Serum Lipids and Polysaccharides in Diabetes Mellitus

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Independent observations indicate that elevations of serum lipids[1-5] as well as serum polysaccharides[6-9] occur in diabetic patients with specific degenerative vascular complications. No comparative study of these groups of substances in the serum has been performed in the same diabetic patients with special reference to the presence or absence of degenerative vascular disease.

This study presents a simultaneous correlative analysis of the serum lipid partition, serum total cholesterol, esterified cholesterol, phospholipids, neutral fats and total lipids, and the serum glucosamine and total polysaccharides bound to serum protein in a group of 66 patients with diabetes mellitus.

SELECTION OF PATIENTS

The patients included in the study did not present any stigmata associated with idiopathic hypercholesteremia or idiopathic hyperlipemia; nor did they exhibit evidences of diseases such as myxedema or biliary cirrhosis, known to affect the concentration of serum lipids, or such diseases as cancer, acute or chronic infections and acute myocardial infarction, known to influence the concentration of serum polysaccharides.[10-15]

Group I consisted of persons who were found to be in good health and were examined as controls during the time of the study. Their ages were comparable with those of the following groups. The control values on serum lipids were obtained from 73 patients between the ages of 48 to 52 years.[16] Similarly the control studies on serum glucosamine and polysaccharides were performed on 15 nondiabetic persons.[8] In contrast to Groups II, III and IV, simultaneous studies were not performed in the controls of Group I.

Group II consisted of 38 patients with uncomplicated diabetes. The average duration of diabetes was 8.4 years, range 1 to 16 years. The average age of the group was 50.2 years. Eighteen of these patients required no insulin, 15 used between 10 to 30 units, 3 used 35 to 50 units and 2 over 55 units daily. Patients with proteinuria, renal insufficiency, retinopathy, neuropathy, coronary artery, and peripheral vascular disease established by clinical, laboratory, and roentgen examination were carefully excluded. The average fasting blood sugar of the group was 128 mg. per 100 cc. The systolic blood pressure exceeded 150 in 7 instances (152 to 170 mm.) and averaged for the group 139 mm. The diastolic blood pressure exceeded 90 mm. in 5 instances only (92 to 104) and the average for the group was 84 mm.

Group III included 12 specially selected patients with early diabetic retinopathy and no evidences of hypertensive retinopathy, proteinuria or renal inadequacy. The duration of diabetes was 2 to 25 years, average 13 years. The average age of the group was 54.3 years. Only one patient of this group required no insulin, 7 used 10 to 30 units, 2 used 35 to 50 units, and 2 used 55 to 60 units daily. The average fasting blood sugar of the group was 137 mg. per 100 cc. The average systolic blood pressure was 155, the average diastolic blood pressure 89 mm.

Group IV consisted of 16 patients with the fully developed Kimmelstiel-Wilson syndrome. Severe retinopathy, hypertension, edema, proteinuria, and doubly refractile lipids in the urine were the outstanding features. In many cases there was evidence of renal insufficiency; six were in congestive heart failure, partially compensated by medication. The known duration of diabetes was 1 to

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29 years, average 13.4 years. The average age of the group was lower than that of the preceding two groups: 45.1 years. Three patients required no insulin while 8 used 10 to 30 units, three 40 to 60 units and two 65 to 90 units daily. The average fasting blood sugar was 134 mg. per 100 cc. The average blood pressure was elevated to 194 mm. systolic and 115 mm. diastolic.

METHODS

Twenty to forty milliliters of blood were drawn from subjects after a fast of 12 to 14 hours. The sera was separated within the next 2 to 6 hours and stored in a refrigerator until they were analyzed within the next 4 to 24 hours. Hemolyzed specimens were not used.

Serum cholesterol and esters were analyzed by the method of Sperry and Schoenheimer,[17] phospholipids by Sperry modification of Fiske-SubBarow method,[18] total lipids by the gravimetric method of Bloor.[19] Neutral fats were estimated by subtracting the sum of total cholesterol and phospholipids from total lipids.

Serum glucosamine was determined by the procedure of West and Clarke.[11] Total polysaccharides bound to serum protein were determined by the method of Graff and co-workers.[20]

RESULTS

The data obtained are summarized in table 1. It is evident that Groups I and II exhibit no striking differences. The average levels of serum cholesterol, esterified cholesterol, neutral fats and total lipids as well as those for serum glucosamine and polysaccharides are somewhat similar in the nondiabetic controls and in the patients with uncomplicated diabetes of similar age. A striking abnormality in both serum lipids and complex carbohydrates is seen in Group IV. The patients of this group show marked elevation of all serum lipid fractions as well as of complex serum carbohydrates. Patients in Group III with early retinopathy without any evidence of renal involvement present apparently statistically significant differences in comparison with patients having uncomplicated diabetes and with nondiabetics. The increases are noted chiefly in neutral fat, total lipids, serum glucosamine, and total serum polysaccharides. In contrast, serum cholesterol and phospholipids remain in normal limits (table 1 and figures 1-4).

DISCUSSION

Although extensive studies of serum cholesterol and lipoproteins have been recently performed in diabetics with and without complications,[3-5] only scant information is available concerning complete serum lipid partitions.[2] Our observations indicate that diabetic patients without complications (Group II) have serum lipid partitions al-

TABLE 1
Summary of chemical data of normal controls, and of diabetics with and without complications

Group	I	II	III	IV
	Normal controls (N)	Uncomplicated diabetes (D)	Diabetes & retinopathy (DR)	Diabetes & Kimmelstiel-Wilson syndrome (DKW)
No. of cases	11	38	12	16
Cholesterol, mg.%				
Total	245±54*	236±49	256±41	318±64
Esterified	——	174±37	187±22	221±59
Phospholipids, mg.%	214±38†	259±47	290±33	326±61
Total lipids, mg.%	629±136†	804±182	958±160	1195±268
Neutral fats,§ mg.%	231	310	411	550
Glucosamine, mg.%	97±8‡	106±16	136±18	168±30
Polysaccharide, mg.%	146±11‡	139±11	158±20	181±23

	Probability values (p)		
	D vs. DR	D vs. DKW	DR vs. DKW
Cholesterol, total	0.23	0.0001	0.02
Phospholipids	0.035	0.0001	Not significant by inspection
Total lipids	0.018	0.0001	0.023
Glucosamine	0.0001	0.0001	0.0025
Polysaccharide	0.00015	0.0001	0.013

*Mean values obtained in 73 controls of age 48 to 52 by Boas, Elster and Adlersberg.[16]
†Mean values of 11 healthy men.
‡Mean values obtained in 15 nondiabetic controls by Berkman, Rifkin and Ross.[8]
§Those are calculated figures; no standard deviation is given.

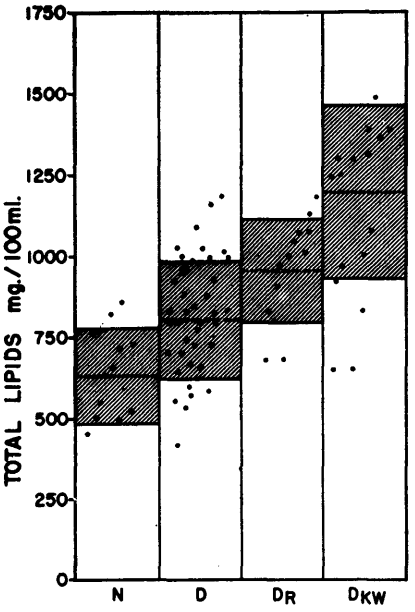

FIG. 1. Serum total lipid (mg. per 100 cc.) in normal controls (N), uncomplicated diabetes (D), diabetes and retinopathy (Dr) and diabetes and Kimmelstiel-Wilson syndrome (Dkw). The interrupted horizontal lines represent the mean of each group and the shaded areas on both sides of the mean represent the standard deviation.

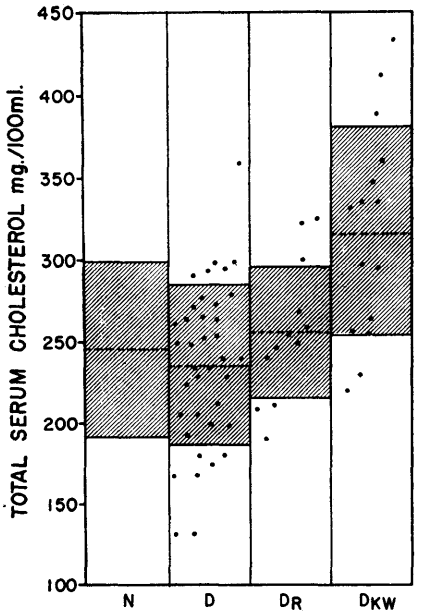

FIG. 2. Total serum cholesterol (mg. per 100 cc.) in the four groups studied. For details see legend to figure 1.

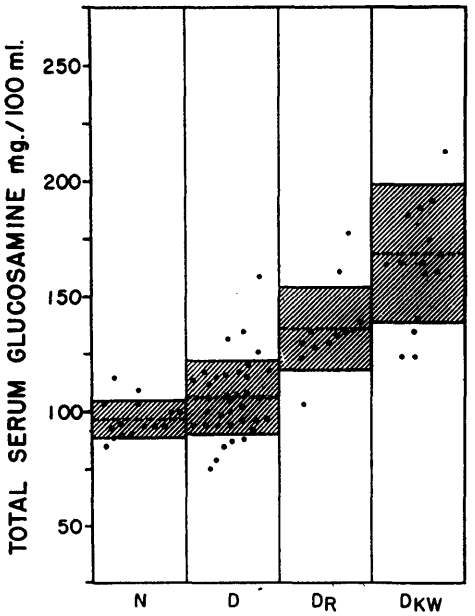

FIG. 3. Serum glucosamine (mg. per 100 cc.) in the four groups studied. For details see legend to figure 1.

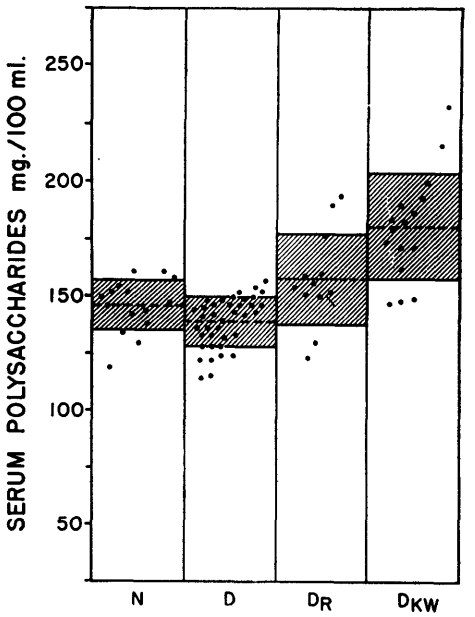

FIG. 4. Serum polysaccharides (mg. per 100 cc.) bound to serum protein in the four groups of persons studied. For details, see legend to figure 1.

most similar to those noted in nondiabetic controls (Group I), except for a slight increase of neutral fats and total lipids. Diabetic patients with early retinopathy (Group III) differ from uncomplicated diabetes (Group II) by significant elevations of serum neutral fats and total lipids without any associated change in serum cholesterol and phospholipids. Keiding *et al.*[4] found a significant correlation between elevated values for the Sf 12 to 20 fraction of lipoproteins and the presence of retinopathy in diabetics. Although there may be pathogenetic

implications, it is difficult to evaluate the marked elevations of all serum lipid fractions in patients with advanced nephropathy (Group IV). These findings may simply reflect profound renal insufficiency or metabolic alteration in the nephrotic syndrome.

As previously observed, the earliest increases of serum glucosamine and polysaccharides are found in diabetic patients with early retinopathy.[8, 9, 21-23] It appears, therefore, that the elevations of both lipids and carbohydrate fractions are found in the earliest clinically detectable specific complications of diabetes mellitus. Although the elevation of serum carbohydrate fractions may be interpreted as a nonspecific index of tissue injury or depolymerization,[7, 24-26] it would be difficult to evaluate the simultaneous increases in serum neutral fat and total lipids in the same light. Although these elevations have been found in diabetic patients with retinopathy,[27, 28] in whom no renal dysfunction exists, it is still possible that more precise evaluation of discrete kidney function would indicate minute functional abnormalities. Preliminary observations by the Montefiore group indicate that this possibility is not a real one.

One must consider, therefore, that the blood changes perhaps precede the degenerative alteration of the tissue and the deposition of protein-carbohydrate and protein-lipid compounds in the retina and in the renal glomerulus. Thus, the increases of serum lipid and carbohydrate components which have been observed may both be pathogenetically related in the development of diabetic retinopathy and diabetic glomerulosclerosis. Whether both the lipids and carbohydrate fractions increase in parallel fashion or one precedes the other remains to be investigated.

SUMMARY

1. Lipid fractions, including cholesterol, phospholipids, total lipids, neutral fats, glucosamine, and total polysaccharides bound to protein, were estimated in 66 patients with and without specific degenerative vascular disease.

2. In patients with uncomplicated diabetes the concentration of these substances in the serum remained within normal limits.

3. Diabetic patients with retinopathy but without evidence of renal involvement showed increases of total serum lipids, neutral fats, glucosamine, and polysaccharides. The serum cholesterol and phospholipids remained within normal limits.

4. Patients with fully developed diabetic glomerulosclerosis (Kimmelstiel-Wilson syndrome) exhibited elevation of all lipid fractions as well as serum glucosamine and polysaccharides.

5. The possible role of these substances in the pathogenesis of diabetic retinopathy and diabetic glomerulosclerosis was discussed.

SUMMARIO IN INTERLINGUA

Seral Lipidos e Polysaccharidos in Diabete Mellite

1. Fractiones lipidic—includente cholesterol, phospholipidos, lipidos total, grassias neutre, glucosamino, e polysaccharidos total ligate a proteina—esseva estimate in 66 patientes con e sin specific morbo vascular degenerative.

2. In patientes con noncomplicate diabete le concentration de iste substantias in le sero remaneva intra limites normal.

3. Patientes diabetic con retinopathia sed sin evidentia de affection renal monstrava augmentos del valores seral total de lipidos, grassia neutre, glucosamino, e polysaccharidos. Le cholesterol e le phospholipidos del sero remaneva intra limites normal.

4. Patientes con perdisveloppate glomerulosclerosis diabetic (syndrome de Kimmelstiel-Wilson) exhibiva elevate nivellos de omne fractiones lipide e etiam del seral glucosamino e polysaccharidos.

5. Es discutite le rolo possibile de iste substantias in le genese de retinopathia diabetic e glomerulosclerosis diabetic.

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DISCUSSION

ARTHUR R. COLWELL, M.D., (*Chicago*): Was there a determination of these substances on only one blood specimen in each patient? Do you know what serial determinations might show on different days or at different times of day? Could you give us any idea of what the relationship of these values was to the state of control of the diabetes in the diabetics?

CHUN-I WANG, M.D., (*New York City*): All determinations were performed simultaneously in fasting blood specimens (groups II-IV). In the nonfasting state the neutral fat content varies with the quantity of dietary fat and the time interval between the last meal and the drawing of blood. On a constant regimen (diet and insulin) the individual lipid fractions remain fairly constant for the same person in the fasting state.

BERNARD A. WATSON, M.D., (*Clifton Springs, New York*): What average diet was given these patients, as far as carbohydrate, protein and fat content is concerned?

DR. WANG: Diabetics with very poor control exhibit marked increases in serum neutral and total fats. It may be stressed that the patients with diabetes included in this study showed adequate control, as may be seen from the presented data.

HAROLD RIFKIN, M.D., (*New York City*): In response to Dr. Colwell, we have measured the serum glucosamine and the total protein-bound polysaccharide serially in a few individuals during a 24-hour period, and there appears to be only slight diurnal variation.

In terms of control, we are a little more disturbed now than we were two years ago. It appears that in some patients who have a very poor level of control, the serum mucopolysaccharides are markedly elevated; then when we controlled them, the polysaccharides became relatively normal. However, the studies presented this morning were based on patients who maintained a fair level of control during the observed period.

Referring to Dr. Watson's question about diet, the carbohydrate intake was in the range of 200-250 gm., protein 70-90 gm., and the rest in fat to make up the necessary caloric intake.

KENNETH STERLING, M.D., (*Syracuse, New York*): I would like to ask either Dr. Wang or Dr. Rifkin whether the reported changes in glucose or polysaccharides have a close relationship to alpha-globulin changes previously reported in diabetic glomerulosclerosis.

DR. RIFKIN: In 1951 Dr. Petermann and I predicted that the highest concentration of carbohydrate would reside in the alpha-2 globulin fraction. Since then, we have measured the carbohydrate concentration of the various protein fractions electrophoretically. I would say, and I believe this is Drs. Keiding's and Tuller's experience in Boston also, that the alpha-2 globulin contains the highest concentration of polysaccharide.