Metabolic Basis of Primary Hypercholesterolemia

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Background. Hypercholesterolemia is a well-established risk factor for coronary heart disease. However, the mechanisms underlying hypercholesterolemia, elevated low density lipoprotein (LDL) in particular, are not well understood. To determine these mechanisms, we studied LDL kinetics in a group of men with primary hypercholesterolemia.

Methods and Results. LDL kinetics in 134 middle-aged men with high-risk levels of LDL cholesterol (more than 160 mg/dl) were compared with kinetics in 16 men with borderline high-risk levels of LDL cholesterol (120–159 mg/dl) and 14 men with heterozygous familial hypercholesterolemia (FH). Patients with primary hypercholesterolemia (non-FH) were further divided into moderate hypercholesterolemia (LDL cholesterol, 160–210 mg/dl; n=108) and severe hypercholesterolemia groups (LDL cholesterol, more than 210 mg/dl; n=26). Four factors contributed to increasing LDL cholesterol concentrations above the borderline range to moderately elevated levels: 37 patients had no increase in LDL apolipoprotein (apo) B levels but had abnormally high LDL cholesterol–to–apo B ratios; 14 patients had very low fractional catabolic rates (FCRs) for LDL, similar to FH patients; 35 patients had FCRs for LDL in the borderline range but high production rates for LDL; and 22 patients had a high flux of LDL (high production rates and high FCRs). In general, patients with severe hypercholesterolemia resembled those with moderate LDL elevations, except that their LDL particles were enriched with cholesterol.

Conclusions. Data from the present study reveal that there are several distinct patterns of LDL metabolism responsible for primary hypercholesterolemia. These patterns can serve as the basis for further investigation to determine the molecular defects responsible for each pattern. (Circulation 1991;84:118–128)

Hypercholesterolemia is a well-established risk factor for coronary heart disease. Recently, the National Cholesterol Education Program (NCEP) identified low density lipoprotein (LDL) cholesterol as the primary target of therapy in patients with hypercholesterolemia.1 A high-risk LDL cholesterol level was defined as a serum level of more than 160 mg/dl, which is present in approximately 25% of adult US men. A borderline high-risk LDL cholesterol level encompasses the range of 130–159 mg/dl and is present in approximately 40% of adult US men. The mechanisms responsible for high-risk LDL cholesterol levels are not well understood, although primary hypercholesterolemia is in general believed to be the result of reduced activity of LDL receptors.2

In the present study, LDL metabolism causing high-risk LDL cholesterol levels was examined using tracer kinetics. Patients with primary forms of moderate and severe hypercholesterolemia were compared with subjects with borderline high-risk levels of LDL cholesterol. The purpose of the study was to identify factors that increase LDL cholesterol concentrations to the high-risk range rather than to the borderline high-risk zone. Among those with high-risk levels, patients with moderate and severe hypercholesterolemia were compared so that factors determining the severity of hypercholesterolemia could be defined.

Methods

Patients

Patients in the present study comprised three groups. The largest group included 134 men with primary hypercholesterolemia in whom clinical evi-
idence of heterozygous familial hypercholesterolemia (FH) was lacking (see below). The second and third groups comprised 14 men with clinical findings of heterozygous FH and 16 men with borderline high levels of LDL cholesterol, respectively. The patients were selected from the patient populations of the Veterans Administration Medical Centers (VAMCs) in Dallas and Bonham, Tex., the University of Texas Southwestern Medical Center at Dallas, and the Aerobics Center and Institute for Aerobics Research, Dallas, Tex. The patients underwent LDL turnover studies during the past 8 years in either the metabolic ward at the Dallas VAMC or the General Clinical Research Center (GCRC), Parkland Memorial Hospital, Dallas. All patients gave written informed consent to participate in the study, which was approved by appropriate institutional review boards. The characteristics of each group are reviewed.

**Primary hypercholesterolemia (non-FH).** The patients in this group were selected by the following criteria. Before entry, they were shown to have estimated LDL cholesterol levels of 160 mg/dl or more, as defined by the NCEP.2 Estimated LDL cholesterol was calculated as follows: total cholesterol minus triglycerides/5 minus high density lipoprotein (HDL) cholesterol. According to Lipid Research Clinics methodology,3 estimated LDL cholesterol corresponds closely to cholesterol in lipoproteins of 1.006–1.063-mg/dl density. Strictly speaking, this density range consists of LDL plus intermediate density lipoprotein (IDL); thus, the true LDL cholesterol level (1.019–1.063-mg/dl density) will be somewhat lower than estimated LDL cholesterol. Before recruitment into this group, estimated LDL cholesterol levels of 160 mg/dl or more were confirmed by taking the average of three determinations obtained while the patient consumed an ad libitum diet. The patients were further divided into those with primary moderate or severe hypercholesterolemia, with the distinction between the two being an estimated LDL cholesterol level of approximately 210 mg/dl (true LDL cholesterol, 200 mg/dl). This distinction was made on the basis of LDL cholesterol levels obtained during the turnover study (see below). All patients had plasma triglyceride concentrations averaging less than 250 mg/dl on three determinations; this value was considered to be the upper limit of the normal range.4

Patients were excluded from the study if they had a history of endocrine disorders, liver dysfunction, renal insufficiency, nephrotic syndrome, diabetes mellitus, or other disorders that cause secondary hypercholesterolemia. None of the patients had classic stigmata for heterozygous FH (i.e., plasma cholesterol levels consistently more than 350 mg/dl, tendon xanthomas, or family history of the same).5 FH was not ruled out by tissue culture studies. None of the patients had taken hypolipidemic drugs for at least 3 months before entering the study, and none of the patients had ever taken probucol.

Mean age of patients in this group was 55.5±12 (±SD) years (range, 30–70 years). Sixty-seven patients had a history of coronary artery disease as manifest by history of angina pectoris, myocardial infarction, or coronary artery surgery, the latter two occurring at least 6 months before entry into the study. Twenty-eight patients had a history of hypertension. Patients with coronary artery disease or hypertension were receiving a variety of drugs for these conditions, the doses of which were not altered during the study. Data from 38 of the 134 patients have been previously presented elsewhere.6–8

**Borderline high LDL cholesterol group.** The patients in this group had estimated LDL cholesterol concentrations ranging from the 25th to the 75th percentile for men 40–54 years old of the Lipid Research Clinics Prevalence Study.9 These percentiles essentially correspond to estimated LDL cholesterol levels of 120–159 mg/dl. Because these values are similar to those defined as borderline high–risk LDL cholesterol by the NCEP1 (i.e., levels of 130–159 mg/dl), patients in this group were designated as having borderline high LDL cholesterol, and the entire group was called the “borderline group.” Mean estimated LDL cholesterol for this group was 137±16 mg/dl; this mean value approximates the 50th percentile for those in the age range of the group, and although it may be slightly lower than the average borderline high–risk LDL cholesterol level for the general middle-age population,1 the designation “borderline” appears to be reasonable. None of the patients in this group had a history of angina pectoris or myocardial infarction. Their average age was 55±12 years. Subjects were generally healthy and not receiving medication. Data from 12 of these men have been previously published elsewhere.10

**Heterozygous FH.** This group contained 14 male patients (mean age, 43±11 years) who were considered to have heterozygous FH. This diagnosis was made on the basis of a history of severe hypercholesterolemia and, in most patients, tendon xanthomas, very premature coronary heart disease, and a strong family history of hypercholesterolemia, tendon xanthomas, and/or premature coronary heart disease.5 The patients were not tested for abnormal binding of LDL to cultured skin fibroblasts. The data for these patients have been previously presented elsewhere.11

**Experimental Design.**

Three weeks before starting the LDL turnover study, the patients were instructed to consume a diet consisting of 40% of total calories as fat (18% saturates, 17% monounsaturates, and 5% polyunsaturates), 45% as carbohydrates, and 15% as protein. Daily intake of cholesterol averaged 300–400 mg/day. The diet was composed mainly of solid foods and has been previously described.6–8 During the third week on this diet, patients underwent a 1-unit plasmapheresis. The plasma obtained was used to isolate autologous true LDL, which was radioiodinated with 125I. The patients were admitted to the metabolic
ward of the Dallas VAMC or the Parkland Memorial Hospital GCRC. They were started on 0.5 g/day potassium iodide, 3 days before injection, and they continued the intake throughout the study. Autologous 125I LDL was injected into the patients 5 days after plasmapheresis. Patients remained on the metabolic ward for 2–3 days after injection, during which multiple blood samples were obtained. Thereafter, most patients completed their study as outpatients while remaining on the same diet for the entire study. In the outpatient setting, fasting blood samples were obtained each morning. Some patients remained on the metabolic ward for the duration of the study after injection of labeled LDL.

**LDL Apolipoprotein B Kinetics**

For determination of fractional catabolic rates (FCRs) and input rates (transport rates) for LDL apolipoprotein (apo B), approximately 200 ml of plasma was collected by plasmapheresis for isolation of LDL. Lipoproteins of 0.109–1.063 g/dl densities, designated true LDL, were isolated by the method of Lindgren et al.12 LDL was resuspended in a salt solution of 1.065 g/ml density and centrifuged to concentrate and remove any contaminating albumin. The lipoprotein was radiolabeled with 125I using the method of McFarlane,13 as adapted for use with lipoproteins.14,15 Excess iodine was removed by extensive dialysis against 150 mM NaCl and 0.27 mM disodium EDTA (pH 7.4). An injection mixture containing 5% human serum albumin and unlabeled autologous LDL was prepared; 20–45 μCi 125I LDL was injected intravenously. Blood was collected into vacuum containing tubes with disodium EDTA, and plasma was isolated for counting. Twenty-three blood samples were collected during the 14-day period of the turnover study, and radioactivity was measured in each sample of plasma. Lipoprotein analyses, detailed below, were carried out on samples obtained on days 1, 4, 8, 12, and 14 of the study. These analyses included determination of LDL apo B levels (see below).

Fraction of injected radioactivity remaining in plasma was plotted as a function of time, and the resulting curve in disappearance of labeled LDL from plasma was used to calculate FCRs for LDL. The curves were consistently biexponential, and they were assumed to conform to a two-pool model16; according to this model, input and exit of LDL occurred from an intravascular pool that was in equilibrium with an extravascular pool. The FCR was estimated by simulation analysis. The size of the intravascular pool of LDL apo B was determined by multiplying the concentration of LDL apo B by the plasma volume. The method for estimating LDL apo B concentration is described below. The plasma pool size for LDL apo B was estimated by isotope dilution from the sample taken at 10 minutes. The input rate (transport rate) for LDL apo B was estimated as the product of pool size and FCR for LDL apo B. The input of LDL is generally believed to be derived from catabolism of triglyceride-rich lipoproteins. Irreversible exits of LDL from plasma can occur via LDL receptor or nonreceptor pathways, the former usually predominating.2 Experience has shown that the FCR for LDL apo B is a reflection of availability of LDL receptors, although this relation does not pertain under all circumstances.

**Plasma Lipid and Lipoproteins**

Plasma concentrations of total cholesterol, triglycerides, and lipoprotein cholesterol were measured enzymatically as described previously.15 Total cholesterol17 and triglycerides18 were determined on whole plasma. HDL cholesterol was measured after precipitation of apo B-containing lipoproteins in whole plasma with heparin manganese.19 Another aliquot of plasma was used to isolate very low density lipoprotein (VLDL) and IDL (density, less than 1.019 g/dl) by ultracentrifugation. Cholesterol was determined in the isolated 1.019 g/ml supernatant and plasma infranatant. Corrections were made for total recoveries that were consistently more than 96%. LDL cholesterol was calculated as the difference between 1.019 g/ml infranatant cholesterol and HDL cholesterol.

**Determination of LDL Apolipoprotein B Concentration**

The true LDL fraction (density, 1.019–1.063 g/ml) was isolated by preparative ultracentrifugation from the five samples selected for lipoprotein analysis. The cholesterol content was determined enzymatically and protein (apo B) was measured by the Lowry method (Lowry et al20), as modified by Markwell et al21 and described in detail previously.22 The absolute plasma level of LDL apo B was 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 taken for each patient represented the mean of the five measurements.

**Phenotyping of Apolipoprotein E**

Phenotyping of apo E was performed on 84 patients according to the methods of Weisgraber et al23,24 and Rall et al.25 Isolated VLDL and IDL were separately treated with three reagents—β-mercaptoethanol,23,24 cysteamine,23,24 or neuraminidase.25 The lipoprotein fraction was delipidated, and the apolipoprotein was resolubilized for isoelectric focusing. Isoforms of apo E were determined by the change in isoelectric point after treatment with cysteamine,23,24 and heterozygosity for apo E isoforms was confirmed after desialylation with neuraminidase.25

**Detection of Familial Defective Apolipoprotein B-100**

Plasma samples of 100 patients with primary hypercholesterolemia were sent to the Gladstone Foundation Laboratories for Cardiovascular Disease, University of California San Francisco, where it was analyzed for familial defective apo B-100. Binding of patients’ LDL to normal cultured fibroblasts was examined by Dr. Thomas Innerarity,26 and DNA was
tested for the presence of a CGG-to-CAG transformation in codon 3500, causing a glutamine-for-arginine substitution, by Dr. Brian J. McCarthy. These 100 patients are part of a larger study, the results of which are presented elsewhere.

Statistical Analysis

Multivariate analyses of different groups and subgroups were carried out using the Newman-Keuls methods for multiple comparisons.

Results

LDL apo B concentrations are plotted in Figure 1 against input rates for hypercholesterolemic (non-FH) patients and for the borderline group. A significant correlation was not observed between concentrations and input rates for LDL apo B for hypercholesterolemic patients as a group. Many patients had input rates in the range of those of borderline patients, whereas others had elevated input rates. In Figure 2, plasma concentrations of LDL apo B are plotted against FCRs for LDL apo B. Horizontal and vertical lines are drawn arbitrarily to enclose the ranges of the borderline group. The upper cutoff level for LDL apo B concentration was set at 110 mg/dl. FCRs for LDL apo B in the borderline group ranged from 0.25 to 0.40 pools per day. This range is similar to values previously obtained for normal middle-aged men by other investigators. On the basis of these cutoff points, the patients were first divided into those in whom concentrations of LDL apo B were in the borderline range (less than 110 mg/dl) and then into those with elevated LDL apo B concentrations (110 mg/dl or more). The latter were next subcategorized into those with low FCRs for LDL (less than 0.25 pools per day), those with midrange FCRs (0.25–0.40 pools per day), and those with high FCRs (more than 0.40 pools per day). Finally, these latter three categories were further divided into patients with moderate hypercholesterolemia (true LDL cholesterol, less than 200 mg/dl) and patients with severe hypercholesterolemia (true LDL cholesterol, 200 mg/dl or more). The characteristics of each of these groups are detailed below.

The essential data related to LDL concentrations and kinetics for the different groups are abstracted in Table 1, which lists concentrations of true LDL cholesterol and LDL apo B, FCRs and input rates for LDL apo B, and LDL cholesterol-to–apo B ratios.

Detailed characteristics of and results for the 16 men with borderline high–risk LDL cholesterol (borderline group) and the 14 men with heterozygous FH are listed (Table 2). Characteristics of and results for the 108 patients with primary moderate hypercholesterolemia and the 26 patients with primary severe hypercholesterolemia are also listed (Tables 3 and 4, respectively). Values are given for age, body mass indexes, plasma total lipids (cholesterol and triglycerides), lipoprotein cholesterol (VLDL plus LDL, true LDL, and HDL), estimated LDL cholesterol, LDL apo B levels, LDL cholesterol–to–apo B ratios, and LDL apo B kinetics (plasma pool sizes, FCRs, and input rates). LDL apo B input rates are expressed as absolute rates (mg/day) and, after normalization, as rates per kilogram of body weight (mg/kg · day).

Compared with the borderline group, FH patients had markedly elevated concentrations of LDL cholesterol and LDL apo B (Table 1). LDL cholesterol–to–apo B ratios also were significantly higher in FH patients. The higher LDL apo B concentrations in FH patients were almost entirely results of a reduction in FCRs; input rates for LDL apo B for the two groups were not statistically different. Very high LDL cholesterol levels in FH heterozygotes were the result not only of high LDL apo B levels resulting from low FCRs for LDL but also of high LDL cholesterol–to–apo B ratios.
TABLE 2. Low Density Lipoprotein Kinetics for Borderline Group and Patients With Heterozygous Familial Hypercholesterolemia

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>LDL apo B kinetics</th>
<th>LDL cholesterol-to-apo B ratio</th>
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<tbody>
<tr>
<td></td>
<td>True LDL cholesterol (mg/dl)</td>
<td>Concentration (mg/dl)</td>
</tr>
<tr>
<td>Borderline (n)</td>
<td>129±15</td>
<td>91±14</td>
</tr>
<tr>
<td>Heterozygous FH (14)</td>
<td>263±52*</td>
<td>159±30*</td>
</tr>
<tr>
<td>Primary hypercholesterolemia Moderate (108)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL apo B &lt;110 mg/dl (37)</td>
<td>165±12*</td>
<td>103±6*</td>
</tr>
<tr>
<td>Low FCR (14)</td>
<td>186±10*</td>
<td>126±9*</td>
</tr>
<tr>
<td>Midrange FCR (35)</td>
<td>176±15†</td>
<td>125±12†</td>
</tr>
<tr>
<td>High FCR (22)</td>
<td>177±12*</td>
<td>125±9*</td>
</tr>
<tr>
<td>Severe (26)</td>
<td>222±21‡</td>
<td>135±16</td>
</tr>
<tr>
<td>Midrange FCR (8)</td>
<td>217±14‡</td>
<td>135±18</td>
</tr>
<tr>
<td>High FCR (4)</td>
<td>216±10‡</td>
<td>130±12</td>
</tr>
</tbody>
</table>

Values are mean±SD.
LDL, low density lipoprotein; FCR, fractional catabolic rates; apo B, apolipoprotein B; FH, familial hypercholesterolemia.
*p<0.001 versus borderline.
†p<0.05 versus group with LDL apo B <110 mg/dl.
‡p<0.05 versus borderline group.
§p<0.001 versus moderate low FCR.
¶p<0.001 versus moderate midrange FCR.
¶p<0.005 versus moderate high FCR.

Essential results for patients with primary moderate hypercholesterolemia (true LDL cholesterol, less than 200 mg/dl) are also given in Table 1. Their data are compared statistically with those of the borderline group. The first subgroup (37 patients) had LDL apo B concentrations less than the cutoff point of 110 mg/dl, even though their estimated LDL cholesterol levels were more than the lower limit for high-risk LDL cholesterol (i.e., 160 mg/dl). Although their LDL apo B levels were somewhat higher than the average values for borderline subjects, their elevated LDL cholesterol concentrations were mainly results of high LDL cholesterol-to–apo B ratios. Their LDL apo B kinetics (FCRs and input rates) were not significantly different from those of borderline patients.

Among the remaining 71 patients with moderate hypercholesterolemia, 14 had reduced FCRs for LDL apo B (less than 0.25 pools per day); these low FCRs were mainly responsible for the patients’ high levels of LDL cholesterol. The average input rate for LDL apo B in this subgroup did not differ significantly from that of borderline patients. LDL cholesterol-to–apo B ratios were neither increased nor decreased for this group; none of the patients had ratios of less than 1.25, which is often seen in patients with hypertriglyceridemia or hyperapobetalipoproteinemia. Two of the 14 patients in this group were found to have familial defective apo B-100 (3500 mutation).

Thirty-five other patients with moderate hypercholesterolemia had midrange FCRs for LDL (0.25–0.40 pools per day). The body mass indexes in this subgroup were significantly higher than those of borderline subjects (Table 2). Compared with the latter, input rates also were significantly elevated, which in large part explains their higher LDL cholesterol level. Their FCRs and LDL apo B–to–cholesterol ratios were on
TABLE 3. Low Density Lipoprotein Kinetics in Primary Moderate Hypercholesterolemia

<table>
<thead>
<tr>
<th></th>
<th>LDL apo B &lt;110 mg/dl</th>
<th>LDL apo B &gt;110 mg/dl</th>
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<tbody>
<tr>
<td></td>
<td>Low FCR</td>
<td>Midrange FCR</td>
</tr>
<tr>
<td>Patients (n)</td>
<td>37</td>
<td>14</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>56±13</td>
<td>60±9</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.3±3.2</td>
<td>25.1±2.5</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>245±14†</td>
<td>258±23†</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>164±55</td>
<td>145±48</td>
</tr>
<tr>
<td>VLDL plus IDL cholesterol (mg/dl)</td>
<td>40±17</td>
<td>35±22</td>
</tr>
<tr>
<td>True LDL cholesterol (mg/dl)</td>
<td>165±12†</td>
<td>186±10†</td>
</tr>
<tr>
<td>Estimated LDL cholesterol (mg/dl)</td>
<td>172±13†</td>
<td>192±10†</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>41±9</td>
<td>37±8</td>
</tr>
<tr>
<td>LDL apo B (mg/dl)</td>
<td>103±6†</td>
<td>126±9†</td>
</tr>
<tr>
<td>Pool size (mg/pool)*</td>
<td>3,326±399</td>
<td>4,201±536$\ddagger$</td>
</tr>
<tr>
<td>FCR (pools per day)*</td>
<td>0.31±0.07</td>
<td>0.21±0.03$\dagger$</td>
</tr>
<tr>
<td>Input rate (mg/day)*</td>
<td>1,022±250</td>
<td>877±194</td>
</tr>
<tr>
<td>Input rate (mg kg/day)*</td>
<td>128±3.3</td>
<td>11.0±2.1</td>
</tr>
<tr>
<td>LDL cholesterol-to-apo B ratio</td>
<td>1.62±0.17†</td>
<td>1.48±0.11</td>
</tr>
</tbody>
</table>

Values are mean±SD.
LDL, low density lipoprotein; FCR, fractional catabolic rates; apo B, apolipoprotein B; FH, familial hypercholesterolemia.

*Pool size, FCRs, and input rates are shown for LDL apo B.
$\dagger$p<0.001, significantly different from borderline group shown in Table 2.
$\ddagger$p<0.01, significantly different from borderline group shown in Table 2.
§p<0.05, significantly different from group with LDL apo B <110 mg/dl.

average similar to those of borderline subjects. They also had FCRs similar to those of hypercholesterolemic subjects with LDL apo B levels of less than 110 mg/dl, but their LDL apo B concentrations were higher because of somewhat higher input rates. They further differed from this latter group in that their LDL apo B-to-cholesterol ratios were not increased. Six patients in this subgroup had LDL cholesterol-to-apo B

TABLE 4. Low Density Lipoprotein Kinetics in Primary Severe Hypercholesterolemia

<table>
<thead>
<tr>
<th></th>
<th>LDL apo B &gt;110 mg/dl</th>
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<tbody>
<tr>
<td></td>
<td>Low FCR</td>
</tr>
<tr>
<td>Patients (n)</td>
<td>14</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>60±5</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.5±3.5</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>301±29†</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>157±62</td>
</tr>
<tr>
<td>VLDL plus IDL cholesterol (mg/dl)</td>
<td>37±18</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>222±21†</td>
</tr>
<tr>
<td>Estimated LDL cholesterol (mg/dl)</td>
<td>228±22†</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>44±11</td>
</tr>
<tr>
<td>LDL apo B (mg/dl)</td>
<td>135±16</td>
</tr>
<tr>
<td>Pool size (mg/pool)*</td>
<td>4,258±607</td>
</tr>
<tr>
<td>FCR (pools per day)*</td>
<td>0.22±0.01</td>
</tr>
<tr>
<td>Input rate (mg/day)*</td>
<td>944±154</td>
</tr>
<tr>
<td>Input rate (mg kg/day)*</td>
<td>12.6±2.5</td>
</tr>
<tr>
<td>LDL cholesterol-to-apo B ratio</td>
<td>1.65±0.14†</td>
</tr>
</tbody>
</table>

Values are mean±SD.
LDL, low density lipoprotein; FCR, fractional catabolic rates; apo B, apolipoprotein B; FH, familial hypercholesterolemia.

*Pool size, FCRs, and input rates are shown for LDL apo B.
$\dagger$p<0.001, significantly different from low FCR group (Table 2).
$\ddagger$p<0.01, significantly different from midrange FCR group (Table 2).
§p<0.05, significantly different from midrange FCR group (Table 2).
$||p<0.05$, significantly different from high FCR group (Table 2).
$\dagger$§p<0.005, significantly different from high FCR group (Table 2).
ratios equal to or less than 1.25. Average LDL levels and kinetic parameters for these six patients were LDL cholesterol, 163±11 (±SD) mg/dl; LDL apo B, 137±16 mg/dl; LDL cholesterol-to-apo B ratio, 1.20±0.07; FCR for LDL, 0.33±0.05 pools per day; and input rate for LDL, 17.1±1.5 mg · kg/day.

Twenty-two additional patients with moderate hypercholesterolemia had elevations in both input rates and FCRs for LDL apo B compared with borderline subjects. Furthermore, these patients had significantly higher triglyceride levels than borderline subjects (Table 3). Their average LDL cholesterol-to-apo B ratios were neither increased nor decreased compared with those of the borderline group; however, two patients in this subgroup had a ratio of less than 1.25.

Table 1 lists essential data for 26 patients with severe hypercholesterolemia (true LDL cholesterol, 200 mg/dl or more). Their values are compared statistically with those of patients with moderate hypercholesterolemia of the corresponding FCR category. Unless otherwise designated, values for the same parameters between each pair of subgroups were not statistically different. The majority of patients with severe hypercholesterolemia (i.e., 54%) had low FCRs for LDL. Besides having higher total cholesterol and LDL cholesterol levels, they primarily differed from patients with moderate hypercholesterolemia by having significantly higher LDL cholesterol-to-apo B ratios. The same was essentially true for the smaller subgroups with midrange and high FCRs. None of the patients had low ratios (1.25 or less). Thus, a higher LDL cholesterol-to-apo B ratio was the major factor responsible for higher LDL cholesterol levels in patients with severe hypercholesterolemia compared with those with moderate hypercholesterolemia, regardless of FCRs for LDL apo B. Two patients in the subgroup with low FCRs were found to have familial defective apo B-100 (3500 mutation).26–28

Apo E phenotypes were determined in 84 patients with both types of hypercholesterolemia. Their frequencies are compared with those reported by Havel43 for the general population (values in parentheses). Percentages of phenotypes were distributed as follows: E-4:E-4, 1% (3%); E-3:E-3, 45% (55%); E-2:E-2, 0% (1%); E-4:E-3, 33% (26%); E-4:E-2, 1% (3%); and E-3:E-2, 20% (12%). Hypercholesterolemic patients of this study appeared to not have an overall enrichment of phenotypes containing E-4. Examination of subgroups did not reveal a selective increase of either E-4 or other E isoforms. Of the 100 patients who were tested for familial defective apo B-100, four unrelated patients were found to have this abnormality.

Discussion

The primary purpose of the present study was to determine the general mechanisms responsible for high-risk LDL cholesterol (termed “primary hypercholesterolemia” in this article and defined as an estimated LDL cholesterol level of more than 160 mg/dl). In the present study, hypercholesterolemia was present while patients consumed a “typical” American diet (i.e., a diet relatively high in saturated fatty acids and cholesterol). This diet resembled that consumed by free-living Americans, and levels of LDL cholesterol and parameters of LDL apo B metabolism thus obtained should be representative of hypercholesterolemic, middle-aged US men. We compared hypercholesterolemic patients with subjects who had borderline high–risk LDL cholesterol levels. Our main purpose was to explain increases of LDL cholesterol above the borderline range, not those above the desirable range or a hypothetical “normal” level. Primary hypercholesterolemia was further divided into moderate and severe forms, and metabolic parameters obtained for these two forms were compared with one another and with patients with heterozygous FH. Next, we separately consider mechanisms for borderline high LDL cholesterol, moderate hypercholesterolemia, and severe hypercholesterolemia.

Borderline High LDL Cholesterol

The NCEP1 defined borderline high–risk LDL cholesterol as a serum level in the range of 130–159 mg/dl. In the present study, one group of subjects had LDL levels essentially in this range and thus served as the reference group. According to epidemiologic studies,44–47 cholesterol levels in the borderline zone increase risk for coronary heart disease above that in the desirable range. Furthermore, in the United States, more adult men have borderline high LDL than any other defined range of LDL cholesterol.1,9 Therefore, mechanisms responsible for borderline increases of LDL cholesterol are of great interest and public health significance. At least three factors have been identified as increasing LDL cholesterol levels to the borderline zone in middle-aged Americans: relatively high intakes of cholesterol and saturated fatty acids,48 the unexplained increase in serum cholesterol with age,9 and obesity.49–51 High intakes of cholesterol52 and saturated fatty acids53–55 appear to depress LDL receptor activity; enrichment of liver cells with dietary cholesterol directly suppresses synthesis of LDL receptors,52 but mechanisms whereby saturated fatty acids reduce activity of LDL receptors are not known. The increase of LDL cholesterol with aging also appears to result in large part from a decrease in LDL receptor activity,10,56 although the precise mechanism is not understood. Finally, the high prevalence of obesity among middle-aged Americans probably contributes to higher LDL levels.58–60 A high intake of cholesterol and saturated fatty acids in many obese people will suppress LDL receptor activity, but obesity can also stimulate overproduction of apo B–containing lipoproteins,57,58 which should increase LDL concentrations. Therefore, a diminished LDL receptor activity appears to be the major factor, although perhaps not the only factor, responsible for borderline high LDL levels; in the present study, we attempted to determine the
extent to which this also is true of patients with LDL cholesterol levels in the high-risk range.

**Primary Moderate Hypercholesterolemia**

Patients in the present study with borderline high concentrations of LDL cholesterol had FCRs for LDL averaging 0.31 pools per day. As indicated previously, this average value was relatively low because of a low activity of LDL receptors secondary to diet and age. One mechanism for development of high-risk LDL cholesterol concentrations could be still further suppression of LDL receptor activity. If this is the major factor responsible for the incremental increase of LDL cholesterol levels above the borderline zone, still lower FCRs for LDL might be expected. Because of the widely held belief that hypercholesterolemia is in large part a result of reduced activity of LDL receptors, it might be expected that the majority of patients with moderate hypercholesterolemia would have a further reduction in FCRs for LDL. This was not observed, however, and the characteristics of different patterns of LDL kinetics for this group will therefore be considered.

**Low FCRs for LDL.** Among patients with moderate hypercholesterolemia and an LDL apo B level of more than 110 mg/dl, 13% had low FCRs for LDL, defined as FCRs of less than 0.25 pools per day (Table 1). A few hypercholesterolemic patients with LDL apo B concentrations less than 110 mg/dl also had low FCRs for LDL (Figure 2). Other patients had FCRs between 0.25 and 0.30 pools per day, but as shown in Figure 2, this range overlaps with several subjects with borderline high LDL cholesterol; hence, it cannot be called "reduced" by current criteria. High-risk levels of LDL cholesterol in patients with reduced FCRs were almost exclusively results of this change; on average, the patients did not have increased LDL apo B inputs or high LDL cholesterol-to–apo B ratios. Two mechanisms can be visualized for the reduced FCRs of LDL in this subgroup, both related to decreased receptor-mediated clearance of LDL. First, the patients could have a still greater suppression of LDL receptor activity, possibly because of a metabolic suppression of LDL receptor synthesis. Alternatively, they could have defective apo B-100 such that LDL interacted poorly with LDL receptors. Two patients in this subgroup were found to have familiar defective apo B-100, with a glutamine-for-arginine substitution at position 3500 of apo B, a defect that interferes with its binding to receptors. A few other patients in this series were previously noted to have unusually slow clearance of LDL compared with normal LDL; they did not have the 3500 mutation, but they may have had other mutations in apo B-100. Thus, poor binding of LDL to receptors could be one cause for low FCRs for LDL. Therefore, additional investigations will be required to distinguish the various causes for low FCRs for LDL and their relative frequencies.

**Midrange FCRs for LDL.** One third of patients with moderate hypercholesterolemia had high concentrations of both LDL apo B and LDL cholesterol and midrange FCRs for LDL (0.25–0.40 pools per day). Thus, compared with borderline levels, high plasma LDL cholesterol in this subgroup cannot be explained by low FCRs. Presumably, these patients did not have suppression of LDL receptor activity beyond that of the borderline group. Instead, their higher LDL cholesterol levels appeared to be secondary to increased input rates for LDL apo B. High input rates for LDL may be explained in part by obesity because on average, this subgroup was heavier than borderline patients; being overweight apparently stimulates the production of apo B–containing lipoproteins, leading ultimately to increased input of LDL apo B. Of course, this subgroup could also have genetic causes for high production of lipoproteins that were not present in the borderline group. Regardless, these patients appeared to have two cholesterol-increasing factors: suppression of LDL receptor activity, increasing LDL cholesterol levels to the borderline zone, and enhanced input of LDL, increasing the level to the moderately high range.

In this subgroup, six patients had unusually low LDL cholesterol-to–apo B ratios (1.25 or less). Consequently, their LDL apo B level was disproportionately elevated compared with LDL cholesterol, and these patients can be said to have hyperapobetalipoproteinemia. Previously, Teng et al reported LDL turnover data in five patients with hyperapobetalipoproteinemia; four of these had midrange FCRs for LDL, and one had a high FCR. However, in contrast to our patients, most of their patients had hypertriglyceridemia. According to Sniderman et al hyperapobetalipoproteinemia occurs only in the absence of hypercholesterolemia, although moderate increases in LDL cholesterol, such as were found in this subgroup, have been allowed in their definition. Thus, by their criteria, our six patients had hyperapobetalipoproteinemia. The latter appears to be a common pattern in patients with familial combined hyperlipidemia, which has also been reported to show midrange FCRs for LDL. Therefore, approximately 20% of our patients with moderate hypercholesterolemia and midrange FCRs for LDL had hyperapobetalipoproteinemia, and detailed family studies might have shown them to have familial combined hyperlipidemia.

**High FCRs for LDL.** An additional 20% of patients with moderate hypercholesterolemia had a pattern of high input rates as well as high FCRs for LDL apo B. Reasons for hypercholesterolemia for this subgroup are not entirely clear, but again they may be related to two factors. First, these patients probably had a relatively low activity of LDL receptors based on their age and diet. However, in addition, they appeared to have a defect in lipoprotein metabolism leading to a high flux of LDL, which further increased LDL cholesterol. Two possibilities for a high flux of LDL can be considered. First, hepatic secretion of apo B–containing lipoproteins could be in-
increased. However, if the total number of lipoproteins entering the circulation were to be increased, a relatively low FCR for LDL would be expected because hepatic LDL receptors should already be overloaded with precursor lipoproteins (i.e., VLDL and IDL). An alternate possibility is that direct removal of these precursor lipoproteins was reduced, possibly because of a defect in VLDL composition that interfered with its binding to receptors. If a defect of this type exists, more VLDL should be converted to LDL, a change that would account for the increased input of LDL; in addition, decreased removal of precursor lipoproteins should leave more LDL receptors available for removal of LDL.

Therefore, an increased availability of LDL receptors could explain high FCRs for LDL. We suggest that this mechanism most likely accounts for the pattern of high input rates and high FCRs for LDL apo B noted in this subgroup. The incremental increase in LDL apo B levels compared with those of the borderline group might then be explained by the relatively poor affinity of LDL for LDL receptors compared with precursor lipoproteins. In other words, LDL particles have a lower affinity for LDL receptors than VLDL; thus, when VLDL is converted to LDL rather than being removed directly, the LDL apo B should increase. Of course, this reasoning represents speculation, and other mechanisms for high flux rates for LDL are conceivable. The nature of the defect in metabolism or composition of VLDL that could account for reduced direct removal of precursor lipoproteins and increased conversion of VLDL to LDL remains to be determined. However, the fact that patients in this subgroup had relatively high triglyceride levels suggests that they had a defect in VLDL metabolism. Of interest, only two patients in this group had a low LDL cholesterol-to-apo B ratio, which is indicative of hyperapoB48 lipoproteinemia. Whether high FCRs for LDL are commonly found in hypercholesterolemic patients from families with familial combined hyperlipidemia remains to be determined.

Overloading of LDL particles with cholesterol. Apparently, a final reason for development of moderate hypercholesterolemia was an overloading of LDL particles with cholesterol. This mechanism appeared to be responsible for high LDL cholesterol levels in an additional one third of patients in this group. These patients did not have elevated LDL apo B concentrations but instead had increased LDL cholesterol-to-apo B ratios. These high ratios were unique among the subgroups with moderate hypercholesterolemia (Table 1). Their FCRs for LDL were essentially identical to those of the borderline group, as were their input rates for LDL apo B. The reason for overloading of LDL particles with cholesterol, presumably with cholesterol ester, is not readily apparent. Overloading could theoretically reflect an increased activity of either cholesterol ester transfer protein or lecithin cholesterol acyl transferase, although no data are available to examine these possibilities. This general mechanism underlying a high LDL cholesterol level has not been widely recognized, and it deserves further investigation.

Primary Severe Hypercholesterolemia

A true LDL cholesterol level of more than 200 mg/dl, which we define as severe hypercholesterolemia, was present in only 20% of patients with elevated LDL cholesterol. This concentration, equal to an estimated LDL cholesterol level of approximately 210–215 mg/dl, corresponds to the 95th percentile for middle-aged US men. In the general population, this level also occurs in approximately 20% of individuals who have high-risk LDL cholesterol. Our findings in patients with severe hypercholesterolemia should thus be representative of middle-aged men with LDL cholesterol levels higher than the 95th percentile. Because only one in 500 people has heterozygous FH, most middle-aged men with LDL cholesterol levels exceeding the 95th percentile do not have FH. In accord, our patients classified as having primary severe hypercholesterolemia did not have heterozygous FH by classic criteria. In general, their levels of total cholesterol, LDL cholesterol, and LDL apo B were lower than those of FH heterozygotes.

Of interest, more than half of the patients in this group had low FCRs for LDL apo B (FCRs, less than 0.25 pools per day). Thus, low FCRs, whether resulting from low activity of LDL receptors or defective apo B-100, appeared to be an important factor responsible for primary severe hypercholesterolemia. On the other hand, the kinetic parameters for LDL apo B in this group were not significantly different from those of corresponding low-FCR patients with moderate hypercholesterolemia. The factor that transformed them into the severe category was a significantly higher LDL cholesterol-to-apo B ratio. In other words, not only did these patients have low FCRs, but their LDL particles were overloaded with cholesterol. The same phenomenon was responsible for severe hypercholesterolemia in the relatively few patients with midrange FCRs and high FCRs. Again, these latter subgroups did not differ in LDL apo B kinetics from the corresponding category with moderate hypercholesterolemia, but instead their LDL particles were overloaded with cholesterol.

These findings in severely hypercholesterolemic patients further emphasize the need to examine the causes of accumulation of excess cholesterol in LDL particles. One cause might be a relatively low FCR for LDL, because the FH heterozygotes also had cholesterol-enriched LDL (Table 1). However, the fact that a subgroup of moderately hypercholesterolemic patients with midrange FCRs and all categories with severe hypercholesterolemia had high LDL cholesterol-to-apo B ratios speaks against the concept that a low FCR is the only factor responsible for overloading LDL particles with cholesterol. The possibility that these patients had abnormalities in activity of cholesterol ester transfer protein or lecithin...
cholesterol acyltransferase is intriguing and worth further exploration.

Conclusions

Results from the present study show that primary hypercholesterolemia can result from several different causes. A suppression of LDL receptor activity, secondary to high intakes of saturated fatty acids and cholesterol and the unexplained increase of LDL with age, appears to drive the LDL level into the borderline high zone in many people. Additional factors are apparently required to increase levels to the high-risk zone in approximately 25% of middle-aged US men. In some individuals, further suppression of LDL receptor activity appears responsible. In others, high inputs of LDL apo B were responsible; in some of these people, high input rates may be related to obesity, but in others, defects in metabolism of precursor lipoproteins (VLDL and IDL) are probably responsible. Finally, one of the more interesting findings of the present study was that enrichment of LDL particles with cholesterol is a significant factor contributing to hypercholesterolemia, particularly in patients with severe elevations of LDL cholesterol.

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