Serum Lipoproteins and Cholesterol Levels in Normal Subjects and in Young Patients with Diabetes in Relation to Vascular Complications.*

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The present study is concerned with the lipoprotein and cholesterol content of the blood serum in relation to diabetes of long duration and its characteristic complications. It has become increasingly evident that although young diabetic patients may survive under insulin treatment for 10 to 15 years without difficulty, thereafter generalized vascular disease, particularly with lesions in the eyes and kidneys, becomes distressingly frequent. These patients offer an unusual opportunity for the study of the nature of arteriosclerosis, uncomplicated by other chronic diseases frequently present in older subjects. A long-term study designed to assess the relationship between the degree of control of diabetes by means of insulin and diet and the frequency or severity of these complications, has been in progress in this clinic for several years.[1,2,3,4] Since the observations by Gofman[5] relating certain of the lipoproteins in the blood serum to such vascular changes as are concerned in coronary atherosclerosis, the application of this method of study to young diabetic patients long known to develop premature atherosclerosis, retinal lesions and nephropathy, seemed a necessary step. It appeared of particular importance to compare this new lipoprotein measurement with the simpler cholesterol determination in respect to their relation to the clinical manifestations of atherosclerosis.

LABORATORY METHODS

Lipoprotein determinations were done by the technic of Gofman and others[6] and cholesterol determinations by the method of Abell, Levy, Brodie and Kendall.[7] The reliability of these methods has been carefully determined by serial introduction of blind replicate samples. Such data collected over the 18 months of the present study reveal the following standard errors of duplicate differences: Total cholesterol 10.7 mg. per cent; lipoproteins, S_f 12-20, 4.9 mg. per cent, S_f 21-35, 3.1 mg. per cent, and S_f 35-100, 7.7 mg. per cent.

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Supported in part by the National Heart Institute, National Institutes of Health, Public Health Service; American Meat Institute; Nutrition Foundation, Inc.; J. M. Kaplan Fund, Inc., and the Nordisk Insulin Fond.

CLINICAL MATERIAL

A group of 218 diabetic patients has been studied. Of this number, 144 patients whose diabetes began between the ages of 1 and 30 years and was of at least 10 years duration, were selected for particular study in order to assess the relationship between laboratory findings, the control of diabetes and the development of complications. It should be pointed out that any such series of patients alive for periods of 10 to 36 years after the onset of diabetes is, by that very fact, a selected group. Follow-up surveys reveal that 25 per cent of 6,000 patients similar in age at onset of diabetes and observed during this same period of years have died chiefly of diabetic complications such as diabetic coma, pyelonephritis, diabetic nephropathy and tuberculosis. The smaller group of 144 patients was obtained by calling back for special study as out-patients those living within a reasonable distance of Boston and, secondly, by utilizing such patients as met the requirements who during this period were admitted to the New England Deaconess Hospital. In the total of 144 cases, 110 were patients who had been thus recalled and 34 were patients seen in the hospital. It is well to point out that these patients were not selected because they had been under our frequent observation. Actually, an outstanding feature of this follow-up was the discovery that patients whom we had first seen 15 or 20 years ago and who we thought were under observation by their own family doctors had, in many cases, ceased to see any doctor. Many patients admitted that, having found that they could feel well and maintain their weight by taking insulin alone without medical advice, had sought no professional care for periods of 5 to 10 or more years.

Each patient came to the office after an overnight fast and blood was drawn for determination of glucose, non-protein nitrogen, serum lipoprotein and cholesterol. In addition, x-ray examination of the arteries of the legs, the pelvis, a lateral film of the aorta and a chest film were made. A physical examination was made with special attention to the presence of neuropathy, exemplified by postural hypotension, loss of reflexes, muscular atrophy and hypesthesia. The patient was sent to an ophthalmologist for a complete examination of the eyes. In addition, a careful and prolonged interview was held with the patient in order to obtain detailed information concerning dietary practices, the amount of insulin taken and the frequency of medical examinations and laboratory tests during each of the 5 year periods of the total duration of diabetes.

CLINICAL CRITERIA

Based on the interview and information from the patient's record the case was classified according to one of three standards of control.

Details concerning the classification are given in a previous article.[4]

GOOD CONTROL 1. The patient must never have been in coma except in those instances in which the initial diagnosis of diabetes was made in coma. 2. Insulin therapy was begun within a few weeks after the onset of diabetes. 3. Urine tests for sugar were made at least once daily ever since onset of diabetes with a conscientious attempt to have the urine sugar free or nearly so before meals. Insulin dosage adjustments were made according to results of urine tests. 4. The diet must have been weighed for the first 6 weeks of treatment and thereafter at intervals with careful measurement of food at all other times since onset of diabetes. 5. Regular physical examinations and laboratory tests were made by a physician at least once annually. The blood and urine tests were satisfactory.

FAIR CONTROL 1. The patient must never have been in coma (except for cases in which the diagnosis was made with the patient in coma, or rare cases in which an unavoidable overwhelming infection or other complication precipitated coma). 2. Insulin therapy was begun within 24 months of onset of symptoms of diabetes. 3. Tests of the urine for sugar were made one or more times weekly in an attempt to maintain freedom from glycosuria. 4. Dietary management by the patient must have been conscientiously attempted, although food was not weighed or measured. Rarely, if ever, was there indulgence in gross dietary indiscretions. 5. Satisfactory blood sugar determinations were made at the time of physical examination by the patient's doctor at least once every 2 years.

POOR CONTROL 1. Avoidable coma one or more times. 2. Insulin therapy was not begun until more than 24 months after the onset of diabetes and in some cases used irregularly. 3. Urine specimens were tested infrequently, or at intervals of months or years. 4. No measurement or weighing of food was made in relation to urine tests or insulin dosage. 5. No regular examinations were made by a physician. At infrequent intervals, office or hospital examination showed marked glycosuria and hyperglycemia.

The diagnosis of retinitis was made in patients in whom many hemorrhages and exudates or actual retinitis proliferans were found. The group of patients with "no retinitis" included those with no hemorrhages whatever or only a minimal number such as 2 or 3 hemorrhages or microaneurisms. Similarly, patients were classified as having calcified vessels when parallel linear

areas of calcification were seen in more than one area and as having "no calcification" when either no evidence of calcification or only the most minimal traces were seen by roentgenogram. The diagnosis of diabetic nephropathy was based upon the finding of proteinuria, edema, hypertension and decreased kidney function; almost invariably there was associated retinitis of moderate or severe degree.

For comparative purposes data obtained from a control group of "normal" individuals have been included. These subjects were selected according to criteria established in the Cooperative Study of Lipoproteins and Atherosclerosis. In brief, subjects were obtained from industrial and business employment rolls and from clinic and hospital contacts of people obtaining annual physical check-up for preventive medical purposes. The clinical classification "normal" was based in each instance on: a negative medical history coupled with no physical and laboratory signs suggesting cardiovascular-renal disease or diabetes; blood pressure under 140/90; a normal electrocardiogram; and absence of protein, sugar or abnormal sediment in the urine. In addition, subjects with such disorders as thyroid disease, xanthomatosis, etc., were excluded when known. This group then serves as a reference for comparison. The term "normal" is applied to these data in the sense of prevalence and without implication of desirability.

RESULTS

The data on lipid levels in the normal group are contained in Table I. It is noteworthy that the values for each quantity are generally lower than the published data of Jones, et al.[8] Furthermore, the differences are larger than can be accounted for by the small but persistent difference of mean values consistently observed between laboratories in the Cooperative Study.*

The lipid levels in the total series of 218 diabetic patients with relation to age, sex, insulin dose and duration of the disease have been summarized in Figures 1a, 1b, 2 and 3. It appears that the concentration of serum lipoproteins of the S_f 12-20 class rises slightly with age but then has a tendency to fall slightly late in life. These changes are not dissimilar from those of the normal group. (Table I) The sex difference is not

TABLE I The serum cholesterol and lipoprotein levels of 704 normal Americans according to age and sex. Values are expressed in mg. per cent followed by standard deviation of distribution.

Age, decades	20-29	30-39	40-49	50-59	60-69	70-79	80-	Total
MALES								
Number of subjects	38	142	225	144	38	12	5	604
S_f 12-20	33.7 ±22.4	37.3 ±21.5	38.1 ±20	39.3 ±18.4	36.6 ±22	37.5 ±25.9	33 ±13.3	
S_f 21-35	21.3 ±21.6	29.9 ±14.4	21.9 ±16.3	22.5 ±13.4	19.1 ±11.7	24.2 ±15	21 ± 8	
S_f 35-100	60 ±51.1	59.3 ±42.2	65 ±52.3	59.1 ±46	50.3 ±37.3	51.7 ±54.9	36 ±21.3	
Total Cholesterol	219.7 ±80	227.5 ±59.5	238.8 ±59.5	236.5 ±51.5	227.6 ±51	195.8 ±51.5	215 ±37.5	
FEMALES								
Number of subjects	19	16	30	16	5	1		87
S_f 12-20	24 ±17.9	26.9 ±10.1	32.7 ±23.2	39.4 ±17.9	25 ±11	35		
S_f 21-35	11.3 ± 9.8	12.5 ± 7.1	15 ±12.9	18.8 ±10.5	11 ±5	35		
S_f 35-100	18.2 ±20.4	22.5 ±11.5	30.3 ±23.8	53.4 ±39.6	19 ± 9.7	90		
Total Cholesterol	196.1 ±75	203.1 ±55.5	223.3 ±50.5	259.4 ±75	215 ±80	275		

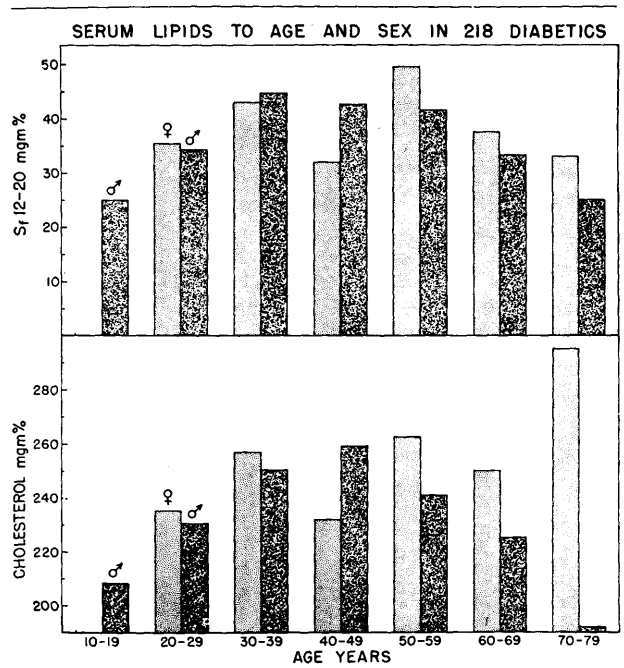

FIGURE 1a Serum lipoprotein of the S_f 12-20 class and cholesterol related to age and sex in 218 diabetic patients.

* Since the subjects measured in each laboratory are obtained from similarly distributed sources (e. g., the Harvard Laboratory measurements include subjects from several California industries and the Donner laboratory group has measured many Massachusetts subjects), it seems unlikely that geographic differences explain the differences observed. For the present comparative purposes, these laboratory differences are of no particular significance.

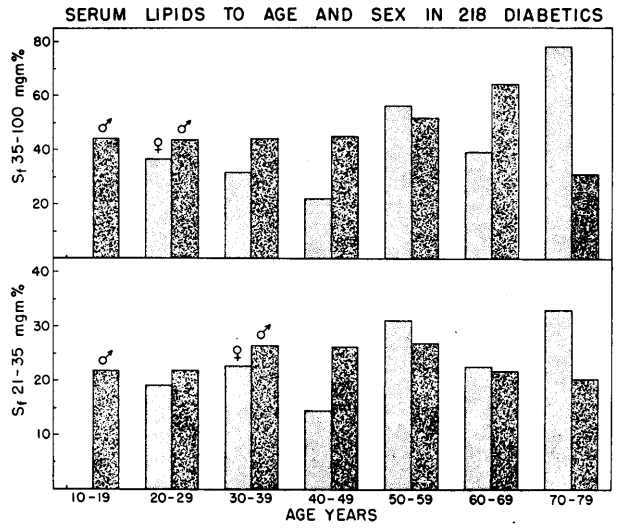

FIGURE 1b Serum lipoprotein of the S_f 21-35 and S_f 35-100 classes related to age and sex in 218 diabetic patients.

striking but in the S_f 21-35 and S_f 35-100 classes of lipoprotein there is persistent evidence that the lipid levels of the males exceed those of females. The relationship between insulin dose and serum lipids is described in Figure 2. The present data do not allow conclusions but there is evidence of some influence of increasing insulin requirement on the serum lipids, although the relationship is apparently not linear and not large. The explanation is especially complicated when it is recalled that it has been established that the degree of diabetic

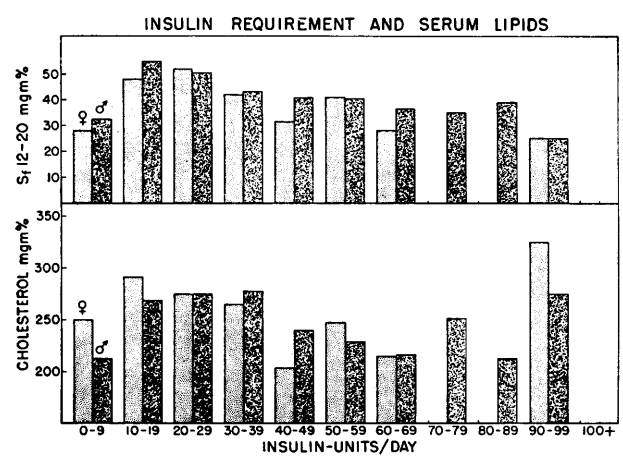

FIGURE 2 Serum lipoprotein of the S_f 12-20 class and cholesterol related to insulin dose in 218 diabetic patients.

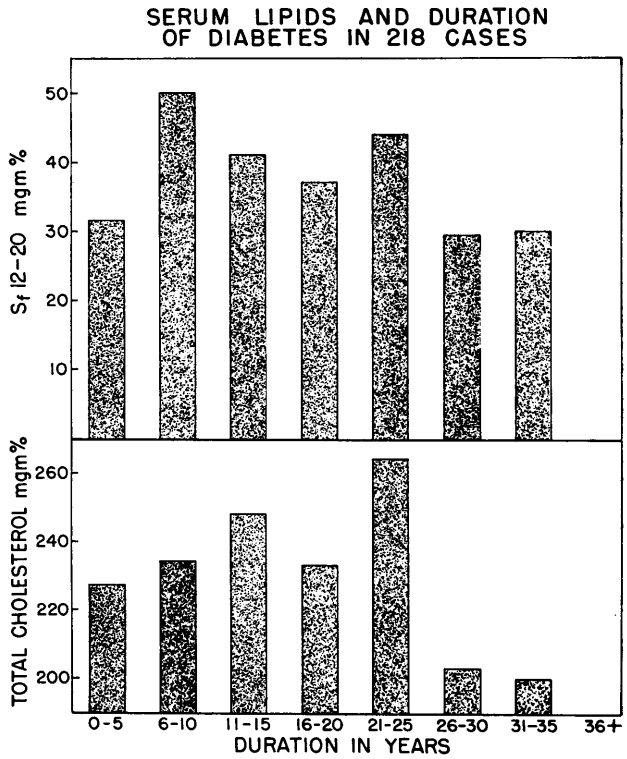

FIGURE 3 The relationship of the S_f 12-20 class of lipoprotein and cholesterol to the duration of diabetes in 218 patients.

control and the incidence of complications is not related to the size of the insulin dose.[4]

In Figure 3 are shown mean values for levels of cholesterol and the S_f 12-20 class of lipoproteins related to duration of diabetes. In the first 25 years of diabetes no definite changes in mean values appear. After 25 years a decrease in mean values, especially in the cholesterol is seen.

The relation between degree of control of diabetes and the distribution of mean lipoprotein and cholesterol values in the group of 144 patients is shown in Table 2. It will be seen that 96 of the total of 144 patients

TABLE 2 Mean lipoprotein and cholesterol values in 144 diabetic patients related to the degree of control of the disease.

Degree of Control	No.	S_f 12-20 mg%	S_f 21-35 mg%	S_f 35-100 mg%	Cholesterol mg%
Good	19	42(31)*	21(16)*	24(22)*	242(231)*
Fair	29	42	20	26	223
Poor	96	55	33	45	256

*One case of familial hypercholesterolemia excluded

were found to have maintained poor control. Twenty-nine had been under fair control and 19 were considered to have been under good control. The S_f 12-20 class of lipoproteins occurred with the same average concentration in the groups of good and fair control but the mean values were higher in patients with poor control of diabetes. A similar increase was shown in the concentration of the lipoproteins of the S_f 21-35 group and the lipoproteins of the S_f 35-100 group. The mean level of plasma cholesterol also was higher in the group with poor control although the difference in terms of percentage was not as great as shown in the levels of lipoproteins. Actually, the figures as given should be corrected for the fact that in the group of patients with good control is included one man, aged 31 years, whose value for lipoproteins of the S_f 12-20 class was 290 mg. per cent. This young man really was under good diabetic control but he suffered from familial hypercholesterolemia. If he is excluded, then the average for the remaining 18 cases under good control would fall to the values shown in parentheses in the tables (31 mg. per cent for the S_f 12-20 group, 16 mg. per cent for the S_f 21-35 and 22 mg. per cent for the S_f 35-100 lipoproteins and the mean cholesterol for that group would fall to 231 mg. per cent). In Figure 4 is shown the frequency distribution of the S_f 12-20 values in relation to good, fair and poor control. This figure serves to indicate the characteristic skewness of the distribution of the S_f 12-20 values. The same skewness is found for the other lipoprotein classes as well as for cholesterol. It is evident, however, that the frequency of high levels of S_f 12-20 values is greater with poorer control. This skewness characteristic of the data minimizes the usefulness of conventional statistical perimeters such as the mean and standard deviation.

It seems clear that a difference in the level of lipoproteins and of cholesterol exists in patients with poor control as contrasted with patients under good diabetic control. The basic and unresolved question is whether these differences in lipoprotein concentrations have etiologic importance in relation to control of the disease or whether they are a reflection of the differnce in incidence of complications in these groups. In other words, are these elevated values to be considered as cause or a consequence of the vascular disease?

DIABETIC NEPHROPATHY

In Figure 5 are contrasted the findings in 26 patients of the study group in whom diabetic nephropathy was present with 118 patients who were without evidence of kidney disease. In the nephropathy group only 4 cases were in the stage of terminal nitrogen retention. The mean value for the S_f 12-20 class of lipoproteins was 100 mg. per cent in the 26 patients with nephropathy and 39 mg. per cent in the 118 cases without nephropathy. For the S_f 21-35 class of lipoproteins the corresponding values were 62 and 21 mg. per cent, respectively. The mean cholesterol values were 318 mg. per cent for the nephropathy group and 234 mg. per cent for the 118 patients without renal disease. The difference between the two groups is striking. Thus in 118 patients without renal disease only 10 per cent had

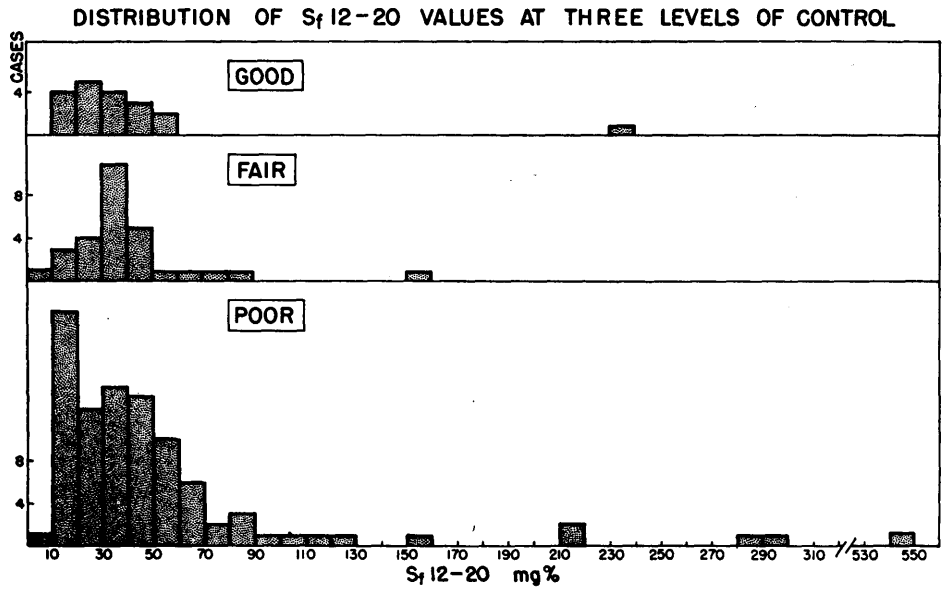

FIGURE 4 Frequency distribution of values for S_f 12-20 class of lipoprotein in 144 diabetics arranged according to degree of control.

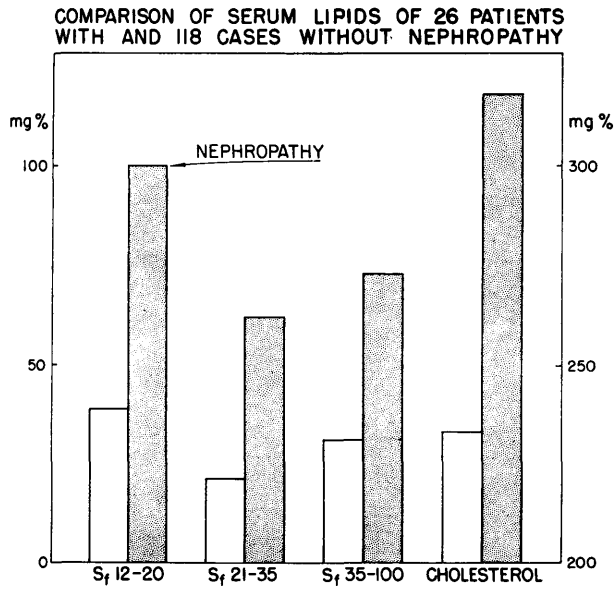

FIGURE 5 Mean serum lipid values in 26 patients with diabetic nephropathy compared with those in 118 diabetic patients without kidney disease.

values of the S_f 12-20 class of lipoproteins exceeding 70 mg. per cent but 31 per cent of the patients with nephropathy had values above this level. Similar differences appeared in the other lipid values. Fifty-eight per cent of the subjects with nephropathy had serum cholesterol values exceeding 260 mg. per cent, whereas only 24 per cent of the subjects without nephropathy had values above this level. In this group of patients the disturbance in protein metabolism with marked proteinuria and changes in the serum protein with an altered albumin-globulin ratio might be expected to lead to alterations in serum cholesterol and other lipids. Since disturbances of protein metabolism are an early sign of nephropathy[9] these serum lipids changes may be a consequence of the disease just as they seem to be in certain other diseases such as nephrosis.[10]

RETINITIS AND ARTERIAL CALCIFICATION

Two other clinical complications in this group of young diabetics are retinitis and arterial calcification. In order to show the relationship of these two complications to the serum lipid values, the patients have been divided into 4 groups. Group 1 includes patients with no or minimal retinal lesions and with no or minimal arterial calcification. Group 2 includes patients with the same

TABLE 3 The relationship of degrees of retinitis and arterial calcification to the mean lipoprotein and cholesterol values of 144 diabetics.*

Group	Number Cases	S_f 12-20	S_f 21-35	S_f 35-100	Cholesterol
			mg%		
1	49	31	15	23	217
2	19	37	13	24	247
3	33	63	43	52	259
4	43	69	41	53	274

*Group 1: no or minimal retinitis and no or minimal arterial calcification; Group 2: no or minimal retinitis but moderate to marked calcification; Group 3: moderate to marked retinitis but no or minimal calcification; Group 4: moderate to marked retinitis and moderate to marked calcification

minimal amount of retinitis but moderate to marked calcification of arteries. In Group 3 are included cases with moderate to marked retinitis but no or minimal calcification. Included in Group 4 are those patients with moderate to marked retinitis and also moderate to marked calcification of the arteries. The mean values for the various serum lipids measured are shown in Table 3. There is a progressive increase from Group 1 to 4 of all the values. However, a striking and characteristic feature of these changes appears in Figure 6 in which the values for the S_f 12-20 class of lipoproteins are placed beside the values for cholesterol in each of the four groups. It is apparent that the S_f 12-20 measurement is most characteristically associated with the increase in the degree of retinitis. The increase in cholesterol values are gradual throughout the four groups. The increase in the level of the S_f 12-20 class of lipoproteins is of much greater degree and seems to be characteristic and specific. The greater extent and

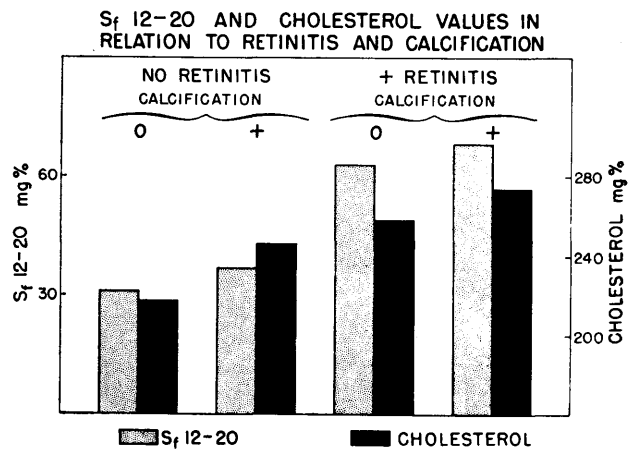

FIGURE 6 Comparison of the increase in S_f 12-20 class of lipoprotein and cholesterol values in 144 diabetic patients arranged according to presence or absence of retinitis and arterial calcification.

significance of the S_f 12-20 change illustrated in Figure 6 is emphasized by a consideration of the greater variability of the cholesterol measure. Since the standard deviation of cholesterol measurements is much larger (50-60 mg. per cent) it follows that mean differences must be large to be significant. Statistical treatment confirmed the finding that there is a significant difference between the S_f 12-20 values for patients with and without retinitis, whereas no significant difference could be found for the cholesterol values in these groups. Significance testing was done after a logarithmic transformation of the data for correction of skewness had been made.

SUMMARY

The serum cholesterol and lipoproteins of the S_f 12-20, S_f 21-35 and S_f 35-100 classes in 218 diabetic patients have been studied and compared with similar data obtained from 691 normal subjects.

It appears that the severity (as measured by insulin dosage) and the duration of diabetes are not important factors in determining the levels of serum lipoproteins and cholesterol in patients under treatment with insulin for periods up to 25 years.

The classification of 144 young patients with diabetes exceeding 10 years duration into 3 classes of control revealed higher mean levels of the serum lipids in the subjects with poor control. Levels of the S_f 12-20 lipoproteins exceeding 50 mg. per cent occurred in 32 per cent of patients with poor control, 17 per cent of patients with fair control and 10 per cent of patients with good control.

In 26 cases with diabetic nephropathy there was a marked elevation in all of the serum lipid components as compared with values obtained in the 118 subjects without renal disease.

A significant finding was the relationship between the presence of retinitis and elevated values of the S_f 12-20 lipoproteins. Arterial calcification showed a much less striking relationship to this lipoprotein. Statistical analysis reveals that the lipoprotein levels alone were significantly associated with retinitis.

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