Apo C Proteins in VLDL of Hyperlipidemic Patients

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SUMMARY  Apo C II and C III of serum VLDL were determined in various types of hyperlipidemias by isoelectric focusing of polyacrylamide gel plate with a pH gradient 4.0–6.0. VLDL from hyperlipidemias of type IIb, III, IV and hypo α-lipoproteinemia had high C II/C III ratio. Percentage of apo C III-O tended to increase in type III and IV, and decreased in type IIa and hypo α-lipoproteinemia. Therefore, apo C II/C III-O was not changed in type III, IV and V and increased type IIa and hypo α-lipoproteinemia. Apo C III-I did not change in every type of hyperlipidemias when compared with normolipidemic control but apo CIII-2 decreased in type IIa and IV. Decrease of apo C III-2 and increase of apo C III-0 in hypertriglyceridemic patients suggests the relative impairment of sialylation of apo C III proteins. E/C ratio was low in type II b, IV and hypo α-lipoproteinemia.

There were no clear relationship between C II/C III or C II/C III-O ratio and serum triglyceride or pre β-lipoprotein concentration. It is concluded that apo C composition of VLDL is not always related to the development of hypertriglyceridemia.

Abbreviations:
VLDL = very low density lipoprotein,
LDL = low density lipoprotein,
HDL = high density lipoprotein,
LPL = lipoprotein lipase.

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Introduction

It has been well documented that apo C lipoproteins have significant role for the metabolism of VLDL or chylomicrons. Breckenridge et al.1) reported a patient with chylomicronemia due to apo CII deficiency and Yamamura et al.2) found a similar patient in Japan. Although their postheparin plasma did not show any LPL activity, they revealed almost normal activity when incubated with normal serum or apo C lipoproteins. These results confirmed clinically that apo CII was a potent activator of LPL and essential for chylomicron metabolism3)–7).

Meanwhile, apo CIII has been considered to be an inhibitor of LPL and compete to the function of apo CII5)–8). Thus, both factors have to collaborate for the regulation of the first step in the metabolism of triglyceride-rich lipoproteins and CII relative to CIII regards to be a modulating factor of serum triglyceride concentration9)10).

In the present experiment, VLDL apo C protein from the patients with various kinds of hyperlipidemias was analyzed by isoelectric focusing of polyacrylamide–ampholine gel plate.

Materials and Methods

Fasting blood samples were obtained from the patients who were admitted into or visited to hospitals. Healthy controls, whose serum cholesterol and triglyceride concentrations were below 200 and 180mg/100ml, and HDL cholesterol were above 40mg/100ml, were confirmed to have no hepatic, renal, endocrine, malignant diseases or other metabolic disturbances by the clinical examinations. As hyperlipoproteinemia, the patients whose serum cholesterol were above 230mg/100ml or triglyceride were 200mg/100ml, were chosen for the subjects for investigation. At the same time, the patients whose serum HDL cholesterol concentrations were below 40 mg/100ml and cholesterol and triglyceride concentrations were within normal ranges, were selected as hypo α-lipoproteinemic subjects.

Serum triglyceride, cholesterol and phospholipids were determined by fully enzymatic methods11). Serum lipoprotein profiles were analyzed by electrophoresis using agarose gel film11). After staining the lipoprotein bands with Fat Red O, the intensities of stained bands were determined by densitometry11). Isoelectric focusing of polyacrylamide gel plate was carried out as reported previously except some compositional modification of polyacrylamide plate and staining solution. The gel plate was made as follows: 7ml of 30% acrylamide, 1.2% N,N’-methylenebisacrylamide, 3.5ml of 40% ampholine (pH 4.0–6.0) and 0.05ml N,N,N’,N’-tetramethylethylenediamine were added to 27.5ml of distilled water and 19.29g urea were dissolved into this solution. Then, 2.0ml of 1.4% ammonium persulfate were added for gel formation. As a staining solution, 0.115g Coomassie brilliant blue R were dissolved into 100ml of fixation soluton (30% methanol water containing 3.45g sulfosalicylic acid and 11.5g trichloroacetic acid). Percentages of apo CII, CIII-0, CIII-1 and CIII-2 to total sum of apo CII and CIII were determined by densitometry.

Apo A was estimated immunologically by anti-apo A rabbit serum (Behring Werke A.G. Marburg, West Germany) with an apparatus of Centrifichem 40013). Cholesterol in the supernatant of serum,
of which LDL and VLDL were removed by the precipitation with phosphotungstic acid and MgCl₂, were determined enzymatically as HDL cholesterol⁴⁴).

Phenotype of hyperlipoproteinemia was classified by the serum cholesterol and triglyceride concentrations with the reference to electrophoretic patterns of lipoproteins⁴³).

**Results**

Table 1 shows serum lipid concentrations and lipoprotein profiles of various hyperlipidemias excluding type 1. Five patients of type III and 2 of type V were found in 193 patients. Although apo A lipoprotein of the patients determined immunologically was not always paralleled with HDL cholesterol, relatively good correlations were observed within each type of hyperlipidemia. Correlation coefficients between both factors in type II a, II b, IV and normolipidemias were 0.703, 0.823, 0.796 and 0.849, respectively.

HDL cholesterol and apo A of the patients with hypertriglyceridemia (type II b, III, IV and V) were lower than those of type II a, as already reported. Apo A/ HDL cholesterol ratios in hypertriglyceridemic patients were higher (6.01 in type II b and 6.29 in type IV) than those in normolipidemic control (3.73) and type II a (4.92).

Subfractions of apo C lipoproteins and E/C ratios of all groups are demonstrated in Table 2. Percentages of apo C II, C III–0, C III–1 and C III–2 and E/C ratio of normolipidemic control were 15.1, 15.6, 31.2, 37.5% and 0.29. A small peak of apo C I–2 was found in 66 cases (34.2% of total) between apo C III–1 and C III–2. In two patients, another peak was present at acidic side of apo C III–2.

Patients of type II b, III, IV and those with low HDL showed high C II ratio than normolipidemic control. Especially, hypo HDL group had the highest C II ratio among them. Apo C II in type II a was not different from control. While, the

<table>
<thead>
<tr>
<th>Patients</th>
<th>Total cholesterol (mg/100 ml)</th>
<th>Triglyceride</th>
<th>HDL cholesterol</th>
<th>Apo A</th>
<th>β (%)</th>
<th>pre-β (%)</th>
<th>α (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normolipidemias (27)</td>
<td>184.9±4.62*</td>
<td>122.1±4.08</td>
<td>52.9±2.17</td>
<td>231.7±3.35</td>
<td>52.3±2.23</td>
<td>13.8±1.00</td>
<td>33.7±2.28</td>
</tr>
<tr>
<td>IIa (74)**</td>
<td>268.7±4.81</td>
<td>148.2±4.31</td>
<td>43.0±1.29</td>
<td>210.1±5.10</td>
<td>64.1±0.82</td>
<td>15.7±0.74</td>
<td>20.1±0.88</td>
</tr>
<tr>
<td>IIb (37)</td>
<td>258.1±4.27</td>
<td>297.3±7.13</td>
<td>37.5±1.85</td>
<td>205.5±7.34</td>
<td>50.9±1.63</td>
<td>32.0±1.33</td>
<td>17.0±0.98</td>
</tr>
<tr>
<td>III (5)</td>
<td>264.0±34.66</td>
<td>249.4±10.93</td>
<td>34.8±4.68</td>
<td>190.7±27.96</td>
<td>84.3±3.26</td>
<td>15.5±3.25</td>
<td></td>
</tr>
<tr>
<td>IV (46)</td>
<td>186.5±6.22</td>
<td>318.8±1.97</td>
<td>28.7±1.22</td>
<td>180.9±5.90</td>
<td>34.9±1.89</td>
<td>50.4±1.96</td>
<td>14.7±0.90</td>
</tr>
<tr>
<td>V (2)</td>
<td>215.5</td>
<td>565.0</td>
<td>25.5</td>
<td>158.0</td>
<td>19.8</td>
<td>63.7</td>
<td>9.4</td>
</tr>
<tr>
<td>Hypo HDL (29)</td>
<td>174.4±4.70</td>
<td>145.2±6.23</td>
<td>28.3±1.27</td>
<td>154.7±5.37</td>
<td>58.2±1.63</td>
<td>24.9±1.59</td>
<td>16.7±5.37</td>
</tr>
</tbody>
</table>

* mean ± S.E.M. ** number of the patients.
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Table 2 Apo C Protein Composition and E/C Ratio of Hyperlipidemic Patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>C II</th>
<th>C III-0</th>
<th>C III-1</th>
<th>C III-2</th>
<th>E/C</th>
<th>C II/C III-0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normolipidemics</td>
<td>15.1 ± 0.76*</td>
<td>15.6 ± 1.70</td>
<td>31.2 ± 2.07</td>
<td>37.5 ± 2.14</td>
<td>0.29 ± 0.041**</td>
<td>0.97</td>
</tr>
<tr>
<td>IIa (74)***</td>
<td>16.1 ± 0.86B</td>
<td>11.4 ± 1.43E</td>
<td>35.3 ± 1.36E</td>
<td>36.9 ± 1.72</td>
<td>0.27 ± 0.041</td>
<td>1.41</td>
</tr>
<tr>
<td>IIb (37)</td>
<td>19.0 ± 1.36B</td>
<td>17.2 ± 1.64</td>
<td>34.2 ± 1.39</td>
<td>29.5 ± 1.29B</td>
<td>0.17 ± 0.031C</td>
<td>1.10</td>
</tr>
<tr>
<td>III (5)</td>
<td>18.3 ± 0.73</td>
<td>18.6 ± 3.10</td>
<td>29.3 ± 6.85</td>
<td>34.1 ± 4.53</td>
<td>0.24 ± 0.070</td>
<td>1.01</td>
</tr>
<tr>
<td>IV (46)</td>
<td>18.9 ± 0.75C</td>
<td>18.4 ± 1.69</td>
<td>32.4 ± 1.47</td>
<td>30.4 ± 1.32B</td>
<td>0.15 ± 0.021B</td>
<td>1.04</td>
</tr>
<tr>
<td>V (2)</td>
<td>13.2</td>
<td>12.9</td>
<td>33.2</td>
<td>40.8</td>
<td>0.20</td>
<td>1.02</td>
</tr>
<tr>
<td>Hypo HDL (29)</td>
<td>23.0 ± 2.36A</td>
<td>9.4 ± 1.57B</td>
<td>33.4 ± 2.17</td>
<td>33.8 ± 2.66</td>
<td>0.17 ± 0.021B</td>
<td>2.45</td>
</tr>
</tbody>
</table>

* mean ± S.E.M. (%). ** mean ± S.E.M. (ratio). *** number of the patients. A : p<0.001, B : p<0.005, C : p<0.01, D : p<0.05, E : p<0.1 to normolipidemics.

Patients of type V, despite of only 2 cases, had lower C II ratios than other hyperlipidemic groups.

Apo C III-0 was low in type IIa, V and hypo HDL groups and tended to be high in type III and IV. Therefore, C II/C III-0 ratios in type III, IV and V were almost identical to that in control, but those in type IIa and hypo HDL patients were higher than that in control.

There were no significant change in apo C III-1 in every type of hyperlipidemia when compared with control, but C III-2 in type IIb and IV patients were lower than that in normolipidemic control.

E/C ratios in type IIb and IV patients were lower than that of normolipidemic control.

Fig. 1 shows the distribution diagram of the numbers of the patients with various apo C II ratios in each type of hyperlipidemia. In type IIb, IV patients and those with low HDL, more cases showed higher apo C II ratio than in normolipidemic control.

Table 3 Sialylation Index of Apo C III in Hyperlipidemic Patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sialylation index ***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normolipidemics (27)***</td>
<td>1.26 ± 0.034 **</td>
</tr>
<tr>
<td>IIa (74)</td>
<td>1.30 ± 0.031 A</td>
</tr>
<tr>
<td>IIb (37)</td>
<td>1.13 ± 0.027 A</td>
</tr>
<tr>
<td>III (5)</td>
<td>1.25 ± 0.040 A</td>
</tr>
<tr>
<td>IV (46)</td>
<td>1.15 ± 0.034 A</td>
</tr>
<tr>
<td>V (2)</td>
<td>1.32 B</td>
</tr>
<tr>
<td>Hypo HDL (29)</td>
<td>1.31 ± 0.031 B</td>
</tr>
</tbody>
</table>

* C III-1 + C III-2 x 2 / C III-0 + C III-1 + C III-2. *** number of the patients. ** mean ± S.E.M. A : p<0.005, B : p<0.01.

Since a C III-1 apoprotein molecule has one sialic acid and C III-2 has two, an index of C III-1 + C III-2 x 2/C III-0 + C III-1 + C III-2 (total apo C without C I) is considered to indicate the tendency of sialylation of apo C proteins. As shown in Table 3, these indeces in type IIb and IV were significantly lower than those in type IIa and hypo HDL patients.

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Discussion

Carlson et al.\textsuperscript{9} and Catapano\textsuperscript{10} reported that apo C\textsubscript{II}/C\textsubscript{III} ratios of triglyceride-rich lipoproteins decreased in type IV and V hyperlipidemias and suggested that the decrease of C\textsubscript{II}/C\textsubscript{III} ratios caused hypertriglyceridemia due to lowering of LPL activity. However, Schofeld et al.\textsuperscript{16} determined apo C\textsubscript{II} and C\textsubscript{III} by radioimmunoassay and showed that both apo C\textsubscript{II} and C\textsubscript{III} increased in hypertriglyceridemic patients and C\textsubscript{II}/C\textsubscript{III} ratio of d<1.006 fraction as well as total plasma were not different from those in healthy control. On the other hand, Stocks et al.\textsuperscript{17} found a patient of chylomicronemia with a variant apo C protein among hypertriglyceridemic patients, whose VLDL contained 45.2% apo C\textsubscript{II} compared with 21.5% of hypertriglyceridemic control in the sum of C apoproteins except C\textsubscript{III}-0. Such triglyceride-rich lipoproteins contained variant apo C\textsubscript{II} activated LPL normally but failed to act as an efficient substrate for LPL.

In the present experiment, patients who had such high C\textsubscript{II} apoprotein was not found in type IV and V hyperlipidemias and their C\textsubscript{II} ratio distributed from 11.7 to 33.9%. Mean of C\textsubscript{II} apolipoproteins in 12 patients of type II\textsubscript{b} and IV who had high serum triglyceridemia more than 400mg/100ml was 18.9% and there were no clear correlation between the concentrations of serum triglyceride and apo C\textsubscript{II} ratios. It is the reason that many factors are related to the determinants of serum triglyceride level. Apo C\textsubscript{III}-0 was
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rather higher and sialylation index was lower in the patients with hypertriglyceridemias than those with normolipidemias except the cases of chylomicronemias. In the 12 cases above described, apo CIII-0 was 22.9% and the sialylation index was 1.02. Apo CIII has been considered to be influenced by dietary factors. Acute ingestion of fat enhances transfer of apo C protein from HDL to chylomicrons. High carbohydrate diet does not only increase serum triglyceride concentrations, but also elevates serum apo C protein levels. Apo CII and CIII concentrations rise proportionally but apo CIII-0 relative to apo CII increases in VLDL fraction by the intake of carbohydrate-rich diet. Increase of apo CIII-0 regards to be derived from the relative reduction of sialylation process of apo CIII proteins in Golgi's apparatus, because of overproduction of apo CIII proteins. In the present experiment, the increase of CIII-0 fraction and the decrease of CIII-2 were observed in hypertriglyceridemic patients, which suggests incomplete sialylation of apo CIII proteins by the increase of triglyceride synthesis in such patients as type IIb and IV. However, a large variations of apo CIII-0 in individual person indicate that sialylation is fluctuated by the degree of triglyceride synthesis.

Apolipoprotein E has a high affinity for hepatocytes as well as LDL receptor on extrahepatic cells. Apo E-rich VLDL or HDL are cleared more rapidly than apo E-poor lipoproteins, and the former are well taken up by hepatocytes in vivo and in vitro. Shellburne et al. showed that the uptake of HDL with apo E by hepatocytes was inhibited by the addition of apo C. In the present experiment, patients with hypertriglyceridemia had low E/C ratio, which might induce the delay of clearance of VLDL and triglyceride from liver and participate partially the elevation of serum VLDL and triglyceride levels.

References

13) T. Kawaguchi, Y. Katayama and N. Takeuchi: to be reported.


