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Pre-Beta Lipoproteinemia

Its Bearing on the Dietary Management of Serum Lipid Disorders as Related to Ischemic Heart Disease[1, 2]

DAVID F. BROWN, M.B., AND JOSEPH T. DOYLE, M.D.

WITH THE USE OF paper electrophoresis of serum lipoproteins, Dangerfield and Smith (1) demonstrated the presence of a characteristic pre-beta lipoprotein band in the majority of patients who had recently sustained an acute myocardial infarction. Besterman (2) indicated that this pre-beta band was present in 99% of subjects with ischemic heart disease. Smith (3) also demonstrated that a moderate pre-beta band was present in almost 70% of men over the age of 50. Recently, by adding human albumin to the buffer and by using plasma rather than serum, Lees and Hatch (4) have been able to achieve a clearer separation of the pre-beta from the beta lipoprotein band, and have also demonstrated that with this technique dietary triglycerides (chylomicrons) remain at the origin of the strip. Fredrickson and Lees (5) have demonstrated as a result of this technique that hyperlipidemic states, or as they more correctly call them, hyperlipoproteinemic states, may be more clearly defined than hitherto. One of the five states that they have defined is characterized by hypercholesterolemia with normal triglyceride levels (*type II*). The remaining four states are characterized by hyperglyceridemia and in two of these (*types I* and *V*) dietary triglyceride is present. In *types III* and *IV*, the triglyceride is endogenous in origin and is present as a distinct pre-beta band. Brown, Kinch and Doyle (6) have recently demonstrated that elevated fasting serum triglyceride levels are common in middle-aged men. Fifty-five percent of men with ischemic heart disease and 42% of apparently healthy men as compared with 5% of healthy medical students were found to exceed a value of 5.2 mEq/liter (7). The nature and source of this increased triglyceride has not been defined. As several studies (8–10) have indicated that subjects with ischemic heart disease exhibit abnormally high and prolonged alimentary lipemia after a fat meal, it has been suggested that the glyceridemia is related to defective clearing of ingested triglyceride. It has been shown, however, that prolonged alimentary lipemia is probably a function of an elevated fasting triglyceride level at the time of fat ingestion (11, 12). As the method of Lees and Hatch (4) differentiates between serum triglyceride of exogenous and endogenous origin, we have applied this method to a study of sera

[1] From the Cardiovascular Health Center and Subdepartment of Cardiovascular Medicine, Albany Medical College, Albany, New York.

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TABLE I

Correlation of the Intensity of Staining of the Pre-Beta Band and Mean Cholesterol and Triglyceride Levels in Health and in Ischemic Heart Disease

Pre-Beta	0		1		2		3		4		5	
Clinical state	Normal	IHD	Normal	IHD	Normal	IHD	Normal	IHD	Normal	IHD	Normal	IHD
Number of men	141	14	138	23	172	15	108	21	28	9	2	1
Cholesterol, mg/100 mg	232	221	242	249	247	263	265	282	273	268	336	225
Triglycerides, mEq/liter	3.1	3.3	4.4	4.5	5.5	5.3	8.0	7.8	13.5	11.3	25.1	16.1

and plasma samples obtained in the fasting state from men who comprise the study population of the Cardiovascular Health Center (CVHC) at Albany, New York (13).

MATERIAL AND METHODS

Fasting sera and plasma samples from 672 men aged 50–65 and from 50 male medical students under 25 years were studied. Eighty-three of the middle-aged men were found to have ischemic heart disease. Serum triglyceride and cholesterol levels were measured with the methods of Van Handel and Zilversmit (14) and of Abell et al. (15), respectively. Plasma lipoproteins were separated by means of paper electrophoresis according to the method of Lees and Hatch (4). The presence or absence of a pre-beta band was noted and when present the intensity of staining visually graded from 1–5 by an observer who was unaware of the serum lipid levels or of the clinical state of the patient. Obesity for each patient was calculated as percentage relative overweight using the Metropolitan Life Insurance tables (16). Obesity, at age 25, was based on the patient's stated weight at that time while obesity at the time of the serum lipid determinations was based on the patient's actual weight at this time.

RESULTS AND DISCUSSION

A pre-beta lipoprotein band was observed in all subjects with fasting triglyceride levels greater than 5.2 mEq/liter, a level previously established as the upper limit of normal and was absent in subjects with a level of less than 2.5 mEq/liter. Its presence was variable between these limits. In the group of 50 healthy medical students, 15 exhibited the presence of a faint pre-beta band not exceeding grade 2 in intensity. The mean triglyceride and cholesterol levels in 83 patients with ischemic heart disease were 6.3 ± 3.6 mEq/liter and 257 ± 52 mg/100 mg, respectively. The former value is significantly higher than the value of 5.5 ± 3.4 mEq/liter ($P < 0.05$) and the latter higher than the value of 247 ± 43 mg/100 mg ($P < 0.1$) found in healthy men. A pre-beta band was observed in 84% of subjects with ischemic heart disease and in 76% of men without ischemic heart disease. The mean cholesterol and triglyceride levels associated with the graded pre-beta bands in patients with and without ischemic heart disease are shown in the table and demonstrate that progressive intensity of staining is associated with increasing elevation in cholesterol and triglyceride levels. Representative samples of plasma with increasing levels of triglyceride which have been subjected to electrophoresis and stained in oil red O are shown in Fig. 1. Strips 1–6 are from subjects in the CVHC population. The pattern in strip 7 is that which we have most commonly encountered in patients referred to us in consultation for gross hyperglyceridemia. If a plasma with normal cholesterol and triglyceride content is run simultaneously with an unknown it is possible at a glance to estimate

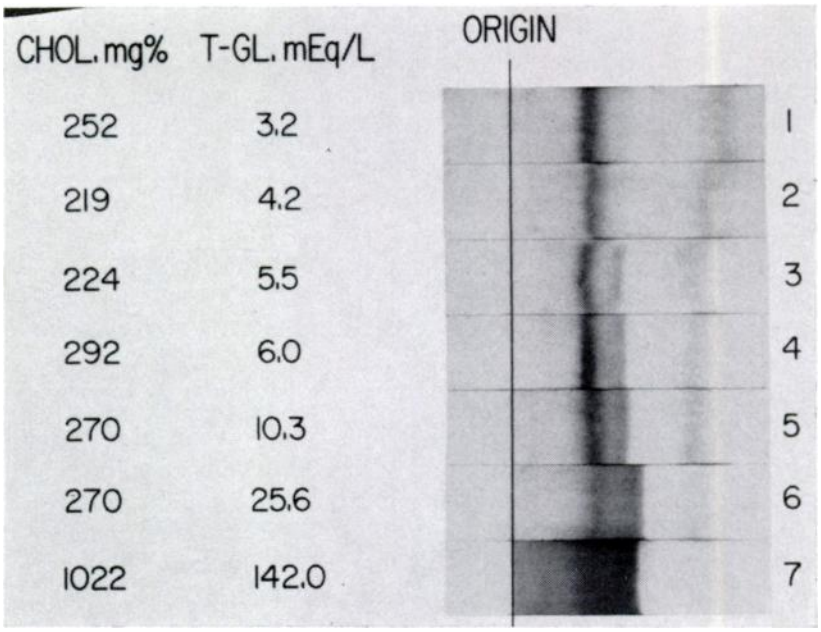

FIG. 1. Plasma lipoprotein patterns associated with increasing serum triglyceride levels.

what lipid abnormalities are present in the latter. In strip 2 with a normal cholesterol and triglyceride level, the normal pattern is present. There is no lipid staining material at the origin; a single beta band and a less densely stained alpha band are visible. Although a similar pattern is shown in strip 1, a more densely stained beta band is present, in keeping with the higher serum cholesterol level in this subject and with the fact that the beta band represents the S_f 0–20 lipoproteins, the major lipid content of which is cholesterol. With a further increase in triglyceride level, a pre-beta band appears in strip 3 and becomes increasingly dense in strips 4, 5, and 6. In strip 7, a very dense beta and pre-beta band cannot be separated and lipid staining material trails to the origin. According to Fredrickson and Lees (5), the pre-beta band consists of very low density lipoproteins with S_f values from 20–100,000 with the majority of these particles in the range 20–400. Smith (3), in defining the S_f classification of the pre-beta lipoprotein band, indicated a more restricted range with a S_f value of 20–100. Lees and Fredrickson (17) have indicated that some trailing from the origin is observed whenever pre-beta lipoproteins are present, and that the degree of trailing appears directly related to their size. Gross trailing is visible in strip 7 and some trailing in strips 5 and 6 of Fig. 1. As the lipoprotein classes which comprise the pre-beta band are high in triglyceride content, the correlation between pre-beta bands and elevated triglyceride levels of endogenous origin demonstrated in the table was predictable.

The high percentage of men with a pre-beta band reported by Smith (3) correlates well with the prevalence of hyperglyceridemia in our population. The observations of Dodds and Mills (18) and of Tibblin and Cramér (19) who demonstrated an increase in triglyceride-rich lipoproteins and total triglycerides, respectively, after myocardial infarction are also in keeping with the high prevalence of a pre-beta band in patients with myocardial infarction. While Smith (3) has indicated that a pre-beta band is uncommon in patients under 30 years of age, the

true prevalence of this band with age must await the collection of a larger series and a comparison of results by different investigators cannot be made unless staining techniques are strictly comparable. In this study, a grade 1 pre-beta band is one that is just visible to the naked eye. The physiological hyperglyceridemia of pregnancy has been associated with the presence of a pre-beta band by Pantelakis et al. (20) and in the same study, they demonstrated its presence in some children with diabetes mellitus.

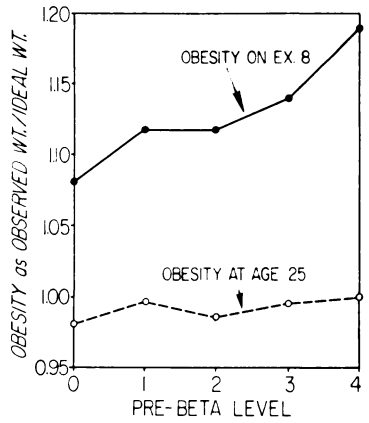

FIG. 2. Correlation between weight gain and intensity of staining of plasma pre-beta lipoproteins.

Weight-Related Changes

Albrink et al. (21) have demonstrated that elevated triglyceride levels are associated not so much with obesity per se, but rather with excessive weight gain. In Fig. 2, the intensity of staining of the pre-beta band is plotted against the calculated value for obesity of the subjects in each group at age 25 and at the time of the present examination in 1965–1966. The small number of subjects in grade 5 precluded their inclusion in this calculation. All groups of subjects appear to be more obese than at age 25 but those subjects with no pre-beta band have gained the least and those with grade 4, the most weight. These findings are in keeping with the endogenous synthesis of the pre-beta lipoprotein bands and the observation that the pre-beta band usually decreases in intensity with caloric restriction and associated weight loss.

Studies in Subjects with Gross Triglyceride Elevation

It has been of interest to compare the relationship of the findings in this population to the more gross defects in lipid levels encountered in 40 patients referred in consultation for grossly elevated triglyceride levels. This latter group was referred after the detection of visibly lipemic sera or by their presenting with eruptive xanthomata, with diabetes mellitus, obesity or, more rarely, manifestations of vascular disease. Approximately 90% of these subjects demonstrated a pre-beta band exceeding grade 5 in intensity. The majority were overweight and diabetes mellitus was common. Although the lipid defect in this group is more severe, it is nevertheless similar to that demonstrated in a large segment of the CVHC population. The findings in this group of referred patients is typified by the patient whose strips are demonstrated in Fig. 3. This patient is a 52-year-old, white male, 60 inches in height and weighing 286 lbs. who appeared with eruptive xanthomata. He was found to have 4+ glycosuria without acetone and an elevated fasting blood sugar level. The initial serum was grossly lipemic and the stained strip revealed the presence of a considerable amount of lipid material at the origin, together with a very dense beta and pre-beta band. Analysis of this patient's serum with a polyvinylpyrrolidone density gradient (22) revealed that a considerable amount of chylomicron triglyceride was present. With calories restricted to 1,000 a day, his serum cleared and his xanthomata disappeared in a period of 2 weeks. On discharge from the hospital, he

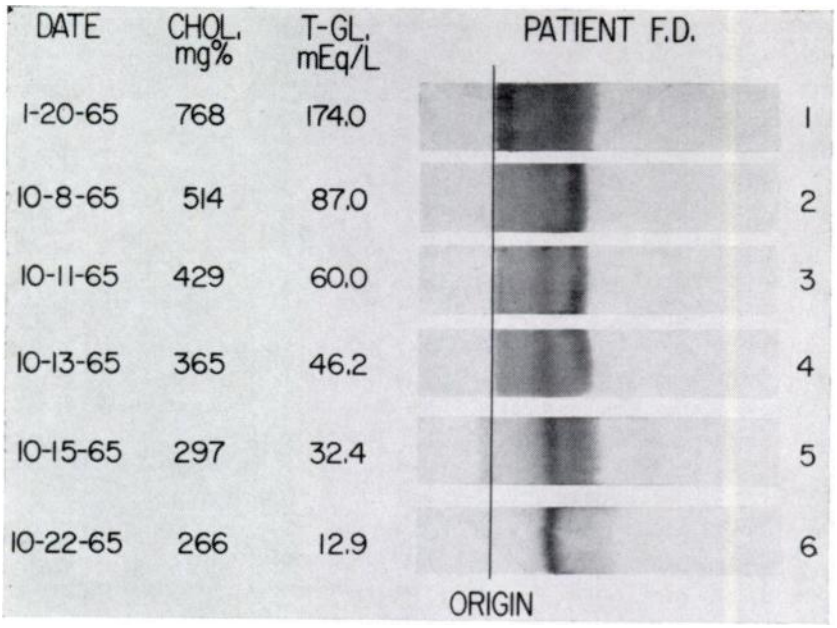

FIG. 3. Effect of caloric restriction on plasma lipoproteins in a patient with gross triglyceride elevation.

was given a 1,500-cal diet consisting of 86 g of protein, 98 g of carbohydrate and 85 g of fat. He did not return for follow-up, however, until September 1965, when his serum was again grossly lipemic. His xanthomata had not recurred. He was admitted to the hospital and given the above 1,500-cal diet. On this diet, the sequence of events depicted in strips 2–6 occurred. No dietary triglyceride appeared, the prebeta band almost disappeared and a beta band which was barely visible in strip 2 became increasingly evident. As this sequence of events occurred during the daily ingestion of 85 g of fat, the dietary fat initially evident in this patient cannot be attributed to a primary defect in handling dietary fat. In a patient with true fat-induced hyperglyceridemia that we have studied, less than 30 g of dietary triglyceride daily was sufficient to produce a progressive increase in serum triglyceride, indicated, both by paper electrophoresis and polyvinylpyrrolidone density gradient, to be due to chylomicron accumulation. We believe that the dietary-fat retention evident in strip 1 of Fig. 3 is a direct consequence of and preceded by an increase in endogenous triglyceride. In other words, when synthesis of endogenous triglyceride is excessive and continued, removal mechanisms eventually are saturated. Ingestion of dietary fat at this time must result in their delayed removal also. When the endogenous component in this patient was reduced, in this instance by preventing its synthesis through carbohydrate restriction (or total calorie restriction), the exogenous triglyceride disappeared from the serum even in the face of 85 g of fat daily. This concept is supported by the fact that feeding an identical radioactively-labeled fat meal (^{3}H palmitate) at the time of strip 2 and again at the time of strip 6 resulted in a slow triglyceride turnover rate at the former time and a normal turnover rate at the latter. This concept is supported by studies of chylomicron metabolism in the alloxan-diabetic rat (23) and from work with labeled fat meals (24) or chylomicrons in human subjects (12) which have shown that accumula-

tion of chylomicrons is directly related to the triglyceride level at the time of ingestion or injection. Bagdade, Porte and Bierman (25) have recently reported cases of marked hyperlipemia in diabetics characterized by large accumulations of dietary fat. They believe that this disorder represents an acquired form of fat-induced lipemia but relate the mechanism to a reduction in postheparin lipase activity in these subjects. Jones et al. (26) have not, however, found any abnormalities in postheparin lipase in diabetics with lipemia. In the alloxan-diabetic rat, Brown and Olivecrona (27) have shown that markedly accelerated clearing of chylomicrons follows intravenous heparin administration and appears no different from that in normal rats.

The similarity of strips 3–6 in Fig. 1 to those in Fig. 3 suggests that a similar biochemical improvement may result in subjects represented in the former by using the type of diet used in the subject represented in the latter. The data in Fig. 1 additionally indicate that if a cholesterol determination alone had been carried out in these subjects, it would not have been possible to predict that this elevation resulted at least in part from elevated S_f 20–400 lipoproteins and that some reduction could reasonably be expected with carbohydrate restriction.

An idea of the number of subjects in the population of this size who might be expected to respond to this type of therapy can be obtained from a study of earlier work (6) carried out in this department which demonstrated that moderate elevation of cholesterol existed in 620 of 1,851 subjects. Of this group, 420 were found to have an associated triglyceride level greater than 5.2 mEq/liter and on the basis of this present study could be expected to have demonstrated pre-beta lipoprotein elevation. On the basis of the cholesterol and triglyceride reductions demonstrated in Fig. 3, it seems possible that of 620 men

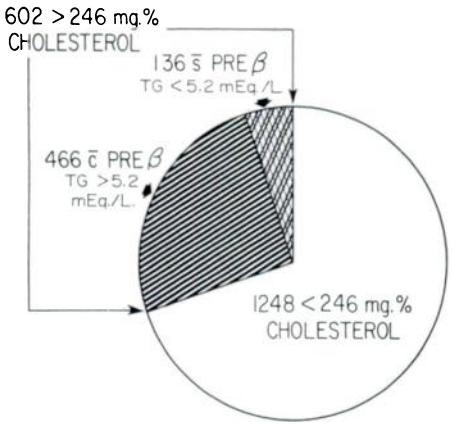

FIG. 4. Prevalence of pre-beta lipoproteinemia in total population studied, 1,850 middle-aged men.

in this middle-aged population with moderate cholesterol elevation, approximately two-thirds might be expected to show some response to a similar type of diet (Fig. 4).

Atromid S

Striking reduction in the intensity of staining of the pre-beta band with associated cholesterol and triglyceride reduction has been achieved in numerous patients with the use of the drug Atromid S (ethyl-α-*p*-chlorophenoxyisobutyrate). In our experience pure hypercholesterolemia, characterized by an increase in the beta band or S_f 0–20 lipoproteins, is seldom affected by this drug. An example of the changes induced by this drug is demonstrated in Fig. 5. This series of strips is again from the patient shown in Fig. 3 who was seen on February 9, 1966, after recurrence of his eruptive xanthomata and gross lipemia. The patient had a gastric ulcer and complained that as his ulcer symptoms were aggravated by the caloric restriction necessary to reduce his weight and to maintain the chemical improvement demonstrated in Fig. 3, he had resumed a normal diet. On this visit, therefore, he was allowed to continue on his regular diet and treated with Atromid S, 1 g twice daily. The eruptive xanthomata disappeared within 2 weeks of adminis-

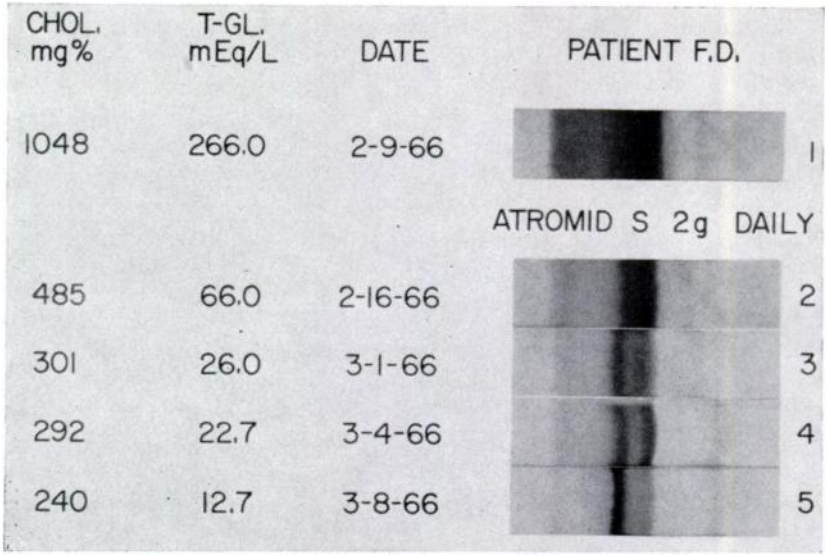

FIG. 5. Effect of Atromid S on plasma lipoproteins in a patient with gross triglyceride elevation.

tration of the drug and the pre-beta band decreased along with the falling triglyceride level. There is tentative evidence (28, 29) to suggest that Atromid S may inhibit free fatty acid mobilization from adipose tissue. If this is borne out, the Atromid-induced disappearance of the pre-beta band we have observed may be taken as indirect evidence of its origin, in some instances, from free fatty acid mobilized from adipose tissue and esterified in the liver. Fredrickson, Lees and Hatch have brought considerable order to a previously confused and complicated field with a simple but effective technique and discerning observation. The method is one which can be established in any laboratory and it is hoped that the contents of this article will encourage many to do so. Its widespread use will define the prevalence of various hyperlipoproteinemias and should provide the impetus for their more detailed study.

SUMMARY

Serum triglyceride and cholesterol levels were determined in 672 men aged 50–65, 83 of whom had ischemic heart disease. Simultaneously obtained samples of plasma were subjected to paper electrophoresis in albumin-containing buffer and stained for lipoproteins. The presence of a pre-beta band indicative of endogenous triglyceride and lipid staining material at the origin, indicative of dietary triglyceride, was observed. These procedures were also carried out in 50 students aged 20–25 and in 40 patients referred in consultation for gross serum-lipid elevations. With the exception of one patient in the last group, who had true fat-induced lipemia, all subjects with elevated triglyceride levels (greater than 5.2 mEq/liter) exhibited a pre-beta band, demonstrating that the commonly encountered triglyceride elevation in middle age is endogenous in origin. A pre-beta band was present in 30% of young men, but was of faint intensity. In middle-aged men, 84% with ischemic heart disease and 76% without it exhibited a pre-beta band of varying intensity. A positive correlation was observed between the intensity of staining of the pre-beta band and the serum triglyceride and cholesterol level. Reduction of serum triglyceride levels in subjects with gross hyperglyceridemia by caloric re-

striction or carbohydrate restriction or with the use of Atromid S alone was associated with a parallel reduction in intensity of the pre-beta band and associated reduction in cholesterol and triglyceride. The occurrence of this type of lipoprotein disorder of less severe degree in a relatively large percentage of the general population suggests that similar therapy may be effective in this group. It is believed that a serum cholesterol determination alone in subjects with elevated cholesterol levels is inadequate when dietary management is contemplated. For rational therapy, this determination should be supplemented with a triglyceride determination or paper electrophoresis of plasma lipoproteins, or both.

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