Vasodilation in resistance arteries is related to the apolipoprotein B/A1 ratio in the elderly

The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study

Lars Lind

Department of Medicine, Uppsala University Hospital, AstraZeneca R&D Mölndal, 751 85 Uppsala, Sweden

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Abstract

Background: Recent studies have shown the apolipoprotein B to apolipoprotein A1 ratio (apoB/A1) to be superior to LDL-cholesterol measurements to predict cardiovascular events.

The present study aims to relate apoB/A1 to endothelium-dependent vasodilation, an early marker of atherosclerosis, in the Prospective Study of the Vasculature in Uppsala Seniors (PIVUS) study.

Methods and results: In this population-based study, 1016 subjects aged 70 years were evaluated by the invasive forearm technique with acetylcholine (EDV), brachial artery ultrasound to assess flow-mediated vasodilation (FMD) and pulse wave analysis with a beta-2 receptor agonist challenge, terbutaline. EDV and the pulse wave response, but not FMD, were related to apoB/A1 levels (r = −0.11, p = 0.0038 for EDV, r = −0.16, p < 0.0001 for the pulse wave analysis and r = 0.01, p = 0.65 for FMD). Neither LDL-cholesterol, nor non-HDL-cholesterol, was significantly related to the measurements of endothelium-dependent vasodilation.

Also endothelium-independent vasodilation (EIDV) evaluated by the invasive forearm technique with sodium nitroprusside was related to apoB/A1 levels (r = −0.12, p < 0.0016).

Conclusion: The apoB/A1 levels, but not LDL-cholesterol, were inversely related to endothelium-dependent vasodilation evaluated by EDV and pulse wave analysis, but not by FMD. Also EIDV showed the same pattern, suggesting a general deterioration in vasoreactivity mainly in resistance arteries in elderly subjects with high apoB/A1 levels.

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1. Introduction

High levels of LDL-cholesterol are one of the best validated risk factors for the development of atherosclerosis and coronary heart disease [1-2]. However, in the recent years some studies have highlighted the importance of the apolipoproteins rather than the LDL-cholesterol level [3-5].

Circulating levels of apolipoproteins reflect the number of, rather than the cholesterol concentration of, lipoprotein particles. Specifically, the level of apolipoprotein B-100 (apoB) reflects the number of triglyceride-rich VLDL particles and the number of LDL particles. It thus gives more weight to the number of small dense LDL particles than the more regular measurement of LDL-cholesterol does. The level of apolipoprotein A1 (apoA1) mainly reflects the number of HDL particles. Thus, the ratio of apolipoproteins B and A1 (apoB/A1) would therefore theoretically be an ideal marker for lipid disturbances, and recently a large cohort study suggested that the apoB/A1 is superior to LDL-cholesterol levels in the prediction of cardiovascular mortality [3].
Endothelium-dependent vasodilation is a characteristic feature of the vasculature and an impaired endothelium-dependent vasodilation is seen early in the atherosclerotic process [6]. A defect endothelium-dependent vasodilation has been shown to predict future cardiovascular events [7,8]. Although several studies have shown endothelium-dependent vasodilation, evaluated with different technologies, to be impaired in subjects with high levels of LDL-cholesterol [9–23], few studies have investigated the relationship between apoB/A1 and endothelium-dependent vasodilation. In a small study in healthy middle-aged subjects, we reported apoB/A1 to be slightly more closely related to endothelium-dependent vasodilation than LDL-cholesterol [24]. However, this finding needs to be evaluated in detail in larger studies.

The Prospective Investigation of the Vasculature in Uppsala Seniors (the PIVUS study) was conducted with the primary aim to evaluate the power of three different tests of endothelium-dependent vasodilation in the peripheral circulation to predict future cardiovascular events in more than 1000 subjects aged 70 years living in the community of Uppsala, Sweden [25]. As this primary aim will demand a long follow-up period, we here report one of the secondary aims of the study, namely to evaluate the relationships between apoB/A1, LDL-cholesterol and measurements of endothelium-dependent vasodilation. It was also investigated if apoB was more closely related to endothelium-dependent vasodilation than non-HDL-cholesterol.

2. Material and methods

2.1. Subjects

Eligible were all subjects aged 70 years living in the community of Uppsala, Sweden. The subjects were chosen from the register of community living and were invited in a randomised order from the start of the study in April 2001 to the last included subject in June 2004. The subjects received an invitation by letter within 1 month of their 70th birthday in order to standardize for age. Of the 2025 subjects invited, 1016 subjects were investigated giving a participation rate of 50.1%.

The study was approved by the Ethics Committee of the University of Uppsala and the participants gave informed consent.

2.2. Baseline investigation

The participants were asked to answer a questionnaire about their medical history, smoking habits and regular medication. All subjects were investigated in the morning after an overnight fast. No medication or smoking was allowed after midnight. After recordings of height, weight, abdominal and hip circumference, an arterial cannula was inserted in the brachial artery for blood sampling and later regional infusions of vasodilators. During the investigation, the subjects were supine in a quiet room maintained at a constant temperature.

Blood pressure was measured by a calibrated mercury sphygmomanometer in the non-cannulated arm to nearest mmHg after at least 30 min of rest and the average of three recordings was used. Cholesterol and triglycerides concentrations were analysed in serum and when HDL was separated by precipitation with magnesium chloride/phototungstate by enzymatic techniques using IL Test Cholesterol Trinder’s Method and IL Enzymatic-colorimetric Method for use in a Monarch apparatus (Instrumentation Laboratories, Lexington, USA). LDL-cholesterol was calculated using Friedewald’s formula. This formula was not applied in the three subjects with serum triglycerides > 4.0 mmol/l. Non-HDL-cholesterol was defined as total serum cholesterol minus HDL-cholesterol. ApoB was determined by a two-site immuno-radiometric assay and apoA1 by a competitive radioimmunoassay, using commercial kits (Pharmacia, Uppsala, Sweden). These assays have a CV in the 5–8% range.

As the participation rate in this cohort was only 50%, we carried out an evaluation of cardiovascular disorders and medications in 100 consecutive subjects who were invited to the study, but denied participation. The prevalences of cardiovascular drug intake, history of myocardial infarction, coro-

**Table 1**

<table>
<thead>
<tr>
<th>Total sample</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females (%)</td>
<td>50.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169 ± 9.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77 ± 14</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.0 ± 4.3</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>91 ± 12</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.90 ± 0.075</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>150 ± 23</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>79 ± 10</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>62 ± 8.7</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>5.4 ± 1.0</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.3 ± 0.68</td>
</tr>
<tr>
<td>Apo B (g/l)</td>
<td>1.06 ± 0.24</td>
</tr>
<tr>
<td>Apo A1 (g/l)</td>
<td>1.40 ± 0.25</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.5 ± 0.42</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/l)</td>
<td>1.3 ± 0.60</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>5.3 ± 1.6</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>11</td>
</tr>
<tr>
<td>EDV (%)</td>
<td>459 (199–809)</td>
</tr>
<tr>
<td>EIDV (%)</td>
<td>328 (149–629)</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>4.4 (0.4−9.7)</td>
</tr>
<tr>
<td>Change in AIX (%)</td>
<td>−26.16</td>
</tr>
</tbody>
</table>

Means are given ±S.D. or as median and 10th and 90th percentiles in parenthesis. SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; EDV, endothelium-dependent vasodilation (invasive forearm technique); EIDV, endothelium-independent vasodilation (invasive forearm technique); FMD, flow-mediated dilatation; AIX, augmentation index.
3. Methods

3.1. The invasive forearm technique

Forearm blood flow (FBF) was measured by venous occlusion plethysmography (Elektromedicin, Kullavik, Sweden) with the strain-gauge technique. FBF was calculated from at least five consecutive recordings.

An arterial cannula was placed in the brachial artery. Resting FBF was measured 30 min after cannula insertion, and local intra-arterial drug-infusions were given during 5 min for each dose with a 20 min washout period between the drugs. The infused dosages were 25 and 50 μg/min for acetylcholine (Clin-Alpha, Switzerland) and 5 and 10 μg/min for SNP (Nitropress, Abbot, UK). The drugs were given in a random order at a maximal rate of 1 ml/min.

3.2. The brachial artery ultrasound technique

The brachial artery was assessed by external B-mode ultrasound imaging 2–3 cm above the elbow (Acuson XP128 with a 10 MHz linear transducer, Acuson Mountain View, California, USA), according to the International Brachial Artery Task Force [27]. A cuff was placed below the elbow and inflated to a pressure at least 50 mmHg above systolic blood pressure for 5 min. FMD was defined as the maximal brachial artery diameter recorded between 30 and 90 s following cuff release minus diameter at rest divided by the diameter at rest, using electronic callipers for measurements. FMD was successfully evaluated in 97% of the participants.

The reproducibility (CV) was 3% for baseline brachial artery diameter and 29% for FMD [28].

3.3. Pulse wave analysis

A micromanometer tipped probe (SphygmoCor, Pulse Wave Medical Ltd., Australia) was applied to the radial artery and 10 pulse waves were used for analyses. After a baseline recording, terbutaline was subcutaneously administered (0.25 mg) and the maximal response after 15 and 20 min was used. In previous studies, the augmentation index (AIx) derived from the first reflected wave in systole was used [29,30]. Here, we report change in AIX as the relative change from baseline following terbutaline. The change in AIx could successfully be evaluated in 86% of the sample. Terbutaline was not given to subjects with frequent ectopic beats or atrial fibrillation. The reproducibility (CV) for the change in AIx was 16% [31].

Each vasodilatory technique was performed and evaluated by one unique technician throughout the study not being aware of other data. A more detailed description of the techniques has previously been presented [25].

3.4. Statistics

Non-normally distributed variables were log-transformed to achieve a normal distribution. Relationships between pairs of variables were evaluated by Pearson’s correlation coefficient. Multiple regression analysis was applied to relate several independent variables to a dependent variable. Two-tailed significance values were given with p < 0.05 regarded as significant. The statistical programme package StatView (SAS Inc., NC, USA) was used.

4. Results

Table 3 summarizes the univariate relationships between the measurements of endothelium-dependent vasodilation, apoB/A1, non-HDL cholesterol and LDL cholesterol.

Table 2

<table>
<thead>
<tr>
<th>Cardiovascular Disease</th>
<th>Total investigated</th>
<th>Not attending</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart attack</td>
<td>1016</td>
<td>100</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>7.1</td>
<td>7.9</td>
</tr>
<tr>
<td>Stroke</td>
<td>3.7</td>
<td>6.7</td>
</tr>
<tr>
<td>Angina pectoris</td>
<td>8.1</td>
<td>13.8</td>
</tr>
<tr>
<td>CABG/PTCA</td>
<td>5.3</td>
<td>5.6</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>3.8</td>
<td>6.9</td>
</tr>
<tr>
<td>Diabetes</td>
<td>8.7</td>
<td>16.9</td>
</tr>
<tr>
<td>Any regular drug</td>
<td>70</td>
<td>64</td>
</tr>
<tr>
<td>Any CV drug</td>
<td>45</td>
<td>52</td>
</tr>
<tr>
<td>Any antihypertensive medication</td>
<td>32</td>
<td>36</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>22</td>
<td>26</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Diuretics</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>ACE-inhibitors</td>
<td>8.5</td>
<td>11</td>
</tr>
<tr>
<td>Angiotensin II-blockers</td>
<td>8.3</td>
<td>9.1</td>
</tr>
<tr>
<td>GTN</td>
<td>3.0</td>
<td>3.4</td>
</tr>
<tr>
<td>Digoxin</td>
<td>2.1</td>
<td>9.2</td>
</tr>
<tr>
<td>Statin</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Other antihypertensive drugs</td>
<td>1.2</td>
<td>4.5</td>
</tr>
</tbody>
</table>

CABG/PTCA, coronary revascularisation; GTN, any nitroglycerine preparation.
In univariate analysis, apoB/A1, but not LDL-cholesterol or non-HDL-cholesterol, was inversely correlated to EDV \( (r = -0.11, p = 0.0038 \text{ for apoB/A1; Fig. 1}) \). No significant interaction was seen between apoB/A1 and gender regarding the relation to EDV \( (p = 0.82) \). When EDV was used as dependent variable in a multiple regression model with the apoB/A1 ratio as an independent variable together with the potentially confounding variables coronary heart disease, stroke and the different cardiovascular medications given in Table 2, including statins and other lipid-lowering agents, the relationship between apoB/A1 and EDV was still significant \( (p = 0.0046) \) after adjustment for the potentially confounding variables. FMD was not significantly related to apoB/A1 \( (p = 0.16) \) or LDL-cholesterol or non-HDL-cholesterol.

In univariate analysis, apoB/A1, but not LDL-cholesterol or non-HDL-cholesterol, was inversely correlated to endothelium-dependent vasodilation evaluated with pulse wave analysis \( (r = 0.16, p = 0.0016 \text{ for change in AIX; Fig. 1}) \). No significant interaction was seen between apoB/A1 and gender regarding the relation to endothelium-dependent vasodilation evaluated with pulse wave analysis \( (p = 0.59) \). The relationship between apoB/A1 and change in AIX was still significant \( (p = 0.0004) \) after adjustment for coronary heart disease, stroke and the different cardiovascular medications in a similar model as described above for EDV.

ApoB/A1, but not LDL-cholesterol or non-HDL-cholesterol, was also inversely correlated to EIDV \( (r = -0.12, p = 0.0016 \text{ for apoB/A1}) \). No significant interaction was seen between apoB/A1 and gender regarding the relation to EIDV \( (p = 0.82) \). The relationship between apoB/A1 and EIDV was still significant \( (p = 0.0052) \) after adjustment for coronary heart disease, stroke and the different cardiovascular medications in a similar model as described above for EDV.

A separate analysis of apoB and apoA1 showed that both of these indices were related to EDV, EIDV and the change in AIX at pulse wave analysis \( (p = 0.0004) \) in the expected ways. However, the apoB/A1 ratio was more closely related to these vasodilatory indices compared to when these indices were related to apoB and apoA1 separately. Serum triglycerides were significantly related to EDV, EIDV and the change in AIX at pulse wave analysis, but not to FMD, in univariate analysis \( (p = 0.0016) \). No significant interaction was seen between serum triglycerides and EDV or EIDV, or the change in AIX at pulse wave analysis, but this might be paralleled by an attenuation of the strength of the relationship between LDL-cholesterol and endothelium-dependent vasodilation by age. It might also be that the

The present investigation supports the only previous study performed showing an inverse relationship between apoB/A1 and endothelium-dependent vasodilation \[24\]. However, contrary to our previous findings in middle-aged subjects \[24\], the present study did not find any relationship between LDL-cholesterol and endothelium-dependent vasodilation. This was somewhat surprising as several studies in the past have documented a clear-cut relationship between high levels of LDL-cholesterol and endothelium-dependent vasodilation evaluated by the invasive forearm technique \[9,10\].

One reason for these discrepant findings might be that most of the previous investigations were performed in middle-aged or young subjects, while the present evaluation was performed in the elderly. LDL-cholesterol has been shown to be reduced in power as a risk factor for coronary heart disease in the elderly compared to middle-aged subjects \[32\].
Fig. 1. Relationships between the apoB/A1 ratio and endothelium-dependent vasodilation evaluated with the invasive forearm technique (ln EDV, relative increase in FBF at the highest dose Ach, top panel, ln-transformed, $r = -0.11, p < 0.0038$), the brachial artery ultrasound technique (ln FMD, middle panel, ln-transformed, $r = 0.02, p = 0.67$) and by pulse wave analysis (augmentation index, AIx, lower panel, $r = 0.16, p < 0.0001$).

5.2. Flow-mediated vasodilation

None of the markers of cholesterol metabolism measured in the present study were related to FMD, in opposition to previous studies conducted in younger samples [15,16,20]. One explanation might be that FMD is rather low in this age-group even in healthy subjects making it hard to show any differences between those with high and those with low levels of different risk factors, as suggested by Wendelhag et al. [33]. It has also recently been shown by Wurz et al. that a reduced arterial compliance in the elderly limits flow-mediated vasodilation to the extent that it is hard to relate FMD to major risk factors in the elderly [34]. Another possibility might be that the effect of apoB/A1 is more pronounced on resistance vessels, as evaluated by the invasive forearm technique and pulse wave analysis, than on capacitance vessels, as evaluated by FMD. In addition to nitric oxide, the most potent vasodilator in conductance vessels, also a yet unidentified hyperpolarization factor is present in resistance vessels and might be effected by the apolipoproteins.

5.3. Pulse wave analysis

Similar to the findings regarding EDV, endothelium-dependent vasodilation evaluated with pulse wave analysis was related to apoB/A1, but not to LDL-cholesterol. This was a likely finding as endothelium-dependent vasodilation evaluated with pulse wave analysis is related to EDV, but not with FMD [25,35]. A reduced change in AIx following beta-2 receptor stimulation has previously been described in subjects with high LDL-cholesterol, but the subjects in that study was younger than in the present investigation [29].

5.4. Endothelium-independent vasodilation

Also EIDV evaluated by the invasive forearm technique was significantly related to apoB/A1, but not to LDL-cholesterol. While many investigators have not found EIDV to be related to coronary heart disease and different cardiovascular risk factors [9], others have [36,37]. EIDV is regarded to mainly represent structural changes in the vascular wall, which are more common in the elderly. Thus, a long-standing
history of high apoB/A1 might not only affect EIDV, but also EDV.

5.5. The EDV/EIDV ratio

The ratio between EDV and EIDV could be used as a way to tease out the independent role of the endothelium in the vasodilatory process in the resistance arteries. This index has been used in previous studies [24,38,39]. In the present study however, no relationship between this index and apoB/A1 or the other evaluated lipids were found, further emphasizing that apoB/A1 not exclusively affects the endothelium, but also EIDV.

5.6. The apoB/A1 ratio and its components

If the apoB/A1 ratio is related to a variable it could be that either apoB or apoA1 is the dominating part of this relation. Therefore, also apoB and apoA1 were separately related to the indices of endothelium-dependent vasodilation. It could then be seen that both of these apolipoproteins were related to the vascular indices in the expected way, but none of them were particularly dominating. Furthermore, the apoB/A1 ratio was more closely related to EDV, EIDV and the change in AIX than apoB and apoA1 alone, further strengthening the use of this lipid index.

5.7. Serum triglycerides

In the present study, EDV, EIDV and the change in AIX, but not FMD, were all significantly related to serum triglycerides. Similar findings have been presented before [38,39].

5.8. Limitation of the study

The present sample is limited to Caucasians aged 70 years. So, caution should be made to draw conclusions to other ethnic and age-groups. The present study had a moderate participation rate. However, an analysis of non-participants showed the present sample to be fairly representative of the total population regarding most cardiovascular disorders and drug intake.

In this study, LDL was calculated by the Friedewald formula and although the three subjects with serum triglycerides above 4 mmol/l were excluded from the analysis, direct measurements of LDL may have resulted in a more accurate evaluation of the relationship between vasodilation and LDL-cholesterol.

EIDV was only assessed by one of the methods for practical and ethical reasons not to prolong the investigation procedure, as we would have had to give GTN before terbutaline and would have had to wait for the withdrawal of the GTN effect. Furthermore, we have previously shown that EIDV evaluated by SNP infusion in the brachial artery and GTN provoked change in brachial artery diameter are closely related [28], so additional measurements of EIDV would probably not add substantial information to the study.

6. Conclusion

The apoB/A1 levels, but not LDL-cholesterol, were inversely related to endothelium-dependent vasodilation evaluated by EDV and pulse wave analysis, but not by FMD. Also EIDV showed the same pattern, suggesting deterioration in vasoreactivity mainly in resistance arteries in elderly subjects with high apoB/A1 levels. This might form the pathophysiological basis for the recent epidemiological findings of an increased predictive power for apoB/A1 when compared to LDL-cholesterol [3].

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References


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