation.

Neither maximal relaxations nor EC$_{50}$ values to SNP were affected by VLDL (0.02 or 0.2 mg protein/ml).

3.2.4. HDL

Mean concentration–response curves to ACh in the absence and presence of HDL 0.02 mg protein/ml are shown in Fig. 2c. Maximal relaxation responses to ACh
histamine and A23187 were all significantly attenuated in the presence of HDL at 0.02 mg protein/ml and at 0.2 mg protein/ml (Table 3), but there was no significant change in the potency of any of these agonists (Table 3).

Responses to SNP were unaltered by HDL (Table 3).

3.2.5. Combined LDL and HDL

Mean maximum relaxation responses ± s.d. mediated by ACh were significantly higher following incubation of LDL combined with HDL (pre-lipoproteins 81.8 ± 5.7 vs plus-LDL/HDL 100 ± 0.0, P < 0.05). There was no difference in the negative log EC50 values for ACh after lipoprotein incubation (pre-lipoproteins 6.69 ± 0.15 vs plus-LDL/HDL, 6.62 ± 0.61).

3.3. Correlations of lipoprotein composition and % maximal inhibition of responses

To investigate whether the inhibition of endothelium-dependent dilatation could be attributed to the composition of the lipoproteins used, correlation analyses were performed comparing the % maximal level of inhibition of all the endothelium-dependent agonists used against the calculated organ bath concentration of each lipoprotein component—cholesterol (total, free and esterified), triglyceride, phospholipid; see data, Section 2.4)—regardless of the density at which each fraction was obtained. The calculated organ bath concentration of phospholipids, but not of cholesterol (free, esterified and total) or triglycerides, correlated positively with the maximal level of inhibition of relaxation obtained to the endothelium-dependent agonists (r = 0.54, P = 0.01).

4. Discussion

The major new finding from the current study is that the inhibitory effect reported of human low-density lipoproteins (LDL) on endothelium-dependent relaxation appears not to be specific to this lipoprotein but is also true of lipoproteins of high density (HDL) and very low density (VLDL). We attribute this non-specific action of lipoproteins to the phospholipid composition of each fraction and suggest that it is this component that is responsible for the inhibitory actions of the lipoproteins on endothelium-derived relaxation.

Our findings with human lipoproteins are entirely consistent with findings using lipoproteins of rabbit origin [8]. In the previous work, the inhibition observed of ACh- and A23187-mediated dilatation was demonstrated to be lipoprotein phospholipid, but not cholesterol, concentration-dependent. It would also appear from both studies that lipoproteins are capable of potently inhibiting endothelium-dependent dilatation without first having to undergo substantial oxidation as previously suggested. Kugiyama et al. [5] found native LDL to have no effect on endothelium-dependent relaxation; however, the concentrations used were half that in the current study and it has been clearly demonstrated that impairment of endothelium-dependent relaxation is concentration-dependent [6]. Organ bath concentrations of LDL used in the current study equate with those observed at physiological plasma levels, those of VLDL are similar to those observed with pathological levels of VLDL and those of HDL somewhat underestimates physiological levels.

We are confident that the non-specific effect of lipoproteins on endothelium-dependent relaxation is not merely a kinetic phenomenon (e.g., binding of the compounds to the protein) since tissue incubation with bovine serum albumin had no effect on endothelium-dependent relaxation mediated by ACh. Nor is it due to time-dependent changes in endothelial function as no change in relaxation responses over time was observed.

The inhibitory effect of LDL on endothelial-dependent relaxation has previously been advocated to be contributory to the atherogenic and possibly vaso-spasmodic actions of this lipoprotein. Following from our data, the corollary would be that both VLDL and HDL are potentially harmful since both these lipoproteins potently inhibit endothelial-dependent relaxation. The latter finding is especially startling since a positive correlation between HDL concentration and ACh-induced relaxation has previously been reported in vivo [15]. HDL is also recognised for its anti-atherogenic properties including reverse cholesterol transport in vivo [16] and protection of LDL oxidation in vitro [17]. To further explore the role of HDL under the current in vitro setting, we studied the combined effect of HDL and LDL. When placed in the organ bath together, endothelial-dependent relaxation to ACh was not impaired, but in fact increased. One explanation may be that the lipoproteins undergo some form of minimal oxidation in the organ bath, which was not detected by the TBARS assay used in the current study, but which is necessary for their inhibitory effect on endothelium-dependent agonists. Co-incubation of HDL with LDL may inhibit this oxidation as has previously been shown in vitro [17].

The in vivo effect of VLDL is not known. Since VLDL is the main carrier for triglycerides, our data suggest that pathological levels of VLDL observed in hypertriglyceridaemia would be inhibitory of endothelium-dependent dilatation.

The exact mechanism by which LDL exerts its inhibitory actions on endothelium-dependent relaxation remain unclear. Since the nitric oxide precursor, L-arginine, reverses the endothelium impairment seen in hypercholesterolaemic rabbits [18] and humans [19], it may be that hypercholesterolaemia depletes L-arginine stores [20] or that LDL blocks binding of nitric oxide synthase enzyme (NOS) to L-arginine or that there is increased metabolism of nitric oxide. Other evidence has implicated free radicals sequestering or inactivating NO after it has been released from the endothelial cell [9,21,22]. Yet other studies sug-
gest that LDL specifically inactivates some part of the G-protein receptor-coupled pathway of bradykinin and ACh [23]. As the disease progresses, however, this selective receptor inactivation becomes less apparent [24]. Our data using LDL are consistent with the hypothesis that at lower concentrations the effect of LDL appears to be selective, only inhibiting responses to ACh and that this selectivity becomes less apparent at the higher concentration of 0.2 mg protein/ml where inhibition is seen to responses mediated not only by ACh but also by histamine which stimulates synthesis of nitric oxide through phospholipase C linked to the G_s protein [25], and the receptor-independent agonist, A23187, which opens calcium channels directly. Our data hence support the premise by Flavahan [24] that the mechanism of LDL action is concentration-dependent such that at lower concentrations there is selective inhibition of the G_s pathway whereas at higher concentrations the inhibition becomes more global affecting further along the nitric oxide pathway. It is interesting that VLDL appears slightly more potent, inhibiting both ACh and histamine at the concentration of 0.02 mg protein/ml and that HDL is even more potent, inhibiting all 3 endothelium-dependent agonists used at this concentration. This may be entirely dependent on phospholipid content; the design of the current study, however, precludes us from confirming this hypothesis.

In conclusion, we have demonstrated that the in vitro inhibitory effect of LDL on endothelium (nitric oxide)-dependent dilatation is not specific to this lipoprotein but is also true of HDL and VLDL. We further conclude that this non-specific action may be attributed to the phospholipid concentration of each fraction.

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References