Metabolism of Apolipoproteins C-II, C-III, and B in Hypertriglyceridemic Men: Changes after Heparin-induced Lipolysis

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The C apolipoproteins are normally transferred to high density lipoproteins (HDL) after lipolysis of very low density lipoprotein (VLDL) triglyceride. In previous studies, a loss of plasma C apolipoproteins was documented after heparin-induced lipolysis in hypertriglyceridemic subjects. The present studies were designed to determine if this decline in plasma C apolipoproteins was due to their clearance with VLDL remnants. Five Type IV hypertriglyceridemic and two normal subjects were injected with $^{125}$I-VLDL and $^{131}$I-low density lipoproteins (LDL) to document kinetically an excess of VLDL apolipoprotein (apo) B flux relative to LDL apo B flux in the Type IV subjects. A mean of 46% VLDL apo B was cleared from the circulation, without conversion to intermediate density lipoprotein (IDL) or LDL. Heparin was then infused (9000 IU over 4 hours) to generate an excess of VLDL remnants that were not converted to IDL or LDL. VLDL triglyceride, apo B, and apo C concentrations fell at a similar rate. VLDL apo B declined by 42% (p<0.01). However, no increases were observed in IDL or LDL apo B in the Type IV subjects. This resulted in a 14% (p<0.01) decline in plasma apo B concentrations, indicating a clearance of VLDL remnants. VLDL apo C-II and C-III concentrations fell by 42% (p<0.025) and 52% (p<0.01), respectively. During the first 2.5 hours of infusion, they were almost quantitatively recovered in HDL. Thereafter, the C apolipoproteins declined in HDL during which time VLDL apo C concentrations continued to decline. This resulted in a delayed fall (C-II, 21%, p<0.025; C-III, 26%, p<0.01) in plasma apo C concentrations. Thus, during enhanced lipolysis in Type IV subjects, C apolipoproteins were not cleared with VLDL remnants, but were initially transferred to HDL. It is possible that C apolipoproteins, along with VLDL surface lipids, form an HDL-like particle that is destined for clearance rather than for recycling to newly formed VLDL.

(Arteriosclerosis 8:471-479, September/October 1988)
high in carbohydrates, which are known to expand the VLDL pool size.\(^{21}\) We also showed that this similarly existed in a non-steady state, since an infusion of heparin in Type V hyperlipoproteinemic subjects caused a proportional shift of apo C-II, C-III, and C-IV from VLDL to HDL.\(^{22}\) An unexpected finding was that a substantial proportion of apo C radioactivity lost from VLDL was not recovered in the HDL fraction. It was not known if this was due to a transfer of C apolipoproteins to IDL or LDL, to a lipoprotein-free fraction, or if it was removed from the circulation. Also, we quantitated changes in apo C by isoelectric focusing gel electrophoresis which was relatively imprecise. Although apo B mass was not quantitated, loss of VLDL apo B radioactivity after heparin administration was not recovered in IDL or LDL, indirectly confirming the existence of the VLDL "shunt" pathway.

The present experiments were designed to test the hypothesis that in Type IV hypertriglyceridemic subjects, the C apolipoproteins leave the circulation with a VLDL remnant and are thus not completely recovered by HDL particles. The kinetics of apo B in VLDL and LDL were measured to document an overproduction of VLDL B relative to LDL B. Subjects were then infused with heparin to initiate rapid VLDL lipolysis and generate VLDL remnants that would not be converted to LDL. Changes in total plasma level and redistribution among the lipoproteins of apo C-II, C-III, and B were quantitated using specific electroimmunoassays.

**Methods**

**Subjects**

Two healthy normolipidemic male subjects (ages 23 and 34 years) and five Type IV hyperlipidemic male subjects (ages 34 to 62 years) who met the classification of Fredrickson et al.\(^{23}\) were studied. All subjects were asked to avoid alcohol during the week before the study. One of the five hyperlipidemic subjects had a slightly abnormal fasting serum glucose (6.45 mM) and a moderate elevation of serum gamma GT (68 units/L) and another had a borderline elevation of serum glutamic oxaloacetic transaminase (35 units/L). Serum alkaline phosphatase and thyroxine levels were normal and urine analyses were normal. None of the subjects was on any lipid-lowering drug during the course of these studies. Each subject received 300 mg/d of potassium iodide daily for 16 days, starting 3 days before the lipoprotein turnover studies. The studies were approved by the University of Western Ontario Health Sciences Standing Committee on Human Research and all subjects gave informed consent. The baseline data are shown in Table 1. Two weeks before the study, all subjects were instructed to consume a balanced, weight-maintaining diet (approximately 40% of calories from fat, 40% from carbohydrate, and 20% from protein). During the study, the subjects received a diet of normal food but with less than 5% fat. The use of this dietary regimen minimized the contribution to plasma of intestinally derived triglyceride-rich lipoproteins, as discussed previously.\(^{9,20}\) Plasma concentrations of VLDL triglyceride, apo B, and apo C before the heparin infusion varied less than 10% and were used as evidence of a steady state.\(^{20}\)

### Lipoprotein Turnover Studies

Most of the procedures used in these studies have been previously described.\(^{9,20,24,26}\) Four days before each study, 200 ml of blood was obtained from each subject after a 14-hour fast. Approximately 30 ml of plasma was ultracentrifuged through 10 ml of buffered saline (0.15 M NaCl; 1 mM Tris, pH 7.4; and 1 mM EDTA) in each of four 60 Ti Beckman Quickseal tubes for 0.5 hours at 17 000 rpm, at 12°C. The absence of chyomicrons was assessed visually and the tubes were ultracentrifuged for another 2 hours at 40 000 rpm at 12°C to isolate VLDL (Sf 60 to 400). After the removal of IDL (Sf 12 to 20) for 16 hours at 40 000 rpm at 12°C, LDL (Sf 0 to 12) was isolated between density 1.019 g/ml and 1.063 g/ml for 24 hours at 40 000 rpm at 12°C.\(^{26}\) Lipoproteins were washed once at the appropriate salt density. VLDL was labeled with \(^{125}\)I and LDL, with \(^{131}\)I by a modification of the iodine monochloride technique.\(^{25}\) The isolation and labeling procedures were performed aseptically and the preparations were filtered through 0.45 \(\mu\)m Millipore filters into a sterile vial containing 100 \(\mu\)g/ml gentamycin sulfate (Parke-Davis). Aliquots were removed for determination of fertility, pyrogenicity, and distribution of the label among

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**Table 1. Baseline Data**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Weight (kg)</th>
<th>Lipoprotein phenotype</th>
<th>Triglyceride</th>
<th>Cholesterol</th>
<th>Apo B</th>
<th>Apo C-II</th>
<th>Apo C-III</th>
<th>Apo E phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>78</td>
<td>IV</td>
<td>636 (mg/dl)</td>
<td>871 (mg/dl)</td>
<td>122</td>
<td>23</td>
<td>20</td>
<td>E4E3</td>
</tr>
<tr>
<td>2</td>
<td>92</td>
<td>IV</td>
<td>352 (mg/dl)</td>
<td>140 (mg/dl)</td>
<td>104</td>
<td>21</td>
<td>24</td>
<td>E4E3</td>
</tr>
<tr>
<td>3</td>
<td>81</td>
<td>IV</td>
<td>608 (mg/dl)</td>
<td>110 (mg/dl)</td>
<td>131</td>
<td>29</td>
<td>20</td>
<td>E4E3</td>
</tr>
<tr>
<td>4</td>
<td>78</td>
<td>IV</td>
<td>794 (mg/dl)</td>
<td>263 (mg/dl)</td>
<td>100</td>
<td>30</td>
<td>22</td>
<td>E4E3</td>
</tr>
<tr>
<td>5</td>
<td>102</td>
<td>IV</td>
<td>960 (mg/dl)</td>
<td>205 (mg/dl)</td>
<td>142</td>
<td>41</td>
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<td>6</td>
<td>82</td>
<td>N</td>
<td>135 (mg/dl)</td>
<td>180 (mg/dl)</td>
<td>81</td>
<td>8</td>
<td>12</td>
<td>E4E3</td>
</tr>
<tr>
<td>7</td>
<td>81</td>
<td>N</td>
<td>114 (mg/dl)</td>
<td>183 (mg/dl)</td>
<td>87</td>
<td>5</td>
<td>13</td>
<td>E4E3</td>
</tr>
</tbody>
</table>

Lipoproteins were separated from plasma obtained from fasting donors and were analyzed for cholesterol, triglyceride, and apolipoproteins (apo) B, C-II, and C-III. Very low density lipoprotein (VLDL) was separated by ultracentrifugation and high density lipoprotein (HDL), by heparin-manganese chloride precipitation; low density lipoprotein (LDL) was calculated by the difference. Lipoprotein phenotypes were classified according to the method of Fredrickson et al.\(^{23}\) and apolipoprotein E phenotypes, according to that of Huff et al.\(^{27}\)
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apolipoproteins and lipids by methods described previously.\textsuperscript{9,20} The VLDL label contained less than 1% free iodine, and approximately 5% to 12% of the label associated with lipid. The percent of iodine bound to apo B was 37% to 43%. Labeled LDL contained less than 1% free iodine, 3% to 4% of the label was bound to lipid, and 90% to 92% was bound to apo B.

On the first day of each study, after a 14-hour fast, subjects were injected with the autologous labeled VLDL (\textsuperscript{125}I-Sf 60 to 400, 60 \mu Ci) and LDL (\textsuperscript{131}I-Sf 0 to 12, 30 \mu Ci). Subsequently, blood samples were collected into tubes containing EDTA (1.5 mg/ml) at 0.5, 1, 2, 4, 6, 8, 10, 14, 24, 24.5, 25, 25.5, 26, 27, 28, 29, 30, 32, 48, 72, and 120 hours. Immediately following the 25.5 hour sample, an infusion of heparin (heparin sodium USP, Organon) was given. Subjects received 4000 IU for 0.5 hour (priming dose) followed by 2000 IU/hour for 3.5 hours. Blood samples were placed on ice and plasma was separated within 10 minutes at 4°C.

Plasma was kept at 4°C for 2 to 12 hours before ultracentrifugation. For those plasma samples taken after the infusion of heparin, no more than 7 hours elapsed at 4°C before ultracentrifugation. After isolation of plasma, m-aminophenylboronic acid (final concentration 0.4 mM) was added to plasma to inhibit lipolysis during storage.\textsuperscript{26} From 10 ml of plasma, VLDL was isolated, followed sequentially by IDL, LDL, and HDL (d-1.063 to 1.21 g/ml).\textsuperscript{20} Samples for electroimmunoassay and lipid analyses were aliquoted after initial isolation, whereas VLDL, IDL, and LDL were washed once at the appropriate salt density before determination of apo B specific activity.

The B apolipoprotein was selectively precipitated with isopropanol, and the protein content, radioactivity, and specific radioactivity were calculated.\textsuperscript{24} The kinetic parameters were calculated from the VLDL or LDL specific activity disappearance curves (Figure 1). The B apolipoprotein kinetics in VLDL for normal subjects could be resolved into two pools, whereas in the hypertriglyceridemic subjects, VLDL apo B specific activity curves were mono-exponential (linear) over the first 24 hours, as found previously.\textsuperscript{20} LDL apo B specific activity curves in all subjects were best fitted to a two-exponential function. The appropriateness of a one-pool or a two-pool model was tested using the Fisher F statistic. Kinetic parameters were calculated from the bi-exponential curves as described by Gurpide et al.\textsuperscript{28} and were applied to apo B kinetics as described previously.\textsuperscript{9,20} Values for flux of material through pool 1, the irreversible fractional catabolic rate from pool 1, and the mass in pool one were obtained. The one-pool model, reflecting a mono-exponential, specific activity-time curve, was analyzed by conventional techniques.\textsuperscript{26} Fractional clearance rate, pool size, and flux through the pool were calculated as described previously for VLDL apo B specific activity curves in hypertriglyceridemic subjects.\textsuperscript{20} Kinetic parameters were calculated before the perturbation by the infusion of heparin (25 hours after injection), which is a shorter time-course than is normally used (36 to 48 hours). This length of time was sufficient to provide good estimates of the apo B kinetic parameters, since in hypertriglyceridemic subjects, VLDL apo B specific activity declined linearly (Figure 1) up to the time of injection. It is possible that in these patients, a second exponential for VLDL apo B was missed; however, in previous studies,\textsuperscript{20} we demonstrated that VLDL apo B specific activity curves in hypertriglyceridemic patients were mono-exponential up to 48 hours. In the normal subjects in this study, the two components of the VLDL apo B specific activity curve were largely resolved during the first 26 hours, since less than 1% of the initial radioactivity remained at that time (Figure 1). Nevertheless, the VLDL apo B kinetic parameters should be regarded as estimates. The full LDL apo B specific activity curve was used for kinetic analysis, since the curves were unaffected by the infusion of heparin.

Analyses

The concentrations of apo B, C-II, C-III, and A-I were determined by modifications of the rocket immunoelectrophoresis assay of Laurell.\textsuperscript{30} Apo B was assayed as described by Reardon et al.\textsuperscript{31} after lipase treatment
Table 2. Metabolism of Apolipoprotein B

<table>
<thead>
<tr>
<th>Subject</th>
<th>Lipoprotein phenotype</th>
<th>Pool size (mg/kg)</th>
<th>FCR (day⁻¹)</th>
<th>Production (Flux) (mg/kg/day)</th>
<th>Flux Direct removal* (mg/kg/day)</th>
<th>Flux LDL apo B (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IV</td>
<td>17.1</td>
<td>1.62</td>
<td>27.7</td>
<td>18.1</td>
<td>9.6</td>
</tr>
<tr>
<td>2</td>
<td>IV</td>
<td>8.4</td>
<td>3.05</td>
<td>23.8</td>
<td>7.4</td>
<td>16.4</td>
</tr>
<tr>
<td>3</td>
<td>IV</td>
<td>26.6</td>
<td>1.18</td>
<td>31.5</td>
<td>8.3</td>
<td>23.2</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>18.0</td>
<td>1.38</td>
<td>24.8</td>
<td>10.7</td>
<td>14.1</td>
</tr>
<tr>
<td>5</td>
<td>IV</td>
<td>17.6</td>
<td>2.25</td>
<td>39.6</td>
<td>24.0</td>
<td>15.6</td>
</tr>
<tr>
<td>6</td>
<td>N</td>
<td>1.0</td>
<td>14.4</td>
<td>14.2</td>
<td>—</td>
<td>17.8</td>
</tr>
<tr>
<td>7</td>
<td>N</td>
<td>0.9</td>
<td>12.78</td>
<td>11.3</td>
<td>—</td>
<td>17.5</td>
</tr>
</tbody>
</table>

125I-VLDL (Sf 60 to 400) and 131I-LDL (Sf 0 to 12) were simultaneously injected into each subject. The kinetic parameters of apolipoprotein metabolism were derived from specific activity vs. time curves of the labeled apolipoprotein (apo) B component in each lipoprotein fraction.

*This was calculated by subtracting the LDL apo B flux from the VLDL apo B flux. The LDL apo B flux was assumed to be entirely derived from VLDL apo B. This assumption may be in error (see Methods) due to the fact that LDL apo B direct synthesis could only be estimated. The values for VLDL apo B direct removal should be regarded as minimal rates.

VLDL = very low density lipoprotein; LDL= low density lipoprotein, FCR = fraction catabolic rate.

Results

The kinetic analyses of apo B metabolism are shown in Table 2. Apo B pool sizes in VLDL in the Type IV hypertriglyceridemic subjects were elevated as expected (mean 17.5 ± 4 mg/kg) as demonstrated previously. This increased pool size was associated with an increased production (flux) rate (29.4 ± 3 mg/kg/day) as well as a reduced fractional catabolic rate (1.9 ± 0.3 day⁻¹). The production rate of LDL apo B was estimated from the 131I-LDL apo B specific activity curve, and in each hyper-
triglyceridemic subject the VLDL apo B flux rate exceeded the flux rate for LDL B.

In the hypertriglyceridemic subjects studied, the $^{125}$I-IDL apo B specific activity reached its peak at or about the point it crossed the precursor VLDL apo B specific activity curve (Figure 1). Similarly, the estimated peak of the LDL specific activity curve occurred close to the point of intersection with the $^{125}$I-IDL apo B specific activity curve. This was interpreted as indicating that the direct synthesis of IDL and LDL (from non-VLDL sources) was minimal. Thus, the greater VLDL apo B flux relative to LDL flux indicated that a substantial proportion (approximately 40%) of VLDL apo B was cleared from the circulation without conversion to LDL. However, because of the difficulty of estimating the peak in LDL apo B specific activity and the intervening heparin infusion, quantitative estimates of direct LDL synthesis were not possible. Therefore, since the direct removal of VLDL apo B to LDL apo B was calculated assuming no direct LDL apo B synthesis (Table 2), values for direct removal of VLDL should be regarded as estimates of minimal rates. Similar assumptions apply to the normal subjects. After the infusion of heparin, rapid VLDL lipolysis was observed. Apo B and C and triglyceride were cleared from VLDL in approximately the same proportions as their pre-infusion concentrations (Figure 2). The percent of VLDL hydrolysis was more extensive for the normal subjects relative to the hypertriglyceridemic subjects. The results of the heparin infusion have been divided into three periods: 1) preheparin, which is a mean of four determinations from 2 hours before until initiation of the infusion; 2) postheparin-1, a mean of four determinations from 0.5 hours until 2.5 hours after the start of the infusion, and 3) postheparin-2, a mean of three determinations from 2.5 hours until 5.5 hours after infusion. The latter included a sample taken 1.5 hours after cessation of the infusion. In the Type IV subjects, there was a significant drop in VLDL apo B during the postheparin-1 period, which continued to drop during the postheparin-2 period (Table 3). This apo B was not recovered in either the IDL or LDL lipoprotein fraction (Table 3 and Figure 3). Therefore, since the direct removal of VLDL apo B to LDL apo B synthesis (Table 2), values for direct removal of VLDL should be regarded as estimates of minimal rates. Similar assumptions apply to the normal subjects. After the infusion of heparin, rapid VLDL lipolysis was observed. Apo B and C and triglyceride were cleared from VLDL in approximately the same proportions as their pre-infusion concentrations (Figure 2). The percent of VLDL hydrolysis was more extensive for the normal subjects relative to the hypertriglyceridemic subjects. The results of the heparin infusion have been divided into three periods: 1) preheparin, which is a mean of four determinations from 2 hours before until initiation of the infusion; 2) postheparin-1, a mean of four determinations from 0.5 hours until 2.5 hours after the start of the infusion, and 3) postheparin-2, a mean of three determinations from 2.5 hours until 5.5 hours after infusion. The latter included a sample taken 1.5 hours after cessation of the infusion. In the Type IV subjects, there was a significant drop in VLDL apo B during the postheparin-1 period, which continued to drop during the postheparin-2 period (Table 3). This apo B was not recovered in either the IDL or LDL lipoprotein fraction (Table 3 and Figure 3). Thus, the generated VLDL remnants were not converted to LDL or HDL. This resulted in a significant loss (14%) of the apo B from plasma during both postheparin periods ($p<0.01$).

In both Type IV and normal subjects, a rise in VLDL apo B specific activity was observed during the heparin infusion (Figure 1). This could arise from the preferential hydrolysis by released lipoprotein lipase of cold nascent VLDL particles in preference to smaller, more slowly turning-over particles still being traced 25 hours after tracer injection. The fall in VLDL apo B mass would be derived mainly from the larger unlabeled pool, resulting in a rise in apo B specific activity. The observation that little or no change in IDL or LDL specific activity occurred in the Type IV subjects after the heparin infusion supports the conclusion that unlabeled apo B disappearing from VLDL does not appear in IDL or LDL.

Apo C-III concentrations in VLDL dropped significantly during both the postheparin-1 period ($9 \pm 1$ vs. $12 \pm 2$ mg/dl, $p<0.02$) and the postheparin-2 period ($7 \pm 1$ vs. $12 \pm 2$, $p<0.05$) compared to the preheparin period (Table 3). Also, apo C-III declined in VLDL in postheparin-1 (15 ± 2 vs. 25 ± 4 mg/dl, $p<0.01$) and postheparin-2 (12 ± 1 vs. 25 ± 4 mg/dl, $p<0.01$). During the postheparin-1 period, HDL concentrations of apo C-II and C-III rose significantly (4.3 ± 0.9 vs. 1.0 ± 0.1 mg/dl, $p<0.01$ and 11 ± 1 vs. 3 ± 1, $p<0.01$) relative to preheparin values (Table 3). However, during the postheparin-2 period, concentrations of both C-II and C-III in HDL dropped significantly ($p<0.05$) relative to the postheparin-1 period at a time when VLDL C-II and C-III concentrations continued to decline. There was no change in the relatively low concentrations of C-II and C-III in either IDL or LDL (data not shown). Also, there was no detectable C-II or C-III in the d>1.21 fraction during any of the time periods. This resulted in a significant 21% and 26% drop in plasma apo C-II and C-III concentrations, respectively, during the postheparin-2 period only. This delayed decline in plasma apo C during the postheparin-2 period is illustrated for one subject in Figure 4. Throughout the heparin infusion periods, there was no significant change in plasma A-I relative to the preheparin period. In the hypertriglyceridemic subjects,

Figure 3. Changes in apolipoprotein B concentrations (mg/dl) in lipoprotein fractions; very low density lipoprotein (VLDL, •—•) (Sf 60 to 400), intermediate density lipoprotein (IDL, o—o) (Sf 12 to 60), and low density lipoprotein (LDL, x—x) (Sf 0 to 12) after an infusion of heparin (9000 IU over 4 hours). Top panel shows data from a normal subject (6), and the lower panel, data from a hypertriglyceridemic subject (5).
Table 3. Redistribution of Apolipoproteins C-II, C-III, and B between Plasma Lipoproteins after Infusion of Heparin

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Apolipoprotein B</th>
<th>Apolipoprotein C-II</th>
<th>Apolipoprotein C-III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VLDL (mg/dl)</td>
<td>HDL (mg/dl)</td>
<td>VLDL (mg/dl)</td>
</tr>
<tr>
<td>Type IV subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preheparin</td>
<td>38±9</td>
<td>12±2</td>
<td>25±4</td>
</tr>
<tr>
<td>Postheparin-1</td>
<td>31±5</td>
<td>4.3±0.9*</td>
<td>15±2*</td>
</tr>
<tr>
<td>Postheparin-2</td>
<td>22±7*</td>
<td>2.8±0.4*†</td>
<td>12±1*†</td>
</tr>
<tr>
<td>Normal subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preheparin</td>
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<td>1.45</td>
<td>4.9</td>
</tr>
<tr>
<td>Postheparin-1</td>
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<td>2.21</td>
<td>5.83</td>
</tr>
<tr>
<td>Postheparin-2</td>
<td>73</td>
<td>4.5</td>
<td>11.8</td>
</tr>
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</table>

Concentrations (mg/dl ± SEM) of apolipoproteins B, C-II, and C-III in plasma (P) and lipoprotein fractions separated by ultracentrifugation. VLDL (Sf 60 to 400), IDL (Sf 12 to 60), LDL (Sf 0 to 12), and HDL (d = 1.062 to 1.21 g/ml). P values were determined by paired t tests.

*Significantly different from preheparin, p<0.025; †significantly different from postheparin-1, p<0.05; ‡mean of data from two normal subjects.

concentrations of HDL A-I during the preheparin and postheparin periods were 109 ± 8 mg/dl and 112 ± 10 mg/dl, respectively. In the normal subjects, the A-I concentrations during the same periods were 125 mg/dl and 128 mg/dl, respectively. In the two normal subjects, apo B concentrations declined during both postheparin periods, in both VLDL and IDL. Apo B was not lost from plasma, but was recovered in LDL (Figure 3 and Table 3) during the postheparin-1 period. However, a small loss from plasma was noted during the postheparin-2 period, which was reflected by a decline in LDL apo B. Apo C-II and C-III shed from VLDL during rapid lipolysis were completely recovered in HDL, with no apparent loss from plasma. Graphically from Figure 2, it appeared that all VLDL components declined in the same proportions as their preheparin concentrations; however, in each Type IV subject, the rate of decline of C-III was greater than that of C-II. Measurement of apo C-II/C-III ratios (Table 3) revealed that C-III was lost more readily from VLDL, resulting in a significant increase in this ratio in VLDL after heparin infusion (preheparin, 0.45 ± 0.1; postheparin-1, 0.57 ± 0.03, and postheparin-2, 0.60 ± 0.02, p<0.025 for both). This was associated with a decrease (although not significant) in the C-II/C-III ratio in HDL during postheparin-1. Thus, after the heparin infusion, the plasma C-II/C-III ratio increased significantly. Neither the ratio of VLDL triglyceride/VLDL apo B nor the ratio of VLDL triglyceride/VLDL cholesterol differed significantly during the postheparin periods relative to the preheparin period (Table 4). As expected, a significant 37% decline in plasma triglyceride was observed in the Type IV subjects which was primarily due to a drop in VLDL triglyceride, although a significant decline in HDL triglyceride was also observed. Plasma cholesterol also declined in these subjects by 16%, which was entirely due to a decline in LDL cholesterol. In the normal subjects, plasma triglyceride and cholesterol also fell, but in contrast to the Type IV subjects, these declines were observed in both VLDL and IDL.

Discussion

The present studies were undertaken to test the hypothesis that in hypertriglyceridemic subjects during rapid VLDL lipolysis induced by heparin, the C apolipoproteins are lost from plasma, as had been previously estimated by gel electrophoresis. In addition, we wanted to determine more precisely by specific electroimmunoassay whether apo C was lost from plasma as part of a VLDL remnant and whether any differences could be observed in the fate of apo C-II relative to C-III during this metabolic perturbation. The results of these experiments have allowed us to make several important observations. In hypertriglyceridemic Type IV subjects having a kinetically defined VLDL
apo B shunt pathway, apo B that was lost from VLDL during accelerated lipolysis was lost from the circulation. This is consistent with the idea that in these subjects conversion of VLDL remnants to IDL and LDL is saturated and rate-limiting (as suggested previously by kinetic analyses) or that the lipolysis products in these patients are not converted to LDL. Eisenberg et al. have reported that large VLDL in hypertriglyceridemic subjects, which would predominate in the Sf 60 to 400 fraction, are relatively enriched in cholesterol ester, interact normally with lipoprotein lipase in vitro, but yield lipolysis products so composed that they do not appear as plasma LDL. The heparin infusion greatly expanded the pool of VLDL remnants so that we could determine if any apo C left the circulation as part of this remnant. We have now documented with immunocassay that plasma concentrations of apo C-II and C-III decline in the circulation in response to an infusion of heparin in hypertriglyceridemic individuals. The decline in the present study is consistent with the in vitro observations of Tam et al. and Tam and Breckenridge who found that during lipolysis of human VLDL in perfused rat hearts, VLDL apo C, along with apo E phospholipid and cholesterol, are converted to an HDL-like spherical particle. Recently, Rubinstein et al. reported that plasma apo C concentrations were unaltered after a bolus heparin injection in Type V subjects. However, the last sample assayed after the injection was at 60 minutes, whereas in our study the C apoprotein concentrations did not begin to decline until 2.5 to 5 hours after infusion.

In the present study, we found that, in contrast to the Type IV subjects, in normal subjects C apolipoproteins were almost completely recovered in the HDL region after heparin-induced lipolysis. It is possible that this difference is related to low HDL levels, abnormal distributions of HDL2 and HDL3, and abnormal distributions of C-II and C-III in HDL2 and HDL3 in hypertriglyceridemic individuals.

In this study we observed that C-III declined more rapidly from VLDL than did C-II during the heparin infusion. This was in contrast to our previous findings that C-II, C-III, and C-III2 shared similar metabolic characteristics. A disparity in response of C-II relative to C-III in the present study is consistent with the in vitro observation of Tam et al. and Tam and Breckenridge. After

### Table 3. (Continued)

<table>
<thead>
<tr>
<th>Apolipoprotein C-II/C-III</th>
<th>VLDL</th>
<th>HDL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.45 ± 0.01</td>
<td>0.38 ± 0.07</td>
<td>0.44 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>0.57 ± 0.03</td>
<td>0.33 ± 0.04</td>
<td>5.1 ± 0.04*</td>
<td></td>
</tr>
<tr>
<td>0.60 ± 0.02</td>
<td>0.37 ± 0.03</td>
<td>5.1 ± 0.04*</td>
<td></td>
</tr>
<tr>
<td>0.47</td>
<td>0.40</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>0.51</td>
<td>0.41</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>0.54</td>
<td>0.432</td>
<td>0.38</td>
<td></td>
</tr>
</tbody>
</table>

We have shown that the C apoproteins do not leave the circulation as part of a VLDL remnant but are first transferred to an HDL-like particle and then are cleared during the late phase of infusion. This is in contrast to our original hypothesis. Also, we had originally argued that the fractional catabolic rate of the C apolipoproteins, even in hypertriglyceridemic subjects, was still two- to threefold faster than that of HDL A-I but slower than VLDL-B, suggesting that at least a portion of apo C was cleared from the circulation as part of a VLDL remnant. However, the results of the present study confirm the earlier idea of Berman et al. that apo C apolipoproteins are cleared from the circulation via HDL. Furthermore, because we observed no change in HDL A-I concentrations, our results suggest that the C apolipoproteins leave the circulation, not with HDL per se but as an HDL-like particle containing apo C and probably VLDL surface lipids. This is consistent with the findings of Tam et al. and Tam and Breckenridge who found that during lipolysis of human VLDL inperfused rat hearts, VLDL apo C, along with apo E phospholipid and cholesterol, are converted to an HDL-like spherical particle. Recently, Rubinstein et al. reported that plasma apo C concentrations were unaltered after a bolus heparin injection in Type V subjects. However, the last sample assayed after the injection was at 60 minutes, whereas in our study the C apoprotein concentrations did not begin to decline until 2.5 to 5 hours after infusion.

In the present study, we found that, in contrast to the Type IV subjects, in normal subjects C apolipoproteins were almost completely recovered in the HDL region after heparin-induced lipolysis. It is possible that this difference is related to low HDL levels, abnormal distributions of HDL2 and HDL3, and abnormal distributions of C-II and C-III in HDL2 and HDL3 in hypertriglyceridemic individuals.

In this study we observed that C-III declined more rapidly from VLDL than did C-II during the heparin infusion. This was in contrast to our previous findings that C-II, C-III, and C-III2 shared similar metabolic characteristics. A disparity in response of C-II relative to C-III in the present study is consistent with the in vitro observation of Tam et al. and Tam and Breckenridge. After

### Table 4. Effect of Infusion of Heparin on Lipoprotein Lipid Concentrations

<table>
<thead>
<tr>
<th>Type IV subjects</th>
<th>Triglyceride (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>VLDL apo B</th>
<th>VLDL TG/CHOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preheparin</td>
<td>419 ± 78</td>
<td>85 ± 6</td>
<td>24 ± 2</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>Postheparin-1</td>
<td>257 ± 50</td>
<td>86 ± 6</td>
<td>30 ± 3*</td>
<td>6 ± 1*</td>
</tr>
<tr>
<td>Postheparin-2</td>
<td>205 ± 33*</td>
<td>84 ± 6</td>
<td>32 ± 3*</td>
<td>7 ± 2*</td>
</tr>
<tr>
<td>Normal subjects†</td>
<td>42</td>
<td>23</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>Postheparin-1</td>
<td>6</td>
<td>18</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>Postheparin-2</td>
<td>12</td>
<td>18</td>
<td>22</td>
<td>4</td>
</tr>
</tbody>
</table>

Concentrations (mg/dl ± SEM) of triglyceride and cholesterol in plasma (P) and lipoprotein fractions separated by ultracentrifuge: V = VLDL (Sf 60 to 400), I = IDL (Sf 12 to 60), L = LDL (Sf 0 to 12), and H = HDL (d = 1.063 to 1.21 g/ml). P values were determined by paired t tests.

*Significantly different from preheparin, p<0.05, †significantly different from postheparin-1, p<0.05, ‡mean of data from two normal subjects.
exposure of human VLDL to lipolytic activity in perfused rat hearts, over twice the amount of C-III relative to C-II was recovered in HDL-like particles. Windler et al. demonstrated that C-III prevented the premature uptake of triglyceride-rich lipoproteins by perfused rat livers. Therefore, one might expect that a more rapid loss of apo C-III from VLDL during lipolysis would then favor either clearance of the remnant or conversion to LDL. Recently, however, Windler observed a greater loss of C-II relative to C-III during in vitro lipolysis of rat chylomicrons. Bukkan et al. reported that human HDL has a significant pool of apo C-III that does not exchange with VLDL apo B. It is possible that clearance of C-III from HDL is more rapid than C-II due to a larger nonexchangeable HDL pool for C-III compared to C-II. Further studies would be required to support this idea.

These changes in apo C may not reflect normal steady-state conditions. However, clearance of C apolipoproteins from plasma in other more physiological situations after lipolysis of triglyceride-rich lipoproteins have been observed. Kashyap et al. demonstrated that, in the late phase of alimentary lipemia in normal subjects, apo C-II and C-III declined significantly in plasma. It is possible that these changes in apo C-III from HDL is more rapid than C-II due to a larger nonexchangeable HDL pool for C-III compared to C-II. Further studies would be required to support this idea.

The present study clearly demonstrates that C apolipoproteins can be cleared from plasma after rapid VLDL lipolysis and that they are not cleared with a VLDL remnant but are first transferred to the HDL density region. The composition and metabolic characteristics of the HDL-like particle with which the C apolipoproteins are cleared requires further study of HDL subpopulations.

Acknowledgments

We are grateful to Dawn Telford and Kim Woodcroft for their expert technical assistance and to Evelyn Geer and Lynn Thomson for preparation of this manuscript.

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Index Terms: lipoproteins • apolipoproteins B, C-II, C-III • lipolysis • heparin
Metabolism of apolipoproteins C-II, C-III, and B in hypertriglyceridemic men. Changes after heparin-induced lipolysis.
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Arterioscler Thromb Vasc Biol. 1988;8:471-479
doi: 10.1161/01.ATV.8.5.471

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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