

Use of Intralipid for kinetic analysis of HDL apoC-III: evidence for a homogeneous kinetic pool of apoC-III in plasma

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Abstract Apolipoprotein C-III (apoC-III) is an important regulator of lipoprotein metabolism. Radioisotope and stable isotope kinetic studies show differing results in relation to the kinetics of apoC-III in HDL. Kinetic analysis of HDL apoC-III may be difficult because of its low concentration, as well as the presence of other apoproteins at higher concentration, in the HDL fraction. We used Intralipid® (IL), known to preferentially extract apoC proteins from plasma, as a means of extracting apoC-III from HDL before apoprotein separation by isoelectric focusing gel electrophoresis for the measurement of tracer enrichment. Protein purity was assessed by an isoleucine-to-leucine (Ile/Leu) ratio, as apoC-III contains no isoleucine. We compared apoC-III kinetics in 14 men using a bolus infusion of deuterated leucine. The Ile/Leu ratio for IL-extracted HDL (IL-HDL) apoC-III ($3.0 \pm 0.7\%$) was not different from that of VLDL apoC-III ($2.6 \pm 0.6\%$) but was significantly lower than that of untreated HDL apoC-III ($9.0 \pm 2.9\%$) ($P < 0.001$). The isotopic enrichment curves and fractional catabolic rates (FCRs) for IL-HDL apoC-III were not different from those of VLDL apoC-III. In contrast, HDL apoC-III had significantly lower isotopic enrichments and FCRs than IL-HDL apoC-III ($P < 0.001$). In conclusion, this simple IL method can be used to isolate apoC-III from HDL with minimal interference from other HDL apoproteins, and it demonstrates that the kinetics of apoC-III in VLDL and HDL are similar, supporting the concept of a single kinetically homogeneous pool of apoC-III in plasma.—Nguyen, M. N., D. C. Chan, K. P. Dwyer, P. Bolitho, G. F. Watts, and P. H. R. Barrett. Use of Intralipid for kinetic analysis of HDL apoC-III: evidence for a homogeneous kinetic pool of apoC-III in plasma. *J. Lipid Res.* 2006. 47: 1274–1280.

Supplementary key words high density lipoprotein • very low density lipoprotein • gas chromatography-mass spectrometry • lipoprotein metabolism • apolipoprotein C-III

Apolipoprotein C-III (apoC-III) is an important regulator of lipoprotein metabolism, in particular, of triglycer-

ide-rich lipoproteins (TRLs) and their remnants. ApoC-III is an 8.8 kDa glycoprotein synthesized by the liver and the small intestine. The majority of apoC-III is associated with TRLs in hypertriglyceridemic subjects; in normolipidemic subjects, the majority is associated with HDLs. ApoC-III is a specific, noncompetitive inhibitor of lipoprotein lipase (1, 2) and also impairs the hepatic uptake of TRLs by the low density lipoprotein receptor (3, 4). Plasma apoC-III is strongly correlated with plasma triglyceride levels (5, 6). Increased apoC-III may cause the accumulation of TRLs in plasma, which is strongly associated with hypertriglyceridemia and the progression of coronary artery disease (7–9).

Compared with TRLs, little is known of the role of apoC-III in HDL metabolism. There is evidence that HDL apoC-III concentration is directly correlated with the concentrations of HDL cholesterol and HDL apoA-I and is inversely correlated with apoA-I catabolism, the major apoprotein of HDL (10, 11).

Although there are three isoforms of apoC-III in human plasma (12), recent isotopic tracer studies have focused on the major isoform, apoC-III₁. Previous studies have shown that apoC-III₁ and apoC-III₂, which account for >90% of plasma apoC-III concentration (6, 13), have similar kinetics in both VLDL and HDL (14, 15). Early kinetics studies using radiolabeled apoC-III demonstrated rapid exchange and equilibration of apoC-III between TRL and HDL fractions in both normal and hyperlipidemic subjects (14, 16–18). Other radioisotope studies have suggested non-equilibrating pools of apoC-III that do not exchange between VLDL and HDL (19–21). Recent endogenous stable isotope tracer studies have also provided evidence of kinetically distinct pools of VLDL and HDL apoC-III (11, 22).

Abbreviations: apoC-III, apolipoprotein C-III; d₃-leucine, deuterated leucine; FCR, fractional catabolic rate; IEF, isoelectric focusing; IL, Intralipid®; Ile/Leu, isoleucine-to-leucine; PR, production rate; PVDF, polyvinylidene difluoride; TRL, triglyceride-rich lipoprotein; TTR, tracer-to-tracee ratio.

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Unlike VLDL, the isolation of apoC-III from HDL is problematic, owing to the low concentration of apoC-III in HDL, together with the presence of other apoproteins at higher concentration in the HDL fraction. Contamination of apoC-III with other proteins may confound the measurement of HDL apoC-III enrichment and hence the determination of kinetic parameters. Thus, the discrepancies in the findings, particularly those observed in stable isotope studies, might be related to methodological limitations of the isolation of apoC-III from HDL.

Astrup and Bengtsson (23) used Intralipid® (IL) to preferentially extract apoC proteins from plasma. Therefore, we investigated whether IL could be used to isolate apoC-III from HDL free of contamination by other proteins. We measured and compared deuterated leucine (d_3 -leucine) enrichment in apoC-III₁ isolated from VLDL and HDL, with and without IL extraction, before isoelectric focusing (IEF) gel electrophoresis. We hypothesized that apoC-III kinetics are similar in both VLDL and HDL, thereby implying the rapid and complete exchange of apoC-III between the VLDL and HDL fractions.

METHODS

Subjects and study protocols

We studied 14 nonsmoking men selected from the community with body mass index ranging from 18 to 47 kg/m². The subject characteristics were (means ± SD): age, 56.5 ± 8.2 years; body mass index, 31.7 ± 7.1 kg/m²; triglyceride, 1.7 ± 1.1 mmol/l; total cholesterol, 5.7 ± 0.5 mmol/l; HDL cholesterol, 1.2 ± 0.5 mmol/l; apoA-I, 127 ± 23 mg/dl; apoC-III, 16.5 ± 3 mg/dl; apoB-100, 115 ± 23 mg/dl. None of the subjects were taking agents known to affect lipid metabolism. After an overnight fast, a bolus of d_3 -leucine (5 mg/kg) was administered intravenously, and blood samples were taken at 0, 0.33, 0.5, 0.67, 1, 2, 3, 4, 6, 8, 10, 24, 48, 72, and 96 h postinjection (24). Plasma was separated from whole blood at 1,000 *g* for 10 min at 4°C and stored at -80°C until analysis. This study was approved by the Ethics Committee of Royal Perth Hospital.

Isolation of lipoproteins and apolipoproteins

Three milliliters of plasma was used for the isolation of 1 ml VLDL (<1.006 kg/l) and 1 ml HDL (1.063–1.21 kg/l) fractions by sequential ultracentrifugation at 40,000 rpm in a Ti 50.4 rotor (Optima LE-80K; Beckman Coulter). HDL fractions were dialyzed to remove salt and preincubated with cysteamine (β -mercaptoethylamine hydrochloride; Sigma) to separate apoA-II from apoC-III (25). ApoA-II comigrates with apoC-III on IEF (26); however, after treatment with cysteamine, which introduces an amino group to its single cysteine residue (27), apoA-II shifts to a higher position on the gel. VLDL (200 μ l) or HDL (100 μ l) from each time point was delipidated with equal volumes of isopropanol and *n*-pentanol (28). The sample was mixed thoroughly and centrifuged for 5 min at 425 *g*. The lower phase was collected and dried in a centrifugal evaporator at room temperature. The dried sample was then reconstituted in 50 ml of sample buffer [8 M urea and 0.001% (w/v) bromophenol blue]. ApoC-III was isolated by preparative IEF gel electrophoresis (8 M urea, 7.5% acrylamide, 1.5% ampholytes, pH 4–6; 16 h; 200 V; 4°C) (29, 30). Gels were electroblotted onto polyvinylidene difluoride (PVDF) membranes (Immobilon; Millipore) at 700 mA for 1 h using a Hoefer

TE 42 transfer unit (Amersham Biosciences) and stained with Coomassie Brilliant Blue R 250.

Measurement of d_3 -leucine enrichment in apoC-III₁

IEF resolved apoC-III into three distinct isoforms: apoC-III₀, apoC-III₁, and apoC-III₂ (13, 31). Because of its greater concentration in plasma (6), and observations from previous studies that the kinetics of apoC-III isoforms were similar, apoC-III₁ was investigated in this study. Note that all references to apoC-III kinetics will correspond to the kinetics of apoC-III₁, unless specified otherwise. The apoC-III₁ protein bands were excised from the PVDF membranes and hydrolyzed in 200 μ l of 6 M HCl overnight at 110°C in pyrolysis-cleaned half-dram vials. Each sample was then dried at 110°C and derivatized using a modified oxazolinone method (300 μ l of cyclohexane was substituted for 500 μ l of benzene) (32). The oxazolinone derivatives were analyzed by negative ion chemical ionization GC-MS. The isotopic enrichment was determined as the tracer-to-tracee ratio (TTR) of monitored selected ions at mass-to-charge ratios of 212 (derived from d_3 -leucine) and 209 (derived from unlabeled leucine). PVDF blanks were also excised from the blots and analyzed by GC-MS, and the isoleucine-to-leucine (Ile/Leu) ratio was measured (means ± SEM).

IL extraction of HDL apoC-III

One milliliter of sodium chloride solution (1.006 kg/l) was added to 1 ml of HDL from each time point. Twenty percent IL emulsion (20% triglyceride, 1.2% phospholipid, and 2.2% glycerol; Fresenius Kabi AB) was diluted to 1% using saline solution, and 1 ml was added to each tube. The suspensions were mixed by inversion and ultracentrifuged for 24 h at 40,000 rpm. After centrifugation, the IL layer (0.5 ml) was aspirated into 15 ml disposable polypropylene tubes (Sarstedt). Ten milliliters of acetone-ethanol (1:1) solution was then added to each sample, mixed at 4°C for 30 min, and delipidated overnight at -20°C (33). The resulting protein precipitates were pelleted by centrifugation for 10 min at 284 *g* (-10°C), and the acetone-ethanol supernatant was aspirated to waste. The delipidated samples were reconstituted in 100 μ l of sample buffer, and the apoproteins were separated by IEF gel electrophoresis as described above.

Serial IL extractions of HDL apoC-III

To assess the effectiveness of IL extraction of apoC-III, we performed serial extractions using 1% IL solution. Three successive IL extractions were performed on HDL samples (96 h time series) from eight subjects using 1% IL solution. After the IL layer was collected, the infranatant was carefully aspirated down to 1 ml using a peristaltic pump and the excess IL was removed from the side of the tube with a cotton swab. The pelleted HDL was resuspended, and the IL extraction procedure was then repeated twice using the same HDL sample. The IL samples were then analyzed as described above. Ten microliter aliquots of the baseline HDL samples were also taken before and after the first, second, and third successive IL extractions and stored at -80°C for quantitation of apoC-III by electroimmunodiffusion. This experiment was also repeated with a 10% IL solution. In contrast to the 1% IL solution, the 10% IL solution is very lipemic and thus difficult to work with in the laboratory.

Reproducibility of the IL method

The reproducibility of the IL extraction method was determined by measuring the TTR, in triplicate, from serial plasma samples (96 h) from three patients. The mean and coefficient of variation for each time point was calculated from the TTR. The

average coefficient of variation for the IL extraction method is $5.4 \pm 0.4\%$. This coefficient of variation represents all laboratory steps, starting from plasma through the measurement of isotopic enrichment.

Effect of apoC-III concentration on the measurement of enrichment

To assess the effect of apoC-III concentration on the measurement of enrichment, apoC-III was isolated from a tracer-enriched plasma sample by extraction using IL. ApoC-III obtained at 6 h postinjection of d_3 -leucine was serially diluted (1, 0.5, and 0.25) and then separated by IEF gel electrophoresis. The enrichment of each apoC-III sample was analyzed by GC-MS, keeping the abundance of leucine loaded constant ($\sim 1.0E6$), and the Ile/Leu ratio was measured (mean \pm SEM).

Quantitation of HDL apoC-III mass by electroimmunodiffusion

ApoC-III mass in VLDL and HDL was quantitated from plasma using a Hydrigel LP CIII Electroimmunodiffusion kit (Sebia) with appropriate standards and quality controls according to the manufacturer's instructions (34). The height of the "rockets" was measured, and the apoC-III concentrations were determined from a standard curve. This method was also used to determine the concentration of apoC-III remaining on the HDL particles after successive IL extractions.

Model of apoC-III metabolism and calculation of kinetic parameters

A model of apoC-III metabolism was developed using the SAAM II program (Resource Facility for Kinetic Analysis, University of Washington, Seattle), with the same four-compartment leucine subsystem (compartments 1–4) used in the apoB model described previously (24). In this study, a single compartment was used to account for the plasma kinetics of apoC-III (compartment 6). A single compartment was also used to account for the intrahepatic delay associated with the synthesis and secretion of apoC-III (compartment 5). This compartment model (Fig. 1) was fit to the apoC-III TTR data to derive the fractional catabolic rate (FCRs) for apoC-III in VLDL, HDL, and IL-extracted HDL (IL-HDL). The production rates (PRs) for apoC-III in VLDL, HDL, and IL-HDL were derived as the product of the FCR and apoC-III concentration data.

Statistical analyses

Paired *t*-test (SPSS 11.5; SPSS, Inc.) was used to assess the differences between the mean values, and statistical significance

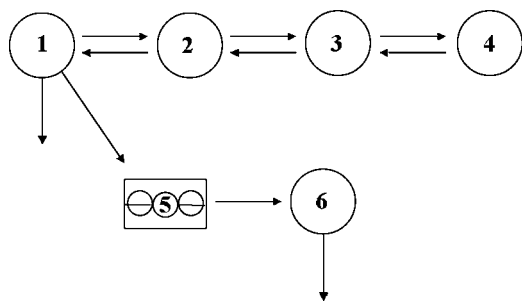


Fig. 1. Compartment model describing apolipoprotein C-III (apoC-III) tracer kinetics. Compartments 1–4 represent the plasma leucine subsystem, compartment 5 represents the intrahepatic delay compartment, and compartment 6 represents the plasma kinetics of VLDL or HDL apoC-III.

was defined at the 5% level using a two-tailed test. Mixed model analysis (SAS Proc Mixed; SAS Institute) was used for comparison of the FCRs derived from the serial IL extractions of HDL apoC-III.

RESULTS

Figure 2 shows the IEF separation of apolipoproteins of VLDL (gel A), HDL (gel B), and IL-HDL (gel C). ApoC-II and the three isoforms of apoC-III were found in the IL emulsion after extraction from HDL. By contrast, apoA-I and apoA-II were not present in the IL-HDL fraction. ApoC-III₁ was isolated from VLDL, HDL, and IL-HDL for each subject over the 96 h time course. As apoC-III contains no isoleucine, we used the Ile/Leu ratio to assess the purity of the apoC-III sample. The PVDF blank was used to account for the background isoleucine accumulated during the entire isolation and derivatization procedure. The Ile/Leu ratio of the PVDF blank was $27.0 \pm 1.0\%$, with a total leucine abundance of $\sim 10\%$ relative to the undiluted apoC-III sample; therefore, the background isoleucine of the procedure would be $\sim 2.7\%$. The Ile/Leu ratio of apoC-III₁ for VLDL of $2.6 \pm 0.6\%$ was not different from that of $3.0 \pm 0.7\%$ for IL-HDL (**Table 1**). In comparison, the Ile/Leu ratio for apoC-III₁ isolated from untreated HDL was significantly higher, $9.0 \pm 2.9\%$, compared with those for VLDL and IL-HDL ($P < 0.001$ for both). The Ile/Leu ratios were not different between obese and nonobese subjects.

Figure 3A shows the apoC-III electroimmunodiffusion rockets after sequential extractions with a 1% IL solution. The initial untreated HDL apoC-III rocket is shown in lane a. After a single extraction with the IL solution, $\sim 57\%$ of apoC-III remained in the HDL fraction (lane b). This decreased to $\sim 25\%$ after the second IL treatment and finally to $< 10\%$ after the third IL treatment

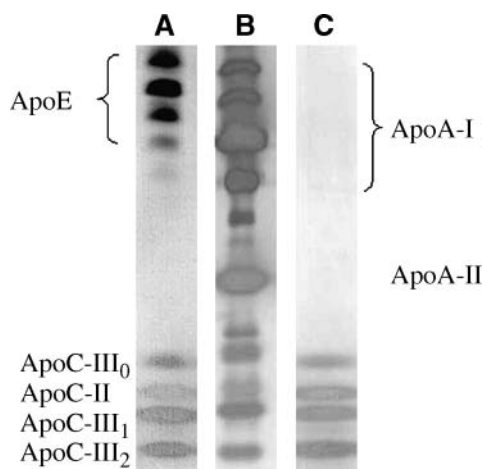


Fig. 2. Isoelectric focusing (IEF) gel electrophoretic analysis of VLDL (A), HDL (B), and Intralipid® (IL)-extracted HDL (C). Samples were subjected to IEF gel electrophoresis, electroblotted onto PVDF membranes, and stained with Coomassie blue, as described in Methods. Note that apoA-II has shifted on the HDL IEF gel (B) as a result of the cysteamine treatment.

TABLE 1. Ile/Leu ratio, apoC-III FCR, concentration, and PR for VLDL, HDL, and IL-HDL fractions (n = 14)

Fraction	Ile/Leu Ratio	FCR	Concentration	PR
	%	pools/day	mg/dl	mg/kg/day
VLDL	2.6 ± 0.6	0.73 ± 0.11	7.2 ± 3.3	2.34 ± 1.07
apoC-III				
HDL	9.0 ± 2.9 ^a	0.62 ± 0.13 ^a	8.1 ± 1.1	2.22 ± 0.47
apoC-III				
IL-HDL	3.0 ± 0.7 ^b	0.74 ± 0.11 ^b	8.1 ± 1.1	2.68 ± 0.47 ^b
apoC-III				

apoC-III, apolipoprotein C-III; FCR, fractional catabolic rate; IL, Intralipid®; Ile/Leu, isoleucine-to-leucine; PR, production rate.

^aStatistical difference compared with VLDL apoC-III ($P < 0.001$).

^bStatistical difference compared with HDL apoC-III ($P < 0.001$).

(lanes c and d, respectively). Figure 3B–D shows the composition of apoproteins associated with the IL fraction after one, two, and three sequential IL extractions. The apoC proteins are predominantly transferred to IL after the first treatment (Fig. 3B). After the second treatment, however, apoA-I was observed in the IL fraction (Fig. 3C), and after the third IL treatment, a substantial quantity of apoA and other HDL apoproteins were associated with the IL fraction (Fig. 3D). This experiment was repeated with a 10% IL solution and showed that ~90% of the apoC-III was removed with a single extraction (data not shown); however, this fraction contained some apoA, similar to that after the third 1% IL extraction (Fig. 3D). Only trace amounts of apoC-III remained after the second extraction using the 10% IL.

The IL-HDL apoC-III isotopic enrichment curves after the sequential IL extractions (Fig. 4) show that the apoC-III₁ enrichments were similar for each extraction. Ile/Leu ratios for the first, second, and third extractions were $2.8 \pm 1.4\%$, $2.7 \pm 1.9\%$, and $2.5 \pm 2.9\%$, respectively. The ratios of the FCRs for apoC-III in the first, second, and third IL extractions were 1.00, 1.00, and 1.01, respectively, and were not different from each other ($P = 0.998$). Thus,

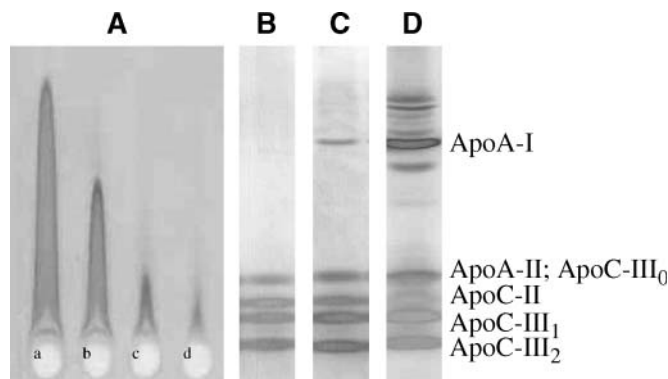


Fig. 3. A: ApoC-III electroimmunodiffusion rockets before (lane a) and after the first (lane b), second (lane c), and third (lane d) successive extractions using 1% IL solution. B–D: IEF gel electrophoretic analysis of the first (B), second (C), and third (D) 1% IL extract. Note that the apoA-II band coincides with the apoC-III₀ band, because the HDL sample was not treated with cysteamine in this procedure.

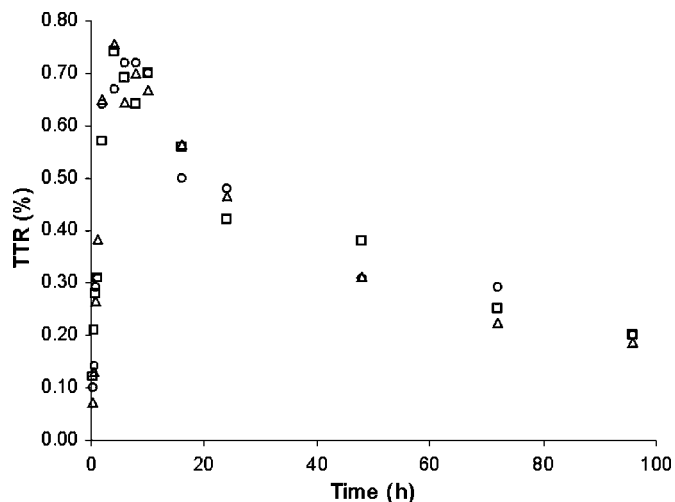


Fig. 4. Isotopic enrichment of apoC-III₁ with deuterated leucine (d_3 -leucine) isolated from HDL after one (triangles), two (squares), and three (circles) successive extractions with 1% IL, expressed as tracer-to-tracee ratio (TTR), up to 96 h after a bolus injection of d_3 -leucine (4 mg/kg).

a single 1% IL extraction of HDL is sufficient for the extraction and analysis of apoC-III enrichment in the HDL fraction.

Figure 5 shows the d_3 -leucine enrichment of apoC-III₁ isolated from VLDL, HDL, and IL-HDL over the 96 h time course in two obese and two lean subjects. Figure 5A, B show representative enrichment curves for two obese subjects. The mean Ile/Leu ratios for these four subjects for VLDL and IL-HDL were $2.5 \pm 1.7\%$ and $3.2 \pm 1.5\%$, respectively. By contrast, the mean Ile/Leu ratio for HDL apoC-III₁ was $11.6 \pm 6.1\%$; furthermore, the shape of the HDL apoC-III₁ enrichment curve was different from that of IL-HDL apoC-III₁. More important, however, is the observation that the VLDL and IL-HDL apoC-III₁ tracer curves were superimposable. This result was independent of whether the plasma samples were frozen or fresh (data not shown). The FCRs and PRs for apoC-III₁ (n = 14) calculated for VLDL, HDL, and IL-HDL are shown in Table 1. The FCR for VLDL apoC-III was significantly different from that for HDL apoC-III ($P < 0.001$) but not from that for IL-HDL apoC-III ($P = 0.421$) and was independent of body mass index. VLDL and HDL apoC-III concentrations were not significantly different ($P = 0.265$). IL-HDL apoC-III PR was significantly higher than HDL apoC-III PR ($P < 0.001$) but not different from VLDL apoC-III PR, as a result of the greater variability of this parameter, which is a function of the variability of VLDL apoC-III concentration.

Figure 6 shows the relationship between apoC-III Ile/Leu ratio and TTR. Serial dilutions of apoC-III were associated with significantly higher Ile/Leu ratios and lower TTRs ($P < 0.001$). This observation was independent of the abundance of leucine on GC-MS. Furthermore, the variability of the measurement variable increased with the dilution of the apoC-III sample.

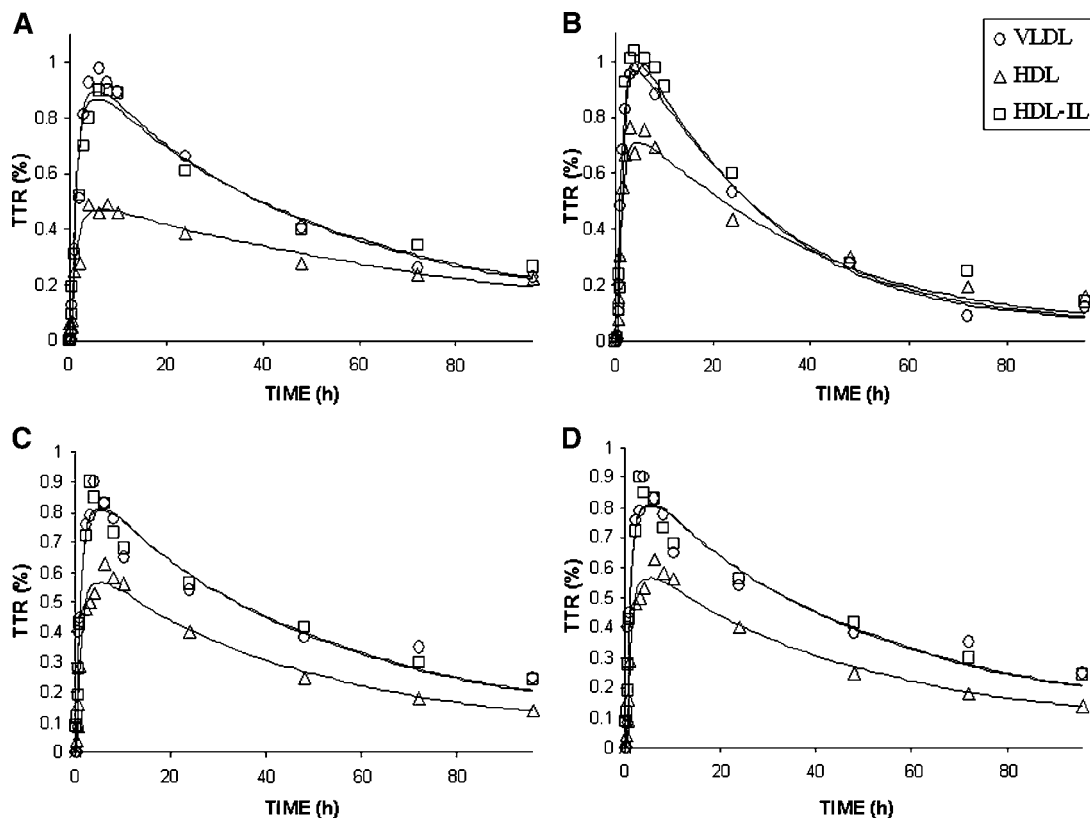


Fig. 5. Isotopic enrichment of apoC-III₁ with d₃-leucine of VLDL (circles), HDL (triangles), and IL-HDL (squares), expressed as TTR, up to 96 h after a single bolus injection of d₃-leucine (4 mg/kg). A, B show representative enrichment curves of obese subjects, and C, D show representative enrichment curves of lean subjects.

DISCUSSION

We describe a simple method that uses IL to isolate HDL apoC-III for kinetic analysis. With the Ile/Leu ratio as a criterion for purity, we demonstrated that IL extraction significantly improves the isolation of apoC-III from HDL compared with previous methods. Using this IL

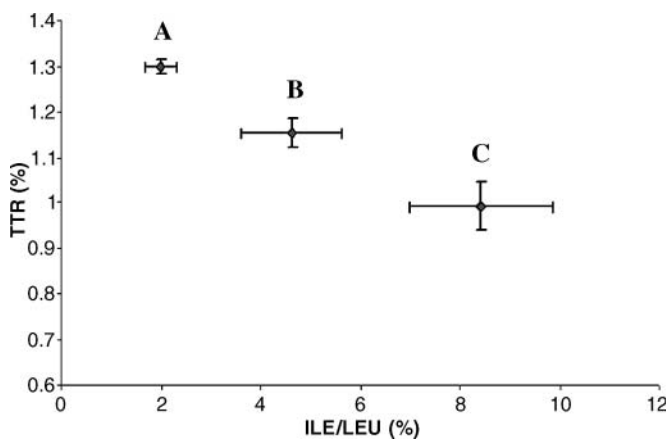


Fig. 6. Relationship between isoleucine-to-leucine (Ile/Leu) ratio and TTR in apoC-III isolated from d₃-leucine-enriched plasma. Data points A, B, and C represent serially diluted concentrations (1, 0.5, and 0.25, respectively) of apoC-III. Error bars represent SEM.

method, we provide new evidence that, contrary to previous isotopic tracer studies, apoC-III in VLDL and HDL has similar enrichment curves. This kinetic finding implies that apoC-III in VLDL and HDL has similar kinetics, supporting the complete and rapid exchange of apoC-III between these lipoproteins.

Previous studies have used preparative IEF to separate the apolipoproteins in VLDL and HDL. We demonstrated that HDL apoC-III isolated without IL had a significantly lower FCR compared with VLDL apoC-III, in accord with several recently conducted stable isotope studies (11, 22), although differences in FCRs reported in these studies were greater than we observed. These studies, however, were conducted using a primed constant infusion of d₃-leucine under the condition of constant feeding and may have produced different metabolic parameters for apoC-III kinetics, owing to different protocols and subject populations. The divergence of the VLDL and HDL apoC-III enrichment curves suggested that there are kinetically distinct pools of apoC-III. Our data for VLDL apoC-III FCR were comparable to these stable isotope studies (22, 35, 36) and also to data from radioisotope studies (14, 37). However, the significantly higher Ile/Leu ratio in HDL apoC-III, compared with VLDL apoC-III, led us to speculate that our measurement of HDL apoC-III enrichment could be inaccurate, owing to amino acid or protein contamination.

We used IL extraction before IEF protein separation to isolate apoC-III from HDL free of other protein contamination. There are two possible sources of contamination: the irreducible background amino acids accumulated during the isolation procedure, and proteins in the sample that are unresolved on the gel. To overcome the irreducible background and acquire sufficient apoC-III for analysis, overloading with HDL would lead to poor resolution of the HDL apoproteins by IEF compared with VLDL (Fig. 2). Some HDL apoproteins, such as isoforms of apoA-II (12% isoleucine), may comigrate with apoC-III isoforms (26). Although cysteamine is used to achieve a single charge shift of apoA-II, its isoforms, which are lower on the gel, may still coincide with apoC-III protein bands. The IL extraction method can be used to preferentially isolate apoC proteins from HDL in sufficient amounts before IEF and thus overcome these problems. The Ile/Leu ratio of IL-extracted apoC-III was significantly lower than the HDL apoC-III isolated by previous methods and was comparable to that of VLDL apoC-III. All of the isoleucine present in VLDL apoC-III ($2.6 \pm 0.6\%$) can be accounted for by the background isoleucine accumulated during the isolation procedures, as seen in the PVDF blank (2.7%). More importantly, we have demonstrated that not only does the Ile/Leu ratio increase with less apoC-III being loaded onto the IEF gel, but that this is associated with lower measures of enrichment.

As with previous studies (38), we used a 1% IL solution for the extraction of apoC from plasma. Although a single extraction using 1% IL does not remove all apoC-III, the enrichment curves resulting from subsequent extractions are not different, nor are the calculated apoC-III FCRs. Thus, we have provided evidence that the apoC-III present on HDL is kinetically homogeneous, and furthermore, that only a single 1% IL extraction is required for kinetic analysis of apoC-III in the HDL fraction. Moreover, a 10% IL solution will remove >90% of HDL apoC-III, as well as other apoproteins, within minutes, demonstrating that the apoC-III pool is readily exchangeable.

Using IL for the isolation of apoC-III from HDL, the FCR of IL-HDL apoC-III was similar to that of VLDL apoC-III, but it was significantly higher than that of untreated HDL apoC-III. As a consequence, IL-HDL apoC-III PR was also significantly higher than untreated HDL apoC-III PR. Although there may be potential inaccuracies associated with the measurement of apoC-III concentration as a result of ultracentrifugation, our measures of HDL apoC-III concentration were from plasma using an electroimmunodiffusion method (34). Some apoC-III may have been lost from HDL during ultracentrifugation; however, the kinetic characteristics of the apoC-III that remained, even after three successive IL extractions, were not different from those observed in the VLDL fraction. We did not examine apoC-III₀ or apoC-III₂ kinetics, because apoC-III₁ is the major isoform (6, 13), and previous studies have shown that the apoC-III isoforms have similar FCRs (14, 15).

This study not only provides an improved method for the isolation of HDL apoC-III for kinetic analysis but also provides new evidence supporting the complete and rapid

equilibration of apoC-III between the VLDL and HDL fractions. The similarity of the enrichment curves and FCRs of apoC-III in VLDL and HDL is consistent with previous radioisotope studies (14–16), which showed superimposable specific activity-time curves. Other radioisotope studies showed parallel specific activity-time curves, which suggested that although VLDL and HDL apoC-III FCRs are similar, differences in specific activity suggested the presence of a nonexchangeable pool of apoC-III (19, 21). These studies, however, used exogenously labeled VLDL and HDL, which may not uniformly label all of the apoC-III present on the lipoprotein particles. Furthermore, the differences between the VLDL and HDL specific activity-time curves were small and may represent limitations in the measurement of apoC-III mass or in apoC-III isolation.

In conclusion, this novel IL method allows for the isolation of apoC-III from HDL free of other protein contamination. Furthermore, we have demonstrated that VLDL and HDL apoC-III have similar tracer curves, thus providing evidence for the existence of a kinetically homogeneous pool of apoC-III, which rapidly and completely exchanges between these lipoproteins. This is consistent with previous radioisotope studies that found that apoC-III is exchangeable and that past discrepancies may be methodological. We found similar results for the obese, non-obese, and lean men in our study population, and we recently published associations between apoC-III and other covariates in a similar population (39). Although our kinetics analysis of VLDL and HDL apoC-III was performed in the fasted state, based on evidence of rapid exchange of apoC-III, we anticipate similar results in the fed state; however, this requires further investigation. Further studies of HDL apoC-III using our IL technique would be informative. ■

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