

# Blood Lipid and Protein Levels in Juvenile Diabetes Mellitus

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In recent years much emphasis has been placed upon the relationship of hyperlipemia to the development of atherosclerosis. The tendency for vascular disease to develop prematurely in patients with diabetes mellitus is well known. Therefore, it seemed pertinent to study the serum lipid and protein levels in patients with juvenile diabetes mellitus in whom there was no clinical evidence of arterial disease. Our findings are presented herewith and discussed in the light of other reports.

## CLINICAL MATERIAL AND METHODS

Fifty patients with juvenile diabetes mellitus were studied in private practice and in the diabetes outpatient clinics and hospitals from which this paper originates. The severity of the diabetes, degree of control, and duration of the disease varied with each patient. There were twenty-four males and twenty-six females, ranging from nineteen months to sixteen years of age. The shortest duration of the disease was three weeks and the longest was eleven and one-half years. The patients were divided into two groups: those whose duration of diabetes was less than five years, and those who had diabetes longer than five years. There were fifteen males and sixteen females in the former group, and nine males and ten females in the latter group.

None of the patients was hospitalized specifically for this study. All patients received Lente or NPH insulin, alone or in combination with Regular Insulin. In a few instances, mixtures of the Lente insulins were administered. The daily insulin dose was given in one injection before breakfast. The patients received a weighed diet of approximately 3 to 4 gm. of protein per kilogram of body weight, with a protein to fat to carbohydrate ratio of approximately 1:1:2. Activity was unrestricted. These patients tested their urine three to five times a day. Estimation of clinical control was based on the patient's well-being, freedom from symptoms, and a normal gain in weight. If acetone appeared

in the urine, or glycosuria appeared in more than half of the daily urine specimens, or mild reactions occurred, diabetes was considered to be unsatisfactorily controlled. In such an instance, readjustment of either the insulin dose and/or diet was made. Details of the routine of management employed may be found elsewhere.<sup>1</sup> Our criteria are similar to Keiding's<sup>2</sup> classification of the degree of control as "good, fair or poor."

Each patient was studied with the following laboratory tests: complete blood count; bleeding, coagulation, clotting, and prothrombin times; twenty-four-hour urine for quantitative albumin and sugar; fasting blood glucose, cholesterol, cholesterol esters, phospholipid, triglyceride, total lipid, lipoprotein (electrophoresis), total protein and A/G ratio, protein (electrophoresis), cephalin flocculation, and vitamin A level. Blood samples were drawn in the morning before the patient received his daily dose of insulin or had his breakfast. This battery of tests was done once in all our diabetic children. The few instances of incomplete determinations were due to technical laboratory difficulties.

## LABORATORY METHODS

Total cholesterol and cholesterol esters were determined by the method of Schoenheimer and Sperry,<sup>3</sup> and triglycerides by the method of Van Handel and associates.<sup>4</sup> Blood for phospholipids was ashed in triplicate by a modification of the method described by Gortner,<sup>5</sup> and the phosphorus measured by the method of Fiske and Subbarow.<sup>6</sup> The total lipids were calculated from the above determinations.

Serum proteins were separated by filter paper electrophoresis in duplicate by the method of Durrum<sup>7</sup> with the following modifications: Samples containing ten lambda of protein were placed on eight strips of Whatman 3-mm. paper and run for sixteen hours at five milliamperes in a 0.075 ionic-strength veronal buffer of pH 8.6. The proteins were stained with bromphenol blue and evaluated in an Analytrol scanner. The values were compared with normal children's values as reported by Oberman and associates.<sup>8</sup>

Lipoproteins were separated by paper electrophoresis

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in duplicate in the same manner with the use of fifty lambda samples that were prestained with Sudan black B by the method described by Bermes and McDonald.<sup>9</sup> The results were read in the Analytrol scanner, and the quantity of lipid present was measured by the area under the curve, the result being a measure of the relative contribution of each lipoprotein component to the total lipid.

All values were compared with those for normal children as determined by Lever<sup>10</sup> and in our laboratory (table 1). Blood sugar was determined by the Nelson-Somogyi method.<sup>11,12</sup>

## RESULTS

Normal values for chemical constituents of blood are given in table 1.

The results obtained for forty-nine juvenile diabetic patients are presented in table 2. One patient of the original group of fifty was omitted from statistical evaluation because chemical studies were performed at the initial phase of her disease prior to treatment. Of significance in the group as a whole is the elevated beta

TABLE 1  
Normal values for chemical constituents of blood

Glucose		60-80 mg. per 100 ml.	Mean
Cholesterol	Age	Range (mg. per 100 ml.)	(mg. per 100 ml.)
	Newborn (cord)	54-120	79.3
	1-30 days	90-160	122.4
	2 months	87-169	126.5
	3 "	106-184	138.4
	4 "	142-178	154.4
	5-12 "	125-198	159.1
	2-6 years	144-188	172.7
6-13 "	150-250	190.2	
13-16 "	148-392	209.8	
Cholesterol esters		120-220	146
Phospholipid		160-310	240
Triglyceride		0-400	180
Total lipid		570-820	700
Cholesterol/phospholipid 0.85-0.94			
Electrophoresis of proteins*			
		Per cent of total protein	
Albumin		63.37±5.7	
Alpha <sub>1</sub> globulin		3.43±0.6	
Alpha <sub>2</sub> "		8.84±1.3	
Beta "		7.34±0.8	
Gamma "		17.02±4.5	
Electrophoresis of lipoproteins*			
		Per cent of total lipoprotein	
0 fraction		42.4±7.8	
Alpha "		15.4±5.6	
Beta "		42.3±4.7	

\*Normal values for lipoproteins and proteins, as determined by paper electrophoresis, expressed as mean and standard deviations.

globulin fraction and the depressed gamma globulin fraction. All other protein determinations were within normal limits.

The mean blood glucose level for the total group was elevated, being 215 mg. per 100 ml. The group with diabetes of less than five years' duration had a mean level of 215 mg. per 100 ml. Those with diabetes of longer than five years' duration had a mean level of 214 mg. per 100 ml.

Complete blood count; bleeding, clotting, coagulation and prothrombin time; and vitamin A level were within normal limits for the entire group.

The cephalin flocculation test was negative in all but one patient. This was an eight-year-old boy with hepatomegaly who had had diabetes for six years. Additional laboratory findings for this patient include a cephalin flocculation of 3+ and hyperlipemia involving all components. Attempts are being made to reverse his present condition by instituting a low fat diet with the addition of safflower oil.

In the group whose duration of diabetes was under five years there were seven instances of hyperlipemia. Six of these were associated with acidosis in patients who are classified as "poorly" controlled. The remaining patient was the previously mentioned new diabetic who was in severe acidosis with a marked hyperlipemia. This girl was omitted from the statistical analysis because of the gross abnormality of her biochemical determinations at this initial phase of her disease. In five of the seven patients the serum lipids reverted to normal after correction of the metabolic acidosis, but in two the hyperlipemia persisted. One was a two and one-half year old boy who had had diabetes for one and one-half years and was well controlled; he is currently receiving safflower oil. The other is a one and one-half year old patient who is poorly managed, in part because of inadequate parental care.

There were four instances of hyperlipemia in the group whose duration of diabetes was each over five years. One case was associated with acidosis, and the lipid values returned to normal after therapy. Another is the patient previously described with an elevated cephalin flocculation. The other two patients are sixteen-year-old girls. One has had diabetes for eight years and is cooperative and well controlled. The other has had diabetes for five years, is uncooperative and poorly controlled. The inclusion of these patients with hyperlipemia is reflected in the somewhat elevated mean total lipid values reported in table 2. Results of lipoprotein electrophoresis and all other lipid determinations were otherwise within normal limits.

Cholesterol/phospholipid ratios were within normal range for the total group as well as for the groups whose duration of diabetes was less than five years and more than five years.

Coefficient of correlation was determined for glucose and cholesterol, and for glucose and beta lipoprotein levels. There was no significant correlation for either.

Quantitative analyses of twenty-four-hour urine specimens for glucose were variable and negative for albumin in all fifty patients.

Means, standard deviations, standard error of the means, standard error of the differences, and tests of significance were calculated. There was no statistically significant difference between the two groups for any parameter measured.

#### DISCUSSION

The results of a large series of standard biochemical determinations in patients with juvenile diabetes mellitus of varying duration indicate that in almost all instances these values were within normal limits. The three exceptions to these findings were the elevation of blood glucose, the elevation of the beta globulin fraction, and the insignificant depression of the gamma globulin fraction. The few instances of hyperlipemia observed were, in the majority of instances, associated with acidosis. Following therapy, in most of these cases there was a return to normal lipid levels. Furthermore, contrary to the report of Wolff and Salt,<sup>16</sup> no relationship between glucose levels and cholesterol or beta lipoprotein levels was demonstrated.

These findings are in agreement with those of Chalkoff and associates,<sup>13</sup> who found normal values for cholesterol, cholesterol esters, phospholipid, fatty acids, and total lipid in pediatric patients with diabetes mellitus regardless of the duration of the disease or amount of insulin taken. The observations of Keiding,<sup>2</sup> Dine and Jackson,<sup>14</sup> and Wolff and Salt,<sup>16</sup> indicating that lipid concentrations are frequently elevated in young diabetic patients with poor control, are substantiated by the findings of the present paper. The publications of Engelberg,<sup>15</sup> DeWind and associates,<sup>17</sup> Adlersberg,<sup>18</sup> and Iannaccone and Kornerup,<sup>19</sup> in which elevated lipid values were reported to occur in presumably nonacidotic adult diabetic patients, are not supported by the present observations of juvenile diabetic patients. These differences may well represent progress of the disease process.

The elevated serum beta globulin and depressed gamma globulin in this study are findings which we are unable to relate directly to any postulated abnormality of lipid metabolism.

TABLE 2

Biochemical results of forty-nine juvenile diabetic patients

Blood constituents	No. determinations	Mean mg. per 100 ml.	Standard deviation
Glucose			
Total group	49	214.7	95.4
0-5 yr. duration	30	215.4	96.2
Over 5 yr. "	19	213.6	94
Cholesterol			
Total group	49	207.7	63.2
0-5 yr. duration	30	219.1	66.4
Over 5 yr. "	19	189.8	60.2
Cholesterol esters			
Total group	49	146.3	45.4
0-5 yr. duration	30	155.0	48.3
Over 5 yr. "	19	132.6	44.2
Phospholipid			
Total group	44	265.3	90.2
0-5 yr. duration	26	264.5	86.4
Over 5 yr. "	18	266.6	91.8
Triglycerides			
Total group	46	84.7	51.8
0-5 yr. duration	28	86.6	48.2
Over 5 yr. "	18	81.7	53.7
Total lipid			
Total group	43	828.2	268.2
0-5 yr. duration	26	832.9	262.8
Over 5 yr. "	17	820.2	256.4
Cholesterol/phospholipid			
Total group	48	0.81	0.05
0-5 yr. duration	30	0.85	0.04
Over 5 yr. "	18	0.76	0.04
		Relative per cent of total protein	
PROTEINS			
Serum albumin			
Total group	49	59.2	8.6
0-5 yr. duration	30	59.4	8.2
Over 5 yr. "	19	59.0	8.8
Alpha <sub>1</sub> globulin			
Total group	49	4.65	1.5
0-5 yr. duration	30	4.80	1.2
Over 5 yr. "	19	4.44	1.4
Alpha <sub>2</sub> globulin			
Total group	49	9.93	3.8
0-5 yr. duration	30	9.67	3.2
Over 5 yr. "	19	10.34	3.8
Beta globulin			
Total group	49	11.76	3.9
0-5 yr. duration	30	11.73	3.5
Over 5 yr. "	19	11.81	2.8
Gamma globulin			
Total group	49	13.38	4.0
0-5 yr. duration	30	13.36	3.6
Over 5 yr. "	19	13.42	3.8
		Relative per cent of lipoproteins	
Lipoproteins			
0 lipoprotein			
Total group	46	47.6	7.6
0-5 yr. duration	27	46.2	6.0
Over 5 yr. "	19	49.7	7.2
Alpha lipoprotein			
Total group	46	15.2	4.6
0-5 yr. duration	27	15.6	4.4
Over 5 yr. "	19	15.1	5.0
Beta lipoprotein			
Total group	46	35.9	6.4
0-5 yr. duration	27	37.1	5.8
Over 5 yr. "	19	34.2	6.8

The failure to substantiate the reported correlation between beta lipoprotein and blood glucose levels would tend to cast doubt on the validity of the hypothesis that hyper-beta-lipoproteinemia develops when insufficient carbohydrate is available for metabolic needs. Furthermore, the postulated relationship between beta lipoprotein levels of juvenile diabetic patients and the development of atheroma<sup>10</sup> would appear to be based on an unsound premise.

While the tendency of diabetic patients to develop premature arterial disease leading to occlusive coronary disease, retinopathy, and nephropathy is well known, the present study of treated diabetic children fails to demonstrate any of the biochemical abnormalities that have been suspected as pathogenetic factors in diabetic vascular disease.

#### SUMMARY

Blood lipid and protein determinations in fifty patients with juvenile diabetes mellitus were recorded. There was no statistical difference between those children whose disease had been present less than five years and those who had diabetes longer than five years. The only abnormal findings were an elevated blood glucose, elevated serum beta globulin fraction, and a decreased serum gamma globulin fraction.

There was no correlation between glucose levels and cholesterol or beta lipoprotein levels.

The question of the relationship of biochemical findings to the pathogenesis of arterial disease in juvenile diabetic patients is discussed.

#### SUMMARIO IN INTERLINGUA

#### *Nivellos Sanguinee de Lipido e Proteina in Diabete Mellite Juvenil*

Le nivellos sanguinee de lipido e proteina esseva determinate in cinquanta patientes con diabete mellite juvenil. Nulle statisticamente significative differentia esseva trovate inter le juveniles con diabete de un duration de minus que cinque annos e illes con diabete de un duration de plus que cinque annos. Le sol constataciones anormal esseva augmentos del glucosa sanguinee e del fraction de globulina beta in le sero e un reduction del fraction de globulina gamma in le sero.

Nulle correlation esseva trovate inter le nivellos de glucosa e le nivellos de cholesterol o de lipoproteina beta. Es discutite le question del relation inter le constataciones biochimic e le pathogenese de morbo arterial in juvenil patientes diabetic.

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#### REFERENCES

- <sup>1</sup> Lussky, R. A., Newcomb, A. L., and Traisman, H. S.: Lente insulin in diabetic children. *Diabetes* 5:124-27, 1956.
- <sup>2</sup> Keiding, N. R., Mann, G. V., Root, H. F., Lawry, E. Y., and Marble, A.: Serum lipoproteins and cholesterol levels in normal subjects and in young patients with diabetes in relation to vascular complications. *Diabetes* 11:434-40, 1952.
- <sup>3</sup> Schoenheimer, R., and Sperry, W. M.: Micromethod for determination of free and combined cholesterol. *J. Biol. Chem.* 106:745-60, 1934.
- <sup>4</sup> Van Handel, E., Zilversmit, D. B., and Bowman, K.: Micro-method for the direct determination of serum triglycerides. *J. Lab. & Clin. Investigation* 50:152-57, 1957.
- <sup>5</sup> Gortner, W. A.: Evaluation of micromethods for phospholipid. *J. Biol. Chem.* 159:97-100, 1945.
- <sup>6</sup> Fiske, C. H., and Subbarow, Y.: Colorimetric determination of phosphorus. *J. Biol. Chem.* 66:375-400, 1925.
- <sup>7</sup> Durrum, E. L.: Continuous electrophoresis and ionophoresis on filter paper. *J. Am. Chem. Soc.* 73:48-75, 1951.
- <sup>8</sup> Oberman, J. W., Gregory, K. O., Burke, F. G., Ross, S., and Rice, E. C.: Electrophoretic analysis of serum proteins in infants and children. I. Normal values from birth to adolescence. *New England J. Med.* 255:743-50, 1956.
- <sup>9</sup> Bermes, Jr., E. W., and McDonald, H. J.: Fractionation and characterization of the lipide stain sudan black B. *Arch. Biochem. & Biophysics* 70:49-57, 1957.
- <sup>10</sup> Lever, W. F., Smith, P. A. J., and Hurley, N. A.: Idiopathic hyperlipemia and primary hypercholesterolemic xanthomatosis. I. Clinical data and analysis of plasma lipids. *J. Invest. Dermat.* 22:33-51, 1954.
- <sup>11</sup> Nelson, H.: Photometric adaptation of Somogyi method for determination of glucose. *J. Biol. Chem.* 153:375-80, 1944.
- <sup>12</sup> Somogyi, D.: Determination of blood sugar. *J. Biol. Chem.* 160:66-73, 1945.
- <sup>13</sup> Chaikoff, I. L., Smyth, F. S., and Gibbs, G. E.: The blood lipids of diabetic children. *J. Clin. Investigation* 15:627-31, 1936.
- <sup>14</sup> Dine, M. S., and Jackson, R. L.: Serial serum cholesterol values in children with diabetes mellitus of recent onset. *Soc. Trans. of Soc. Ped. Research. Am. J. Dis. Child.* 86:660, 1953.
- <sup>15</sup> Engelberg, H., Gofman, J., and Jones, H.: Serum lipids and lipoproteins in diabetic glomerulosclerosis. *Diabetes* 11:425-33, 1952.
- <sup>16</sup> Wolff, O. H., and Salt, H. B.: Serum lipids and blood sugar levels in childhood diabetes. *Lancet* 1:707-10, 1958.
- <sup>17</sup> DeWind, L. T., Michaels, G. D., and Kinsell, L. W.: Lipid studies in patients with advanced diabetic atherosclerosis. *Ann. Int. Med.* 37:344-51, 1952.
- <sup>18</sup> Adlersberg, D.: Serum lipids and polysaccharides in diabetes mellitus. *Diabetes* 5:116-29, 1956.
- <sup>19</sup> Iannaccone, A., and Kornerup, T.: Plasma lipids and diabetic retinopathy. *Acta med. Scandinav.* 148:411-16, 1954.