The Effects of Acetyl-L-Carnitine and Sorbinil on Peripheral Nerve Structure, Chemistry, and Function in Experimental Diabetes

John I. Malone, Saul Lowitt, Angela F. Salem, Candy Miranda, Jan K. Korthals, and Jane Carver

Nerve conduction velocity (NCV) increased with age in nondiabetic male Wistar rats for the first 26 weeks of life. The NCV of animals made hyperglycemic at age 6 weeks by administration of streptozotocin (STZ) also increases, but at a slower rate. Animals with 4 weeks of hyperglycemia and reduced NCV treated with an aldose reductase inhibitor (sorbinil) or a short-chain acyl-carnitine (acetyl-L-carnitine [ALC]) daily for 16 weeks showed an improvement in NCV. Morphometric studies of tibial nerves collected from animals after 20 weeks of hyperglycemia (age 26 weeks) showed a consistent reduction in the width of the myelin sheath and little change in axon area. The number of large myelinated fibers (>6.5 μm) found in nerves collected from hyperglycemic animals was less than the number found in nondiabetic animals. Treatment of hyperglycemic rats with either sorbinil or ALC was associated with increased NCV, myelin width, and large myelinated fibers. The apparent metabolic effect of these agents was similar for fatty acid metabolism, but different for polyol pathway activity. We conclude that in animals hyperglycemic long enough to slow NCV, sorbinil and/or ALC treatment reduces the functional, structural, and biochemical changes associated with hyperglycemia that occur in the myelin sheath.

EXPERIMENTAL DIABETES reduces the conduction velocity of peripheral nerves.[1,2] Reduced nerve conduction velocity (NCV) has been associated with paranodal axon swelling 3 weeks after induction of diabetes in the Bio-Breeding (BB) rat.[3] More permanent impairment of NCV was noted after irreversible axo-glial dysjunction was documented at 26 weeks of diabetes.[4] Rats with streptozotocin (STZ)-induced diabetes first show reduced myelin width after 6 weeks of hyperglycemia.[5] These structural abnormalities have been associated with increased activity of the polyol pathway and prevented with administration of an aldose reductase inhibitor.[6] Increased polyol pathway activity is deduced from the accumulation of sorbitol and fructose in peripheral nerves, which are freely permeable to the elevated glucose concentrations of diabetes mellitus. It has also been noted that increased nerve sorbitol is associated with reduced concentrations of inositol and taurine in nerves.[2,7] Aldose reductase inhibitors correct the biochemical abnormalities of sorbitol and inositol[2] in conjunction with improved NCV. The structural abnormalities associated with diabetes mellitus also improve in laboratory animals[3] and in humans[8] taking aldose reductase inhibitors. Abnormalities in fatty acid metabolism have been noted in association with diabetes mellitus.[9-11] Low levels of arachidonic acid have been reported in neuronal tissues from animals with diabetic neuropathy.[12] Cameron et al[13] have reported that an increase in plasma arachidonic acid levels is associated with prevention of nerve conduction deficits in STZ diabetic rats.[13]

Acetyl-L-carnitine (ALC) enhances regeneration of rat sciatic nerve after transection and microsurgical repair.[14] ALC has also been shown to improve NCV in STZ diabetic rats.[15,16] We document the functional, morphologic, and biochemical changes that occur in association with long-standing (20 weeks' duration) STZ diabetes, and evaluate the effects of treatment with either sorbinil or ALC on nerve function, structure, and metabolism in animals that were hyperglycemic 4 weeks before starting therapy.

MATERIALS AND METHODS

The animal experiments reported herein were reviewed and approved by the Laboratory and Animal Medicine Committee of the University of South Florida College of Medicine. The animals were housed and cared for in facilities built and managed according to the *Guide for the Care and Use of Laboratory Animals* (Department of Health, Education, and Welfare publication no. NIH 85-23).

Induction of Hyperglycemia

Fasted male Wistar rats (Charles River, Wilmington, MA) weighing 150 to 200 g were made diabetic by intraperitoneal (IP) injection of STZ 50 mg/kg (Upjohn Laboratories, Kalamazoo, MI) in cold buffered sodium citrate (pH 4.5). Seven days after STZ injection, tail blood was collected from nonfasting rats, and glucose content was measured with an Ames Glucometer 2 (Miles Laboratories, Elkhart, IN). Hyperglycemia was defined as plasma glucose greater than 16.7 mmol/L; subsequent tests for hyperglycemia were performed every 4 weeks, and glycosylated hemoglobin (HbA_{1C}) was determined at the end of the study. Ultra Lente insulin (Eli Lilly & Co, Indianapolis, IN) 3 to 5 U/kg was injected subcutaneously 3 times per week at a dose designed to prevent weight loss. The insulin dose (units per kilogram) was determined weekly based on the mean weight of all STZ diabetic rats. Thus, all diabetic rats received the same insulin dose based on body weight. This dose ensured weight gain (Fig 1) but did not normalize blood glucose, as indicated by the HbA_{1C} values at the end of each experiment (Table 1). Animals were provided free access to food and water.

Food-Limited Animals

Six nondiabetic rats were weighed daily and provided a limited number of food pellets so that their body weight was identical to that of the age-matched hyperglycemic rats.

Four weeks after STZ injection, hyperglycemic animals were

From the Diabetes Center and the Departments of Pediatrics and Neurology, University of South Florida, Tampa, FL.

Submitted September 8, 1995; accepted December 27, 1995.

Supported in part by grants from The Health Program Office, Health and Rehabilitative Services, State of Florida, and Sigma Tau s.p.a. Rome, Italy.

Address reprint requests to John I. Malone, MD, USF College of Medicine, 12901 Bruce B. Downs Blvd, MDC Box 45, Tampa, FL 33612-4799.

0026-0495/96/4507-0019$03.00/0

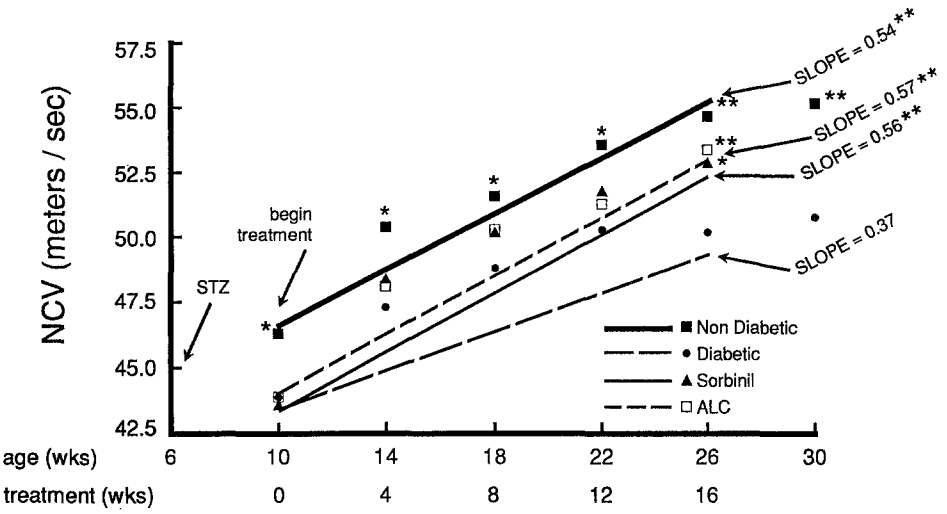

Fig 1. NCV progression for nondiabetic and diabetic rats that were untreated or treated with sorbinil or ALC. *$P < .01$ *v* age-matched diabetic rats. **$P < .005$ *v* age-matched diabetic rats.

divided into three groups for 16 weeks of daily IP injections of (1) ALC 300 mg/kg, (2) sorbinil 40 mg/kg, or (3) vehicle alone.

Biochemical Measurements

HbA_{1C} was assayed by affinity chromatography using a Glyc-Affin column (Isolab, Akron, OH). Sciatic nerve sorbitol and inositol levels were measured by gas-liquid chromatography of acetate derivatives as previously described.[15]

Total lipids were extracted from nerve homogenates by the method of Folch et al.[17] Solvents were glass-distilled with butylated hydroxytoluene (50 mg/L) added to methanol as an antioxidant. Fatty acids were methylated according to the procedure of Morrison and Smith.[18] Fatty acid methyl esters were separated and quantified by gas-liquid chromatography on a Hewlett Packard model 5890 gas chromatograph using a 30-m × 0.25-mm capillary column with a 0.2-μm stationary phase of SP.2330 (Supelco, Bellefonte, PA). Helium was used as the carrier gas, and the temperature was increased from 50°C to 150°C at 20°/min, and then to 210°C at 2°/min. Fatty acids were identified by comparison to retention times established for authentic standards (Nu-Check Prep, Elysian, MN). This qualitative analytical method permits an estimate of the percentage of each fatty acid in a nerve total lipid extract.

Nerve Conduction Studies

NCV was measured for each rat 4 weeks after STZ treatment and at monthly intervals thereafter. The animals were anesthetized with sodium pentobarbital (60 mg/kg IP). Rear-limb temperature was maintained at 36°C with a radiant heater controlled by a needle thermistor placed subcutaneously in the area of the gastrocnemius muscle. Stimulating needle electrodes were inserted close to the sciatic nerve at the sciatic notch (for the "proximal latency" measurement) and the tibial nerve at the ankle (for the "distal latency" measurement). Subcutaneous recording electrodes were placed transversely over the plantar intrinsic foot muscles. Stimuli were supramaximal (10 to 20 V) square-wave pulses of 0.05 milliseconds' duration. The rear limb was extended, and the distance between the two points of stimulation was measured. Proximal and distal latencies were measured to the negative peak of the compound muscle action potentials, and conduction velocities were calculated. NCVs reported herein were determined on each of the animals used for the biochemical measures reported in Table 1.

Morphometry

Animals were anesthetized with sodium pentobarbital 100 mg/kg, and the tibial nerve in the leg opposite that used for NCV was exposed and fixed in situ with 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer for 20 minutes. The middle segment of the tibial nerve was then dissected and immersed in 2.5% buffered glutaraldehyde overnight at 4°C. Transverse samples were taken from the nerve and postfixed with 1% chrome osmium tetroxide.

Morphometric studies were performed on 0.75-μm epoxy-embedded sections stained with toluidine blue. Slides were positioned on the stage of an Olympus (Tokyo, Japan) model BH-2 microscope, and the image was projected through a Panasonic (Osaka, Japan) WV 1500-X television camera to a computer display. Tracings of the outlines of axons and their myelin sheaths were made using a Hipad digitizer (Houston Instrument, Austin, TX) under a final magnification of ×2,500. These tracings were analyzed for myelinated-fiber diameter, myelin width, and axon area by a computer software package (Frederick Haer & Co,

Table 1. Sciatic NCV (m/s)

	Animal Age (wk)				
	10	14	18	22	26
Diabetes duration (wk)	4	8	12	16	20
Treatment (wk)	0	4	8	12	16
Nondiabetic (n = 12)	46.3 ± 0.5*	50.4 ± 0.6*	51.6 ± 0.5*	53.6 ± 0.5*	54.7 ± 0.5†
Diabetic (n = 12)	43.9 ± 0.6	47.3 ± 0.4	48.8 ± 0.5	50.3 ± 0.6	50.2 ± 0.5
Diabetic + sorbinil (n = 12)	43.6 ± 0.4	48.4 ± 0.4	50.2 ± 0.03	51.8 ± 0.5	52.9 ± 0.03*
Diabetic + ALC	43.9 ± 0.5	48.1 ± 0.4	50.3 ± 0.5	51.3 ± 0.5	53.4 ± 0.5†

*$P < .05$ *v* diabetic.

†$P < .01$ *v* diabetic.

Brunswick, ME). The number of large myelinated fibers (LMF; >6.5 μm) or small myelinated fibers (SML; <6.5 μm) fibers per nerve was determined by counting the fibers in a number of regularly spaced frames (containing ≥ 600 fibers per frame) within each nerve according to a method described by Dyck et al.[19]

Electron microscopic morphometry was performed on three diabetic and three nondiabetic rats. Measurements were made on photographic enlargements (×49,400 and ×13,650) of 50 myelinated fibers encountered serially in transverse nerve sections examined under an electron microscope. Using a programed morphometric system, tracings of the outlines of axons and their myelin sheaths were made and axon area and myelin width were calculated. The number of major dense lines of myelin were counted through a dissecting microscope.

Statistics

The data are expressed as the mean ± SEM. Differences among three or more means were tested by ANOVA (PROC GLM; SAS Institute, Cary, NC) coupled with the least-significant difference multiple-range test. Differences in slopes of straight lines representing the progression of NCV over time were analyzed by analysis of covariance. Repeated-measures ANOVA was also performed with PROC GLM. Data analyses were performed using SAS-PC (SAS Institute).

RESULTS

Rats used in this experiment were 6 weeks old at the time of STZ injection. As nondiabetic animals grew older, NCV increased at a rate of 0.54 m/s/wk. The NCV of rats hyperglycemic for 4 weeks was reduced (Table 1), and increased at a slower rate of 0.37 m/s/wk ($P < .005$; Fig 1). Rats with STZ-induced hyperglycemia of 4 weeks' duration began treatment with either ALC or sorbinil, which was continued for 16 weeks. The NCV of diabetic animals when treated with ALC (0.57 m/s/wk) and/or sorbinil (0.56 m/s/wk) (Fig 1) increased at a rate similar to the nondiabetic rate. Repeated-measures ANOVA showed that the increasing NCVs of nondiabetic and diabetic animals treated with either ALC or sorbinil were greater than the NCVs found in untreated diabetic animals (.37 m/s/wk, $P < .005$). Diabetic animals required 16 weeks of treatment with sorbinil or ALC to attain NCVs significantly greater than those found in untreated diabetic rats (Table 1).

Body weights of food-limited nondiabetic rats were the same as those of the hyperglycemic age-matched littermates (Fig 2). The progression of NCV in food-limited nondiabetic rats (0.58 m/s/wk) did not differ from that in the nondiabetic (fed ad libitum) age-matched littermates (Fig 3).

The morphometry of tibial nerves collected from diabetic animals showed no changes in axon area, but myelin width was significantly reduced (Table 2). Morphologic results determined by light microscopy were confirmed by electron microscopy comparing sections from the same nerve. Myelin width in hyperglycemic rats (0.91 ± 0.06 μm) was less than that found in age-matched nondiabetic animals (1.16 ± 0.02 μm, $P < .01$). This reduction in myelin substance was confirmed by the number of myelin lamellae counted on sections magnified by electron microscopy (diabetic, 57.9 ± 2.2, n = 3; nondiabetic, 70.8 ± 2.3, n = 3; $P < .005$).

A greater number of myelinated fibers less than 6.5 μm in diameter and fewer fibers greater than 6.5 μm were found in nerve fascicles from STZ diabetic rats versus nondiabetic animals (Table 2).

Treatment with either ALC or sorbinil for 16 weeks was associated with increased myelin width and a greater percentage of large myelinated fibers (Table 2). Morphometric analysis of axon area and myelin width in food-limited nondiabetic rats showed no difference when compared with nerves from age-matched nondiabetic (fed ad libitum) rats (Table 2).

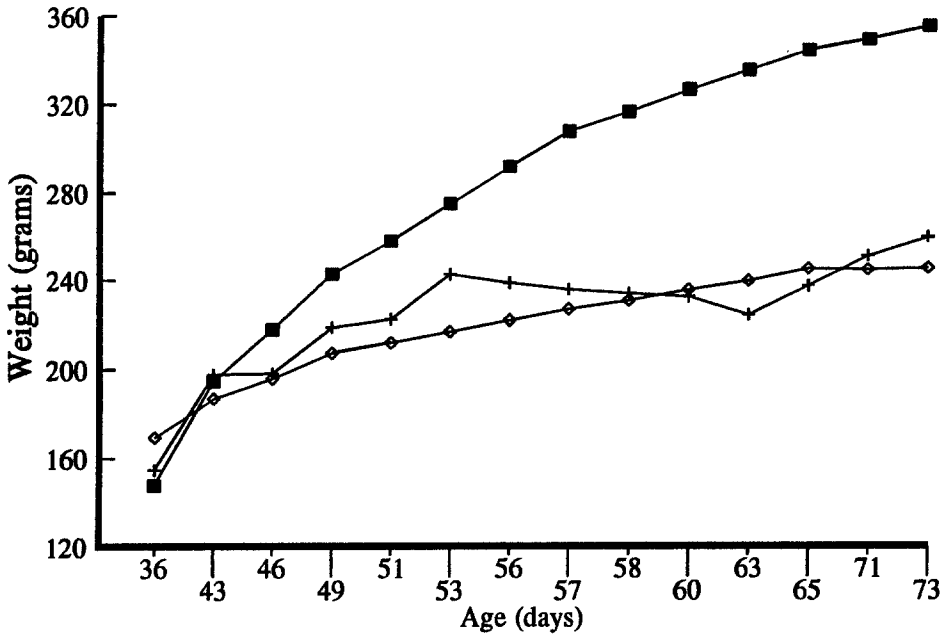

Fig 2. Progression of body weight in nondiabetic rats fed ad libitum (■), nondiabetic food-limited rats (+) and diabetic rats (◇).

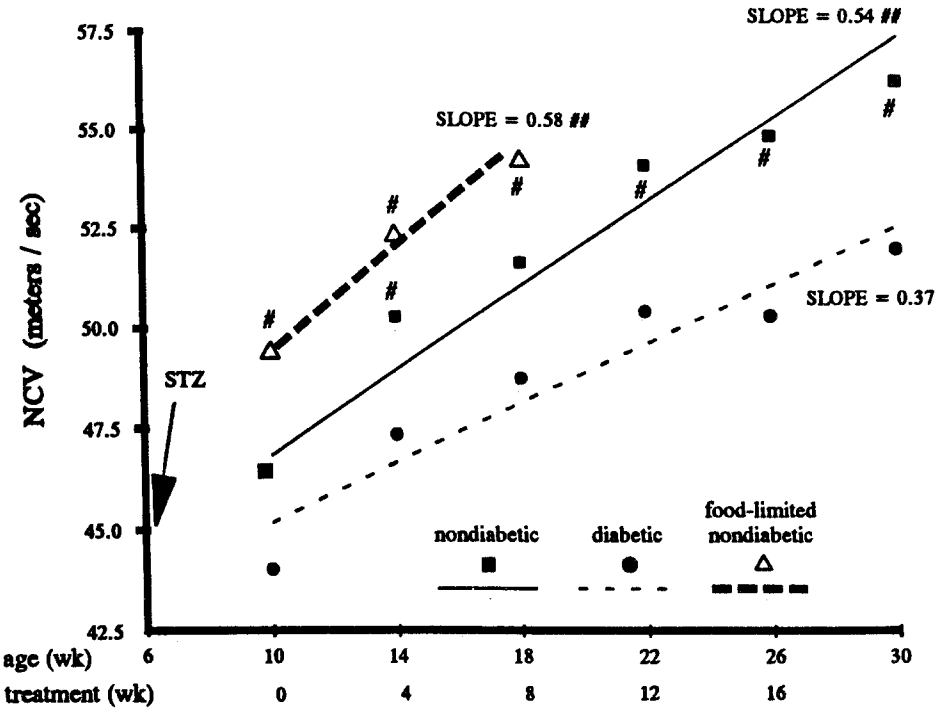

Fig 3. NCV progression for nondiabetic rats fed ad libitum, nondiabetic food-limited rats, and diabetic rats. #$P < .01$ *v* age-matched diabetic rats. ##$P < .005$ *v* age-matched diabetic rats.

Sciatic nerve sorbitol was increased but nerve inositol was not reduced in untreated diabetic animals that were hyperglycemic for 20 weeks (Table 2). Diabetic animals treated with sorbinil had nerve sorbitol and inositol levels not different from those in control animals. Nerve sorbitol and inositol levels were unchanged in diabetic animals treated with ALC versus untreated diabetic animals. There was no apparent change in inositol levels in any diabetic rats. The fatty acid content of sciatic nerves indicated an increase in linoleic acid in STZ diabetic animals. This, coupled with a slight reduction in arachidonic acid, resulted in an increase in the linoleic/arachidonic acid ratio in diabetic animals, which was corrected by both sorbinil and ALC.

DISCUSSION

Wistar rat sciatic NCV continues to increase for the first 30 weeks of life (Fig 1). The rate of increase was measured at 4-week intervals from 10 to 26 weeks of age in STZ diabetic animals. The greatest increase was noted in both control and diabetic animals from 10 to 14 weeks. The rate of increase changed little from 14 to 26 weeks, and then began to slow. This reflects the functional maturation of the sciatic nerve. During the course of this experiment, NCV in

Table 2. 26-Week-Old Wistar Rat Tibial Nerve Morphometry With and Without 20 Weeks of STZ Diabetes

	Nondiabetic		Diabetic		
Parameter	Ad Libitum–Fed	Food-Limited	Untreated	+Sorbinil	+ALC
No. of rats	12	6	12	12	18
HbA_{1c} (%)	5.8 ± 0.2	ND	16.7 ± 0.3*	14.7 ± 0.5*	14.6 ± 0.4*
Nerve sorbitol (nm/mg dry weight)	0.63 ± 0.1	ND	3.6 ± 0.97*	.53 ± 0.17	3.9 ± 0.27*
Nerve inositol (nm/mg dry weight)	6.14 ± 0.53	ND	5.96 ± 0.30	5.58 ± 0.37	6.97 ± 0.41
Axon area (μm^2)	14.75 ± 0.38	15.13 ± 0.04	14.0 ± 0.44	14.9 ± 0.39	14.22 ± 0.28
Myelin width (μm)	1.16 ± 0.02‡	1.12 ± 0.02‡	0.95 ± 0.02*	1.03 ± 0.02*‡	1.05 ± 0.02*‡
Linoleic acid (%)	5.2 ± 0.45	—	7.2 ± 0.71*	4.6 ± 0.33	5.4 ± 0.69
Arachidonic acid (%)	2.6 ± 0.2	—	2.2 ± 0.08	2.3 ± 0.3	2.6 ± 0.4
L/A	2.24 ± 0.24	—	3.5 ± 0.42*	1.9 ± 0.16	2.6 ± .45
LMF per nerve (%)	2,015 ± 51 (56)‡	2,059 ± 113 (60)‡	1,353 ± 91 (39)*	1,566 ± 78 (49)†	1,582 ± 64 (48)†
SMF per nerve (%)	1,573 ± 44 (44)	1,388 ± 91 (40)	2,107 ± 106 (61)*	1,658 ± 72 (51)‡	1,704 ± 68 (52)‡
Index of circularity	0.90 ± 0.01	0.87 ± 0.01	0.88 ± 0.01	0.90 ± 0.01	0.89 ± 0.01
Weight change (wt 26 wk − 6 wk)	438 ± 6	198 ± 12	216 ± 7	206 ± 12	205 ± 10

Abbreviations: ND, not determined; L/A, ratio of linoleic to arachidonic acid.

*$P < .01$ *v* nondiabetic rats.

†$P < .05$ *v* diabetic rats.

‡$P < .01$ *v* diabetic rats.

nondiabetic rats increased at a rate of 0.54 m/s/wk. A group of animals (n = 42) had hyperglycemia induced with STZ. Each of these animals received the same dose of insulin for the next 16 weeks. Twelve of these animals received no other treatment, and NCV increased at a slower rate (0.37 m/s/wk, $P < .005$). This suggests that STZ-induced hyperglycemia impairs but does not stop the maturational development of peripheral nerve function. STZ diabetic rats fail to gain weight and grow (Fig 2) because of restricted calorie utilization caused by insulin insufficiency. We included in this study a group of nondiabetic animals that were age- and weight-matched (by calorie restriction) to the STZ diabetic rats to show the effect of reduced nutrition without hyperglycemia on developing peripheral nerve structure and function. Since no difference in either NCV (Fig 3) or morphometry (Table 2) was noted in food-limited nondiabetic animals, it appears that the pathology demonstrated in the STZ rat is related to hyperglycemia rather than to compromised nutrition.

Daily ALC or sorbinil treatment of rats that were hyperglycemic for 4 weeks with reduced NCV was associated with an evolution of NCV greater than that found in untreated diabetic rats (Fig 1). This improvement in nerve functional maturation did not result in an absolute increase in NCV until the rats had been treated for 16 weeks (Table 1). This required a "catch up" in the delayed nerve maturation acquired during the unmodified hyperglycemia that occurred between 6 and 10 weeks of age. This differs from the apparent normalization of NCV in STZ diabetic rats treated with other agents for a shorter time but starting at the initiation of hyperglycemia.[15,16] This indicates that earlier treatment may prevent the toxic effect of hyperglycemia.

Although STZ-induced hyperglycemia was decreased in sorbinil- and ALC-treated animals, as indicated by HbA_{1C} values at the end of the experiment (Table 1), it was significantly greater than the nondiabetic mean level ($P < .05$). Sciatic nerve sorbitol content, believed to have some influence on peripheral neuropathy, was reduced in STZ diabetic rats treated with sorbinil but not in STZ diabetic rats treated with ALC. This suggests that the apparent reduction of HbA_{1C} in STZ diabetic rats treated with ALC was not sufficient to reduce the activity of the polyol pathway—a glucose metabolic pathway believed to be important in the pathogenesis of diabetic neuropathy. Nerve inositol was unchanged in all of the diabetic animals, which has been observed by others in animals with long-standing hyperglycemia.[20] Sorbinil and ALC had no effect on myositol levels, which indicates that the reduced NCV of diabetes that increased in association with both sorbinil and ALC is not primarily influenced by nerve inositol content.

Peripheral nerve dysfunction has been linked to morphologic changes. Axonal atrophy has been reported to be associated with reduced NCV.[21] Axon atrophy can be recognized morphologically as a reduction of the index of circularity.[22] Atrophic axons may have reduced areas, but shrinkage is manifest by a reduction of axon circularity. Axonal atrophy may represent tissue injury caused by biochemical changes resulting from hyperglycemia or axon shrinkage in response to tissue dehydration[23] commonly found in diabetic animals unable to replace the massive fluid loss associated with glucosuria. The axon area and index of circularity of nerves from diabetic animals did not differ from those found in nondiabetic control animals. The state of hydration of our animals may explain why we did not see axon atrophy or shrinkage. The STZ diabetic animals in this study, although hyperglycemic, gained weight and did not appear clinically dehydrated. Indeed, the greater number of small myelinated nerve fibers less than 6.5 μm found in diabetic nerve fascicles indicates maturational arrest, since the axon area of these 26-week-old animals did not differ from that of nondiabetic control animals but the myelin width was reduced.[24]

Animals hyperglycemic for 4 weeks and manifesting reduced NCV were treated for 16 weeks with either sorbinil or ALC. Functional improvement in NCV was documented by the rate of increase of NCV during the 16 weeks of treatment (with either sorbinil or ALC). NCV of treated animals became significantly greater than that of untreated diabetic animals after 16 weeks of treatment. The first 4 weeks of unmodified hyperglycemia produced a significant reduction in NCV, which required 16 weeks of improving function to demonstrate an absolute increase in NCV. This is consistent with observations made on the BB rat comparing early and late initiation of therapy.[4] During the interval of improving function (NCV), there was an apparent increase in myelin width and the number of LMF. Each of these agents (sorbinil and ALC) apparently promoted tibial nerve function and structural maturation in the face of hyperglycemia. The increased myelin width suggests a mechanism that influences development of the myelin sheath.

Sorbinil has been reported[3] to correct morphologic and functional abnormalities associated with experimental diabetes. This effect has been attributed to reducing the activity of the polyol pathway, documented in this report by reduced nerve sorbitol levels.

We have previously reported a reduction (not normalization) of nerve sorbitol in STZ diabetic rats treated with ALC.[15] Our more recent experience[16] and that of others[24] have not shown a reduction of nerve sorbitol in diabetic animals treated with ALC. Our earlier report[15] did not show any reduction in erythrocyte sorbitol, another indicator of tissue polyol pathway activity in STZ diabetic rats,[25] in animals treated with ALC. Hyperglycemic animals in the current study treated with ALC for 16 weeks did not show any reduction in nerve sorbitol. This collective evidence indicates that ALC does not reduce polyol pathway activity in tissues of hyperglycemic animals. However, ALC treatment of STZ diabetic animals in this study was associated with increased tibial nerve function and myelin width.

Fatty acid abnormalities noted by others[13] in the sciatic nerve of diabetic animals were also noted in these animals. The normalization of linoleic and arachidonic acids in diabetic animals treated with ALC and/or sorbinil suggests that these agents have a common mechanism involving fatty

acid metabolism and possibly myelin development, since myelin is largely a lipid tissue. The observations in this study do not indicate whether this mechanism is a direct or indirect influence on fatty acid metabolism. More studies will be required to clarify this issue.

In summary, rats that become hyperglycemic during maturation of peripheral nerve function have reduced NCV and smaller nerves with reduced myelin width and normal axon area. These same nerves manifest a defect in fatty acid metabolism, noted as an increase in the linoleic/arachidonic acid ratio, indicating a block in the conversion of linoleic acid to arachidonic acid. This has been noted by others,[13] who have demonstrated normalization of peripheral nerve function by artificially increasing arachidonic acid with oral supplementation. This study shows that treatment of STZ diabetic rats that have a reduced NCV with an aldose reductase inhibitor and/or ALC (which has no effect on aldose reductase activity) promotes improved peripheral nerve function (NCV) and correction of the nerve linoleic to arachidonic acid ratio. This effect was noted in association with increased myelin width, indicating an influence on lipid metabolism. This suggests that changes in the myelin sheath possibly influenced by altered fatty acid metabolism may play an important role in the pathogenesis of diabetic neuropathy.

ACKNOWLEDGMENT

We thank Lewis A. Barness for reviewing the manuscript, N.J. Hutson, Pfizer Central Research (Groton, CT) for providing sorbinil, and Thomas R. Klobucar, Upjohn Laboratories (Kalamazoo, MI), for providing STZ (Zanosar).

REFERENCES

1. Eliasson SG: Nerve conduction changes in experimental diabetes. J Clin Invest 43:2353-2358, 1964

2. Greene DA, Lattimer SA: Action of sorbinil in diabetic peripheral nerve: Relationship of polyol (sorbitol) pathway inhibition to a *myo*-inositol–mediated defect in sodium-potassium ATPase activity. Diabetes 33:712-716, 1984

3. Sima AAF, Prashar A, Zhang W-X, et al: Preventive effect of long-term aldose reductase inhibition (Ponalrestat) on nerve conduction and sural nerve structure in the spontaneously diabetic Bio-Breeding rat. J Clin Invest 85:1410-1420, 1990

4. Brismar T, Sima AAF, Green DA: Reversible and irreversible nodal dysfunction in diabetic neuropathy. Ann Neurol 21:504-507, 1987

5. Thomas PK, Fraher JP, O'Leary D, et al: Relative growth and maturation of axon size and myelin thickness in the tibial nerve of the rat (effect of streptozotocin-induced diabetes). Acta Neuropathol 79:375-386, 1990

6. Gillon KRW, Hawthorne JN, Tomlinson DR: *Myo*-inositol and sorbitol metabolism in relation to peripheral nerve function in experimental diabetes in the rat: The effect of aldose reductase inhibition. Diabetologia 25:365-371, 1983

7. Malone JI, Harvey RC, Benford S, et al: Taurine deficiency may cause nerve dysfunction in streptozotocin diabetic rats. Diabetes Res Clin Pract 1:S356, 1985 (suppl 1, abstr)

8. Sima AAF, Bril V, Nathaniel V, et al: Regeneration and repair of myelinated fibers in sural nerve biopsy specimens from patients with diabetic neuropathy treated with sorbinil. N Engl J Med 319:548-555, 1988

9. Holman RT, Johnson SB, Gerrard JM, et al: Arachidonic acid deficiency in streptozotocin-induced diabetes. Proc Natl Acad Sci USA 80:2375-2379, 1983

10. Tilvia RS, Miettinen TA: Fatty acid composition of serum lipids, erythrocytes and platelets in insulin-dependent diabetic women. J Clin Endocrinol Metab 61:741-745, 1985

11. Dang AQ, Faas FH, Lee JA, et al: Altered fatty acid composition in the plasma, platelets and aorta of the streptozotocin-induced diabetic rat. Metabolism 37:1063-1072, 1988

12. Zhu X, Eichberg J: 1,2-Diacylglycerol content and its archidonyl-containing molecular species are reduced in sciatic nerve from streptozotocin-induced diabetic rats. J Neurochem 53:1087-1090, 1990

13. Cameron NE, Cotter MA, Robertson S: Essential fatty acid diet supplementation. Effect on peripheral nerve and skeletal muscle function and capillarization in streptozotocin-induced diabetic rats. Diabetes 40:532-539, 1991

14. Fernandez E, Pallini R, Gangitano C, et al: Studies on the degenerative and regenerative phenomena occurring after transection and repair of the sciatic nerve in rats: Effects of acetyl-L-carnitine. Int J Clin Pharmacol Res 10:85-99, 1990

15. Malone JI, Lowitt S, Corsico N, et al: Altered neuroexcitability in experimental diabetic neuropathy: Effect of acetyl-L-carnitine. Int J Clin Pharmacol Res 12:237-241, 1992

16. Lowitt S, Malone JI, Salem AF, et al: Acetyl-L-carnitine corrects the altered peripheral nerve function of experimental diabetes. Metabolism 44:677-680, 1995

17. Folch J, Lees M, Stanley GH: A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497-509, 1957

18. Morrison WR, Smith LM: Preparation of fatty acid methyl esters and dimethylacetals. J Lipid Res 5:600-608, 1964

19. Dyck PJ, Giannini C, Lais A: Pathological alterations of nerves, in Dyck PJ, Thomas PK, Griffin JW, et al: (eds): Peripheral Neuropathy. Philadelphia, PA, Saunders, 1993, pp 514-595

20. Yagikashis S, Kamijo M, Ido Y, et al: Effects of long-term aldose reductase inhibition on development of experimental diabetic neuropathy. Ultrastructural and morphometric studies of sural nerve in streptozotocin-induced diabetes rats. Diabetes 39:690-696, 1990

21. Jirmanova I: Giant axonopathy in streptozotocin diabetes of rats. Acta Neuropathol 86:42-48, 1993

22. Fraher JP, O'Leary D, Moran MA, et al: Relative growth and maturation of axon size and myelin thickness in the tibial nerve of the rat (normal animals). Acta Neuropathol 79:364-374, 1990

23. Dyck PJ, Lambert EH, Windebank AJ, et al: Acute hyperosmolar hyperglycemia causes axonal shrinkage and reduced nerve conduction velocity. Exp Neurol 71:507-514, 1981

24. Ido Y, McHowat J, Chang KC, et al: Neural dysfunction and metabolic imbalances in diabetic rats. Prevention by acetyl-L-carnitine. Diabetes 43:1469-1477, 1994

25. Malone JI, Leavengood H, Peterson MJ, et al: Red blood cell sorbitol as an indicator of polyol pathway activity. Inhibition by sorbinil in insulin-dependent diabetic subjects. Diabetes 33:45-49, 1984