Peripheral Neuropathy in Diabetic Monkeys

DAVID R. CORNBLATH, MICHAEL A HILLMAN, JOHN S. STRIFFLER, CHARLES N. HERMAN, AND BARBARA C. HANSEN

Peripheral neuropathy is a significant complication of human diabetes and a source of morbidity. Appropriate experimental models may aid in understanding its pathogenesis and in developing therapeutic strategies. We sought to determine whether spontaneously diabetic obese adult monkeys developed peripheral neuropathy and whether it occurred early or late in relation to the onset of hyperglycemia. We studied nerve conduction in both motor (peroneal, median, and ulnar) and sensory (median and ulnar) nerves in 13 adult male rhesus monkeys, 4 overtly diabetic and 9 nondiabetic (mean age 21 ± 2 and 16 ± 2 yr, respectively, NS; mean fasting plasma glucose 14.5 ± 3.4 and 4.4 ± 0.6 mM, P = .001). The diabetic animals had significantly reduced motor conduction velocities and prolonged F-wave latencies. Motor-evoked amplitudes did not differ. In the diabetic monkeys, nerve conduction times were increased in motor fibers, which could be identified as early as 2 yr after the onset of hyperglycemia. These abnormalities are similar to those seen in humans and suggest further study of these animals as a primate model of human diabetic neuropathy. *Diabetes* 38:1365–70, 1989

Peripheral neuropathy is a serious complication of human diabetes. Symptomatic diabetic neuropathy develops in up to 50% of those with diabetes of 25 yr duration, and to date, no specific treatment exists for this condition (1). Therapy consists of achieving good control of the underlying metabolic condition with the assumption that with improved laboratory measurements associated with glucose intolerance, such as reductions in fasting glucose and glycosylated hemoglobin levels, the neuropathy will improve.

Experimental models of diabetic neuropathy lack many features of the human disease, because small laboratory animals are usually used and are rendered diabetic by injection of pancreatic β-cell toxins (2). The experimental studies usually include acute development of disease; young, growing animals; and short study times. This contrasts with clinical observations in humans suggesting that diabetic neuropathy develops over many years of sustained hyperglycemia in mature adults (3) and that the severity of the neuropathy is related to diabetic control (1).

A more appropriate model of human diabetic neuropathy may provide clues to pathogenesis and experimental paradigms for the development and testing of potential therapies. We recently reported findings from neurophysiological studies with a single diabetic monkey, which were indicative of peripheral neuropathy (4). Therefore, we performed electrophysiological studies with a larger group of healthy and diabetic monkeys (5). Our results indicate that diabetic monkeys develop reduced motor nerve conduction velocities (MNCVs) compared with controls, identical to findings previously reported in humans, and that these defects can be detected as early as 2 yr after the onset of hyperglycemia. These studies suggest that diabetic monkeys may provide an appropriate experimental model of human diabetic neuropathy.

RESEARCH DESIGN AND METHODS

Study population. Thirteen adult male rhesus monkeys (*Macaca mulatta*) were studied. These monkeys are part of a larger colony of monkeys under study as a model of obesity-associated type II (non-insulin-dependent) diabetes mellitus (5). These 13 were chosen because they were representative of the spectrum of metabolic phases that are seen as the animals progress from healthy to overtly diabetic (5). All animals were reared under laboratory conditions and housed individually in stainless steel cages. The monkeys were maintained on a 12-h light-dark cycle with constant ambient tem-

From the Department of Neurology, The Johns Hopkins University School of Medicine, and the Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland.

Address correspondence and reprint requests to Dr. David Cornblath, Meyer 2-147, The Johns Hopkins Hospital, Baltimore, MD 21205.

Received for publication 27 February 1989 and accepted in revised form 26 June 1989.

perature (~22°C). Food was available ad libitum and was either monkey chow, containing 15% protein, 59% carbohydrate, and 26% fat (Ralston-Purina, St. Louis, MO), or a liquid diet, containing 14% protein, 54.5% carbohydrate, and 31.5% fat (Ensure, Ross, Columbus, OH). All animals were maintained in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, and the protocols were approved by the Institutional Animal Care and Use Committee.

Metabolic studies. Blood sampling and monitoring of basal levels of glucose, lipid, and hormones in plasma and glucose tolerance were performed as described previously (5–7). Briefly, after a 16-h overnight fast, blood samples were collected with peripheral venous catheters from animals anesthetized with ketamine (10 mg/kg body wt). Individual hormone and glucose concentrations recorded for each monkey correspond to values assayed in plasma pooled from four samples of blood drawn 3 min apart to average out the 10- to 12-min large-amplitude oscillations that occur spontaneously in monkeys and humans fasted overnight (8). Intravenous glucose tolerance tests (IVGTTs) were performed on each monkey as described previously (samples obtained at 1, 3, 5, 10, 15, 20, 30, and 60 min after a 0.227-g/kg dose), and the rate of glucose disappearance (K_G) was calculated with the 5- to 20-min plasma glucose period of the IVGTT, previously shown to be the most linear portion of the $\log_e$ slope (6). Fasting plasma glucose (FPG), fasting plasma insulin (IRI), and K_G values for individual monkeys represent averages of determinations performed on samples of blood drawn on 3–6 separate occasions over 4- to 12-mo periods close to the time of the nerve conduction studies.

Electrophysiological procedures. Under light ketamine anesthesia, all monkeys were studied by electrophysiological methods similar to those used for humans (9). The protocol is analogous to that in use in the Diabetes Control and Complications Trial (10). With surface stimulating and recording electrodes, sensory conduction was measured orthodromically in the median and ulnar nerves, and peak-to-peak sensory potential amplitudes (SAMPs) and sensory nerve conduction velocities (SNCVs) were recorded. Motor conduction was measured in the peroneal nerve recording over the intrinsic toe extensor muscles, the median nerve recording over the thenar muscles, and the ulnar nerve recording over the hypothenar muscles. Motor distal latencies (MDLs), baseline-to–negative peak motor-evoked compound muscle action potential amplitudes (MAMPs), MNCVs, and minimum F-wave latency (F) values were recorded. Amplitudes were recorded in microvolts, latencies in milliseconds, and velocities in meters per second. Rectal and skin temperatures were monitored and did not differ between groups.

Statistical analysis. In our primary analysis, we tested the hypothesis that diabetic monkeys differ from nondiabetic monkeys in their physiological characteristics. Analysis of variance was performed to compare the means of the four variables (MDL, MAMP, MNCV, and F) in each of the three motor nerves (peroneal, median, and ulnar) and the two variables (SAMP and SNCV) in the two sensory nerves (median and ulnar). Each nerve group was considered separately, and each variable was evaluated as a univariate case, because the sample size was small. Because of the known effects of aging on peripheral nerve function (11), we performed an analysis of covariance with age as the covariate. Regression analysis was then performed with motor conduction data and the metabolic variables IRI, FPG, and K_G. A secondary analysis was undertaken to determine if nondiabetic monkeys had different results depending on their metabolic phase of progression toward overt diabetes (5). Statistical analysis was performed with the SAS-PC (12). All results are expressed as means ± SE.

RESULTS

Characteristics of experimental animals. The 13 animals were assigned to either a diabetic or nondiabetic group in accordance with criteria of the National Diabetes Data Group (13) and the World Health Organization (14) for obesity-associated type II diabetes (Table 1). After a 16-h overnight fast, the 4 overtly diabetic monkeys (phase 8 or 9; 5,15) had hyperglycemia (mean FPG 14.5 ± 3.4 mM) and normal plasma insulin levels (mean IRI 208 ± 36 pM). The mean age of the monkeys in this group was 21 ± 2 yr. All had been severely obese but experienced weight loss at the onset of clinically overt diabetes. Before the electrophysiological studies, the mean duration of diabetes was 38 mo (range 20–59 mo), and the mean duration of insulin therapy was 23 mo (range 4–55 mo). Insulin therapy consisted of daily subcutaneous injections of 5–30 U/day exogenous hormone (either beef-pork or humulin insulin); insulin was withheld on the morning of the study. The 9 nondiabetic monkeys did not differ significantly in age from the diabetic monkeys (16 ± 2 vs. 21 ± 2 yr). Metabolic data were significantly different in the diabetic and nondiabetic groups for FPG (P = .001), IRI (P = .02), and K_G (P = .004).

Based on metabolic data and previously outlined criteria, we separated the nondiabetic animals into two groups (Table 2). The lean subgroup consisted of four younger adult animals aged 9–13 yr. These monkeys were normoglycemic and normoinsulinemic (phase 2). The five monkeys in the obese subgroup were characterized by fasting hyperinsulinemia: two were normoglycemic (phase 4), and three had slight fasting hyperglycemia (phase 7; FPG range 5.9–7.6 mM). Compared with the lean group, monkeys in the obese group were older (P = .01) and heavier (P = .01) and had higher IRI (P = .002) and lower K_G (P = .008) values but did not differ significantly in FPG levels from the lean group.

Electrophysiological studies. The results of the nerve conduction studies of diabetic and nondiabetic groups are pre-

TABLE 1
Clinical and laboratory features of diabetic and nondiabetic rhesus monkeys

	Diabetic (n = 4)	Nondiabetic (n = 9)
Age (yr)	21 ± 2	16 ± 2
Weight (kg)	10 ± 1	14 ± 1
Fasting plasma glucose (mM)	14.5 ± 3.4	4.4 ± 0.6*
Fasting plasma insulin (pM)	208 ± 36	610 ± 101†
K_G (%/min)	0.8 ± 0.2	2.7 ± 0.3‡

Results are means ± SE. K_G, glucose disappearance.
*P = .001, †P = .02, ‡P = .004, vs. diabetic. All other comparisons not significant.

TABLE 2
Clinical and laboratory features of nondiabetic monkeys based on metabolic characteristics

	Lean (n = 4)	Obese (n = 5)
Age (yr)	11 ± 1	30 ± 1*
Weight (kg)	11 ± 1	17 ± 1†
Fasting plasma glucose (mM)	3.2 ± 0.2	5.3 ± 1
Fasting plasma insulin (pM)	244 ± 93	1012 ± 115‡
K_G (%/min)	3.3 ± 0.4	1.8 ± 0.2§

Results are means ± SE. K_G, glucose disappearance.
*P = .0002, †P = .01, ‡P = .002, §P = .008, vs. lean. All other comparisons not significant.

sented in Table 3. Significant differences were determined by univariate analysis in all motor nerves for MNCV and F and in the median and ulnar nerves for MDL. We then performed analysis of covariance with age as the covariant. MNCV was still highly significant in the peroneal and ulnar nerves but was not as significant in the median nerve. F was still significantly different between the diabetic and the nondiabetic animals in both the median and ulnar nerves. MDL was no longer significant. In all cases, the diabetic monkeys showed increased conduction times in motor fibers. Figures 1 and 2 depict motor conduction values MNCV and F for the diabetic and nondiabetic groups.

We then used regression analysis to determine whether FPG, IRI, or K_G was related to MNCV. Only FPG was statistically significant in all motor nerves: peroneal nerve, P = .04; median nerve, P = .005; and ulnar nerve, P = .0002. The regression plot for FPG and MNCV in the ulnar nerve is depicted in Fig. 3. In this figure, individual animals are indicated by their phase of progression from normal to overt diabetes (phases 2–9), with phases 8 and 9 representing overt and severe advanced diabetes, respectively. The curve, representing all monkeys, suggests a trend in the progression of values from lean (phase 2) to diabetic (phases 8 and 9) animals, with the obese (phases 4–7) animal values falling in the middle.

Figure 4 is a three-dimensional plot of the motor conduction values for MNCV, MDL, and F by diabetic and nondiabetic groups and motor nerve. This illustrates the interdependence among the three variables, with values for nondiabetic animals clustering in the region of the graph reflecting faster conduction times. MAMP was not significantly different between the groups and therefore was not included in the plot (Table 3).

For the sensory nerves, median SAMP and ulnar SNCV were significantly different before correction for age (P < .009 and P < .03, respectively), with lower values in the diabetic animals. After covariate analysis, only MAMP retained statistical significance but at a borderline level (P < .04).

There were no significant differences in nerve conduction in the lean and obese nondiabetic monkeys either with or without age as a covariate (data not shown).

DISCUSSION

This study shows that there are reduced MNCVs in diabetic monkeys compared with nondiabetic monkeys. Increased conduction time was also present in the related parameter motor F. In addition, we found a significant correlation between MNCV and FPG in the three motor nerves studied. However, there was no significant relation between MNCV

TABLE 3
Results of nerve conduction studies in diabetic and nondiabetic monkeys

Nerve and variable	Diabetic (n = 4)	Nondiabetic (n = 9)	P (univariate)	P (covariate)
Motor				
Peroneal				
MDL	2.7 ± 0.3	2.0 ± 0.2	NS	NS
MAMP	8500 ± 2000	11,000 ± 1400	NS	NS
MNCV	43 ± 6	77 ± 4	.001	.002
F	24 ± 1.6	19 ± 1.1	.04	NS
Median				
MDL	3.9 ± 0.3	3.0 ± 0.2	.04	NS
MAMP	5800 ± 1000	7100 ± 700	NS	NS
MNCV	51 ± 4	68 ± 3	.006	.03
F	19 ± 0.8	14 ± 0.6	.0008	.003
Ulnar				
MDL	3.4 ± 0.3	2.2 ± 0.2	.01	.05
MAMP	4800 ± 1200	6900 ± 800	NS	NS
MNCV	50 ± 5	76 ± 3	.0006	.008
F	19 ± 0.7	14 ± 0.4	.0001	.003
Sensory				
Median				
SNCV	37 ± 5	45 ± 3	NS	NS
SAMP	13 ± 3	26 ± 2	.009	.04
Ulnar				
SNCV	39 ± 7	58 ± 4	.03	NS
SAMP	10 ± 4	19 ± 2	NS	NS

Results are means ± SE. MDL, motor distal latency (ms); MAMP, motor amplitude (μV); MNCV, motor nerve conduction velocity (m/s); F, F-wave latency (ms); SNCV, sensory nerve conduction velocity (m/s); SAMP, sensory amplitude (μV).

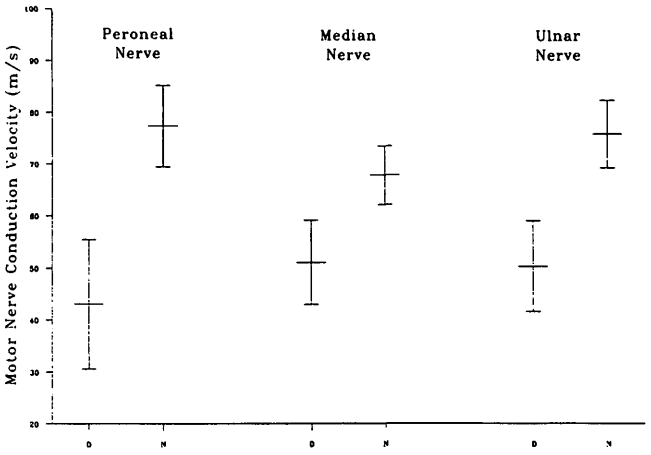

FIG. 1. Motor nerve conduction velocities in each of 3 motor nerves for diabetic (D) and nondiabetic (N) rhesus monkeys. Values for D and N are significantly different in all 3 nerves (see Table 3). Values are means ± 2SE.

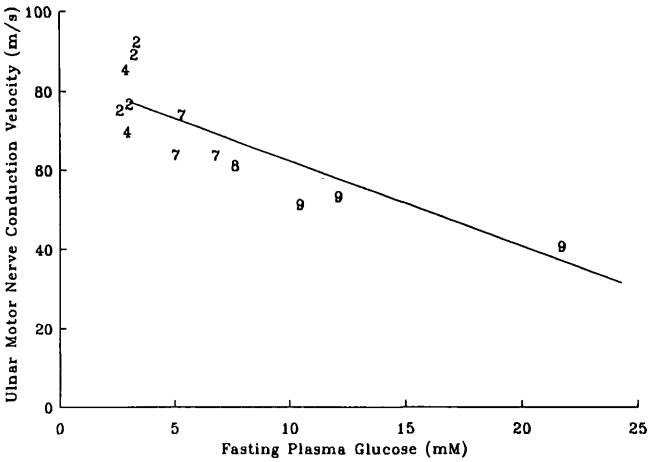

FIG. 3. Ulnar motor nerve conduction velocity plotted against fasting plasma glucose for 13 rhesus monkeys. Value for each animal is represented by number (2–9) that is metabolic phase of progression from normal to diabetic (5). Regression line is significant at P = .0002.

and IRI or K_G for the total group. Motor-evoked amplitudes and sensory conductions did not differ between groups.

Our study also demonstrated that certain measurements of nerve conduction studies are affected by age. In humans, MNCV falls with age beginning at age 29 yr (11). In humans, little is known about the effects of age on other nerve conduction values. When we corrected our analysis for age, there was an age effect on several parameters, indicating that age is an important factor. Despite this effect of age, MNCV and F were still highly significantly different between the diabetic and nondiabetic animals (Table 3).

These results support previous studies showing that nerve conduction velocity is reduced in diabetic individuals (16,17) and diabetic experimental animals (18) compared with nondiabetic subjects. Our study also supports the hypothesis ,that the reduction in nerve conduction is correlated with the level of fasting hyperglycemia, as has been shown in humans (16). We have extended these observations to include the monkey model of obesity-associated type II diabetes, a primate model that exhibits other features of human diabetes and its complications. Although we did not determine glycosylated hemoglobin, others have found a relationship between that value and MNCV (16,19).

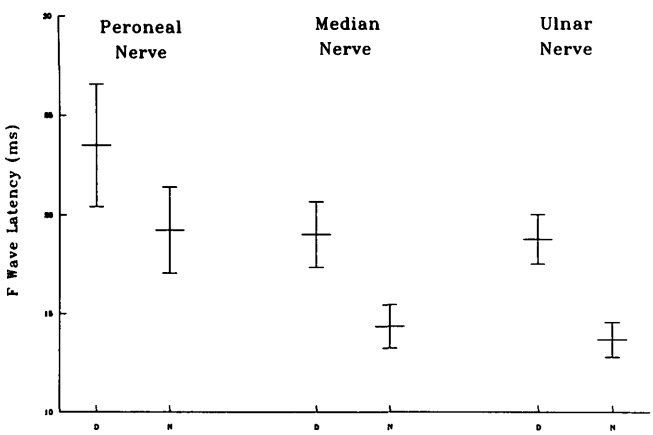

FIG. 2. F-wave latencies in each of 3 motor nerves for diabetic (D) and nondiabetic (N) rhesus monkeys. Values for D and N differ significantly in all 3 motor nerves (see Table 3). Values are means ± 2SE.

The exact mechanisms by which MNCV is reduced in diabetes are not known (20–24). Several hypotheses exist: *1*) increased levels of sorbitol and decreased levels of *myo*-inositol in nerve leading to altered phosphoinositide metabolism and subsequently to axoglial dysjunction (23,24) and reduction in conduction times along nerve fibers (25), *2*) thickening of endoneurial blood vessels leading to chronic hypoxia and nerve ischemia (21,26,27), and *3*) nonenzymatic glycosylation in nerve tissue accelerated by hyperglycemia (28). Further studies are needed to determine the precise pathogenesis of diabetic neuropathy so that specific treatments can be developed (20).

Our study suggests that further investigation of the peripheral nervous system of the aging monkey with obesity-associated type II diabetes is warranted. Available experimental models of human diabetic neuropathy do not fully reproduce the human disease. Most models involve small growing laboratory animals (mainly rats) made diabetic by alloxan or streptozocin injection or by genetic predisposition (2). The aging monkeys described here offer several advantages for study: they have characteristics similar to those found in humans with obesity-associated type II diabetes (5,15), they are a good model on which to perform longitudinal serial studies with mature animals, and complications such as hyperlipidemia and atherosclerotic plaques are known to occur in this model (7).

The precise pathology that underlies the nerve conduction changes in this and other studies of diabetes is uncertain. This uncertainty in part relates to the stage of the disease and the conduction abnormality present at that time. Early in the course of diabetes, conduction velocity is below that of control values (29), but it can be corrected rapidly with insulin therapy. Reduced conduction velocity presumably represents a metabolic defect, possibly resulting in nodal and/or paranodal swelling, because it is easily reversible (35). With diabetes of longer duration, conduction velocities continue to fall, reflecting either loss of the largest myelinated fibers or changes in myelin surrounding nerve fibers (36).

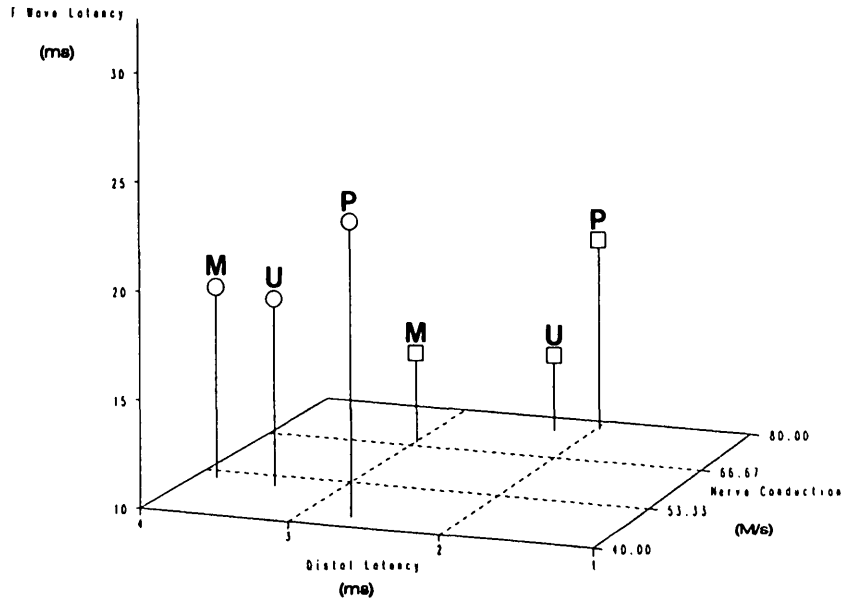

FIG. 4. Three-dimensional plot of motor nerve conduction velocity, motor distal latency, and F-wave latency for each motor nerve (P, peroneal; M, median; U, ulnar) for diabetic (○) and nondiabetic (□) rhesus monkeys.

Correlative pathological studies of our monkeys would be of interest.

Substantial evidence suggests that hyperglycemia plays an important role in the pathogenesis of diabetic neuropathy (37). A continuing question has been the effect of insulin deficiency on the process. Regression analysis of MNCV data did not reveal a significant relationship to IRI, yet confirmed the relationship between MNCV and FPG. Thus, our data do not support a primary role for insulin deficiency in the conduction abnormalities seen in diabetes.

In summary, we demonstrated that the aging diabetic monkey has prolonged motor nerve conduction times compared with nondiabetic animals. The degree of reduction of MNCV was inversely correlated with FPG but was not related to IRI or K_G. Our studies suggest that aging diabetic monkeys, previously shown to be an excellent primate model of obesity-associated type II diabetes, develop peripheral neuropathy early after the onset of hyperglycemia, which is similar to that seen in human diabetes and which may serve as a model of human diabetic neuropathy.

ACKNOWLEDGMENTS

The collaborative effort of Dr. N. Bodkin and the staff of the Obesity and Metabolism Research Laboratory, University of Maryland, is gratefully acknowledged for the metabolism studies that led to this work. Dr. Pamela Talalay provided expert editorial assistance. We thank Dr. E. David Mellits for statistical advice. Rod Graham prepared the manuscript.

This study was supported by National Institutes of Health Grant DK-37717.

REFERENCES

1. Pirart J: Diabetes mellitus and its degenerative complications: a prospective study of 4,400 patients observed between 1947 and 1973. *Diabetes Care* 1:168–88, 252–63, 1978
2. Sharma AK, Thomas PK: Animal models: pathology and pathophysiology. In *Diabetic Neuropathy*. Dyck PJ, Thomas PK, Asbury AK, Winegrad AJ, Porte D, Eds. Philadelphia, PA, Saunders, 1987, p. 237–52
3. Gregersen G: Diabetic neuropathy: influence of age, metabolic control and duration of diabetes on motor conduction velocity. *Neurology* 17:972–80, 1967
4. Cornblath DR, Dellon AL, MacKinnon SE: Spontaneous diabetes mellitus in a rhesus monkey: neurophysiological studies. *Muscle Nerve* 12:233–35, 1989
5. Hansen BC, Bodkin NL: Heterogeneity of insulin responses: phases leading to type II (non-insulin dependent) diabetes mellitus in the rhesus monkey. *Diabetologia* 29:713–19, 1986
6. Jen K-LC, Hansen BC: Glucose disappearance rate in rhesus monkeys: some technical considerations. *Am J Primatol* 14:153–66, 1988
7. Hannah JS, Verdery RB, Bodkin NL, Hansen BC: Changes in lipoproteins during the development of NID diabetes in Rhesus monkeys. In *Lessons From Animal Diabetes II, 2nd Int. Workshop*. London, Libby, 1988, p. 321
8. Hansen BC, Jen K-LC, Pek SB, Wolfe RA: Rapid oscillations in plasma insulin, glucagon, and glucose in obese and normal weight humans. *J Clin Endocrinol Metab* 54:785–92, 1982
9. Kimura J: *Electrodiagnosis in Diseases of Nerve and Muscle: Principles and Practice*. Philadelphia, PA, Davis, 1983
10. The DCCT Research Group: Factors in development of diabetic neuropathy: baseline analysis of neuropathy in feasibility phase of diabetes control and complications trial (DCCT). *Diabetes* 37:476–81, 1988
11. Norris AH, Shock NW, Wagman IH: Age changes in maximum conduction velocities of motor fibers of human ulnar nerves. *J Appl Physiol* 5:589–93, 1953
12. *SAS/STAT User's Guide. Release 6.03 Ed.* Cary, NC, SAS, 1988
13. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 28:1039–57, 1979
14. World Helath Organization: *Diabetes Mellitus, Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser., no. 727)
15. Bodkin NL, Metzger BL, Hansen BC: Hepatic glucose production and insulin sensitivity preceding diabetes in monkeys. *Am J Physiol* 256:E676–81, 1989
16. Graf RJ, Halter JB, Halar E, Porte D: Nerve conduction abnormalities in untreated maturity-onset diabetes: relation to levels of fasting plasma glucose and glycosylated hemoglobin. *Ann Intern Med* 90:298–303, 1979
17. Graf RJ, Halter JB, Pfeifer MD, Halar E, Brozovich F, Porte D Jr: Glycemic control and nerve conduction abnormalities in noninsulin-dependent diabetic subjects. *Ann Intern Med* 94:307–11, 1981
18. Eliasson SG: Nerve conduction changes in experimental diabetes. *J Clin Invest* 43:2353–58, 1964
19. Gallai V, Firenze C, Mazzotta G, Del Gatto F: Neuropathy in children and adolescents with diabetes mellitus. *Acta Neurol Scand* 78:136–40, 1988
20. Asbury AK: Understanding diabetic neuropathy. *N Engl J Med* 319:577–78, 1988
21. Johnson PC, Doll SC, Cromey DW: Pathogenesis of diabetic neuropathy. *Ann Neurol* 19:450–57, 1986
22. Greene DA, Lattimer SA, Sima AAF: Sorbitol, phosphoinositides, and sodium-potassium ATPase in the pathogenesis of diabetic complications. *N Engl J Med* 316:599–606, 1987
23. Greene DA, Lattimer SA, Sima AAF: Are disturbances of sorbitol, phosphoinositide, and Na^+-K^+-ATPase regulation involved in pathogenesis of diabetic neuropathy? *Diabetes* 37:688–93, 1988
24. Greene DA, Lattimer SA, Sima AAF: Pathogenesis and prevention of diabetic neuropathy. *Diabetes Metab Rev* 4:201–21, 1988
25. Brismar T, Sima AAF, Greene DA: Reversible and irreversible nodal dysfunction in diabetic neuropathy. *Ann Neurol* 21:504–507, 1987

26. Low PA, Schmelzer JD, Ward KK: Experimental chronic hypoxic neuropathy: relevance to diabetic neuropathy. *Am J Physiol* 250:E94–99, 1986
27. Yasuda H, Dyck PJ: Abnormalities of endoneurial microvessels and sural nerve pathology in diabetic neuropathy. *Neurology* 37:20–28, 1987
28. Brownlee M, Cerami A, Vlassara H: Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N Engl J Med* 318:1315–21, 1988
29. Daube JR: Electrophysiological testing in diabetic neuropathy. In *Diabetic Neuropathy*. Dyck PJ, Thomas PK, Asbury AK, Winegrad AJ, Porte D, Eds. Philadelphia, PA, Saunders, 1987, p. 162–76
30. Fraser DM, Campbell IW, Ewing DJ, Murray A, Neilson JMM, Clarke BF: Peripheral and autonomic nerve function in newly diagnosed diabetes mellitus. *Diabetes* 26:546–50, 1977
31. Gregersen G: Variations in motor conduction velocity produced by acute changes of the metabolic state in diabetic patients. *Diabetologia* 4:273–77, 1968
32. Ward JD, Barnes CG, Fisher DJ, Jessop JD, Baker RWR: Improvement in nerve conduction following treatment in newly diagnosed diabetics. *Lancet* 1:428–31, 1971
33. Greene DA, DeJesus PV, Winegrad AI: Effects of insulin and dietary *myo*-inositol on impaired peripheral motor nerve conduction velocity in acute streptozotocin diabetes. *J Clin Invest* 55:1326–36, 1975
34. Jakobsen J: Early and preventable changes of peripheral nerve structure and function in insulin-deficient diabetic rats. *J Neurol Neurosurg Psychiatry* 42:509–18, 1979
35. Sima AAF, Brismar T: Reversible diabetic nerve dysfunction: structural correlates to electrophysiological abnormalities. *Ann Neurol* 18:21–29, 1985
36. Sima AAF, Lattimer SA, Yagihashi S, Greene DA: Axo-glial dysjunction: a novel structural lesion that accounts for poorly reversible slowing of nerve conduction in the spontaneously diabetic Bio-breeding rat. *J Clin Invest* 77:474–84, 1986
37. Dyck PJ, Brown M, Greene D, Jakobsen J, Pfeifer M, Porte D, Thomas PK: Does improved control of glycemia prevent or ameliorate diabetic polyneuropathy? *Ann Neurol* 19:288–90, 1986