Familial HDL Deficiency Characterized by Hypercatabolism of Mature ApoA-I but Not ProApoA-I

Rami Batal, Michel Tremblay, Larbi Krimbou, Orval Mamer, Jean Davignon, Jacques Genest, Jr, Jeffrey S. Cohn

Abstract—We have previously described patients with familial high density lipoprotein (HDL) deficiency (FHD) having a marked reduction in the plasma concentration of HDL cholesterol and apolipoprotein (apo) A-I but lacking clinical manifestations of Tangier disease or evidence of other known causes of HDL deficiency. To determine whether FHD in these individuals was associated with impaired HDL production or increased HDL catabolism, we investigated the kinetics of plasma apoA-I and apoA-II in two related FHD patients (plasma apoA-I, 17 and 37 mg/dL) and four control subjects (apoA-I, 126±18 mg/dL, mean±SD) by using a primed constant infusion of deuterated leucine. Kinetic analysis of plasma apolipoprotein enrichment curves demonstrated that mature plasma apoA-I production rates (PRs) were similar in patients and control subjects (7.9 and 9.1 versus 10.5±1.7 mg · kg⁻¹ · d⁻¹). Residence times (RTs) of mature apoA-I were, however, significantly less in FHD patients (0.79 and 1.66 days) compared with controls (5.32±1.05 days). Essentially normal levels of plasma proapoA-I (the precursor protein of apoA-I) in FHD patients were associated with normal plasma proapoA-I PRs (7.8 and 10.4 versus 10.9±2.6 mg · kg⁻¹ · d⁻¹) and proapoA-I RTs (0.18 and 0.15 versus 0.16±0.03 day). The RTs of apoA-II were, however, less in patients (3.17 and 2.92 days) than control subjects (7.24±0.71 days), whereas the PRs of apoA-II were similar (1.8 and 1.9 versus 1.7±0.2 mg · kg⁻¹ · d⁻¹). Increased plasma catabolism of apoA-II in FHD patients was associated with the presence in plasma of abnormal apoA-II–HDL (without apoA-I). These results demonstrate that FHD in our patients is characterized, like Tangier disease, by hypercatabolism of mature apoA-I and apoA-II, but unlike Tangier disease, by essentially normal plasma catabolism and concentration of proapoA-I. (Arterioscler Thromb Vasc Biol. 1998;18:655-664.)

Key Words: cholesterol ■ kinetics ■ atherosclerosis ■ reverse cholesterol transport ■ hypoalphalipoproteinemia

Epidemiological studies have consistently demonstrated that low plasma HDL levels are associated with the presence of coronary artery disease.¹² This association has been attributed to a role of HDL in mediating reverse cholesterol transport.³ HDL has other potentially antiatherogenic functions, however, such as inhibiting LDL oxidation,⁴ reducing the expression of endothelial cell adhesion molecules,⁵ and inhibiting platelet aggregation.⁶

ApoA-I and apoA-II are the major structural proteins of HDL.³ ApoA-I (28 kDa) is synthesized in the liver and intestine as a pre-propeptide.⁷ It is processed cotranslationally to form proapoA-I, which is secreted into the circulation. A metalloenzyme rapidly cleaves six amino acids from its amino terminus, leading to the formation of mature apoA-I (243 amino acids),⁸ which represents the majority (>95%) of total plasma apoA-I. ApoA-I is believed to be cleared from the circulation in an almost lipid-free form by glomerular filtration and tubular reabsorption in the kidney.⁹ ApoA-II (17 kDa) is synthesized predominantly by the liver¹⁰ and is secreted in plasma either as a propeptide, which is readily cleaved, or directly as mature apoA-II.¹¹ ApoA-II is found in association with apoA-I in the plasma of normolipidemic subjects (on particles designated LpA-I:A-II), whereas apoA-I is also present in particles not containing apoA-II (LpA-I).¹² Kinetic studies in humans have shown that the variation in plasma apoA-I level is determined by the FCR of apoA-I,¹³,¹⁴ whereas the variation in plasma apoA-II levels, as well as the distribution of apoA-I between LpA-I and LpA-I:A-II particles,¹⁵ is determined by the rate of production of apoA-II. At the same time, FCRs of both apoA-I and apoA-II have been found to be inversely correlated with HDL cholesterol levels.¹⁶

Human HDL deficiency (hypoalphalipoproteinemia) defines a group of dyslipidemias characterized by an HDL cholesterol level below the 10th percentile for age- and sex-matched subjects.¹⁷ HDL deficiency has been shown to be the result of: (1) apoA-I gene abnormalities caused by deletion, inversion, insertion, nonsense, or missense mutations; (2) apoA-II deficiency; (3) complete or partial absence of LCAT activity, as seen in classic LCAT deficiency or fish-eye disease; (4) increase in cholesteryl ester transfer protein activity; or (5) severe hypertriglyceridemia (reviewed

Received August 7, 1997; revision accepted December 11, 1997.
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in References 18 and 19). HDL deficiency is also a major characteristic of Tangier disease, wherein an abnormality in either HDL-mediated cholesterol and phospholipid efflux from peripheral cells20,21 and/or a defect in HDL particle interconversion22 have been suggested as possible metabolic causes.

Severe familial FHD has recently been described in three French-Canadian kindreds as a trait with autosomal codominant inheritance.23 Two members of one particular kindred, who were extensively investigated, had normal fasting triglyceride concentrations with HDL cholesterol levels below the 5th percentile. One of them had evidence of coronary artery disease. Both patients had a 50% to 80% reduction in plasma apoA-I concentration, a decrease in average HDL particle size, and a relative increase in plasma proapoA-I levels. No evidence was obtained for the presence of an apoA-I or apoA-II gene abnormality, and the FHD patients had none of the lipoprotein lipase gene mutations commonly found in French Canadians.24 LCAT activity was normal in the FHD patients. Finally, none of the patients had clinical manifestations of Tangier disease,25 eg, cholesteryl ester deposition in reticuloendothelial tissues, hyperplastic orange tonsils, splenomegaly, or relapsing neuropathy.

To investigate the plasma kinetics of HDL apolipoproteins (proapoA-I, mature apoA-I, and apoA-II) in the aforementioned FHD patients, we carried out a stable-isotope kinetic study in two FHD and four normolipidemic control subjects. Our aim was to determine whether catabolism of apoA-I was greatly increased in these individuals, as previously documented for Tangier disease patients25 and other HDL deficiency states,26–32 or whether production of apoA-I was impaired.

**Methods**

Six male subjects were investigated in the present study: two brothers with FHD and four healthy control subjects (Table 1). The medical history of the two affected brothers has been described previously.23 In brief, patient 1 (proband 24430–301) was diagnosed with coronary artery disease at the age of 42. When diagnosed, he had a history of high blood pressure and he had been a smoker. His HDL cholesterol concentration had been known to be low, but he had no signs of abnormal liver function or clinical manifestations of Tangier disease. He underwent percutaneous transluminal coronary angioplasty at the age of 42 years and subsequently had coronary artery bypass surgery at the age of 48. Patient 2 (24430–313) did not have evidence of coronary artery disease, nor did he show clinical signs of Tangier disease. HDL cholesterol concentrations in the two patients, measured during routine visits to the lipid clinic of the Clinical Research Institute of Montreal, were consistently found to be <0.25 mmol/L. Both patients were not taking any medications known to affect plasma lipid levels, and their HDL deficiency was not due to known causes.23 Four healthy males acted as controls subjects. They had no evidence or history of dyslipidemia, diabetes mellitus, or any other metabolic disorder and were not taking any medications known to affect plasma lipid levels. Only one of them, subject 4, was a smoker (<1 pack/d). All six subjects gave informed consent to the study protocol, which was approved by the ethics committee of the Clinical Research Institute of Montreal.

**Infusion Protocol**

The in vivo measurement of plasma apolipoprotein kinetics was carried out as described previously.33 After a 12-hour overnight fast, subjects were given an injection of 10 μmol/kg of body weight of [D-3]leucine ([D-3]leucine 98%, Cambridge Isotope Laboratories) dissolved in physiological saline (0.9% NaCl) via an intravenous line attached to a left forearm vein. After the bolus injection, subjects were infused for 12 hours with 10 μmol·kg⁻¹·h⁻¹ of [D-3]leucine dissolved in physiological saline. The infusion was carried out using a volumetric pump (Life Care Pump model 3, Abbott) set to deliver 48 mL of infusate per hour. Subjects were not given food during the time course of the infusion but had free access to drinking water. They were encouraged to move around to maintain good blood

**TABLE 1. Characteristics of FHD Patients and Control Subjects**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age, y</th>
<th>BMI, kg/m²</th>
<th>ApoE phenotype</th>
<th>TC, mmol/L</th>
<th>HDL-C, mmol/L</th>
<th>TG, mmol/L</th>
<th>VLDL-TG, mmol/L</th>
<th>ApoA-I, mg/dL*</th>
<th>ApoA-II, mg/dL</th>
<th>ApoB, mg/dL</th>
<th>ApoE, mg/dL</th>
<th>ApoC-III, mg/dL</th>
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<tr>
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<tr>
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<td>52</td>
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<td>3/3</td>
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<td>1.04</td>
<td>1.29</td>
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<td>5.1</td>
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</tr>
<tr>
<td>SD†</td>
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<td>±0.9</td>
<td>...</td>
<td>±1.08</td>
<td>±0.15</td>
<td>±0.74</td>
<td>±0.52</td>
<td>±18</td>
<td>±2.4</td>
<td>±32</td>
<td>±0.9</td>
<td>±5.6</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; TC, total cholesterol; -C, cholesterol; and TG, triglyceride. Plasma concentrations for individual subjects represent the average of five measurements made at 3-hour intervals during the stable-isotope infusion.

*Includes all apoA-I isoforms.

†Mean data for control subjects ± standard deviation.
circulation. Blood samples (20 mL) were collected from an antecubital vein at regular intervals (0, 15, 30, and 45 minutes and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 hours) in tubes containing EDTA to a final concentration of 0.1%. Samples were kept on ice, and plasma was immediately separated by centrifugation at 3500 rpm for 15 minutes at 4°C. An anticoagulant agent (Na2EDTA) and a protease inhibitor (aprotinin) were added to plasma to give a final concentration of 0.02% and 1.67 µg/mL, respectively.

Isolation of Lipoproteins and Apolipoproteins

VLDL, IDL+LDL, and HDL were isolated from 5 mL plasma by sequential ultracentrifugation in an XL-90 ultracentrifuge using a 50.4 Ti rotor (Beckman) at 50 000 rpm for 10 hours at densities (d) of 1.006, 1.019, and 1.21 g/mL, respectively. Total lipoproteins were isolated from plasma by ultracentrifugation (50 000 rpm, 10 hours) of 1 mL of plasma, adjusted to d = 1.25 g/mL with KBr. Lipoproteins were recovered in the supernatant by tube slicing. VLDL apoB-100 was isolated by preparative SDS-polyacrylamide gel electrophoresis on a 4% to 22.5% gradient gel.33 Plasma apoA-I and apoA-II were isolated by preparative IEF on 7.5% polyacrylamide–urea (8 mol/L) gels (pH gradient, 4 to 7) of apolipoproteins in total plasma isolated by preparative IEF on 7.5% polyacrylamide–urea (8 mol/L) containing lipoproteins were performed as described 36 by using blocking, and immunolocalization steps of apoA-I– and apoA-II–immunopurified polyclonal anti-human apoA-I antibody and monoclonal anti-apoA-II antibody (125I labeled). Plasma without apoA-I–containing lipoproteins was prepared by immunoaffinity chromatography by using anti-apoA-I latex (Genzyme). Plasma (200 µL) was added to 1 mL of anti-apoA-I latex suspension, gently mixed for 15 minutes at room temperature, and then centrifuged at 12 000 rpm for 10 minutes. The infranatant, which contained non–latex-bound plasma now devoid of apoA-I, was concentrated by using Centri- con-10 concentrators (Amicon) before separation by electrophoresis.

Plasma Lipids and Apolipoproteins

Plasma and lipoprotein fractions were assayed for total (free and esterified) cholesterol and triglyceride with a Cobas Mira-S automated analyzer (Hoffman–La Roche) using enzymatic reagents. Plasma apoB concentration was measured by noncompetitive ELISA using an immunopurified goat anti-human apoB antibody and horseradish peroxidase–conjugated monoclonal antibody.37 Plasma apoA-I concentration was measured by nephelometry on a Behring nephele meter 100 (Behring) using the Behring protocol and reagents. Plasma apoA-II was measured by nephelometry in the laboratory of Dr Linda Bausserman (Meriam Hospital, Brown University, Providence, RI).38 Plasma apoE and apoC-III concentrations were measured by ELISAs developed in our laboratory.39,40 ApoE phenotype was determined by IEF of delipidated VLDL.41

Quantification of ProApoA-I

Mature apoA-I and proapoA-I concentrations in plasma of normolipemic and FHD subjects were derived from total plasma apoA-I concentrations measured by nephelometry. The proportion of each apoA-I isoform contributing to total plasma apoA-I was determined by GC-MS and densitometric scanning of IEF gels. The amount of leucine associated with the major mature apoA-I IEF band (apoA-L1) and the major proapoA-I band (isofrom apoA-I-2)42 was then estimated by measuring the relative amounts of these isoforms by IEF gel scanning densitometry. Plasma concentration of proapoA-I was then calculated as

\[
\text{ProApoA-I (mg/dL)} = \frac{\text{ApoA-I Protein (µg)} \times \text{Total Weight of Leucine Residues in ApoA-I Isoform (Da)}}{\text{Leucine (µg)} \times \text{MW of ApoA-I Isoform (Da)}}
\]

The amount of protein in minor mature apoA-I bands (isosforms apoA-I-1 and apoA-I-2) and the minor proapoA-I band (isofrom apoA-I-1)42 was then estimated by measuring the relative amounts of these isoforms by IEF gel scanning densitometry. Plasma concentration of proapoA-I was then calculated as

\[
\frac{\text{ProApoA-I (mg/dL)} = \text{ApoA-I-1} + \text{ApoA-I-2}}{\text{ApoA-I-1} + \text{ApoA-I-2}} \times \frac{\text{ApoA-I-1} \times \text{ApoA-I-2}}{\text{Leucine (µg)} \times \text{MW of ApoA-I Isoform (Da)}}
\]
Determination of Isotopic Enrichment in Isolated Apolipoproteins

Apolipoprotein bands as well as blank (non–protein-containing) gel slices were excised from polyacrylamide gels (VLDL apoB-100 from SDS-polyacrylamide and apoA-I and apoA-II from IEF)\(^4\) Each slice was added to a borosilicate sample vial containing 600 \(\mu\)L of 6N HCl and an internal standard of 250 ng norleucine (Sigma-Aldrich) dissolved in 50 \(\mu\)L double-distilled water. Gel slices were hydrolyzed at 110°C for 24 hours, cooled to ~20°C for 20 minutes, and centrifuged at 3500 rpm for 5 minutes. Free amino acids in the hydrolysate were separated from precipitated polyacrylamide, purified by cation-exchange chromatography using AG 50 W-X8 resin (Bio-Rad), and derivatized by treatment with 200 \(\mu\)L of acetyl chloride-acidified 1-propanol (1:5, vol/vol) for 1 hour at 100°C and 50 \(\mu\)L of heptfluorobutyric anhydride (Supelco) for 20 minutes at 60°C\(^3\). Enrichment with deuterated leucine was determined by GC-MS (Hewlett-Packard, 5988 GC-MS) using negative chemical ionization and methane as the reagent gas. Selective ion monitoring at \(m/z\) 352 and 349 (ionic species corresponding to derivatized deuterated and derivatized nondeuterated leucine, respectively) was performed, and the tracer to tracee ratio was calculated from the isotopic ratio in each sample according to the formula derived by Cobelli et al\(^4\)

\[ z(t) = \frac{r(t) - r_{R}}{r(t) + r_{R}} \times \frac{1 + r_{I}}{1 + r_{N}} \]

where \(z(t)\) is the tracer to tracee ratio at time \(t\), \(r(t)\) is the isotopic ratio at time \(t\), and \(r_{R}\) and \(r_{I}\) are the naturally occurring and infused isotopic ratios, respectively. \(r(t)\) was obtained from the ratio of the areas under the peak of leucine ionic species \(m/z=352\) and 349. The ratios of the areas under the peak of the ionic species \(m/z=349\) of leucine and the internal standard (norleucine) detected in blank samples were used to correct for background (method-introduced) leucine, which was 0.44±0.27\%, 6.04±2.06\%, and 4.12±2.96\% (mean±SD) of total leucine in mature apoA-I, proapoA-I, and apoA-II samples, respectively.

Kinetic Analysis

Tracer to tracee ratios of VLDL apoB-100, mature plasma apoA-I, proapoA-I, and apoA-II were fitted to a monoeponential function using SAAM II computer software (SAAM II Institute). The function was defined as \(z(t) = Zp [1-\exp(-kt)]\), where \(Zp\) was the tracer to tracee ratio at time \(t\), \(Zp\) was the tracer to tracee ratio of the tissue precursor amino acid pool from which the protein in question was derived (estimated from VLDL apoB-100 and proapoA-I enrichment curves at plateau; see below), \(d\) was the delay time in hours, and \(k\) was the FPR (pools per hour). RT was calculated as the reciprocal of FPR (1/FPR), and the absolute PR in (in milligrams per kilogram per day) was calculated as

\[ PR = \frac{FPR \times \text{Pool Size (mg)}}{\text{Body Weight (kg)}} \]

where pool size=plasma concentration (mg/dL)×plasma volume (0.045 L/kg).

The tracer to tracee ratios (Zp) of the intestinal and hepatic precursor amino acid pools from which proapoA-I and hence apoA-I were derived were taken to be the enrichment of proapoA-I at plateau. Since apoA-II, like apoB-100, is predominantly of hepatic origin, the enrichment of the apoA-II precursor pool was taken to be the enrichment of VLDL apoB-100 at plateau.

Results

Plasma Lipids and Apolipoproteins

Plasma concentrations of lipids and apolipoproteins in subjects on the day of the infusion are shown in Table 1. The two brothers with FHD had plasma HDL cholesterol concentrations of 0.19 and 0.27 mmol/L, which were, on average, 18% and 26% of HDL cholesterol concentrations in control subjects. Their plasma concentrations of apoA-I and apoA-II were 14% and 29% (for apoA-I) and 46% and 44% (for apoA-II) of control values, respectively. Total plasma triglyceride, VLDL triglyceride, and apoB concentrations were slightly higher in FHD patients than in control subjects, whereas plasma apoE levels were slightly lower. Total plasma cholesterol and apoC-III levels were within the normal range.

The separation by IEF gel electrophoresis of proapoA-I isoforms, mature apoA-I isoforms, and apoA-II from total plasma lipoproteins (d<1.25 g/mL) of control and FHD subjects separated by IEF gel electrophoresis. Gels are shown for two control and two FHD patients, corresponding to control subjects 1 and 2 and FHD patients 1 and 2 in Table 1. Different isoforms of proapoA-I and mature apoA-I are indicated. Similar amounts of total protein (600 \(\mu\)g) were separated on each gel.

Figure 2. Comparison of relative amounts of proapoA-I, mature apoA-I, and apoA-II in total plasma lipoproteins (d<1.25 g/mL) of control and FHD subjects separated by IEF gel electrophoresis. Gels are shown for two control and two FHD patients, corresponding to control subjects 1 and 2 and FHD patients 1 and 2 in Table 1. Different isoforms of proapoA-I and mature apoA-I are indicated. Similar amounts of total protein (600 \(\mu\)g) were separated on each gel.
amounts of proapoA-I (apoA-I-2 isoform). As is evident from Fig 2, the minor proapoA-I isoform (apoA-I-1, isoform) was somewhat reduced in FHD patients compared with that in control subjects; however, quantification of total plasma mature apoA-I and total proapoA-I revealed that the decrease in plasma apoA-I concentration in patients was due to a decrease in mature apoA-I and not proapoA-I concentration (Table 2). The relative contribution of proapoA-I to total plasma apoA-I was thus higher in FHD patients (18.2% and 9.5% for patients 1 and 2, respectively) compared with control subjects (2.9% and 0.3% for patients 1 and 2, respectively) with controls (Fig 4), which corresponded to a twofold reduction in total plasma apoA-II concentration (measured by nephelometry) (Table 2). Larger α-migrating HDLs containing apoA-II were virtually absent from the plasma of FHD patients, which meant that the average particle size of α-LpA-II in FHD patients was smaller than that of control. As shown in gels C and D in Fig 4, these apoA-II-containing HDLs were unique, in that they did not contain apoA-I, unlike the majority of apoA-II–containing HDLs in control subjects, which was removed by apoA-I affinity chromatography; the efficiency of removal of apoA-I–containing lipoproteins was >97% for patients and control subjects, as assessed by an essentially complete absence of apoA-I in electrophoretically separated samples after affinity chromatography. This result is consistent with the observation that all HDL apoA-II is (under normal circumstances) bound to apoA-I in LpA-I:A-II particles in normolipidemic subjects.12

Stable-Isotope Enrichment of Plasma ApoA-I

Newly synthesized mature apoA-I, enriched with deuterated leucine, was detected in the plasma of all subjects within 2 hours of the start of the stable-isotope infusion experiment.

Table 2. Kinetic Parameters for Mature ApoA-I, ProapoA-I, and ApoA-II

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Mature ApoA-I</th>
<th>ProapoA-I</th>
<th>ApoA-II</th>
</tr>
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<tbody>
<tr>
<td>Conc. mg/dL</td>
<td>FPR, pools/day</td>
<td>RT, days</td>
<td>PR, mg · kg⁻¹ · d⁻¹</td>
</tr>
<tr>
<td>FHD</td>
<td>1</td>
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<td>1.266</td>
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<tr>
<td>Control</td>
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<td>33.5</td>
<td>0.602</td>
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</table>

Table 3. Two-dimensional gel electrophoretic separation of apoA-I–containing lipoproteins from the plasma of control and FHD subjects. Gels (A and B) are shown for two control and two FHD patients, corresponding to control subjects 1 and 2 and FHD patients 1 and 2 in Table 1. Plasma (200 μL) was separated in the first dimension (left to right) by agarose gel electrophoresis and in the second dimension (top to bottom) by 3% to 24% polyacrylamide gradient gel electrophoresis. Lipoproteins containing apoA-I were detected with 125I-labeled polyclonal anti-human apoA-I antibody after electrotransfer to nitrocellulose membranes. Different apoA-I–containing HDL subpopulations are indicated with vertical arrows. Molecular size markers were separated between plasma samples (in the center of each gel): thyroglobulin (17 nm), ferritin (12.2 nm), catalase (9.5 nm), lactate dehydrogenase (8.2 nm), and albumin (7.1 nm), from top to bottom.
The rate of appearance of deuterium-labeled mature apoA-I measured as percentage tracer (deuterated leucine) to tracee (nondeuterated leucine) ratio was linear for the 12-hour duration of the study in all six subjects. The fractional rate of appearance of labeled mature apoA-I (the slope of the enrichment curve) was sevenfold and threefold higher in the two FHD patients compared with control, corresponding to significantly increased FPRs (measured in pools per day; Table 2). Because mature apoA-I pool sizes were proportionately decreased, absolute apoA-I PRs were similar in patients and control subjects (Table 2), ranging between 7.9 and 12.9 mg kg$^{-1}$ d$^{-1}$. The RT of mature apoA-I in plasma was, however, significantly shorter in patients (0.79 and 1.66 days) than in control subjects (5.32±1.05 days), demonstrating that mature apoA-I catabolism was significantly increased in FHD patients. Thus, in the case of FHD patient 1, each molecule of mature apoA-I remained in the plasma for a period that was 6.7 times less than that of control subjects, corresponding to a plasma apoA-I concentration that was 8.8 times lower than that in control. In the case of FHD patient 2, each molecule of mature apoA-I remained in the plasma 3.2 times less than that of control subjects, corresponding to a plasma apoA-I concentration that was 3.7 times lower than control.

The time course of enrichment of plasma proapoA-I for the four control subjects (A) and two FHD patients (B) is shown in Fig 6. The tracer to tracee ratio of plasma proapoA-I increased monoexponentially and reached a plateau level during the time course of the infusion. The mean level of proapoA-I enrichment (Fig 5). The rate of appearance of deuterium-labeled mature apoA-I measured as percentage tracer (deuterated leucine) to tracee (nondeuterated leucine) ratio was linear for the 12-hour duration of the study in all six subjects. The fractional rate of appearance of labeled mature apoA-I (the slope of the enrichment curve) was sevenfold and threefold higher in the two FHD patients compared with control, corresponding to significantly increased FPRs (measured in pools per day; Table 2). Because mature apoA-I pool sizes were proportionately decreased, absolute apoA-I PRs were similar in patients and control subjects (Table 2). Because mature apoA-I pool sizes were proportionately decreased, absolute apoA-I PRs were similar in patients and control subjects (Table 2).
at plateau for the control subjects was 6.7±2.4% compared with 8.2±2.2% for VLDL apoB-100. Because the proapoA-I plateau varied somewhat from one individual to another (being a function of the amount of deuterated leucine reaching the proapoA-I precursor amino acid pool and the level of unlabeled leucine in this pool), curves in Fig 6 have been normalized to allow the data from individual subjects to be visually compared (this measure was unnecessary for slower-turning-over mature apoA-I). ProapoA-I ratios have thus been expressed as a percentage of the tracer to tracee ratio of proapoA-I at plateau in each individual. As shown in Fig 6B, the time course of enrichment of proapoA-I for the two FHD patients was not different from that of the control subjects; mean data (±SD) for the four control subjects are shown with open diamond symbols. Kinetic analyses of these curves demonstrated that neither the RT nor the PR of proapoA-I was different from that of the control subjects; mean data (error bars are SDs). Tracer to tracee ratios were expressed as percentages of those at plateau to allow direct comparison between subjects.

**Stable-Isotope Enrichment of Plasma ApoA-II**

The time course of plasma apoA-II enrichment with deuterated leucine for the four control subjects and two FHD patients is shown in Fig 7. As was the case for mature apoA-I, the tracer to tracee ratio of apoA-II increased linearly, and the fractional rate of appearance of newly synthesized apoA-II was about twofold higher in patients than in control subjects. Because plasma apoA-II concentration (and hence apoA-II pool size) was approximately two times less, apoA-II PRs in FHD patients (1.8 and 1.9 mg kg⁻¹ d⁻¹) were not significantly different from those of control (1.7±0.2 mg kg⁻¹ d⁻¹). ApoA-II RTs, on the other hand (3.17 and 2.92 days), were significantly less compared with controls (7.24±0.71 days), demonstrating that the FCR of plasma apoA-II was significantly increased in FHD patients.

**Discussion**

Our results have demonstrated that the FHD patients in the present study (with no clinical symptoms of Tangier disease or evidence of other known causes of HDL deficiency) had significantly reduced levels of plasma apoA-I and apoA-II, which were not caused by reduced apoA-I or apoA-II production. Reduced HDL levels were instead associated with increased plasma catabolism of mature apoA-I and, to a lesser extent, of apoA-II but not increased catabolism of proapoA-I. We have therefore concluded that hypercatabolism of mature plasma apoA-I, but not proapoA-I, was responsible for the HDL deficiency in these patients.

Two previous studies have investigated the plasma kinetics of HDL apolipoproteins in patients with severe genetic HDL deficiency of unknown origin. Emmerich et al described a 46-year-old man with coronary artery disease who had severely reduced levels of plasma HDL cholesterol (5.0 mg/dL) and total plasma apoA-I (4.5 mg/dL). His brother and two children had reduced HDL levels, suggesting codominant inheritance of the abnormality. His apoA-I was structurally normal, he had no clinical features of Tangier disease, and he was found to have marked hypercatabolism of apoA-I. Rader et al similarly identified two male and three female probands with very low HDL levels (of apparently familial origin), who had no evidence of premature coronary heart disease. They had no clinical or biochemical characteristics typical of known HDL deficiency states, and all five individuals had increased catabolism of HDL apoA-I and apoA-II. These reported cases of severe HDL deficiency of unknown etiology resemble those described in the present study, in that an increase in plasma apoA-I catabolism, rather than a reduction in apoA-I production, was responsible for reduced
HDL levels. This situation is analogous to the one in patients with less severe reductions in HDL, in whom the FCR of plasma apoA-I has consistently been shown to be the primary metabolic predictor of intersubject variability in plasma apoA-I and HDL cholesterol concentrations. It has been hypothesized that increased fractional catabolism of apoA-I in these individuals is caused by triglyceride enrichment and cholesterol depletion of HDL particles, the formation of smaller HDL, increased interaction of HDL with lipoprotein and hepatic lipases, greater dissociation of apoA-I from HDL, and subsequent clearance of “free” apoA-I by the kidney. Increased renal clearance of apoA-I in a lipid-free or greatly lipid-depleted form may also be responsible for apoA-I hypercatabolism in our more severely affected FHD patients. This may be the result of impaired HDLc and apoA-I–mediated cellular cholesterol and phospholipid efflux, which we have found to be a characteristic of fibroblasts from FHD patients (data not shown), similar to that of fibroblasts from Tangier patients. A decrease in availability of tissue-derived cholesterol in precursor HDL particles could reduce cholesteryl ester formation in HDL (catalyzed by LCAT), resulting in impaired maturation of these particles into larger, cholesteryl ester–rich HDLs and formation of relatively small, lipid-depleted apoA-I particles, which are rapidly cleared. Support for this concept is provided by our two-dimensional electrophoretic analysis of apoA-I–and apoA-II–containing HDL-size lipoproteins (Figs 3 and 4), showing that larger α-migrating HDLs, particularly those containing apoA-II, were greatly reduced in FHD patients, whereas small pre-β-LpA-I, representing lipid-poor or lipid-free apoA-I particles, were present in similar amounts compared with controls and at significantly elevated levels relative to other apoA-I subfractions in FHD plasma.

A distinguishing feature of the present investigation is the measurement (for the first time) of the plasma kinetics of proapoA-I with an endogenous, stable-isotope labeling technique and the finding that the plasma proapoA-I PR and RT were essentially normal in FHD patients compared with control subjects (Table 2). The in vivo kinetics of plasma proapoA-I has been previously studied in two normolipidemic and two Tangier disease patients by Bojanovski et al. using purified and radioactively labeled proapoA-I. The two normolipidemic subjects with plasma proapoA-I concentrations of 5.5 and 6.0 mg/dL had proapoA-I RTs of 0.19 and 0.27 day and proapoA-I PRs of 11.6 and 8.8 mg/kg·d⁻¹, respectively. These values are similar to those obtained in the present study for control subjects (RT, 0.16±0.03 day; PR, 10.9±2.6 mg·kg⁻¹·d⁻¹; Table 2), providing evidence that exogenous and endogenous tracer techniques give comparable results.

In view of this similarity, we have made a direct comparison between kinetic parameters obtained previously for Tangier patients and those obtained for our control and FHD patients (Fig 8) (average results for two subjects are shown in the case of FHD and Tangier patients). As depicted by the relative sizes of shaded circles in Fig 8, FHD patients had mean plasma mature apoA-I concentrations of 24 mg/dL compared with 1.0 mg/dL in Tangier disease patients and 123 mg/dL in control subjects. Plasma pools of mature apoA-I were thus five times smaller, on average, in FHD patients than in control subjects and were >100 times smaller in Tangier disease patients (reflecting the greater severity of HDL deficiency in Tangier disease). In absolute terms, plasma concentrations of proapoA-I were increased in FHD and Tangier disease patients (ie, proapoA-I represented 14% of total apoA-I in FHD patients and 60% in Tangier disease patients compared with 3% in control subjects). In absolute terms, however, average plasma concentrations of proapoA-I were 3.3, 1.5, and 3.7 mg/dL, respectively, and proapoA-I pool sizes were thus reduced in Tangier disease patients though not in patients with FHD (Fig 8). It is significant that these relatively normal levels of proapoA-I in FHD patients were associated with essentially normal rates of proapoA-I production and fractional catabolism unlike Tangier disease patients, who were characterized by slightly reduced rates of proapoA-I production and significantly increased rates of proapoA-I fractional clearance from plasma (9.3 pools/d versus 0.3 and 0.3 pool/d in FHD and control subjects, respectively). Thus, as depicted by the relative widths of arrows in Fig 8, 3% to 7% of proapoA-I is cleared from plasma and escapes conversion to mature apoA-I in control and FHD patients, whereas a significant proportion (~70%) of proapoA-I in Tangier disease patients is removed in unconverted form from plasma. Mature apoA-I is subsequently catabolized at an increased fractional rate in both FHD and Tangier disease, with hypercatabolism being six
times greater in Tangier than FHD patients. ApoA-II fractional catabolism is also increased in FHD patients (Table 2), though not to the same extent as in Tangier disease patients (plasma apoA-II RT in FHD versus Tangier, 3.0 versus 0.8 days).

These data taken together demonstrate that from an HDL kinetic perspective, FHD patients are clearly dissimilar from control subjects. Furthermore, they are distinguishable from patients with Tangier disease, since (1) reduction in plasma apoA-I and hypercatabolism of plasma apoA-I are significantly more severe in Tangier disease, (2) increase in fractional catabolism of plasma apoA-II is also more severe in Tangier disease, and (3) plasma catabolism and concentration of proapoA-I are essentially normal in FHD but not in Tangier disease. The reason for this latter difference in proapoA-I metabolism and its pathophysiological significance deserve further investigation.

As shown by the data in Fig 4 and as demonstrated previously,

nearly all apoA-II in the plasma of normolipidemic subjects is associated with HDL containing apoA-I. We have found, however, that our patients with FHD had abnormal apoA-II–containing HDL, which did not contain apoA-I. It is significant that similar particles have been detected in the plasma of patients with Tangier disease.

These lipoproteins, designated Lp(A-II), were found to be equally effective as Lp(A-I) in promoting cholesterol efflux from cholesterol-loaded human fibroblasts, and therefore, no evidence has been obtained suggesting that these lipoproteins are of particular atherogenic potential. Nevertheless, they represent a characteristic feature of HDL deficiency states, and rapid turnover of plasma apoA-I is perhaps responsible for abnormal conversion of LpA-I:A-II to LpA-II or simply prevents the normal formation of LpA-I:A-II particles. Alternatively, impaired HDL3- and apoA-I–mediated cellular cholesterol and phospholipid efflux, shown to be a characteristic of our FHD patients (as mentioned previously), leads to the formation of abnormal apoA-II–only–containing HDL. A third possibility is that the absence or deficiency (as shown for Tangier disease patients) of a plasma factor responsible for the formation of mature HDL (by converting pre-β-LpA-I to α-LpA-I) results in the abnormal formation of LpA-II. The reason for the existence of LpA-II thus remains speculative, although we have assumed that these lipoproteins represent a consequence rather than a cause of HDL deficiency.

We have found in the present study that the tracer to tracee ratio of plasma proapoA-I reaches a plateau during the time course of a 12-hour infusion experiment (Fig 6). This is of methodological significance, since the tracer to tracer ratio of proapoA-I at plateau can be assumed to represent the enrichment of the intestinal and hepatic precursor amino acid pools from which proapoA-I is derived, in the same way that plateau enrichment of VLDL apoB-100 represents the enrichment of hepatic precursor amino acid pools. We have found that the level of plasma proapoA-I enrichment at plateau was less than that of VLDL apoB-100 in FHD patients as well as all four control subjects (proapoA-I plateau enrichment, 6.7 ± 2.4% versus VLDL apoB-100, 8.2 ± 2.2%), suggesting that intestinal precursor leucine pools were enriched with deuterated leucine to a lesser extent than were hepatic leucine pools. This idea is consistent with previous data showing that VLDL apoB-48 of intestinal origin plateaus at lower enrichment than does VLDL apoB-100 of hepatic origin.

We therefore contend that using proapoA-I enrichment at plateau rather than VLDL apoB-100 enrichment at plateau to calculate apoA-I kinetic parameters is more accurate and results in an average RT for mature apoA-I in plasma of 5.3 days for control subjects compared with values of 3.4 and 4.5 days for total apoA-I and 6.5 days for mature apoA-I that have been obtained in exogenous apoA-I radioiodination studies. A somewhat higher RT is expected for mature apoA-I than for total apoA-I, since total apoA-I includes both mature apoA-I and proapoA-I (the latter having a faster rate of turnover, ie, shorter RT).

In conclusion, although a specific gene defect has not yet been identified as the cause of HDL deficiency in our FHD patients, the present results demonstrate that these patients are kinetically distinguishable from control subjects and from patients with Tangier disease. The gene responsible for low HDL levels and associated hypercatabolism of HDL in FHD patients may therefore be different from that of Tangier disease, or it may represent a less severe abnormality of the same gene. Further investigation of this kindred is thus warranted, since it has the potential to provide new insight into genetic factors affecting HDL metabolism.

Acknowledgments

This study was supported by a joint university-industry grant from the Medical Research Council (MRC) of Canada and Parke-Davis (PA-14006) and by an MRC operating grant (MT 12884) to J.G., Jr. R.B. received a scholarship and J.S.C. was supported by a grant-in-aid from the Heart and Stroke Foundation of Quebec. We would particularly like to acknowledge the help of Denise Dubreuil and the other nurses of the Lipid Clinic of the Clinical Research Institute of Montreal and the excellent technical assistance of Hélène Jacques and Nancy Doyle. The generous gift of anti–apoE antibody and anti–apoA-I latex gel from Tom Pasani of Genzyme Corp was also very much appreciated.

References


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doi: 10.1161/01.ATV.18.4.655

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

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