Recent Progress in the Development of Radioimmunoassays for Human Serum Lipoproteins

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ABSTRACT

A review of radioimmunoassays for measuring human apolipoprotein B (apo B), the A apolipoproteins of high density lipoprotein (apo A-I and apo A-II) and apolipoprotein C-II (apo C-II) in human plasma and in isolated lipoproteins is presented. The sensitivity, specificity and validity of each of these assays is discussed. In normolipidemic subjects the reported serum apo B concentrations ranged between 0.83 ± 0.16 and 0.92 ± 0.21 g per l (m ± SD). Serum apo B concentrations were highest in Type II subjects (Type IIa homozygotes 3.83 ± 0.43 g per l; Type IIa heterozygotes 2.37 ± 0.47 g per l) and were less elevated in patients with Type IV and Type V disorders (1.32 ± 0.21 g per l and 1.26 ± 0.30 g per l, respectively). Preliminary data on the relationship between plasma apo B and cholesterol, the distribution of apo B amongst the lipoprotein classes and a comparison of the lipoprotein lipid-apo B ratios in the various hyperlipidemic disorders are summarized.

In contrast to apo A-II, the immunoreactivity of apo A-I was not fully exposed in whole sera and in isolated lipoproteins. The different methods used to measure the apo A-I immunoreactivity are discussed. In normolipidemic subjects the serum apo A-I concentration in males and females was 1.13 ± 0.061 and 1.24 ± 0.068 g per l (m ± SD), respectively, while the corresponding serum apo A-II values were 0.35 ± 0.038 g per l and 0.41 ± 0.046 g per l. In subjects with Tangier’s disease, the serum apo A-I and apo A-II concentrations were < 1 percent and 5 to 7 percent of that found in controls. The serum apo A-I level was also reduced in two subjects with abetalipoproteinemia (0.38 g per l and 0.30 g per l) and Type II hyperlipoproteinemia (range 0.54 to 0.86 g per l).

In normotriglyceridemic subjects and those with Type IIa hyperlipoproteinemia, the total plasma apo C-II concentrations were 0.0497 ± 0.0040 g per l and 0.0562 ± 0.0054 g per l (m ± SE). Plasma apo C-II levels in Type IIb, Type IV and Type V lipoproteinemic subjects were 0.0899 ± 0.0046, 0.0854 ± 0.0069 and 0.1328 ± 0.0021 g per l, respectively, and were significantly higher than in the normotriglyceridemic subjects. An analysis of the relationship between the apo C-II content and the lipoprotein lipase activator properties of VLDL isolated from normo- and hypertriglyceridemic plasma samples is presented.
Introduction

Until recently it has been technically difficult to measure accurately the specific apoproteins which are associated with the lipid moiety of circulating lipoproteins. As a consequence, the investigation of the various dyslipoproteinemias and their mode of inheritance has been limited. The recent availability of specific and sensitive antibodies to the individual apolipoproteins has permitted the development of immunological methods of quantitating these molecules. Although a number of these assays have been developed, the radioimmunoassay technique is becoming popular for the quantitation of apolipoproteins because of its high degree of sensitivity, specificity, reproducibility and its ability to handle efficiently a large number of samples. Moreover, these assays are capable of measuring the apolipoprotein in the isolated lipoprotein molecules as well as in small volumes of sera and other biological fluids. The immunoassays thus far developed effectively complement the ultracentrifugal, electrophoretic and chromatographic methods commonly employed in the isolation and analysis of the various lipoproteins and have already been useful in basic, clinical and epidemiological studies.

In this review, the principles and methods are summarized of the radioimmunoassays for human apolipoproteins B, A-I, A-II and C-II and their application to epidemiological studies described.

Measurement of Apolipoprotein B in Very Low Density Lipoprotein and Low Density Lipoprotein by Radioimmunoassay

The RADIOIMMUNOASSAY

Double antibody radioimmunoassays for human apolipoprotein B (apo B) have been established in several laboratories (table I). The good agreement among these assays suggests that investigators have a certain latitude in establishing this assay. First, the antisera (raised in rabbits) may be prepared against whole low density lipoprotein (LDL) or its isolated apo B. It has been demonstrated that either of these two antisera may be used for the determination of the immunoreactive apo B concentration in serum, the results being

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Type of Immunoassay*</th>
<th>Preparation of Low Density Lipoprotein</th>
<th>Iodination of Low Density Lipoprotein</th>
<th>Population Size (n)</th>
<th>Mean Age (Years)</th>
<th>Total Serum apo B (m ± SD) (g per l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lees et al25</td>
<td>SRID</td>
<td>Dextran sulfate precipitation + ultracentrifugation</td>
<td>---</td>
<td>64</td>
<td>34</td>
<td>0.83 ± 0.25</td>
</tr>
<tr>
<td>McConathy et al27a</td>
<td>EIA</td>
<td>d 1.030 - 1.043 g/ml†</td>
<td>---</td>
<td>12</td>
<td>---</td>
<td>0.89 ± 0.13</td>
</tr>
<tr>
<td>Schonfeld et al37</td>
<td>RIA</td>
<td>d 1.025 - 1.050 g/ml†</td>
<td>Chloramine-T</td>
<td>42</td>
<td>42 ± 13</td>
<td>0.83 ± 0.16</td>
</tr>
<tr>
<td>Albers et al1</td>
<td>RIA</td>
<td>d 1.030 - 1.050 g/ml†</td>
<td>Iodine</td>
<td>349</td>
<td>(20 - 65)</td>
<td>0.81</td>
</tr>
<tr>
<td>Bautovich et al6</td>
<td>RIA</td>
<td>d 1.020 - 1.050 g/ml†</td>
<td>Chloramine-T</td>
<td>82</td>
<td>(20 - 35)</td>
<td>0.90 ± 0.24</td>
</tr>
<tr>
<td>Karlin et al19</td>
<td>RIA</td>
<td>d 1.019 - 1.063 g/ml†</td>
<td>Chloramine-T</td>
<td>82</td>
<td>(20 - 39)</td>
<td>0.92 ± 0.21</td>
</tr>
</tbody>
</table>

*SRID = single radial immunodiffusion assay; EIA = electroimmunoassay; RIA = radioimmunoassay.
†Low density lipoprotein (LDL) isolated from normolipidemic sera by sequential ultracentrifugation.
(Adopted from Karlin et al21 with permission.)
equivalent provided that whole LDL was used as the standard and tracer.1,19 Second, either a broad (d 1.019 to 1.063 g per ml) or narrow (d 1.030 to 1.040 g per ml) cut of LDL may be used for the standard and tracer. Though the broad cut of LDL may contain some Lp(a) lipoprotein (d 1.050 to 1.12 g per ml), its quantitative contribution to LDL is small.2 Third, the 125I-LDL tracer may be prepared by the iodine monochloride1,15 or chloramine-T method.6,19,37 The specific radioactivity of the 125I-LDL ranged from 1 to 8 μCi per μg protein. Lipid extraction of the 125I-LDL revealed that between 87 and 95 percent of the label was associated with the protein component. Fourth, the immunoreactive apo B in normolipidemic sera was stable for at least one year when stored at −20°C.19 Since several different batches of the LDL standard were prepared during the course of these experiments, it was encouraging that the intraassay variation remained as low as 10 percent.19 Apo B in normolipidemic sera was also stable in the lyophilized state for at least four months.1

Prior to the development of radioimmunoassays for human apo B, plasma LDL had been quantitated by a number of methods including single radial immunodiffusion25 and electroimmunoassay.27 The apo B concentrations in fasting healthy subjects as determined by the single radial immunodiffusion (0.83 ± 0.25 g per l)25 and electroimmunoassay (0.89 ± 0.13 g per l)27 are similar to those measured by radioimmunoassay (0.92 ± 0.21 g per l)19 (table I). A serious limitation of the single radial immunodiffusion method is that the large lipoprotein molecules, such as chylomicrons and very low density lipoproteins (VLDL), do not readily diffuse into the solid agarose gel and are not measured.1,42 Although the quantitation of apo B in chylomicrons and VLDL by the electroimmunoassay method has not been reported, it is possible that this method may not easily be applied to these large molecules.24 In the radioimmunoassay, however, the immunoreactive apo B of lymph chylomicrons (d < 1.006 g per ml) gave a displacement curve parallel to that of the LDL standard.37

It has been shown previously that VLDL gives displacement curves parallel to LDL (figure 1).1,6,19,37 An average of 35 to 38 percent of the VLDL protein was accounted for by immunoreactive apo B,37 a result which agrees with the chemical
assessment of the quantity of apo B in VLDL.\textsuperscript{1,37} Further confirmation of the specificity of the apo B radioimmunoassay has been the observation that high density lipoprotein (d 1.090 to 1.21 g per ml) displaced less than 0.2 percent of the antibody bound \textsuperscript{125}I-LDL.\textsuperscript{37} This was probably due to contamination with small amounts of LDL and Lp(a) because the C apoproteins, apo A-I and apo A-II (all isolated from high density lipoprotein [HDL]), did not crossreact even when added in 1000-fold excess (figure 1).\textsuperscript{19} Also, sera from patients with abetalipoproteinemia did not react in the assay even when added in 8000-fold excess compared to normal serum.\textsuperscript{6,19,37} For these reasons, the apo B radioimmunoassay should prove to be an excellent epidemiological tool as well as being applicable to studies of the physiology and metabolism of this apolipoprotein.

**APOLIPOPROTEIN B VALUES IN SERA**

The serum apo B value (0.92 ± 0.21 g per l) in our population of healthy subjects\textsuperscript{19} is similar to that measured by Bautovich et al\textsuperscript{6} (0.90 ± 0.24 g per l) and by McConathy et al\textsuperscript{27} (0.89 ± 0.13 g per l) (table I). In laboratories where the LDL concentration was adjusted according to the Lowry value, serum apo B levels were somewhat lower (0.83 ± 0.16 g per l;\textsuperscript{37} 0.81 g per l\textsuperscript{1}) (table I). A difference was not observed by us in apo B concentrations in males and females, a finding which has also been reported by others.\textsuperscript{1,6,37} The total serum apo B has been shown to correlate with both total serum cholesterol\textsuperscript{1,6} and LDL cholesterol.\textsuperscript{1} Serum apo B, however, did not correlate with serum triglyceride\textsuperscript{1,6} but did correlate significantly with serum phospholipids (p < 0.05). The presence of 87 ± 5 percent of the serum apo B in LDL is in agreement with studies in which the serum was fractionated by preparative ultracentrifugation\textsuperscript{6} and those in which apo B was determined in both the d < 1.006 g per ml and d > 1.006 g per ml fractions.\textsuperscript{1,37} Serum apo B concentrations have been determined by radioimmunoassay in subjects with different types of hyperlipoproteinemias. The apo B values were highest in Type II subjects (Type IIa homozygotes 3.83 ± 0.43 g per l; Type IIa heterozygotes 2.37 ± 0.47 g per l)\textsuperscript{1,19,37} and less elevated in patients with Type IV and Type V disorders (1.32 ± 0.21 g per l and 1.26 ± 0.30 g per l, respectively).\textsuperscript{6,37} In hyperlipoproteinemic individuals, the precise relationship between plasma apo B and cholesterol levels differed depending on the particular disorder.\textsuperscript{1,6} Plasma apo B and cholesterol were significantly correlated in Types IIb, IV and V. The slopes of the regression lines for all the hyperlipoproteinemias, except Type V, were similar to that of normolipidemic subjects.\textsuperscript{5} There was no significant correlation between plasma triglycerides and apo B in hyperlipidemic subjects.\textsuperscript{1,6,19} When the concentrations of plasma apo B and triglyceride of Type IV subjects were compared, the existence of two subpopulations, which may represent different genetic or biochemical subgroups, was apparent.\textsuperscript{37} So that the distribution of apo B among the various lipoprotein classes could be determined, the sera under investigation were fractionated by either preparative or single-spin ultracentrifugation.\textsuperscript{6,19} In both cases, the results were comparable to those in which apo B was determined in the d < 1.006 g per ml and d > 1.006 g per ml fractions.\textsuperscript{1,37} More than 90 percent of the serum apo B of healthy and Type II subjects was confined to LDL.\textsuperscript{6,19} In contrast, 83 percent and 51 percent of serum apo B was found in the LDL of Type IV and Type V subjects, respectively. In normal and Type II subjects, VLDL apo B comprised 2.5 to 5.0 percent of plasma apo B.\textsuperscript{6,19} In Types IIb and IV, the VLDL apo B increased to approximately 15 percent of the total; in Type V individuals, about
28 percent of the total serum apo B was in VLDL and 27 percent in chylomicrons. In addition, there were small, but significant \((p < 0.01)\) increases of apo B in the HDL region for all hyperlipoproteinemias, except in Type IIa homozygotes. Total serum apo B and LDL cholesterol were significantly correlated in normolipidemic subjects \((r = 0.929, p < 0.001)\). On the other hand, Bautovich et al observed that apo B and LDL cholesterol correlated significantly in Type IIb patients but not in normal or Type IIa, IV and V subjects.

A comparison of the lipoprotein lipid—apo B ratios revealed important changes in patients with hyperlipidemia. For example, the cholesterol: apo B ratio of VLDL in Type IIa homozygotes was 15.6; in Type IIa heterozygotes, 9.3; and in Type IV, 4.7 \((\text{normal value: 7.4})\). The cholesterol: apo B ratio of LDL was 0.7 in Type V as compared to 1.3 in normals. The triglyceride: apo B ratio of VLDL was 39.5 in Type V and 16.1 in normals, while in the LDL of Type V this ratio was 0.9 compared to a value of 0.3 in normals. The data on the distribution of apo B, together with the compositional changes in the low density lipoproteins, provide support for the lack of correlation between the apo B of total serum and LDL cholesterol in some hyperlipoproteinemias.

**Measurement of Apolipoprotein A-I of High Density Lipoprotein by Radioimmunoassay**

**THE RADIOIMMUNOASSAY**

Another major group of serum lipoproteins is represented by the high density lipoproteins (HDL) which contain a large portion of the plasma cholesterol and approximately 50 percent protein by weight. It is now established that the protein moiety of HDL (apo HDL) is composed of several polypeptides which are distinct in their physical, chemical, and immunological properties. Between 60 to 70 percent of this protein is apolipoprotein A-I \((\text{apo A-I})\) which, in addition to being important to the structure of HDL, is also an activator of lecithin-cholesterol acyl transferase. Apolipoprotein A-II \((\text{apo A-II})\), the other major apolipoprotein of HDL, comprises about 20 percent of apo HDL.

To date, no biological activity has been described for apo A-II. It appears that this is a structural protein which avidly binds lipids. The C-apolipoproteins \((\text{apo C})\) represent 3 to 10 percent of apo HDL and are composed of at least three major polypeptides, apo C-I, apo C-II and apo C-III. The apo C proteins, also present in VLDL, modulate the activity of lipoprotein lipase. The D apolipoprotein \((\text{apo D})\), whose function is not yet known, contributes 5 percent or less to the weight of apo HDL. Currently radioimmunoassays for apo A-I, and apo A-II and apo C-II have been developed.

Four double-antibody radioimmunoassays for human plasma apolipoprotein A-I have been reported (table II). A major difficulty in these assays has been that the direct measurement of apo A-I in plasma or in isolated high density lipoprotein fractions yielded variable results. Apo A-I concentrations were maximized when the lipids were extracted prior to assay. Although 95 percent of apo A-I was recovered, this procedure was found to be time-consuming and cumbersome. The present authors and Fainaru et al were able to avoid the delipidation step.

It was reported by us that heating dilutions of whole sera or HDL at 52°C for three hours resulted in maximal increases in apo A-I immunoreactivity; the increases were comparable to those found in delipidated specimens (table III). Instead of heating samples prior to assay, Fainaru et al raised the temperature from 4°C to 37°C for two hours at three different times during the incubation period of the assay. These simple procedures did not alter the parallelity of the sera and HDL to the
standards and permitted multiple sera to be assayed efficiently with full recovery of apo A-I.

For the purification of apo A-I, the preparation of monospecific antisera and quantification of the standard, very similar methods have been used. Human apo A-I was isolated from HDL which was delipidated with ethanol-ether and fractionated on Sephadex G-200 columns equilibrated in Tris-urea buffer. Apo A-I was standardized by the Lowry method. This method gave comparable results to that determined by amino acid analysis.

The working range of the assay was between 3 and 20 ng apo A-I and the interassay variation was 12 percent. Immunoass-

**TABLE II**

### Measurement of Human Apolipoprotein A-I by Immunoassay in Normolipidemic Subjects

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Immunoassay*</th>
<th>Subjects (n)</th>
<th>Age (Mean)</th>
<th>Total Serum Apo A-I (g per l)</th>
<th>High Density Lipoprotein-Cholesterol (mg per dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albers et al[^3]</td>
<td>SRID</td>
<td>Males (263)</td>
<td>39</td>
<td>1.20 ± 0.20†</td>
<td>45 ± 11†</td>
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<tr>
<td></td>
<td></td>
<td>Females (99)</td>
<td>36</td>
<td>1.29 ± 0.25</td>
<td>56 ± 13</td>
</tr>
<tr>
<td>Curry et al[^10]</td>
<td>EIA</td>
<td>Males (19)</td>
<td>37</td>
<td>1.46 ± 0.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Females (19)</td>
<td>29</td>
<td>1.04 ± 0.34</td>
<td></td>
</tr>
<tr>
<td>Schonfeld et al[^18]</td>
<td>RIA</td>
<td>Males (41)</td>
<td>37</td>
<td>1.00 ± 0.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Females (34)</td>
<td>29</td>
<td>1.30 ± 0.20§</td>
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<tr>
<td>Karlin et al[^20]</td>
<td>RIA</td>
<td>Males (17)</td>
<td>27</td>
<td>1.30 ± 0.20§</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Females (35)</td>
<td>33</td>
<td>1.49 ± 0.30</td>
<td></td>
</tr>
</tbody>
</table>

*SRID = single radial immunodiffusion assay; EIA = electroimmunoassay; RIA = radioimmunoassay.
†Apo A-I of males < females (p < 0.01).
§Apo A-I of males < females (p < 0.05).

**TABLE III**

### Patients with Mixed Pattern in the Urea Denaturation Test

<table>
<thead>
<tr>
<th>Case</th>
<th>ALP[^†]</th>
<th>Urea Activity (Percentage)</th>
<th>LPA[^†]</th>
<th>LPA Activity (Percentage)</th>
<th>GGTP[^®]</th>
<th>HOP[^x]</th>
<th>Discharge Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
<td>27</td>
<td>93</td>
<td>117</td>
<td>23</td>
<td></td>
<td>Temporal arteritis, viral infection</td>
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<tr>
<td>2</td>
<td>200</td>
<td>28</td>
<td>92</td>
<td>97</td>
<td>14</td>
<td></td>
<td>Chronic diarrhea</td>
</tr>
<tr>
<td>3</td>
<td>218</td>
<td>26</td>
<td>93</td>
<td>84</td>
<td>26</td>
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<td>Chronic cholecystitis</td>
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<tr>
<td>4</td>
<td>321</td>
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<td>91</td>
<td>203</td>
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<td>250</td>
<td>26</td>
<td>94</td>
<td>220</td>
<td>21</td>
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<td>Cirrhosis, alcoholic hepatitis</td>
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<td>6</td>
<td>212</td>
<td>26</td>
<td>90</td>
<td>105</td>
<td>35</td>
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<td>Laennec's cirrhosis</td>
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<td>7</td>
<td>271</td>
<td>26</td>
<td>90</td>
<td>105</td>
<td>35</td>
<td></td>
<td>Bone &amp; liver-metastatic adenocarcinoma</td>
</tr>
<tr>
<td>8</td>
<td>215</td>
<td>30</td>
<td>85</td>
<td>220</td>
<td>27</td>
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<td>Chronic cholecystitis</td>
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<tr>
<td>9</td>
<td>330</td>
<td>31</td>
<td>96</td>
<td>199</td>
<td>15</td>
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<td>24</td>
<td>76</td>
<td>800</td>
<td>47</td>
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<td>Bone-metastatic adenocarcinoma</td>
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<td>11</td>
<td>470</td>
<td>19</td>
<td>85</td>
<td>190</td>
<td>97</td>
<td></td>
<td>Bone-metastatic adenocarcinoma</td>
</tr>
<tr>
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<td>340</td>
<td>26</td>
<td>90</td>
<td>335</td>
<td>46</td>
<td></td>
<td>Osteoporosis</td>
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<tr>
<td>13</td>
<td>215</td>
<td>28</td>
<td>95</td>
<td>145</td>
<td>33</td>
<td></td>
<td>Acute cholecystitis</td>
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<td>14</td>
<td>280</td>
<td>24</td>
<td>82</td>
<td>165</td>
<td>15</td>
<td></td>
<td>Acute allergic urticaria</td>
</tr>
<tr>
<td>15</td>
<td>224</td>
<td>33</td>
<td>93</td>
<td>65</td>
<td>59</td>
<td></td>
<td>Atherosclerotic heart disease</td>
</tr>
</tbody>
</table>

*Figures are percent of original activity remaining after denaturation with urea or inhibition with L-phenylalanine. GGTP values are in IU per L (normal up to 45) and HOP in mg per 24 hours (normal up to 45). For interpretation of denaturation patterns, see text.
[^†]: Serum alkaline phosphatase
[^®]: Gamma-glutamyl transpeptidase
[^x]: Hydroxyproline
sayable apo A-I accounted for less than 0.1 percent and one percent of the protein of VLDL and LDL, respectively, and probably represented contamination with small amounts of HDL and/or apo A-I (figure 2). Apo A-II and the C-apoproteins (prepared from apo HDL) contained less than 0.1 percent apo A-I.

The concentration of apo A-I, expressed as a percentage of the protein in whole, heated human HDL (65 ± 2 percent, 68 percent), was similar to that in unheated apo HDL (74 ± 2 percent). In the radioimmunoassay reported by Schonfeld, apo A-I represented 51 percent of the human apo HDL protein. The concentration of apo A-I in heated HDL and apo HDL was in good agreement with that found after chromatography on Sephadex columns. It is likely that the discrepancy between the A-I detected in whole HDL (65 ± 2 percent) and apo HDL (74 ± 2 percent) does not indicate that the radioimmunoassay is incapable of detecting all of the apo A-I present, but rather that selective protein losses occurred in the delipidation procedure, which involved very small volumes of HDL.

The distribution of serum apo A-I by molecular size was assessed by fractionation of fresh human sera on a Sephadex G-200 column equilibrated with 0.05 M Tris buffer, pH 8.0. Apo A-I eluted in three broad peaks corresponding to molecular weights of approximately 320,000, 155,000 and 50,000. Peaks 1, 2 and 3 contained 42, 45 and 13 percent of the total apo A-I, respectively. The peaks were assayed before and after lipid extraction; this procedure increased the immunoreactivity of peaks 1, 2 and 3 by 17-, 14-, and 2-fold, respectively. The significance of these findings is not completely understood at present.

**APOLIPOPROTEIN A-I VALUES IN SERA**

The apo A-I values of normolipidemic subjects were similar when measured by single radial immunodiffusion, electroimmunoassay or radioimmunoassay and within the range of 0.95 to 1.99 g per l (table II). In the populations studied by Albers et al and Karlin et al, the serum apo A-I of males was significantly lower than that in females (1.30 ± 0.03 g per l versus 1.54 ± 0.06 g per l). In other studies, the serum apo A-I values in males and females were found to be in the same range. Variation in the criteria for selecting the subjects and/or differences in the age distribution of the populations studied may account for the observed differences.

Schonfeld and Pfleger reported a significant correlation between the apo A-I value and HDL cholesterol but not total serum cholesterol. Interestingly, a sig-
significant positive correlation between apo A-I and total serum cholesterol was present in both our female and male populations (p < 0.01 and p < 0.05, respectively). At present, the significance of this correlation is uncertain. Although approximately 27 percent of HDL is phospholipid by weight, the apo A-I and serum phospholipid concentrations were not correlated. In the populations sampled, apo A-I did not correlate with serum apo B or LDL cholesterol.

If several assumptions are made, the HDL concentration (mg per dl) in serum can be approximated from the apo A-I value. Using the apo A-I content (65 percent) of isolated HDL (d 1.063 to 1.21 g per ml) as representative of the ratio of apo A-I to protein in all the HDL subclasses, and a protein: lipid ratio of 51:49 (as the weighted average of HDL, and HDL, ratio 1:3 for males), the calculated mean HDL concentration in males was 392 mg per dl (range 286 to 498) and females 465 mg per dl (range 324 to 600). These values are in agreement with the determination of HDL concentration by ultracentrifugation and heavy metal precipitation.

The serum level of apo A-I in subjects with abetalipoproteinemia (ABL) (n = 2; 0.38 g per l and 0.30 g per l), Type II hyperlipoproteinemia (n = 3; mean 0.72 g per l; range 0.54 to 0.86 g per l) and Tangier’s disease (0.01 g per l) was significantly lower than those in healthy males and females. The lower level of apo A-I in these subjects is consistent with previous reports showing lower values for whole HDL. These preliminary results indicated that significant alterations in the LDL concentrations and changes in the physico-chemical properties of HDL in the ABL patients (and Tangier patient) do not affect the optimum conditions for measuring apo A-I. Though this result will have to be substantiated for other dyslipoproteinemias, it seems probable that the apo A-I assay can readily be adapted for analyzing sera from patients with various diseases and thus improve our understanding of the role of this polypeptide and HDL in lipid transport.

Measurement of Apolipoprotein A-II of High Density Lipoprotein by Radioimmunoassay

Three radioimmunoassays for human plasma apolipoprotein A-II (apo A-II) have been reported. In contrast to apo A-I, it appears that the immunoreactivity of apo A-II is maximally exposed in dilutions of whole HDL and of plasma. Similarly to human apo A-I, apo A-II was isolated from HDL which was delipidated with ethanol-ether and fractionated on Sephadex G-200 columns equilibrated in Tris-urea buffer. Apo A-II was standardized by the Lowry method, which gave comparable results to that determined by amino acid analysis.

The working range of this assay was between 0.5 and 50 ng and the intraassay and interassay coefficients of variation were 7 ± 4 and 11 ± 6 percent, respectively. Immunoadassayable apo A-II accounted for less than 2 percent of the total protein of VLDL and LDL. No immunoreactive apo A-II was detected in the individual C-apolipoproteins or albumin when added in amounts of up to 200 ng protein.

HDL (d 1.083 to 1.21 g per ml), HDL, (d 1.083 to 1.124 g per ml) and HDL (d 1.124 to 1.195 g per ml) (prepared from normolipidemic plasma) each produced displacement curves which were parallel to the apo A-II standard. The apo A-II content of these whole and delipidated HDL fractions ranged from 20.1 to 24.8 percent of the total protein. These values agreed well with the apo A-II contents determined by column chromatography.

Similarly to intact and apo HDL, whole and delipidated normolipoproteinemic plasma gave displacement curves parallel to the apo A-II standard. Again, delipidation did not alter the apo A-II value measured in whole normo- and hyperlipoproteinemic (Type II, Type III and Type
Varying amounts of apo A-II standard added to diluted plasma samples were fully recovered.

**Apolipoprotein A-II Values in Sera**

Apo A-II values of 9 normolipoproteinemic women and 20 normolipoproteinemic men were $0.418 \pm 0.107$ and $0.392 \pm 0.070$ g per l, respectively. These values are in good agreement with the plasma apo A-II values reported by Assmann et al (normolipoproteinemic females and males: $0.39 \pm 0.035$ and $0.34 \pm 0.031$ g per l, respectively). These apo A-II values are consistent with those determined by single radial immunodiffusion. The plasma apo A-I and apo A-II concentrations determined from the respective radioimmunoassay and single radial immunodiffusion measurements yielded a 3:1 weight ratio of apo A-I:apo A-II, a result which is in good agreement with the apo A-I:apo A-II weight ratio (between 3:1 and 4:1) determined for HDL.

The normolipidemic plasma apo A-II values measured by radioimmunoassay are one-half of that determined by the electroimmunoassay method of Curry et al. The reasons for this difference are not clear. However, Schonfeld et al have described interesting experiments which may in part explain this discrepancy. Firstly, plasma samples measured in the radioimmunoassay with four different anti-apo A-II antisera had the same apo A-II value with three of these antisera. The fourth antiserum gave apoA-II values which were approximately 15 percent higher. No further experiments were done to resolve this difference. Secondly, Schonfeld et al compared his radioimmunoassay method with that of electroimmunoassay. They found that isolated apo A-II was not an appropriate standard for the electroimmunoassay, since under these conditions the apo A-II content of HDL ranged from 77 to 93 percent, clearly an overestimate.

These results suggested that isolated apo A-II and HDL migrated at different rates and that apo A-II could not be used as the standard for measuring the apo A-II contents of plasma. Using whole HDL (in which the apo A-II content had been determined by radioimmunoassay or column chromatography) as the standard, the apo A-II values of plasma were in good agreement with those determined by radioimmunoassay. Similar results were obtained using delipidated HDL with delipidated plasma. However, apo HDL was not the appropriate standard for whole plasma samples and vice versa, because of the reduced migration of the delipidated sample. Since the apo A-II values of samples measured in the radioimmunoassay are not altered by delipidation, the radioimmunoassay method is clearly more versatile, allowing isolated polypeptides and lipid-bearing molecules to be assayed simultaneously.

In three adult subjects affected with Tangier's disease, the apo A-I and apo A-II plasma concentrations were < 1 percent and 5 to 7 percent, respectively of that found in normolipidemic plasma. Ultracentrifugation (at density 1.21 g per ml KBr) of the Tangier plasma revealed that more than 90 percent of the apo A-I sedimeted. By contrast, more than 95 percent of the apo A-II floated under these conditions. The dissociation of the A-I and A-II apolipoproteins in Tangier's disease was confirmed by immunoelectrophoresis of fresh plasma with monospecific antisera. The reasons for the abnormal concentration and distribution of the A-apolipoproteins in these patients is not yet completely understood.

**Measurement of Apolipoprotein CII by Radioimmunoassay**

**The Radioimmunoassay**

Apo CII, which is present in both VLDL and HDL, is generally accepted as
the major activator of lipoprotein lipase (LPL) in various tissues including human adipose tissue,\textsuperscript{11} the human heart,\textsuperscript{43} human and rat post-heparin plasma,\textsuperscript{16,23} bovine milk,\textsuperscript{16,31} and rat adipose tissue.\textsuperscript{23} Other apolipoproteins in VLDL and HDL viz CI, CIII, AI and the arginine-rich polypeptide (E) inhibit human adipose tissue LPL.\textsuperscript{11} Thus the C apolipoproteins play an important role in the metabolism of triglycerides.

In order to understand better the possible role of apo CII in the pathophysiology of hypertriglyceridemic states, a sensitive, accurate, precise and specific method for its measurement is necessary. Miller and Schonfeld, in a preliminary report, described a radioimmunoassay for apo CII.\textsuperscript{30} Subsequently, Kashyap et al\textsuperscript{22} published a more detailed account of a double antibody radioimmunoassay for apo CII. The radioimmunoassay, as compared to quantitative bioassay, has the advantage that it is specific for apo CII, while the bioassays measure the net LPL activator concentration in whole or fractionated serum. Quantitation of apo CII by the method developed by Kane et al\textsuperscript{18} gives comparable results to that of the radioimmunoassay,\textsuperscript{22} but the tetramethyl urea (TMU) delipidation and separation of the constituent polypeptides by polyacrylamide gel electrophoresis is only applicable to serum fractions and not to whole sera. The generally higher degree of sensitivity of radioimmunoassays as compared to single radial immunodiffusion and electroimmunoassay methods is an important advantage, because the serum apo CII concentration is very low.

For the radioimmunoassay, apo CII was purified from VLDL isolated from healthy fasting normolipidemic subjects. Monospecific antisera were raised in rabbits. Apo CII was standardized by amino acid analysis and the \textsuperscript{125}I-apo CII was prepared by the chloramine-T method (specific radioactivity 87 to 107 mCi per mg).\textsuperscript{22}

The assay was sensitive to 0.6 ng apo CII and the working range lay between 1.56 and 50 ng. No appreciable (less than 1 percent) immunoreactive apo CII was present in apolipoproteins C1, CIII\textsubscript{1}, CIII\textsubscript{2}, AI and AII, in LDL (d 1.024 to 1.050 g per ml) and in lipoprotein free plasma (d > 1.25 g per ml). VLDL, intermediate low density lipoprotein (I-LDL), HDL\textsubscript{2}, HDL\textsubscript{3} and VHDL fractions gave displacement curves that were parallel to the apo CII standard. The content of apo CII (as percent of the total lipoprotein) ranged from an average of 12.3 percent for VLDL to 0.12 percent for VHDL. The apo CII content of VLDL determined by the radioimmunoassay (range 5.1 to 16.9 percent) was in good agreement\textsuperscript{22} with the values reported by Kane et al\textsuperscript{18} (range 4.5 to 12.0 percent), who calculated the apo CII content from the densitometric scans of TMU soluble VLDL apolipoproteins which had been separated by polyacrylamide gel electrophoresis.

Further evidence that the apo CII immunoreactivity in whole plasma and isolated lipoprotein samples is fully exposed is based on the observations that delipidation of plasma and VLDL with ethanol-diethyl ether followed by solubilization in 8 M urea did not alter the apo CII content. Also, unlike the immunoreactivity of human apo A-I in plasma and HDL, heating plasma samples at 26°C or 52°C for three hours prior to assay did not change the apo CII concentration.

**Apolipoprotein CII Values in Sera**

The total plasma apo CII concentration (mean ± SE) in 32 normolipoproteinemic subjects (0.0497 ± 0.0040 g per l) was not significantly different from that in 15 subjects with Type IIA lipoproteinemia (0.0562 ± 0.0054 g per l).\textsuperscript{22} Plasma apo CII levels in nine subjects with Type IIB, 14 with Type IV and five with Type V lipoproteinemia were 0.0899 ± 0.0046, 0.0854 ± 0.0069 and 0.1328 ± 0.0021 g
per l, respectively, and were significantly higher than in the normotriglyceridemic subjects (p < 0.001).22

In both normo- and hypertriglyceridemic subjects, the fasting plasma triglycerides correlated positively with plasma apo CII. However, there was an inverse correlation between total plasma triglyceride and the apo CII content of VLDL-free plasma (d > 1.006 g per ml), suggesting a transfer of apo CII from HDL to VLDL as the plasma triglycerides increased.22 The relationship between the apo CII content and the LPL activator properties of the VLDL isolated from normo- and hypertriglyceridemic plasma samples was further investigated. The apo CII content of VLDL (as a percent of total VLDL protein) was significantly greater for normotriglyceridemic than did normotriglyceridemic individuals (145.6 ± 11.3 μU per mg) (p < 0.01). From measurements of the apo CII content and the LPL activator concentration of VLDL, the LPL activator potency per microgram of VLDL apo CII was calculated. For the hypertriglyceridemic subjects the LPL activator potency was significantly less than for the normotriglyceridemic subjects (1.02 ± 0.04 versus 1.54 ± 0.13 μU per μg; p < 0.025). This analysis suggests that the impaired catabolism of triglycerides in these subjects may be due in part to the lower LPL activator potency of their VLDL.22 Further studies on the apolipoprotein content of VLDL as well as of the other lipoproteins are needed in order to more fully understand the mechanisms by which the LPL activator potency of VLDL is reduced in these hypertriglyceridemic subjects.

References


