The effect of decreasing renal function on lipoprotein profiles

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Abstract

Background. Progressive chronic kidney disease (CKD) is accompanied by dyslipidemia that is characterized by increased concentrations of intact and partially metabolized ApoB and ApoC-III-containing triglyceride-rich lipoproteins in very low-density lipoprotein, intermediate density lipoprotein (IDL) and low-density lipoprotein. The purpose of the present study was to characterize the distribution of individual discrete lipoprotein subclasses in relation to the glomerular filtration rate (GFR) in nondiabetic CKD subjects.

Methods. Fifty-one subjects (33 patients with CKD and 18 asymptomatic healthy subjects) with GFR ranging from 12 to 120 mL/min were studied. Individual ApoA- and ApoB-containing lipoprotein subclasses (Lp) were determined in plasma by sequential immunoaffinity chromatography and subsequent determination of apolipoprotein composition. Based on the apolipoprotein compositions, there are two major groups of lipoprotein subclasses. One group consists of ApoA-containing lipoprotein subclasses and the other group of ApoB-containing lipoprotein subclasses. The individual ApoA-containing lipoproteins are almost exclusively found in high-density lipoprotein (HDL) as Lp-A-I, Lp-A-I:A-II and Lp-A-II. The ApoB-containing lipoproteins are distributed throughout very low-density lipoprotein (VLDL), IDL and low-density lipoprotein (LDL) ranges. The five distinct ApoB-containing lipoprotein subclasses are Lp-B, Lp-B:E, Lp-B:C:E, Lp-B:C and Lp-A-II:B:C:D:E.

Results. There were no changes in concentrations of ApoA-containing lipoproteins with decreasing GFR. The levels of ApoB-containing lipoproteins increased significantly with decreasing GFR. There was a moderate increase of cholesterol-rich LpB and a 3-fold increase of ApoB- and ApoC-III-containing lipoproteins in subjects in the two lowest quintiles of GFR. This was accompanied by a significant increase of plasma ApoC-III.

Conclusions. Reduced renal function is associated with a complex alteration of the lipoprotein profile that is predominantly characterized by increased concentrations of triglyceride-rich lipoprotein subclasses containing both ApoB and ApoC-III.

Keywords: chronic kidney disease; glomerular filtration rate; lipoprotein subclasses

Introduction

Progressive chronic kidney disease (CKD) is accompanied by the development of specific alterations of the lipoprotein metabolism [1, 2]. The renal dyslipidemia is characterized by increased concentrations of intact and partially metabolized apolipoprotein (Apo)B- and ApoCIII-containing triglyceride-rich lipoproteins in very low-density lipoprotein (VLDL) and intermediate density lipoproteins (IDLs). Depending on the degree of delipidization of lipoproteins, serum triglyceride levels may be normal or elevated.

Plasma lipoproteins consist of discrete lipoprotein subclasses (Lp) with varying physical and metabolic properties [3]. They can be categorized and identified by their apolipoprotein composition. Based on the apolipoprotein compositions, there are two major groups of lipoprotein subclasses. One group consists of ApoA-containing lipoprotein subclasses and the other group of ApoB-containing lipoprotein subclasses. The individual ApoA-containing lipoproteins are almost exclusively found in high-density lipoprotein (HDL) as Lp-A-I, Lp-A-I:A-II and Lp-A-II. The ApoB-containing lipoproteins are distributed throughout VLDL, IDL and low-density lipoprotein (LDL) ranges. The five distinct ApoB-containing lipoprotein subclasses are Lp-B, Lp-B:E, Lp-B:C:E, Lp-B:C and Lp-A-II:B:C:D:E.

Several studies in nonrenal patients have clearly shown that ApoB-containing lipoproteins are atherogenic [4–6]. Recent studies strongly indicate that lipoproteins that in addition to ApoB also contain ApoC-III could be particularly harmful [7, 8].

We have previously shown that renal dyslipidemia is already present at mildly reduced renal function. The alterations are primarily seen in a characteristic apolipoprotein pattern but are not necessarily manifested as hyperlipidemia [9]. The purpose of the present study was to characterize the distribution of individual discrete lipoprotein subclasses in relation to the glomerular filtration rate (GFR) in nondiabetic subjects with varying degree of renal function.

Patients and methods

Thirty-three nondiabetic and non-nephrotic patients with CKD due to primary renal disease and 18 asymptomatic healthy subjects were studied. The subjects were divided into quintiles according to their GFR; Group I: GFR ≥ 90 mL/min, n = 11 subjects; Group II: GFR 76–89 mL/min, n = 10 subjects; Group III: GFR 38–75 mL/min, n = 10 subjects; Group IV: GFR 24–37 mL/min, n = 10 subjects and Group V: GFR ≤ 23 mL/min, n = 10 subjects (Table 1).

The patients were recruited from the Department of Nephrology at Sahlgrenska University Hospital, Göteborg, Sweden. No patient was on
any lipid-lowering treatment or any other therapy known to affect lipoprotein metabolism. The asymptomatic subjects were recruited as a control group from an intervention study [10].

The study was conducted in accordance with the ethical standards of the institutional committee on human experimentation and the Helsinki Declaration as revised in 2000. Written consent was obtained from all participants.

All blood samples were taken after an overnight fast. Total cholesterol and triglyceride concentrations were determined by enzymatic methods [11]. Apolipoproteins A-I, B, C-III and E were measured by electroimmunoassays using monospecific antisera as previously described [12]. The distribution of ApoC-III in heparin-Mn++ supernates (i.e. in HDL) and precipitates (i.e. in VLDL + LDL), and the Apo-C-III ratio (Apo-C-III in HDL/Apo-C-III in VLDL + LDL) was calculated. This ratio reflects the removal of triglyceride-rich lipoproteins; the lower the Apo-C-III ratio, the less efficient the removal [12].

The LP-A-I and LP-A-I:A-II subclasses and the major classes of ApoB-containing lipoprotein subclasses, including cholesterol-rich, LP-B and triglyceride-rich LP-B:C, LP-B:E and LP-B:C:E and LP-A-II:B:C:D:E were measured by sequential immunoaffinity chromatography as previously described [13].

GFR was measured as plasma clearance of iohexol or $^{51}$Cr-ethylenediaminetetraacetic acid. In patients with mild renal insufficiency and in healthy subjects, blood was drawn for determination of concentration of the filtration marker at 1.2 and 4 h after the injection. In patients with moderate renal insufficiency, samples were taken after 2.3 and 5 h, and in patients with severe renal insufficiency, samples were taken at 5 and 24 h after the injection.

Statistical methods

Standard statistics were used to illustrate the salient features of data. The patients were categorized according to their degree of renal function. They were divided into subgroups of equal size and we used quintiles of GFR as the cutoff points. Differences between groups were tested with analysis of variance. A P-value < 0.05 was regarded as statistically significant.

Results

The plasma concentrations of lipids, apolipoproteins and individual lipoprotein subclasses are presented in Tables 2 and 3.

All plasma lipids changed with decreasing renal function. The linear regression analyses revealed that these changes were all highly statistically significant. However, it is evident from Table 2 that subjects with GFR > 75 mL/min had a normal lipid profile, whereas the major alterations in the lipid pattern were observed in the two lower quintiles of GFR, i.e. < 38 mL/min.

There were no changes in the ApoA-containing lipoproteins with decreasing GFR (Table 3). Apolipoprotein A-I and the individual lipoprotein subclasses Lp-A-I and Lp-A-I:A-II as well as the complex Lp-A-I:A-II:B:C:E subclass showed no correlation with GFR, and the plasma concentrations were similar in subjects with the lowest GFR and in those with normal renal function. The ApoB-containing lipoproteins increased with lower GFR. Similar to the plasma lipids, the major alterations were observed in the two lowest quintiles of renal function. While concentrations of the cholesterol-rich lipoprotein LpB increased by only ~ 30% in the two lowest quintiles compared to subjects with GFR > 75 mL/min, the plasma concentration of the triglyceride-rich lipoprotein subclass Lp-B:C was almost three to four times higher. Similarly, the triglyceride-rich lipoprotein subclass Lp-B:C:E was twice as high in subjects with GFR < 38 mL/min in comparison to subjects with GFR > 75 mL/min.

The plasma concentration of ApoC-III was also almost twice as high in patients with GFR < 38 mL/min compared to subjects with GFR > 75 mL/min (Groups I and II). This was entirely due to increased ApoC-III concentrations in the lower density ranges, i.e. in the ApoB-containing lipoproteins with no change of ApoC-III levels in HDL. This is also reflected in a low ApoC-III ratio.

Discussion

The main finding of this study was a marked increase in the plasma concentrations of two of the lipoprotein subclasses that contain both ApoB and ApoC-III, i.e. Lp-B:C and Lp-B:C:E, in subjects with CKD and a GFR < 75 mL/min.

Table 1.

<table>
<thead>
<tr>
<th>Gender, age and GFR in patients in Groups I–V. Mean and SD (in parenthesis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
</tr>
<tr>
<td>GFR ≥ 90 mL/min</td>
</tr>
<tr>
<td>n = 11</td>
</tr>
<tr>
<td>Male/ female</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>GFR (mL/min × 1.73 m$^2$ BSA)</td>
</tr>
</tbody>
</table>

Table 2.

<table>
<thead>
<tr>
<th>Plasma lipids (mmol/L)</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR ≥ 90 mL/min</td>
<td>n = 11</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.3 (0.7)</td>
<td>5.3 (0.8)</td>
<td>5.5 (2.2)</td>
<td>6.6 (1.2)</td>
<td>7.2 (1.3)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.2 (0.3)</td>
<td>0.9 (0.2)</td>
<td>1.8 (0.8)</td>
<td>1.9 (1.0)</td>
<td>2.2 (0.8)</td>
</tr>
<tr>
<td>VLDL-cholesterol</td>
<td>0.5 (0.2)</td>
<td>0.4 (0.1)</td>
<td>0.8 (0.3)</td>
<td>0.8 (0.5)</td>
<td>1.2 (0.5)</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>3.3 (0.7)</td>
<td>3.4 (0.7)</td>
<td>4.0 (1.3)</td>
<td>4.4 (0.7)</td>
<td>4.8 (1.3)</td>
</tr>
<tr>
<td>Non-HDL-cholesterol</td>
<td>3.8 (0.7)</td>
<td>3.8 (0.7)</td>
<td>4.8 (1.4)</td>
<td>5.3 (1.3)</td>
<td>6.0 (1.2)</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>1.5 (0.3)</td>
<td>1.5 (0.4)</td>
<td>1.1 (0.2)</td>
<td>1.2 (0.3)</td>
<td>1.2 (0.3)</td>
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</tbody>
</table>

*Mean and SD (in parenthesis) and P-value (analysis of variance).
Previous studies from this and other laboratories have shown that progressive loss of renal function is accompanied by increasing concentrations of ApoB-containing lipoproteins, while there is no or only minor changes in the levels of major ApoA-containing lipoproteins [1, 2]. To our knowledge, the present study is the first to measure individual lipoprotein subclasses in CKD patients and relate them to the degree of renal functional impairment. We acknowledge that the cutoff points used to categorize the patient group according to their degree of CKD is not equivalent to the CKD staging generally used in clinical practice. However, the purpose of the categorization in the current paper is not to study the grade of CKD itself but to investigate the relationship with lipoproteins. From a statistical point of view, it is preferred to have categorized groups of equal size. In order to achieve this, the patients were divided into percentiles of the GFR value; we have used quintiles of GFR as the cutoff points. Furthermore, Quintile V in the present analyses is about the same as CKD Stages 4 and 5 together, and Quintile I is equal to CKD Stage 1.

When renal function, expressed as GFR, decreased <75 mL/min, there was a progressive increase of both cholesterol-rich LpB and triglyceride-rich Lp-B:C and Lp-B:C:E. However, in relative terms, there is much more pronounced increase in the latter two triglyceride-rich lipoprotein subclasses. The change in both the concentrations and distribution of these individual lipoprotein subclasses can be explained by the previously described characteristic apolipoprotein profiles of renal dyslipidemia, with increased levels of ApoC-III, a reduced ratio between ApoC-III in HDL and ApoC-III in VLDL and LDL and a reduced ApoA-I/ApoC-III ratio [1].

We and other investigators have shown that one of the main pathophysiological mechanisms underlying these lipoprotein abnormalities of CKD is a delayed catabolism and removal of triglyceride-rich ApoB-containing lipoproteins [14, 15]. Our present results indicate that patients with mildly reduced renal function have already increased concentrations of lipoprotein subclasses, such as Lp-B:C and Lp-B:C:E, and that this becomes more pronounced during the course of progressive loss of renal function.

Several observational and interventional studies have clearly indicated that intact or partially delipidized ApoB-containing lipoproteins with ApoC-III have an atherogenic potential similar to, or even greater than, ApoB-containing lipoproteins without ApoC-III [7, 8, 16–19]. The atherogenic potential of ApoC-III has recently been described in a series of experimental studies by Kawakami et al. [20] showing that ApoC-III can trigger a cascade of proinflammatory events, which result in endothelial dysfunction and vascular damage. Furthermore, these events seem to be closely associated with decreased insulin activity and insulin resistance [20]. The insulin resistance that develops early in renal disease [21] may therefore be a pathogenetic link between CKD and elevated levels of ApoC-III [22, 23].

Cardiovascular disease (CVD) is the main cause of morbidity and premature mortality in patients with advanced CKD. It is still not clarified to what extent renal dyslipidemia may contribute to the development of CVD in renal patients. Studies from Stenvinkel et al. [24] and others have clearly demonstrated that increased levels of circulating markers of chronic inflammation herald premature CVD in patients with CKD. The findings that increased levels of ApoC-III-containing lipoproteins can trigger inflammation in the vasculature are intriguing and support the notion that it is not the lipid content of the lipoprotein particles but the apolipoprotein composition that is of importance for the development of vascular damage. This may be one of the explanations why plasma lipids have not been associated with CVD in prospective follow-up studies of renal patients [25, 26].

Several controlled trials have indicated that lipid-lowering treatment may reduce CVD in subjects with mildly reduced renal function [1]. In contrast, two large, randomized controlled trials failed to show any benefit of statin treatment on CVD in dialysis patients [27, 28]. There are several possible explanations for this lack of treatment effects of statins in advanced renal failure. One explanation may be the altered lipoprotein profile of CKD with the predominate increase of...
atherogenic triglyceride-rich Lp-B:C and Lp-B:C:E subclasses shown in the present study. The statins are very effective in lowering plasma concentrations of cholesterol-rich ApoB-containing lipoproteins [28], whereas their effects on the triglyceride-rich lipoprotein subclasses that accumulate in CKD patients are modest [13]. It is possible that the lack of a beneficial effect of statins on CVD in advanced renal failure may partially be related to unaffected increased levels of atherogenic ApoC-containing lipoproteins. One may speculate that drugs that increase the insulin sensitivity, such as peroxisome proliferator-activated receptor agonists, may be more effective in normalizing renal dyslipidemia and, thereby, improve the prognostic outlook in terms of atherosclerotic complications [10, 29].

In conclusion, the present study has shown that reduced renal function is associated with a complex dyslipoproteinemia primarily characterized by increased concentrations of ApoC-III-containing triglyceride-rich lipoproteins. When GFR is reduced to <75 mL/min, there is an increase in atherogenic lipoprotein subclasses, which in addition to ApoB also have ApoC-III as their protein moieties. The importance of these lipoproteins for the development of CVD and further progression of renal insufficiency remains to be established in controlled intervention studies.

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Conflict of interest statement. None declared.

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