THE REGULATION OF PLASMA LIPOPROTEIN CONCENTRATIONS AS AFFECTED IN HUMAN MUTANTS

BY DONALD S. FREDRICKSON

MOLECULAR DISEASE BRANCH, NATIONAL HEART INSTITUTE, BETHESDA, MARYLAND

Introduction.—The preceding three papers in this symposium have dealt with the definition, structure, and interrelationships of the plasma lipoproteins. It remains to discuss the regulation of lipoprotein concentrations, a subject of interest particularly because it is evident that abnormal lipoprotein concentrations accelerate the development of atherosclerosis, which is now the greatest single cause of premature death in a highly industrialized society like the United States. The plasma lipoprotein concentrations are determined by environmental and genetic factors, many operating remote from the immediate metabolism of lipid or formation and disposal of lipoproteins. Some of the more obvious genetic determinants can be illustrated by certain genetic abnormalities in man.

Previous reviews have described in detail the studies in our laboratory from which evidence has been accumulated that there are at least five different mutants causing hyperlipoproteinemia and one leading to high-density lipoprotein (HDL) deficiency (Tangier disease). When these are added to mutants causing absence or decrease in low-density lipoprotein (LDL), the number of abnormal genotypes that are now recognizable and expressed primarily by an alteration in lipoprotein concentrations is not less than ten (Table 1).

Recent research on the structure of lipoproteins has kindled new interest in some of these mutants. I have particular reference to the expansion of the number of known polypeptide chains (apolipoproteins) in the lipoproteins from two to five. As identified by their COOH-terminal residues, an apoHDL-Gln has been added by the work of Shore and Shore to the apoHDL-Thr previously considered the only apolipoprotein in high density lipoproteins. Brown et al. have recently described an apoVLDI-Ala and apoVLDI-Val in the polypeptides associated with very-low-density lipoproteins (VLDL). Thus far, the polypeptides in low-density lipoproteins consist mainly of an apoLDI-Ser which also is a significant contributor to apoVLDL. In Figure 1, these apolipoproteins are designated R-Thr, etc.

Magnitude of Fat Transport in Lipoproteins.—The magnitude of fat transported by the several classes of lipoproteins is summarized in Table 2. The estimates are of net transport; the major tasks involve movement of fatty acids, and the amounts transported during a given period often considerably exceed the amount oxidized. The largest single contributor to fatty acid transport is the free fatty acid-albumin complex. This is not a lipoprotein but free fatty acid flux exerts an effect on lipoprotein concentrations. This is particularly true of the VLDL, one of whose functions is to return free fatty acid released in excess of calorie demand back to the adipose tissue in the form of triglycerides. Transport in chylomicrons is regulated by dietary intake, since it is in this form that ingested fats reach the tissues through the blood. Only a fraction of these glyce-
TABLE 1. Recognizable phenotypes primarily expressed in abnormal lipoprotein concentrations.

<table>
<thead>
<tr>
<th>Designation</th>
<th>Lipoprotein abnormalities</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abetalipoproteinemia</td>
<td>LDL, VLDL, chylomicrons absent</td>
<td>6, 7</td>
</tr>
<tr>
<td>Hypobetalipoproteinemia</td>
<td>LDL deficient, more than one mutation likely</td>
<td>8, 9</td>
</tr>
<tr>
<td>Tangier disease homozygote</td>
<td>Normal HDL absent, abnormal HDL (HDL_T) present</td>
<td>4</td>
</tr>
<tr>
<td>Tangier disease heterozygote</td>
<td>HDL deficient, HDL_T present</td>
<td>4</td>
</tr>
<tr>
<td>Type I hyperlipoproteinemia</td>
<td>Chylomicrons persist in post-absorptive state</td>
<td>2</td>
</tr>
<tr>
<td>Type II hyperlipoproteinemia,</td>
<td>LDL ca. 2 X normal; VLDL increased ±</td>
<td>2</td>
</tr>
<tr>
<td>homozygote</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type III hyperlipoproteinemia</td>
<td>VLDL increased by LDL of abnormally low density</td>
<td>2</td>
</tr>
<tr>
<td>Type IV hyperlipoproteinemia</td>
<td>VLDL increased</td>
<td>2</td>
</tr>
<tr>
<td>Type V hyperlipoproteinemia</td>
<td>VLDL increased, chylomicrons persist in post-absorptive state</td>
<td>2</td>
</tr>
</tbody>
</table>

Abbreviations as in Fig. 1.

eride fatty acids is oxidized immediately. Many are stored and latter supplied as free fatty acid when demanded. Of the several lipoprotein classes, chylomicrons and VLDL have the more rapid turnover and are subject to acute changes in concentration. The net movement of cholesterol and phospholipids is much less. These lipids are principally found in the LDL and HDL, and these lipoproteins seem to act mainly as solubilizers for glycerides (in the VLDL.

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Fig. 1.—The plasma lipoprotein spectrum as segregated by the ultracentrifuge (center), in which Sf or flotation rates are inversely related to density, and by paper electrophoresis. The apolipoproteins in high-density or α-lipoproteins (HDL), low-density or β-lipoproteins (LDL), and very-low-density or pre-β-lipoproteins (VLDL) are designated as R followed by their COOH-terminal residues. The apolipoproteins shown in parentheses are apparently present only in very small quantities.
and chylomicrons). LDL and HDL, however, also may subtend more subtle functions in maintaining the lipids in plasma and the cells bathed by this medium in equilibrium.

The Major Determinants of Lipoprotein Concentrations.—The most obvious factors that regulate lipoprotein concentrations fall into three categories (listed in probable order of their importance): (1) demand for fatty acid transport, (2) sufficiency of the catabolic mechanisms that dispose of lipids (or lipoproteins), and (3) adequacy of the apoproteins. It is easiest in discussing mutational errors in regulation to begin with the apoproteins. Either a decrease in quantity or quality of the apoproteins may affect lipoprotein concentrations.

A striking example of apparent apoprotein insufficiency is provided by the mutation leading to the disease, abetalipoproteinemia6 (Fig. 2). In the plasma of the presumed homozygote, neither normal LDL16 nor soluble apoLDL16 is immunochemically detectable.

Abetalipoproteinemia is also associated with defective fat absorption, fatty liver, abnormalities in the central nervous system, eyes, and erythrocytes. Chylomicrons and VLDL are completely missing from plasma.16 It will now be relevant to screen the remaining lipoproteins in the plasma of patients with abetalipoproteinemia for traces of the newly discovered apoVLDL-Ala and apo-VLDL-Val which are found in VLDL in amounts about equal to the apoLDL-

Table 2. Magnitude of major fat transport tasks in plasma.

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Lipoprotein or carrier</th>
<th>Grams/day*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free fatty acids</td>
<td>Albumin</td>
<td>50–150</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>Chylomicrons</td>
<td>70–150</td>
</tr>
<tr>
<td>Exogenous</td>
<td>VLDL</td>
<td>25–50</td>
</tr>
<tr>
<td>Endogenous</td>
<td>Chylomicrons, VLDL, LDL, HDL</td>
<td>1</td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Estimates for an adult.5,13
Abbreviations as in Fig. 1.
Ser (Figs. 1 and 2). Should the R-Ala and R-Val be present, it will make it more likely that the mutation for the abetalipoproteinemia causes a deletion of apo-LDL-Ser synthesis and that the latter is indeed critical for glyceride transport from cells as has been suggested.18 On the other hand, Lees has presented evidence that an altered apoLDL may be circulating in this disease.17 The molecular basis of abetalipoproteinemia remains an open question. Its solution may also bring answers to a number of other outstanding problems relevant to VLDL and chylomicon formation. Several reported families with low LDL appear to represent one or more mutations at loci different from the one affected in abetalipoproteinemia.7–9

**Deficiency of apoHDL:** Tangier disease is the antithesis of abetalipoproteinemia. In the centrifuge HDL appears to be completely missing,18 but Levy and Fredrickson have found that an abnormal HDL antigen (HDLt) is present in homozygotes; heterozygotes have about half of the usual amounts of normal HDL and quantities of the abnormal "HDLt" similar to those in homozygotes.19 It will now be possible to determine if the mutation selectively affects apoHDL-Thr or apoHDL-Gln. Tangier disease has thus far not revealed the functions of apoHDL but has indicated that apoHDL does not appear necessary for VLDL and chylomicon formation. The homozygote accumulates cholesteryl esters in many tissues of the body, but the manner in which HDL maintain cholesterol equilibrium between tissue and plasma is not known. One possibility is a role of HDL in plasma lecithin: cholesterol acyltransferase activity,20 a genetically determined deficiency of this enzyme is associated with low HDL concentrations.21 It has been reported that a small amount of a COOH-terminal apolipoprotein is present in the HDL density region,22 and LaRosa in our laboratory has discovered that antigens identical to apoVLDL-Ala and apoVLDL-Val are present in HDL and that their amounts increase upon activation of lipolytic activity in plasma.23 Tangier disease thus also offers an opportunity to explore suggested relationships between apoVLDL and apoHDL.

**Type III hyperlipoproteinemia:** Of mutations leading to excessive concentrations of certain lipoproteins, one is suspected but not proved to be due to a change in the structure of an apoprotein. This is the disorder first described as "xanthoma tuberosum"24 and proved to be a genetic variant in the NIH studies in which it is called type III hyperlipoproteinemia.2 In Mendelian terms the mutant behaves as an autosomal recessive with incomplete penetrance. The characteristic lipoprotein anomaly (Fig. 3) is due to the presence in VLDL of an ab-

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**Fig. 3.—**The lipoprotein pattern in type III hyperlipoproteinemia.18 Abbreviations as in Fig. 1.
normal complex of apoLDL and triglycerides.\textsuperscript{25, 26} The removal of triglyceride
is retarded and the net effect is a decrease in normal LDL and increase in total
VLDL, the latter being of abnormal composition. The patients have accelerated
atherosclerosis and peculiar skin xanthomas, and the phenotype can only
be ascertained by lipoprotein analyses.\textsuperscript{26} Treatment lowers lipoprotein con-
centrations to normal levels although the "floating LDL" is always detectable
amidst the VLDL. Although the abnormal LDL appears to contain apoLDL-
Ser, the normalcy of its structure and the quantity and quality of apoVLDL-Ala
and apoVLDL-Val in the plasma of patients with type III remains to be estab-
lished. It is also possible that the cause of type III ultimately may prove to be
more related to one of the following determinants of lipoprotein concentrations.

\textit{Mutants affecting Catabolism}.—There are two heritable disorders that illustrate
probable defects in pathways involved in catabolism of lipid transported in lipop-
roteins if not in the handling of lipoproteins \textit{per se}. One involves severe rate-
limitation in removal of triglycerides (type I). Another is associated with per-
sistent elevations in LDL (type II).

\textit{Familial hyperchylomicronemia}: Type I hyperlipoproteinemia is a rare pheno-
type, less than 50 examples having been reported.\textsuperscript{2} They appear to be homozy-
gous for a mutant allele controlling the activity of a lipolytic system required in
the removal of exogenous glyceride. Chylomicrons tarry in plasma and triglyc-
erides may reach spectacular concentrations of 15,000 mg per decaliter or higher.
HDL and LDL are depressed and VLDL concentrations are normal or slightly
elevated.

Chylomicon glyceride is normally removed by hydrolysis at, or close to, the
capillary membrane followed in re-esterification within the cell. Lipolysis is
catalyzed by lipoprotein lipase,\textsuperscript{27} an enzyme abundant in adipose tissue and
vascular walls. Adipose tissue lipoprotein lipase has been reported to be low in
type I.\textsuperscript{28} Recent studies of plasma post-heparin "lipoprotein lipase" suggest
that a specific triglyceride lipase is decreased in the type I homozygote.\textsuperscript{29} Pheno-
copies of type I have also been discovered in patients with abnormal circulating
globulins that preferentially bind heparin,\textsuperscript{30} an essential cofactor for tissue
lipoprotein lipase.

\textit{Familial hyperbetalipoproteinemia}: Possibly the single most common mutant
primarily affecting lipoprotein concentrations is that responsible for the increase
in LDL identified as type II in the NIH system.\textsuperscript{2} Cholesterol concentrations
tend to be continuous variables in the general population.\textsuperscript{31} Those at the higher
end, having LDL concentrations above the 95 per cent confidence limits in sam-
plest adjusted by age,\textsuperscript{2} include a group of patients who also have either or both of
the following: (1) lipid deposits in the skin or tendons (xanthomas) of certain
types, and (2) similar LDL elevations in about half of their first-degree relatives.
In a recent analysis of 377 parents, sibs, and children of 99 propositi so defined,
48.6 per cent had type II lipoprotein patterns, 48.6 per cent were normal, and 2.8
per cent had an isolated increase in VLDL (type IV).\textsuperscript{4} Familial type II thus ap-
ppears to be a disorder due to an autosomal mutant nearly always detectable in
single dosage; but ascertainment of phenotype is not absolute and more than one
mutation may be involved.
The frequency of the type II gene(s) in the general population is unknown; it is probably greater than 1:10,000 and could be as high as 1:1,000. The life span of heterozygotes may be reduced by a tendency to accelerated atherosclerosis. Homozygotes often do not live to the child-bearing age.

The metabolic error in type II is unknown. In heterozygotes, LDL concentrations can be lowered by restricting cholesterol intake and increasing the polyunsaturated fatty acids in the diet, and administering drugs that increase excretion of bile acids. Once therapy is suspended, the LDL pool expands again to its abnormal level. Recent measurements of the disappearance of LDL tagged in the apoprotein have indicated that the fractional turnover rate of LDL in type II is decreased. It is not possible to deduce whether this is due to a rate limitation in the catabolism of a component of LDL or the lipoprotein as a whole.

Demand for Fat Transport.—The most important determinant of acute and chronic changes in lipoprotein concentrations is the demand for fatty acid transport. Carbohydrates spare the utilization of fatty acids in most tissues. The glyceryl-PO₄ derived from carbohydrate breakdown is also essential for facilitating the re-esterification in adipose tissue necessary for disposal of chylomicon and VLDL triglycerides. Insulin is almost the sole force opposing many lipolytic factors that act to increase FFA flux from adipose tissue. Insulin deficiency also decreases the lipolytic activity required for glyceride removal. Thus, a mutant or mutants affecting insulin activity may also be determinants of various lipoprotein abnormalities.

Caloric supply also regulates lipoprotein levels. An acute excess, far exceeding that demanded for energy balance, elevates lipoprotein concentrations in normal men. Negative caloric balance promptly causes abnormal concentrations of VLDL to fall.

Triglyceride concentrations are also rapidly responsive to the metabolic mixture consumed. This is illustrated in Figure 4 in a simple experiment involving normal young adults fed three different diets for each of three 7-day periods. Triglyceride concentrations on the last day of each diet are plotted in relation to the three meals. A diet containing mainly fat and almost no carbohydrate (II, Fig. 4) halves the fasting glyceride (VLDL) concentrations and induces wide oscillations in glycerides (chylomicrons) following each meal. Reversal of the content of fat and carbohydrate (III, Fig. 4) trebles the fasting glyceride (VLDL) concentration and leaves it the highest seen throughout the 24-hour period. This last is an illustration of what is called "carbohydrate induction" of hyperglyceridemia. It is a normal phenomenon that may be exaggerated in some genetic abnormalities.

There is evidence of several mutants in the American population that have defective responses to fat transport demand. These fall into the type IV and type V summarized in Table 1 and illustrated in Figure 5. Evidence for their segregation has been given elsewhere. Type IV behaves as though it were a single autosomal mutant, about 50 per cent of first-degree adult relatives of propositi being similarly affected. The involvement of relatives in type V is more extensive. Children are rarely affected; more than half of the patients have
The influence of preceding diet on diurnal plasma triglyceride concentrations. Three subjects, ages 18–21, were each fed three isocaloric diets for one week. On the seventh day of each diet, blood was obtained at the indicated time intervals. The mean triglyceride concentrations are plotted. In diet I, 20, 40, and 40 per cent of calories were provided by protein (P), carbohydrate (C), and fat (F), respectively; diet II, 20, 10, and 70 from P, C, and F; diet III, 20, 79, and 1 from P, C, and F.

Summary and Conclusions.—The determinants of plasma lipoprotein concentrations can be illustrated by certain human mutants that appear to represent a defect in one of three major categories of control: (1) adequacy of the apolipoproteins; (2) sufficiency of mechanisms for catabolism of lipids (or lipoproteins); and (3) demands for fat transport. Abetalipoproteinemia and Tangier disease present stark examples of apoprotein inadequacy and suggest or eliminate specific functions for LDL and HDL. Type III hyperlipoproteinemia offers a possible example of altered chemical reactivity of LDL or apoLDL. Type I exemplifies the effects of a mutational defect in the lipolytic systems responsible for triglyc-
eride removal. The common mutation(s) giving rise to the hyperbetalipoproteinemia that is characteristic of type II hyperlipoproteinemia probably causes a rate-limiting defect in the catabolism of either cholesterol or LDL. Finally, there are other diseases leading to hyperlipoproteinemia, grouped as type IV or type V hyperlipoproteinemia, that seem clearly due to mutations but the defects are obscure. Most likely they are related to adjustments required by changing demands for fat transport. The paramount importance of insulin activity threads through several of these disorders and it is obvious that the gene or genes for diabetes cannot now be clearly separated from some of those causing the latter forms of hyperlipoproteinemia. Recently acquired new knowledge of the structure of lipoproteins can be expected to assist in study of the regulation of fat transport in lipoproteins and mutations affecting these processes. Most notable is the recent discovery of three new polypeptides among the apoproteins: apo-HDL-Gln, apoVLDL-Ala, and apoVLDL-Val, all of whose functions and inter-relationships with each other and the more established apolipoproteins remain to be determined.

10 Shore, B., and V. Shore, Biochemistry, 7, 3396 (1968).
17 Lees, R. S., J. Lipid Res., 8, 396 (1967).
26 Levy, R. I., and D. S. Fredrickson, manuscript in preparation.