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

Murray W. Huff, W. Carl Breckenridge, Wendy L.P. Strong, and Bernard M. Wolfe

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 $^{151}$Mow 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.

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The C apolipoproteins in human plasma are an integral part of triglyceride-rich very low density lipoproteins (VLDL). VLDL are synthesized and secreted by the liver to transport endogenously synthesized triglycerides. The metabolism of VLDL in peripheral tissues is initiated by the action of lipoprotein lipase. This enzyme is normally bound to capillary endothelial cell surfaces but can be released by heparin administration. Studies in vivo, in situ, and in vitro have shown that VLDL exposed to lipoprotein lipase lose triglyceride, resulting in smaller, denser particles. The reduction of VLDL core results in excess surface material, including the C apolipoproteins, phospholipids, unesterified cholesterol, and some apolipoprotein (apo) E, which are transferred to high density lipoproteins (HDL). Apo B-100 remains with the VLDL remnant particle intermediate density lipoprotein (IDL), which subsequently become low density lipoproteins (LDL). Some VLDL are removed from plasma, particularly in patients with hypertriglyceridemia.

The group of C apolipoproteins includes apo C-II, which is required to achieve maximal activation of lipoprotein lipase both in vitro and in vivo. Apo C-III appears in three different polymorphic forms (C-III₀, C-III₁, and C-III₂) because of differing sialic acid content. It is thought that C-III may inhibit the premature clearance of triglyceride-rich lipoproteins until sufficient triglyceride has been hydrolyzed and removed from the core. The distribution of the C apolipoproteins between VLDL and HDL reflects the steady-state concentrations of these lipoproteins. Consequently, hypertriglyceridemic subjects demonstrate an increased pool size of C apolipoproteins of which up to 90% are found to be associated with VLDL. Huff et al. have shown previously that in humans in a steady state, the kinetics of metabolism of the individual C apolipoproteins (C-II, C-III₁, and C-III₂) were similar, indicating a common metabolic fate. This was shown for normal individuals, and Type IV hypertriglyceridemic subjects as well as for normal subjects consuming diets.
five hyperlipidemic subjects had a slightly abnormal fasting 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. 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.

**Lipoprotein Turnover Studies**

Most of the procedures used in these studies have been previously described. 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 chylomicrons 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. Lipoproteins were washed once at the appropriate salt density. VLDL was labeled with 125I and LDL, with 131I by a modification of the iodine monochloride technique. The isolation and labeling procedures were performed aseptically and the preparations were filtered through 0.45 µm Millipore filters into a sterile vial containing 100 µg/ml gentamycin sulfate (Parke-Davis). Aliquots were removed for determination of sterility, pyrogenicity, and distribution of the label among

**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. 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. 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.

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**Table 1. Baseline Data**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Weight (kg)</th>
<th>Lipoprotein phenotype</th>
<th>Plasma Triglyceride (mg/dl)</th>
<th>Plasma Cholesterol (mg/dl)</th>
<th>Apo B (mg/dl)</th>
<th>Apo C-ll (mg/dl)</th>
<th>Apo C-lll (mg/dl)</th>
<th>Apo C-llll (mg/dl)</th>
<th>Apo E phenotype</th>
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<td>IV</td>
<td>636</td>
<td>148</td>
<td>112</td>
<td>38</td>
<td>18</td>
<td>16</td>
<td>E3E3</td>
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<tr>
<td>2</td>
<td>92</td>
<td>IV</td>
<td>352</td>
<td>140</td>
<td>104</td>
<td>21</td>
<td>11</td>
<td>5</td>
<td>E3E3</td>
</tr>
<tr>
<td>3</td>
<td>81</td>
<td>IV</td>
<td>608</td>
<td>394</td>
<td>131</td>
<td>29</td>
<td>17</td>
<td>11</td>
<td>E3E4</td>
</tr>
<tr>
<td>4</td>
<td>78</td>
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<td>794</td>
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<td>100</td>
<td>30</td>
<td>12</td>
<td>11</td>
<td>E3E3</td>
</tr>
<tr>
<td>5</td>
<td>102</td>
<td>IV</td>
<td>960</td>
<td>756</td>
<td>142</td>
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<td>81</td>
<td>N</td>
<td>114</td>
<td>68</td>
<td>87</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>E3E3</td>
</tr>
</tbody>
</table>
apolipoproteins and lipids by methods described previously. 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 (125I-Sf 60 to 400, 60 μCi) and LDL (131I-Sf 0 to 12, 30 μCi). 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. From 10 ml of plasma, VLDL was isolated, followed sequentially by IDL, LDL, and HDL (d-1.063 to 1.21 g/ml). 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. The kinetic parameters were calculated from the bi-exponential curves as described by Gurpide et al. and were applied to apo B kinetics as described previously. 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. 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. 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, 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. Apo B was assayed as described by Reardon et al. after lipase treatment.
Table 2. Metabolism of Apolipoprotein B

<table>
<thead>
<tr>
<th>Subject</th>
<th>Lipoprotein phenotype</th>
<th>VLDL Apo B</th>
<th>LDL apo B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pool size (mg/kg)</td>
<td>FCR (day⁻¹)</td>
<td>Production (Flux) (mg/kg/day)</td>
</tr>
<tr>
<td>1</td>
<td>IV</td>
<td>17.1</td>
<td>1.62</td>
</tr>
<tr>
<td>2</td>
<td>IV</td>
<td>8.4</td>
<td>3.05</td>
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<tr>
<td>3</td>
<td>IV</td>
<td>26.6</td>
<td>1.18</td>
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<tr>
<td>4</td>
<td>IV</td>
<td>18.0</td>
<td>1.38</td>
</tr>
<tr>
<td>5</td>
<td>IV</td>
<td>17.6</td>
<td>2.25</td>
</tr>
<tr>
<td>6</td>
<td>N</td>
<td>1.0</td>
<td>14.4</td>
</tr>
<tr>
<td>7</td>
<td>N</td>
<td>0.9</td>
<td>12.78</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.

(Rhizopus arrhizus, Boehringer-Mannheim, Montreal, Quebec), and apo A-I was assayed according to the method of Fidge et al. Apo C-II and C-III were assayed by the modifications reported by Tam et al. Apo C-II and C-III assays were initially standardized by using purified apolipoproteins, and subsequently a secondary serum standard was prepared. Validity was originally determined by comparing the values for VLDL C-II and C-III to protein determinations of C-II and C-III isolated by ion exchange chromatography. The values for C-II and C-III were within 6% and 5%, respectively, of the electroimmunoassay values. The limit of detection for apo C-II and apo C-III electroimmunoassays were 10 ng per 10 µl of assay volume or 1 µg per ml. The apo C-II and C-III assays were unaffected by triglyceride concentration in plasma or lipoprotein fractions when run in the presence of 0.016% Triton X-100. In the presence of Triton X-100, the results obtained for both delipidated and undelipidated samples were within 5%. Samples for each patient were run in the same assay. The intra-assay coefficient of variation for both C-II and C-III was 3%, and the interassay coefficient of variation was 9%.

The total cholesterol and triglyceride concentrations in plasma and lipoprotein fractions were determined enzymatically (Boehringer-Mannheim, Montreal, Quebec). Apo E phenotypes were determined by analytical isoelectric focusing polyacrylamide gel electrophoresis of VLDL apolipoproteins, as described previously.

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-

Figure 2. Changes in very low density lipoprotein (VLDL) components, triglyceride (TG, O—O), apolipoproteins B (●—●), C-II (x—x), and C-III (△—△) after an infusion of heparin (6000 IU over 4 hours). The results are expressed as the percent of their pre-infusion concentrations. The upper panel depicts data from a normal subject (6) and the lower panel, data from a hypertriglyceridemic subject (1).
triglyceridemic subject the VLDL B flux rate exceeded the
flux rate for LDL B.

In the hypertriglyceridemic subjects studied, the 125I-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 125I-IDLapo B specific activity curve.
This was interpreted as indicating that the direct synthesis
of LDL apo B was 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
apo B 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 cessa-
tion 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).
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.

Also, apo C-III declined in VLDL in postheparin-1 (15 ± 2
mg/dl, p<0.01) and postheparin-2 (12 ± 1 vs. 25 ± 4 mg/dl, p<0.01). During the postheparin-1 period,
LDL 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 signifi-
cant 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>Type IV subjects</th>
<th>Preheparin</th>
<th>Postheparin-1</th>
<th>Postheparin-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preheparin</td>
<td>Postheparin-1</td>
<td>Postheparin-2</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>38±9</td>
<td>27±8*</td>
<td>22±7*t</td>
</tr>
<tr>
<td>IDL</td>
<td>5.0</td>
<td>0.1</td>
<td>0.9</td>
</tr>
<tr>
<td>LDL</td>
<td>30±5</td>
<td>31±5</td>
<td>29±4</td>
</tr>
<tr>
<td>Apolipoprotein C-II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>12±2</td>
<td>1.0±0.1</td>
<td>14±2</td>
</tr>
<tr>
<td>HDL</td>
<td>25±4</td>
<td>3±1</td>
<td>31±2</td>
</tr>
<tr>
<td>Apolipoprotein C-III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>14±2</td>
<td>11±1.5+</td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>4.5</td>
<td>4.4</td>
<td>4.5</td>
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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; t-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 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 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

Discussion

The present studies were undertaken to test the hypothesis that in hypertriglyceridemic subjects during rapid VLDL

Figure 4. Changes in apolipoprotein C-III concentrations in plasma (x-x), very low density lipoprotein (VLDL, o-o) (Sf 60 to 400), and high density lipoprotein (HDL, ---) (d = 1.063 to 1.21 g/ml) after an infusion of heparin (9000 IU over 4 hours). Data are expressed as change in mg/dl from pre-infusion values. Top panel shows data from a normal subject (6) and the lower panel, data from a hypertriglyceridemic subject (5).
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.22 Also, we had originally argued23 that the fractional catabolic rate of the C apoproteins, even in hypertriglyceridememic 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 original idea of Berman et al.42 that C apoproteins are cleared from the circulation via HDL. Furthermore, because we observed no change in HDL A-I concentrations, our results suggest that the C apoproteins 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.6 and Tam and Breckenridge7 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.4 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 apoproteins 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 HDL_{2} and HDL_{3}, and abnormal distributions of C-II and C-III in HDL and HDL_{3} in hypertriglyceridemic individuals.41

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-III_{2} shared similar metabolic characteristics.20,21,22 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.6 and Tam and Breckenridge.7 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>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>0.50 ± 0.03*</td>
<td></td>
</tr>
<tr>
<td>0.60 ± 0.02*</td>
<td>0.37 ± 0.03</td>
<td>0.51 ± 0.04*</td>
<td></td>
</tr>
</tbody>
</table>

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 analyses23-26) or that the lipolysis products in these patients are not converted to LDL. Eisenberg et al.30 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.36 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 immunossay 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 studies (average 25% decrease) was considerably less than the apparent losses (up to 90%) we had reported previously.22 The reason for this is not known, although this difference may be related to differences in the metabolic disorders in the two groups of patients; Type V subjects were used previously, whereas in this study Type IV subjects were investigated. The losses may be greater with the former subjects.

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

<table>
<thead>
<tr>
<th>Type IV subjects</th>
<th>Triglyceride</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preheparin</td>
<td>VLDL 419±78</td>
<td>IDL 85±6</td>
</tr>
<tr>
<td>Postheparin-1</td>
<td>LDL 24±2</td>
<td>HDL 15±1</td>
</tr>
<tr>
<td></td>
<td>TG/ VLDL 618±84</td>
<td>CHOL 77±13</td>
</tr>
<tr>
<td>Postheparin-2</td>
<td>VLDL 257±50</td>
<td>IDL 86±6</td>
</tr>
<tr>
<td></td>
<td>LDL 30±3*</td>
<td>HDL 6±1*</td>
</tr>
<tr>
<td></td>
<td>TG/ VLDL 487±80*</td>
<td>CHOL 57±11*</td>
</tr>
<tr>
<td>Normal subjects</td>
<td>VLDL 205±33</td>
<td>IDL 84±6</td>
</tr>
<tr>
<td>Postheparin-1</td>
<td>LDL 32±3*</td>
<td>HDL 7±2*</td>
</tr>
<tr>
<td></td>
<td>TG/ VLDL 391±36*</td>
<td>CHOL 40±6*</td>
</tr>
<tr>
<td>Postheparin-2</td>
<td>VLDL 12±18</td>
<td>IDL 22±4</td>
</tr>
<tr>
<td></td>
<td>LDL 6±63</td>
<td>HDL 6±63</td>
</tr>
<tr>
<td></td>
<td>TG/ VLDL 192±18</td>
<td>CHOL 52±18</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. Bukberg 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 hypertriglyceridemic subjects have an elevated steady-state and postprandial clearance of apo C related to their low HDL concentrations. In addition, we have recently followed a hyperglycemic, insulin-deficient diabetic patient with severe hypertriglyceridemia during an infusion of insulin. The initially high plasma concentrations of C apolipoproteins declined by over 60% within 12 hours of initiating the insulin infusion (Huff MW, Breckenridge WC, Strong WLP, McDonald TJ, unpublished observations).

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.

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References

EFFECT OF HEPARIN INFUSION ON APO C AND B IN MEN  Huff et al.  479


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Metabolism of apolipoproteins C-II, C-III, and B in hypertriglyceridemic men. Changes after heparin-induced lipolysis.

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