Lipoprotein Electrophoresis Should Be Discontinued as a Routine Procedure

R. M. Iammarino

Reviewing our five-year experience with more than 1000 lipid profiles (assays for cholesterol and triglycerides in plasma of fasting patients and lipoprotein electrophoresis of their plasma lipoproteins), I conclude that lipoprotein electrophoresis is superfluous for routine clinical laboratory use, being generally redundant to the other parts of the profile. This conclusion is also prompted in part by recent information that changes our conceptions of lipoprotein metabolism and the clinical expressions of genetic hyperlipoproteinemia. A different approach to lipid profiling is suggested.

Diagnosis and assessment of atherosclerosis has been based on a succession of kinds of procedures during the past 25 years: measurement of lipoproteins separated by analytical ultracentrifugation (1), of plasma cholesterol (2) or triglycerides (3, 4), and most recently by interpreting the electrophoretic pattern of plasma lipoproteins (5, 6). In 1970 the World Health Organization offered guidelines for diagnosis and therapy of the six “Fredrickson types” (7) of hyperlipoproteinemia. Most clinical laboratories throughout the world now evaluate plasma lipids by collating the results of assays for plasma cholesterol and triglycerides plus an interpretation of the pattern of lipoprotein electrophoresis. In my opinion, the use of lipoprotein electrophoresis should be restricted to those specimens for which triglyceride concentrations are >350 mg/dl; this procedure would then be done only about a tenth as frequently as it is now. Because lipoprotein electrophoresis accounts for about half the cost of lipid profiles, such a curtailment would effect substantial savings.

This paper gives my reasons for this argument.

Materials and Methods

In the vast majority of cases, lipids were analyzed as an ordered test by the attending physician because of a clinical suspicion of a lipid disorder. Blood was collected, after an overnight fast, in 7-ml Vacutainers (Becton-Dickinson, Rutherford, N. J. 07070) containing 10.5 mg of ethylenediaminetetraacetic acid. The blood was centrifuged shortly after collection and the supernatant plasma removed and stored at 4 °C. Generally the lipids were analyzed within one to four days. Freezing was avoided because of its known deleterious effect on lipoproteins.

For most of the study, plasma cholesterol and triglycerides were assayed with a dual-channel Auto-Analyzer (Technicon Instruments Corp., Tarrytown, N. Y. 10591) system (8) adapted from Noble and Campbell (9). During the last year, cholesterol has been measured by a method adapted from Parekh and Jung (10), and triglycerides by an enzymatic procedure (11). Lipoproteins were electrophoresed in agarose gel by a previously reported system (12) as modified by Noble (13). The values for cholesterol and triglycerides, the appearance of the plasma from these fasting patients and a summary of the visual inspection of the lipoprotein electrophoresis pattern were then collated and abnormal results were placed on a report form, summarized by a written statement, “Consistent with Type____.” The printed report form has on it a chart relating cholesterol and triglycerides to the age of the patient, and an admonition to the physician to rule out “secondary diseases.” I seldom attempted to follow up abnormal assays by reviewing other information on the patient.

Table 1 gives the criteria used in classifying lipid disorders, together with the frequency and percentage of the various lipoprotein disorders I found in
Table 1. Distribution of Lipoprotein Types

<table>
<thead>
<tr>
<th>Diagnostic classification</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Appearance of lipoprotein electropherogram</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low lipid concentration</td>
<td>&lt;120</td>
<td>&lt;150</td>
<td>Faint bands</td>
<td>345</td>
</tr>
<tr>
<td>Normal</td>
<td>121-260</td>
<td>&lt;150</td>
<td>Chylomicrons</td>
<td>7280</td>
</tr>
<tr>
<td>Type I</td>
<td>&lt;260</td>
<td>&gt;1000</td>
<td>Dense β band</td>
<td>1</td>
</tr>
<tr>
<td>Type IIa</td>
<td>261-299</td>
<td>&lt;150</td>
<td>Clear separation</td>
<td>680</td>
</tr>
<tr>
<td>Borderline</td>
<td>&gt;300</td>
<td>&lt;150</td>
<td>between β and pre-β</td>
<td>320</td>
</tr>
<tr>
<td>Overt</td>
<td></td>
<td></td>
<td>Broad β band</td>
<td>160</td>
</tr>
<tr>
<td>Type IIb</td>
<td>261-299</td>
<td>151-350</td>
<td>(about 50 of these cases were studied by follow-up)</td>
<td></td>
</tr>
<tr>
<td>Borderline</td>
<td>&gt;300</td>
<td>151-350</td>
<td>&quot;definitive&quot; tests</td>
<td></td>
</tr>
<tr>
<td>Overt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Possible Type III</td>
<td>351-500</td>
<td>351-500</td>
<td>Prominent pre-β band</td>
<td>1850</td>
</tr>
<tr>
<td>Type IV</td>
<td>&lt;260</td>
<td>151-200</td>
<td></td>
<td>2090</td>
</tr>
<tr>
<td>Borderline</td>
<td>&gt;260</td>
<td>201-999</td>
<td>Prominent chylomicron and pre-β band</td>
<td>74</td>
</tr>
<tr>
<td>Overt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type V</td>
<td>&lt;300</td>
<td>&gt;1000</td>
<td></td>
<td>14380</td>
</tr>
</tbody>
</table>

This segregation into "Fredrickson types" is somewhat arbitrary, and does not take age and sex into account. The limits for "borderline" values of Type IIa, IIb, and IV are conservative. Most cases were adults who were more than 40 years old. The numbers represent close approximations to total cases studied after an allowance (projected from a sampling of computer data file) for duplicate analyses. Not all cases of "possible Type III" were studied by ultracentrifugation. "Low lipids" (cholesterol <120 mg/dl and triglycerides <150 mg/dl) were segregated because there is a possible association between low lipids and certain disease states, e.g., hyperthyroidism or intestinal malabsorption (16).

more than 14 000 assays performed in this institution during the past five years.1

Discussion

The Fredrickson system for typing hyperlipoproteinemias has stimulated much published work on lipid disorders associated with atherosclerosis, and is used by clinical centers throughout the world. The possibility of categorizing lipid disorders according to a few genetic expressions is a most appealing one and has been generally accepted.

Although the typing system is basically sound, I am not convinced it is being used as efficiently as it should be. I propose changes in the way it is used, because of the following observations:

(a) There is significant overlap between the interpretation given to data generated by lipid analyses and the interpretation of lipoprotein electrophoresis.

(b) Types I, III, and V are rarely seen.

(c) There are problems associated with the study of lipids in hospitalized patients.

(d) Newer concepts of lipoprotein metabolism have changed our views on the interrelationships of Fredrickson types.

I will discuss each of these individually.

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1 This study was aided by a computer file of lipoprotein profiles, with use of a specially designed program with an IBM System 370/145. The data were retrieved by use of an SPSS program (14). The technical help with this of Karen Troy, Dave Libenson, and Jerry Grunert is gratefully acknowledged.

Redundancy. There is virtually complete agreement between the conclusions one makes from the analytical values for cholesterol and triglycerides, and those one makes from the appearance of the lipoprotein electropherogram. Because lipoprotein electrophoresis is so hard to control, particularly with regard to uniform sample application and irregular dye uptake by lipids, we in this laboratory have gradually come to rely more and more on the analytical data. Whenever significant discrepancies were noted between the conclusion based on lipoprotein electrophoresis pattern and that based on chemical assays, such disagreement could almost always be traced to sampling errors. Some rare instances of true analytical discrepancies were noted, but most often these could be resolved and the lipidemia classified on the basis of lipid assay alone.

Rarity of Types I, III, and V. These types collectively account for less than 5% of those plasmas studied. During the five years being reviewed here we encountered one case of Type I and two of documented Type III, confirmed by ultracentrifugation of the plasma (40 000 X g, 15 h, 15 °C) and electrophoresis of the supernatant fraction. The two cases of Type III were secondary to hypothyroidism and reverted to normal on appropriate thyroid replacement. Seventy-four cases of Type V hyperlipidemia were detected during the study. Because not all cases of "possible Type III" were studied and ruled out by ultracentrif-
ugation, this figure may be spuriously low. Still, almost without exception, electrophoresis needed to differentiate these types would be performed if one restricts electrophoreses to those plasmas having triglyceride concentrations greater than 350 mg/dl.

Problems with patients. The hospitalized patient is a poor subject for lipid determinations. Proper typing requires good baseline studies. In the United States, hospital laboratory testing is done after the patient is admitted; no baseline values are generally available on him. Most patients are in the hospital because they are ill, but if a patient has been admitted primarily for a diagnostic workup, his altered setting (dietary changes, more rest, etc.) and the stress associated with his need for hospitalization can adversely affect the result. Lipid values are often severely decreased under these conditions (16). Overt or occult "secondary diseases" are frequent in hospitalized patients, but the clinician may not take this into consideration unless he is specifically alerted to them. Obviously, the typing done by the laboratory in the absence of such clinical information may be in error, no matter how elegant the lipid studies have been. False normals are as frequent as false abnormalities under these conditions. If used improperly, the existence of the typing system blunts thinking—the attending physician may accept the report uncritically, attributing to the laboratory a diagnostic potential that it very often does not have. These precautions are not new; they were clearly pointed out by Fredrickson et al. (6) in their earliest publications.

Newer concepts. The primary object of lipid assays is to discover genetic disorders, and I believe studies of the hospitalized patient are generally unsuited to this object. A more substantive criticism of the basis of the Fredrickson typing system has recently been raised. Initially it was thought that the Fredrickson types would be inherited in a consistent fashion; that is, there would be "Type II families" and "Type IV families," etc. The lipid disorders found in survivors of myocardial infarction and their immediate families were the subject of an extensive study (17) in the Seattle, Washington, area. It was shown that various Fredrickson types can be expressed within single family units; but Types IIa, IIb, IV, and V were randomly scattered in 47 of 168 families who were found to have a "genetic" lipid disorder that was called "combined familial hyperlipidemia." In smaller numbers, families of Type II and Type IV were found consistently, as had been anticipated. The authors offered explanations for the altered physiologic parameters involved. They found that elevated plasma triglyceride concentrations appeared to be the single common genetic expression forming the possible biochemical basis for "combined familial hyperlipidemia."

You will note an overwhelming frequency of Types II and Type IV in our study, Types IIb and IV accounting for 38% (over 5500 cases) of all studies and 78% of those that proved to be abnormal. These types are characterized by supranormal concentrations of plasma triglycerides, with or without concomitantly increased plasma cholesterol. Cases presenting primarily with increased cholesterol—i.e., Type IIa—were found in 7% (1000 cases) of all lipid profiles and in 14% of those that proved to be abnormal. Thus supranormal plasma triglycerides were the single most frequent expression of a lipid disorder in our sample of hospitalized patients. In trying to account for this it becomes necessary to discuss some of the more recent concepts concerning lipoprotein metabolism.

Figure 1 summarizes some current concepts regarding synthesis, release, and turnover of lipoprotein moieties. The intestine and liver are primarily responsible for most lipoprotein synthesis. The intestinal cell is chiefly responsible for the synthesis of chylomicrons, the liver cell for VLDL and HDL. Lipoprotein lipase (diacylglycerol acylhydrolase, EC 3.1.1.34) catalyzes release of the lipid moieties of both VLDL and chylomicrons. A protein moiety of HDL is one activator of lipoprotein lipase (18). LDL are formed outside of the liver after partial catabolism of VLDL (19). LDL have a significantly prolonged half-life compared with VLDL and therefore the concentration of lipids during fasting favors LDL, which apparently cannot be re-utilized in the synthesis of VLDL and is catabolized (20). Whether the liver cell is the primary site for the catabolism of LDL is not certain (21).

Figure 2 depicts some of the recognized primary and secondary hyperlipoproteinemias in terms of lipoprotein synthesis and turnover. The most common causes of hyperlipoproteinemia, primary or secondary, are those associated with augmented synthesis and (or) release of VLDL. Depending on the activity

2 Nonstandard abbreviations used: HDL, high-density lipoproteins; LDL, low-density lipoproteins; VLDL, very-low-density lipoproteins.
of lipoprotein lipase transformation and whether LDL is catabolized normally, it can be seen that augmented synthesis and release of VLDL could, as has been suggested (22), be the basic defect in "combined familial hyperlipidemia," allowing for the expression of Types IIa, IIb, IV, and V. Figures 1 and 2 are based on present concepts and are of course subject to modification or radical change as further knowledge is added.

A hypothetical example might clarify some of these points. A 50-year-old man is admitted to the hospital with an acute myocardial infarction. Twelve or sixteen hours after the acute event, blood is drawn for determination of lipids, the values for which by this time may well be normal. It should again be emphasized that acute stress will often decrease lipid values (23). A few weeks after the myocardial infarct the patient has already received dietary instructions that favor elimination of cholesterol-rich food, and a transition from saturated to polyunsaturated fats; his diet now begins to contain a larger proportion of carbohydrates. If his plasma is studied at this time, it may show a mild Type IV pattern, attributable to this changed diet. If the patient resumes his previous dietary ways, he may be back to a Type IIa or Type IIb pattern in three to six months. Possibly the laboratory may never detect an abnormal lipid pattern for this patient after his myocardial infarction, but this is far from saying that one never existed. In this type of patient the myocardial infarction may well have been induced as the result of long-standing dietary indiscretions: too high a caloric intake, too much carbohydrates, and too much saturated fat in his diet. The only hope for delineation of a genetic disorder would be to study the lipid patterns of the patient's first-degree kin. In this way some indication of the primary or genetic nature of the lipid disorder can be obtained, even in the face of "normalized" lipid values.

A further criticism of the rigid definition of Fredrickson was raised by Davignon (23a). He showed that there was a difference in response to diet and drugs, depending on an arbitrary cholesterol-triglyceride ratio. Individuals presently classified as Type IIa responded generally with a more significant lowering of cholesterol concentration after treatment with ethyl p-chlorophenoxyisobutyrate (Clofibrate) than do individuals in their group presently identified as Type IIb. They concluded that the two subtypes of Type II do not constitute a single disease entity. It would be interesting to see if these findings would relate to the "combined familial hyperlipoproteinemia" as identified by the Seattle group.

Is any information obtained uniquely by lipoprotein electrophoresis? The answer appears to be a qualified yes. There are three reports of a pre-beta fraction occurring in a high percentage of patients with myocardial infarction (24–26). Although with our agarose gel system we have noted occasional split pre-beta fractions, we could not confirm these reports. Seidel et al. (27) recently has approached the diagnosis of Type III by agarose electrophoresis coupled with post-electrophoretic polyanionic precipitation of the lipoproteins within the gel. "Lipoprotein X" is an abnormal plasma lipoprotein associated with obstructive jaundice and lecithin:cholesterol acyltransferase (EC 2.3.1.43) deficiency. Seidel et al. (28) have described a system of agar gel electrophoresis that causes lipoprotein X to move to the cathode by electroendosmosis. Although more widely used for lipoprotein electrophoresis, the purified agar marketed as "agarose" causes lipoprotein X to migrate to the anode and thus will not discriminate as effectively as agar. We use Agar Difco Noble (Difco Laboratories, Detroit, Mich. 48232) in the electrophoretic identification of lipoprotein X. While this test may have an application for the screening of this rare transferase deficiency, the role of lipoprotein X as a determinant in obstructive jaundice remains to be clarified. We also found lipoprotein electrophoresis of pleural fluid to be useful in confirming the diagnosis of chylos effusion. We have previously reported (29) a heparin effect in routine lipoprotein electrophoresis, in which the patient undergoing heparin treatment has an increased migration of all lipoproteins, probably owing to the protein binding of the highly negatively charged heparin molecule. Because the migration of lipoproteins is so altered, one should be cautious of any interpretation of abnormalities when heparin has been clinically administered. Lipoprotein electrophoresis has been used (30) in cases of suspected lipoprotein lipase deficiency associated with fasting chylomicronemia. Here, a baseline sample is drawn, a second sample is drawn 10 min after a standard dose of heparin, and both samples are subjected to lipoprotein electrophoresis. Disappearance of the chyloheparin chylicromic band is presumptive evidence of a functioning lipoprotein lipase system. Polyacrylamide gel electrophoresis has
been described for the confirmation of Type III and for allowing some quantification of the LDL fraction (31).

Figure 3 shows the report form we now use. It places the burden of typing on the attending physician by asking him to interpret lipid data himself. In a busy hospital service it is virtually impossible for the laboratory to do the appropriate clinical follow-up and the attending physician is best situated to interpret these data.

As for the future, lipid surveys in my opinion would be more profitably done in a younger, ambulatory, healthy population. We have studied such a group—sophomore medical students—and my experience convinces me that a group such as this provides a more rational study population than do hospitalized patients. Identification is cleaner and treatment, if indicated, is more likely to succeed. Younger school populations have been surveyed here (32) and elsewhere (33, 34), with similar conclusions. An even more rational group for screening may prove to be the neonate (35-37) for, although the data are initially somewhat conflicting (38), some evidence suggests that a change in diet during the first few months of life may beneficially and permanently alter the blood lipid expression of this genetic disorder (39).

At a basic science level, better delineation of the control points for lipoprotein synthesis and release will have important clinical applications. Studies concerning bile acid metabolism in hypercholesterolemia (40), feedback control of cholesterol synthesis in genetic hyperlipoproteinemia (41), accelerated atherosclerosis during renal dialysis (42), VLDL → LDL transformation, and a better understanding of the hypolipoproteinemia associated with the acute phase protein reaction are some examples of current interest.

References
5. Lees, R. S., and Hatch, F. C., Sharper separation of lipoprotein
8. Technicon Method N37a (Cholesterol) and N7OP (Triglycerides), AutoAnalyzer Manual, Technicon Corp., Tarrytown, N.Y. 10591.


Two persons were asked to comment on this opinion:

Gerd Assmann:

Hyperlipidemia, cigarette smoking, and hypertension have been identified as major risk factors in the development of coronary artery disease (1). Abnormalities in plasma lipids and lipoproteins, therefore, have received much attention in the diagnosis and treatment of atherosclerotic heart disease.

The diagnosis of hyperlipidemia is established by recognition of elevated plasma cholesterol or triglyceride concentrations, while the diagnosis of hyperlipoproteinemia depends on the determination of increased individual lipoprotein fractions.

To date, the physician involved in primary health care, clinical laboratories, the epidemiologist, and...