HDL in the coronary survivors suggests an impairment in the process of reverse cholesterol transport in which this fraction has been demonstrated to be implicated in animal studies [8]. The results of this study therefore suggest that measurements of these HDL subfractions may provide better indicators of coronary risk than that of total plasma cholesterol which is frequently employed for this purpose but was indistinguishable between the two groups in the present study.

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A quantitative immunoassay for apolipoprotein B in plasma lipoproteins and subcellular fractions of rat liver

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Very-low-density lipoproteins (VLDL) are the vehicles by which triacylglycerol is transported from the liver to other tissues. Morphological studies have already demonstrated that VLDL-like particles first appear in hepatic endoplasmic reticulum (ER) and then move to the trans-Golgi region, from where VLDL are secreted into the bloodstream [1-3]. The polypeptide apolipoprotein B (apo B) has been shown to be essential for the hepatic assembly and secretion of VLDL [4]. However, the kinetics of apo B transfer between the subcellular organelles and the mechanisms of apo B association with lipid have yet to be fully elucidated. Studies of the kinetics of synthesis and assembly of VLDL in hepatocytes are clearly dependent upon the availability of a sensitive quantitative assay for apo B. However, until recently, little attention has been given to the quantification of the principal B apo-proteins: apo B-100 and apo B-48. Here we have employed a new immunoblotting/radio-iodination technique to quantify apo B-100 and apo B-48 in plasma lipoproteins and hepatic ER and Golgi fractions in the rat.

A purified apo B standard was prepared from rat plasma total precipitated by precipitation with tetrathiomethyl urea (TMU) [5], and separated into apo B-100 and apo B-48 by SDS-PAGE [6] on 3-20% (w/v) gradient gels. The gel slices containing apo B were solubilized in hydrogen peroxide and assayed for protein by the Lowry method [7]. Of the total apo B protein applied to gels, 42.5% was derived from apo B-100 and 57.5% from apo B-48. The purified standard was employed in the quantification of apo B in hepatic subcellular fractions. ER and Golgi (trans- and cis-elements) were prepared from whole rat livers, as previously described [8], and a cocktail of proteinase inhibitors added to minimize enzymic degradation of apo B [9]. The subcellular fractions were then detergent-solubilized [10], and the apo B immunoprecipitated using sheep anti-human apo B as a primary antibody, and donkey anti-sheep/goat IgG as a secondary antibody [8]. The immunoprecipitates and their supernatants were delipidated, solubilized in SDS/PAGE sample buffer, and applied to 3-20% (w/v) gradient gels, together with a range of concentrations (1-40 μg) of the purified apo B standard. The apo B from duplicate gels was electroblotted on to nitrocellulose membrane [11]. One blot was immunostained for apo B, and the other was taken for apo B quantification.

For specific immunostaining of apo B (Fig. 1), blots were incubated in anti-human apo B (primary antibody), followed by biotinylated anti-sheep IgG (secondary antibody) and then avidin/alkaline phosphatase which was detected colorimetrically (Vectastain). For apo B quantification, blots were first stained with Amido Black, and then incubated in anti-human apo B, followed by anti-sheep IgG, and finally, 125I-protein A (1 μCi). Blots were washed and air-dried, and the nitrocellulose chips, containing the Amido Black-stained apo B-100 and apo B-48, were cut out and counted in a γ-counter. To allow for changes in the specific activity of 125I-protein A, a new apo B standard curve was prepared for each assay.

Preliminary results of the apo B concentrations (μg/mg of subcellular fraction protein) from four separate livers were as follows [values represent mean (± S.E.M.)]. ER: B-100 = 6.9 (0.3), B-48 = 20.7 (1.4); Golgi (trans): B-100 = 27.7 (1.6), B-48 = 72.9 (3.4); Golgi (cis): B-100 = 21.2 (0.9), B-48 = 50.9 (2.8). In the immunoprecipitates from subcellular fractions (Fig. 1), the fine immunostained bands lying between apo B-100 and apo B-48 were not apparent in the Amido Black-stained preparations. These did not account for a significant proportion of total apo B, and were probably due to a small proportion of apo B-100 and apo B-48 from duplicate gels was electroblotted on to nitrocellulose membrane [11]. One blot was immunostained for apo B, and the other was taken for apo B quantification.

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amount of apo B degradation. Recovery of total apo B from ER and Golgi was about 50% of the amount assayed in the immunoprecipitate from an equivalent weight of whole liver homogenate. This was consistent with our previous finding of 50% recovery of ER and Golgi, as determined by marker enzyme assays [12-14]. Apo B immunoprecipitations from subcellular fractions was highly efficient, since no apo B was detectable in the supernatants (Fig. 1), and about 98% of the 125I counts were found in the immunoprecipitates.

Our preliminary data indicate that all of the hepatic apo B is located in the ER/Golgi system. We suggest that this immunoblotting/radioiodination technique is a sensitive and reproducible method for apo B quantification in subcellular fractions and secreted lipoproteins.

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Inhibition of endothelial-dependent relaxation by oxidized low-density lipoproteins

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Endothelium-dependent relaxation is mediated by the release of nitric oxide (EDNO) derived from the vascular endothelium [11]. In atherosclerotic arteries and those exposed to hypercholesterolaemia, the EDNO response is attenuated [2, 3]. Studies in our laboratory [4], have shown that Cu2+-oxidized low-density lipoprotein (OXLDL), inhibits endothelium-dependent relaxations mediated by nitric oxide in the rabbit aorta and may therefore account for the attenuation of vasodilation in atherosclerotic arteries. This conclusion is consistent with the presence of OXLDL in atherosclerotic lesions [5]. The aim of this work was to determine whether the inhibitor was lysophosphatidylcholine (LPC) as postulated by Kugiyama et al. [6].

LDL and high-density lipoproteins (HDL) were prepared separately from the plasma of each volunteer by density gradient ultracentrifugation [4]. HDL were further purified by heparin--Sepharose chromatography [7]. Oxidation of LDL was achieved by incubation of fresh LDL for 2 h at 37°C with 5 μM-Cu2+ [5]. Phospholipase A2 (PLA2)-treated LDL containing LPC was prepared by the incubation of fresh LDL (1 mg of protein/ml) with 10 units of PLA2 (Sigma, Naja naja venom) in 1 ml of 100 mM-Tris buffer (pH 7.4) containing 2 mM-CaCl2. The enzyme itself had no effect.

The bioassay of EDNO from isolated rabbit aortic rings was performed as previously described [4]. Aortic rings were precontracted with phenylephrine and relaxed by cumulative concentrations of acetylcholine (ACh). Preincubation with OXLDL (2 mg of protein/ml for maximum inhibition [4]) for 30 min inhibited the maximum relaxation (at 1 μM-ACh) irreversibly by 60% whereas fresh LDL caused no inhibition.

Addition of HDL at concentrations of 1 and 2 mg of protein/ml, with the OXLDL, reduced the inhibition by 50% and 100%, respectively. Similarly, serum albumin (1.5 mg/ml) caused a 66% decrease in the inhibition by OXLDL (2 mg-protein/ml) whereas inhibition by a lower concentration of OXLDL (1 mg of protein/ml) was completely abolished.

The inhibition by HDL and serum albumin suggested that lipid or fatty acid component(s) of the OXLDL might be the factor(s) implicated in the inhibition of relaxation. As LPC is generated by an endogenous PLA2 during the modification of LDL [5], the effect of PLA2-treated LDL on relaxations was investigated. The finding that PLA2-treated LDL (0.1 mg of protein/ml) whereas inhibition by a lower concentration of OXLDL (1 mg of protein/ml) was completely abolished.

The effect of 1-palmitoyl-LPC on relaxations evoked by ACh was also examined. This lysolipid abolished the relaxations at a concentration of 10 μM and the effect was reversible. At concentrations of 30-50 μM, inhibition was irreversible due to removal of the endothelium.

The degree of inhibition of relaxation by OXLDL and its reversibility varied reproducibly with the donor of the plasma from which LDL was prepared [4]. If LPC is the inhibitory component of OXLDL, its concentration in the lipoproteins should correlate with the inhibitory potency of the lipoproteins.

Abbreviations used: EDNO, endothelial nitric oxide; LPC, lysophosphatidylcholine; HDL, high-density lipoprotein; OXLDL, oxidized low-density lipoproteins; PLA2, phospholipase A2; ACh, acetylcholine.

Table 1. Comparison of the inhibition of relaxation by oxidized LDL with LPC content

The inhibition of relaxation was assayed as in [4] and the LPC content determined by the method used in [8]. Oxidized LDL was prepared separately from the plasma of two different donors [4]. Each value is expressed as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Inhibition of maximal relaxation (%)</th>
<th>LPC content (μmol of LPC/mg of protein)</th>
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<tbody>
<tr>
<td>Donor A</td>
<td>15 ± 1.4</td>
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<tr>
<td>Donor B</td>
<td>97 ± 2.5</td>
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