Characterization of a New Form of Inherited Hypercholesterolemia
Familial Recessive Hypercholesterolemia


Abstract—We previously described a Sardinian family in which the probands had a severe form of hypercholesterolemia, suggestive of familial hypercholesterolemia (FH). However, low density lipoprotein (LDL) receptor activity in fibroblasts from these subjects and LDL binding ability were normal. The characteristics of the pedigree were consistent with an autosomal recessive trait. Sitosterolemia and pseudohomozygous hyperlipidemia were ruled out. A second Sardinian kindred with similar characteristics was identified. Probands showed severe hypercholesterolemia, whereas their parents and grandparents were normolipidemic. FH, familial defective apoprotein (apo) B, sitosterolemia, and cholesteryl ester storage disease were excluded by in vitro studies. We addressed the metabolic basis of this inherited disorder by studying the in vivo metabolism of LDL in 3 probands from these 2 families. $^{125}$I-LDL turnover studies disclosed a marked reduction in the fractional catabolic rate (0.19 ± 0.01 versus 0.36 ± 0.03 pools per day, respectively; P < 0.001) and a significant increase in the production rate [20.7 ± 4.4 versus 14.0 ± 2.4 mg·kg$^{-1}$·d$^{-1}$, respectively; P < 0.01] of LDL apoB in the probands compared with normolipidemic controls. We then studied the in vivo biodistribution and tissue uptake of $^{99m}$technetium-labeled LDL in the probands and compared them with those in normal controls and 1 FH homozygote. The probands showed a significant reduction in hepatic LDL uptake, similar to that observed in the FH homozygote. A reduced uptake of LDL by the kidney and spleen was also observed in all patients. Our findings suggest that this recessive form of hypercholesterolemia is due to a marked reduction of in vivo LDL catabolism. This appears to be caused by a selective reduction in hepatic LDL uptake. We propose that in this new lipid disorder, a recessive defect causes a selective impairment of LDL receptor function in the liver. (Arterioscler Thromb Vasc Biol. 1999;19:802-809.)

Key Words: hypercholesterolemia ■ genetics ■ LDL turnover ■ LDL receptor ■ apoB

Several conditions have been identified causing severe primary hypercholesterolemia.$^1$ The best characterized are familial hypercholesterolemia (FH) and familial defective apoB (FDB). FH is an autosomal dominant disease caused by mutations in the LDL receptor (LDLR) gene.$^2$ The LDLR is a cell surface transmembrane protein that mediates the specific uptake of plasma LDL. Receptor defects markedly impair LDL catabolism, and FH patients typically show severe elevations of plasma LDL cholesterol (LDL-C), tendon xanthomata, and premature coronary atherosclerosis.$^3$ FDB is a genetic disorder caused by point mutations in the gene encoding for apoB100 that reduces the binding ability of LDL particles to the LDLR; as a consequence, LDL removal is reduced, and affected individuals develop high plasma cholesterol concentrations.$^4$ Two other rare, autosomal recessive diseases, sitosterolemia and cholesteryl ester storage disease (CESD), can result in severe hypercholesterolemia. Sitosterolemia is characterized by an accumulation of plant sterols, mainly $\beta$-sitosterol, in the plasma,$^5$ whereas CESD results from a massive deposition of cholesteryl esters in most tissues due to a deficient lysosomal acid lipase (LAL) activity.$^6$

We recently described an unusual pedigree in which several cases of severe primary hypercholesterolemia and/or premature sudden death were present.$^7$ The probands showed the clinical features of FH homozygotes, including severely elevated plasma LDL-C, tuberous and tendon xanthomata, and premature atherosclerosis. However, the LDLR activity measured in skin fibroblasts was normal, as was LDL binding ability. Haplotype segregation analysis further excluded in-
volvement of the LDLR and apoB genes in the pathogenesis of the disease. Consanguinity, absence of vertical transmission, and bimodal distribution of plasma cholesterol levels in the kindred were consistent with an autosomal recessive trait.

In this report, we describe 2 other subjects with similar characteristics belonging to a second kindred, and we investigated the metabolic mechanisms underlying this lipid disorder. To this aim, the in vivo kinetic parameters and the tissue distributions of radiolabeled, autologous LDL were evaluated in 3 probands from the 2 kindreds. Our results demonstrate that this form of hypercholesterolemia is due to a marked reduction in LDL catabolism caused by a selective reduction of LDL uptake by the liver. We hypothesize that a recessive defect in these patients selectively causes an impairment of LDLR function in the liver.

Methods

Patients

Table 1 summarizes the clinical and biochemical characteristics of the subjects included in this study. The 2 probands of kindred No. 1 (G.F.C. and J.C.) have been previously described in detail.7 Probands of kindred No. 2 (A.P. and G.P.) are of Sardinian origin and were referred to the Lipid Clinic of the University of Rome for evaluation of primary hypercholesterolemia (ranging between 400 and 600 mg/dL) and tendon xanthomata. A.P. had exertional angina, and a coronary angiogram showed 3-vessel disease; G.P. had no clinical signs of coronary atherosclerosis. Neither showed abnormal liver or endocrine function tests. At the time of the LDL turnover study, average TC and LDL-C values were 502 and 446 mg/dL, respectively.

Genetic Analysis

Genomic DNA Preparation

Blood samples from probands A.P. and G.P. were collected in 10-mL tubes containing Na-EDTA and stored at -20°C. Genomic DNA was extracted by the salting-out method16 and stored in Tris-EDTA buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.0).

Cell Culture Studies

LDLR Activity Assay

In vitro LDLR activity was determined in probands A.P. and G.P. by evaluating the specific 125I-LDL internalization and degradation in confluent cultured fibroblasts from skin biopsies, as previously described.7

Cellular Metabolism of Cholesterol

Intracellular accumulation of [3H]cholesterol esters was evaluated after incubation of skin fibroblasts of proband G.P. with recombinant [3H]cholesterol-enriched human LDL ([3H]rLDL). Skin fibroblasts from a normolipidemic subject and an LDLR-negative FH homozygote were used as controls. [3H]rLDL was prepared according to Brown et al.9 In brief, 20 μCi of [3H]cholesterol linoleate was dried under an N2 stream and resuspended in 200 μL of acetone. Two milliliters of lipoprotein-deficient serum (LPDS) was added as a source of cholesterol ester transfer protein, and the acetone was evaporated under N2. Blood samples from probands A.P. and G.P. were collected in 10-mL tubes containing Na-EDTA and stored at -20°C. Genomic DNA was extracted by the salting-out method16 and stored in Tris-EDTA buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.0).

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Table 1. Clinical and Biochemical Characteristics of Probands, FH Homozygote, and Control Subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age, y</th>
<th>Sex</th>
<th>BMI</th>
<th>TC, mg/dL</th>
<th>TG, mg/dL</th>
<th>LDL-C, mg/dL</th>
<th>HDL-C, mg/dL</th>
<th>LDLR Activity*</th>
<th>FDB</th>
<th>β-Sitosterolemia</th>
<th>CESD</th>
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<tr>
<td>Kindred No. 1</td>
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<td></td>
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<tr>
<td>GFC</td>
<td>29</td>
<td>M</td>
<td>23.1</td>
<td>581</td>
<td>86</td>
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<td>56</td>
<td>80</td>
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<td>NE</td>
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<tr>
<td>JC</td>
<td>26</td>
<td>F</td>
<td>19.6</td>
<td>434</td>
<td>84</td>
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<tr>
<td>AP</td>
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<td>F</td>
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<tr>
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<td>M</td>
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<tr>
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<td>M</td>
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<tr>
<td>27.5 (9.7)</td>
<td>4M, 1F</td>
<td>24.4 (1.3)</td>
<td>210.0 (49.1)</td>
<td>88.2 (24.8)</td>
<td>132.7 (52.3)</td>
<td>49.0 (12.0)</td>
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<td>NE</td>
<td>NE</td>
<td>NE</td>
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</tr>
<tr>
<td>Controls (2) (n=12)</td>
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<tr>
<td>58.5 (7.1)</td>
<td>10M, 2F</td>
<td>25.4 (3.9)</td>
<td>233.7 (53.1)</td>
<td>197.6 (80.1)</td>
<td>153.7 (44.1)</td>
<td>46.5 (16.5)</td>
<td>NE</td>
<td>NE</td>
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</table>

*BMI indicates body mass index; TC, total cholesterol; TG, total triglycerides; and NE, not evaluated.

125I-LDL uptake and degradation are % of normal values. FDB refers to the apoB 3500 mutation. LDLR activity refers to the apoB 3500 mutation. LDL-C reflects cholesterol concentration in the 1.019 to 1.063 g/mL density fraction. Controls (1) and (2) are control groups for 125I-LDL and 99mTc-LDL metabolic studies, respectively. Data for control subjects are reported as mean±SD.

*Plasma lipid values are those at the time of diagnosis. During the LDL turnover study, average TC and LDL-C values were 502 and 446 mg/dL, respectively.

In conclusion, the findings of this study suggest that the LDLR and apoB genes are involved in the pathogenesis of familial hypercholesterolemia in this Sardinian kindred. These patients were found to have a recessive defect in LDL catabolism caused by a selective reduction in LDL uptake by the liver. The specific defect in LDLR activity in these patients selectively causes an impairment of LDLR function in the liver.

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Familial Recessive Hypercholesterolemia

LDLR Gene Analysis
A combination of polymerase chain reaction (PCR), single-strand conformation polymorphism (SSCP), and direct sequencing was used to screen the LDLR gene in probands from kindred No. 2. The promoter region and each exon of the LDLR gene were amplified from genomic DNA by using 25–bp primers, and the fragment was analyzed by the SSCP method. In brief, 0.1 μg genomic DNA was amplified in 20 μl (final volume) containing 1.75 mmol/L of each oligonucleotide primer, 50 mmol/L of each dNTP, 50 mmol/L KCl, 1.5 mmol/L MgCl2, 10 mmol/L Tris-HCl (pH 8.3), 10 μCi of [32P]dCTP (3000 Ci/mmole), and 2 U of Taq DNA polymerase. Each PCR was subjected to 30 cycles at 94°C for 1 minute and 68°C for 5 minutes in a thermocycler (Perkin Elmer Cetus). One microliter of the PCR mixture was diluted in 30 μL of formamide dye (98% vol/vol; 50 mmol/L Na-EDTA, 0.01% wt/vol xylene cyanol, and 0.01% wt/vol bromophenol blue) and boiled for 5 minutes. A 3–μL aliquot was subjected to electrophoresis (250 V for 16 hours at room temperature) on a 6% to 7% nondenaturing gel in 2X Tris/borate/EDTA and 10% glycerol (EM Science). Each gel was dried and sequenced on an adenine G-50, Pharmacia) and 0.1 mol/L NaHCO3 in saline (pH 8). The serum sterol fraction was subjected to gas-liquid chromatographic analysis, as reported. Measurements of LAL activity in the difference between the 1.019 g/mL infranatant cholesterol and 1.019 g/mL infranatant fractions, and the concentrations were corrected for weight.

Detection of Defective Apo B-100
Classic FDB (apoB 3500) was detected by PCR andMspI restriction enzyme digestion as previously described. Direct sequencing of 1000 bases around codon 3500 of the apoB gene was also performed by following standard methods. The ability of LDL to bind to the LDLR was further examined by an in vitro cell surface binding assay, as previously described. In brief, LDL from proband G.P. was isolated and used in competition experiments on normal human fibroblasts against 2 mg/mL of 125I-LDL obtained from normal subjects.

In Vivo Study of LDL Metabolism
125I-LDL Turnover Study
LDL turnover studies were performed at the University of Rome (Rome, Italy) for probands J.C. and A.P. and at the National Institutes of Health (Bethesda, Md) for proband G.F.C. and following similar protocols. The studies had been approved by the appropriate ethics committees at both institutions. In brief, LDL (1.019 to 1.063 g/mL) was isolated from 30 to 50 mL of blood by sequential ultracentrifugation, and ~2 to 4 mg of dicycylized LDL was radiiodinated by the McFarlane method as adapted for lipoproteins. The LDL preparation was sterilized by filtration through a 0.22-mm Millipore filter and tested for pyrogenicity by a procedure of Lee. The LDL preparation was sterilized by filtration through a 0.22-mm Millipore filter and tested for pyrogenicity by a procedure of Lee.12

Biodistribution of 99mTc-LDL
Native LDL preparations were radiolabeled with 99mTc after reduction of technetium atoms with sodium dithionite, as previously described. In brief, 1 to 2 mg LDL was mixed with 40 to 50 mCi of 99mTc pertechnetate solution; 0.1 mL of 0.5 mol/L glycine buffer, pH 10, containing 10 mg sodium dithionite was then added. The mixture was gently mixed and incubated for 30 minutes at room temperature. After incubation, 99mTc-LDL was separated from free 99mTc by gel filtration chromatography on a PD-10 column (Sephadex G-50, Pharmacia) and 0.1 mol/L NaHCO3 in saline (pH 8). LDLs were eluted with the void volume. Labeled LDLs were sterilized by filtration through a 0.22-mm Millipore filter. Each patient was positioned supinely under the collimator of an Elscint SP4 gamma camera, and 10 mCi of 99mTc-LDL was injected intravenously as a bolus. Dynamic gamma-camera images of the chest and upper abdomen were acquired every 15 seconds for the first 60 minutes. Static anteroposterior images of the abdomen were also acquired at 1, 4, and 24 hours after injection. Blood time-activity curves were generated from the dynamic images with subsequent biexponential fitting to obtain the half-life of the slow distribution phase (S1) and the half-life of the rapid distribution phase (S2). From the static images, we drew regions of interest (ROIs) over the heart, liver, spleen, and kidneys, and time-activity curves were generated after normalization for injected dose, isotope decay, and ROI area. In particular, total liver uptake was calculated by drawing an ROI over the whole organ and measuring total liver radioactivity (expressed in counts per minute). By using an internal standard, counts were then converted to activity (MBq), and liver uptake was expressed as a percentage of the injected dose (data not shown). Liver size was estimated as the number of pixels within the ROI area. Unitary liver uptake was calculated by dividing the total liver uptake by the liver size, thus allowing the comparison of 99mTc-LDL liver uptake in subjects with different liver sizes.

Plasma Lipid and Lipoprotein Measurements
Blood samples were collected in the morning after an overnight fast in EDTA-containing tubes. Plasma was immediately separated by low-speed centrifugation, and total cholesterol, triglycerides, and HDL-C were determined by standard methods. During the 125I-LDL turnover study, lipoproteins were quantified in the plasma samples taken on days 1, 4, 8, 12, and 14 of the turnover period. The VLDL+LDL (d<1.019 g/mL) and LDL (d=1.019 to 1.063 g/mL) fractions were obtained by sequential ultracentrifugation, as described; cholesterol was measured in the VLDL+LDL and LDL (d=1.019 to 1.063 g/mL) fractions, and the concentrations were corrected for total recoveries. Plasma LDL-C concentrations were calculated as the difference between the 1.019 g/mL infranatant cholesterol and HDL-C. Cholesterol and total protein (apoB) were also assayed in isolated LDL, and the plasma concentrations of LDL apoB were derived by multiplying the cholesterol-to-apoB ratio in isolated LDL by the absolute plasma concentration of LDL-C, determined as described above.

Other Laboratory Measurements
The serum sterol fraction was subjected to gas-liquid chromatographic analysis, as reported. Measurements of LAL activity in proband G.P. (kindly performed by Dr R. Anderson at the Bowman Gray School of Medicine, Winston-Salem, NC) was carried out using both the [3H]triolein and [14C]oleate methods and following a previously described procedure. Apo E genotyping was determined according to Hixon and Vernier.

Statistical Analysis
Data were assessed for significance with Student’s unpaired, 2-tailed t test. In all cases, statistical significance was set at P<0.05. Analysis was carried out using the Stat-View for Macintosh package.

Results
Cellular and Genetic Studies in Kindred No. 2
The pedigree of family No. 2 spanning 5 generations is shown in Figure 1. Besides the probands (IV-2, A.P.; and IV-3, G.P.), 6 other family members were tested for plasma lipid and lipoprotein concentrations (III-1, III-2, III-19, IV-4, IV-6, and V-1). Subject III-19 presented with severe hypercholesterolemia (520 mg/dL), but the others showed total chole-
terol levels <95th age-matched percentile of cholesterol distribution in the Italian population. In particular, the father (III-1) showed total cholesterol levels of 257 mg/dL and the mother (III-2), 245 mg/dL. The mother after the time of measurement showed poorly compensated type II non-insulin-dependent diabetes mellitus (blood glucose 170 mg/dL). An accurate anamnesis revealed 3 other cases of severe hypercholesterolemia in the family (III-7, III-10, and III-23) with reported values >500 mg/dL. Subject III-19 showed evidence of coronary artery disease, whereas subject III-10 died of a documented myocardial infarction at the age of 50 years. Like kindred No. 1,7 pedigree No. 2 was characterized by the absence of vertical transmission of the hypercholesterolemic trait and by the presence of consanguinity between the parents of affected individuals.

To determine whether hypercholesterolemia in this kindred was due to FH, we first measured LDLR activity in cultured fibroblasts. In both probands, 125I-LDL internalization was between 80% and 100% of the normal range (Table 1). These values were not compatible with the diagnosis of FH, in which LDLR activity is usually 40% to 60% of normal. It has been suggested that mutations in the cytoplasmic tail of the LDLR gene may produce an FH phenotype that is undetectable by a125I-LDL study in isolated fibroblasts.24 To exclude this possibility, we performed SSCP analysis of exon 17 (coding for the cytoplasmic tail of LDLR) as well as of other exons and the promoter of the LDLR gene (data not shown). Other than common polymorphisms, this analysis did not disclose any alteration in the DNA sequences, once again suggesting that structural defects in the LDLR gene were not the cause of this form of hypercholesterolemia.

We also ruled out the possibility of defective binding of LDL by excluding the presence of FDB and other mutations near the binding domain of the apoB gene, as well as by demonstrating that LDL particles isolated from proband G.P. were able to compete normally with control LDL (data not shown).

Plasma concentrations of sitosterol in A.P. and G.P. were 0.78 and 0.62 mg/dL, respectively. These values were within the normal range (0.17 to 0.78 mg/dL), thus allowing us to exclude the presence of sitosterolemia. Measurements of LAL activity were performed for proband G.P., and these analyses showed values of 220 (pmol/h)/mg cell protein by the triolein method and 207 (nmol/h)/mg cell protein by the oleate method. Both values were within the normal limits.25

The results of the experiments carried out with [3H]rLDL are reported in Figure 2. The amount of [3H]cholesterol incorporated into cellular cholesteryl esters in cultured fibroblasts from proband G.P. was similar to that observed in normal fibroblasts at all [3H]rLDL concentrations in the medium. As expected, the ability to metabolize [3H]cholesterol was completely abolished in the fibroblasts obtained from the receptor-negative FH homozygote. These data provide additional strong support for the observation that LDL uptake was not impaired in the proband’s fibroblasts, an observation incompatible with the diagnosis of FH. Further-
more, these experiments demonstrated the ability of the proband’s fibroblasts to esterify cholesterol to a normal extent.

**In Vivo Metabolic Studies**

Composition and in vivo kinetic parameters of LDL in the 3 probands are shown in Table 2 and are compared with those obtained in 5 age-matched, normolipidemic controls. The mean FCR (0.36±0.03 pool/d) and production rate (14.0±2.4 mg·kg⁻¹·d⁻¹) of LDL apoB in controls were very similar to values previously reported in young adults 26. As expected, probands showed markedly elevated plasma concentration of LDL apoB, which were 1.6 to 3 times higher than in controls. Moreover, LDL particles in probands G.F.C. and J.C. appeared to be significantly cholesterol enriched, as suggested by the high LDL-C-to-apoB ratio (2.10 and 1.76, respectively, compared with 1.40±0.11 in controls). The FCRs for LDL apoB were significantly (P<0.001) reduced in all probands and were ‘50% lower than control values. Individual figures ranged from 0.18 to 0.21 pool/d and were only slightly higher than those typically seen in FH homozygotes.26 In the turnover study, proband G.F.C. was simultaneously injected with autologous ¹²⁵I-LDL and homologous ¹³¹I-LDL. Both tracers showed a similar, marked reduction in the FCR (data not shown), further confirming the absence of binding defects in LDL. The probands also showed increased LDL apoB production rates, but although these values were higher than in controls (P<0.01), they were lower than those typically seen in FH homozygotes.26 Nevertheless, we observed wide differences among probands; indeed, whereas the LDL apoB production rate in J.C. (15.1 mg·kg⁻¹·d⁻¹) was within the normal range, in G.F.C. and A.P. it was >95th percentile of control subjects (23.4 and 22.0 mg·kg⁻¹·d⁻¹, respectively).

The tissue distributions of autologous ⁹⁹mTc-LDL in the probands, the homozygous FH patient, and the normal controls are shown in Figure 3. As confirmed by the data obtained in the ¹²⁵I-LDL turnover study, the plasma half-life of ⁹⁹mTc-LDL (estimated by the biexponential fitting of the heart time-activity curves) was significantly longer in all probands compared with controls and very similar to that obtained in the FH homozygote (A). Half-life values of (1) and (2) were 189.4 minutes for G.F.C., 178.4 minutes for J.C., and 182.6 minutes for A.P., versus 110.1±25.7 minutes for controls.

So far as the ⁹⁹mTc-LDL tissue uptake is concerned, the probands showed a severe reduction in liver uptake (B) compared with controls. This reduction was already significant at early time points (P<0.05) and was of the same magnitude as that observed in the FH homozygous subject. After 24 hours, 3- to 10-fold less radioactivity was taken up by the liver in the probands compared with controls (P<0.001). Even though the probands and patient H.F.H. showed 1.6 to 3 times higher LDL concentrations than

### Table 2. Kinetic Parameters of LDL (¹²⁵I-LDL) Metabolism

<table>
<thead>
<tr>
<th>Parameters</th>
<th>J.C.</th>
<th>G.F.C.</th>
<th>A.P.</th>
<th>Controls (n=5)</th>
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<tr>
<td>LDL-C, mg/dL</td>
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<td>339.0</td>
<td>461.0</td>
<td>132.7±52.3</td>
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<td>LDL apoB, mg/dL</td>
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<td>FCR, pools/d</td>
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<td>0.20</td>
<td>0.21</td>
<td>0.36±0.03</td>
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<tr>
<td>Production rate, (mg/kg)/d</td>
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<td>15.1</td>
<td>22.0</td>
<td>14.0±2.4</td>
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<tr>
<td>LDL-C/apoB</td>
<td>1.76</td>
<td>2.10</td>
<td>1.43</td>
<td>1.40±0.11</td>
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</table>

Data for control subjects are reported as mean±SD.

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Figure 3. Biodistributions of autologous ⁹⁹mTc-LDL in the heart (A), liver (B), spleen (C), and kidneys (D) in study patients. Data are reported as percentage of radioactive dose injected corrected for the area of ROI. □ indicates controls; △, proband J.C.; ▲, proband G.F.C.; ○, proband A.P.; and ●, the homozygote FH patient.
normals, their liver uptake of LDL was 3- to 10-fold less, indicating a much greater reduction than that predicted simply on the basis of the dilution of the tracer in the larger LDL pool of the patients. In the hypercholesterolemic patients, moreover, liver uptake of $^{99m}$Tc-LDL peaked within 4 to 6 hours and then reached a plateau, whereas in the controls, it showed a steady increase up to 24 hours. The spleen and kidney also showed reduced uptake of $^{99m}$Tc-LDL in the probands compared with controls (C and D). At 2 and 4 hours, no significant differences were found between patients and controls, but at 24 hours after injection, kidney and spleen $^{99m}$Tc-LDL uptake was significantly lower in patients ($P<0.05$). Kidney uptake in proband A.P. and in the FH patient was completely abolished. Unlike that for the liver, the $^{99m}$Tc-LDL activity curves of these organs did not suggest the presence of a specific, saturable mechanism of uptake. Finally, by comparing in different organs the time-activity curves before and after normalization of radioactivity per organ area, we observed that the total liver uptake and the unitary liver uptake were both reduced in G.F.C. and J.C., whereas the total liver uptake was normal in A.P. and in the FH homozygote, despite a reduced unitary uptake. This observation suggests that in these 2 subjects, liver hypertrophy and the increased Kupffer cell uptake compensated for the hepatocyte defect; G.F.C. and J.C. also had increased liver size, but it was not sufficient to compensate for the hepatocyte defect.

Discussion

This article reports the metabolic characteristics of an unusual form of familial hypercholesterolemia that was detected in 2 families of Sardinian origin. A common feature of the 2 families was that the probands exhibited the clinical phenotype of FH homozygotes while having normal LDLR activity in cultured fibroblasts. Furthermore, genetic analysis did not reveal any sequence alteration in the LDLR gene, thus excluding the possibility that structural defects in the LDLR protein might be responsible for the hypercholesterolemia. In addition, abnormalities in apoB100 binding ability, as well as the presence of sitosterolemia and CESD, were ruled out.

Plasma LDL-C levels are determined by the balance between the production and the catabolic rate of LDL particles. Therefore, to investigate the metabolic mechanisms underlying this lipid disorder, we first determined the in vivo kinetics of autologous $^{125}$I-LDL in 3 probands from the 2 families. In all probands we found an average 50% reduction in the FCRs of LDL apoB and a 40% increase in the production rates of LDL apoB compared with controls. These findings are different from those obtained in patients with pseudohomozygous type IIa hyperlipoproteinemia, which also shows severe hypercholesterolemia without evidence of vertical transmission. Indeed, a 4-fold increase in LDL production rate has been found in this disorder while LDL catabolism is normal. Moreover, these patients are very sensitive to a low-cholesterol diet and lipid-lowering drugs, whereas our probands did not respond to either of these treatments. Sirtori et al described the case of an Italian woman with severe hypercholesterolemia, xanthomatosis, coronary heart disease, and normal LDLR function. An LDL turnover study showed a 3-fold increase in the LDL apoB production rate (36.8 mg · kg$^{-1}$ · d$^{-1}$) while the FCR was within normal limits. This pattern is clearly different from that observed in our probands. Harada-Shiba et al recently reported about 2 hypercholesterolemic Japanese brothers with normolipidemic parents, clinical features of FH homozygotes, and normal LDLR activity in fibroblasts. These authors estimated from the rebound curve of plasma cholesterol after plasmapheresis that cholesterol FCR was reduced (0.102 pool/d) while cholesterol synthesis was normal. The most reasonable explanation for the association between low FCRs and increased LDL production rates in our probands is a reduced LDLR activity. Indeed, in the case of a reduction in LDLR activity, fewer VLDL remnants are removed from the circulation and hence, more are converted to LDL; such a phenomenon has been advanced for FH homozygous patients. Therefore, the results of our $^{125}$I-LDL turnover study seem to be consistent with an abnormal LDLR activity. Nevertheless, this conclusion is in contrast with the finding that the probands showed a normal LDLR activity in cultured fibroblasts.

Because the liver is the major site of LDLR-mediated catabolism, we investigated the possibility of a liver-specific defect in LDL catabolism. To this aim, we injected autologous $^{99m}$Tc-LDL, a procedure that has been previously demonstrated to allow the in vivo evaluation of lipoproteins. All probands showed a reduction in LDL liver uptake, as did the FH homozygous subject. At the later time points, we also observed a reduced LDL uptake by the spleen and kidneys. It has been reported in animals that the spleen and kidney may catabolize LDL. However, in our patients, the contribution of these organs to LDL catabolism was small and might be related, at least partially, to a scavenger receptor pathway.

On the whole, the results of this study are consistent with the hypothesis that hypercholesterolemia in these kindreds is caused by an inherited defect in LDL catabolism, which appears to be expressed in hepatocytes but not in fibroblasts. This defect cannot be explained by a disorder in the LDLR gene itself but might be due to aberrant regulation of LDLR gene expression. LDLR expression largely depends on the intracellular cholesterol content: an overaccumulation of cholesterol in the hepatocytes suppresses LDLR activity. This accumulation could be due to abnormalities in (1) cholesterol discharge and degradation through bile acids or (2) regulation of cholesterol esterification.

The liver is the only organ that can excrete relatively large amounts of cholesterol from the body, either directly or after conversion to bile acids. This process is considered an important regulatory step in the determination of cholesterol balance. The synthesis of bile acids is carried out in the liver by the action of at least 10 different enzymes, and cholesterol 7α-hydroxylase is the rate-limiting enzyme of this pathway. By SSCP analysis, we were able to show that the 2 probands from pedigree No. 1 (G.F.C. and J.C.) inherited different alleles for the 7α-hydroxylase gene (H.H. Hobbs, personal communication, 1998), excluding the possibility that a mutation in this gene might be responsible for their disease. Nevertheless, we could not definitively exclude the hypothesis that an alteration in bile acid synthesis might be associated with this inherited disease. On the other hand, in a previous description of Japanese siblings with similar characteristics, no abnormality in whole-body cholesterol synthesis, as estimated by urinary mevalonate excretion, could be
demonstrated. Also, an abnormality in the regulation of cholesterol esterification seems to be unlikely, because we demonstrated that the esterification rate of cholesterol in cultured fibroblasts from 1 of the probands was normal, thus implying normal acyl coenzyme A cholesterol acyltransferase activity.

The possibility that the defect we identified is due to some alteration in the way that the hepatocytes “sense” intracellular cholesterol is more intriguing. In this regard, an involvement of the regulatory mechanism of LDLR gene expression cannot be completely excluded, because alterations in different trans-acting factors might affect this pathway independently of the integrity of the LDLR gene itself. A pivotal role in this pathway is played by sterol regulatory element binding proteins 1 (SREBP1) and 2 (SREBP2).35 Defects in these proteins might be responsible for an impairment of LDLR gene expression. Nevertheless, by SSCP analysis, we have demonstrated that G.F.C. and J.C. were heterozygous for different alleles at both SREBP1 and SREBP2 loci (H.H. Hobbs, personal communication, 1998), and this finding would not be expected if the mutation were located in these genes. Alternatively, the LDLR pathway might not be directly involved, and the disease may result from a defect in another step of the intracellular metabolism of cholesterol that specifically affects hepatocytes. An example of the feasibility of this hypothesis is provided by the model (HepG2 cell) in which LDLR activity is only slightly reduced when 25-hydroxycholesterol or LDL is added to the medium, (HepG2 cell) in which LDLR activity is only slightly reduced feasibility of this hypothesis is provided by the model that specifically affects hepatocytes. An example of the another step of the intracellular metabolism of cholesterol directly involved, and the disease may result from a defect in this pathway independently, of the LDLR gene activity. A defect in this complex mechanism could lead to an accumulation of cholesterol in the hepatocytes, with consequent downregulation of LDLR activity.

In conclusion, we observed an unusual form of familial hypercholesterolemia in 2 families of Sardinian origin, which was caused by a selective reduction in the hepatic uptake of LDL. On the basis of our data, we suggest that this disease results from a suppression of LDLR activity in hepatocytes, possibly due to a recessive defect in the regulation of cellular cholesterol metabolism. Identification of the molecular basis of this dyslipidemia is under active investigation. We propose to identify this new clinical condition as “familial recessive hypercholesterolemia.”

Note Added in Proof
At present, 4 new Sardinian families with the characteristics of “familial recessive hypercholesterolemia” have been identified. In all probands, the LDLR activities in fibroblasts as well as the binding ability of LDL to the LDLR are normal.

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References


Characterization of a New Form of Inherited Hypercholesterolemia: Familial Recessive Hypercholesterolemia


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