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Thomas J. Merimee, … , Judith D. Hall, S. Edwin Fineberg

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Research Article

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Capillary Basement Membrane Structure: a Comparative Study of Diabetics and Sexual Ateliotic Dwarfs

THOMAS J. MERIMEE, MARVIN D. SIPERSTEIN, JUDITH D. HALL, and S. EDWIN FINEBERG

From the Evans Division of Clinical Research, Boston University Hospital, Boston, Massachusetts 02118, Boston University Medical Service, Boston City Hospital, Boston, Massachusetts 02118, and the Department of Internal Medicine, The University of Texas, Southwestern Medical School, Dallas, Texas 75235

ABSTRACT A group of 32 sexual ateliotic dwarfs with an isolated deficiency of human growth hormone (HGH) were shown previously to resemble subjects with genetic diabetes mellitus in terms of hyperlipemia, carbohydrate intolerance, and patterns of insulin secretion. 11 of these dwarfs had needle biopsies of the quadriceps femoris carried out and tissue fixed for electron microscopy. Capillary basement membrane thickness was measured and compared with measurements previously obtained in diabetics and normal controls. Measurements were similar in controls and dwarfs (1080 ±27 A and 1086 ±90 A, respectively) and significantly less than in diabetics (2403 ±119 A). Placed in juxtaposition with the absence of retinopathy in dwarfs and the high incidence in the diabetic group (41%), the data support the thesis that these anatomical abnormalities are largely independent of serum lipid and carbohydrate abnormalities. The data are consistent with a supportive, if not causative role of growth hormone in the pathogenesis of these lesions.

INTRODUCTION

The majority of dwarfs with a monotropic deficiency of growth hormone exhibit abnormalities of insulin secretion, glucose intolerance, hypercholesterolemia, and hypertriglyceridemia without having diabetic retinopathy (1). With a recently described technique, capillary basement membrane thickness can be measured in quadriceps femoris needle biopsy specimens by electron microscopy (2).

Friedenwald, on the basis of light microscopic studies, indicated that an abnormality of the capillary basement membrane in retinal and renal vessels might be a unique abnormality of diabetes (3, 4). More recently, basement membrane thickening in muscle capillaries was reported in 53% of prediabetics studied and in 98% of diabetic patients with fasting hyperglycemia (2). In the latter study, the presence or absence of basement membrane thickening appeared to bear little relation in either group to age, weight, or the duration and severity of diabetes. There are reports, however, that microangiopathic lesions occur in patients with abnormal glucose tolerance from other causes, such as chronic pancreatitis and diabetes secondary to hemochromatosis (5, 6).

In the present study, capillary basement membrane width was measured in patients with a monotropic deficiency of growth hormone and compared with normal and diabetic subjects. Despite metabolic similarities between diabetes mellitus and ateliotic dwarfism, thickening of the basement membrane of capillaries did not occur in dwarfs lacking growth hormone. The data are consistent with the thesis that thickening of the capillary basement membranes and hence the microangiopathy of diabetes may not be the result of carbohydrate intolerance or hyperlipemia. Rather, this abnormality may be an independent variable that is dependent upon the presence of growth hormone for its full manifestation.

METHODS

11 sexual ateliotic dwarfs lacking only human growth hormone (HGH) were studied. They were compared with normal and diabetic patients previously reported in detail (2). Normal subjects were defined as those having normal fasting plasma glucose and a normal glucose tolerance test. In addition, each normal subject was required to have a negative family history for diabetes in a family sufficiently

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large enough to give this history validity. Patients with diabetes mellitus were defined as those having two fasting plasma glucose values in excess of 140 mg/100 ml. Carbohydrate intolerance was grossly abnormal in this group. Both groups, diabetics and prediabetics, have previously been reported in detail (2). Of the 11 sexual ateliotic dwarfs, 7 showed insulinopenia after arginine and glucose and increased sensitivity to exogenous insulin. Four dwarfs showed greater than normal insulin responses to glucose and resistance to exogenous insulin. The two groups thus corresponded to type 1 and type 2 sexual ateliotic dwarfs, respectively, as initially described by Merimee et al (7, 8).

Biopsy technique. Muscle biopsies were obtained from the lateral aspect of the quadriceps muscle at a point midway between the knee and the hip. After infiltration of the skin, underlying tissue and muscle with 1% xylocaine, a 10–20 mg specimen of muscle was obtained with a modified Franklin-Silverman biopsy needle. No complications of the procedure were noted.

Electron microscopy and measurement of capillary basement membrane width. Muscle biopsies were obtained in duplicate and fixed in Palade's buffered osmium tetroxide fixative (9). After 60 min of fixation, the specimens were placed in 3.7% formaldehyde for 30 min and then were dehydrated successively through 70, 95, and 100% ethanol and two changes of propylene oxide. The tissue was then embedded in Maraglass (Allied Products Corp., Chicago, Ill.) according to the procedure of Spurlock, Kattine, and Freeman and sections approximately 300–500 A thick were cut with a glass knife on a Porter-Blum microtome (10). All electron micrographs were obtained with an RCA-EMU-3 Electron Microscope. The instrument was calibrated at monthly intervals with a carbon grid (2160 lines/mm), and when necessary, suitable corrections were made on the calculated basement membrane widths. All electron micrographs used in this study were obtained at a single tap setting of six (approximately times 8200) to eliminate possible errors on recording the magnification. The routine technique for assaying the basement membrane width was adhered to strictly without knowledge of the source of the biopsy. This entire procedure has been described in detail in a previous publication (2).

Laboratory tests. Glucose tolerance tests were performed with the oral administration of 100 g of glucose to patients at 8:00 a.m. after an overnight fast. All patients received a high carbohydrate diet for at least 3–5 days before the test. Plasma glucose determinations were carried out using a glucose oxidase method, the upper limits or normal of the fasting plasma glucose being 84.5 ±8.5 (mean ±SD). Immunoreactive insulin levels were obtained during the glucose tolerance test (11). Serum cholesterol and triglycerides were measured by methods previously described (1).

TABLE I

Plasma or Serum Constituents Measured in Sexual Ateliotic Dwarfs

	Cholesterol	Triglycerides	Fasting blood sugar
	mg/100 ml	*mg/100 ml*	*mg/100 ml*
A. Type 1 sexual ateliotic dwarf (Insulinopenia after glucose, hypersensitivity to exogenous insulin)			
1. D. S.	253	191*	71
2. R. S.	350	237*	86
3. E. S.	476*	357*	108‡
4. A. S.	424*	360*	104‡
5. C. S.	324*	118	110‡
6. N. S.	175	75	100
7. D. S.	204	89	106‡
B. Type 2 sexual ateliotic dwarf			
8. R. Sh.	332*	117	104‡
9. A. M.	360*	310*	88
10. L. L.	380*	400*	107‡
11. B. A.	289	91	101

* Denotes a value greater than 2 SD from the mean value of the appropriate age group.

‡ Denotes a value 2 SD greater than the control mean ±SD of 84.5±8.5 mg/100 ml.

RESULTS

Blood lipids and glucose. Of the 11 sexual ateliotics having biopsies performed, 8 had abnormally elevated concentrations of either serum cholesterol, serum triglyceride, (>2 SD above the mean), or both. The normal age corrected values of serum cholesterol and triglyceride were taken from the work of Albrink, Meigs, and Man (12).

Basal values of plasma glucose were within the normal range in five sexual ateliotics and exceeded 2 SD from the mean in six. These individual results are given in Table I.

Glucose and insulin responses to oral glucose ingestion were grossly abnormal in all of the sexual ateliotic dwarfs selected for muscle biopsies. Seven of these sexual ateliotics with peak glucose concentrations after oral glucose between 164 and 274 mg/100 ml and a delayed return to basal level, had maximal plasma insulin responses ranging from 21 to 80 μU/ml. 4 sexual ateliotics (patients 8 through 11) had greater than normal absolute insulin and glucose responses after the ingestion of glucose. As indicated in a previous publication, in neither group could insulin secretion be correlated with obesity, age, or sex (7, 8). Results of the glucose tolerance tests are given in Table II for each sexual ateliotic dwarf.

Biopsy data. Basement membrane widths were determined in muscle capillaries from each of the 11 ateliotic dwarfs.

In eight of the patients, the muscle capillary basement membrane widths were well within the normal range. Only three showed slight thickening of the basement membrane above 1325 A. None of the HGH-deficient dwarfs had values equal to or greater than 1500 A. Female and male growth hormone–deficient subjects did not significantly differ, (1033 ±120 versus 1149 ±132 A, respectively). It should be noted that the *average* base-

TABLE II

Plasma Glucose and Insulin of Biopsied Patients after Oral Glucose

	Sex	Age	Glucose					Insulin					Capillary basement membrane width, $\bar{X} \pm SE$
			0	30	60	90	120	0	30	60	90	120	
		yr	*mg/100 ml*					*μU/ml*					*A*
Type 1													
1. D. T.	M	36	71	174	249	278	254	3.0	10.0	35.0	30.0	34.0	1481 ±109
2. R. S.	M	65	86	158	218	243	259	12.0	38.0	54.0	46.0	78.0	1442 ±169
3. E. S.	F	64	108	167	194	151	139	10.0	23.0	56.0	50.0	40.0	1318 ±103
4. M. S.	F	55	110	160	208	200	164	13.0	37.0	41.0	44.0	31.0	998 ±88
5. A. S.	M	57	104	128	164	144	154	6.0	17.0	21.0	20.0	20.0	860 ±60
6. D. S.	F	20	106	184	196	208	296	12.0	38.0	42.0	60.0	80.0	710 ±49
7. N. S.	F	23	100	—	238	—	120	11.0	—	21.0	—	23.0	761 ±71
Type 2													
8. R. Sh.	M	34	104	184	176	172	156	19.0	108	153	204	161	1180 ±109
9. A. M.	F	62	88	195	213	191	175	38.0	200	200	200	220	973 ±91
10. L. L.	F	49	107	127	204	229	229	24.0	43	90	100	68	1442 ±107
11. B. A.	M	48	101	133	191	142	146	20.0	60	94	140	90	784 ±79

ment membrane width of the 11 ateliotic dwarfs with either abnormal glucose tolerance tests or elevated fasting blood sugars was 1086 A (±90 SE) (Table III), a figure that is not significantly different from that noted previously in a large nondiabetic population (2).

DISCUSSION

The vascular complications of genetic diabetes mellitus have usually been classified according to the size of the arterial vessels involved. Lesions involving the smaller arterial vessels or capillaries, the so-called microangiopathic lesions, occur in skeletal muscle and several organs, most notably, the kidney and eye (13, 14). These vessels are characterized anatomically by increased width of the basement membrane and deposition of periodic acid-Schiff (PAS) positive material. Some reports indicate quantitative abnormalities in glycoprotein structure of the basement membranes from such patients (15). Although human growth hormone as well as other factors may modify the synthetic rate of glycoprotein formation, whether this relates to the anatomical abnormalities of the basement membranes is not known (15).

TABLE III

Average Muscle Capillary Basement Membrane Width in Normal, Diabetic, and High-Deficient Dwarfs

Subjects	No. of subjects	Average basement membrane width
Normal	50	1080 ±27
Diabetics	51	2403 ±119
Dwarfs	11	1086 ±90

Normal and diabetic subjects are those previously reported in detail (2). Diabetics differ from both normals and dwarfs with $P < 0.01$. Normals and dwarfs do not significantly differ.

In a separate study recently reported, 38 diabetics were selected to match 31 dwarfs deficient only in HGH as closely as possible in physical characteristics (age, sex, body habitus) and insulin secretion (1). It was particularly difficult to satisfy this latter requirement and approximately 200 diabetics from two clinics were screened for this purpose. Both diabetics and dwarfs showed gross glucose intolerance and an increased incidence of abnormally elevated serum lipids. Both groups also showed similar secretory patterns of insulin, which were abnormally low in the majority or increased in a smaller number. Despite these metabolic similarities, no HGH-deficient dwarf had the retinal complications of diabetes, whereas 41% of the diabetic group had multiple retinal complications (1). It is worth noting that in this latter group of diabetics, the mean plasma glucose was 120 mg/100 ml with nine diabetics having fasting sugars between 100 and 115 mg/100 ml. Of these nine, three had typical diabetic retinopathy.

In the present study, 11 dwarfs were chosen for biopsies. The majority had abnormal serum lipids, and in addition, all exhibited glucose intolerance.

The HGH-deficient subjects were diverse as regards insulin secretion. Four dwarfs listed under the category of type 2 sexual ateliotic dwarfs (1) were biopsied. Although lacking HGH, the latter group of dwarfs consistently have high insulin output after the ingestion of

glucose and the infusion of arginine, which contrasts with the insulinopenia seen in the majority of HGH-deficient dwarfs. At least two dwarfs secreting greater than normal amounts of insulin (A. M. and L. L.) inherited dwarfism as a dominant trait, which also contrasts with the typical pattern of inheritance for sexual ateliotic dwarfs.

If carbohydrate intolerance alone, or in the presence of low insulin output, caused the membrane abnormalities of diabetes mellitus, these abnormalities should have been detected in the dwarf population. Similarly, if lipid abnormalities contributed to this condition, one would have expected a significant increase of basement membrane width. In true diabetes mellitus, 98% of the patients with elevated fasting blood sugar showed thickening of capillary basement membrane above 1325 A. Approximately 90% had widths greater than 1500 A. Not a single dwarf biopsied had a capillary basement membrane width as great as 1500 A. All had glucose intolerance and six had basal plasma glucose values greater than 2 SD above the mean normal plasma glucose concentration. Although most of the dwarfs had significant elevations of the basal plasma glucose concentrations, this was not to the same degree as seen in the diabetics. However, thre are three lines of evidence that suggest the differences in basal plasma glucose concentrations were probably of little significance in affecting the results of the present study. In prediabetics, fasting plasma glucose concentrations are normal, yet the mean capillary basement membrane thickness is decidedly abnormal, significantly greater than seen in sexual ateliotics (12). Secondly, our own data previously reported, indicated that diabetic retinopathy occurred in diabetics with normal to high insulin output, most of whom had fasting plasma glucose concentrations between 100 and 120 mg/100 ml (1). One would predict that this group would also be likely to have vascular lesions elsewhere. More importantly, preliminary data on basement thickness in diabetics with fasting plasma glucoses less than 120 mg/100 ml has been obtained. Capillary basement membrane thickness is likewise significantly increased in this group.[1]

Although the fasting plasma glucose concentrations may be a factor contributing to the vascular abnormalities of diabetes mellitus, it is probably dependent upon the presence of relatively normal HGH secretory responsiveness. This last statement is supported by the data that, although much less common than in true genetic diabetes mellitus, microangiopathic lesions do occur in hemochromatosis and other conditions when carbohydrate intolerance is observed (5, 6).

It is possible that people with small body size have capillary basement membranes less thick than normal-sized individuals. Ideally, one should compare sexual ateliotic dwarfs without the metabolic abnormalities of diabetes with sexual ateliotic dwarfs having these metabolic abnormalities. Unfortunately, we have been unable to obtain "nondiabetic" dwarfs, since all over the age of 30 have shown abnormal carbohydrate tolerance.

We are unable to define the precise role or significance of carbohydrate intolerance and other metabolic abnormalities to the complications of diabetes mellitus. It seems reasonable to postulate that HGH plays at least a supporting role in the progressive development of these lesions.

[1] Siperstein, M. D. In preparation.

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REFERENCES

1. Merimee, T. J., S. E. Fineberg, V. A. McKusick, and J. D. Hall. 1970. Diabetes mellitus and sexual atellotic dwarfism: a comparative study. *J. Clin. Invest.* **49**: 1096.
2. Siperstein, M. D., R. H. Unger, and L. L. Madison. 1968. Studies of muscle capillary basement membranes in normal subjects, diabetics, and prediabetic patients. *J. Clin. Invest.* **47**: 1973.
3. Friedenwald, J. S. 1949. A new approach to some problems of retinal vascular disease. *Amer. J. Ophthalmol.* **32**: 487.
4. Friedenwald, J. S. 1950. Diabetic retinopathy. *Amer. J. Ophthal.* **33**: 1187.
5. Becker, D., and M. Miller. 1960. Presence of diabetic glomerulosclerosis in patients with hemochromatosis. *N. Engl. J. Med.* **263**: 367.
6. Ennis, G., M. Miller, and F. M. Ungor. 1968. Intercapillary glomerulosclerosis in diabetes secondary to chronic relapsing pancreatitis. *Diabetes.* **18**: 333.
7. Merimee, T. J., J. D. Hall, D. L. Rimoin, and V. A. McKusick. 1968. Isolated growth hormone deficiency. IV. A metabolic and hormonal basis for classifying ateliotic dwarfs. *Lancet.* **1**: 963.
8. Merimee, T. J., D. Rabinowitz, D. L. Rimoin, and V. A. McKusick. 1968. Isolated human growth hormone deficiency. *Metabolism.* **17**: 1005.
9. Palade, G. E. 1952. A study of fixation for electron microscopy. *J. Exp. Med.* **95**: 285.
10. Spurlock, B. O., V. C. Kattine, and J. A. Freeman. 1963. Technical modifications in maraglass embedding. *J. Cell. Biol.* **17**: 203.
11. Yalow, R. S., and S. A. Berson. 1960. Immunoassay of endogenous plasma insulin in man. *J. Clin. Invest.* **39**: 1157.
12. Albrink, M. J., J. W. Meigs, and E. B. Man. 1961. Serum lipids, hypertension and coronary artery disease. *Amer. J. Med.* **31**: 4.
13. Bloodworth, J. B., Jr. 1963. Diabetic microangiopathy. *Diabetes.* **12**: 99.
14. Warren, S., P. M. LeCompte, and M. A. Legg. 1966. The pathology of diabetes mellitus. Lea and Febiger, Philadelphia, 4th edition. 528.
15. Spiro, R. G. 1969. Glycoproteins: biochemistry, biology and role in disease. *N. Engl. J. Med.* **281**: 1043.