Libyan Family with Hypercholesterolemia and Increased High-Density Lipoprotein Cholesterol in Plasma

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Genetic deficiencies of cholesteryl ester transport protein (CETP) and hepatic lipase activities have been associated with hyperalpha-lipoproteinemias. Here we present a family of 11 members, of which 9, including the father, mother, 5 sons, and 2 daughters, show a marked increase in high-density lipoprotein (HDL) cholesterol alone with low plasma concentrations of triglycerides. Analyses of lecithin:cholesterol acyltransferase (LCAT) activity, cholesteryl ester transfer between HDL fractions, hepatic lipase (HL) activity, and lipoprotein lipase (LPL) activity in these cases showed that a decrease in the heparin-releasable HL activity was the possible cause of the marked increase of HDL2 fractions observed in nine of them. Such a defect in HL activity could significantly affect HDL metabolism in particular and lipoprotein metabolism in general. Evidently, a marked increase in serum total cholesterol due to abnormal metabolism of HDL cholesterol, separate from known causes of altered low-density lipoprotein cholesterol metabolism, e.g., a clearance or a receptor defect, is not uncommon. The coordinated action of HDL, LCAT, LPL, and CETP may be essential for normal metabolism of plasma lipoproteins.

Indexing Terms: heritable disorders/metabolism/lipoproteinemia

Fatty streaks and fibrous plaques, which appear in the coronary arteries during the second decade of life, have also been identified in aortas of younger children (1). These early atherosclerotic lesions are associated with the blood concentrations of lipids and lipoprotein cholesterol fractions in children and adults (2). Thus, screening the plasma lipid profile in children seems appropriate as a routine procedure to identify subjects potentially susceptible to atherosclerosis as adults. Such a screening program was undertaken in a cross-section of the local Libyan population of Benghazi by determining the plasma lipid profile in children up to age 7 years. During this study, a Libyan family with 11 members showed hyperlipoproteinemia with a marked increase in high-density lipoprotein (HDL) cholesterol and very low concentrations of triglycerides. Genetic deficiencies of cholesteryl ester transport protein and of hepatic lipase (HL) activities and hyperalpha-lipoproteinemia are associated with high HDL cholesterol concentrations (3), and in certain cases high HDL cholesterol (HL deficiency) does not protect against coronary artery disease (4).

Previously, we had reported a family with hypercholesterolemia associated with beta-thalassemia minor (5). The present case study provides another example of a family with a marked increase in HDL cholesterol, possibly linked to a decrease in heparin-releasable HL activity.

Materials and Methods

Blood was withdrawn after 12 h of overnight fasting from a family consisting of a father, mother, 6 sons, and 3 daughters, including a 9-month-old girl. The samples were subjected to the following analyses:

1) Total cholesterol and triglycerides, estimated by specific enzyme assays (total cholesterol: CHOD-PAP; triglycerides: PERIDOCHROM, both from Boehringer, Mannheim, Germany; CVs of 4.9% and 7.6%, respectively).

2) Total HDL cholesterol measured in the supernate after precipitation of apolipoprotein (apo) B-containing lipoproteins—very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL)—by addition of heparin and manganese chloride.

3) HDL2 precipitated from the HDL fraction with dextran sulfate of low molecular mass (15–20 kDa, lot no. 2576: Sochibo, Boulogne, France), and HDL2 concentration is derived by subtracting the HDL3 cholesterol from the total HDL cholesterol value.

4) Derived LDL cholesterol, estimated according to the Friedewald formula (6) as total cholesterol – HDL cholesterol – (triglycerides/5), where the HDL cholesterol is a measured value and the triglyceride/5 is an estimate of VLDL cholesterol.

In addition, postheparin plasma was obtained by collection into cooled heparin-containing tubes 5 and 15 min after intravenous injection of 100 IU/kg heparin. Postheparin plasma lipoprotein lipase (LPL) and HL activities were determined by a selective immunochromatic method (7) after extraction and purification. The postheparin plasma was incubated at 37°C for 15 min with 1/20 volume of 20% Intralipid® (Vitrum, Stockholm, Sweden). The fat cake obtained after centrifuging
at 60 000g for 60 min contained 50% of the HL activity and was delipidated. The residue was dissolved in 5 mmol/L sodium barbital buffer, pH 7.4/glycerol (80/20, by vol) and subjected to chromatography on Sepharose (Pharmacia, Piscataway, NJ) with covalently bound heparin. Use of a linear NaCl gradient to elute the enzymes yielded three major peaks, eluting at 0.7, 0.85, and 1.15 mol/L NaCl. Peaks I and II contained the HL activity; peak III had LPL activity, which was inactivated by the high salt concentration and required plasma and apo C-II for full activity. The 50 to 100 μg of protein in peak I was injected with complete Freund’s adjuvant into rabbits. The rabbits were reimmunized at 2-week intervals until the antisera titer was adequate to precipitate the peak II activity with no appreciable cross-reaction with LPL activity.

LPL was measured by inactivating HL with specific antiserum. Postheparin plasma (10 μL) was incubated with 10 μL of HL antiserum for 2 h at 4°C, then mixed with 500 μL of assay mixture—per liter, 3.2 mmol of [acyl-l-14C]triolein emulsion (see below), 40 mmol of Tris-HCl (pH 8.4), 0.1 mol of NaCl, 2.5 g of bovine serum albumin—and 50 μL of normal human serum. After incubation at 28°C for 60 min, the radioactivity of the free fatty acids was measured in a liquid scintillation counter. HL activity was measured after inhibiting the LPL activity by increasing the NaCl to 1.0 mol/L and omitting the serum activator. The HL activity was determined by using triolein emulsion under the same experimental conditions but without preincubation with antiserum.

The triolein emulsion was prepared from 100 mg of [acyl-l-14C]triolein (specific activity 0.070 Ci/mol) stored in benzene. The solvent was evaporated under nitrogen and 7.5 mL of 50 g/L gum arabic was added; the mixture was then sonicated in an ice bath. A fresh batch of emulsions was prepared each day.

Lecithin:cholesterol acyltransferase (LCAT) activity was determined by the method of Stokke and Norum (8). The fractional esterification rate was expressed as the percentage of total radioactivity incorporated into the cholesterol fraction per hour (%h⁻¹). The molar LCAT rate (μmol·h⁻¹·L⁻¹) was obtained by multiplying the %h⁻¹ by the plasma concentration of free cholesterol. Free and esterified cholesterol were assayed by an enzyme assay. We also tested the inhibitory effects of 5,5′-dithiobis-(2-nitrobenzoic acid) on LCAT (8). Lipid phosphorus was measured by the method of Bartlett (9).

The apo-B-containing lipoproteins (VLDL and LDL) were precipitated from fresh human plasma by the method of Burstein et al. (10) with the modification of Warnick and Albers (11). The supernate, which contained HDL, was washed twice with 0.15 mol/L NaCl and 0.02 mol/L Tris-HCl, pH 7.4, and used in a series of experiments studying the decrease of cholesterol in HDL and increases of cholesterol in VLDL and LDL under different conditions (e.g., changes in speed of rotation at which the plasma was mixed, temperature, and duration of the incubation). We incubated each subject’s plasma (0.5 to 1.0 mL) with constant mixing by rotation at 40 rpm for 4 h. The 4-h incubation period gave the maximum measurable decrease in HDL cholesterol while keeping the rate of the reaction nearly linear. After 4 h, the incubation was stopped by adding heparin and MnCl₂ to precipitate VLDL and LDL. The HDL cholesterol in the supernate was measured before (HDL₀) and after incubation (HDL₄). We calculated the rate of cholesterol transfer (RCT) in the 4 h (12) from the difference between the two HDL cholesterol determinations:

\[ \text{RCT} = \frac{(\text{HDL}_0 - \text{HDL}_4)}{4} \]

RCT was expressed in milligrams per hour per 100 mL of plasma or, after multiplying by 25.96, in micromoles per hour per liter. Statistical analyses were carried out by Student’s t-test or one-way analysis of variance with a Statview program on a Macintosh computer.

Results

The mean concentrations of total cholesterol, LDL cholesterol, HDL cholesterol, HDL₁ and HDL₂ subfractions, and triglycerides of the family members and control subjects (age- and sex-matched controls) are listed in Tables 1 and 2. The VLDL triglyceride concentrations were markedly decreased in all the family members except the 3-year-old and 9-month-old daughters. The control subjects selected for the present study and the family members had no history of endocrine abnormalities or drug intake, including contraceptives. All had a simple lifestyle with a diet usually rich in polyunsaturated fatty acids. Ten family members had a greatly increased HDL cholesterol, the youngest child had a normal value. The three youngest children had LDL cholesterol concentrations twice that of the control children, and the adults had values exceeding the 95th percentile for their age and gender. The LPL and HL activities are shown in Table 3. HL activity was significantly decreased in nine members of the family, with the 13-year-old son and the 9-month-old daughter having normal activity. The LPL activity, however, appeared normal in all the family members, with values in the upper normal range, possibly suggesting a nonsignificant increase.

The LCAT activity (fractional rate: percent of labeled cholesterol esterified per hour) for controls and patients was 3.65 ± 1.15 and 3.36 ± 1.80 %h⁻¹, respectively, or, expressed as cholesterol esterified per hour, 65.0 ± 12.5 and 63.0 ± 4.5 μmol·L⁻¹·h⁻¹ (not significantly different). Cholesteryl ester transport protein activities remained normal in the family members studied (Table 4).

Discussion

In the present study 9 of the 11 Libyan family members showed a great increase in HDL cholesterol, some increase in LDL cholesterol, and a marked decrease in triglycerides and VLDL. Further analyses demonstrated that these nine family members had a very low activity of heparin-releasable HL and normal plasma LCAT activity. The relative transfer of cholesterol be-
between the two HDL fractions was normal, indicating a normal cholesteryl ester transfer protein activity. The fractionation of HDL cholesterol into its HDL2 and HDL3 fractions demonstrated that the increase in HDL cholesterol was due mainly to increases in HDL2. Recent studies have indicated a major role for hepatic endothelial lipase in the uptake and catabolism of HDL2 lipids in the liver (13). The lipid on the luminal surface of the hepatic endothelial cells binds to HDL and hydrolyzes the phospholipid found on HDL2 (14). HL, therefore, may play a role in the hepatic uptake of cholesterol and in the conversion of HDL2 to HDL3 (15).

In the present study, 9 of the 11 family members showed low activity of HL, which may be one of the causes for the observed increase in serum HDL2 cholesterol; however, such an increase in HDL cholesterol was also seen in the 13-year-old son and the 9-month-old daughter, who had normal HL activity. The lower activity of HL, combined with LPL activity in the upper range of normal, may have led to the accumulation of HDL2, HL activity being inversely related to concentrations of HDL2 cholesterol (15). Tall (16) demonstrated that an estradiol-induced increase in HDL lipids is associated with a decrease in HL activity and, conversely,
that androgen-derived anabolic steroids and progester-
onal steroids cause a decrease in HDL lipids accompa-
nied by an increase in HL activity. Nine of the family
members seemed to exhibit such an inverse relation-
ship between HDL concentrations and HL activity.
However, because none of the family members had any
other history of endocrine abnormalities or drug intake
(including contraceptive steroids), the abnormality
observed in their HL activity may be due to other
causes.

Unfortunately, we were unable to conduct further
studies on the patients to determine the probable cause
for such lowered HL activity. We hypothesize that re-
duced expression of the HL gene or an altered posttran-
scriptional or posttranslational regulation of HL ac-
tivity may account for the observed decrease in HL activity
in this family. Or perhaps the subjects' plasma con-
tained an inhibitor that could lower the measured HL
activity. The grandparents of the family were alive but
their medical history could not be traced. However, con-
trols and the family members reported here were Arabs
with a long tradition of following a consanguineous
marriage system.

The parents in the family had an active lifestyle with
a normal body mass index. The low plasma triglyceride
concentrations seen in some of the family members can-
not be explained on the basis of increased LPL activity,
because the activity of this enzyme was in the upper
range of normal. A reduced HL activity, with other
enzymes' activities being normal, might affect the over-
all metabolism and possibly the turnover of lipopro-
teins. Thus, a concerted action of HL, LPL, LCAT, and
cholesteryl ester transport protein may be necessary to
maintain a normal turnover and metabolism of lipopro-
teins. An abnormal activity of any one of the enzymes
might seriously impair the lipoprotein metabolism, par-
ticularly HDL metabolism.

Genetically determined variation in HL activity may
also influence the inheritance of HDL cholesterol (16).
Such genetic variation could be influenced by the race
and region of the population being studied. Studies car-
rried out in different races in different regions could help
explain the molecular mechanisms involved in meta-
bolism of plasma lipoproteins. In the midst of the sim-
mmering controversy as to whether too much or too little
emphasis is laid on reducing plasma cholesterol concen-
trations (17), such studies may help us to understand
the effects of reducing plasma cholesterol and to know
and rectify the factors that affect circulating concentra-
tions of plasma cholesterol so as to help reduce the
susceptibility to coronary artery disease.

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