Serum lipids and lipoproteins

Clinical relevance

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Few topics receive more thought than fats and lipids. Yet the answers the physician must be able to provide relative to lipids, and what the patient must do about them for best health often seem elusive.

Two major factors can account for the increased interest in lipids: (1) much improved methods for analysis of blood lipids, including cholesterol,[1] triglycerides,[2] and lipoproteins,[3] and (2) from extensive studies in scientific centers throughout the world it has been concluded that there is a close correlation between the serum lipid levels and incidence of coronary heart disease.[4] Since heart disease is a major cause of death in the United States, information concerning blood lipid levels and the institution of appropriate treatment to bring them to desirable levels are essential.

Since blood is the most readily available tissue for analysis, it is usually the material analyzed. All fats in the blood are transported bound to protein—the most rapidly metabolized fraction, a ready, and for some tissues the preferred source of energy, the free fatty acids are loosely bound to albumin. The other lipids, cholesterol, phospholipids, and triglyceride, are combined with specific polypeptides to form the lipoproteins. In normal human serum there are four major classes of lipoproteins. The chylomicra normally present

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in very low concentration in fasting serum are composed chiefly of triglyceride, with a very low percentage of cholesterol, phospholipid, and protein. On electrophoresis the chylomicra do not migrate from the point of application. The other three lipoprotein fractions have been named according to their relative speed of migration during electrophoresis; the most rapidly migrating fraction has a mobility of α_1-globulin and contains a relatively large proportion of phospholipid and protein and about 25% of the molecule is cholesterol. The next fraction, identified as pre-β-lipoprotein, has a mobility between that of α- or β-lipoprotein, is rich in triglyceride and contains relatively less protein and cholesterol than the α- or β-lipoprotein. The slowest migrating fraction, the β-lipoprotein fraction, is rich in cholesterol (about 40% of the molecule) and phospholipid and contains relatively little triglyceride.

The chemical composition and characteristics of the lipoproteins were first determined on material isolated at different densities in the ultracentrifuge. Many of the basic concepts concerning lipoproteins were developed by Gofman's group using ultracentrifugal techniques,[5, 6] but time and expense made them unsuitable for general use. The paper electrophoretic technique developed by Hatch and Lees[3] has made lipoprotein studies practical for large scale screening of populations as well as for study of patients. Automated techniques[1] in the clinical laboratory and improved standards of accuracy provide good lipid analyses for the physician when he feels that they are needed.

Who should have lipid studies? All adults having complete physical examinations, such as the annual physical examinations recommended by many industries and physicians, should have serum cholesterol and triglyceride determinations. If these lipid levels are abnormal it is important to know which lipoprotein fraction is involved. Lipid determinations should also be ordered on all children from a very early age, of known hyperlipemic parents, or of families with a strong history of heart disease or atherosclerosis. Lipid analyses should be ordered when indicated by clinical findings; for example, in coronary disease, diabetes, renal or thyroid disease.

What preparation should a patient have for lipid studies? Most meaningful information can be obtained when the blood is collected after a 12 to 16 hour fast, when the subject has been eating his regular diet for at least 2 weeks and is maintaining a constant weight. It is important to know if any drugs are being taken, since some, such as the contraceptive "pill", cause major changes in the lipoprotein pattern, and some interfere with accurate chemical determinations.

In interpreting the results of serum lipid and lipoprotein studies, consideration of the age and sex of the subject is important. It is also necessary to know the values considered as normal by the laboratory doing the study, because there is some difference in levels depending on the method of estimation, whether colorimetric or fluorometric, type of standard material used, etc.

Serum lipoprotein levels have been found to be partially under genetic control. Classification of the various types of genetically determined hyperlipoproteinemias has been simplified and amplified by Fredrickson and his

group.[7] *Figure 1* shows the typical lipoprotein patterns obtained on normal serum, and on the serum of patients with each of the five major types of hyperlipoproteinemia. Type I hyperlipoproteinemia is an autosomal recessive type, characterized by milky serum containing very high levels of chylomicra and triglyceride. The serum cholesterol, β- and α-lipoprotein levels are low normal or low. This type of abnormality is found in children who are frequently the long, lean type. The postheparin lipoprotein lipase activity of plasma is low.

Type II-A hyperlipoproteinemia, frequently called primary hypercholesteremia, is an autosomal dominant trait which may be manifest from a very early age.[8] There are high concentrations of serum β-lipoprotein and cholesterol and normal or low levels of α-lipoprotein. The triglyceride and pre-β-lipoprotein levels are normal or only slightly elevated.

Type II-B, mixed type hyperlipidemia, is characterized by increased levels of β- and pre-β-lipoprotein, of cholesterol and moderate increase in triglyceride. Because of the much more favorable response of patients with this pattern to cholesterol lowering diets than of those of type II-A, Brown, Lewis, and Page[9] have stated these patients should be in a separate group, type VI.

Type III is a relatively rare type of hyperlipidemia in which both cholesterol and triglyceride levels are elevated and in which the β-lipoprotein migrates as a broad band of slightly faster than normal mobility and floats in the preparative ultracentrifuge at the density of serum. This is not the case with normal β-lipoprotein. An abnormal glucose tolerance is frequently found in patients with type III hyperlipidemia.

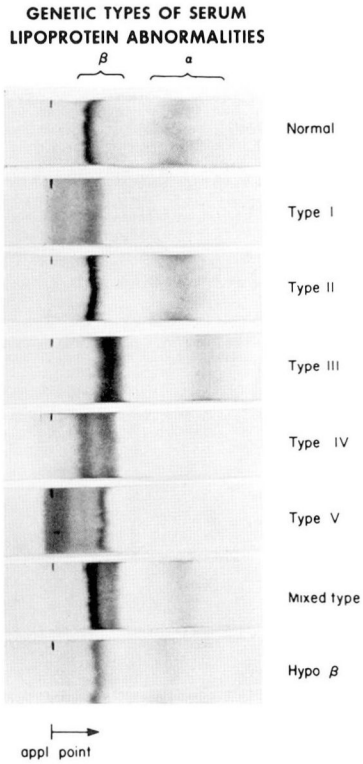

Fig. 1. Serum lipoprotein patterns of human sera. Electrophoretic separation using albuminated buffer pH 8.6; Oil Red-O stain.

Type IV hyperlipoproteinemia usually is not demonstrable until maturity. It is characterized by increased levels of triglyceride and pre-β-lipoprotein with little change in cholesterol and β-lipoprotein levels. It is often found in middle-aged individuals with a plethoric, trunk type of obesity. Doctor Gennes of Paris has described it as developing when peripheral lipogenesis ceases and weight accumulation occurs in the trunk area. It is frequently accompanied by an abnormal glucose tolerance.

Type V hyperlipoproteinemia is characterized by increased triglyceride,

Table 1.—Clinical features associated with hyperlipoproteinemia

1. Xanthomatosis	Found in all types
2. Vascular disease	Found in types II, III, IV
3. Hyperuricemia	Found in hyper-β-lipoproteinemia, type II
4. Corneal arcus	
5. Lipemia retinalis	Found with hyper-pre-β-lipoproteinemia, type IV or type V
6. Marrow foam cells	
7. Abnormal glucose tolerance	
8. Abdominal pain	Found with hyperchylomicronemia, type I
9. Pancreatitis	
10. Hepatosplenomegaly	
11. Low postheparin lipoprotein lipase	

normal or slightly increased cholesterol, and increased chylomicra and pre-β-lipoprotein levels. Usually it is first manifest in the young adult. It is frequently carbohydrate and alcohol dependent.

Clinical features associated with the various types of hyperlipidemia are summarized in *Table 1*. Xanthomatosis at all ages and atherosclerosis in the adult are common characteristics of all except type I. Patients with types I and V patterns having increased levels of chylomicra frequently show hepatosplenomegaly and unexplained abdominal pain. When hyper-pre-β-lipoproteinemia is present, abnormal glucose tolerance and lipemia retinalis are often found. Complications found in type II hypercholesteremics are corneal arcus and hyperuricemia.

After finding hyperlipidemia, and establishing that it is a primary disease, not secondary to some other disease, such as hypothyroidism, diabetes, liver disease, etc., which will be discussed briefly later, the question is, "What is the best way of correcting the abnormal serum lipidemia?" For all types, the initial treatment is an appropriate diet. If the subject is overweight, the starting diet should be limited in calories and be continued until ideal weight is achieved. At the Cleveland Clinic two major dietary patterns have been used;[10, 11] both contain less than 300 mg cholesterol/day (*Table 2*). One is low in fat, the other rich in unsaturated but low in saturated fat (VO diet). The low fat diet is necessary for all patients with hyperchylomicronemia, as in type I hyperlipidemias. It is also prescribed for type V subjects. Types II, III, and IV patients usually respond to either the low fat or the vegetable oil food pattern. The latter is preferred for type III and type IV patients after they have reached desirable weight, as the low fat diet necessarily is high in carbohydrate, which is definitely contraindicated for many hyperglyceridemics of these types. Type II patients find the vegetable oil food pattern easier to adhere to over the years than the low fat diet, and as a result they usually show better response to the VO diet.[12]

Figure 2 shows the excellent response of a patient with type IV hyperlipidemia to dietary treatment and the relatively desirable levels of serum

Table 2.—Composition of diets

	Calories, %				Cholesterol, mg/day
	Fat	Poly-unsat. fat	Prot.	Carbohydrate	
1. Low fat	15	4	13	72	200
2. Vegetable oil	38	17	13	49	200
3. Average American	44	5	13	43	750

lipids as long as his dietary adherence was maintained.

When a patient is found to have type II hyperlipidemia it is important to study other members of the family for lipid abnormality. The incidence

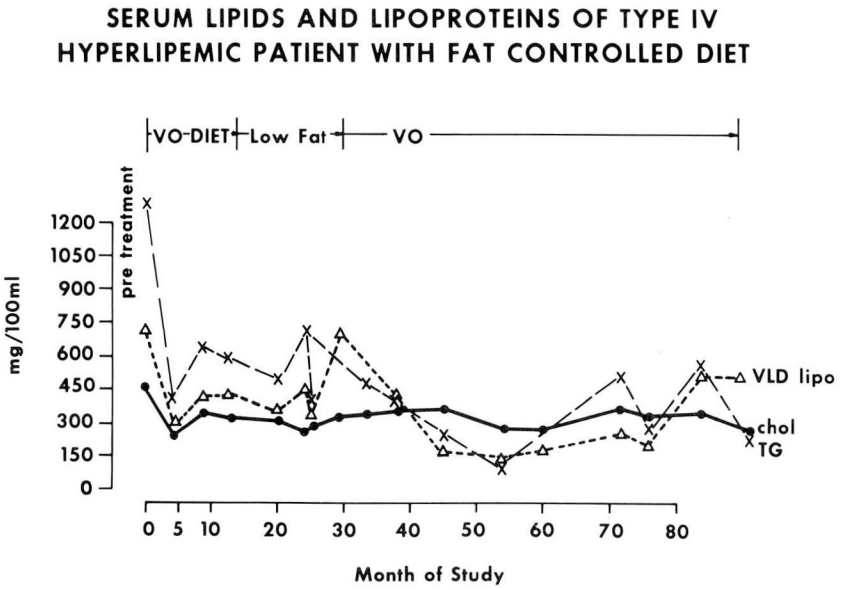

Fig. 2. Changes in levels of serum lipids and lipoproteins of patient with type IV hyperlipidemia with fat controlled diet.

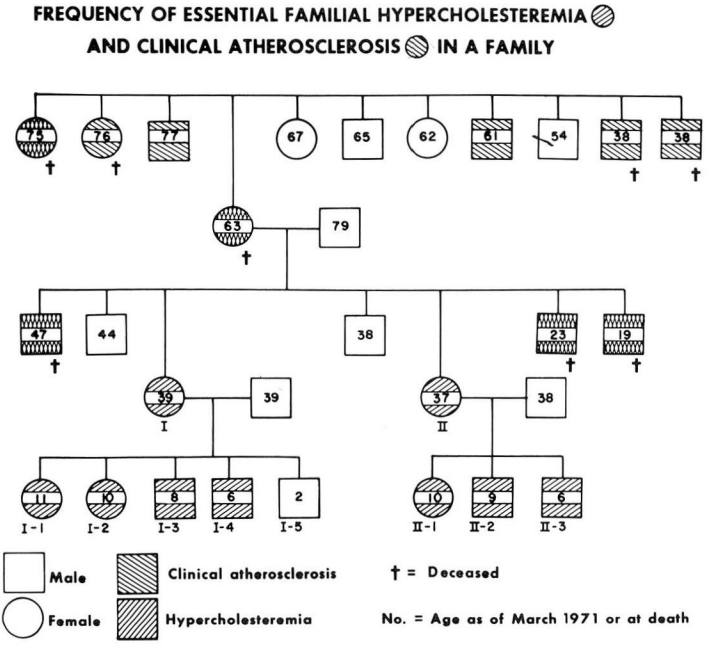

Fig. 3. Frequency of familial hypercholesteremia, (type II) hyper-β-lipoproteinemia, in a family.

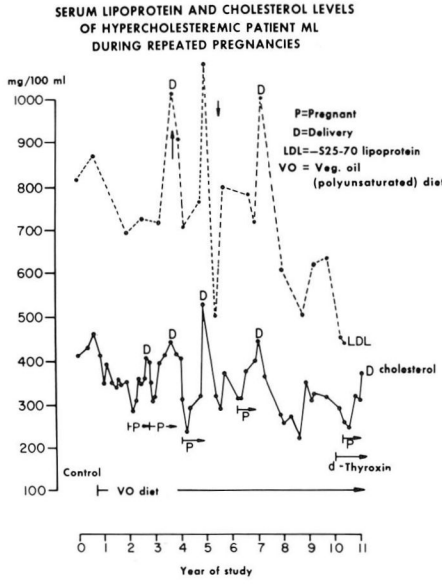

Fig. 4. Changes in serum cholesterol and β-lipoprotein levels of type II hypercholesteremic patient during repeated pregnancies.

of hypercholesteremia in a family studied at the Clinic for nearly 20 years is shown in *Figure 3*. Heart attacks at an early age were common. Two sisters in this family have been studied during pregnancies (six and four respectively) and the changes observed in one are shown in *Figure 4*. As is true in subjects with normal serum cholesterol levels, the cholesterol levels of hypercholesteremic sister I increased very significantly in the latter part of pregnancy. There was no correlation between the level of the mother's serum cholesterol at delivery and that of the infant's cord blood (*Table 3*). Similar lack of correlation between mother's and cord blood cholesterol levels have been observed in the other sister of this family and in another hypercholesteremic (type II) subject of a different family who have been studied during three pregnancies each. The infants' cord blood levels ranged from the normal, i.e., 83 mg/100 ml, found in our laboratory for infants of normal cholesteremic mothers, to slightly elevated levels. Within a few days of birth, some infants had significantly elevated serum cholesterol levels, concentrations exceeding 163 mg/100 ml within 4 days in one, 4 months in three, and 18 months in another.[8] Three children, first studied when 13, 16, and 29 months old, had serum cholesterol levels ranging from 1½ to 4 times normal. When the children were given a modified food pattern (*Table 4*) with fat and cholesterol content controlled, their elevated serum cholesterol levels decreased 25% or more.

Some subjects with type II hyper-

Table 3.—Serum cholesterol of cord blood and of mother at delivery

Family	Child	Sex	Chol. at delivery, mg/100 ml		Age, yrs July, 1970
			Cord blood	Mother's level	
A	I-2	F	111	440	9
	I-3	M	89	526	7
	I-4	F	78	450	5½
	I-5	M	132	196	1½
	II-2	M	97	454	9
B	V-1	M	109	534	4
	V-2	M	82	600	1½
	V-3	M	141	540	½
Normal			83 ± 3	295	

cholesteremia fail to achieve desirable serum cholesterol levels when treated with diet or with available cholesterol lowering drugs. Studies of such a patient are shown in *Figure 5*. While some decrease was obtained by diet, the resulting cholesterol level of 400 mg or more was not desirable. Since the patient had severe angina pectoris, an ileal bypass operation was performed in hopes of improving serum lipid levels and delaying progression of the disease. We had observed that when a jejunocolic shunt operation was performed for reducing body weight of the excessively obese individual the serum cholesterol levels consistently decreased 40%.[13] Buckwald,[14] at the University of Minnesota, has applied this observation and modified the operation to an ileal bypass which results in reduction of serum cholesterol and β-lipoprotein levels without change in body weight. The serum cholesterol level of this patient decreased after ileal bypass operation approximately 40% below the level achieved by diet alone, and without any change in body weight. It should be noted that dietary adherence is also important even after the shunt operation. The patient's serum cholesterol levels have been maintained around 300 mg/100 ml during periods of good adherence, and about 350 mg/100 ml when poor. It is now 5 years since the shunt operation.

Table 4.—Recommended foods for hypercholesteremic children

Skim milk
Low fat cheeses, sherbets, and ices
Lean meat, chicken, and fish
Egg whites only
Unsaturated margarines
Salad oils
All fruits and vegetables
Breads and cereals

Regulation of serum lipid levels of patients with type V hyperlipidemia may be especially difficult. A balance between a low fat and not excessive carbohydrate intake and adequate calories is achieved only by the careful counseling of nutritionist and excellent cooperation of the patient and

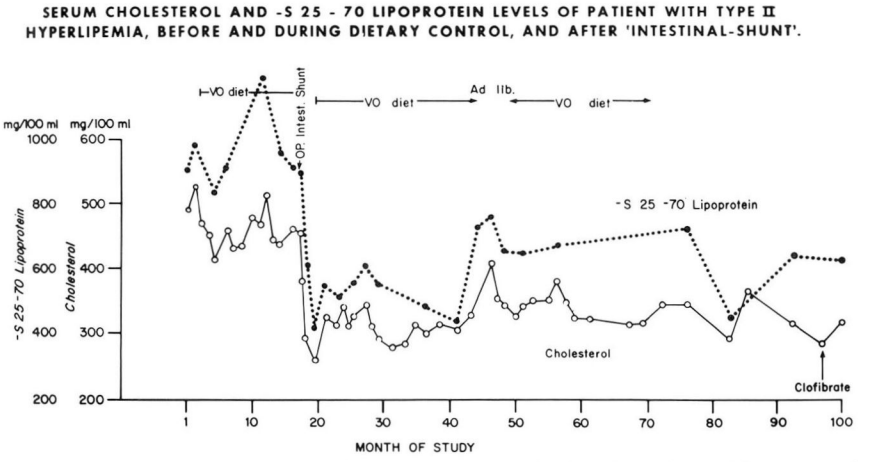

Fig. 5. Serum cholesterol and LDL (-S25-70) lipoprotein levels of patient with type II hyperlipemia, before and during dietary treatment and after ileal-by-pass operation.

Table 5.—Change in serum lipids of type V hyperlipemic child during dietary program

Duration of diet	Total chol., mg/100 ml	Trig., mg/100 ml	Lipoprotein electrophoresis			
			Chylo.	β-	Preβ-	α-
Prediet	500	3165	4+	2+	4+	±
3 months*	410	2246	3+	3+	4+	±
6 months*	420	1800	±	2+	4+	+
Normal	178	80	±	2+	±	3+
S.D.	±15	±20				

* Only partial adherence to low fat—low cholesterol, reducing diet.

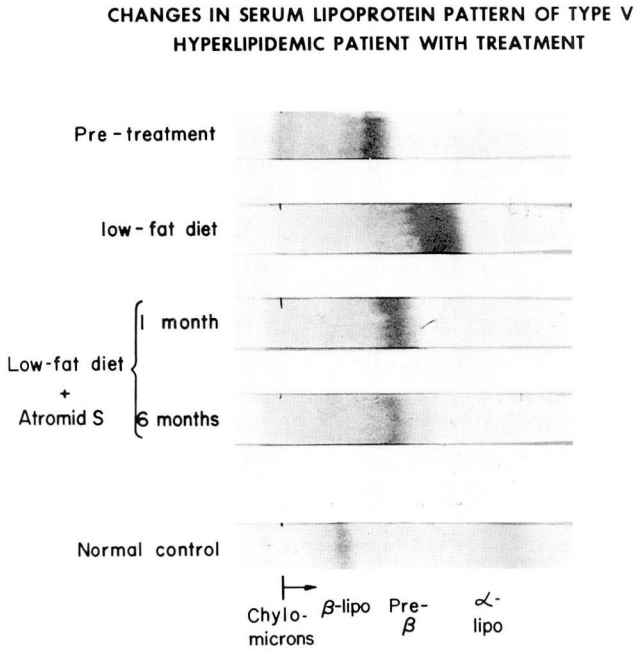

Fig. 6. Changes in serum lipoprotein pattern of patient with type V hyperlipemia with treatment.

family. *Table 5* summarizes the studies on a young boy who had very high serum triglyceride levels and elevated cholesterol. Definite improvement in the lipoprotein and lipid pattern occurred with dietary treatment, but desirable levels were not reached; this was partially due to inconsistent adherence to the diet. *Figure 6* shows the changes in the serum lipoprotein pattern of another patient with type V hyperlipidemia. With dietary treatment, which was a very low fat diet (less than 15% calories as fat), serum chylomicra levels became normal but the pre-β-lipoprotein was elevated. When clofibrate* was used in addition to diet, serum triglyceride levels became nearly normal. The lipoprotein

* *Atromide-S.*

pattern, while greatly improved was not normal, having lower than normal β-lipoprotein and slightly increased pre-β-lipoprotein levels.

The person with a genetically determined lipoprotein abnormality of types II, III, or IV which is associated with development of atherosclerosis at an early age, can be pictured as being on a balance (*Table 6*). When one of these genetic factors is present the usual diet high in cholesterol, saturated fat, and simple carbohydrates causes excessively high levels of β-lipoproteins or pre-β-lipoproteins. These result in accelerated lipid deposition in coronary and other arteries. On the other side of the balance with the same genetic factor, a modified dietary pattern low in cholesterol and containing unsaturated rather than saturated fat may maintain a more nearly desirable serum lipoprotein and lipid pattern and, hopefully, delay the development or progression of coronary heart disease and atherosclerosis. Evidence accumulated in Scandinavian countries in recent years strongly suggests that dietary regulation can cause such a change.[15]

It might be concluded that cholesterol and β- and pre-β-lipoproteins are undesirable serum constituents. While too high levels are harmful, too little may be even worse. In 1950 Bassen and Karnzweig[16] described a syndrome characterized by neuromuscular disturbances simulating Friedreich's ataxia, atypical retinitis pigmentosa, and abnormal erythrocytes with a thorny appearance. Subsequent studies have shown that patients with the disease have an absence or extremely low levels of β-lipoprotein.[17] It now appears that there are varying degrees of β-lipoprotein deficiency

Table 6.—The balance sheet

High β-Lp synthesis (genetic)	High β-Lp synthesis (genetic)
+	+
Excess cholesterol-sat. fat intake	Low cholesterol-low sat. fat intake
↓	↓
High blood lipoprotein, lipids	Desirable blood lipoprotein, lipid levels
↓	↓
Accelerated development of atherosclerosis	Delayed development of atherosclerosis
	Low β-Lp synthesis (genetic)
	High cholesterol-sat. fat intake
	↓
	Low or low normal blood lipoproteins, lipid levels
	↓
	No atherosclerosis

which are genetically determined, and that the degree of neurologic involvement varies greatly. We have studied a patient with very low levels of β-lipoprotein who has a demyelinating type of disease.[18] Her serum cholesterol levels during the 5 years of the study have been consistently between 75 and 90 mg/100 ml and the β-lipoprotein concentration about 10% of the normal level (*Fig. 7*). The lipoprotein deficiency is genetically determined and was found in 12 of her kindred, including her two brothers and her four children. Attempts to increase the serum lipid and lipoprotein levels of the patient have been unsuccessful. We feel that the extremely low serum β-lipoprotein levels may be an etiologic factor in some of the bizarre groups of demyelinating diseases, and serum lipids should be determined. As

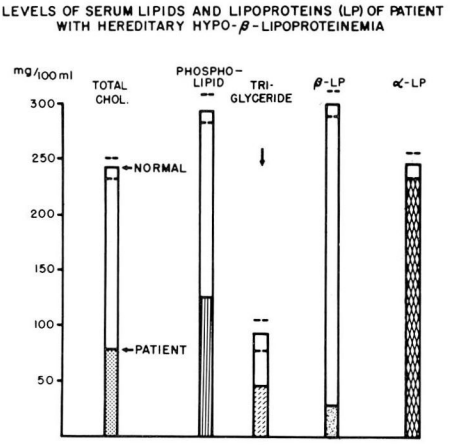

Fig. 7. Serum lipid and lipoprotein levels of patient with familial hypo-β-lipoproteinemia.

Williams[19] has expressed it so well, "Perhaps by screening all such patients for abnormal lipoproteins a more complete understanding of the etiology of demyelinating diseases will be obtained."

In addition to the genetically determined hyper- and hypolipidemias abnormal serum lipid and lipoprotein patterns are associated with a number of diseases; some of the most important are summarized in *Table 7*.

It has been known for many years that serum cholesterol and triglyceride levels are abnormal in a number of endocrine diseases. Since the thyroid regulates the metabolic rate in general, changes in the serum lipid patterns of patients with thyroid disease could be expected. *Figure 8* presents the serum cholesterol and lipoprotein pattern of a patient with myxedema and shows the high cholesterol and β-lipoprotein levels typical of this disease.[20] When the patient's basal met-

Table 7.—Diseases with secondary hyper- or hypolipidemia

Thyroid diseases
Diabetes
Renal diseases
Liver diseases
Pancreatitis
Multiple myeloma
Autoimmune diseases
Crohn's disease

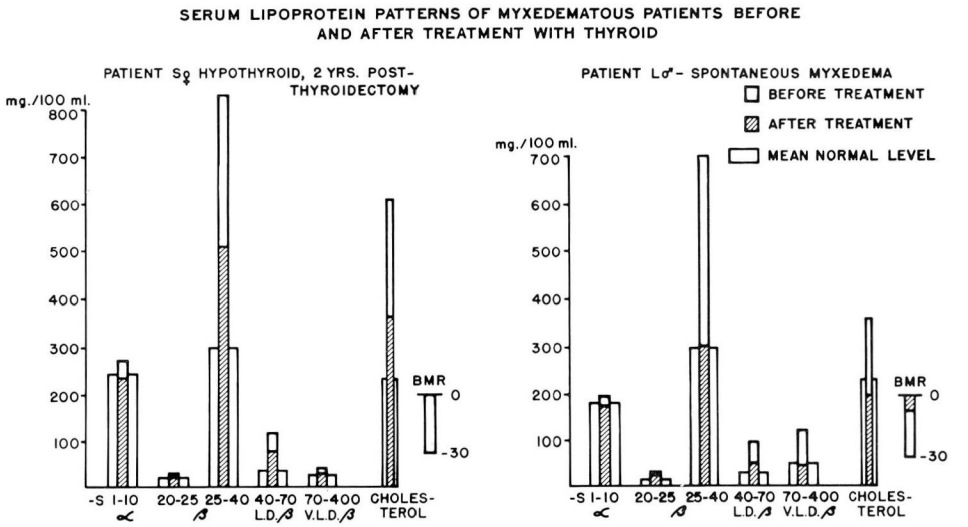

Fig. 8. Serum lipoprotein patterns of myxedematous patients before and after treatment with thyroid.

abolic rate was restored to normal by administration of thyroxin, the serum lipids and lipoproteins became normal. In some hypothyroid patients extremely high cholesterol levels occur with only mild clinical evidence of hypothyroidism. In hyperthyroidism serum cholesterol and β-lipoprotein levels are decreased and return to normal following thyroidectomy or treatment with ^{131}I.[20]

In uncontrolled diabetes mellitus severe hyperlipidemia is common. High levels of serum triglyceride and cholesterol occur and a lipoprotein pattern similar to a severe type IV hyperlipidemia is found. With control of the diabetes, the lipids and lipoprotein pattern improve. Lipid levels in well controlled diabetics may be normal, although as a group the cholesterol and β-lipoprotein levels tend to be at the high normal or slightly elevated level.[20] In general the lipid and lipoprotein levels of the diabetics showed little correlation with the incidence of vascular disease unless there was renal involvement. The lipoprotein pattern of diabetics with intercapillary glomerular sclerosis showed a high concentration of β- and pre-β-lipoprotein, while the α-lipoprotein levels were low.

Very severe hyperglyceridemia and chylomicronemia may occur in pancreatitis with a lipoprotein pattern similar to that of a primary type I or type V hyperlipidemia. Treatment with a very low fat diet (less than 15% calories as fat) is indicated.

In the nephrotic phase of renal disease severe hyperlipidemia is commonly found. Triglyceride and cholesterol levels of serum are greatly increased, and the lipoprotein pattern shows high pre-β, β and sometimes chylomicron levels; α-lipoprotein level is very low. Observing changes in the lipoprotein pattern gives an excellent index of the progress of the disease process. When patients in renal failure are being maintained on the artificial kidney α-lipoprotein levels may be very low, while β-lipoprotein concentration is normal.[21] Following renal transplantation when renal function is good, α-lipoprotein levels increase to normal, but decrease at the time of an acute rejection crisis, to increase again when the crisis subsides. The lipoprotein patterns of a patient before, 1 and 9 months after renal transplantation showed the presence of normal α-lipoprotein levels when renal function was good.

The liver plays a vital role in regulation of lipid metabolism and lipoprotein composition. Many studies have reported changes in various types of liver disease.[22, 23] *Figure 9* shows the pattern found in obstructive liver disease in which an abnormal type of lipoprotein, not normally present in demonstrable amount, is present.[23, 24] It is antigenically different from normal lipoproteins and may be identified by special immunologic techniques. In liver failure, serum cholesterol and both α- and β-lipoprotein levels decrease to very low levels. In infectious hepatitis and hepatic cirrhosis α-lipoprotein levels are decreased and the concentration and composition of β and pre-β, and chylomicron and abnormal types of lipoprotein vary from very high to very low depending on the degree of hepatic involvement.[20]

In Crohn's disease, and other conditions in which fat absorption from the intestinal tract is impaired, β-lipoprotein and serum cholesterol levels are low. They are also usually low in pa-

tients with multiple myeloma and with Waldenstrom's macroglobulinemia. When the levels of immunoglobulin decrease as a result of appropriate drug therapy, the serum cholesterol and β-lipoproteins increase to nearly normal levels (*Fig. 10*). Occasionally in hyperimmunoglobulinemic patients

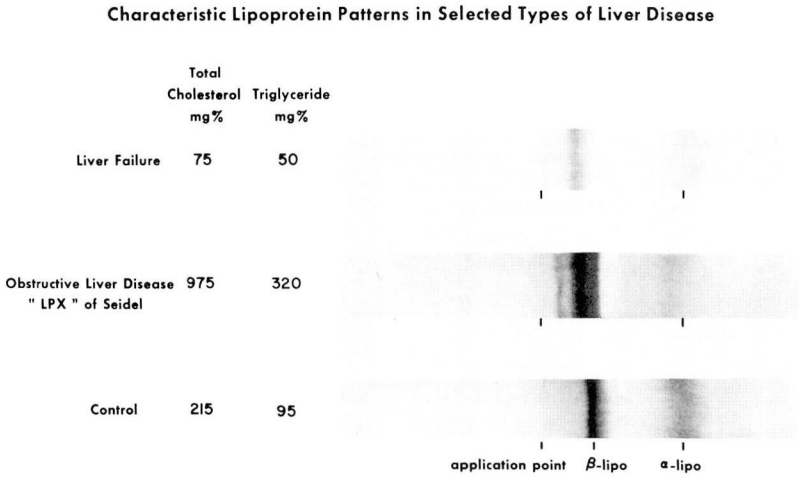

Fig. 9. Characteristic serum lipoprotein patterns in patients with liver failure or with obstructive liver disease.

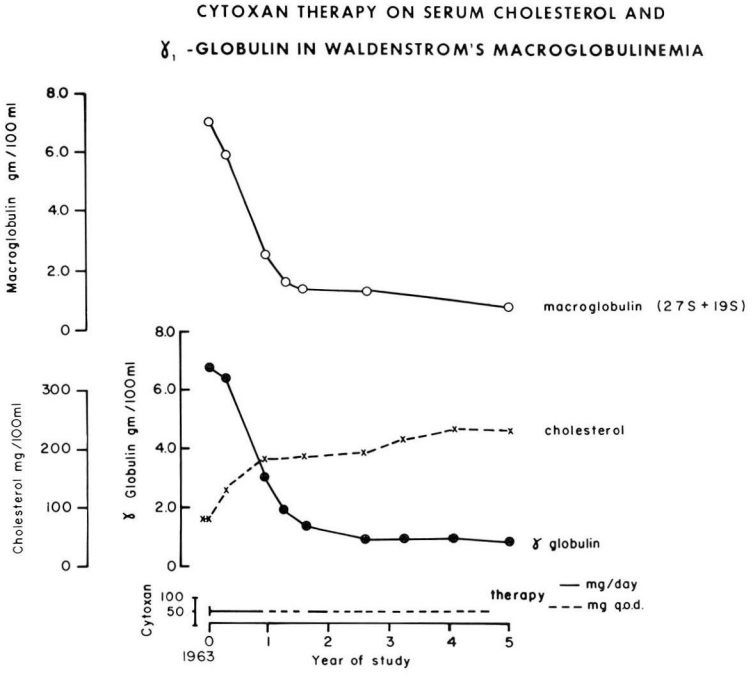

Fig. 10. Changes in serum cholesterol and gamma globulin levels in a patient with Waldenstrom's macroglobulinemia during treatment with cytoxan.

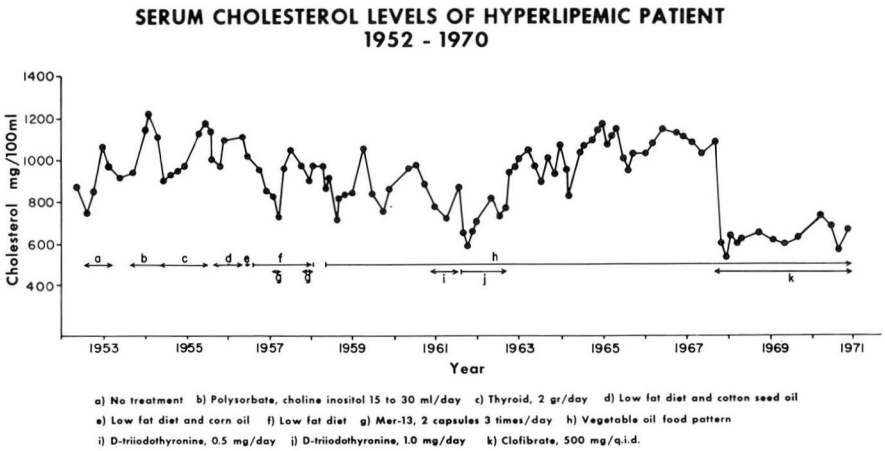

Fig. 11. Serum cholesterol changes in hyperlipemic-hyper-IgA globulinemic patient during 18 years of study.

an autoimmune situation exists in which the abnormal globulin interacts with the lipoprotein in the patient's serum. In such cases extremely high cholesterol and β-lipoprotein levels are found. One patient who has been studied for 20 years has consistently maintained serum cholesterol levels over 800 mg and high levels of β- and pre-β-lipoproteins (*Fig. 11*). He has a high level of IgA globulin which is more than 2 g/100 ml; it is very firmly bound to the lipoproteins.[25] Dietary treatment has been ineffective in reducing his serum lipids to desirable levels. His bone marrow shows between 12 and 18% plasma cells, which show immunofluorescence when interacted with anti-IgA globulin antisera. He has no clinical evidence of multiple myeloma. During the last 2 years his treatment has included clofibrate in addition to diet and his serum cholesterol has been about 600 to 700 mg/100 ml. An aortic aneurysm developed for which he was operated on 3 years ago; he made an excellent recovery. Cine coronary angiography showed very little evidence of coronary atherosclerosis. Another patient studied for more than 3 years has an autoimmune type of hyperlipidemia in which greatly elevated levels of serum lipoprotein are combined with IgG, type K, globulin. She has multiple myeloma and has responded favorably to treatment.[26] On electrophoresis all of her serum lipoproteins migrated with the γ-globulin. Separation of the complex was accomplished by ultracentrifugation at a high salt concentration, the lipoprotein floating while the immune globulin concentrated at the bottom of the tube. When her serum IgG concentration decreased to nearly normal levels, her lipoprotein, cholesterol, and triglyceride levels also normalized. When there was an exacerbation of the disease serum IgG and lipoprotein levels increased. Treatment of the primary disease with immunosuppressive therapy not only reduced IgG, but also serum lipoprotein and lipid levels.

Summary

The five primary types of hyperlipidemia have been described and the

clinical findings associated with them have been listed. Recommended treatment for all types of primary hyperlipidemia is an appropriate diet. For those types with increased levels of chylomicra a low fat, low cholesterol diet is recommended; for other types of hyperlipidemia, after ideal weight has been attained, a low cholesterol diet, low in saturated, rich in unsaturated fatty acids is recommended. Clofibrate, cholestyramine, and nicotinic acid may be useful drugs in cases which do not achieve desirable levels with diet alone. Ileal bypass operation can be considered for those patients who fail by other methods to achieve desirable serum lipid and lipoprotein levels.

Familial hypo-β-lipoproteinemia may in some neurologic cases be an important etiologic factor.

Serum lipid and lipoprotein levels may provide important additional information concerning the activity of the disease process and the efficacy of treatment in certain diseases in which lipid changes are important characteristics of the primary disease. These include thyroid diseases, diabetes, pancreatitis, liver and renal disease, and some cases of macroglobulinemia and multiple myeloma.

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