# Metabolism of C-apolipoproteins: kinetics of C-II, $C\text{-}III_1$ and $C\text{-}III_2$, and VLDL-apolipoprotein B in normal and hyperlipoproteinemic subjects

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**Abstract** The turnover and metabolism of the individual C apolipoproteins (C-II, $C\text{-}III_1$, and $C\text{-}III_2$) were studied following the injection of $^{125}$I-labeled VLDL into 15 normal and hyperlipoproteinemic subjects. The C apolipoproteins from very low density lipoprotein (VLDL) and high density lipoprotein (HDL) were separated by analytical isoelectric focusing, and subsequent densitometric scanning and radioassay of the stained bands yielded values for specific activity. In 13 of 15 subjects, kinetics of C-II, $C\text{-}III_1$, and $C\text{-}III_2$ were best described by a one-pool model, whereas two subjects showed biexponential kinetics. The specific activity-time curves for VLDL and HDL were superimposable, indicating rapid exchange of all C apolipoproteins in hyperlipidemic as well as in normal subjects. In each subject the half-life was similar for C-II, $C\text{-}III_1$, and $C\text{-}III_2$, which suggests similar synthesis and catabolic mechanisms for each C apolipoprotein. The mass of exchangeable C-II (range 1.0–5.8 mg/kg), $C\text{-}III_1$ (2.6–20 mg/kg), and $C\text{-}III_2$ (2.0–13 mg/kg) increased with plasma triglyceride concentrations. Values for flux of C-II were 1.0–2.8 mg/d per kg, for $C\text{-}III_1$ 1.6–5.6 mg/d per kg, and for $C\text{-}III_2$ 1.2–3.1 mg/d per kg, but they were not related to levels of plasma triglyceride. However the irreversible fractional catabolic rates were inversely related to C apolipoprotein (and triglyceride) mass, suggesting that expansion in C apolipoprotein pool size is related to slower removal, due to the longer residence time of triglyceride-rich VLDL particles in plasma. This was confirmed by similar findings for B apolipoprotein kinetics in VLDL carried out simultaneously.—**Huff, M. W., N. H. Fidge, P. J. Nestel, T. Billington, and B. Watson.** Metabolism of C-apolipoproteins: kinetics of C-II, $C\text{-}III_1$ and $C\text{-}III_2$, and VLDL-apolipoprotein B in normal and hyperlipoproteinemic subjects. *J. Lipid Res.* 1981. **22:** 1235–1246.

**Supplementary key words** VLDL · HDL · isoelectric focusing

The C group of apolipoproteins consists of small molecular weight peptides apoC-I, apoC-II, and apoC-III. Together, the C-apolipoproteins make up approximately 50% and 10% of VLDL and HDL proteins, respectively, and in normal subjects are equally distributed between VLDL and HDL (1, 2).

Of the C group of apolipoproteins, apoC-II has been shown to be a potent activator of lipoprotein lipase (3). Its central role in lipoprotein catabolism has been demonstrated by Breckenridge et al. (4) who reported a subject with apoC-II deficiency and severe hypertriglyceridemia. ApoC-I is thought to be an activator of a specific lipoprotein lipase (5) and to stimulate the activity of lecithin:cholesterol acyltransferase (6), an enzyme which, like lipoprotein lipase, has a crucial role in lipoprotein lipid transport.

Little is known of the function of the C-III group of peptides which contain between zero and two or more sialic acid residues per mole of protein (7), and are denoted as $C\text{-}III_0$, $C\text{-}III_1$, etc. ApoC-III has been reported to inhibit lipoprotein lipase in vitro (8, 9). Shelburne et al. (10) have shown that human apoC-III inhibits the apoE-stimulated uptake of rat lymph chylomicrons in an isolated rat liver perfusion system. However Windler, Chao, and Havel (11) have shown that rat apoC-III, as well as rat apoC-II, is equally effective in inhibiting the hepatic clearance of rat lymph chylomicrons. The role of apoC-III has been more difficult to assess in vivo, although reports suggest that the ratio of apoC-II to apoC-III modulates the catabolism of triglyceride-rich lipoproteins (12, 13). Furthermore, the distribution of C peptides between HDL and triglyceride-rich particles is different in normolipidemic and hypertriglyceridemic subjects (14–16).

Information on the metabolic fate of the C-apolipoproteins is sparse. In vitro studies have shown that C-apolipoproteins from VLDL are rapidly transferred to HDL during the lipolysis of VLDL triglyceride (17). Berman et al. (18) have provided some information about the in vivo kinetics of the C-apolipoproteins as a group, but little is known of the metabolism of the individual C peptides.

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Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; apo, apolipoprotein.

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Because each of the C-apolipoproteins may have an independent function, we have developed a method for measuring their individual kinetics using analytical isoelectric focusing. This study provides details of that method and then compares the metabolism, pool sizes, fractional catabolic rates, and distribution among VLDL and HDL of C-II, C-$III_1$, and C-$III_2$ between normal individuals and subjects with increasing concentrations of plasma triglycerides. Since apolipoprotein B in VLDL remains with the particle throughout its catabolic cycle, the kinetics of VLDL-apo B metabolism were determined simultaneously in the same subjects, using methods developed previously in this laboratory (19, 20). This approach enabled the study of the interrelationships between VLDL-apo B, the individual C-apolipoproteins, and plasma triglyceride.

## METHODS

### Subjects

Fifteen subjects, 13 men and 2 women, were studied; there were 8 normal subjects and 7 with various forms of hyperlipoproteinemia (**Table 1**). Subjects were admitted to the metabolic ward 2 days prior to each study and those who were hypertriglyceridemic were placed on minimal fat intake 48 hr prior to collection of plasma for the labeling of VLDL with $^{125}I$. During the 2 days of the study, all subjects were given this diet as described below. As found previously (19), this practice led to minimal variations in the concentrations of plasma lipids and body weight which did not alter more than 0.5 kg in any subject over the 4-day period. The subjects were given potassium iodide, 180 mg/day in divided oral doses, to reduce the uptake of radioiodide by the thyroid. Informed consent was obtained from all subjects.

### Lipoprotein turnover studies

The procedures used in these studies have been described in detail elsewhere (19). Briefly, VLDL ($S_f$ 20–400) was isolated from fasting subjects by ultracentrifugation at d 1.006 g/ml after removal of any chylomicrons. VLDL was then washed once by flotation through 0.15 M NaCl, pH 7.4, containing 1 mM EDTA and 1 mM Tris buffer. The VLDL was radioiodinated with $^{125}I$ as described previously (19) using a modification of the MacFarlane method (24). Approximately 90–95% of the $^{125}I$ was attached to the protein moiety and labeling efficiency was between 6 and 12%.

The labeled VLDL was sterilized by passage through an 0.45 $\mu$m Millipore™ filter and checked for the absence of pyrogens, and reinjected within 96 hr. The labeled VLDL (45–180 $\mu$Ci) was injected in the morning after an overnight fast. Subsequently, blood samples were collected over 48 hr during which time subjects received a minimal fat diet consisting of 4% fat, 80% carbohydrate, and 16% protein, providing approximately 85% of energy requirements. We have found that this procedure minimizes the contribution of particles of intestinal origin and leads to a relatively constant concentration of total VLDL apolipoproteins (19).

VLDL and HDL were isolated from plasma obtained from each time-course sample after removal of chylomicrons (25). VLDL (d < 1.006 g/ml) and HDL (d 1.063–1.21 g/ml) were washed once by recentrifugation at the appropriate salt densities.

### Separation of the C apolipoproteins from VLDL and HDL

The total soluble VLDL apolipoprotein was isolated by the isopropanol method of Holmquist, Carlson, and Carlson (26); 250-$\mu$l aliquots of VLDL (1.0 mg protein per ml) were transferred to small plastic tubes to which were added equal volumes of isopropanol. The precipitated B apolipoprotein was pelleted by centrifugation and the soluble isopropanol–water phase was extracted with chloroform, methanol, and ether to precipitate soluble proteins and remove the lipid phase as follows. Methanol, 2 volumes, chloroform, 3 volumes, and diethyl ether, 5 volumes were added sequentially and mixed; the tubes were stoppered and chilled for 1 hr. The C apolipoprotein was precipitated by centrifugation (2000 rpm for 2 min), washed with ether, dried under $N_2$, and the pellet was redissolved in 200 $\mu$l of buffer containing 8 M urea in 0.1 M Tris-HCl, pH 8.2, and applied to gels for isoelectric focusing.

The precipitated B protein was apparently free of all soluble proteins when redissolved in 0.1 M SDS and applied to polyacrylamide gels containing SDS. Also, the soluble proteins showed no apolipoprotein B when run on SDS polyacrylamide gels. The apolipoprotein B specific radioactivities were determined as described previously (19).

HDL samples were dialyzed against 0.05 M $NH_4HCO_3$ buffer, pH 8.0, and aliquots containing 2 mg of HDL protein were freeze-dried, redissolved in 200 $\mu$l of the same buffer, and delipidated as described above.

### Separation of C apolipoproteins by isoelectric focusing in polyacrylamide gels

Isoelectric focusing was chosen to separate the VLDL soluble apolipoproteins because the separation of the C-$III_0$ from the C-II apoprotein cannot be

TABLE 1. Clinical and lipid status of subjects studied

| Subject | Lipoprotein Phenotype[a] | Sex | Age | Weight | Plasma | | Lipoproteins[b] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | VLDL | | LDL | HDL |
| | | | | | Triglyceride | Cholesterol | TG[c] | CH[c] | CH | CH |
| | | | *Yr* | *Kg* | *mg/dl* | | | | | |
| 1 | Normal | M | 23 | 72 | 98 | 172 | 67 | 16 | 112 | 44 |
| 2 | Normal | M | 21 | 71 | 101 | 172 | 25 | 17 | 118 | 37 |
| 3 | Normal | M | 20 | 67 | 110 | 170 | 55 | 20 | 115 | 35 |
| 4 | Normal | M | 24 | 76 | 110 | 164 | 38 | 16 | 105 | 43 |
| 5 | Normal | M | 51 | 82 | 166 | 224 | 84 | 22 | 167 | 35 |
| 6 | Normal | M | 44 | 103 | 176 | 152 | 97 | 33 | 89 | 30 |
| 7 | Normal | M | 46 | 92 | 195 | 218 | 46 | 25 | 150 | 40 |
| 8 | Normal | M | 29 | 106 | 198 | 220 | 96 | 27 | 164 | 29 |
| 9 | Hypertriglyceridemia (Type IV) | M | 49 | 65 | 298 | 226 | 169 | 43 | 135 | 48 |
| 10 | Combined hyperlipoproteinemia (Type IIb) | F | 59 | 82 | 490 | 340 | 316 | 104 | 207 | 29 |
| 11 | Hypertriglyceridemia (Type IV) | M | 63 | 85 | 530 | 288 | 360 | 131 | 137 | 20 |
| 12 | Hypertriglyceridemia (Type IV) | M | 56 | 72 | 710 | 273 | 428 | 107 | 141 | 25 |
| 13 | Hypertriglyceridemia (Type IV) | M | 51 | 47 | 980 | 278 | 797 | 187 | 37 | 5.4 |
| 14 | Hypertriglyceridemia (Type V) | F | 51 | 78 | 1030 | 308 | 593 | 131 | 154 | 23 |
| 15 | Hypertriglyceridemia (Type V) | M | 62 | 84 | 1105 | 342 | 697 | 199 | 177 | 26 |

[a] World Health Organization classification (21).
[b] VLDL was isolated by ultracentrifugation and LDL in the infranate was precipitated with $MnCl_2$ (92 mM) and heparin (22). Cholesterol and triglyceride concentrations were determined in the whole plasma, VLDL, and HDL, and calculated in LDL by difference (23).
[c] TG, triglyceride; CH, cholesterol.

accomplished by other conventional electrophoretic systems (27). Polyacrylamide gels (7.5%) containing 6.8 M urea and 2% ampholine (LKB, pH 4–6) were prepared, focused, and stained essentially as described by Swaney and Gidez (28). VLDL apolipoproteins (100 $\mu$l of the delipidated isopropanol-soluble peptides) and HDL apolipoprotein (50 $\mu$l of the delipidated HDL) were added per gel with 20 $\mu$l of a 1:5 dilution of ampholine (with 8 M urea) and 50 $\mu$l of 80% (w/v) sucrose. The gels were scanned at 560 nm in a Pye-Unicam SP 1800 spectrophotometer using a scanning attachment and the scans were quantitated with a Hewlett-Packard model 1201 integrator.

### Determination of C apolipoprotein specific activity

To quantitate C apolipoprotein mass, standard curves were prepared by electrophoresis of C-II, C-$III_1$, and C-$III_2$ apolipoproteins in the 5–40 $\mu$g range. As found previously by others (27), the chromogenicity of C-$III_1$ and C-$III_2$ peptides was identical whereas that for C-II showed an approximate 20% increase in the amount of dye bound per unit of protein. Densitometric response was found to be linear between 5 and 40 $\mu$g of protein.

After scanning the gels, the bands corresponding to C-II, C-$III_0$, C-$III_1$, and C-$III_2$ were sliced and the gel segments were counted in a Packard Autogamma spectrometer. Radioactivity was associated only with stained bands. Samples were run in duplicate and a high degree of accuracy was achieved as follows. The VLDL and HDL samples from a given subject were run simultaneously on a 24-gel electrophoresis apparatus (nine VLDL samples, nine HDL samples, three C-II standards [5, 10, and 20 $\mu$g] and three C-III standards [5, 10, and 20 $\mu$g]). Duplicates were

run at the same time, on an additional group of 24 gels from the same batch. Sufficient apolipoprotein was loaded so that each was within the linear range of the assay. Each gel slice was counted until the error of counting was <1%. Specific activity values were accepted if the duplicates varied by <10%. The intra-assay variation determined from quadruplicates was less than 5%. Specific activity coefficient of variation for the samples containing the lowest acceptable level of radioactivity and lower limit of protein (<5 μg per band) was 3.9% for C-II, 4.5% for C-$III_1$, and 2.7% for C-$III_2$. Specific activities calculated from the 48-hr time-course plasma samples invariably met the above criteria, but at 72 hr and beyond the counting errors exceeded acceptable limits.

### Preparation of C-II and C-III standards

Soluble proteins of apo VLDL were obtained by gel filtration through Sephacryl S-300 (Pharmacia, Sweden) columns (2.5 × 100 cm) (29). The C apolipoprotein peak was dialyzed to remove sodium decyl sulfate and urea, and fractionated by flat bed isoelectric focusing (Pharmacia Preparative IEF kit) on thin layers of Ultradex (LKB, Sweden) in 6 M urea containing Pharmalytes (Pharmacia, pH 2.5–5) (30). The sample mixed with the gel was focused for 16 hr (8W constant power), after which the section of the gel containing the separated C proteins (located by the paper print technique) was refocused (pH 4–5) to obtain better resolution. The proteins were eluted by addition of 6 M urea to liquefy the gel and by titrating to pH 8.9 with a concentrated Tris solution. After centrifugation to remove the granular gel, the concentration of each peptide was determined, free of interference from Pharmalytes, by a modification of the Lowry assay (31). C apolipoproteins purified by the above procedure were identified by pI values (27) and amino acid composition (29).

### Analytical methods

The kinetic analyses required precise determination of injected radioactivity in apoB and each C-apolipoprotein. This was determined for the B-apolipoprotein by adding a small amount (15 μl) of radiolabeled VLDL to 250 μl of unlabeled VLDL (1 mg/ml of VLDL protein) and precipitating the B-protein with an equal volume of isopropanol. The precipitate was washed and counted. The washes were pooled and counted, thus giving the percentage of the injected radioactivity in apoB. A mean of 49 ± 2 (S.E.) % of the protein-bound $^{125}I$ was found to associate with apoB and the remainder with the soluble proteins. The injected dose of each C-apolipoprotein was determined by delipidating approximately 150 μg of unlabeled VLDL (1 mg/ml of VLDL protein) with chloroform–methanol–ether. The delipidated material was dissolved in 200 μl of 8 M urea, 0.01 M Tris, pH 8.0, to which approximately 5 μl of the injected $^{125}I$-labeled VLDL preparation was added, and the mixture was applied to an analytical isoelectric focusing gel. The bands containing C-II, C-$III_0$, C-$III_1$, and C-$III_2$ were sliced from the stained gel and their radioactivity was determined to provide the percentage of each C peptide in the injected dose. C-$III_0$ was not sufficiently labeled.

After isoelectric focusing, each stained C-apolipoprotein band was associated with a peak of radioactivity and areas between bands were found to have negligible radioactivity, indicating the identity of the iodinated C apolipoproteins with the native peptides. Furthermore, the amount of radioactivity in the top 2-mm segment of the gel (after correcting for the amount of lipid labeling) corresponded exactly to the percent of radioactivity in apoB determined by the isopropanol–water precipitation technique.

Protein content was determined by the method of Lowry et al. (32). Plasma cholesterol and triglyceride concentrations were determined in whole plasma by enzymatic methods adapted for a Technicon II Autoanalyzer (23).

### Kinetic analyses

There was rapid isotopic equilibration between corresponding C-apolipoproteins within VLDL and HDL (**Fig. 1**), indicating that the various apoC pools within the plasma may be considered homogeneous for the purposes of kinetic analysis; LDL contained negligible radioactivity. Semilogarithmic plots of specific activity versus time were fitted to a mono- and biexponential function and the curve parameters were calculated by computer, utilizing a non-linear least squares technique. The Fisher F statistic was used to test the appropriateness of a one-pool model rather than a two-pool model. In 13 of 15 subjects, the addition of a second pool did not result in significant reduction of the residual mean squares. The kinetic parameters for total mass, fractional catabolic rate, and flux through the pool were calculated as detailed below. In subjects 2 and 3, each C-apolipoprotein was best described by a two-pool model; addition of a third pool did not result in a significantly better fit. Kinetic parameters calculated from the biexponential curves yielded values for flux of material through pool 1, the irreversible fractional catabolic rate from pool 1, and the mass in pool 1. It is recognized that studies were not carried out beyond 48 hr due to insufficient radioactivity in samples obtained at 72 hr and

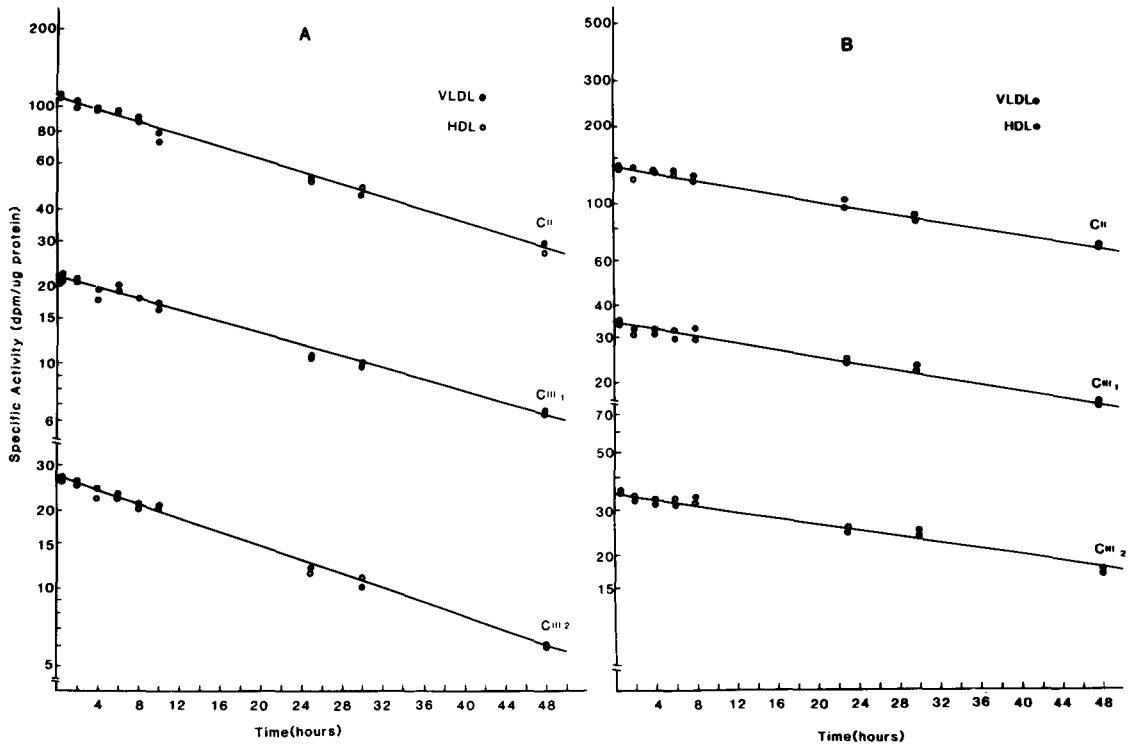


**Fig. 1.** The specific activity-time curves of $^{125}$I-labeled apolipoproteins C-II, C-III$_1$, and C-III$_2$ in VLDL (d < 1.006 g/ml) and HDL (d 1.063–1.21 g/ml). The curves exhibit monoexponential decay. Note that the Y-axis is broken and in fact the C-III$_1$ and C-III$_2$ curves are almost superimposable. The curves in A are from subject 6 (normal) and the curves in B are from subject 12 (Type IV).

beyond, and that a more complex pattern of removal has not been excluded.

The B-apolipoprotein kinetics in VLDL for subjects 1–8 could be clearly resolved into two pools as described previously (19) and kinetic parameters were calculated accordingly. In subjects 9–14, VLDL-B apolipoprotein kinetics conformed to a one-pool model and kinetic parameters were calculated as described for the C-apolipoproteins. In the two-pool model, the rapidly equilibrating, larger pool is envisaged either as exchanging with a smaller extravascular compartment or representing two populations with dissimilar fractional turnover rates. In the one-pool model the entire pool is envisaged as being intravascular.

### Analysis of kinetic data

The one-pool model, reflecting the monoexponential specific activity-time curve was analyzed by conventional techniques (33). Fractional removal rate (FCR) = 0.693/t½; pool size = Injected dose/Specific activity at $t_0$; flux = FCR × pool size. This method of analysis was applied to all VLDL-B and C-apolipoprotein studies in which monoexponential removal occurred.

The two-pool model of analysis of Gurpide, Mann, and Sandberg (34) was used for apoC studies in subjects 2 and 3 and for apoB studies in subjects 1–9 in whom removal occurred biexponentially. The model describes the relationship between pools 1 and 2 and allows for independent entry and exit from both pools.

Since there are no definitive data in man on the magnitude of apolipoprotein C production or catabolism in different organs, we have to allow that both pools 1 and 2 may contain tissues that participate both in synthesis and removal. However, we have assumed that direct input into pool 2 and irreversible loss from pool 2 are likely to be far less than from pool 1 which includes plasma, since in the majority of studies only a single pool (plasma) applied.

The transport of material through pool 1, representing the influx of apolipoproteins is given by the equation: Flux = $R_1.\alpha.\beta/\beta.C_1 + \alpha.C_2$ and equals transport through pool 1, other than material recycled from pool 2. $R_1$ = dose of radioactivity reinjected into pool 1; $\alpha$ and $\beta$ are the rate constants of the exponentials describing the biexponential specific radioactivity-time curve of B- or C-apolipoprotein. $C_1$ and $C_2$ are the intercepts of the two rate constants $\alpha$ and $\beta$ on the Y-axis of the specific radioactivity-time curve.

The mass of B- or C-apolipoprotein in pool 1 ($M_1$)

can be derived from the equation: $M_1 = R_1/C_1 + C_2$. The fractional removal rate from pool 1 is an index of total removal, including that of material leaving pool 1 irreversibly ($K_{0,1}$) plus material that is transferred from pool 1 to pool 2 ($K_{2,1}$). It is calculated as $-(\alpha.C_1 + \beta.C_2)/C_1 + C_2$. The removal rate of material irreversibly lost from pool 1 ($K_{0,1}$) was calculated as follows: $K_{0,1} = \alpha.\beta/K_{1,2}$; $K_{1,2} = \alpha + \beta + (K_{0,1} + K_{2,1})$; $K_{2,1}$ = total removal rate $- K_{0,1}$.

Comparisons that include kinetic parameters derived both from a two-pool model and a one-pool model pose difficulties. However, since the mass of VLDL B-protein in pool 1 from the two-pool analysis accounts for about 90% of the total mass (19), it was combined with values for total mass from one-pool analyses. The irreversible fractional removal rate is comparable to the fractional removal rate of the whole system obtained by one-pool analysis. By making the same assumption of negligible catabolism from pool 2, the flux (or transport) through a two-pool system is analogous to flux through a one-pool system. Therefore, for the purposes of the present study we have combined kinetic parameters derived from studies analyzed by the one- and two-pool models.

## RESULTS

Separation of the C-peptides associated with plasma VLDL or HDL is shown in **Fig. 2.** Despite the relatively larger load of HDL apoprotein, the method

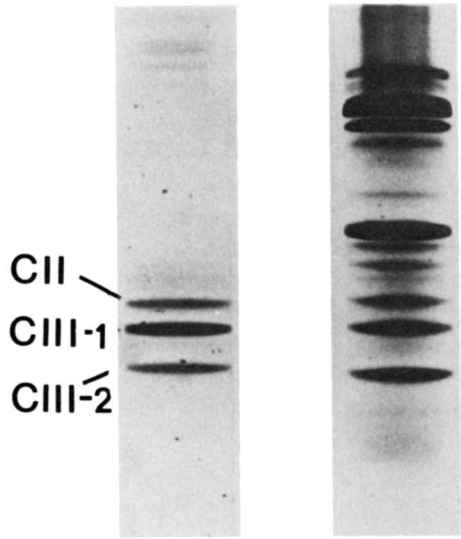


**Fig. 2.** Comparison of the separation of C-apolipoproteins of VLDL (left) and HDL (right) by isoelectric focusing on polyacrylamide gels (pH 4–6 range) as described in Methods. Peptides of both lipoproteins migrate to same position as C-apolipoprotein standards.

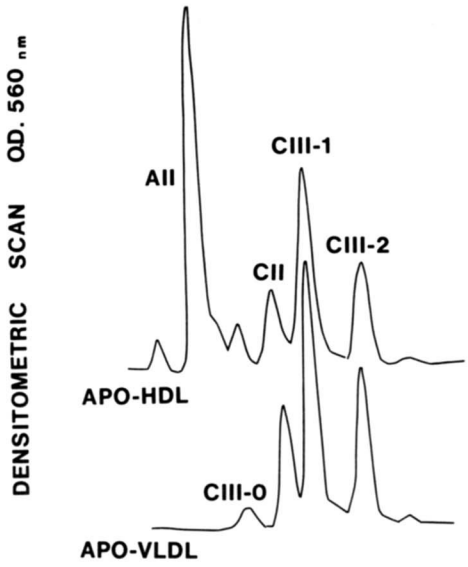


**Fig. 3.** Densitometric scan of C-apolipoprotein region of isoelectric focusing gels (polyacrylamide gel) of VLDL (bottom) and HDL (top). Proteins were stained with Coomassie blue and scanned at 560 nm as described in text.

provided excellent resolution of C-apolipoproteins and the migration in HDL samples corresponded closely with that in VLDL. A representative profile of scanned gels is shown in **Fig. 3** which again illustrates the exact correspondence in position of C peptides from both lipoproteins.

In 13 of the 15 subjects, the specific activity-time curves, resolved by computer, were monoexponential for apoC-II, C-$III_1$, and C-$III_2$ (Fig. 1) over the first 48 hr. In five normal subjects and five hyperlipidemic subjects, specific activity-time curves for apoC-II, C-$III_1$, and C-$III_2$ in HDL were also determined. For each subject and for each C-apolipoprotein, the corresponding specific activities in VLDL and HDL were similar at each time point resulting in similar fractional catabolic rates (e.g., for C-II, VLDL 0.022 $\pm$ 0.003 $hr^{-1}$ (mean $\pm$ SE) and HDL 0.023 $\pm$ 0.003 $hr^{-1}$; similar results were observed for C-$III_1$ and C-$III_2$). This rapid equilibration of radioactivity in the injected C-apolipoproteins between VLDL and HDL was unaffected by plasma triglyceride concentration and allowed us to consider the C-apolipoproteins in VLDL and HDL as one kinetic pool.

Kinetic parameters of C-II, C-$III_1$, and C-$III_2$ are listed in **Table 2.** The mass of apoC-II ranged from 1.0 to 2.6 mg/kg in normal subjects; in subjects with hypertriglyceridemia it was significantly higher (3.8–5.8 mg/kg; $P < 0.001$). The mean fractional catabolic rate (FCR), the fraction of the pool of apoC-II removed irreversibly per hr, was significantly higher in normals (0.029 $\pm$ 0.002 $hr^{-1}$) relative to those subjects

TABLE 2. Kinetic parameters of C-apolipoprotein metabolism[a]

| Subject | Plasma Triglyceride | C-II | | | | $C\text{-}III_1$ | | | | $C\text{-}III_2$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mass | t½ | FCR | Flux | Mass | t½ | FCR | Flux | Mass | t½ | FCR | Flux |
| | *mg/dl* | *mg/kg* | *hr* | $hr^{-1}$ | *mg/day/kg* | *mg/kg* | *hr* | $hr^{-1}$ | *mg/day/kg* | *mg/kg* | *hr* | $hr^{-1}$ | *mg/day/kg* |
| 1 | 98 | 1.0 | 17 | 0.04 | 1.0 | 2.6 | 17 | 0.04 | 2.1 | 1.9 | 26 | 0.027 | 1.2 |
| 2 | 101 | 1.2 | 21 | 0.033 | 1.0 | 2.6 | 22 | 0.031 | 1.9 | 2.8 | 21 | 0.033 | 2.4 |
| 3 | 110 | 1.7 | 24 | 0.029 | 1.2 | 4.5 | 24 | 0.029 | 3.2 | 4.3 | 22 | 0.031 | 3.2 |
| 4 | 110 | 1.9 | 30 | 0.023 | 1.0 | 3.3 | 36 | 0.019 | 1.6 | 3.2 | 32 | 0.022 | 1.7 |
| 5 | 166 | 2.0 | 22 | 0.032 | 1.5 | 6.4 | 27 | 0.026 | 4.0 | 4.4 | 29 | 0.024 | 2.5 |
| 6 | 176 | 1.9 | 24 | 0.028 | 1.3 | 6.1 | 26 | 0.027 | 3.0 | 4.9 | 21 | 0.033 | 3.8 |
| 7 | 195 | 2.6 | 27 | 0.026 | 1.7 | 6.7 | 29 | 0.024 | 3.9 | 4.6 | 29 | 0.024 | 2.7 |
| 8 | 198 | 2.4 | 39 | 0.018 | 1.0 | 3.5 | 43 | 0.016 | 1.3 | 3.4 | 41 | 0.017 | 1.4 |
| $\bar{x}$ | 144 | 1.8 | 26 | 0.029 | 1.2 | 4.5 | 28 | 0.027 | 2.6 | 3.7 | 28 | 0.026 | 2.4 |
| ± SE | 15 | 0.2 | 2.4 | 0.002 | 0.096 | 0.6 | 3.0 | 0.003 | 0.36 | 0.36 | 2.4 | 0.002 | 0.32 |
| 9 | 298 | 4.4 | 50 | 0.014 | 1.5 | 7.7 | 50 | 0.014 | 2.6 | 7.5 | 43 | 0.016 | 2.9 |
| 10 | 490 | 3.9 | 46 | 0.015 | 1.3 | 8.1 | 58 | 0.012 | 2.3 | 5.3 | 50 | 0.014 | 1.8 |
| 11 | 530 | 5.8 | 46 | 0.015 | 2.1 | 14.3 | 50 | 0.015 | 5.2 | 8.1 | 50 | 0.014 | 2.8 |
| 12 | 710 | 5.8 | 38 | 0.018 | 2.6 | 12.6 | 38 | 0.018 | 5.6 | 6.4 | 40 | 0.017 | 2.6 |
| 13 | 980 | 5.6 | 62 | 0.010 | 1.3 | 19.0 | 62 | 0.010 | 4.6 | 11.0 | 62 | 0.010 | 2.6 |
| 14 | 1030 | 4.5 | 58 | 0.012 | 1.3 | 6.6 | 53 | 0.013 | 2.1 | 6.2 | 41 | 0.017 | 2.5 |
| 15 | 1105 | 3.8 | 87 | 0.008 | 0.7 | 20.3 | 87 | 0.008 | 3.9 | 13.2 | 87 | 0.008 | 2.5 |
| $\bar{x}$ | 734 | 4.8 | 55 | 0.013 | 1.5 | 12.7 | 57 | 0.013 | 3.7 | 8.2 | 53 | 0.014 | 2.5 |
| ± SE | 117[b] | 0.32[b] | 6[b] | 0.001[b] | 0.2 | 2.1[c] | 5.7[b] | 0.0012[b] | 0.6 | 1.1[b] | 6.2[c] | 0.002[b] | 0.13 |
| % Change relative to normals | | +166 | +111 | −55 | +25 | +182 | +103 | −51 | +42 | +130 | +89 | −46 | +4.1 |

[a] Calculations based on a one-pool model for all subjects (33), except for subjects 2 and 3 whose data best fitted a two-pool model (34). For subjects 2 and 3, mass refers to mass of pool 1; FCR, irreversible fractional catabolic rate; and flux of material (production and removal) through pool 1. Data are based on VLDL apolipoprotein C specific activity-time curves. In 10 of 15 subjects HDL apoC specific activity-time curves were also determined and the kinetic parameters calculated from these curves were similar to the data derived from the VLDL curves. Thus the C-apolipoproteins in VLDL and HDL can be considered as one kinetic pool.

[b] Different from normal subjects, $P < 0.001$.

[c] Different from normal subjects, $P < 0.005$.

with elevated plasma triglycerides ($0.013 \pm 0.001$ $hr^{-1}$; $P < 0.001$). The parameters for mass and FCR of $C\text{-}III_1$ and $C\text{-}III_2$ showed a similar trend. Compared to the normal subjects, in individuals with hypertriglyceridemia the mean mass of $apoC\text{-}III_1$ and of $apoC\text{-}III_2$ was significantly higher ($P < 0.005$ for both) and mean FCRs were significantly lower ($P < 0.001$ for both). Thus, the mass for each of apoC-II, $C\text{-}III_1$, and $C\text{-}III_2$ was positively correlated with the level of plasma triglyceride ($r = 0.78$, 0.82, and 0.83, respectively, $P < 0.001$ for each) whereas the FCR for each C-apolipoprotein was negatively correlated with plasma triglyceride concentration ($r = -0.81$, $-0.75$ and $-0.77$, respectively, $P < 0.001$ for each).

The flux through the pool of apoC-II was found to be between 0.7 and 2.6 (mg/day per kg) for all subjects and showed no relationship with plasma triglyceride concentration ($r = 0.11$). Fluxes for $apoC\text{-}III_1$ and $C\text{-}III_2$ ranged from 1.3 to 5.6 and from 1.2 to 3.8 (mg/day per kg), respectively, and also showed no correlation with triglyceride concentration ($r = 0.42$ and 0.13).

The average half-lives (t½) of apoC-II, $C\text{-}III_1$, and $C\text{-}III_2$ were similar in normal subjects (26, 28, and 28 hr, respectively) and in the hypertriglyceridemic subjects (55, 57, and 53 hr, respectively), although the values were significantly higher with hypertriglyceridemia ($P < 0.005$, Table 2). The FCRs for apoC-II in normal subjects were slightly higher than those for $apoC\text{-}III_1$ when analyzed by a paired *t*-test ($P < 0.05$). However no difference was found between FCRs for these two apolipoproteins in hypertriglyceridemic subjects or between FCRs for apoC-II and $apoC\text{-}III_2$ in normal or hyperlipidemic subjects. The percent increments in the mass and percent reductions in FCR that accompanied hypertriglyceridemia were similar for each C-apolipoprotein (Table 2).

The total mass of apoC calculated from the kinetic data, and the percentage distribution of C-II, $C\text{-}III_1$, and $C\text{-}III_2$ are listed in **Table 3** and averaged 20, 46, and 35%, respectively. The ratios of mass of C-II/$C\text{-}III_1$ (Table 3) were variable (range 0.19–0.69) but were unrelated to plasma triglyceride levels. The mass ratios of C-II/$C\text{-}III_1$, measured from the areas of the scanned analytical isoelectric focusing gels were found to be similar for VLDL and HDL in normal subjects

TABLE 3. Percent distribution of C-apolipoproteins and the calculated plasma concentration of apoC-II

| Subject | Mass of Total ApoC[a] | % | | | Mass Ratio C-II/C-III$_1$ | | |
|---|---|---|---|---|---|---|---|
| | | C-II | C-III$_1$ | C-III$_2$ | Total[b] | VLDL[c] | HDL[c] |
| | *mg* | | | | | | |
| Normal 1–8 ($\bar{x}$ ± SE) | 855 ± 126 | 19 ± 1.3 | 44 ± 1.6 | 37 ± 1.1 | 0.44 ± 0.05 | 0.39 ± 0.05 | 0.41 ± 0.05 |
| Hypertriglyceridemic 9–15 ($\bar{x}$ ± SE) | 1861 ± 253[d] | 20 ± 2.0 | 48 ± 2.5 | 32 ± 1.8 | 0.44 ± 0.06 | 0.42 ± 0.025 | 0.29 ± 0.025 |
| Total ($\bar{x}$ ± SE) | 1325 ± 188 | 20 ± 1.2 | 46 ± 1.5 | 35 ± 1.2 | 0.44 ± 0.04 | 0.40 ± 0.029 | 0.35 ± 0.034 |

[a] Sum of the kinetically defined mass of apoC-II, C-III$_1$, and C-III$_2$.
[b] Ratio of the kinetically defined mass of C-II and C-III$_1$.
[c] Calculated from the ratio of the scanned areas of C-II and C-III$_1$ from isoelectric focusing gels.
[d] Significantly different from normal subjects $P < 0.01$.

and resembled the ratios derived from the kinetic analyses. However, in hypertriglyceridemic individuals the C-II/C-III$_1$ ratio, obtained from the gels, was higher in VLDL than in HDL ($P < 0.01$). The kinetically determined ratio was similar to that in VLDL, reflecting the greater proportion of C-apolipoprotein in VLDL in these subjects. The plasma concentration of apoC-II was established by dividing the total mass of C-II (from the kinetic analysis, Table 2) by the plasma volume (assumed to be 4.5% of body weight), and was found to be 4.2 ± 0.5 mg/dl (mean ± SE) for normals and 10.7 ± 0.7 mg/dl for hypertriglyceridemic individuals.

VLDL apolipoprotein-B kinetics were also determined in each subject (**Table 4**). The kinetics of subjects 1–9 were best described by a two-pool model

TABLE 4. Comparison of kinetic parameters for apoC-II[a] and VLDL apoB[b] with plasma triglyceride concentrations

| Subject | Plasma Triglyceride | Mass[c] | | Irreversible Fractional Catabolic Rate[d] | | Flux[e] | |
|---|---|---|---|---|---|---|---|
| | | C-II | VLDL-B | C-II | VLDL-B | C-II | VLDL-B |
| | *mg/dl* | *mg/kg* | | *hr*$^{-1}$ | | *mg/day/kg* | |
| 1 | 98 | 1.0 | 3.4 | 0.040 | 0.258 | 1.0 | 21 |
| 2 | 101 | 1.2 | 3.2 | 0.033 | 0.195 | 1.0 | 15 |
| 3 | 110 | 1.7 | 4.2 | 0.029 | 0.266 | 1.2 | 27 |
| 4 | 110 | 1.9 | 2.6 | 0.023 | 0.234 | 1.0 | 12 |
| 5 | 166 | 2.0 | 2.6 | 0.032 | 0.200 | 1.5 | 18 |
| 6 | 176 | 1.9 | 2.8 | 0.028 | 0.261 | 1.3 | 23 |
| 7 | 195 | 2.6 | 5.8 | 0.026 | 0.169 | 1.7 | 17 |
| 8 | 198 | 2.4 | 5.1 | 0.018 | 0.126 | 1.0 | 16 |
| $\bar{x}$ ± S.E. | 144 ± 15 | 1.8 ± 0.02 | 3.7 ± 0.42 | 0.029 ± 0.003 | 0.214 ± 0.02 | 1.2 ± 0.1 | 19 ± 1.7 |
| 9 | 298 | 4.4 | 7.4 | 0.014 | 0.083 | 1.5 | 14 |
| 10 | 490 | 3.9 | 19.3 | 0.015 | 0.058 | 1.3 | 27 |
| 11 | 530 | 5.8 | 18.0 | 0.015 | 0.058 | 2.1 | 31 |
| 12 | 710 | 5.8 | 23.0 | 0.018 | 0.058 | 2.6 | 26 |
| 13 | 980 | 5.6 | 26.8 | 0.010 | 0.048 | 1.3 | 30.6 |
| 14 | 1030 | 4.5 | 20.3 | 0.012 | 0.053 | 1.3 | 20 |
| 15 | 1105 | 3.8 | 21.4 | 0.008 | 0.039 | 0.7 | 37 |
| $\bar{x}$ ± S.E. | 734 ± 117 | 4.8 ± 0.32[f] | 19.5 ± 2.3[f] | 0.013 ± 0.002[f] | 0.057 ± 0.005[f] | 1.6 ± 0.2 | 27 ± 2.7[g] |
| Correlation coefficient ($r$) | | 0.88, $P < 0.001$ | | 0.87, $P < 0.001$ | | 0.12, N.S. | |

[a] Calculation based on a one-pool model (33) for all subjects except 2 and 3, where a two-pool model was used (34).
[b] Calculation based on a two-pool model for subjects 1–9 (34) and a one-pool model for subjects 10–15 (33).
[c] In subjects where a two-pool model was used values for mass refer to mass of pool 1.
[d] In subjects where a two-pool model was used values refer to irreversible fraction of pool 1 removed per hr.
[e] In subjects where a two-pool model was used values refer to flux through pool 1.
[f] Significantly different from normal subjects $P < 0.001$.
[g] Significantly different from normal subjects $P < 0.01$.

whereas the kinetics of hypertriglyceridemic subjects 10–14 were best described by a one-pool model. As in previous studies from this laboratory (19, 20), the mass of VLDL-apoB (range 2.6–27 mg/kg) was positively correlated with the level of plasma triglyceride ($r = 0.95$; $P < 0.001$). The irreversible FCR was higher in normal subjects ($0.214 \pm 0.02$ hr$^{-1}$) relative to hypertriglyceridemic subjects ($0.057 \pm 0.005$; $P < 0.001$) and showed a negative correlation with plasma triglyceride concentration ($r = -0.83$; $P < 0.001$). The flux of VLDL-apoB through pool 1 was significantly higher in individuals with elevated plasma triglycerides ($27 \pm 2.7$ mg/day per kg, $P < 0.01$) than in normal subjects ($19 \pm 1.7$ mg/day per kg).

A comparison of the kinetic parameters of apoC-II and VLDL-apoB determined simultaneously in each subject is also shown in Table 4. The rationale for the comparison of values derived from a one-pool model (most of the apoC studies) and values from a two-pool model (VLDL-apoB in subjects 1–9) was discussed in the Methods section. The significantly elevated mass of both total apoC-II and VLDL-apoB in hypertriglyceridemic subjects (Table 4) is illustrated by the strong positive correlation between the amounts of these two apoproteins ($r = 0.88$; $P < 0.001$). The concomitant decrease of apoC-II FCR and the FCR of VLDL-apoB with increasing plasma triglyceride is also shown in Table 4 ($r = 0.87$; $P < 0.001$). There was no relationship between the flux of apoC-II and VLDL-apoB.

## DISCUSSION

The need to study the kinetics of individual C-apolipoproteins is essential to enable a full understanding of the role of each peptide in lipoprotein metabolism. This study demonstrates for the first time the use of analytical isoelectric focusing to determine the specific radioactivity and subsequent kinetic parameters of the individual C-apolipoproteins in both VLDL and HDL.

Redistribution of radiolabeled C-apolipoproteins as a group between VLDL and HDL has been described in vitro (17, 35, 36) and in vivo (18, 37–39). In contrast to these experiments, which consider only the transfer of radioactivity, this study clearly shows rapid equilibration of radiolabeled apoC-II, C-III$_1$, and C-III$_2$ giving virtually identical specific activities in the corresponding apolipoproteins of VLDL and HDL (Fig. 1) in both normal and hypertriglyceridemic individuals. This is despite the substantial difference in distribution of C-apolipoproteins in hypertriglyceridemic subjects that results in more than 80% of the plasma C-apolipoproteins being found in the VLDL density range (15, 16). Thus, in both normal and hypertriglyceridemic subjects, C-apolipoproteins undergo very rapid exchange between VLDL and HDL which appears to be distinct from the mass distribution of these peptides. It is now generally accepted that as chylomicrons and VLDL are catabolized by the hydrolysis of triglyceride, the C-apolipoproteins are then transferred back to newly secreted triglyceride-rich lipoproteins.

The predominantly monoexponential decline in VLDL and HDL C-apolipoprotein specific radioactivities indicates metabolism within a homogeneous pool. Since the amount of apoC-II that we have calculated to reside in the equilibrated pool is similar to that measured directly within the plasma by specific radioimmunoassay by Schonfeld et al. (15) and by Kashyap et al. (16), it is likely that the C-apolipoproteins are largely confined to plasma and pools that may readily exchange with plasma, such as the lymphatics. The biexponential decline observed in two subjects might indicate slower equilibration between C-peptides in plasma lipoproteins and those in extravascular spaces such as lymphatics. Alternatively this might represent the catabolism of a more heterogeneous population of particles in these subjects.

Our findings differ from those reported by Berman et al. (18) who developed a multicompartmental model for the metabolism of C-apolipoproteins. However, measurements of only total C-apolipoprotein radioactivity were made in their studies which showed, in addition to an apparently biexponential removal rate, a recycling of radioactivity 8–12 hr after reinjection which we have not observed. Our value for the half-life of each C-peptide was 28 hr in normal subjects compared to 10–18 hr reported by Berman et al. (18). This may be related to the fact that virtually no fat was eaten during our studies (thus reducing the production of lymphatic chylomicrons and the transfer of plasma C-apolipoproteins to these newly secreted triglyceride-rich particles).

The present experiments also demonstrate for the first time the similarity in metabolism for the three major C-apolipoproteins. The similar fractional catabolic rates for C-II, C-III$_1$, and C-III$_2$ in normal and hypertriglyceridemic subjects suggest that they have similar removal mechanisms. It also implies that they have related functions. To date only the role of apoC-II has been established (3, 4), although the recent report by Shelburne et al. (10) suggests that C-III$_1$ might prevent the premature removal of chylomicron remnants from the plasma. Thus the C-II and C-III$_1$ peptides might act in unison, the former initiating the hydrolysis of triglyceride and the latter ensuring that the particle remains in the circulation until the

required amount of triglyceride has been removed. The precise functions of C-$III_0$ and C-$III_2$ are as yet unknown.

A small but significant difference was seen in normal subjects between FCRs for apoC-II and apoC-$III_1$. However, since this difference did not exist in hypertriglyceridemic subjects nor between the FCRs for apoC-II and apoC-$III_2$, we concluded that the above difference probably had little or no physiological significance.

The data in Table 4 confirm that in both normal and hypertriglyceridemic subjects the rate of removal of VLDL-B is much faster than that of VLDL apoC (18). Since apoB remains with the VLDL particle during catabolism, the higher apoC-II specific activity (longer plasma residence time) clearly indicates that during delipidation apoC-II is transferred either directly or via HDL to newly secreted VLDL. It is therefore likely that catabolism of the C and VLDL-B apolipoproteins is controlled by different mechanisms.

When the irreversible fractional catabolic rates per hour for the C-apolipoproteins and for VLDL-B protein determined in this study are compared with those for HDL-apoA-I and apoA-II determined in previous studies from this laboratory (40), it is evident that those for the C-apolipoproteins are intermediate, in normal subjects (0.029 $hr^{-1}$) between values for VLDL-B (0.214) and HDL-A-I (0.012) and A-II (0.012). At the same time, while the fractional removal rates for the C-apolipoproteins are closer to those for HDL apolipoproteins A-I and A-II, the values still appear too high to suggest that all C-apolipoprotein catabolism is mediated through HDL. It is therefore possible that the C-apolipoproteins may be removed with both VLDL and HDL particles.

Since the C-apolipoproteins do not remain with the VLDL particle during its conversion to LDL, some catabolism of apoC may occur through the direct catabolism of VLDL. This "shunt" pathway has been described for VLDL apoB in hypertriglyceridemic individuals (19) and shown to be operative to a lesser extent in normal subjects (41). VLDL-B can be degraded in a variety of cultured cells (42, 43) and a previous study from this laboratory has demonstrated uptake and degradation of both VLDL apoB and apoC by cultured human lymphocytes (44).

If, on the other hand, C-apolipoproteins were removed predominantly within HDL, it would be necessary to postulate a faster rate of removal of the C peptides from HDL than of A-apolipoproteins (45). Alternatively, the C-apolipoproteins may have a higher affinity for a subclass of HDL particle with a faster catabolic rate (1). Subclasses of HDL separated by ultracentrifugation show no major differences in apolipoprotein composition or in vivo metabolism (45). However, studies of HDL subclasses by ion-exchange chromatography have demonstrated that whereas all three subfractions contain apoA-I and A-II, the C-apolipoproteins are confined mainly to one fraction (46). It is therefore possible that this subfraction is removed at a faster rate than the remainder of the HDL particles. We have carried out one study (data not shown) in a subject with hyperalphalipoproteinemia in whom HDL accounted for approximately three-quarters of the plasma apoC concentration. This was the only study in which the removal rates of each C peptide were slower in HDL (FCR = 0.016 $hr^{-1}$) than in VLDL (FCR = 0.021) suggesting that C-apolipoprotein catabolism occurred mainly via HDL.

The kinetic analyses clearly demonstrate that subjects with hypertriglyceridemia have increased amounts of each C-apolipoprotein, C-II, C-$III_1$, and C-$III_2$. This is in agreement with the conclusions of others (15, 16) based only on plasma concentrations determined directly by radioimmunoassay. This is, however, the first demonstration of decreased fractional removal from the expanded pools and an absence of an increase in C-apolipoprotein flux (production and catabolism) with increasing plasma triglyceride. Thus the fractional removal rate of the C-apolipoproteins is related to triglyceride concentration and the expansion in their pool size may stem from the prolonged residence time of triglyceride-rich VLDL particles that is characteristic of hypertriglyceridemia (19, 20). Interestingly, all three major C-apolipoproteins are similarly affected.

The simultaneous determination of VLDL apoB kinetics has allowed a direct comparison of both C and B apolipoprotein kinetics with plasma triglyceride concentrations. We have confirmed the findings of previous studies from this laboratory (19, 20) and elsewhere (41, 47, 48) that apoB flux is increased and the fractional catabolic rate decreased in hypertriglyceridemia (Table 4). This suggests that C-apolipoprotein metabolism is not as tightly coupled to triglyceride metabolism as is VLDL apoB. This conclusion is substantiated by the fact that while the mass for both total apoC and VLDL-apoB is increased and fractional removal rates are reduced in hypertriglyceridemia, the flux (formation and total removal) of VLDL-B, but not of apoC, is increased.

In the present study, the mass ratio of total apoC-II/apoC-$III_1$ or the relative percentages of C-II, C-$III_1$, and C-$III_2$ were not significantly different between normal and hypertriglyceridemic subjects (Table 3). This is consistent with the data of Schonfeld et al. (15) who also failed to find any difference between normal and hypertriglyceridemic subjects in the

ratio of plasma C-II/C-III, determined in this case by radioimmunoassay. This point remains controversial, as other reports have suggested that the plasma ratio of apoC-II/C-III may be significantly lower in hypertriglyceridemic subjects and that this may be a factor in the development of hypertriglyceridemia (12, 13, 49).

The precise physiological significance of an altered C-II/C-III$_1$ ratio is not clear, as rat lymph chylomicrons with low C-II/C-III$_1$ ratios have been found to inhibit the activity of solubilized lipoprotein lipase but had no effect on the endothelium-bound enzyme in the perfused rat heart (50). It should however be noted that our study, as well as that of Schonfeld et al. (15), has shown that whereas the C-II/C-III$_1$ ratio may be similar in VLDL and HDL in normal individuals, it is lower in HDL than in VLDL in hypertriglyceridemic subjects (Table 3). It is possible that apoC-II, C-III$_1$, and C-III$_2$ have different affinities for the triglyceride-rich lipoproteins and HDL, although, as shown in our studies, this did not affect the rates of exchange between the lipoproteins nor influence their fractional catabolic rates. Thus our data suggest that apoC-II, C-III$_1$, and C-III$_2$ are metabolized as a group and are removed from circulation by a common mechanism in hypertriglyceridemic as well as in normal subjects.

This project was supported by the National Heart Foundation of Australia and the National Health and Medical Research Council of Australia. The technical assistance of Robyn Paynter, Sandra Menson, Patricia Astwood, and Margaret O'Connor is gratefully acknowledged.

*Manuscript received 5 September 1980, in revised form 13 February 1981, and in re-revised form 29 June 1981.*

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