Plasma Lipoprotein Distribution of Apolipoprotein E in Familial Hypercholesterolemia


Although familial hypercholesterolemia (FH) has been well characterized in terms of the etiology of the major lipoprotein abnormality, that of low density lipoproteins (LDL), less information is available on changes in other lipoproteins which could influence the atherogenic process in this disorder. The present study has focused on such potential abnormalities by studying in detail the lipoprotein association of apolipoprotein E (apo E) in a large group of subjects homozygous for FH. Total plasma apo E levels in homozygous subjects were significantly elevated ($p < 0.001$) relative to heterozygous subjects which were, in turn, significantly greater ($p < 0.001$) than controls (137.6 μg/ml, 69.4 μg/ml, 46.5 μg/ml respectively). After separation of plasma lipoproteins by 4% agarose chromatography, an increased mass of apo E in lipoproteins of intermediate size was present; this may reflect the absence of LDL receptors that normally mediate their clearance. Homozygous FH subjects also demonstrated an increased mass of apo E-enriched high density lipoproteins (HDL) of large size, but a reduction in HDL cholesterol and apo A-I. The increase in the potentially atherogenic remnant lipoproteins and the decrease in HDL are associated with an Increased risk for atherosclerosis, even in the absence of the LDL elevation, which is characteristic of FH. The increase in apo E-enriched HDL could reflect a compensatory mechanism that permits reverse cholesterol transport in the absence of LDL receptors.

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Familial hypercholesterolemia (FH) is a well characterized disorder caused by an inherited deficiency or absence of active low density lipoprotein (LDL) receptors. As a result, LDL catabolism is impaired, LDL synthesis is increased, and patients homozygous for this gene (FHH) manifest a massive accumulation of LDL cholesterol. Although this LDL elevation is the most obvious lipoprotein abnormality, several studies have described subtle abnormalities in the metabolism of other lipoproteins or apolipoproteins. For example, very low density lipoprotein (VLDL) metabolism and structure appear normal, but intermediate density lipoprotein (IDL) catabolism has been shown to be retarded. High density lipoprotein (HDL) abnormalities have also been documented in FHH. Specifically, reduced levels of HDL that are nearly devoid of the HDL2 subclass have been documented.

With respect to the plasma apolipoproteins, apolipoprotein E (apo E) is of particular interest. It is known that this apolipoprotein binds with high affinity to the LDL receptor and also to an hepatic receptor specific for apo E. In familial hypercholesterolemia, researchers have reported that total plasma apo E levels were elevated and proportional to the concentration of plasma total cholesterol. The Watanabe heritable hyperlipidemic (WHHL) rabbit, an animal model of FHH, also has elevated plasma apo E levels. The lipoprotein association of apo E, however, has not been described in this disorder. Apo E is normally a component of VLDL, IDL, and HDL subclasses. Apo E-enriched lipoproteins in VLDL and IDL have been linked to enhanced atherogenesis in humans and in experimental animals. Apo E-containing HDL (HDL-E), on the other hand, may function as intermediates in peripheral cholesterol mobilization and transport to the liver. In FHH, the described abnormalities in IDL metabolism and in HDL composition would be consistent with a profile of apo E-containing lipoproteins that could alter the flux of cholesterol between peripheral tissues and hepatic sites and thus contribute to the atherosclerotic complications of this disease. In the present study, we have pursued this possibility by studying in detail the lipoprotein association of apo E in a large group of subjects homozygous for familial hypercholesterolemia.

Methods

Patients

The diagnosis of the heterozygous and homozygous states of FH were based upon the criteria of Fredrickson and Levy. Fifteen of the homozygous FH patients (Pa-
patients 4 through 18) attended the Lipid Disorders Clinic of the Johannesburg Hospital. All except one were of African descent. There is a particularly high incidence of FH in this population group because of the effect of a founder gene resulting from a mutation of the receptor-defective type.9 Nine of the 15 subjects studied expressed the receptor-defective mutation in fibroblast cultures.

Two of the other homozygous FH patients attended the Lipid Clinic at the Mount Sinai School of Medicine in New York, and the third was a patient of Dr. Aubie Angel at the Hospital for Sick Children in Toronto, Canada. These three patients were of Lebanese descent with clinical or biochemical evidence that both parents are heterozygous for familial hypercholesterolemia. LDL apo B kinetic studies performed by Henry Ginsberg were compatible with the homozygous state in Patient 1. No receptor studies were performed in these latter subjects, so the precise defect is not known. The fact that the South African homozygous patients did not differ qualitatively or quantitatively from the Lebanese group in any parameter tested suggests, however, that the nature of the defect had little impact upon the apolipoprotein profiles reported. At the time of study, Patients 10 and 11 were receiving cholestyramine and probucol and Patient 12 had had a portacaval shunt and was receiving probucol. No other patients were receiving any drug for hypercholesterolemia at the time of study.

All 16 patients with heterozygous FH were relatives of the homozygote subjects and attended the Lipid Disorders Clinic of the Johannesburg Hospital. The controls were 35 normolipidemic subjects, including 14 from the South African population; these were also studied for total plasma lipid and apolipoprotein levels. The U.S. subset of the control group (n = 21) was studied in detail by column chromatography and provided the control data shown in Tables 2 and 3.

Laboratory Investigation

Blood samples were obtained from all patients on the morning of their clinic visit after an overnight fast. Total cholesterol and triglyceride levels were measured by enzymatic methods and HDL cholesterol was similarly quantified in the supernatant after precipitation of VLDL and LDL by heparin/manganese or by dextran sulfate/magnesium.18,19 LDL cholesterol was determined by the Friedewald equation.20 Aliquots of EDTA plasma from the South African population and from the Canadian patient were packed in crushed ice without freezing and airfreighted for analysis within 24 hours.

Apolipoprotein levels were quantified by specific double antibody radioimmunoassays described in detail elsewhere.10,22,23,24

Column Chromatography

Glass columns (2.5 x 100 cm) were packed with 4% agarose beads (Biogel A15M, 200 to 400 mesh) and equilibrated in 0.01 M sodium barbital, 0.15 M sodium chloride, 0.01% EDTA, 0.02% sodium azide (pH 7) containing 50 KIU Trasylol/ml to inhibit proteolytic activity. For lipoprotein fractionation, 3 to 5 ml of EDTA plasma was applied to a column at 4°C and lipoproteins were eluted at 10 ml/hr with the same buffer. Fractions of approximately 7 ml were collected and exact volumes were determined gravimetrically for calculation of precise elution volumes.

Since three different agarose columns were used at different times, each was standardized so that elution volumes of apo E fractions could be compared (Table 2). This standardization involved the chromatography of normal plasma samples on the three columns with monitoring of cholesterol, triglyceride, and apolipoproteins B, A-I, and E. The difference in the void volume as determined by blue dextran and the elution volume of HDL (determined by cholesterol and apo A-I elution profiles) was assigned a relative elution volume of 1.0. Apo E fraction I was then defined as the eluate from the void volume to the end of the small VLDL range as defined by the triglyceride distribution after chromatography of normal plasma. A relative elution volume (REV) of 0.33 identified the end of this fraction:

\[
REV = \frac{V_{O} - V_{X}}{V_{O} - V_{HDL}}
\]

where \(V_{O}\) is the void volume, \(V_{X}\) is the elution volume of interest, and \(V_{HDL}\) is the elution volume of HDL. Fraction I was not always present as a peak distinct from fraction II. Fraction II was the apo E in the eluate from REV of 0.33 (the end of fraction I) to the second minimum of the apo E distribution. This had an REV of approximately 0.62. Fraction III comprised the remaining apo E eluate, containing apo E containing particles smaller than LDL.

Statistical Evaluation

Data were analyzed by one-way analysis of variance when homoygote, heterozygote, and control populations were compared (Table 1), and by Student's t test when FHH were compared with the control populations (Tables 2 and 3).

Results

Table 1 presents the clinical data and total plasma lipid and apolipoprotein levels of 16 patients homozygous for familial hypercholesterolemia, 16 obligate heterozygote relatives, and 35 normolipoproteinemic controls. Homozygous patients were significantly younger (mean \(\pm\) SEM = 18 ± 3 years) than heterozygote (30 ± 3 years) or control (32 ± 3 years) subjects. Three of the homozygotes, however, were adults; eight were teenagers. Age did not correlate with any lipoprotein parameter. Homozygous patients had markedly elevated levels of total plasma and LDL cholesterol and of apo B. HDL cholesterol and apo A-I concentrations, on the other hand, were significantly reduced relative to both heterozygotes and controls (\(p<0.001\)). Total plasma apo E concentrations were significantly higher in the homozygote group (137.6 ± 10.3 \(\mu g/ml\)) than in the heterozygotes (69.4 ± 5.6 \(\mu g/ml\)) which were, in turn, higher than the control population (46.5 ± 2.7 \(\mu g/ml\)) (\(p<0.001\)) (Figure 1). Apo C-III concentrations were slightly, but significantly, elevated in FH homozygotes relative to controls (\(p<0.03\)). Plasma triglyceride levels were normal.

Experiments to determine the distribution of the elevated apo E among the various lipoproteins were performed.
Fasting plasma samples from 12 of the homozygotes were separated into lipoprotein components by gel filtration on 4% agarose columns. Analysis of apo E across the spectrum of eluted lipoproteins revealed three apo E-containing fractions as defined under Methods. Figure 2 compares representative elution profiles of cholesterol, apo E, and apo C-III of one control and one homozygous subject to illustrate the unusual pattern of the homozygous subjects.

As detailed in Table 2, only a small proportion of apo E was associated with lipoproteins of VLDL size (fraction I). The major portion (48.7 ± 3.0%) of the plasma apo E of these patients was associated with fraction II, an apo E-containing lipoprotein class that includes particles of IDL and large LDL size. In three of the nine, the size of fraction II as determined by elution volume was very similar to that of LDL. The remaining apo E (46.5 ± 4.0%) was associated with fraction III, namely lipoproteins smaller than LDL. This percentage distribution contrasts with that of a series of 21 normolipoproteinemic control subjects who transported the major portion (64.0 ± 4.0%) of their plasma apo E in fraction III and a minor proportion (23.5 ± 2.7%) in fraction II (Table 2). Reflecting the increased plasma concentration of apo E in homozygous subjects, the actual mass of apo E associated with fraction II was elevated in homozygous subjects to a level seven times that of the normal subjects (Figure 1). As inferred from the ratio of the elution volume of the LDL cholesterol peak to that of apo E in fraction II, this fraction appeared to be smaller in size and more congruent with LDL in homozygous subjects than the corresponding fraction in normal subjects (Table 2). The elution volume of LDL cholesterol relative to LDL cholesterol, however, was not different from that seen in normal subjects, suggesting that the proximity of the apo E peak to LDL in FH resulted from an alteration in the elution position of the apo E fraction, rather than LDL. The mass of apo E in fraction III, while lower than normal on a percentage basis, was twice normal in terms of mass (Figure 1), again reflecting the higher total plasma apo E levels in the patients. The apparent particle size of this HDL-E was, on average, similar to that of controls as inferred from the mean elution volume relative to that of the HDL cholesterol peak.

Table 3 details the lipoprotein distribution of apo C-III in homozygous subjects. Apo C-III moves as readily between lipoproteins as does apo E, but appears to behave independently of apo E metabolically. Apo C-III is normally distributed in plasma in three lipoprotein fractions distinct from apo E (Figure 2), corresponding to VLDL, LDL, and HDL sized particles. Similar to the observed distribution of apo E, only a minor fraction of the total plasma apo C-III was associated with lipoproteins of LDL size in homozygous subjects (Figure 2). A major fraction of the plasma apo C-III of homozygotes (62.2 ± 3.6%) was present in a lipoprotein particle that co-chromatographed with LDL cholesterol. This contrasts with normolipidemic, fasting subjects in whom the major lipoprotein fraction transporting apo C-III was of HDL size (Figure 2). In terms of apo

Table 1. Clinical Features and Total Plasma Lipids and Lipoproteins

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<th>Subject</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>Apo A-I (mg/ml)</th>
<th>Apo B (mg/ml)</th>
<th>Apo C-III (µg/ml)</th>
<th>Apo E (µg/ml)</th>
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<td>13.4</td>
<td>10.3</td>
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</table>

Heterozygotes (n = 16)

| Mean    |                   |     | 332                 | 134                 | 41           | 248          | 1.05           | 1.11          | 125.0           | 69.4          |
| SEM     |                   |     | 17                   | 33                   | 3            | 14           | 0.26           | 0.06          | 21.7            | 5.6           |

Controls (n = 35)

| Mean    |                   |     | 190                 | 73                   | 53           | 129          | 1.42           | 0.73          | 119.4           | 46.5          |
| SEM     |                   |     | 7                    | 6                    | 2            | 12           | 0.05           | 0.04          | 5.8             | 2.7           |

ND = Not determined.
C-III mass, the LDL fraction of homozygotes contained
times the normal amount of apo C-III, but the apo
C-III mass in HDL was proportionately reduced in parallel
with the reduction of total HDL mass.

Radioimmunoassay analysis provided profiles of apo B
and apo A-I, as well as of apo E and apo C-III, across the
spectrum of eluted lipoproteins. Apo B co-eluted with the
major cholesterol peak, confirming its identification as
LDL, and apo A-I eluted coincident with the second HDL
cholesterol peak. Integration of apo E and apo B masses
under the apo E peak representing fraction II (between
REV = 0.33 and the minimum in the apo E profile after LDL
in Figure 2), revealed that the molar apo E/apo B ratios in
fraction II were not different in FHH subjects and controls
(mean ± SEM = 0.336 ± 0.075 and 0.336 ± 0.051, re-
spectively). Thus, there appeared to be increased num-
bers of particles of normal composition (with respect to
these apoproteins) in the patient population. This
approach, however, can provide no data on whether all parti-
cles are similar in composition or whether a subpopulation
of apo E-containing particles of abnormal composition may
exist. Using the same approach and integrating apo E and
apo A-I areas under fraction III, a significant difference
between FHH and normolipoproteinemic subjects was ap-
parent (p< 0.001). This analysis indicated the presence of
an HDL fraction enriched with apo E relative to apo A-I in
FHH subjects (apo E/apo A-I molar ratios = SEM
= 0.114 ± 0.013 and 0.026 ± 0.004, respectively, in FHH
and controls).

Discussion

The present study was undertaken to investigate the
possible abnormalities in apo E metabolism that accompa-
ny defects in the LDL receptor by analysis of a series of
patients homozygous for familial hypercholesterolemia.
This disease has been well characterized as involving a
disorder of LDL metabolism but few studies have focused
on other lipoprotein abnormalities that could influence the
atherogenic process.

In fasting normolipoproteinemic subjects, gel filtration of
the plasma lipoproteins coupled with apolipoprotein and
lipid quantification of column eluates has identified apo E
as a constituent of VLDL (fraction I), intermediate sized
lipoproteins (fraction II), and large HDL (fraction III).10 Nor-
molipidemic subjects have a major proportion of their plas-
ma apo E in the HDL fraction, while hypertriglyceridemic

Figure 1. Comparison of the mass of apo E in the plasma of
control, heterozygous, and homozygous subjects and in apo E
fractions I, II, and III after separation of plasma of control and
homozygous subjects by gel filtration. Bars represent mean-
± SEM. The total plasma mass of apo E is significantly increased
in familial hypercholesterolemia (p< 0.001) and the mass of apo E
in both fractions II (IDL-E) and III (HDL-E) is significantly elevated
in homozygous familial hypercholesterolemia (FHH) relative to
control subjects (p< 0.001). Fractions I, II, and III are defined in
the text.

Figure 2. Cholesterol, apo E, and apo C-III distribution in normo-
lipoproteinemic control and homozygous familial hypercholester-
olemic (FHH) subjects. A. The massive increase in LDL cholesterol
in homozygous subjects. B. The increased proportion of apo E in
lipoproteins of intermediate size (fraction II) and increased
mass of apo E in both fractions II and III. C. The apo C-III profiles
and the increase in apo C-III in lipoproteins of LDL size.
individuals typically express a predominance of apo E in chylomicrons and/or VLDL.

By contrast, Type III hyperlipoproteinemia—a condition that predisposes to premature atherosclerosis—is characterized by strikingly elevated plasma apo E levels that accumulate in intermediate sized lipoproteins (fraction II). An increased mass of apo E in partially catabolized VLDL, IDL, and LDL is also found in certain animal models of atherosclerosis such as the WHHL rabbit and cholesterol-fed monkeys, dogs, and swine. It has been suggested that apo E-containing intermediate sized lipoproteins are atherogenic by virtue of their capacity to deliver cholesterol to macrophage precursors of atherosclerotic foam cells via surface receptors specifically recognizing apo E. The finding that patients homozygous for FH also have very high total plasma apo E concentrations contained largely in intermediate sized lipoproteins raises the possibility that these particles may have an important role in the development of the premature atherosclerosis characteristic of this disorder.

In addition to providing inferential evidence for a contribution by IDL-E to atherogenesis, these studies also suggest that different mechanisms contribute to the catabolism of apo E-containing VLDL and IDL in humans. While the present data have demonstrated that VLDL apo E and triglyceride levels are low in FHH, implying normal metabolism of these particles, the large proportion and mass of apo E in lipoproteins of intermediate size suggest that an abnormality in IDL catabolism exists. In support of this, kinetics analysis of VLDL apo E in FH showed normal VLDL kinetics in homozygous and heterozygous FH, but a retarded clearance of IDL apo E. Apo E-VLDL catabolism, therefore, does not appear to depend upon the LDL receptor, since these are essentially absent in FH homozygotes, but may be effected by nonreceptor intravascular pathways mediated by the postheparin plasma lipases or by binding to the apo E receptor. In contrast to VLDL, catabolism of IDL-E depends, at least in part, upon binding of apo E (or apo B) to an intact LDL receptor, and in its absence these lipoproteins accumulate in the plasma. In particular, studies using monoclonal antibody probes and thrombin in vitro indicated that while the catabolism of VLDL and large remnants of VLDL depends largely upon their apo E content, apo B receptor interaction assumes increasing significance as catabolism proceeds through small VLDL to IDL and LDL. The finding that the apo E peak in fraction II elutes closer to the LDL-cholesterol peak in FHH than in controls raises the possibility that IDL in FHH are smaller than normal. Despite this, the apo-protein composition of these particles does not differ from intermediate-sized E-containing lipoproteins eluting in fraction II in normal subjects.

Another possibility for the increased apo E in the inter-
mediate-sized lipoproteins is that this represents accumulated LDL containing apo E. Although these studies cannot totally exclude this possibility, the fact that the apo E peak was generally distinct from the cholesterol and apo B peaks of LDL, and the apo E/B ratio of this apo E-rich fraction was identical to that in normal subjects favors the notion that the apo E is associated with LDL rather than LDL. Moreover, a correlation between the apo E and apo B levels would be expected in either the heterozygote or homozygote groups if the apo E increase was simply a function of the LDL level. No such correlation was detected.

While the data fit with the concept that VLDL catabolism is the source of IDL-E in FHH, other non-VLDL sources should be considered. Kinetic studies, for example, have described LDL synthesis independent of VLDL catabolism in FHH subjects. In the African green and rhesus monkeys, perfused liver studies have documented the synthesis and secretion of lipoproteins of LDL size and density which are unusually enriched in apo E. Specific radioactivity data indicated that in the rhesus monkey, this LDL apo E was not solely a product of VLDL remodeling. The possibility, therefore, exists that in FHH there is increased de novo input of apo E-containing particles into the plasma pool of fraction II lipoproteins.

Despite the observation that FHH subjects transport proportionately less of their plasma apo E than normal in the fraction III lipoproteins of large HDL size, the absolute mass of apo E in these lipoproteins is twice normal. Apo A-I levels, on the other hand, are reduced as reported previously and the apo C-III mass of HDL is decreased. Reflecting this pattern, the calculation of the apo E/A-I molar ratios in this fraction indicates a substantial enrichment of the average particle with apo E. These data are consistent with the presence in FHH plasma of an HDL subclass enriched in apo E at the expense of HDL2 and HDL3. This observation may have potential significance in terms of cholesterol transport in FHH. It has been suggested that large, apo E- and cholesterol-enriched HDL are important intermediates in the transport of cholesterol between peripheral sites and the liver. Such HDL particles have been documented in interstitial fluid and in vitro, large HDL form as products of cholesterol and apo E acquisition by normal plasma HDL2 and HDL3. Furthermore, these HDL-E can interact with an hepatic receptor by virtue of their apo E content. The apo E contributing to these HDL may come from intravascular processing of VLDL and IDL or from de novo synthesis and secretion, or from both. With respect to the latter source, apo E newly synthesized and secreted by human or murine macrophages has been localized to an HDL fraction of large size and the formation of these HDL. Their role as vehicles for reverse cholesterol transport in FHH is speculative but attractive in view of the dependence on apo E as a mediator of cell lipoprotein interaction that exists in this disorder.

This study has documented several abnormalities in apo E metabolism that are potentially related to the absence of LDL receptors or due to tissue cholesterol overload. Although levels of apo E in VLDL are normal, there is an increased mass of apo E-containing lipoproteins of intermediate size which may directly reflect the absence of the LDL receptor that in normal circumstances mediates their clearance. Accumulation of these apo E-enriched intermediates may contribute to accelerated atherogenesis in this disorder. FHH is also characterized by a mass of apo E in particles the size of large HDL which is increased at the apparent expense of HDL and HDL. Whether this pattern reflects a compensatory mechanism for permitting reverse cholesterol transport in the absence of LDL receptors remains to be determined.

Acknowledgments

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References
4. Pittas RE, Innerarity TL, Arnold KS, Mahley RW. Rate and equilibrium constants for binding of apo E HDL (a cholesterol induced lipoprotein) and low density lipoproteins to human fibroblasts: evidence for multiple receptor binding of apo E-HDL. Proc Natl Acad USA 1979;76:2311-2315
13. Seftel HC, Baker RG, Sandor MP. A host of hypercholester-


40. Basu SK, Brown MS, Ho YK, Havel RJ, Goldstein JL. Mouse macrophages synthesize and secrete a protein resembling apolipoprotein E. Proc Natl Acad Sci USA 1981;78:7545–7549
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