Increased atherogenicity of low-density lipoprotein in heavy proteinuria

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Abstract

Background. Heavy proteinuria is associated with marked abnormalities of lipoprotein metabolism and increased risk of atherogenesis. It is possible that qualitative as well as quantitative changes occur in lipoproteins to contribute to increased cardiovascular risk; for example, it is known that LDL exhibits heterogeneity, with small, dense LDL III particles being more atherogenic.

Methods. We investigated LDL subfractions (measured by density gradient ultracentrifugation), VLDL subfractions, and post-heparin lipases in 12 patients with primary glomerular disease and 24-h albuminuria >2.5 g. These were compared to 23 age- and sex-matched controls.

Results. Total LDL concentrations were similar in proteinuric patients and controls; however, there was a shift in subfraction distribution. The larger LDL I and LDL II particles were lower in the proteinuric group (29 ± 24 vs 62 ± 26 mg/dl P = 0.011 and 121 ± 80 vs 197 ± 74 mg/dl P = 0.028), whereas the concentration of atherogenic LDL III (small dense) was higher (135 ± 64 vs 75 ± 71 mg/dl P = 0.0016). The concentration of total VLDL and both its subfractions were increased in the patients with proteinuria. Post-heparin hepatic and lipoprotein lipase levels were similar to normal.

Conclusions. These findings suggest that the atherogeneity of LDL is increased in patients with heavy proteinuria because of the redistribution towards smaller denser particles. Since small, dense LDL has a lower affinity for the LDL receptor, the altered nature of the lipoprotein in proteinuria may decrease its clearance by the receptor-mediated pathway and contribute to the reduced clearance of LDL observed in this population. This may contribute to progression of renal failure or the accelerated vascular disease found in patients with heavy proteinuria.

Key words: cardiovascular risk; glomerulonephritis; LDL subfractions; lipoprotein metabolism; low-density lipoprotein; proteinuria

Introduction

Hyperlipidaemia has long been recognized as a consistent feature in patients with nephrotic-range proteinuria [1]. The most common lipid abnormality is a raised serum cholesterol, which is thought to contribute to the accelerated atherosclerosis found in these patients [2]. Low-density lipoprotein (LDL) cholesterol is usually elevated, and in patients with severe nephrotic syndrome (serum albumin <15 g/l), serum triglyceride and very low-density lipoprotein (VLDL) rise dramatically [3].

Metabolic studies have indicated that in patients with proteinuria, the transformation of VLDL to LDL is delayed [4] and clearance of LDL by the receptor-mediated pathway is reduced [5]. High-density lipoprotein (HDL) cholesterol concentration has been reported as being normal [6], increased [7], or decreased [3]. HDL subclass distribution is abnormal with low HDL2 levels being reported. Lipoprotein lipase activity has been found to be reduced in patients with the nephrotic syndrome and nephrotic-range proteinuria [8]; however, some authors have not confirmed this, finding normal levels [9].

LDL exhibits marked heterogeneity in terms of size and density, being subdivided into large, light LDL I (d 1.025–1.034 g/ml) and LDL II (d 1.034–1.044 g/ml), and small, dense LDL III (d 1.044–1.063 g/ml). LDL particle size is influenced by many factors the most important of which is the serum triglyceride level [10]; however, genetic background, age, gender, hormonal status, obesity, diet, and diabetes all have an effect [11]. An increasing number of studies have demonstrated a connection between small, dense LDL and increased risk of coronary artery disease [11,12]; a plasma level of LDL III >100 mg/dl has been shown to confer a sevenfold increased risk of myocardial infarction [12]. Recently two prospective studies have added to the evidence in favour of the increased atherogenicity of small, dense LDL.
Subjects

activity of 30 Subjects and methods

were included. All patients had a biopsy-proven diagnosis. 

Patients attending the renal unit at 9 a.m., having fasted since midnight. An intravenous cannula was inserted and samples were taken for the estimation of lipids and lipoproteins, LDL and VLDL subfractions, serum creatinine, and albumin. Seventy units/kg body-weight of i.v. heparin was then given and 10 min later samples taken for hepatic and lipoprotein lipase measurement [19]. A 24-h urine collected over the preceding day was analysed for urinary albumin excretion.

Methods

Plasma cholesterol, triglyceride, VLDL cholesterol, LDL cholesterol, and HDL cholesterol were analysed by modification of the standard Lipid Research’s Clinics protocol [20]. VLDL₁ (S<sub>1</sub>, 60–400) and VLDL₂ (S<sub>2</sub>, 20–60) were isolated from plasma by a modification of the cumulative-gradient ultracentrifugation procedure. The triglyceride, free cholesterol, cholesterol ester, phospholipid, and protein contents of the lipoproteins were assayed and lipoprotein concentrations were calculated as the sum of these components [21]. Three LDL subfractions were isolated from fresh plasma by non-equilibrium density gradient ultracentrifugation as previously described [21]. Following centrifugation for 24 h at 40 000 r.p.m. and 23°C in a swinging bucket rotor, the contents of the centrifuge tube were eluted by upward displacement and the presence of three fractions (LDL₁, d 1.025–1.034 g/ml; LDL₂, d 1.034–1.044 g/ml; and LDL₃, d 1.044–1.063 g/ml) were detected by continuous monitoring at 280 nm. The individual subfraction areas were quantified, corrected for previously calculated extinction coefficients, and expressed as percentage of total LDL.

The lipoprotein mass of LDL (d 1.019–1.063 g/ml) was determined as above and used to generate individual subfraction concentrations in milligrams lipoprotein per 100 ml plasma. The activities of LPL and HL were measured in post-heparin plasma incubated with a gum-arabic-stabilized triglyceride emulsion containing glycerol tri(14C) oleate at a specific activity of 30 μCi/mmol triglyceride fatty acids as substrate [19]. For the specific assay of LPL, plasma was preincubated with sodium dodecyl sulphate to inhibit HL, and pooled pre-heparin plasma was added to the incubation as a source of cofactor apo CII to activate the LPL enzyme. HL was assayed in the presence of 1.0 mol/l NaCl to inactivate LPL. Enzyme activities are expressed in μmol of fatty acids released per hour per millilitre of plasma. Apo E phenotypes were

Subjects and methods

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Twelve patients with primary glomerular disease (10 male, 2 female) aged between 18 and 65 years consented to participate in the study. They were recruited from consecutive attendees at the outpatient clinic of the Glasgow Royal Infirmary Renal Unit according to the following inclusion criteria: (a) serum creatinine < 300 μmol/l, and (b) urinary albumin excretion >2.5 g/24 h.

Patients suffering from other diseases or on treatment that might influence their lipid profile were excluded, specifically patients with a diagnosis of diabetes mellitus, amyloid, any neoplastic disorder, systemic lupus erythematosus, or taking thiazide diuretics, fat-soluble beta-blockers, corticosteroids, or any other immunosuppressive agent. Treatment with other antihypertensives or diuretics was permitted. In addition, patients being followed up who were already on lipid-lowering therapy were excluded, although newly diagnosed patients who had not yet commenced lipid-lowering therapy were included. All patients had a biopsy-proven diagnosis. The clinical and biochemical features of the patients studied are listed in Table 1. They were compared to 23 age- and sex-matched controls with no history of arterial disease, extracted from a database of the Department of Pathological Biochemistry at the Royal Infirmary. The study was approved by the Ethics committee of Glasgow Royal Infirmary.

Heavy proteinuria is known to be a risk factor for progression of chronic renal failure; there is also experimental evidence suggesting that lipids may contribute directly to glomerular and tubulointerstitial injury. These same mechanisms that lead to increased atherosclerosis with small, dense LDL could contribute to glomerular injury. The increased filtration of LDL by the endothelium would result in greater quantities of LDL being present in the mesangium. Oxidized LDL has been found in the glomeruli of experimental nephrotic rats and is known to be cytotoxic to mesangial cells [16]; also, mesangial matrix itself has a high capacity to bind LDL with a 10-fold increase in glomerular localization of oxidized LDL compared with LDL [17].

LDL subfraction profiles in patients with nephrotic-range proteinuria have not been studied but clearly abnormal profiles may play an important role in a population with increased risk of both cardiovascular disease and development of renal failure. The primary aim of this study therefore was to determine whether LDL particles in patients with nephrotic-range proteinuria were more atherogenic in type compared to age- and sex-matched controls with no history of ischaemic heart disease. Our secondary aim was to investigate the possible origins of any abnormal LDL profiles found. Therefore, post-heparin lipase activity was measured to establish if any catabolic defect present could be attributable to impaired lipase activity. VLDL subfractions were analysed to establish if the origin of any abnormal LDL profiles differed from that found in patients with dyslipidaemia of different origin. It has previously been suggested that the E4 isoform of apoE as was more common in patients with the nephrotic syndrome and may contribute to the hyperlipoproteinaemia [18]. Apo E phenotyping was therefore also performed.

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Table 1. Characteristics of patients studied

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>BMI*</th>
<th>Smoker</th>
<th>Vascular disease</th>
<th>Serum creat. (µmol/l)</th>
<th>24-h UAE** (g)</th>
<th>Serum albumin (g/l)</th>
<th>Diagnosis</th>
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<td>77</td>
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<tr>
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<td>yes</td>
<td>273</td>
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<td>42</td>
<td>IgA nephropathy</td>
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<tr>
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<td>60</td>
<td>64</td>
<td>24</td>
<td>yes</td>
<td>no</td>
<td>193</td>
<td>4.0</td>
<td>34</td>
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<tr>
<td>7 m</td>
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<td>72</td>
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<td>71</td>
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<td>no</td>
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<td>19</td>
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<td>24</td>
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</tr>
<tr>
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<td>21</td>
<td>yes</td>
<td>no</td>
<td>80</td>
<td>3.0</td>
<td>29</td>
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<tr>
<td>Mean (SD)</td>
<td>46 (16)</td>
<td>82 (20)</td>
<td>28 (6.8)</td>
<td>182 (70)</td>
<td>4.3 (1.8)</td>
<td>35 (5.6)</td>
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</tr>
</tbody>
</table>

*Body mass index; **urinary albumin excretion.

determined by isoelectric focusing followed by Western blotting using an adaptation of the method of Havekes et al. [22].

Statistics

Statistical analysis and manipulations were performed by using Minitab 10X for Windows (Minitab Inc.). The following factors which were not normally distributed were subjected to log transformation as previously described [23]: BMI, plasma TG, HDL-C, LPL, HL, VLDL total, VLDL1, VLDL2, LDLI, LDLL, and LDLLIII. Plasma VLDL-C was normalized by taking the square root [23]. All other variables were examined untransformed. Results are shown as means and standard deviations (Tables 2–4), samples were compared using the two-sample t test. In some instances the *P < 0.005; **P < 0.01; ‡P < 0.05.

Results

Anthropometry, renal function, lipids and lipoproteins

Table 1 summarizes the details of the patients studied. The mean serum creatinine was moderately elevated at 182 µmol/l, with a urinary albumin excretion of 4.3g/24 h (corresponding to a urinary protein of >6g/24 h). Despite this, the mean serum albumin was remarkably well maintained at 35 g/l. Table 2 summarizes the anthropometry, lipid, and lipoprotein results. The control population’s data corresponded very closely to that obtained in other population studies with much larger numbers [23]. No significant difference in age, weight or body mass index was obtained. The proteinuric group had mildly increased serum cholesterol levels 6.5 ± 1.4 mmol/l vs 5.3 ± 1.1 mmol/l (P = 0.023) and substantially higher serum triglyceride levels 3.2 ± 2.7 mmol/l vs 1.2 ± 0.4 mmol/l (P = 0.009). LDL cholesterol and HDL cholesterol showed no significant difference; however, VLDL cholesterol levels were markedly raised 1.7 ± 1.3 mmol/l vs 0.5 ± 0.2 mmol/l (P = 0.003).

LDL subfractions

There was no difference in total LDL mass between the two groups (Table 3). The proteinuric group had slightly lower amounts of LDL at 285 ± 97 mg/dl compared with controls 334 ± 88 mg/dl, but the difference was not significant (P = 0.15). Small, dense LDL III was present in much greater quantities in proteinuric patients compared with controls 135 ± 64 mg/dl vs 75 ± 71 mg/dl (P = 0.0016), and eight of 12 patients Table 3. LDL subfractions (Mean ± SD)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL total (mg/dl)</td>
<td>285 (97)</td>
<td>334 (88)</td>
</tr>
<tr>
<td>LDL I (mg/dl)</td>
<td>29 (24)*</td>
<td>62 (26)</td>
</tr>
<tr>
<td>LDL II (mg/dl)</td>
<td>121 (80)</td>
<td>197 (74)</td>
</tr>
<tr>
<td>LDL III (mg/dl)</td>
<td>135 (64)</td>
<td>75 (71)</td>
</tr>
</tbody>
</table>

*One patient’s LDL I = 0, therefore unable to log transform; comparative statistics done on 11 patients.
had LDL III levels greater than 100 mg/dl (Figure 1). The opposite was found with the larger, lighter LDL subtypes, LDL I levels being significantly less in proteinuric patients $29\pm 24$ mg/dl vs $62\pm 26$ mg/dl ($P = 0.011$), as were levels of LDL II $121\pm 80$ mg/dl vs $197\pm 74$ mg/dl ($P = 0.028$). In the case of LDL I, statistical analysis was performed on 11 samples instead of 12 as the LDL I levels of one patient were undetectable and therefore could not be log transformed.

**VLDL subfractions, lipases, and Apo E phenotype**

Total VLDL mass was significantly raised in the patients with proteinuria (Table 4) $339\pm 280$ mg/dl vs $103\pm 63$ mg/dl ($P = 0.014$). This was due to increases in both VLDL subfractions in the study population VLDL$_1$ $192\pm 178$ mg/dl vs $58\pm 45$ mg/dl ($P = 0.028$) and VLDL$_2$ $147\pm 117$ mg/dl vs $45\pm 22$ mg/dl ($P = 0.049$). Post-heparin lipoprotein and hepatic lipase analysis showed no significant difference between proteinuric and control groups and no patients had lipoprotein lipase activity below the normal range of $2–10$ µmol FA/ml/h. There was no observed correlation between lipoprotein lipase activity and plasma total cholesterol, triglyceride, serum creatinine, LDL, or VLDL subfraction concentrations; however, hepatic lipase activity was observed to have a positive correlation with plasma triglycerides and the VLDL$_1$ subfraction. There was no observed correlation between hepatic lipase activity and plasma total cholesterol, serum creatinine, or LDL subfractions.

Apo E phenotyping was performed in 11 patients. The commonest phenotype (3/3) was found in four, another four had 3/2. The E4 isoform was found in only three (4/3 in two and 4/4 in the other) and therefore had an allele frequency of 0.18. This is similar to previous findings where the E4 frequency was 0.19 [4], and is not different from that expected in the normal population.

**Discussion**

This is the first study to demonstrate that there is an increase in atherogenic LDL subfractions in patients with nephrotic-range proteinuria and reasonably well preserved renal function. We have also shown that post-heparin lipoprotein and hepatic lipase levels in proteinuric patients did not differ from controls, and that total VLDL levels were increased due to increases in both VLDL subfractions.

The mean plasma total cholesterol and LDL cholesterol were lower than have been found previously in patients with the nephrotic syndrome [24]. There are two main reasons for this: firstly, most of the patients studied had nephrotic-range proteinuria but normal albuminaemic; Joven et al. [6] have shown previously that patients with nephrotic-range proteinuria but well-maintained serum albumin have a less severe dyslipidaemia than those with hypoalbuminaemia. This has been confirmed recently, and it was further shown that VLDL cholesterol is raised in patients with nephrotic-range proteinuria but normal albuminaemia [25]. The mean plasma total cholesterol and LDL cholesterol in our patients are comparable to published data [6]. Secondly, because of the renal unit’s policy of actively following up and treating hypercholesterolaemia, the patients with the worst dyslipidaemia were already on active treatment and were therefore excluded. The fact that the patients studied had only modest dyslipidaemia makes the finding of significantly raised levels of atherogenic LDLIII more remarkable.

The precise biochemical mechanisms that lead to the formation of small, dense LDL are not yet fully understood. It is known that LDL structure is strongly influenced by plasma triglyceride and individuals with a plasma triglyceride $>1.5$ mmol/l have a predominant of small, dense LDL [10]. A model for the formation of LDL III has been proposed: this suggests that in the presence of hypertriglyceridaemia, excess large, light VLDL$_1$ is produced which takes part in neutral lipid exchange with LDL via cholesterol ester transfer protein (CETP). Triglyceride is passed to LDL in exchange for cholesterol, which is transferred to VLDL; this creates triglyceride-enriched LDL, which acts as the ideal substrate upon which hepatic lipase acts. The enzyme delipidates the LDL particle, thus

![Fig. 1. Dot plot showing distribution of LDL III subfractions in patients and controls.](image)
Increased atherogenicity of low-density lipoprotein in heavy proteinuria

shrink ing it and forming small, dense LDL [26]. The findings in this study are concordant with this hypothesis. Plasma triglyceride levels were significantly raised, excess large, light VLDL1 was present, and hepatic lipase levels, although not raised, were sufficient to hydrolyse any triglyceride-enriched LDL species present.

VLDL subfractions were both raised; however, the proportion of VLDL1: VLDL2 was abnormal for the observed level of plasma triglyceride. In hypertriglyceridaemia without renal disease, the main defect is increased production of VLDL1, thus the ratio of VLDL1 to VLDL2 is increased markedly; the ratio of VLDL1 to VLDL2 at plasma triglycerides of 0.5 mmol/l is 1:1, and the ratio at triglycerides of 2.0 mmol/l is 2:1 [23]. The serum triglyceride level in the patients studied was >3.0 mmol/l, and thus, if the origin of the raised serum triglycerides was similar to that in patients without renal disease, one would expect a ratio of VLDL1: VLDL2 of greater than 2:1; however, the ratio of VLDL1: VLDL2 was close to unity. This, we speculate, reflects a different origin to the excess VLDL subfractions in patients with proteinuria.

Post-heparin lipase levels were similar in proteinurics and controls. It has previously been suggested that there may be important differences between chylomicron-lipoprotein lipase and VLDL-lipoprotein lipase interactions due to the paradoxical finding of normal clearance of chylomicron particles but severely impaired clearance of VLDL particles in the nephrotic syndrome [4]. It has been postulated that this may be due to loss of cofactors in the urine [27]. This would certainly explain our normal laboratory measurements accompanied by abnormalities in VLDL subfractions but normal lipase activity. We suggest that the altered nature of the LDL particle may contribute to the decreased clearance of LDL by the receptor mediated pathway that is found in this condition, and that the raised LDL III levels are likely to contribute to the increased vascular disease found in this population.

In conclusion, in this pilot study involving a small non-representative sample, we have shown that in patients with heavy proteinuria, reasonably well-preserved renal function, and well-maintained serum albumin, there are marked abnormalities in LDL subfraction distribution due to a shift in LDL particle size towards an atherogenic phenotype. This is accompanied by abnormalities in VLDL subfractions but normal lipase activity. We suggest that the altered nature of the LDL particle may contribute to the increased vascular disease found in this population.

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References


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