Two Patterns of LDL Metabolism in Normotriglyceridemic Patients With Hypoalphalipoproteinemina

Gloria Lena Vega and Scott M. Grundy

The objective of this study was to determine whether normotriglyceridemic patients with low levels of high density lipoprotein (HDL) cholesterol have concomitant defects in the metabolism of low density lipoproteins (LDLs). To address this question, measurements of turnover rates of apolipoprotein A-I (apo A-I) and LDL apolipoprotein B (apo B) were made in 36 middle-aged men with low HDL cholesterol (<40 mg/dL), normal triglyceride (<250 mg/dL), and normal total cholesterol (≤90th percentile) levels. Similar measurements were made in eight hypertriglyceridemic men having low HDL levels. For control, turnover rates of LDL apo B were measured in 24 healthy, normolipidemic men, and apo A-I kinetics were determined in 20 other healthy men with normal HDL cholesterol levels. In all patients with low HDL levels, fractional catabolic rates (FCRs) for apo A-I were increased compared with control subjects; in contrast, input rates for apo A-I in low-HDL patients were similar to control. Hypertriglyceridemic patients had significantly higher FCRs for LDL (0.463±0.040 pool/day, [mean±SEM]) than control subjects (0.328±0.008 pool/day, p<0.001). In normolipidemic patients having low HDL, a bimodal pattern of LDL–apo B kinetics was observed. For 23 low-HDL patients, FCRs for LDL apo B averaged 0.450±0.017 pool/day and were significantly higher than control values. Additionally, in these patients, levels of very low density lipoprotein plus intermediate density lipoprotein (VLDL+IDL) cholesterol and VLDL+IDL apo B were higher than in control subjects (54±3 versus 32±3 mg/dL and 25±2 versus 18±1 mg/dL, respectively). The remaining 13 low-HDL patients had lower and essentially normal FCRs for LDL (0.300±0.009 pool/day); these patients also had relatively low levels of cholesterol and apo B in VLDL+IDL. Thus, two patterns of LDL kinetics were present in normotriglyceridemic patients with low HDL levels. One pattern was indistinguishable from that typically present in patients with hypertriglyceridemia, whereas the other was similar to normal control subjects. These two patterns of LDL–apo B kinetics may reflect different mechanisms for the causation of low HDL cholesterol concentrations. (Arteriosclerosis and Thrombosis 1993;13:579–589)

KEY WORDS • hypoalphalipoproteinemina • low HDL • LDL–apo B kinetics • apo A-I kinetics

Serum levels of high density lipoprotein (HDL) cholesterol are correlated inversely with rates of coronary heart disease (CHD). The mechanisms underlying this inverse relation unfortunately are not well understood. According to one hypothesis, a low HDL level per se promotes coronary atherosclerosis; it could do this by failure to remove excess cholesterol from arterial wall cells or by failure to prevent oxidation or self-aggregation of low density lipoprotein (LDL). Another possibility is that a low HDL level reflects increased concentrations of other atherogenic lipoproteins, most likely remnants of very low density lipoproteins (VLDLs). Certainly, hypertriglyceridemia frequently co-exists with low levels of HDL and an inverse relation between HDL cholesterol levels and concentrations of triglyceride-rich lipoproteins (TGRLPs) has been observed in several population studies. Thus, it is difficult to know whether the atherogenic factor is an increase in TGRLP levels or a decrease in HDL levels. Many patients with low HDL cholesterol levels nonetheless have normal triglyceride concentrations, and in them, no obvious connection between TGRLP and HDL levels exists. Still, normolipidemic patients with low HDL concentrations appear to be at increased risk for CHD and thus, we must keep in mind the possibility that a low HDL level predisposes to CHD independent of TGRLPs.

The mechanisms whereby high levels of TGRLPs reduce HDL levels have not been fully determined. One possibility is that excess TGRLPs receive increased amounts of cholesterol ester from HDL, thereby partially depleting HDL particles of cholesterol. Besides a reduction in the cholesterol content of HDL, however, high triglyceride levels usually are accompanied by low levels of apolipoprotein A-I (apo A-I), indicating a
reduction in the number of HDL particles. Recent studies suggest that low apo A-I concentrations in hypertriglyceridemic patients are due primarily to enhanced catabolism of apo A-I and not to decreased production of apo A-I. An increase in TGRLP levels in some way may destabilize HDL particles, and this could enhance their clearance from the plasma.

Patients with hypertriglyceridemia not only have enhanced catabolism of apo A-I, but they typically also have increased fractional clearance rates (FCRs) for LDL apolipoprotein B-100 (apo B) as well.11,12 The latter is frequently but not always accompanied by high input rates for LDL apo B.11,12 Consequently, abnormally high FCRs for both LDL apo B and apo A-I typically coexist in patients with hypertriglyceridemia. Recently, we have reported that near normalization of elevated triglyceride concentrations by weight reduction failed to correct these abnormalities in metabolism of apo A-I and LDL apo B in a group of patients with preexisting hypertriglyceridemia; i.e., the abnormalities in apolipoprotein catabolism persisted despite restoration of normotriglyceridemia.13 This finding raises the possibility that some normotriglyceridemic patients with low HDL levels have subclinical defects in metabolism of apo B-containing TGRLPs that account for their low HDL concentrations.

The current study, therefore, was performed to determine whether normotriglyceridemic patients with low levels of HDL cholesterol carry concomitant defects in metabolism of apo B-containing lipoproteins, particularly abnormalities consistent with a subclinical defect in TGRLP metabolism. Specifically, the protocol examined the frequency of simultaneous abnormalities in the kinetics of apo A-I and LDL apo B in normotriglyceridemic patients with low HDL cholesterol levels, both of which would point to a defect in TGRLP metabolism. A subsidiary question was whether low HDL cholesterol levels are invariably associated with enhanced catabolism of apo A-I, regardless of whether LDL–apo B kinetics are abnormal.

Methods

Patients

Thirty-six men with normotriglyceridemia and hyperapolipoproteinemia were recruited from outpatient clinics of the Veterans Affairs Medical Center, Dallas, Tex. Normotriglyceridemia was defined as a plasma triglyceride level below 250 mg/dL, which generally is below the 90th percentile cutoff for men of comparable age as reported in the Lipid Research Clinics Population Survey.14 This definition is consistent with that set forth by various expert panels.15,16 Hypoapolipoproteinemia was defined as an HDL cholesterol level below 40 mg/dL; this value was chosen because of data of the Framingham Heart Study17 that showed a substantial increment in risk for CHD when HDL cholesterol levels were below this level. During this study, 30 of 36 patients in this group had HDL cholesterol concentrations below 35 mg/dL, the latter being defined as “low HDL cholesterol” by the National Cholesterol Education Program.16 To be eligible for the study, patients were required to have total cholesterol levels (and LDL cholesterol levels) below the 90th percentile for their age.14 A decision for patient inclusion in the study was made from the average of measurements of plasma lipids and lipoproteins taken on 3 consecutive days. Patients were excluded if they had clinically significant disorders of the liver, gastrointestinal tract, kidneys, lungs, or endocrine system, or if they had chronic congestive heart failure or severe angina pectoris. Additional patients were excluded if they had myocardial infarction, coronary artery surgery, or coronary angioplasty in the 6 months before the study. Further exclusion criteria included poorly controlled hypertension or use of steroid hormones.

Ages of the 36 patients ranged from 35 to 68 years (mean age, 58±2 [SEM] years). Their mean body mass index (BMI) was 26.0±0.6 kg/m². Sixteen patients had a history of CHD. Thirteen patients were under treatment for hypertension, but none were taking β-adrenergic–blocking agents. Any medication used was continued without a change in dosage throughout the study. Twenty-two patients were cigarette smokers at the time of study and continued to smoke throughout.

Another eight patients were recruited who had hypoalphalipoproteinemia and primary moderate hypertriglyceridemia, the latter being defined as fasting plasma triglyceride concentrations between 250 and 500 mg/dL.15,16 Except for the presence of elevated plasma triglycerides in these patients, inclusion and exclusion criteria were identical to those for the group described above. The mean age of the hypertriglyceridemic group was 53±3 years, their BMIs averaged 28.4±2 kg/m², and three of the eight had a history of CHD. None were taking β-adrenergic–blocking agents.

Two additional groups consisting of healthy subjects underwent apolipoprotein-turnover studies and served as control subjects. One group of 24 normal and healthy men served as control subjects for the LDL-turnover studies (see below). Data from several of these men have been reported previously.18 The average age of this control group was 59±2 (mean±SEM) years, and their total cholesterol and LDL cholesterol concentrations were below the 90th percentile for age and sex according to the Lipid Research Clinics Prevalence Study.14 The BMI for the group averaged 23.7±0.6 kg/m². Another group of 20 normal men served as control subjects for apo A-I–turnover measurements (see below). Their ages averaged 56±2 (mean±SEM) years, and their BMIs averaged 23.6±0.7 (mean±SEM) kg/m². The men in this second control group had normolipidemia (total cholesterol <250 mg/dL [mean, 213±6 mg/dL] and triglycerides <250 mg/dL [mean, 99±8 mg/dL] and normal HDL cholesterol levels [>40 mg/dL; mean, 54±3 mg/dL]). The results of these patients as a control group have been presented recently.13 None of the patients in the control groups had a history of CHD, were smokers, or were taking β-adrenergic–blocking agents.

Experimental Design

The study was carried out for a period of 5 weeks. At entrance into the study, patients were instructed to follow a weight-maintenance, solid-food diet consisting of 40% of total calories as fat (18% saturates, 17% monounsaturates, and 5% polyunsaturates), 45% as carbohydrate, and 15% as protein. Daily cholesterol intake averaged 450 mg/dL. Alcohol was proscribed for the duration of the study. This diet has been used
previously in our lipoprotein kinetic studies.\textsuperscript{11,19,20} The patients consumed this diet at home for 3 weeks before entering the metabolic ward. They were instructed to maintain their exercise at a constant level throughout the study. They generally had a sedentary lifestyle, and none were engaged in an exercise program. Previous metabolic studies\textsuperscript{21–23} have shown that plasma lipid and lipoprotein concentrations reach a steady state after 2 weeks of a constant diet.

At the beginning of the third week of the metabolic diet, 100 mL blood was drawn after a 12-hour fast. Subsequent procedures are described in detail in the sections to follow. Briefly, LDL and HDL were isolated from 50 mL plasma by ultracentrifugation, and autologous apo A-I was isolated from HDL. LDL apo B and apo A-I were radioiodinated with \textsuperscript{125}I and \textsuperscript{131}I, respectively. Patients were admitted to the metabolic ward, and the next morning the two isotopic tracers were simultaneously injected by vein. These tracers were injected 1 week after removal of plasma and at least 3 weeks after starting the metabolic diet. Twenty-three blood samples were obtained during the 2 weeks. All samples were collected after patients had been in the sitting position for 10 minutes. Patients began taking supersaturated potassium iodide (0.5 mg/day) 3 days before isotope injection and remained on this dose throughout the entire turnover study. Most of the patients stayed in the metabolic ward for 1 week after injection and then completed the turnover study as outpatients. A minority remained on the metabolic ward for the full 2 weeks. Plasma die-away curves for the two tracers were constructed, and FCRs for each were estimated by multicomartmental analysis (see below). The control subjects for LDL–apo B- and apo A-I–turnover measurements underwent identical studies as the patients with hypoalphalipoproteinemia.

Analysis of Lipid and Lipoproteins

Fresh plasma was preserved with 0.005% gentamicin sulfate, 0.005% chloramphenicol, 0.01% NaN\textsubscript{3}, and 100 IU/mL aprotinin. Total cholesterol and triglycerides were measured enzymatically,\textsuperscript{24,25} and lipoprotein cholesterol levels were determined by Lipid Research Clinics techniques,\textsuperscript{26} except that HDL cholesterol was determined enzymatically. Lipoproteins of \textit{d} < 1.019 g/mL were isolated by preparative ultracentrifugation,\textsuperscript{27} and cholesterol was measured in the supernatant and infranatant. Recoveries of cholesterol exceeded 98%, and corrections were made for recovery. Lipoproteins of \textit{d} < 1.019 g/mL were designated VLDL plus intermediate density lipoprotein (IDL). LDL cholesterol was taken as the difference between total cholesterol and the sum of VLDL+IDL plus HDL cholesterol. In Lipid Research Clinics terminology,\textsuperscript{28} the fraction called "LDL" actually consists of LDL+IDL, whereas in this study, “true” LDL represents lipoproteins of \textit{d} = 1.019–1.063 g/mL.

Procedures for LDL–Apo B Kinetics

For LDL–apo B kinetics, lipoproteins of \textit{d} = 1.019–1.063 g/dL, representing true LDL, were isolated ultracentrifugally from 25 mL plasma according to Lindgren et al.\textsuperscript{27} LDL was resuspended in a salt solution of \textit{d} = 1.065 g/mL and recentrifuged to concentrate and remove any contaminating albumin. The isolated lipoprotein (3 mg LDL protein) was radiolabeled with \textsuperscript{125}I by a modification\textsuperscript{11,28} of the method of McFarlane.\textsuperscript{29} Excess iodine was dialyzed against 150 mM NaCl and 0.27 mM disodium EDTA (pH 7.4). Over 98% of the remaining radioactivity was precipitable by trichloroacetic acid. A mixture of 5% human serum albumin, unlabeled autologous LDL, and 20–45 \textmu Ci \textsuperscript{125}I-LDL was prepared for intravenous injection. The radiolabeled LDL was filtered through a pyrogen-free, sterile filter of 0.22 \mu m before injection. The injection mixture was tested to document the absence of pyrogens by a Limulus assay. One milliliter of the mixture containing 20–30 \mu Ci of \textsuperscript{125}I-LDL was injected intravenously.

Twenty-three blood samples were taken over a 14-day period, and the radioactivity in plasma was measured in each sample. Lipoprotein analyses, as detailed above, were done on samples from days 1, 4, 8, 12, and 14. LDL–apo B levels were also determined on the true LDL fraction (\textit{d} = 1.019–1.063 g/mL). For the latter, LDL cholesterol was determined enzymatically, and LDL protein (apo B) was measured colorimetrically,\textsuperscript{30} as modified for lipoproteins.\textsuperscript{11,31} LDL–apo B concentration was then estimated by multiplying the absolute concentration of LDL cholesterol (as determined above) by the LDL–apo B–to-cholesterol ratio (determined on the isolated LDL fraction). The LDL–apo B concentration used was the average of 5 days' measurements. VLDL+IDL–apo B levels were estimated simultaneously by a similar procedure on the VLDL+IDL fraction. The fraction of \textsuperscript{125}I remaining in plasma was plotted against time to obtain a die-away curve for calculation of FCRs for LDL. The volume of distribution of radiolabeled LDL was estimated by isotope dilution of \textsuperscript{125}I from the sample taken at 10 minutes. The intravascular pool size of LDL apo B was taken as the volume of distribution times the concentration of LDL apo B.

Model Selection for LDL–Apo B Kinetics

The FCRs for LDL apo B were estimated by the two-pool model of Matthews\textsuperscript{32} as described previously\textsuperscript{18}; standard deviations for FCRs were also calculated during curve fitting. This model assumes that LDL is homogeneous in affinity for LDL receptors, the predominant pathway of LDL removal. However, evidence of several types has shown that all LDL particles do not have identical affinities for LDL receptors\textsuperscript{33–36}; it seems likely that the receptor-binding domain of apo B on various LDL particles may have slightly different degrees of exposure to the receptors, and hence, the ability of different particles to bind to receptors will vary somewhat. Consequently, the FCR obtained by the two-pool model represents a "weighted average" of the catabolism of LDL particles.\textsuperscript{37} At present, there are no available methods to isolate and study the multiple different "kinetic" species of LDL, nor would such an attempt be feasible in a sizable number of patients. Nonetheless, previous investigation has revealed that significant metabolic defects in LDL metabolism can be detected by isotope kinetic techniques despite the "kinetic heterogeneity" of LDL. Examples include familial hypercholesterolemia,\textsuperscript{38} in which removal of LDL is retarded, and the hypertriglyceridemic state,\textsuperscript{34} in which catabolism of LDL is accelerated. The LDL-turnover measurements in this study were based on the hypoth-
esis that if significant alterations in LDL metabolism exist in patients with hypoalphalipoproteinemia, then they will be reflected by abnormalities in the FCRs calculated for LDL in the patients; it is our postulate that the presence of an abnormality will be detected despite the kinetic heterogeneity of LDL. In this study, the input rate (transport rate) for LDL apo B was estimated as the product of intravascular pool size and FCR for LDL apo B. The estimated input rate is of course model dependent, and the true input rate could deviate from the estimated value depending on the degree of kinetic heterogeneity of LDL.

**Procedures for Apo A-I Kinetics**

The procedure for estimating turnover rates of apo A-I has been described recently. Briefly, HDL (d = 1.090–1.21 g/mL) was isolated ultracentrifugally from plasma. Isolated HDL was resuspended at its native density and recentrifuged in a TV-865B vertical rotor (Dupont Sorvall, Wilmington, Del.) for 2.5 hours at 65,000 rpm to remove contaminating proteins. An aliquot of purified HDL was used to isolate autologous apo A-I according to the following procedure. HDL containing approximately 9 mg protein was dialyzed against 150 mM NaCl, 10 mM tris(hydroxymethyl)aminomethane (Tris) chloride, and 0.01% disodium EDTA, pH 8.0. The dialyzed solution containing HDL was transferred into a sterile, pyrogen-free vial, and 1.146 mg guanidine hydrochloride was added to bring the solution to 4 M. This solution was incubated for 3 hours at 37°C and then dialyzed against 150 mM NaCl, 10 mM Tris-chloride, and 0.01% EDTA, pH 8.0. After dialysis, the solution was transferred into a sterile 6-mL ultracentrifuge tube, and the density was adjusted to 1.21 g/mL with solid sodium bromide. The solution was overlaid with an equal volume of sodium bromide solution of similar density, and the mixture was subjected to ultracentrifugation at 50,000 rpm for 24 hours in a 30.3 fixed-angle rotor (Beckman Instruments, Palo Alto, Calif.) at 10°C. The bottom 1.5 mL was collected and dialyzed extensively against 150 mM NaCl and 0.01% EDTA, pH 7.4. The protein concentration was determined. The purity of apo A-I was verified immunologically by demonstrating an absence of immunoactivity for apo B, apo A-II, and albumin; on polyacrylamide electrophoresis, no protein bands other than apo A-I were detected.

Approximately 1 mg autologous apo A-I was radiolabeled with 131I. Free iodine was removed by dialysis against 150 mM NaCl, 10 mM Tris-chloride, and 0.01% EDTA, pH 8.0. A mixture of 4% human serum albumin and 25–30 µCi of tracer was injected intravenously. Twenty-three blood samples were collected over 14 days, as described above. Four milliliters of plasma was counted for radioactivity at each time point. Concentrations of plasma apo A-I were determined by a modification of the electroimmunoassay of Laurell. Polycrylamide gel electrophoresis of apo A-I antisera were obtained from Boehringer-Mannheim Corp., Biochemical Products, Indianapolis, Ind. The electrophoresis gel consisted of 1.25% antisera, 2% (wt/vol) agarose (Seakem agarose, 0.16–0.19 relative mobility electroendoosmosis; Marine Colloids Division, FM Corp., Rockland, Me.), 2% (wt/vol) dextran (molecular weight = 70,000; Sigma Chemical Co., St. Louis, Mo.), and a nonbarbital buffer. The standard used was a serum calibrator for apo A-I obtained from Boehringer-Mannheim Diagnostics. This standard was recalibrated in our laboratory by electroimmunoassay using pure apo A-I as the primary standard. Concentrations were calculated from peak heights. The coefficient of variation for each assay was determined; each assay had 5% or less variation within and between assays.

**Model Selection for Apo A-I Kinetics**

FCRs for apo A-I were estimated by the two-pool model of Matthews. As indicated above, this model assumes that plasma apo A-I is homogeneous in its catabolism. However, previous investigators have presented evidence for inhomogeneity in the catabolism of apo A-I. For example, it has been reported that the urine-to-plasma ratio of radioactivity is not constant at every time point after injection of tracer apo A-I, as would be predicted by the two-pool model. This and other evidence has led to the development of various multicompartamental models to account for potential heterogeneity of apo A-I catabolism. One such model, proposed by Zech et al, postulates the existence of two intravascular pools of apo A-I, one of which is rapidly catabolized and the other that decays more slowly. The rapidly catabolized, intravascular pool of apo A-I might be related in part to HDL particles that contain only apo A-I, in contrast to particles having both apo A-I and apo A-II. Indeed, Rader et al recently reported that the apo A-I contained in HDL particles having only apo A-I is catabolized about 15% more rapidly than that in particles having both apo A-I and apo A-II. These findings suggest that the Matthews two-pool model may not be an adequate representation of apo A-I metabolism, although a 15% difference in catabolism of apo A-I in the two types of particles probably would introduce only a small error in the results of a model having only a single intravascular compartment.

Furthermore, in our view, models that assume two (or more) intravascular compartments of apo A-I have not been adequately validated. A major question about the experimental basis of these models is whether the observed kinetics of the isolated tracer are identical to those of the natural traced compound. Several studies suggesting more complex models have employed traces prepared from very small quantities of isolated apo A-I. However, using this labeling technique, Osborne et al were able to separate radioiodinated apo A-I into two fractions; the first was indistinguishable in physical properties from unlabeled apo A-I, whereas the second seemingly was partially denatured. In vivo kinetic studies revealed that the second, partially denatured fraction was catabolized more rapidly than the first, suggesting that it did not reassociate completely with lipoproteins. Results of a similar type have been reported by Patterson and Lee. The method of isolation of apo A-I in the present study differed from those of previous reports in that relatively large amounts were isolated for labeling; this should minimize the overlap and denaturation of apo A-I as may occur when only small quantities of apoprotein are labeled. Indeed, in the report describing our current method, it was shown that the isolated, pure apo A-I decayed at the same rate as apo A-I on whole HDL particles when both were labeled and injected intravenously. Thus, until the
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FIGURE 1. Histogram of fractional catabolic rates (FCRs) for low density lipoprotein apolipoprotein B (LDL apo B) in control subjects and low-HDL patients. The frequency distributions for each are shown. Data for control patients are most consistent with a unimodal distribution, whereas data for low-HDL patients best fit a bimodal distribution (see “Results” section). HDL, high density lipoprotein.

The possibility of introducing artificial isotopic heterogeneity in the tracer has been ruled out, we suggest that the two-pool model is adequate, and in fact, may be more valid than those models that assume heterogeneity in apo A-I catabolism. Since apo A-I rapidly equilibrates among the various lipoprotein fractions, it seems likely that apo A-I is largely homogeneous in its kinetics. Indeed, Atmeh et al were not able to detect a difference in catabolism of apo A-I on HDL having apo A-I only versus that on HDL having apo A-I and apo A-II. It is probable that the rates of exchange are much more rapid than the rates of decay of the two types of particles. If so, the two-pool model would be the appropriate model for estimating apo A-I kinetics. In this study, the input rate for apo A-I was calculated by multiplying the intravascular pool size of apo A-I by FCR. The former was estimated as the product of plasma apo A-I concentration and the volume of distribution of labeled apo A-I, as determined by the 10-minute sample.

Statistical Analysis

Means of each variable were compared between each group by analysis of variance. Because of skewed distributions and unequal variances for some variables, non-parametric tests were implemented. To compare the three groups, the Kruskal-Wallis test was performed. Multiple comparisons were made with the Mann-Whitney U test, using the Bonferroni inequality to adjust for multiplicity of testing (α=0.0167).

To compare distribution functions for FCRs for LDL apo B in patients and control subjects, the Kolmogorov-Smirnov two-sample test was used. A bimodality coefficient for each of these distributions was computed. The bimodality coefficient is

\[ b = \frac{(m_3^2+1)[(n-3)^2]}{[m_4+3(n-1)^2(n-2)(n-3)]} \]

where \( m_3 \) is skewness (asymmetry) and \( m_4 \) is kurtosis (peakedness or flatness). The maximum value for \( b \), 1.0, which is obtained for the Bernoulli distribution, represents a population with only two distinct values. Values of \( b \) greater than 0.555 (the value for a uniform population) are most consistent with bimodal or multimodal marginal distributions. Values of \( b \) less than 0.555 suggest a unimodal distribution in the population. When a bimodal distribution was observed, the cutpoint between the two populations was determined by inspection. For each population, a normal distribution curve was constructed on the basis of the calculated mean±SD.

Results

The primary question under consideration in this study was whether most patients with low HDL cholesterol levels have concomitant defects in their LDL metabolism that could reflect an abnormality in triglyceride metabolism. Therefore, the FCRs for LDL apo B

<table>
<thead>
<tr>
<th>Group</th>
<th>Total number</th>
<th>Number with CHD</th>
<th>Number of smokers</th>
<th>Age (years, mean±SEM)</th>
<th>BMI (kg/m², mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-HDL groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower FCRs for LDL</td>
<td>13</td>
<td>6</td>
<td>8</td>
<td>60±3</td>
<td>26.4±1.0</td>
</tr>
<tr>
<td>Higher FCRs for LDL</td>
<td>23</td>
<td>10</td>
<td>9</td>
<td>55±2</td>
<td>26.4±0.5</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>8</td>
<td>3</td>
<td>5</td>
<td>53±3</td>
<td>28.4±1.7</td>
</tr>
</tbody>
</table>

HDL, high density lipoprotein; CHD, coronary heart disease; BMI, body mass index; FCRs, fractional catabolic rates; LDL, low density lipoprotein.
in the current patients were compared with those of 24 healthy men of normal weight with normal plasma lipids (Figure 1). The FCRs for low-HDL patients suggested a bimodal distribution. The bimodality coefficient for the low-HDL group ($b=0.877$) was statistically significant ($p<0.004$), indicating bimodality of distribution. The cutpoint between the two populations was determined by inspection to be an FCR of 0.36 pool/day. Thirteen patients fell into the population having lower FCRs (mean, 0.300±0.009 pool/day), whereas 23 patients were in the population with higher FCRs (mean, 0.328±0.028 pool/day). Control subjects had a distribution of FCRs (mean, 0.328±0.028 pool/day) that was consistent with a normal (unimodal) distribution ($b=0.383$). The standard deviations for FCRs for LDL apo B were calculated for individual patients as described under "Methods." For the low-HDL group, the standard deviations for FCRs averaged ±0.011 ±0.007, whereas those for the control group averaged ±0.008±0.006. These low values support the validity of the distributions presented (Figure 1). A detailed comparison of these groups is presented below.

The demographic characteristics of the two subgroups of low-HDL patients are shown in Table 1; their data are compared with the characteristics of the group of patients with hypertriglyceridemia. Although low-HDL patients with lower FCRs for LDL were somewhat older than subjects in the other two groups, the differences were not statistically significantly. On the average, patients in these three groups had somewhat higher BMIs than control patients (see "Methods" section). Percentages of study subjects having established CHD in the three groups were similar to each other.

Levels of plasma lipids and lipoprotein cholesterol for all groups are presented in Table 2. HDL cholesterol levels in the three patient groups were abnormally low and significantly below those of the control groups. In low-HDL patients with lower FCRs for LDL, levels of total cholesterol were similar to those of the control group undergoing LDL turnover studies; concentrations of triglycerides and VLDL+IDL cholesterol in these patients likewise were similar to those of the same control group. Low-HDL patients with higher FCRs for LDL in contrast had relatively higher triglyceride levels, and VLDL+IDL cholesterol levels were significantly above those of low-HDL patients with lower FCRs for LDL and the two control groups. Still, VLDL+IDL cholesterol levels in low-HDL patients with higher FCRs for LDL were not as high as those in the hypertriglyceridemic group.

Concentrations of apo B in total plasma, VLDL+IDL, and LDL are given in Table 3 for low-HDL patients and LDL-turnover control subjects. LDL cholesterol/apo B ratios also are presented. Low-HDL patients having higher FCRs for LDL and hypertriglyceridemic patients both had significantly higher levels of total apo B than the control group but not higher than the other low-HDL group. VLDL+IDL–apo B levels were significantly lower than both control groups: $p<0.001$.

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma total (mg/dL, mean±SEM)</th>
<th>Lipoprotein cholesterol (mg/dL, mean±SEM)</th>
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<tbody>
<tr>
<td></td>
<td>Chol</td>
<td>TG</td>
</tr>
<tr>
<td>Low-HDL groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower FCRs for LDL</td>
<td>223±8</td>
<td>124±10</td>
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<tr>
<td>Higher FCRs for LDL</td>
<td>231±10</td>
<td>166±8‡</td>
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<td>Hypertriglyceridemia</td>
<td>239±11</td>
<td>392±40‡</td>
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<tr>
<td>Normal subjects</td>
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<td></td>
</tr>
<tr>
<td>LDL–apo B–turnover control subjects</td>
<td>212±5</td>
<td>140±12</td>
</tr>
<tr>
<td>Apo A-I–turnover control subjects</td>
<td>213±6</td>
<td>99±8</td>
</tr>
</tbody>
</table>

Chol, cholesterol; TG, triglyceride; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; FCR, fractional catabolic rate; apo, apolipoprotein.

*Significantly lower than both control groups: $p<0.001$.
†Significantly higher than both control groups: $p<0.001$.
‡Significantly higher than the low-HDL group with normal LDL FCR.

Table 3. Concentrations of Apolipoprotein B-100

<table>
<thead>
<tr>
<th>Group</th>
<th>Apolipoprotein B-100 (mg/dL, mean±SEM)</th>
<th>LDL-chol/apo B (mean±SEM)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>VLDL+IDL</td>
</tr>
<tr>
<td>Low-HDL groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower FCRs for LDL</td>
<td>121±5</td>
<td>18±1</td>
</tr>
<tr>
<td>Higher FCRs for LDL</td>
<td>126±5*</td>
<td>25±2†</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>131±5*</td>
<td>37±3†</td>
</tr>
<tr>
<td>LDL–apo B–turnover control subjects</td>
<td>107±3</td>
<td>15±2</td>
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VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; chol, cholesterol; apo, apolipoprotein; HDL, high density lipoprotein; FCR, fractional catabolic rate.

*Significantly higher than control: $p<0.005$.
†Significantly higher than control and low-FCR groups: $p<0.001$. 

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higher in low-HDL patients with higher FCRs for LDL than in either the control or the other low-HDL group; hypertriglyceridemic patients likewise had high VLDL+IDL–apo B levels. There were no differences among the four groups in either LDL–apo B levels or LDL cholesterol/apo B ratios. The latter ratios appeared to be lower in hypertriglyceridemic patients than those of other groups, although the differences were not statistically significant.

The kinetic parameters for LDL–apo B in the two low-HDL groups are compared to hypertriglyceridemic and normal subjects in Table 4. The low-HDL group with higher FCRs for LDL had increased input rates for LDL apo B, whereas the low-HDL group with lower FCRs for LDL had normal input rates. Kinetic parameters in hypertriglyceridemic patients resembled those of low-HDL patients having higher FCRs for LDL. Both FCRs and input rates were abnormally high in hypertriglyceridemic patients. There was not a significant correlation between FCRs for LDL and HDL cholesterol levels for the entire low-HDL group, nor were these correlations significant for either low-HDL group.

Table 5 presents kinetic data for apo A-I. The two normotriglyceridemic groups with low HDL concentrations as well as the hypertriglyceridemic group had decreased levels of apo A-I compared with control. Of these three groups, average FCRs for apo A-I were significantly higher than those of the control group. In contrast, input rates for apo A-I in the three patient groups were not significantly different from those of normal control subjects.

Discussion

This investigation revealed that a group of normotriglyceridemic patients with low HDL cholesterol levels had two different patterns of kinetics of LDL apo B. In approximately two thirds of the patients, the FCRs for LDL apo B were abnormally high, whereas in about one third, FCRs were similar to those of normal men of the same age. This observation raises the possibility that low concentrations of HDL cholesterol may occur by different mechanisms and could have a different clinical significance. When considering the reasons for reduced HDL levels in the current patients, it may be of interest to compare their data with those of patients with distinct hypertriglyceridemia; patients of the latter type have been previously shown to have abnormalities in kinetics of both apo A-I and LDL apo B. In the discussion to follow, therefore, we will consider and compare lipoprotein kinetics in the three study groups: 1) primary hypertriglyceridemia, 2) normotriglyceridemia with low HDL levels and higher FCRs for LDL, and 3) normotriglyceridemia with low HDL levels and lower FCRs for LDL.

Hypertriglyceridemic Patients

A low level of HDL cholesterol frequently is present in patients with hypertriglyceridemia. This association appears to result from two factors: 1) exchange of triglyceride and cholesterol ester between HDL and TGRLPs, resulting in cholesterol-poor HDL particles, and 2) enhanced clearance of apo A-I, seemingly reducing the number of circulating HDL particles. Another common abnormality observed in hypertriglyceridemic patients is an increased FCR for LDL apo B. This latter abnormality typically is accompanied by an increased rate of formation of LDL particles. The latter could arise in either of two ways: 1) by an increased hepatic secretion of apo B-containing lipo-

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<th>Table 4. Kinetic Parameters of LDL Apolipoprotein B</th>
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<td>Group</td>
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<tr>
<td>Low-HDL groups</td>
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<tr>
<td>Lower FCRs for LDL</td>
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<td>Higher FCRs for LDL</td>
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<tr>
<td>Hypertriglyceridemia</td>
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<tr>
<td>Normal subjects</td>
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<tr>
<td>LDL–apo B turnover control subjects</td>
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<td>Values are expressed as mean±SEM.</td>
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<tr>
<td>—Significantly higher than control group; p&lt;0.01.</td>
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<td>†Significantly higher than control; p&lt;0.001.</td>
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<th>Table 5. Kinetic Parameters of Plasma Apolipoprotein A-I</th>
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<td>Group</td>
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<td>Normal subjects</td>
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<tr>
<td>Apo A-I turnover control subjects</td>
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<td>FCR, fractional catabolic rate; HDL, high density lipoprotein; LDL, low density lipoprotein; apo, apolipoprotein.</td>
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<tr>
<td>*Significantly lower than control; p&lt;0.01.</td>
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<tr>
<td>†Significantly higher than control; p&lt;0.01.</td>
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<tr>
<td>‡Significantly higher than the low-HDL group with lower FCRs for LDL; p&lt;0.001.</td>
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proteins that are precursors of LDL (i.e., VLDL and IDL) or 2) by a decrease in direct removal of these precursors, allowing for more of them to be converted to LDL. We have postulated previously that a decreased direct removal of precursor lipoproteins is the more likely mechanism because of the associated high FCR for LDL; to wit, if fewer VLDL particles are removed directly by hepatic LDL receptors, then more receptors should be available for uptake of LDL and hence, a consequent higher FCR for LDL. On the other hand, if secretion rates of apo B-containing lipoproteins were increased, hepatic lipoprotein receptors should be relatively "saturated" by precursor lipoproteins, and FCRs for LDL should be either normal or decreased. The former mechanism thus appears to be more consistent with the observed high FCRs for LDL, although increased secretion of VLDL apo B cannot be ruled out with certainty. Potential mechanisms for the decrease in direct hepatic uptake of VLDL and IDL will be discussed below. The hypertriglyceridemic patients of the current study consistently revealed the kinetic findings reported previously; i.e., they had abnormally high FCRs for both apo A-I and LDL apo B. Therefore, should similar abnormalities in lipoprotein kinetics be noted in normotriglyceridemic patients with low HDL levels, this would suggest the presence of an underlying defect in triglyceride metabolism.

Normotriglyceridemia, Low HDL Levels, and Higher FCRs for LDL

Twenty-three patients of the current study had the lipoprotein pattern of this type. The coexistence of low HDL cholesterol levels and higher FCRs for LDL in this group resembled the pattern observed in patients with primary hypertriglyceridemia, despite the fact that triglyceride levels were in the normal range. Even so, this group had higher levels of triglycerides, VLDL+IDL cholesterol, and VLDL apo B than both control groups, and these relatively high levels again are consistent with a defect in metabolism of TGRLPs. Once more, we propose that the abnormally high FCRs for LDL in this group were due to defective direct removal of TGRLPs, resulting in a greater conversion of the latter to LDL; still, an increased secretion of VLDL apo B leading to increased flux of LDL apo B cannot be ruled out with certainty.

Recently, our laboratory reported apolipoprotein kinetic studies in a group of moderately obese patients having primary hypertriglyceridemia. Before undergoing kinetic studies, these patients had lost weight and thereby had nearly normalized their plasma triglyceride concentrations. Of note, apolipoprotein kinetics were examined after weight reduction and after reestablishment of a new steady state in body weight at a normal level. Despite a fall in triglycerides with weight loss, FCRs for both apo A-I and LDL apo B remained abnormally high. Since these patients were previously hypertriglyceridemic, the persistence of abnormalities in apolipoprotein kinetics after normalization of body weight suggested that the defect in TGRLP metabolism was not completely corrected by weight reduction. The kinetics of apolipoproteins in our current group having higher FCRs for LDL in many ways resembled those of the previous patients after weight loss, except that average triglyceride levels in the current patients were somewhat lower. Along the same lines, Kissebah et al have reported on LDL kinetics in a group of normolipidemic patients with non–insulin-dependent diabetes mellitus (NIDDM); in these patients, FCRs for LDL were markedly raised. Certainly, abnormalities in triglyceride metabolism are common in NIDDM patients, and these abnormalities could be present in latent form even when triglyceride levels are in the normal range. Patients with NIDDM often have low HDL cholesterol levels as well, and abnormally high FCRs for apo A-I have been reported. Recently Austin, Krauss, and associates described a syndrome characterized by high-normal triglycerides, low HDL cholesterol, and small, dense LDL particles. Patients with this syndrome were said to have an "atherogenic lipoprotein phenotype" because they seemingly were at increased risk for CHD. These workers have speculated that this syndrome results from a defect in triglyceride metabolism. McNamara et al and Campos et al have described a similar lipoprotein pattern in patients at risk for CHD. Our current patients having higher FCRs for LDL in many ways resemble those with the atherogenic lipoprotein phenotype, although we did not carry out a thorough study of their LDL particle sizes to define the LDL pattern. It is not unreasonable to speculate that a common defect in metabolism of TGRLPs, yet to be defined, accounts for the abnormalities in LDL and HDL metabolism in our patients as well as in those described by the aforementioned workers.

Finally, what mechanisms might explain the abnormalities in apolipoprotein metabolism in this group of patients? In our view, most likely are defects in the catabolism of TGRLPs; such abnormalities are consistent with the high-normal levels of triglycerides and VLDL+IDL cholesterol noted in these patients. One possible catabolic defect is a decreased activity of lipoprotein lipase. Although a decreased activity of lipoprotein lipase might be expected to markedly raise triglyceride levels, several studies have shown that many patients who are heterozygous for lipoprotein lipase deficiency do not manifest hypertriglyceridemia; nonetheless, these individuals frequently have low HDL cholesterol levels. Several other studies suggest that low HDL cholesterol levels are directly correlated with a low activity of lipoprotein lipase. Just why a reduced activity of lipoprotein lipase would retard direct removal of TGRLPs by the liver and secondarily promote conversion of VLDL to LDL is not entirely clear. One possibility may relate to the findings of Felts et al that lipoprotein lipase attaches to chylomicron remnants and to VLDL remnants and may serve as the signal for the liver to recognize these lipoproteins. Recently, Beisiegel et al have shown that lipoprotein lipase enhances the binding of chylomicron remnants to the LDL-related protein, a potential receptor for apo E–containing lipoproteins. If a similar mechanism exists for VLDL remnants and if lipoprotein lipase activity is low, direct uptake of VLDL remnants by the liver might be impaired, and more VLDL would be converted to LDL.

What might be responsible for low HDL cholesterol levels in patients with an increased flux of LDL? Schwartz et al have reported that a sizeable fraction of HDL cholesterol ester is transferred to LDL, and this
transfer might be enhanced in the presence of a high flux of LDL. When the flux rate of LDL is increased, each LDL particle probably carries less cholesterol ester and, hence, may be a better acceptor for HDL cholesterol ester. Thus, a high flux of LDL may "bleed" cholesterol ester away from HDL and thereby 1) lower the HDL cholesterol level and 2) destabilize HDL particles to promote catabolism of apo A-I. If so, the high flux of LDL could play a direct role in lowering the HDL level.

**Normotriglyceridemia, Low HDL, and Lower FCRs for LDL**

This group differed from the previous one in that it did not have high FCRs for LDL. Concentrations of triglycerides and VLDL+IDL cholesterol furthermore were not increased compared with controls, and these levels were lower than those of low-HDL patients with high FCRs for LDL. Despite these differences, patients of this group still had reduced concentrations of HDL cholesterol and apo A-I and high FCRs for apo A-I. Differences in LDL metabolism between the two groups raise the possibility that low HDL cholesterol levels in the group with lower FCRs for LDL did not have the same origins as in the previous group. There is no evidence that normal-FCR patients had a defect in TGRLP metabolism, at least as reflected by levels of triglycerides or VLDL+IDL cholesterol or by FCRs for LDL.

The question might be raised whether patients in this category could have an abnormality in the metabolism of chylomicrons that was not reflected in the metabolism of VLDL+IDL or LDL. Previously, Patsch et al. reported that there is an inverse correlation between postprandial triglyceride concentrations and HDL cholesterol levels over a broad range of HDL concentrations. On the other hand, Cohen and Grundy recently examined the specific question of whether normotriglyceridemic men with distinctly low HDL cholesterol levels have an abnormally high response in postprandial triglycerides after consumption of a high-fat meal. In fact, the data showed that only two of 22 patients with low HDL levels had an abnormal postprandial lipemic response to the high-fat meal; it was thus concluded that only rarely do normotriglyceridemic men with low HDL levels have such a response. Therefore, low HDL concentrations in patients with lower FCRs for LDL probably cannot be explained by postprandial hypertriglyceridemia.

What might cause low HDL cholesterol levels that are independent of a defect in the metabolism of apo B-containing lipoproteins? A strong candidate for such a metabolic defect is an increased activity of hepatic triglyceride lipase. A high activity of hepatic triglyceride lipase has been implicated by several investigators as a cause of low HDL levels. A proposed mechanism is that the surface-coat phospholipids of larger HDL particles, i.e., HDL2, are a substrate for hepatic triglyceride lipase, and a high hepatic triglyceride lipase activity thus would promote catabolism of HDL particles. Enzymatic degradation of HDL2 could account for the increased clearance rate for apo A-I as well. This mechanism would not be expected to be accompanied by high levels of triglycerides or VLDL+IDL cholesterol, consistent with the results in the patients of this group. On the basis of previous studies, a high activity of hepatic triglyceride lipase seems the most likely defect in this group of patients, but we cannot rule out an alternate mechanism, namely, a high level of cholesterol ester transfer protein. For example, a rise in cholesterol ester transfer protein has been reported to explain the decrease in HDL cholesterol levels in patients treated with probucol. To date, however, elevated cholesterol ester transfer protein levels have not been reported in patients with isolated low HDL cholesterol concentrations.

**Conclusions**

In summary, this study shows two patterns of LDL metabolism in patients with low HDL cholesterol levels. In one of these, FCRs (and flux rates) of LDL apo B were elevated, and when this pattern was present, levels of triglycerides and VLDL+IDL cholesterol (and apo B) were higher than in control subjects. These abnormalities suggest a defect in metabolism of TGRLPs. Other patients with low HDL cholesterol levels, however, had a relatively normal catabolism of LDL and normal concentrations of TGRLPs; these patients probably have metabolic defects other than an abnormal catabolism of TGRLPs. An increased activity of hepatic triglyceride lipase appears to be the most likely cause of this category of defect, although a high cholesterol ester transfer protein level is another possibility. Recognition of these two different patterns of LDL metabolism in patients with low HDL levels seems important, because ultimately the most effective therapy for each type could differ and may depend on correcting the underlying abnormality.

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Two patterns of LDL metabolism in normotriglyceridemic patients with hypoalphalipoproteinemia.

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