Memoranda

CLASSIFICATION OF HYPERLIPIDAEMIAS AND HYPERLIPOPROTEINAEMIAS *

Many studies of atherosclerosis have indicated hyperlipidaemia as a predisposing factor to vascular disease. The relationship holds even for mild degrees of hyperlipidaemia, a fact that underlines the importance of this category of disorders. Both primary and secondary hyperlipidaemias represent such a variety of abnormalities that an internationally acceptable provisional classification is highly desirable in order to facilitate communication between scientists with different backgrounds.

The present memorandum presents such a classification; it briefly describes the criteria for diagnosis of the main types of hyperlipidaemia as well as the methods of their determination. Because lipoproteins offer more information than analysis of plasma lipids (most of the plasma lipids being bound to various proteins), the classification is based on lipoprotein analyses by electrophoresis and ultracentrifugation. Simpler methods, however, such as the observation of plasma and measurements of cholesterol and triglycerides, are used to the fullest possible extent in determining the lipoprotein patterns.

INTRODUCTION

Many studies in different countries have shown that hyperlipidaemia is often present in patients with clinical manifestations of atherosclerosis. In families with genetic forms of severe hyperlipidaemia the commencement of complications of atherosclerosis is commonly premature. Prospective studies also indicate that even milder degrees of hyperlipidaemia predispose to atherosclerotic vascular disease.

Most of the lipids in plasma [1] are present as lipoproteins and hyperlipidaemia almost always means that the concentrations of one or more lipoproteins are elevated (hyperlipoproteinaemia). Determination of lipoprotein concentration offers more information than lipid analyses alone in assessing the many abnormalities collectively considered under hyperlipidaemia. The most rational basis for describing hyperlipidaemia at present is to do so in terms of lipoproteins. Quantitative determination of lipids, however, is much easier than quantitative determination of lipoprotein families; for practical purposes it may often be enough, after one lipoprotein determination, to make lipid analyses thereafter.

The principal reasons for attempting to adopt an internationally acceptable system of classifying the different types of hyperlipoproteinaemia are:

(1) *Diagnostic*—to aid in diagnosis and in evaluation of prognosis.

(2) *Therapeutic*—to guide the most rational choice of intervention and to evaluate its effectiveness.

(3) *Epidemiological*—to enhance the ability to compare different populations.

(4) *Genetic*—to permit better recognition and assessment of the prevalence of mutations causing hyperlipidaemia.

(5) *Etiological*—to provide a better framework for discovery of different causes and mechanisms of hyperlipidaemia.

The measurement of cholesterol and triglycerides is the most practical means of detecting hyperlipoproteinaemia and provides some information as to the type of hyperlipoproteinaemia. The lipoprotein pattern offers the best basis for a classification of hyperlipoproteinaemia at present. There are many pitfalls in the use of lipoproteins, however, and some important practical problems have not yet been satisfactorily solved. Three premises underlie the use of lipoproteins to describe the great hetero-

* This memorandum was prepared by the signatories listed on page 907, who took part in an informal consultation arranged by the World Health Organization.

[1] Throughout this memorandum, statements referring to plasma may be applied, in general, to serum and *vice versa*.

geneity concealed within the general problem of hyperlipidaemia:

(1) It is not necessary to use any one particular technique for defining lipoprotein patterns, and, in fact, definition can be achieved only if more than one technique is used.

(2) To achieve useful classification, one must go beyond lipoprotein patterns and consider etiology.

(3) Any classification used today is necessarily an open-ended and incomplete one.

The classification systems described below are, in principle, reasonably simple. With the use of appropriate methods they should permit the adoption of a common language in describing different forms of hyperlipidaemia. However, as no one particular technique is recommended, it is of the utmost importance that future reports and publications should carefully describe the methods and standards used to define hyperlipidaemia and hyperlipoproteinaemia.

DESCRIPTION OF PLASMA LIPIDS AND LIPOPROTEINS

LIPIDS

The lipids in serum are mixtures of many constituents. The usefulness of cholesterol and triglycerides in the clinical classification of hyperlipidaemia and hyperlipoproteinaemia at present far outweighs that of the other lipids.

Cholesterol and triglycerides

Cholesterol occurs as the alcohol ("free cholesterol") and in the esterified form, in a proportion that is fairly constant. About 70% of the cholesterol is esterified, except in obstructive disease of the liver and some rare diseases. Practically all of the sterol in plasma is $\Delta 5$,6-cholestene-3β-ol. Cholesterol is usually determined as total cholesterol and it is recommended that this be expressed in mg/dl.

Glycerides in human plasma occur mainly as triglycerides. Relatively insignificant concentrations of di- and monoglycerides are also present. Triglycerides are usually determined by estimation of the glycerol content; however, the results are calculated as amounts of triglyceride. The concentrations may be expressed either as mg/dl or as mmol/litre. The molecular weight of the standard used for the calculations should be stated when the result is given on a weight basis, e.g., as mg/dl.

Other lipids

The phospholipids in plasma include mainly phosphatidyl-choline, sphingomyelin, lysophosphatidyl-choline and phosphatidyl ethanolamine. The proportions of these vary in the different lipoproteins; this information is not generally used in clinical classification. Fatty acids are present mainly as glyceryl, phosphoglyceryl and cholesteryl esters; in a small, but metabolically very active, fraction they are not esterified and are called free fatty acids (FFA). Small amounts of glycosphingolipids are also present in plasma.

The fat soluble vitamins (A, D, E, K) and carotenoids are present in plasma. Vitamins A and E have been used to study absorption and transport of dietary fat.

LIPOPROTEINS

General description

All the lipids in plasma circulate in combination with protein (Fredrickson et al., 1967; Schumaker & Adams, 1969; Tria & Scanu, 1969). The free fatty acids (FFA) are bound to albumin, the other lipids aggregate with other proteins and these latter complexes are called lipoproteins. They range in size from small "soluble" lipoproteins of about 100 Å in diameter, having molecular weights of less than 400 000, to "particles" larger than 1 μ in diameter and having molecular weights that are proportionally greater. The smaller the lipoprotein, the greater is the relative amount of protein present. The lipid component decreases the density of the lipoproteins. The differences in the content of lipids and proteins among the several lipoproteins give them different densities and permit their further separation in the ultracentrifuge. The lipoproteins also differ in electrical charge. This property, in combination with differences in size, also permits separation of the lipoproteins by electrophoresis.

Major lipoprotein families

Electrophoresis and ultracentrifugation are the principal methods for both separation and identification of lipoproteins (Ewing et al., 1965; Hatch & Lees, 1968). By either technique, the plasma lipoproteins are usually considered as representing 4 major families. Since pure lipoproteins can best be obtained by ultracentrifugation, the basic nomen-

clature is usually derived from this technique. Electrophoresis is more practical, and the terminology derived from this method is more familiar to clinicians. Both sets of nomenclature are used interchangeably in this report and their relationship is illustrated in the accompanying figure. The 4 major lipoprotein families are as follows:

Chylomicrons (the same name is used whether ultracentrifugal or electrophoretic separation is employed). These large particles have a density of about 0.9 (S_f values > 400). They collect at the top of plasma left standing for 16–24 hours at 4°C, they remain at the origin on paper or agarose electrophoresis or in the loading gel on polyacrylamide gel, and they behave in a certain way in PVP (polyvinylpyrrolidone) gradients or on starch-gel electrophoresis.

Most chylomicrons are considered to represent exogenous triglycerides. As useful as this metabolic definition is, however, it is not an absolute one. Some dietary triglycerides also appear soon after eating, in particles having the properties of very low density lipoproteins (VLDL). Some VLDL also have the immobility of chylomicrons on electrophoresis.

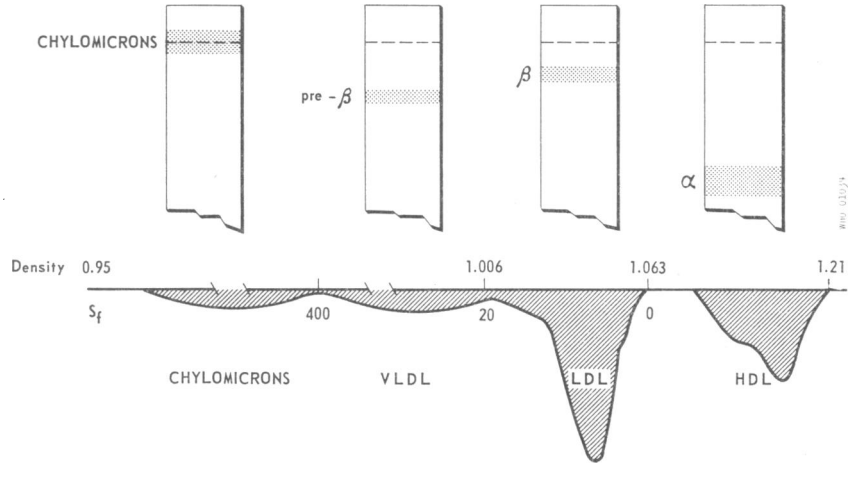
Very low density lipoproteins (VLDL) (now called pre-β lipoproteins in most of the systems for electrophoresis used clinically). These lipoproteins are isolated in the ultracentrifuge in the fraction of density < 1.006 and have S_f values of 20–400. VLDL (pre-β) consist mainly of glycerides that are "endogenous", i.e., newly synthesized or derived from body stores rather than directly from the diet.

On some electrophoretic systems, several pre-β bands may appear in plasma. No pathological significance has yet been assigned to these. In about 10% of subjects, including those with different types of hyperlipoproteinaemia and their normal relatives, some lipoproteins of pre-β mobility are present that sediment rather than float at density 1.006. These "sinking pre-β" correlate well with a lipoprotein fraction of density 1.050–1.080 that appears to contain the "Lp antigen (Berg)", a marker of genetic polymorphism that, as yet, is not known to have pathological implications.

Low density lipoproteins (LDL) (called β-lipoproteins in all systems for electrophoresis). The lipoproteins are isolated between the densities of 1.006 and 1.063 and have S_f values of 0–20. Their major constituents are cholesterol and cholesteryl esters; the remaining components are mainly phospholipids, protein and small amounts of glyceride.

High density lipoproteins (HDL) (called α-lipoproteins, or more specifically α_1-lipoproteins, in all systems for electrophoresis). HDL, including the HDL_2 and HDL_3 classes as defined by de Lalla & Gofman (1954) are isolated between the densities of 1.063–1.21 and contain about 50% protein; the lipid component consists mainly of cholesterol and phospholipids in a ratio of about 0.5 (by weight).

THE PLASMA LIPOPROTEIN SPECTRUM AS SEGREGATED BY PAPER ELECTROPHERESIS (ABOVE) AND BY THE ULTRACENTRIFUGE (BELOW) IN WHICH S_f OR FLOTATION RATES ARE INVERSELY RELATED TO DENSITY



Abnormal lipoproteins

A few abnormal lipoproteins that may appear in plasma have now been recognized. These include:

"*Floating β-lipoproteins*". These have also been called "broad β" or "β-VLDL". They are now identified as either (*a*) lipoproteins of β-mobility floating at density 1.006 after 16 hours at 100 000 *g* or (*b*) an inversion of the usual concentrations of S_f 0–20 and S_f 20–100 lipoprotein as measured in the analytical ultracentrifuge. On starch-block electrophoresis the VLDL become clearly bimodal, having peaks of both β- and α_2-mobility. An absolute definition of this anomaly at present requires the ultracentrifuge. Possible simpler methods are described below. "Floating β" may prove to be a normal lipoprotein that is present only in trace concentrations. By the use of paper electrophoresis after ultracentrifugation, it has been observed so far only in patients homozygous for familial HDL deficiency (Tangier disease) or with an unusual form of hyperlipoproteinaemia (Type III). The latter is usually "primary" in etiology and often familial. In rare instances "floating β" may be secondary to other diseases such as uncontrolled diabetes.

"*Lipoprotein-X*" *(Lp-X)*. These are complexes mainly of phospholipid, unesterified cholesterol and VLDL apoproteins (the delipidated proteins from VLDL, see below) that form in patients with obstructive liver disease. The lipoprotein is separable by Cohn-fractionation and is identified by an antibody that also reacts with apo-VLDL (Seidel et al., 1969). The accompanying lipoprotein pattern is variable. On paper and agarose, Lp-X migrates slower than β and, because it contains free cholesterol and phospholipids, it stains poorly with lipid stains (Oil-Red-O or Sudan). Often α_1-migrating lipoproteins are decreased or absent.

Complexes of "normal" lipoproteins with other globulins. Complexes, some representing antigen–antibody reactions, occur between lipoproteins and immunoglobulins or macroglobulins. It is now known that certain IgA and IgG myeloma proteins react with lipoproteins and it has been suggested that such complexes may be models of other kinds of "auto-immune hyperlipoproteinaemia" (Beaumont, 1965, 1969). There are no definitive electrophoretic or ultracentrifugal tests for such complexes and lipoprotein patterns consistent with all of the major abnormal patterns (see below) may be obtained. It is at present necessary to isolate the immunoglobulin and test its reactivity with lipoprotein antigens using sensitized erythrocytes.

HDL_T or α_T-lipoproteins. The small amounts of HDL in homozygotes for Tangier disease have now been shown to contain abnormalities in the proportions and possibly the structure of the apoproteins. The anomaly is detectable only immunochemically or chemically and arises from one or more rare mutations.

Other abnormal lipoproteins. These include HDL and VLDL having abnormal chemical composition in familial lecithin: cholesterol acyltransferase (LCAT) deficiency, abnormal HDL in abetalipoproteinaemia, chemically abnormal VLDL in Tangier disease, and VLDL of β-mobility in severe parenchymal liver disease. Lipoproteins having abnormal apoprotein content have thus far been demonstrated only in Tangier disease; others probably exist in some forms of hyperlipidaemia.

Apolipoproteins

The proteins that form complexes with lipids to produce the lipoproteins appear to be specific and presumably they serve primarily or exclusively to facilitate fat transport or the metabolism of lipoproteins. The proteins, once the lipid has been removed, are called apolipoproteins or, more simply, apoproteins. The total number that may be present in all the lipoprotein families is not yet known. There is also no standard nomenclature for the apoproteins. One terminology describes those proteins in HDL as apo-A and those in LDL as apo-B. In VLDL apo-B and possibly apo-A are present in addition to apo-C, which includes at least 3 different proteins. At least 4, probably many more, proteins are present in VLDL, and there are at least 5 in HDL. Therefore another common practice is to refer to them according to the lipoprotein family from which they are isolated, i.e., apo-LDL, apo-VLDL, apo-HDL, sometimes followed by their carboxyterminal amino acids, e.g. apo-HDL—threonine, apo-HDL—glutamine, etc. Some of the apoproteins in VLDL are also present in HDL and LDL, indicating interrelationships between the lipoprotein families that no doubt are significant in regard to their metabolism. The apoproteins confer some degree of immunological specificity, and aid in the identification and measurement of lipoproteins.

No type of hyperlipidaemia has yet been recognized to be due to an abnormal apoprotein, although the possibility of this, especially if the abnormal

protein were to arise through a mutation, offers an attractive basis for the possible etiology of some forms of hyperlipoproteinaemia.

Metabolism—turnover

Generally speaking the concentration of a lipoprotein class in plasma depends on two processes: (1) the rate of entry into plasma (turnover rate, synthesis or production rate), and (2) the rate of efflux from plasma (fractional turnover rate, clearance, catabolism). When the concentration is constant (steady state conditions) these two rates are equal. To understand the pathogenesis of any hyperlipidaemia the primary question is whether the increased plasma levels are due to increased turnover rate (production) or decreased fractional turnover rate (clearance). Methods for measurement and evaluation of turnover rate and fractional turnover rate of various plasma lipids and lipoproteins are at present being worked out in several laboratories. They involve different techniques such as the measurement of the rate of disappearance of intravenously injected, labelled lipoproteins and fat emulsions (fat tolerance) or the determination of the rate of entry into plasma (production) of labelled triglycerides from various precursors. Furthermore, methods are available for estimating the total cholesterol (sterol) balance and turnover. At present such functional tests do not form the basis for classification of hyperlipidaemias, but they may ultimately be valuable for the classification of hyperlipoproteinaemias.

BIOLOGICAL VARIATION

The fasting levels of the different plasma lipids vary considerably between different individuals within a population but also from hour to hour and day to day in one and the same individual. Some of the factors that have a definite influence on plasma levels of cholesterol and triglycerides will be mentioned below. Some factors that have a less well-documented effect, but may be of importance, will also be considered. It is highly desirable to try to standardize the conditions for sampling blood in order to minimize the influence of such factors on lipid and lipoprotein levels. When the sample is obtained under other conditions, the influence of these factors should be considered.

INTRA-INDIVIDUAL VARIATION

Effect of meals

Within hours after eating, several changes occur in the plasma lipids. If the meal contains fat, the most relevant change for classification of hyperlipoproteinaemia is the appearance of chylomicrons in plasma: the so-called alimentary hyperlipaemia. The course of alimentary lipaemia and its concomitant hypertriglyceridaemia is very variable. It is influenced by the composition of the meal, by the absorptive processes and by the metabolism of the chylomicra. The duration and degree is often exaggerated in hyperlipaemic states. In normal subjects after the consumption of 1 g–2 g of fat per kg of body weight, the peak lipaemia is reached after 2–4 hours and the hypertriglyceridaemia persists for 6–8 hours. After the appearance of chylomicronaemia, there may be increases in the VLDL (pre-β-lipoproteins) followed by slight changes in both LDL (β-lipoproteins) and HDL (α-lipoproteins). Even non-fat carbohydrate meals may change the lipoprotein levels.

Effect of diet

The habitual diet of a person has a considerable influence on the lipid and lipoprotein concentrations; the calorie intake from fat and carbohydrates is an important factor in this regard. The influence of protein intake is less well known. The type of fatty acids (chain length, degree of unsaturation) and the amount of cholesterol in the diet are also of great importance, especially for the LDL (β-lipoprotein) levels. The kind of carbohydrate in the diet may also influence lipoprotein concentrations, in particular the VLDL (pre-β-lipoproteins). It is also possible that a number of other constituents of the diet may influence the lipid levels. Alcohol, for example, in "excessive" amounts may have pronounced effects in particular on VLDL (pre-β-lipoproteins).

Changes in habitual dietary habits such as altered caloric intake or shifts in the ratio of fat to carbohydrate may induce both permanent and transitory changes in lipid and lipoprotein levels.

Effect of posture and venostasis

The plasma volume changes with posture; concomitantly the plasma protein and lipoprotein con-

centrations vary. Increases of the order of magnitude of 10% may sometimes be seen when the subject moves from the recumbent to the standing position. Venostasis, for example during a difficult venepuncture, can elevate the serum cholesterol by up to 15% (Koerselman, Lewis & Pilkington, 1961).

Other factors

There are several environmental and other factors and conditions that may acutely or chronically have an influence on the plasma lipid and lipoprotein levels (Carlson & Lindstedt, 1969). Examples of such factors are acute trauma (one week after an acute myocardial infarction, plasma cholesterol and LDL (β-lipoprotein) may be lowered by 25%–50%), pregnancy, exercise, emotional stress, and smoking. In some places seasonal variation occurs in cholesterol and triglyceride concentrations. Lipid-lowering drugs particularly, but also other drugs such as estrogens and other hormones (including certain oral contraceptives) and salicylates, affect the concentration of lipids in plasma.

INTER-INDIVIDUAL VARIATION

Age

Age is one of the more important factors influencing plasma lipid levels. From birth to middle-age cholesterol and triglyceride levels (in several countries) increase 4-fold–5-fold (Brody & Carlson, 1962; Carlson, 1960a). The greatest rise usually occurs during the first years of life. In some countries there is no further rise after about 20 years of age, in others there is a steady rise also from 20 years to about 50 or 60 years of age.

Sex

Significant differences between the sexes in both cholesterol and triglyceride levels have been reported. At birth the levels are the same (Brody & Carlson, 1962). Men and women often have similar values for cholesterol from 20–50 years of age, whereafter the females tend to have higher levels. The triglyceride level is in general lower in females than in males (Carlson & Lindstedt, 1969). From the lipoprotein point of view, women characteristically tend to have higher HDL (α-lipoproteins) and lower VLDL (pre-β-lipoproteins) levels than men.

Other factors

Genetic and ethnic factors have to be considered in relation to plasma lipids. Cultural factors, including nutrition and physical activity, may have a pronounced influence on the concentration of lipids and lipoproteins.

CONDITIONS OF SAMPLING

It is evident from the above that a number of factors may cause considerable changes in the plasma levels of lipids and lipoproteins. Some of these are amenable to standardization, others are not. For classification of hyperlipidaemia it is necessary to take the blood in the fasting state, i.e., 10–14 hours after the last meal. Under certain conditions, the composition of the last meal may be of importance.

The composition of the patient's diet several days or weeks before the study should also be taken into consideration. It is important that the caloric balance be maintained constant, i.e., that no weight changes occur. For genetic studies it is desirable that the patient, for at least the last 2 weeks, has been eating the diet usual for his race.

It is recommended that each laboratory should take care to standardize its conditions for taking the blood sample. Venostasis should be avoided and the subjects should be resting.

DEFINITION OF NORMAL VALUES

Hyperlipidaemia implies that the concentration of one or more lipids is increased above the "normal" level. The basic requirement for establishing the presence of hyperlipidaemia is a definition of what is the normal concentration of lipids. In this regard, a number of factors, including some discussed above, must be considered when defining what is "normal" or "abnormal". The concept of normality will be briefly considered from two aspects.

Physiological (biological) normality

Increased levels of plasma lipids are associated with increased risk of developing atherosclerotic vascular disease. Genetic absence of HDL (α-lipoprotein) or of LDL (β-lipoprotein), VLD (pre-β-lipoprotein) and chylomicrons are associated with typical clinical pictures. Although complete absence of all lipoprotein families appears to be an unhealthy state, the minimum levels compatible with good health are not known.

Several populations are known to live in apparently good health with much lower lipid values than

those generally encountered in heavily industrialized communities. It is probable that in certain of these latter populations the statistically normal lipoprotein levels are higher than is required for normal physiological function and carry with them a generally increased risk of premature vascular disease.

Statistical normality

It is relatively easy, for a given population, to establish the frequency distribution of the values for various lipid and lipoprotein fractions. Based on such data, statistical methods can be applied and the population can be defined in statistical terms. It should be emphasized in this connexion that the distribution of plasma lipids is very seldom confined to the normal or Gaussian distribution curve. The distribution is usually skewed to the right with the mean greater than the median. This is especially true for triglyceride values, both in adults (Carlson, 1960a; Carlson & Lindstedt, 1969) and in the newborn (Brody & Carlson, 1962). The non-Gaussian distribution often calls for the use of special methods, such as log-transformation (Carlson, 1960a; Carlson & Lindstedt, 1969), before the application of ordinary statistical formulae.

A definition of " abnormally high " based on statistical normality may be constructed in various ways, all of which at present have to be arbitrarily chosen. Values above a certain upper limit are often used. One may then consider those above this limit as abnormal. Sometimes subjects outside twice the standard deviation of the mean are called abnormal. The definition of such " cut-off points " or " upper limits of normality " will vary with the problem under investigation. The epidemiologist, the clinician, and the geneticist, for example, may use different limits of " normal ". It is therefore meaningless here to supply specific normal limits for the concentrations of plasma lipids or lipoproteins for use in all populations and all studies. In this report the term " increased " implies concentrations that exceed whatever arbitrary limit has been selected.

The classification described below uses relative definitions and is therefore applicable to populations which may have widely different distributions of the concentrations of lipids and lipoproteins.

METHODS FOR ANALYSIS OF LIPIDS AND LIPOPROTEINS

Cholesterol measurement and estimation of the triglyceride concentration are the minimum requirements for the detection of hyperlipidaemia. However, accurate measurements of cholesterol and triglycerides, separation and identification of lipoproteins including qualitative examination for chylomicrons, and estimation of LDL and VLDL concentrations are recommended for the classification for hyperlipidaemias. In the future, apolipoproteins, free fatty acids, the fatty acid composition of certain lipid fractions, the individual phospholipid classes, and the activities of various lipid transferases and hydrolases may have more value in the classification of altered states of lipid metabolism. Any classification employed today will be necessarily limited and open-ended because of current limitations in knowledge and inability to measure the levels of some lipids and lipoproteins, and the activities of different enzymes in cells as well as tissues.

LIPIDS

Reliable manual and automated methods are available for the determination of cholesterol and triglycerides. The stability of performance and ease of standardization of different methods vary considerably.

The sample

Serum obtained in the fasting state is preferred for the measurement of both cholesterol and triglycerides. Plasma is acceptable provided correction is applied for any dilution caused by the anticoagulants. Samples stored for analysis must be kept under sterile conditions. Freezing does not affect either cholesterol or triglyceride analysis. Turbidity, haemolysis, and elevated bilirubin content of samples make them unacceptable for some methods, and in others necessitate special blanks or other corrections.

Samples collected at 10–14 hours postprandially are recommended for the determination of triglycerides. Some investigators believe that triglyceride concentrations obtained in the non-fasting state can be corrected to, or correlated with, those in the fasting state. Diurnal variations in cholesterol are usually ignored.

Cholesterol

Many methods have been developed for the determination of total cholesterol. Most methods

utilize acetic anhydride and acetic acid or ferric chloride to develop colour; specificity is enhanced by prior extraction techniques. The highly specific method devised by Abell et al. (1952) is recommended as a reference method. It can also be semi-automated as a routine procedure, but has not been fully automated because of the 2-phase extraction procedure and the use of highly corrosive reagents.

Most direct methods (i.e., with colour-developing reagent added directly to the serum) and automated methods usually give higher values than the Abell–Kendall method. Since the cholesterol values obtained and the slope of the standard curve vary with different procedures, care is required to assure accuracy, stability of performance and comparability of results obtained with different methods.

The determination of free and esterified cholesterol is more arduous; fortunately the measurement of total cholesterol suffices for the classification of nearly all known forms of hyperlipidaemia.

Triglycerides

Three basic methods are now used successfully (Witter & Whitner, 1970) for the determination of triglyceride: (1) an enzymatic procedure, (2) a colorimetric procedure in which the glycerol is oxidized to formaldehyde which is then determined by one of a number of colorimetric reactions, and (3) a fluorometric procedure that measures the formed formaldehyde. Almost all triglyceride methods gain specificity by initial extraction of lipids from serum or plasma, separation of phospholipids and glucose, and hydrolysis of the isolated triglycerides prior to determination of glycerol. Most enzymatic, fluorometric and colorimetric methods give comparable results since all are based on the determination of glycerol and all include provisions to control interfering substances. Automation of some steps in all three types of methods is possible, but it is necessary to maintain constant control of reproducibility and performance relative to known standards.

Standardization of cholesterol and triglyceride methods

Acceptable assay of cholesterol and triglycerides depends upon use of specific and reproducible analytical procedures and care in the collection, transport and storage of samples. These procedures also must be controlled by proper use of primary lipid standards and serum reference samples. Adequate standards and reference materials are available for the determination of both cholesterol and triglycerides (Witter et al., 1970).

Cholesterol. Primary standards of cholesterol of at least 99% purity are available from several commercial sources (Williams et al., 1970). The US National Bureau of Standards also provides a primary cholesterol standard of known purity. These crystalline standards should be stored in small aliquots over a desiccant such as silica gel at −20°C in the dark. Useful criteria of purity are provided by the melting point (when determined in evacuated capillary tubes or on melting-point blocks under cover slips), chromatography in gas–liquid systems or on thin layers of silicic acid and alumina–silver nitrate, constancy of dry weight when heated at 80°C *in vacuo* and comparison of absorbency in the Lieberman–Burchard reaction with known standards. The odour of cholesterol preparations is a sensitive index of the accumulated oxidized products.

A serum reference material for the cholesterol determination may be prepared by filtering pooled human serum through clarifying and " sterilizing " filters. Stable serum preparations containing concentrations of cholesterol from 100 mg/dl to 400 mg/dl may be made by the addition of an alcohol-precipitated cholesterol-rich protein fraction from human serum to either bovine, horse or human serum (Williams, Taylor et al., 1970). Aliquots should be stored in properly sealed vials or ampules at temperatures not higher than −20°C. These preparations have the same stability as human sterile serum. Preliminary data indicate that these sterile preparations may be shipped at room temperature for periods as long as 5 days without change in cholesterol content as determined by the Abell–Kendall method. Lyophilized samples are also best stored in the frozen state, but can be distributed to distant points without refrigeration.

The cholesterol concentrations of some commercial reference sera may vary significantly with the values obtained with the reference Abell–Kendall method. All such samples should be checked against serum reference materials and standardized with the reference cholesterol method.

Triglycerides. Acceptable primary standards and serum reference materials also are available for the standardization of the triglyceride determination. Purified preparations of either triolein or tripalmitin are currently used as primary standards. If the results are expressed in mg/dl, differences will be obtained in final concentrations depending upon the molecular weight employed in calculating the results. The primary standard used in analysis should be reported with the results. The use of

corn oil or other cooking oils as standards is unacceptable. These preparations are not pure triglycerides and the glyceride content varies from one lot to another.

Triglycerides are usually measured as glyceride glycerol, but the results are often calculated as mass concentration of whole glyceride. For this purpose, an " average " molecular weight is often arbitrarily selected, and standards must be either prepared or computed to this assumed base. This practice has resulted in such confusion and multiplicity of values for mass concentration units that clarity and precision demands a world-wide uniform basis for reporting glyceride values. It is therefore recommended that the millimolar concentration (mmoles/litre) be adopted for reporting triglyceride results.

Stable serum reference materials of different concentrations can be prepared for triglyceride standardization by the addition of a concentrated extract of egg yolk to animal or human serum (Williams, Taylor et al., 1970). The triglyceride content of these samples, sealed under pure nitrogen, is stable at −20°C for at least 1 year whether stored in the frozen or lyophilized state. Human pooled serum is also suitable as control material when stored at −20°C, but unlike the above reference materials it usually forms a turbid suspension when thawed. Lyophilized sera, with triglyceride levels in the lower range of normal, are available from a few commercial suppliers, but sometimes the stated concentrations do not agree with those obtained in reference laboratories.

WHO International Reference Centre for Lipid Determination in Cardiovascular Research

This Centre has been established in the Lipid Standardization Laboratory of the Center for Disease Control (CDC), Atlanta, Ga., USA. At the present time, the Centre operates International Co-operative Cholesterol and Triglyceride Standardization Programmes. These programmes provide several services to co-operating international laboratories. The Centre (1) issues a standard operating protocol; (2) distributes reference materials; (3) evaluates data obtained from analyses of their reference material; (4) consults on problems of lipid analyses; (5) provides training; and (6) disseminates information to the different laboratories through collaborative reports prepared by the World Health Organization and the Lipid Standardization Laboratory.

The International Co-operative Cholesterol Standardization Programme has been active since 1961 and has served laboratories in more than 30 countries. The International Co-operative Triglyceride Standardization Programme has been operating since 1968 and has served laboratories in more than 12 countries.

LIPOPROTEINS

There are many methods for measuring or estimating the quantities of the different lipoprotein families. Those now used most widely are based on three techniques: observation of the plasma, electrophoresis and ultracentrifugation. Observation of the plasma under standard conditions is highly recommended because of its simplicity and relative accuracy in detecting chylomicrons. Electrophoresis and ultracentrifugation are used because they have the widest range: electrophoresis is cheaper and simpler; ultracentrifugation can be more easily quantified.

Samples

Either plasma or serum may be used for lipoprotein analyses. Plasma is preferred since it can be kept cold from the time of sampling. The ideal sample is plasma, kept constantly at 0°C–4°C, and analysed the same day the blood is withdrawn; it should be used within 7 days at the latest. Some properly refrigerated, sterile samples may be used as long as 30 days after their collection, but samples containing high concentrations of VLDL or chylomicrons are stable for only a few days. Frozen samples should generally not be used. Samples thawed only once may sometimes be used to study LDL and HDL. VLDL and chylomicrons aggregate and undergo deterioration when frozen. Samples must be shipped at 0°C–4°C. Various anticoagulants may be used. Disodium ethylenediaminetetraacetate (EDTA), 1 mg/ml of blood, is one of the most commonly employed, because it binds divalent cations that catalyse oxidation of lipoproteins. After the blood cells have been sedimented, care must be taken to collect any floating creamy material (chylomicrons) with the plasma or serum.

Observation of plasma

If chylomicrons or VLDL are present in sufficient concentration (usually representing a triglyceride concentration of 300 mg/100 ml or greater) they will scatter light and impart turbidity or lactescence to the sample (lipaemia). When plasma stands in a tube at 0°C–4°C (without freezing) for 18–24 hours, chylomicrons will rise to the top of the tube and are

visible there as a layer of " cream ". The density of the VLDL is relatively greater than that of the chylomicrons. These particles remain suspended throughout the tube. Such diffuse turbidity indicates an increase in VLDL concentration. Minimal elevations in TG and VLDL may be present in the absence of turbidity. By this test (referred to hereinafter simply as " standing plasma "), chylomicrons can be detected with greater sensitivity than by electrophoresis.

Electrophoresis

Any medium used for zone electrophoresis should be capable of sharply defining 4 major lipoprotein bands. The strips are usually stained for lipids after electrophoresis. Sometimes the plasma is pre-stained with a lipid dye before electrophoresis. Only the lipoproteins are thus visible, although albumin occasionally also takes a faint stain because of its free fatty acid content. Two lipoprotein bands, β- (LDL) and α- (HDL) lipoproteins are visible in all normal plasmas; often a pre-β-lipoprotein band is also visible in normal plasma. Chylomicrons are not seen in normal plasma drawn under the fasting conditions recommended above (page 897).

The media most commonly used for lipoprotein electrophoresis are paper (albumin usually being present in the buffer), agarose and cellulose acetate. The relative position of the 4 bands is the same on these media (accompanying figure), although all chylomicrons are not held at the origin on some cellulose acetate preparations. Large concentrations of VLDL tend to trail from the pre-β region towards the origin on all media.

On polyacrylamide gel, the migration of the plasma lipoproteins is not the same as on other media. Chylomicrons remain at the origin (in the loading gel), VLDL (pre-β) migrate behind instead of in front of LDL (β-lipoproteins), HDL (α-lipoproteins) migrate to the most advanced position, as they do on other media. Similar migration of the lipoproteins occurs on starch-gel electrophoresis.

It is possible to quantify the lipoprotein bands resulting from electrophoresis by densitometry or by elution of bands followed by chemical determinations. Densitometry requires rigid and continuous standardization of the uptake and staining properties of the dye.

Electrophoresis is of little value unless some estimates of the concentrations of VLDL and LDL are obtained independently. For practical purposes, it may be assumed that the level of TG in plasma is a direct reflection of VLDL concentrations, when chylomicrons are absent. On these premises, abnormal TG concentrations are interpreted as abnormal VLDL concentrations, and normal TG levels as normal VLDL concentrations. LDL can be roughly equated to the plasma cholesterol concentration only when VLDL and HDL are normal. Another method for estimation of LDL is described below.

It should be noted that one important abnormal lipoprotein pattern (type III) can only be suspected and never unequivocally diagnosed from electrophoresis on one medium alone. Determination of this pattern is also described in detail below.

The often used ratio of α- to β-lipoproteins is a relative quantity that is valueless in typing patterns unless another reference is provided.

Ultracentrifugation

There are two principal ways of using ultracentrifugation to determine lipoproteins. They employ two different instruments, the preparative and analytical ultracentrifuges.

Preparative ultracentrifuge. Plasma has a salt density of about 1.006. Ultracentrifugation of plasma, without adjustment of its density, for a short period (approx. 1 min at 100 000 *g*) brings the chylomicrons rapidly to the top of the tube. Longer ultracentrifugation at this density allows the VLDL to be collected on the surface. Addition of salt or D_2O to plasma will further raise the density to selected levels and permit isolation of other lipoprotein families or " subclasses " of them. LDL are commonly isolated between densities 1.006 to 1.063 and HDL between 1.063 to 1.21. Some " very high density lipoproteins ", consisting mainly of lysolecithin possibly bound to albumin, and perhaps some lipid-poor apoprotein complexes may be present in the fraction of density >1.21.

Such lipoprotein isolates are then quantified by chemical measurement of one or more of their components. Since the cholesterol content of LDL, for example, is usually constant and the measurement is easy to make, isolated LDL is often quantified in terms of its cholesterol content.

Analytical ultracentrifuge. In this instrument plasma fractions, usually prepared at density 1.063, are centrifuged at high speeds and the moving boundaries of the floating lipoproteins are serially photo-

graphed and later used to determine concentrations that are referred to certain standard conditions. The lipoproteins of density <1.063 are described in terms of flotation rate (S_f values). Chylomicrons are not usually measured as such in the analytical ultracentrifuge but must first be separated and analysed separately. Very few laboratories provide analyses over the full range of the lipoprotein spectrum from chylomicrons to HDL (see the accompanying figure). A number of laboratories can provide quantitative measurements by the analytical ultracentrifuge of LDL (S_f 0–20), VLDL (S_f 20–400) and several subclasses within these S_f ranges.

Other techniques

Precipitation. A number of polyfunctional ions like heparin and dextran sulfate will form complexes with LDL, VLDL and chylomicrons that will precipitate in the presence of certain cations, such as Mg^{++} and Mn^{++}. This forms the basis for a rapid separation of the HDL, which remain soluble, while all the other lipoproteins are precipitated. If cholesterol or some other lipoprotein component is measured in the plasma and in the supernatant, precipitation can be made a quantitative test. Methods have been proposed for recognition of all lipoprotein families (and hence provision of lipoprotein patterns) by differential precipitation.

Other polymers such as polyvinylpyrrolidone (PVP) have been used to separate large particles by flocculation or flotation. Separation of different chylomicrons and VLDL fractions is possible and the method can be used as a sensitive " chylomicron test ".

Nephelometry. The measurement of light scattering of the chylomicrons and VLDL can be used to estimate TG. If the plasma is forced through filters of different pore size, concentrations of chylomicrons and VLDL, and possibly LDL (if one assumes the HDL concentration is constant), can be estimated.

Immunochemical. Present techniques permit fairly quantitative estimation through immunoprecipitation of HDL or the combined amounts of LDL, VLDL and chylomicrons. LDL can be measured if the lipoproteins with a salt density <1.006 have first been removed by ultracentrifugation.

Combination of methods. Several methods may be combined for lipoprotein analyses. Preparative ultracentrifugation may be combined with precipitation and cholesterol determinations to obtain concentrations of VLDL, LDL and HDL. For example, plasma is separated into two fractions by one ultracentrifugation at a salt density of 1.006 (requiring no adjustment of plasma density), and cholesterol concentrations are determined in plasma (P), the whole 1.006 infranatant fraction (B) and in the soluble lipoproteins remaining in this fraction after all lipoproteins except HDL have been precipitated by heparin and Mn^{++} (A). LDL (as cholesterol) = B–A; VLDL (cholesterol) = P–B. Electrophoresis and ultracentrifugation are also combined to define the type III pattern (see below).

Calculated values. Nomograms have been suggested for deriving lipoprotein patterns from plasma cholesterol and triglycerides alone. The results do not correlate well with the assignment of types by more specific methods.

LDL concentrations can be calculated from the following formula:

$$LDL = P - (TG/5 + A)$$

where P = plasma cholesterol
TG = plasma triglyceride concentration (mg/dl)
A = HDL cholesterol concentrations obtained by rapid precipitation with heparin – Mn^{++} of all lipoproteins from the plasma except HDL

This formula is applicable to all plasma samples in which TG is < 400 and in which the type III anomaly has been excluded.

Standardization

The standardization of laboratory techniques for determining the type of lipoprotein pattern is more difficult. No plasma reference material is stable for all types of lipoproteins. Substitute approaches are therefore used. Each procedure receives independent checks of equipment, reagents and technique.

Observation of serum. Ideal standardization has not been adopted for this procedure. Since the purpose (of this procedure) is to demonstrate the presence or absence of chylomicrons, non-fasting plasma or milk added to serum can be used as a positive reference serum. (A standardized fat emulsion—available for parenteral nutrition—may be more suitable for this purpose because of the wide range of fat content in different kinds of milk.) Even though the procedure is qualitative, the volume of the sample, the dimensions and type of tube used, and the time of reading should be kept fairly constant within each laboratory. Specimens should be placed

near the bottom of the refrigerator away from the cooling unit to prevent any chance of freezing. Turbidity due to fibrin must not be confused with lactescence due to chylomicrons and VLDL.

Electrophoresis. Electrophoretic analyses should be performed under standard conditions. The ability to effect sharp separations and to stain reproducibly requires regular surveillance of apparatus, the current and voltage employed, the aging of buffers and dye and careful application of starting material. Perhaps the most sensitive guide to satisfactory analysis is the sharpness of the β band.

Freezing will destroy the chylomicrons, change severely the pre-β fraction and cause variable spreading of other zones. Evaporation tends to distort the electrophoretic pattern. Normal human plasma without a significant pre-β band, kept either liquid or frozen, may be used as a continuous control, both to confirm that electrophoresis is operating as expected and to control the intensity of staining with the dye. Lipoprotein fractions prepared with the ultracentrifuge are also useful to test electrophoretic separation of the several lipoprotein families.

Ultracentrifugation. Standard conditions, now universally accepted, must be followed in both preparatory and analytical ultracentrifugal analyses. In particular, a precise pycnometer should be used to confirm accurately the density of the medium used in ultracentrifugation. As with electrophoresis, sterile specimens stored for less than 6 hours at 0°C–4°C are best for controls. Shipped samples must not be subjected to dilution or evaporation and must be kept stored at 0°C–4°C until analysed. Precautions should be exercised to ascertain the validity and applicability of chemical measurements of any lipoprotein components to the ultracentrifugal fraction.

Other techniques. Precipitation, immunological, and nephelometric procedures are being introduced in some lipid laboratories. Standardization of each of these requires both calibration and surveillance of equipment, reagents, and the basis of calculation. Fresh normal human serum and fresh serum from individuals with established abnormal lipoprotein patterns are the best reference materials.

CLASSIFICATION OF HYPERLIPIDAEMIAS AND HYPERLIPOPROTEINAEMIAS

As discussed earlier, (1) the plasma lipids circulate in lipoproteins, (2) each of the 4 main lipoprotein families, chylomicrons, pre-β (VLDL), β (LDL), and α (HDL) contains cholesterol, triglycerides and phospholipids; and (3) the metabolism of the 4 lipoprotein families is different. These facts provide keys to the classification of hyperlipidaemias, because they indicate that (1) hyperlipoproteinaemia very seldom occurs without hyperlipidaemia and, consequently, hyperlipidaemia may be used to detect hyperlipoproteinaemia; (2) a classification based on lipoproteins offers more information than one based on lipids alone; (3) a classification should distinguish between disorders in the metabolism of lipoproteins as well as lipids.

The proposed classification described here includes, step by step, the use of lipid analyses, lipoprotein analyses and other clinical and biological data. It provides an approach to the etiological and to the pathogenic classification by which the former will ultimately be replaced. The classification for genetic purposes is based on the assumption that the patient has been on a standard diet prior to the analyses.

HYPERLIPIDAEMIA

Cholesterol (Chol) and triglyceride (TG) analyses are the simplest means for detecting hyperlipoproteinaemia. They also provide some information about the type of hyperlipoproteinaemia because the proportion of these lipids varies from one lipoprotein family to another.

Knowledge of the concentrations of cholesterol and triglycerides permits the distinction of three general types of hyperlipidaemia that roughly correspond to certain types of hyperlipoproteinaemias:

(1) High cholesterol concentrations and normal triglyceride concentrations—this group, sometimes called " pure hypercholesterolaemia ", usually corresponds to hyper-β-lipoproteinaemia.

(2) High triglyceride and normal cholesterol concentrations—this group usually corresponds to either " pure hyperchylomicronaemia " or hyperpre-β-lipoproteinaemia.

(3) High cholesterol and high triglyceride concentrations—all of the major types of hyperlipoproteinaemia, except " pure " hyper-β-lipoproteinaemia, may occur in this group.

The heterogeneity of the third group particularly emphasizes the need for a classification based on lipoproteins.

It is possible to refine a little the classification of hyperlipidaemias by adding a total phospholipid (PL) measurement and also by calculating the following ratios: Chol/TG and Chol/PL.

The ratio Chol/TG indicates whether the predominant elevation is in cholesterol or in triglyceride. The ratio Chol/PL often indicates elevation of HDL (α-lipoproteins) when it falls under 0.5. These refinements are not necessary to detect hyperlipidaemia but do offer some assistance in classification if lipoproteins are not determined.

HYPERLIPOPROTEINAEMIA

Hyperlipidaemia can usually be resolved into one of the abnormal lipoprotein patterns summarized in Table 1. For the sake of simplicity, these patterns or types can be numbered according to the system of Fredrickson and colleagues (Fredrickson, Levy & Lees, 1967; Fredrickson & Levy, in press). These patterns are not to be equated with single diseases and each may have multiple causes. Most, but not all, hyperlipidaemia is represented by the six patterns described. The methods of diagnosis described are arranged in the order of practicality. Some tests are diagnostic (definitive) of a given type; others are not.

TABLE 1

THE MAJOR ABNORMAL LIPOPROTEIN PATTERNS [a] AND THEIR TYPE NUMBERS

Type	Chylomicrons	LDL (β-lp)	VLDL (pre-β-lp)	Floating β-lipoproteins [b]
I	+			
IIa		+		
IIb		+	+	
III				+
IV			+	
V	+		+	

[a] + indicates which lipoprotein " family " (families) occurs in concentration above " normal " in the different abnormal patterns.

[b] Also known as " broad β-lipoproteins ".

Type I—Hyperchylomicronaemia

Criteria

(1) Chylomicrons present.

(2) VLDL (pre-β-lipoproteins) normal or only slightly increased.

Methods of diagnosis

(1) Standing plasma contains a " cream " layer over a clear infranatant layer (diagnostic test).

(2) Plasma cholesterol usually increased; plasma triglyceride increased; Chol/TG less than 0.2; a ratio of less than 0.1 occurs only in type I.

(3) Electrophoresis—a heavy chylomicron band is present and is distinct from any lipoproteins trailing from the pre-β region; sometimes α- (HDL) and β- (LDL) lipoprotein bands are not visible; a pre-β (VLDL) band may be absent or it may appear with diminished, normal or slightly increased intensity and with trailing into the massive chylomicron band (usually diagnostic).

(4) Ultracentrifugation—chylomicrons, markedly increased; VLDL, usually increased (separation from chylomicrons incomplete); LDL markedly decreased; HDL markedly decreased.

Comment. It must be noted that in Type I, chylomicrons may sometimes be accompanied by an apparent modest increase in VLDL (pre-β). This is partly due to the difficulty of separating these two lipoprotein families. The amount of excess VLDL, however, is always far less than the overwhelming amount of chylomicrons.

Recommended tests for diagnosis

(1) Examination of standing plasma.

(2) Electrophoresis.

Type II—Hyper-β-lipoproteinaemia

Criterion. Abnormal increase in LDL (β) concentration.

Note. For some purposes it may be convenient to distinguish between two subtypes of this pattern. These are referred to here as IIa and IIb. In both, the criterion for Type II, an increase in LDL (β), is present, but in one (IIb) an increase in VLDL (pre-β) is also present. Recognition of IIb is important because it may require treatment additional to that required for " pure " hypercholesterolaemia. Both patterns may occur in the same kindreds affected with familial hyper-β-lipoproteinaemia; it

is mainly for this reason that they must at present be considered under the main rubric of Type II.

Type IIa

Criteria

(1) Increase in LDL (β).

(2) Normal VLDL (pre-β) concentrations.

Methods of diagnosis

(1) Standing plasma clear (very helpful; not always diagnostic).

(2) Plasma cholesterol usually increased; plasma triglycerides normal; Chol/TG always > 1.5.

(3) Electrophoresis—an intensely stained β-lipoprotein band is present; a pre-β band is either not present or, if present, is of normal intensity. Chylomicrons are not visible, α-lipoproteins are usually normal. (Diagnostic only if accompanied by estimation of LDL concentration.)

(4) Ultracentrifugation—LDL (S_f 0–20) is increased. VLDL (S_f 20–400) is normal, HDL is usually normal, and chylomicrons are not increased (diagnostic).

Type IIb

Criteria

(1) Increase in LDL (β).

(2) Increase in VLDL (pre-β).

Methods of diagnosis

(1) Standing plasma either clear, or faintly turbid throughout, without a chylomicron (" cream ") layer on the top (not diagnostic).

(2) Plasma cholesterol usually increased; plasma triglyceride always increased; Chol/TG is variable (not diagnostic).

(3) Electrophoresis—β-lipoprotein band is intensely stained; pre-β band is increased in intensity. Chylomicrons are not visible, α-lipoproteins are usually normal. (Diagnostic only if accompanied by estimations of LDL and VLDL concentrations.)

(4) Ultracentrifugation—LDL (S_f 0–20) is increased, VLDL (S_f 20–400) increased, chylomicrons are not increased, HDL is usually normal (diagnostic).

Comment. Definite ascertainment of Type II depends upon the establishment of an abnormal increase in LDL (β) concentrations. This is most precisely obtained by analytical or preparative ultracentrifugation. It may also be estimated from the cholesterol, triglyceride and HDL–cholesterol concentrations as described above.

LDL can also be measured by immunochemical analysis of the 1.006 infranatant fractions using anti-LDL sera. (Such antisera also react with VLDL and therefore do not permit accurate LDL determinations on whole plasma.)

The Type IIa pattern can usually be ascertained by the cholesterol and triglyceride analyses alone, especially when the Chol/TG ratio is > 2. The exceptions are those patients who may have abnormally increased LDL concentrations in the presence of a normal plasma cholesterol concentration.

The Type IIb pattern is difficult to ascertain from plasma lipids alone.

Recommended tests

(1) Chol plus TG plus electrophoresis, when Chol/TG > 2.

(2) Chol plus TG plus HDL (cholesterol measurements after precipitation) for calculation of LDL (applicable only when Type III is excluded and TG < 400). If estimated LDL is increased, assignment of subtypes is: IIa when TG is normal, IIb when TG is increased.

(3) Ultracentrifugal analyses.

Type III—" floating β " or " broad β " pattern

Criterion. Presence of VLDL having abnormally high cholesterol content and abnormal electrophoretic mobility (" floating-β "; " β-VLDL ").

Methods of diagnosis

(1) Standing plasma usually turbid, frequently with a faint chylomicron " cream " layer (helpful but not diagnostic).

(2) Plasma cholesterol nearly always increased, plasma triglycerides nearly always increased, Chol/TG frequently about 1, may vary from 0.3 to > 2.0.

(3) Electrophoresis—on paper, agarose or cellulose acetate, there is usually a " broad β " band extending from the β position into the pre-β position. This occurs in about two-thirds of plasma containing " floating β ". A distinct pre-β band is sometimes present and may be increased in intensity: α-lipoproteins usually appear normal. A faint chylomicron band is often present even during periods of very low fat intake (helpful but not diagnostic). On polyacrylamide gel electrophoresis (PGE) a broadened pre-β (VLDL) band is present,

and no lipoproteins are seen in the usual position occupied by β-lipoproteins (LDL) on this medium. The concomitant presence of β-migrating lipoproteins on paper, agarose or cellulose acetate and their absence on polyacrylamide gel is a presumptive test for the Type III anomaly and is about 95% accurate. (The combination electrophoretic test is considered diagnostic.)

On starch-block electrophoresis of isolated VLDL, two bands are obtained, one in the usual α_2 position (sometimes called "α_2-VLDL") and one in an abnormal β position ("β-VLDL") (diagnostic).

Paper, agarose, cellulose acetate or starch electrophoresis of the supernatant fraction of plasma after ultracentrifugation at its unadjusted salt density of 1.006 reveals β-migrating lipoproteins. Normally only pre-β migrating lipoproteins are present in the lipoprotein fraction of density < 1.006. (The demonstration of "floating β" is at present the definitive standard against which other diagnostic tests must be compared.)

(4) Ultracentrifugation—in the analytical ultracentrifuge the normally predominating LDL subclass of density 1.010–1.063 (S_f 0–12) is greatly decreased and the LDL subclass of density 1.006–1.019 (S_f 12–20) is disproportionately increased. The VLDL subclass (S_f 100–400) is also increased. Chylomicrons may be increased. This inversion of the usual concentrations of LDL and VLDL usually provides a characteristic pattern in Type III; however it is possible to have similar changes in total S_f 0–20 and S_f 20–400 subclasses in other types. (Very helpful but not always diagnostic.)

The combination of preparative ultracentrifugation and electrophoresis described above may be augmented by a measurement of cholesterol and triglycerides in the VLDL (density < 1.006) ultracentrifuge fraction (the latter may possibly be substituted for the former). Normally, the Chol/TG ratio in VLDL is 0.2 or less. Significantly higher ratios (> 0.4) are indicative of Type III (probably diagnostic).

Comment. The Type III anomaly indicates the presence of abnormal VLDL, or more precisely, of abnormal LDL in the VLDL fraction of plasma lipoproteins. It may be suspected from a Chol/TG ratio of 1, especially when repeated analyses show marked lability of both Chol and TG concentrations, and a "broad β" band appears on conventional electrophoresis. This combination may permit a presumptive diagnosis; however, the diagnosis should never be made alone from conventional electrophoresis on a single medium.

The definitive test is the demonstration of "floating β" but an analysis of equivalent value may prove to be the measurement of cholesterol and triglyceride in VLDL; combining electrophoresis on PGE and one other medium permits a presumptive diagnosis. A simpler, accurate diagnostic test is still desired.

Recommended tests. When the plasma Chol/TG ratio is close to 1 and a "broad β" band is suspected on electrophoresis:

(1) Plasma lipoprotein patterns obtained on polyacrylamide gel and on either paper, agarose or cellulose acetate should be compared. Absence of β-migrating lipoproteins on PGE and their presence on the other systems permits a presumptive diagnosis.

(2) When possible, confirmation of "floating β" (or VLDL having a high Chol/TG ratio) should be made after preparative ultracentrifugation.

Type IV—Hyperpre-β-lipoproteinaemia

Criteria

(1) Increased VLDL (pre-β).

(2) No increase in LDL (β).

(3) Chylomicrons absent.

Methods of diagnosis

(1) Standing plasma clear or turbid throughout with no overlying chylomicron layer (helpful but not diagnostic).

(2) Plasma cholesterol normal or increased; plasma triglycerides increased; plasma Chol/TG, variable (very helpful, sometimes diagnostic).

(3) Electrophoresis—increased intensity of pre-β-lipoprotein band; β band normal or decreased; α-band may be normal, often decreased; chylomicrons not visible. There may be trailing of lipoproteins from the pre-β region to the origin (helpful, but not diagnostic without some quantification—see comments).

All of the isolated VLDL on starch-block electrophoresis has the usual α_2 mobility (α_2-VLDL). There is no β-VLDL, or "floating β", and VLDL has usual Chol/TG ratio of 0.2 or less.

(4) Ultracentrifugation—VLDL (S_f 20–400) is increased; LDL (S_f 0–20) is normal or decreased; HDL is normal or decreased, and chylomicrons are not increased (diagnostic).

Comments. If the plasma cholesterol is definitely normal, triglycerides are clearly increased, and there are no chylomicrons visible on standing plasma, the ascertainment of type IV is fairly certain. The accuracy of assignment is enhanced if electrophoresis reveals a distinct pre-β band and a distinct and diminished β band. Plasma TG is always used to assess pre-β concentrations with electrophoresis, and it is always elevated in Type IV. Conversely, an apparent increase in pre-β lipoproteins on electrophoresis will *not* be accompanied by an increase in plasma TG if most of the pre-β represents " sinking pre-β " (see above). This is a normal phenomenon and its frequent occurrence emphasizes the need for TG concentrations to monitor electrophoresis. One should look for signs of the " Type III anomaly "; it is not necessary to exclude the anomaly by specific tests in most instances of Type IV.

Recommended tests

(1) For most samples, Chol plus TG plus observation of plasma plus electrophoresis permits a diagnosis.

(2) Estimate LDL and exclude Type III in doubtful cases.

(3) The ultracentrifuge can be very helpful in certain cases.

Type V—Hyperpre-β-lipoproteinaemia and chylomicronaemia

Criteria

(1) VLDL increased.

(2) Chylomicrons present.

Methods of diagnosis

(1) Standing plasma—chylomicron (" cream ") layer overlying a turbid infranatant layer (diagnostic, if Type III anomaly excluded).

(2) Plasma cholesterol increased, plasma triglyceride increased, plasma Chol/TG usually >0.15 and <0.6 (helpful but not diagnostic).

(3) Electrophoresis—pre-β band is increased and frequently trails to origin where a distinct accentuation indicates concomitant presence of chylomicrons: β- and α-lipoprotein bands are usually decreased, often markedly so. There is no " floating β " (can be diagnostic, if trailing pre-β does not obscure a chylomicron band).

(4) Ultracentrifugation—chylomicrons and VLDL (S_f 20–400) increased. LDL, particularly subclass S_f 0–12, and HDL usually decreased (diagnostic).

Comments. The major diagnostic problem, that of discerning chylomicrons by electrophoresis when pre-β (VLDL) concentrations are extremely high, can usually be overcome by observation of standing plasma. The latter is a very good test for Type V.

Recommended test

(1) Examine standing plasma and measure Chol plus TG. The typical appearance in Type V may be imitated in two situations. One is a Type I pattern with enough VLDL to impart faint turbidity to the infranatant layer. The Chol/TG ratio is usually below 0.15 in Type I, usually above this in Type V. The other situation is Type III. Here the Chol/TG ratio is often close to 1 but may be as low as 0.3. Test for " floating β " should be done if any uncertainty remains.

ADDITIONAL USEFUL CLINICAL DATA

Certain clinical signs, and other information that is relatively easy to obtain, are valuable for the detection of hyperlipidaemia and can sometimes be used to predict the type of hyperlipoproteinaemia that is present. Xanthomas and other lipid deposits and the familial history are the most valuable.

Lipid deposits—xanthomas

Tendon xanthomas are not rare; they are easy to detect, and are especially informative because they almost invariably indicate hyperlipoproteinaemia of long duration. They usually indicate hyper-β-lipoproteinaemia and almost always imply familial Type II.

Tuberous xanthomas occur with Type II and Type III hyperlipoproteinaemia. Somewhat similar " tubero-eruptive " lesions appear with Types III and IV. Eruptive xanthomas always indicate severe hyperglyceridaemia (usually Types I or V).

Planar xanthomas occur with several kinds of hyperlipoproteinaemia. In the familial disorders, they occur on the palms of the hands in Type III and in homozygotes for Type II. They also may occur with obstructive liver disease. More widely distributed planar lesions, on the trunk and elsewhere, are rare and occur especially in hyperlipoproteinaemia associated with dysglobulinaemias.

Xanthelasma is frequent in Type II and sometimes occurs in Type III, but often may be seen in the

absence of hyperlipidaemia or hyperlipoproteinaemia.

Arcus corneae (arcus senilis) is significant only when it appears before the age of 40 years. In younger people it usually implies familial Type II hyperlipoproteinaemia.

Other clinical signs

Pancreatitis or recurrent abdominal pain should lead to a suspicion of severe hyperglyceridaemia (Types I or V).

Family history

The family history often leads to the detection of hyperlipidaemia.

Ischaemic heart disease and other vascular accidents in young relatives are usual in familial Type II and Type IV hyperlipoproteinaemia.

Diabetes is often seen in families of patients with Type IV and Type V hyperlipoproteinaemia, even if the patient himself is not the diabetic.

Other useful laboratory data

These include some common laboratory tests, such as those for: thyroid function; glucose tolerance; urinary protein; plasma protein electrophoresis; immunoglobulin quantification; liver function; and uric acid.

Certain special analyses may also be useful and include: plasma post-heparin lipolytic activity; proportion of plasma cholesterol in the esterified form; lecithin cholesterol acyltransferase activity (LCAT); fat tolerance; and vitamin A tolerance.

ETIOLOGY OF HYPERLIPOPROTEINAEMIA

Once the type pattern of hyperlipoproteinaemia has been established, it is necessary to consider etiology. One approach is to consider etiology as falling into two main categories, *secondary* and *primary* hyperlipoproteinaemias.

Secondary to known diseases

Common diseases that are often associated with hyperlipoproteinaemia and that must always be excluded in considering etiology are: (1) hypothyroidism, (2) diabetes, (3) nephrotic syndrome, (4) biliary obstruction, (5) pancreatitis, and (6) dysglobulinaemia (including auto-immune hyperlipoproteinaemia). The lipoprotein patterns that may be associated with these diseases are shown in Table 2.

TABLE 2

TYPES OF HYPERLIPOPROTEINAEMIA ASSOCIATED WITH SELECTED COMMON DISEASES[a]

Disorder	Types of hyperlipoproteinaemia
Hypothyroidism	II, IV
Insulin-dependent diabetes (uncontrolled)	I, IV, V (II, III)
Nephrotic syndrome	II, IV, V
Biliary obstruction	Does not conform predictably to any of the major types
Pancreatitis	IV, V
Dysglobulinaemia	I, II, IV, V (III)
Auto-immune hyperlipoproteinaemia	I, III, IV, V (II)

[a] Secondary hyperlipoproteinaemias are shown in parentheses.

Primary

These are hyperlipoproteinaemias that are due to genetically determined defects in lipid or lipoprotein metabolism or are caused by some environmental factors through an unknown mechanism.

All 5 major types of hyperlipoproteinaemia may be familial and probably represent many different mutations.

Environmental factors that may cause primary hyperlipoproteinaemia include: (1) diet, including alcohol intake; and (2) drugs—many drugs cause hyperlipidaemia, particularly the oestrogens, as contained in contraceptive medications, and steroid hormones.

A proper classification of hyperlipoproteinaemia should include reference to both lipoprotein pattern and etiology.

* * *

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Annex

GLOSSARY OF TERMS

Abetalipoproteinaemia	Absence of β-lipoprotein
α-lp (α_1-lp)	Lipoproteins appearing in the α (α_1) electrophoresis band (same as HDL)
β-lp	Lipoproteins having β-mobility on electrophoresis band (same as LDL)
" β-VLDL "	Lipoproteins of β-mobility floating at density 1.006 (same as " Floating β-lp ")
Broad β-lp	Same as " Floating β-lp "
Floating β-lp	Lipoproteins of β-mobility floating at density 1.006 (same as " broad β-lp ")
HDL	High-density lipoproteins, isolated between density 1.063 to 1.21 (same as α-lp)
Hyperlipaemia	A lactescent appearance of plasma due to increased concentrations of glycerides in either VLDL or chylomicrons
Hyperlipidaemia	An increase in concentration of any plasma lipid constituent; for practical purposes usually confined to cholesterol or triglycerides or both
Hyperlipoproteinaemia	An increase in plasma concentration of one or more lipoprotein families; nearly always accompanied by hyperlipidaemia
LDL	Low-density lipoproteins (same as β-lp)
Lp antigen (Berg)	A form of genetic polymorphism of lipoprotein
Lp-X	Lipoprotein-X (complexes mainly of phospholipid, unesterified cholesterol and VLDL apoprotein seen in obstructive liver disease)
Pre-β-lp	Lipoproteins appearing in the pre-β electrophoresis band (same as VLDL)
S_f value	Svedberg unit of flotation
" Sinking " pre-β-lp	Pre-β lipoproteins that sediment at density 1.006
Tangier disease	Familial deficiency of HDL
VLDL	Very low density lipoproteins (pre-β-lipoproteins)

RÉSUMÉ

CLASSIFICATION DES HYPERLIPIDÉMIES ET DES HYPERLIPOPROTÉINÉMIES

De nombreuses études, dans différents pays, ont montré qu'une hyperlipidémie est souvent présente chez les malades ayant des manifestations cliniques d'athérosclérose. Dans les familles où il existe une forme héréditaire d'hyperlipidémie sévère, les premières manifestations d'athérosclérose apparaissent souvent très précocement. Des études prospectives indiquent aussi qu'une hyperlipidémie même modérée prédispose à des complications cardio-vasculaires par athérosclérose.

La plupart des lipides du plasma [1] sont sous forme de lipoprotéines, et une hyperlipidémie signifie presque toujours que les concentrations d'une ou de plusieurs lipoprotéines sont élevées (hyperlipoprotéinémie). La détermination de la concentration des lipoprotéines donne toujours plus d'informations que l'analyse des lipides seule, dans l'estimation des nombreuses anomalies désignées globalement par le terme d'hyperlipidémie. La base la plus rationnelle pour décrire une hyperlipidémie consiste actuellement à déterminer à quel type d'hyperlipoprotéinémie elle correspond. Une détermination quantitative des lipides étant cependant plus facile qu'une détermination quantitative des familles de lipoprotéines, on peut souvent en pratique se contenter de faire seulement l'analyse des lipides, après identification des lipoprotéines en cause.

Le dosage du cholestérol et des triglycérides est le moyen le plus pratique pour détecter une hyperlipoprotéinémie et fournit des informations quant au type même de l'hyperlipoprotéinémie. Le type lipoprotéique est actuellement la meilleure base d'une classification des hyperlipoprotéinémies. Il y a cependant beaucoup d'inconnues dans l'utilisation des lipoprotéines et certains problèmes pratiques importants n'ont pas encore été résolus de manière satisfaisante. Trois notions préalables sont à la base de l'utilisation des lipoprotéines; elles feront comprendre la grande hétérogénéité qui se dissimule à l'intérieur du problème général de l'hyperlipidémie:

1) Il n'est pas nécessaire d'utiliser une technique unique pour définir les types lipoprotéiques et, en fait, une définition ne peut être complète si elle repose sur une seule technique.

2) Pour parvenir à une classification utile, on doit aller au-delà des types lipoprotéiques et considérer l'étiologie.

3) Toute classification actuellement utilisée est nécessairement imparfaite et incomplète. Les systèmes de classification décrits plus loin sont en principe raisonnables et simples. Par l'usage de méthodes appropriées, ils doivent permettre l'adoption d'un langage commun pour la description des différentes formes d'hyperlipidémies. Cependant, étant donné qu'on ne recommande pas une technique unique, il est de la plus haute importance que chaque rapport ou publication décrive soigneusement les méthodes et les normes utilisées pour définir l'hyperlipidémie et l'hyperlipoprotéinémie.

DESCRIPTION DES LIPIDES ET LIPOPROTÉINES DU PLASMA

LIPIDES

Les lipides du sérum sont sous forme de mélanges de nombreux constituants. Pour la classification clinique des hyperlipidémies et hyperlipoprotéinémies, le cholestérol et les triglycérides sont de loin les plus importants à connaître.

Cholestérol et triglycérides

Le cholestérol (Chol) se rencontre en tant qu'alcool (« cholestérol libre ») et sous forme estérifiée dans une proportion pratiquement constante. Environ 70% du cholestérol est estérifié, sauf dans les maladies obstructives des voies biliaires et certaines maladies rares. Pratiquement tous les stérols du plasma sont Δ5,6-cholestène-3β-ol. Le cholestérol est habituellement dosé en tant que cholestérol total et il est recommandé de l'exprimer en mg/dl.

Les glycérides se rencontrent principalement dans le plasma humain sous forme de triglycérides (TG). Des concentrations relativement faibles de di- et monoglycérides sont aussi présentes. On mesure généralement les triglycérides par détermination de leur partie glycérol; cependant, les résultats sont calculés comme triglycérides. Les concentrations sont exprimées soit en mg/dl, soit en millimoles par litre. Le poids moléculaire du standard utilisé pour les calculs doit être établi quand le résultat est donné sur une base pondérale (c'est-à-dire en mg/dl).

Autres lipides

Les phospholipides du plasma comprennent principalement la phosphatidyl-choline, la sphingomyéline, la lysophosphatidyl-choline et la phosphatidyl-éthanolamine. Les proportions de ces substances varient dans les différentes lipoprotéines; cette donnée n'est généralement

[1] Tout au long de ce texte, tout ce qui est dit concernant le plasma peut aussi bien s'appliquer au sérum et vice versa.

pas utilisée pour la classification clinique. Les acides gras sont présents, surtout comme esters de glycéryle, de phosphoglycéryle et de cholestéryle; une petite fraction d'acides gras, métaboliquement très active, n'est pas estérifiée: ce sont les acides gras libres (FFA). De petites quantités de glycosphingolipides sont aussi présentes dans le plasma.

Les vitamines liposolubles (A, D, E, K) et les caroténoïdes sont présents dans le plasma. Les vitamines A et E sont utilisées pour étudier l'absorption et le transport des graisses alimentaires.

LIPOPROTÉINES

Principales familles de lipoprotéines

L'électrophorèse et l'ultracentrifugation sont les principales méthodes pour la séparation et l'identification des lipoprotéines. Par ces deux techniques, les lipoprotéines du plasma sont généralement groupées en quatre grandes familles. Etant donné que les lipoprotéines pures sont obtenues de la meilleure façon par ultracentrifugation, la nomenclature de base est habituellement dérivée de cette technique. L'électrophorèse est plus pratique et la terminologie dérivée de cette méthode est plus familière aux cliniciens. Ces deux nomenclatures sont utilisées indifféremment dans ce rapport et leur correspondance est illustrée dans la figure de la page 893. Les quatre principales familles de lipoprotéines sont les suivantes:

Chylomicrons (on emploie le même nom, qu'ils soient le produit d'une séparation par ultracentrifugation ou par électrophorèse). Ces grosses particules ont une densité d'environ 0,9 (valeurs de $S_f > 400$). On les obtient aussi en prélevant le dessus du plasma qui a été laissé à reposer pendant 16 à 24 heures à +4°C; ils restent au point de départ en électrophorèse sur papier ou sur agarose ou sur le gel en gel de polyacrylamide, et ont un certain comportement en gradients de PVP (polyvinylpyrrolidone) ou en électrophorèse en gel d'amidon.

Lipoprotéines de très basse densité (VLDL) (maintenant appelées pré-β-lipoprotéines dans la plupart des systèmes d'électrophorèse utilisés par les cliniciens). Ces lipoprotéines sont isolées en ultracentrifugation dans la fraction de densité <1,006 et ont des valeurs de S_f de 20 à 400. Les VLDL (pré-β) contiennent surtout des glycérides « endogènes », c'est-à-dire nouvellement synthétisés ou dérivés des réserves de l'organisme, plutôt que directement de l'alimentation.

Dans certains systèmes électrophorétiques, plusieurs bandes de pré-β peuvent apparaître dans le plasma. On ne leur a encore attribué aucune signification pathologique. Chez environ 10% des sujets, y compris dans différents types d'hyperlipoprotéinémies, des lipoprotéines de mobilité pré-β sont présentes et sédimentent plutôt qu'elles ne flottent à la densité 1,006. Ces pré-β sédimentées correspondent bien à une fraction lipoprotéique de densité 1,050 à 1,080 qui paraît contenir « l'antigène Lp (Berg) », marque de polymorphisme génétique qui jusqu'à maintenant n'a pas eu d'implications pathologiques connues.

Lipoprotéines de basse densité (LDL) (appelées β-lipoprotéines dans tous les systèmes électrophorétiques). Ces lipoprotéines sont isolées entre les densités 1,006 et 1,063 et ont des valeurs de S_f de 0 à 20. Leurs constituants majeurs sont le cholestérol et des esters de cholestérol; comme autres constituants, on trouve principalement des phospholipides, des protéines et une petite quantité de glycérides.

Lipoprotéines de haute densité (HDL) (appelées α-lipoprotéines, ou plus spécifiquement α_1-lipoprotéines dans tous les systèmes d'électrophorèse). Les HDL, comprenant les classes HDL_2 et HDL_3 selon la définition de Lalla & Gofman, sont isolées entre les densités de 1,063 et 1,21, et contiennent environ 50% de protéines. Les composants lipidiques sont principalement le cholestérol et les phospholipides, dans un rapport pondéral d'environ 0,5.

Lipoprotéines anormales

Quelques lipoprotéines anormales pouvant apparaître dans le plasma ont été identifiées. Elles comprennent:

β-lipoprotéines flottantes (« floating β-lipoproteins »). On les appelle aussi « β larges (*broad* β) » ou « β-VLDL ». Elles sont maintenant identifiées *a*) soit comme des lipoprotéines de mobilité β flottant à la densité de 1,006 après 16 heures à 100 000 *g*, *b*) soit comme une inversion des concentrations habituelles en lipoprotéines de S_f 0-20 et S_f 20-100, mesurées par ultracentrifugation analytique. En électrophorèse sur gel d'amidon, les VLDL deviennent clairement bimodales, ayant à la fois des pics de mobilité β et α_2. Une définition absolue de cette anomalie exige l'ultracentrifugation. Des méthodes plus simples sont décrites plus loin. Une « β flottante » peut se révéler être une lipoprotéine normale, présente seulement sous forme de traces. En utilisant l'électrophorèse sur papier après ultracentrifugation, on n'a observé ce fait jusqu'à présent que chez des patients homozygotes dans la carence familiale en HDL (maladie de Tangier) ou présentant une forme rare d'hyperlipoprotéinémie (type III). Cette dernière est habituellement « primitive » et souvent familiale. Dans de rares exemples, la β flottante peut être secondaire à d'autres maladies, telles que le diabète non équilibré.

Lipoprotéines X (Lp-X). Ce sont des complexes composés principalement de phospholipides, de cholestérol libre et d'apoprotéines VLDL (voir plus loin les protéines délipidées des VLDL) qui se forment chez des patients ayant une maladie obstructive des voies biliaires. La lipoprotéine est séparable par fractionnement de Cohn et est identifiée par un anticorps qui réagit également avec l'apo-VLDL. Le type lipoprotéique est variable: sur papier et agarose, la Lp-X migre plus lentement que la β et, parce qu'elle contient du cholestérol libre et des phospholipides, elle se colore peu par les colorants des

lipides (Oil-Red-O ou Noir Soudan). Souvent les lipoprotéines migrant en α_1 sont diminuées ou absentes.

Complexes de lipoprotéines « normales » avec d'autres globulines. Des complexes, dont certains représentent des réactions antigène-anticorps, se forment entre lipoprotéines et immunoglobulines ou macroglobulines. On sait maintenant que certaines protéines d'IgA et IgG myélomes réagissent avec les lipoprotéines et il a été suggéré que ceci puisse être le modèle d'autres sortes d'« hyperlipidémie par auto-anticorps ». Il n'y pas de tests absolus en électrophorèse ou ultracentrifugation pour de tels complexes et les types de lipoprotéines compatibles avec tous les principaux types anormaux (voir plus loin) peuvent être obtenus. Actuellement, il est nécessaire d'isoler l'immunoglobuline et de tester sa réactivité avec l'antigène lipoprotéique en utilisant des hématies sensibilisées.

HDL_T ou α_T-lipoprotéines. On a montré que les petites quantités de HDL décelées chez les homozygotes de la maladie de Tangier présentent des anomalies dans la proportion et peut-être la structure de leurs apoprotéines. Cette anomalie est détectable seulement immunochimiquement et chimiquement, et provient de mutations rares.

Autres lipoprotéines anormales. Elles comprennent: les HDL et VLDL ayant une composition chimique anormale dans la déficience familiale en lécithine-cholestérol-acyltransférase (LCAT), les HDL anormales dans les a-β-lipoprotéinémies, les VLDL chimiquement anormales dans la maladie de Tangier et les VLDL de mobilité β dans plusieurs maladies sévères du parenchyme hépatique. Des lipoprotéines ayant un contenu apoprotéique anormal ont été démontrées seulement dans la maladie de Tangier, mais d'autres existent probablement dans d'autres formes d'hyperlipidémies.

Apolipoprotéines

Les protéines qui en formant des complexes avec des lipides produisent les lipoprotéines apparaissent spécifiques, et vraisemblablement elles servent surtout ou exclusivement à faciliter le transport des graisses et le métabolisme des lipoprotéines. Les protéines séparées des lipides sont appelées apolipoprotéines ou plus simplement apoprotéines. Le nombre total qui peut être présent dans toutes les familles de lipoprotéines n'est pas encore connu. Il n'y a pas non plus de nomenclature standard pour les apoprotéines. Une terminologie décrit ces protéines dans les HDL comme apo-A et dans les LDL comme apo-B. Dans les VLDL, les apo-A et apo-B sont également présentes; en plus cette terminologie décrit aussi une autre apolipoprotéine dans les VLDL appelée apo-C. Au moins 4 protéines, ou probablement beaucoup plus, sont présentes dans les VLDL, et pas moins de 5 dans les HDL. Cependant, il est de pratique courante de se référer à ces apoprotéines en les reliant à la famille lipoprotéique d'où elles sont isolées: c'est-à-dire apo-LDL, apo-VLDL, apo-HDL, parfois suivies de l'amino-acide fixé sur leur dernier atome de carbone, par exemple: apo-HDL-thréonine, apo-HDL-glutamine, etc. Certaines des apoprotéines des VLDL sont aussi présentes dans les HDL et LDL, montrant qu'il y a des relations entre les familles de lipoprotéines qui, sans aucun doute, ont une signification lorsqu'on considère leur métabolisme. Les apoprotéines fournissent un certain degré de spécificité immunologique et facilitent l'identification des lipoprotéines.

Aucun type d'hyperlipidémie n'a encore été trouvé qui aurait pour cause une apoprotéine anormale, bien que cette possibilité, principalement à la suite de mutations, offre une base attrayante pour une étiologie possible de certaines formes d'hyperlipoprotéinémies.

VARIATIONS BIOLOGIQUES

Les taux des lipides plasmatiques varient non seulement d'une personne à l'autre mais aussi chez un même individu. La prise d'un repas, de même que toute modification du régime alimentaire habituel, peut être à l'origine de fluctuations marquées des concentrations. La position du corps, la stase veineuse et d'autres facteurs (traumatismes, grossesse, exercice, émotions, usage du tabac, etc.) influent également sur les taux. Les différences entre individus sont fonction de l'âge, du sexe, des caractéristiques génétiques et ethniques, de l'état de nutrition et de l'activité physique. D'où la nécessité de normaliser les conditions dans lesquelles sont pratiqués les prélèvements: le sang doit être recueilli 10-14 heures après le dernier repas, chez le sujet au repos, en évitant la stase veineuse.

Pour définir l'hyperlipidémie, il est indispensable de préciser au préalable la notion de « valeurs normales ». Les taux minimaux de lipides plasmatiques compatibles avec un bon état de santé (valeurs normales biologiques) ne sont pas connus. En revanche, il est relativement aisé d'établir pour une population donnée la distribution de fréquence des taux de lipides et de lipoprotéines. Les valeurs normales statistiques sont définies arbitrairement et leur niveau variera selon l'usage auquel elles sont destinées. Dans le présent document, le terme « augmentées » s'applique aux concentrations qui excèdent la limite fixée, quels que soient les critères qui ont déterminé le choix.

MÉTHODES D'ANALYSE DES LIPIDES ET DES LIPOPROTÉINES

Pour le dosage du cholestérol, il est conseillé d'utiliser la méthode d'Abell et al. (1952), très spécifique, qui doit être considérée comme une méthode de référence. Pour le dosage des triglycérides, on dispose de trois méthodes de base qui donnent de bons résultats: *a*) une méthode enzymatique; *b*) un procédé au cours duquel le glycérol est transformé par oxydation en formaldéhyde que l'on dose par colorimétrie; *c*) un procédé dans lequel la formaldéhyde formée est dosée par fluorimétrie. Les problèmes que pose la normalisation des méthodes d'analyse du cholestérol et des triglycérides sont examinés et l'on mentionne à cette occasion les activités du Centre international OMS de référence pour le dosage des lipides dans la recherche sur les maladies cardio-vasculaires.

L'observation du plasma dans des conditions normalisées, l'électrophorèse et l'ultracentrifugation sont les trois techniques les plus fréquemment utilisées à l'heure actuelle pour l'évaluation et le dosage des lipoprotéines. Ces méthodes, ainsi que quelques autres (précipitation, néphélométrie, méthode immunochimique) sont décrites et la question de leur normalisation est brièvement évoquée.

Il faut insister sur le fait qu'à l'avenir l'étude des apolipoprotéines et des acides gras libres, de la composition de certaines fractions lipidiques, des classes de phospholipides et de l'activité de certaines enzymes permettra peut-être de classer avec davantage de précision les altérations du métabolisme des lipides. En raison des lacunes présentes des connaissances et des techniques, les classifications en usage ne peuvent être que limitées et sujettes à révision.

CLASSIFICATION DES HYPERLIPIDÉMIES ET DES HYPERLIPOPROTÉINÉMIES

Comme il a été indiqué précédemment, 1) les lipides du plasma circulent sous forme de lipoprotéines; 2) chacune des 4 principales familles de lipoprotéines, chylomicrons, pré-β (VLDL), β (LDL) et α (HDL) contient du cholestérol, des triglycérides et des phospholipides; 3) le métablisme des 4 familles de lipoprotéines est différent. Ces faits fournissent les clés pour la classification des hyperlipidémies, car ils indiquent que 1) l'hyperlipoprotéinémie survient très rarement sans hyperlipidémie et par conséquent que l'hyperlipidémie peut être utilisée pour détecter l'hyperlipoprotéinémie; 2) une classification basée sur les lipoprotéines offre plus de renseignements qu'une autre basée sur les lipides seuls; 3) une classification devrait faire la distinction entre les désordres du métabolisme des lipoprotéines aussi bien que des lipides.

La classification proposée ici inclut, étape par étape, l'utilisation des analyses de lipides, des analyses des lipoprotéines et d'autres données cliniques et biologiques. Elle fournit une approche de la classification étiologique ou pathogénique par laquelle elle sera finalement remplacée. On doit mentionner que cette classification est basée sur la présomption que le patient était à un régime standard avant les analyses.

HYPERLIPIDÉMIE

L'analyse du cholestérol et des triglycérides est le moyen le plus simple pour détecter une hyperlipoprotéinémie. Elle fournit aussi des informations sur le type d'hyperlipoprotéinémie, car la proportion de ces lipides varie d'une famille de lipoprotéines à l'autre.

Les concentrations de cholestérol et de triglycérides permettent de distinguer trois types généraux d'hyperlipidémie qui correspondent approximativement à certains types d'hyperlipoprotéinémie:

1) Taux de cholestérol élevés et taux de triglycérides normaux. Ce groupe, appelé parfois « hypercholestérolémie essentielle », correspond habituellement à l'hyper-β-lipoprotéinémie.

2) Taux de triglycérides élevés et taux de cholestérol normaux. Ce groupe correspond habituellement soit à l'« hyperchylomicronémie essentielle », soit à l'hyper-pré-β-lipoprotéinémie.

3) Taux de cholestérol et de triglycérides élevés. Tous les principaux types d'hyperlipoprotéinémie, excepté l'« hyper-β-lipoprotéinémie essentielle », peuvent se trouver dans ce groupe.

L'hétérogénéité de ce troisième groupe souligne particulièrement la nécessité d'une classification basée sur les lipoprotéines.

Il est possible d'affiner un peu la classification des hyperlipidémies en ajoutant la mesure des phospholipides totaux (PL) et aussi en calculant les rapports suivants: Chol/TG et Chol/PL.

Le rapport Chol/TG indique si l'élévation prédominante concerne le cholestérol ou les triglycérides. Le rapport Chol/PL indique souvent une élévation des HDL (α-lipoprotéines) quand il tombe au-dessous de 0,5. Ces raffinements ne sont pas nécessaires pour détecter l'hyperlipidémie, mais peuvent offrir une aide pour la classification si les lipoprotéines ne sont pas déterminées.

HYPERLIPOPROTÉINÉMIE

L'hyperlipidémie peut habituellement être classée dans l'un des types de lipoprotéines anormales résumés dans

le tableau 1 (voir page 903). Dans un but de simplification, ces types sont numérotés selon le système de Fredrickson et collaborateurs. Ces types ne doivent pas être considérés comme des maladies simples et chacun peut avoir des causes multiples. La plupart des hyperlipidémies, mais pas toutes, sont représentées par les six types décrits. Les méthodes de diagnostic décrites ici sont établies dans un but pratique. Certains tests sont utiles au diagnostic d'un type donné, les autres pas.

Type I — Hyperchylomicronémie

Critères

1) Présence de chylomicrons.

2) VLDL (pré-β-lipoprotéines) normales ou seulement légèrement augmentées.

Méthodes de diagnostic

1) Le plasma reposé contient une couche de « crème » au-dessus d'un sous-nageant clair (test diagnostique).

2) Le cholestérol est habituellement augmenté; les triglycérides sont augmentés; Chol/TG<0,2. Un rapport inférieur à 0,1 se rencontre seulement dans le type I.

3) Electrophorèse. On note la présence d'une forte bande de chylomicrons, distincte des traînées des lipoprotéines de la région des pré-β; parfois les bandes des α (HDL) et β (LDL) ne sont pas visibles; la bande des pré-β (VLDL) peut être absente ou peut apparaître avec une intensité diminuée, normale ou légèrement augmentée et avec une traînée vers la bande massive des chylomicrons (habituellement caractéristique).

4) Ultracentrifugation. Chylomicrons très fortement augmentés; VLDL habituellement augmentées (la séparation des chylomicrons est incomplète); LDL très diminuées; HDL très diminuées.

Commentaires. Il faut noter que, dans le type I, les chylomicrons peuvent parfois être accompagnés par une augmentation apparemment modérée des VLDL (pré-β). Ceci est dû en partie à la difficulté de séparer ces deux familles de lipoprotéines. La quantité de VLDL en excès, cependant, est toujours beaucoup moindre que la quantité énorme de chylomicrons.

Tests recommandés pour le diagnostic

1) Examen du plasma reposé.

2) Electrophorèse.

Type II — Hyper-β-lipoprotéinémie

Critère. Elévation anormale de la concentration en LDL (β).

Note. Dans certains cas, il peut être utile de distinguer deux sous-groupes dans ce type. Ils seront appelés IIa et IIb. Pour tous les deux, le critère du type II, élévation des LDL (β), est présent, mais dans l'un (IIb), on note aussi une élévation des VLDL (pré-β). Les deux formes peuvent être rencontrées dans la même famille affectée d'hyper-β-lipoprotéinémie familiale; c'est principalement pour cette raison qu'on les classe actuellement sous la même rubrique du type II.

Type IIa

Critères

1) Elévation des LDL (β).

2) VLDL (pré-β) normales.

Méthodes de diagnostic

1) Plasma reposé clair (très utile, pas toujours formel).

2) Cholestérol habituellement augmenté; triglycérides normaux; Chol/TG toujours >1,5.

3) Electrophorèse. On observe une bande de β-lipoprotéines très fortement colorée; la bande pré-β peut être absente ou présente et alors d'intensité normale. Les chylomicrons ne sont pas visibles, les α-lipoprotéines sont habituellement normales. (Permet le diagnostic, mais seulement si elle est accompagnée de l'estimation du taux de LDL).

4) Ultracentrifugation. Les LDL (S_f 0-20) sont augmentées. Les VLDL (S_f 20-400) sont normales, les HDL sont habituellement normales et les chylomicrons ne sont pas augmentés (test diagnostique).

Type IIb

Critères

1) Augmentation des LDL (β).

2) Augmentation des VLDL (pré-β).

Méthodes de diagnostic

1) Plasma reposé soit clair, soit légèrement trouble, mais sans couche de chylomicrons (« crème ») à la partie supérieure. (Ce test n'est pas diagnostique.)

2) Le cholestérol est habituellement augmenté; les triglycérides toujours augmentés; Chol/TG variable. (Ces tests ne sont pas diagnostiques.)

3) Electrophorèse. La bande des β-lipoprotéines est intensément colorée; la bande de pré-β est augmentée en intensité. Les chylomicrons ne sont pas visibles; les α-lipoprotéines sont habituellement normales. (Ce test n'est diagnostique que s'il est accompagné d'une estimation des concentrations en LDL et VLDL.)

4) Ultracentrifugation. LDL (S_f 0-20) augmentées, VLDL (S_f 20-400) augmentées, chylomicrons non augmentés, HDL habituellement normales (test diagnostique).

Commentaires. La définition certaine du type II dépend de la mise en évidence d'une élévation anormale de la concentration en LDL (β). On l'obtient avec le maximum de précision par ultracentrifugation analytique et préparative. On peut aussi l'estimer à partir des concentrations de cholestérol, des triglycérides et du cholestérol des HDL, comme décrit ci-dessus.

Les LDL peuvent aussi être mesurées par analyse immunochimique des fractions sous-nageantes à 1,006 en utilisant des sérums anti-LDL. (De tels antisérums réagissent aussi avec les VLDL et c'est pourquoi ils ne

permettent pas une détermination sûre des LDL dans le plasma complet.)

La forme de type IIa peut habituellement être déterminée avec certitude par l'analyse du cholestérol et des triglycérides seulement, particulièrement quand le rappor Chol/TG est supérieur à 2. Les exceptions sont les patients qui ont un taux de LDL anormalement élevé en présence d'une concentration normale de cholestérol plasmatique.

La forme de type IIb est difficile à déterminer avec certitude à partir des lipides plasmatiques seuls.

Tests recommandés

1) Chol+TG+électrophorèse quand Chol/TG>2.

2) Chol+TG+HDL (mesure du cholestérol après précipitation) pour calcul des LDL (applicable seulement quand le type III est exclu et TG<400). Si les concentrations de LDL sont augmentées, la répartition des sous-types est: IIa quand les triglycérides sont normaux, IIb quand les triglycérides sont augmentés.

3) Analyses par ultracentrifugation.

Type III — Forme à « β flottantes » ou « β larges »

Critères. Présence de VLDL ayant un contenu en cholestérol anormalement élevé et de mobilité électrophorétique anormale (« β flottantes », « β-VLDL»).

Méthodes de diagnostic

1) Plasma reposé habituellement trouble avec, fréquemment, une faible couche « crémeuse » de chylomicrons (utile, mais ne permettant pas le diagnostic).

2) Cholestérol plasmatique presque toujours augmenté, de même que les triglycérides plasmatiques; Chol/TG fréquemment aux environs de 1, mais pouvant varier de 0,3 à >2,0.

3) Electrophorèse (sur papier, agarose ou acétate de cellulose). On note habituellement une bande de « β larges », s'étendant de la position β jusque dans la position pré-β. Ceci apparaît dans environ les 2/3 des plasmas qui contiennent des « β-flottantes ». Une bande pré-β distincte est parfois présente et peut être augmentée en intensité. Les α-lipoprotéines sont habituellement normales. Une légère bande de chylomicrons est souvent présente, même durant les périodes de régime pauvre en graisses (utile, mais ne permettant pas le diagnostic). En électrophorèse sur gel de polyacrylamide (PGE), une bande de pré-β (VLDL) élargie est présente, et on ne voit pas de lipoprotéines dans la position habituellement occupée par les β-lipoprotéines (LDL) dans ce milieu. La présence simultanée de lipoprotéines « β migrantes » sur papier, agarose ou acétate de cellulose et leur absence en gel de polyacrylamide est un test de présomption pour une anomalie de type III, qui s'avère exacte dans près de 95% des cas. (La combinaison de ces deux tests électrophorétiques est considérée comme diagnostique.)

En électrophorèse en gel d'amidon des VLDL isolées, on obtient deux bandes, une dans la position habituelle α_2 (parfois appelée « α_2-VLDL ») et une dans une position β anormale (« β-VLDL ») (test diagnostique).

L'électrophorèse sur papier, en agarose, en acétate de cellulose ou en gel d'amidon de la fraction surnageante du plasma, après ultracentrifugation à densité saline non ajustée de 1,006, révèle des lipoprotéines « β migrantes ». Normalement, seules des lipoprotéines migrant en pré-β sont présentes dans la fraction lipoprotéique de densité <1,006. (La démonstration de « β flottantes » est actuellement le test étalon définitif avec lequel les autres tests diagnostiques doivent être comparés.)

4) Ultracentrifugation. En ultracentrifugation analytique, la sous-classe normalement prédominante des LDL de densité 1,010-1,063 (S_f 0-12) est fortement diminuée et la sous-classe des LDL de densité 1,006-1,019 (S_f 12-20) est augmentée de façon disproportionnée. La sous-classe des VLDL (S_f 100-400) est aussi augmentée. Les chylomicrons peuvent être augmentés. Cette inversion des concentrations habituelles des LDL et VLDL fournit habituellement un schéma caractéristique pour le type III; cependant, il est possible d'avoir des changements semblables des sous-classes totales S_f 0-20 et S_f 20-400 dans d'autres types (très utile, mais ne permet pas toujours le diagnostic).

La combinaison de l'ultracentrifugation préparative et de l'électrophorèse décrite ci-dessus peut être améliorée et on peut la remplacer par une mesure du cholestérol et des triglycérides dans la fraction de VLDL (densité <1,006) obtenue par ultracentrifugation. Normalement, le rapport Chol/TG dans les VLDL est inférieur ou égal à 0,2. Des taux significativement plus élevés (>0,4) sont caractéristiques du type III (donnée probablement diagnostique).

Commentaires. L'anomalie de type III indique la présence de VLDL anormales, ou plus précisément, de LDL anormales dans la fraction VLDL des lipoprotéines du plasma. On peut la suspecter à partir d'un rapport Chol/TG=1, particulièrement quand des analyses répétées montrent une labilité marquée des concentrations de Chol et de TG, et qu'une bande de « β larges » apparaît en électrophorèse classique. Cette combinaison doit permettre une présomption de diagnostic; ce dernier ne devrait jamais être établi uniquement sur la base d'une électrophorèse classique dans un seul milieu.

Le test définitif est la démonstration des «β flottantes », mais une preuve ayant une valeur équivalente peut être trouvée dans la mesure du cholestérol et des triglycérides dans les VLDL; en combinant électrophorèse en gel de polyacrylamide (PGE) et dans un autre milieu, on peut avoir une présomption de diagnostic. Un test plus simple, fournissant un diagnostic plus précis, serait cependant très utile.

Tests recommandés. Quand le rapport Chol/TG dans le plasma est proche de 1 et qu'une bande de « β larges » est suspectée en électrophorèse:

1) Des schémas de lipoprotéines plasmatiques obtenus par électrophorèse en PGE et, soit sur papier, soit en agarose, soit en acétate de cellulose, doivent être comparés. L'absence de lipoprotéines « β migrantes » en PGE et leur présence dans un autre système permet une présomption de diagnostic.

2) Quand cela est possible, une confirmation des « β flottantes » (ou « VLDL » ayant un rapport Chol/TG élevé) doit être faite après ultracentrifugation préparative.

Type IV — Hyper-pré-β-lipoprotéinémie

Critères

1) VLDL (pré-β) augmentées.

2) LDL (β) non augmentées.

3) Absence de chylomicrons.

Méthodes de diagnostic

1) Plasma au repos clair ou trouble dans son ensemble, sans couche surnageante de chylomicrons (utile mais ne suffit pas au diagnostic).

2) Cholestérol plasmatique normal ou augmenté; triglycérides plasmatiques augmentés; Chol/TG variable (test très utile, parfois diagnostique).

3) Electrophorèse. Intensité augmentée de la bande des pré-β-lipoprotéines; bande β normale ou diminuée, bande α normale, souvent diminuée; chylomicrons non visibles. Il peut y avoir des traînées de lipoprotéines depuis la région pré-β jusqu'à l'origine (test utile, mais non diagnostique sans une mesure quantitative; voir commentaires).

Toutes les VLDL isolées par électrophorèse en gel d'amidon ont la mobilité habituelle α_2 (α_2-VLDL). Il n'y a pas de β-VLDL ou de « β flottantes » et les VLDL ont habituellement un rapport Chol/TG $\leqslant 0{,}2$.

4) Ultracentrifugation. Les VLDL (S_f 20-400) sont augmentées; les LDL (S_f 0-20) sont normales ou diminuées; les HDL sont normales ou diminuées et les chylomicrons ne sont pas augmentés (diagnostic).

Commentaires. Si le cholestérol est absolument normal, si les triglycérides sont nettement augmentés, et s'il n'y a pas de chylomicrons visibles sur le plasma au repos, le diagnostic de type IV est quasi certain. La certitude est renforcée si l'électrophorèse révèle une bande distincte de pré-β et une bande de β bien distincte et diminuée. Les triglycérides plasmatiques sont toujours utilisés pour estimer les concentrations de pré-β par l'électrophorèse et sont toujours élevés dans le type IV.

Réciproquement, une augmentation apparente des pré-β-lipoprotéines en électrophorèse ne sera pas accompagnée par une élévation des triglycérides plasmatiques si la plupart des pré-β représentent des « pré-β qui sombrent » (*sinking pre-β*). Il s'agit ici d'un phénomène normal et sa fréquence souligne la nécessité de contrôler par les concentrations de triglycérides les données de l'électrophorèse. On devrait rechercher les signes d'une « anomalie de type III »; il n'est pas nécessaire d'exclure l'anomalie par des tests spécifiques dans la plupart des cas de type IV.

Tests recommandés

1) Pour la plupart des échantillons: Chol+TG+observation du plasma+électrophorèse permettent un diagnostic.

2) Estimer les LDL et exclure le type III dans les cas douteux.

3) L'ultracentrifugation peut être très utile dans certains cas.

Type V — Hyper-pré-β-lipoprotéinémie et chylomicronémie

Critères

1) VLDL augmentées.

2) Présence de chylomicrons.

Méthodes de diagnostic

1) Plasma au repos. Couche « crémeuse » de chylomicrons, recouvrant un sous-nageant trouble (test diagnostique si une anomalie de type III est exclue).

2) Cholestérol augmenté, triglycérides augmentés; rapport Chol/TG habituellement $>0{,}15$ et $<0{,}6$ (test utile mais non diagnostique).

3) Electrophorèse. Bande pré-β augmentée et fréquemment traînées vers l'origine où une accentuation distincte indique la présence concomitante de chylomicrons. Les bandes d'α- et β-lipoprotéines sont habituellement, et souvent fortement, diminuées. Il n'y a pas de « β-flottantes ». (Ce test peut être diagnostique si une traînée de pré-β n'obscurcit pas une bande de chylomicrons.)

4) Ultracentrifugation. Chylomicrons et VLDL (S_f 20-400) augmentés; LDL, particulièrement la sous-classe S_f 0-12, et HDL habituellement diminuées (test diagnostique).

Commentaires. Le principal problème de diagnostic, qui est de discerner les chylomicrons par électrophorèse quand les concentrations en pré-β (VLDL) sont extrêmement fortes, peut habituellement être surmonté par l'observation du plasma reposé. Ce dernier test est très bon pour le type V.

Tests recommandés

Examiner le plasma et mesurer Chol+TG. L'aspect typique du type V peut apparaître dans deux autres situations. L'une est une forme de type I lorsqu'il y a assez de VLDL pour donner un faible trouble à la couche sous-nageante. Le rapport Chol/TG est habituellement supérieur dans le type V. La seconde est le type III. Là le rapport Chol/TG est souvent proche de 1, mais il peut aussi descendre jusqu'à 0,3. Le test des « β flottantes » doit être pratiqué si une quelconque incertitude demeure.