Reduced Motor Nerve Conduction Velocity and Na^+-K^+-ATPase Activity in Rats Maintained on L-Fucose Diet

Reversal by *myo*-Inositol Supplementation

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L-Fucose is a monosaccharide that occurs in low concentrations in normal serum but has been shown to be increased in diabetic individuals. In cultured mammalian cells, L-fucose is a potent competitive inhibitor of *myo*-inositol transport. Abnormal *myo*-inositol metabolism has been proposed to be a factor in the development of diabetic complications. To test the hypothesis that *myo*-inositol deficiency may be responsible for the electrophysiological and biological defects in diabetic neuropathy, rats were fed a diet containing 10 or 20% L-fucose for a period of 6 wk. After 3 wk, the L-fucose diets in two groups of rats were supplemented with 1% *myo*-inositol. At the end of the study protocol, motor nerve conduction velocity, sciatic nerve tissue Na^+-K^+-ATPase activity, and *myo*-inositol content were determined. These results were compared with those of STZ-induced diabetic rats fed either a normal diet or a diet containing 1% *myo*-inositol or with those given 450 mg/kg body wt of sorbinil. Serum L-fucose levels were significantly increased in rats fed a diet containing 10 or 20% L-fucose. In comparison, the serum L-fucose levels in the diabetic rats were increased to a lesser extent. Motor nerve conduction velocity was significantly slower in rats fed a 10 or 20% L-fucose diet. Sciatic nerve composite and ouabain-sensitive Na^+-K^+-ATPase activity and *myo*-inositol content was also significantly decreased. Supplementation of 1% *myo*-inositol to the L-fucose-containing diet restored nerve *myo*-inositol levels and significantly improved Na^+-K^+-ATPase activity and motor nerve conduction velocity. In diabetic rats, similar changes were prevented by treatment with *myo*-inositol or sorbinil. These observations suggest that *myo*-inositol deficiency may be a major factor in the development of neural defects associated with acute diabetic neuropathy. *Diabetes* 42:1401–406, 1993

In diabetic animal models, the acute nerve conduction slowing has been linked to metabolic abnormalities associated with hyperglycemia (1–3). In peripheral nerve, the flux of glucose through the polyol pathway is increased, leading to sorbitol accumulation that is accompanied by decreased *myo*-inositol content and metabolism (4–6). Decreased Na^+-K^+-ATPase activity and slowing of nerve conduction velocity, two associated defects in the diabetic peripheral nerve, are prevented by treatment with ARIs or dietary *myo*-inositol supplementation (6–15), suggesting that a deficiency in *myo*-inositol metabolism may be responsible for these acute changes.

Hyperglycemia may cause a decrease in *myo*-inositol uptake by various mechanisms. In neural preparations and cultured cells, increased glucose concentrations have been shown to competitively inhibit *myo*-inositol transport (16–18). In cultured mammalian cells, glucose has also been shown to cause a noncompetitive inhibition of *myo*-inositol transport by a mechanism reversible by ARIs (19–23). Being an intracellular osmolyte, *myo*-inositol may be depleted reciprocally to increased intracellular sorbitol levels consequent to activation of the polyol pathway by hyperglycemia (24). Decreased *myo*-inositol metabolism may lead to abnormal phosphoinositide metabolism, contributing to decreased neural Na^+-K^+-ATPase activity (13,25). Some investigators have shown that Na^+-K^+-ATPase activity is regulated by a specific pool of phosphatidylinositol that is dependent on *myo*-inositol uptake for synthesis (26,27). This phospholipid may be the source of diacylglycerol, which is

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STZ, streptozocin; MNCV, motor nerve conduction velocity; ARI, aldose reductase inhibitor; ANOVA, analysis of variance.

important in the regulation of Na^+-K^+-ATPase directly or through a protein kinase-C mechanism (13). Impaired Na^+-K^+-ATPase activity contributes to other defects in the diabetic peripheral nerve including decreased nerve conduction velocity (25).

To explore further whether *myo*-inositol deficiency contributes to the early abnormalities in diabetic peripheral nerve, nondiabetic rats were fed a diet containing 10 or 20% L-fucose for a 6-wk period. L-Fucose is a monosaccharide that is present in low concentrations in normal plasma and is elevated in plasma of diabetic subjects (18,28,29). In previous studies, we have shown that L-fucose is a potent competitive inhibitor of *myo*-inositol transport by mammalian cells (18,30,31). Furthermore, exposure of cultured neuroblastoma cells to L-fucose causes a decrease in Na^+-K^+-ATPase transport activity (30). In this study, the effect of an L-fucose–containing diet on MNCV, rat sciatic nerve *myo*-inositol content, and Na^+-K^+-ATPase activity were compared with the same parameters in STZ-induced diabetic rats.

RESEARCH DESIGN AND METHODS

Male Sprague-Dawley rats 8–9 wk of age were used. Rats were divided into 8 experimental groups. One group of nondiabetic control rats was fed a normal diet, repelleted from ground rat chow containing 1% gum xanthan as a binding agent. Two groups were fed a diet containing either 10 or 20% L-fucose (Pfanstiehl, Waukegan, IL). Diets were made by thoroughly blending the desired amount of L-fucose (by weight) with ground rat chow and 1% gum xanthan. This mixture was pelleted and dried in a vacuum oven to a constant weight. The diets were then stored in a cold room (4°C). Two additional experimental groups received the above-described L-fucose diets for 3 wk, after which the diets were supplemented with 1% *myo*-inositol (Sigma, St. Louis, MO) by weight and fed to these 2 groups for an additional 3 wk.

Diabetes was induced by intravenously injecting STZ (50 mg/kg in 0.9% NaCl, adjusted to a pH 4.0 with 0.2 M sodium citrate). The rats were anesthetized with methoxyflurane before injection. Diabetes was verified 24 h later by evaluating blood glucose levels with the use of glucose oxidase reagent strips (Boehringer-Mannheim, Indianapolis, IN). Samples for blood and urine glucose measurements were also taken on the day of the experiments. Diabetic animals were divided into 3 groups. One group received a normal diet, a second group received a diet containing 1% *myo*-inositol, and the final group received a diet containing 450 mg sorbinil/kg body wt (Pfizer, Groton, CT). The *myo*-inositol- and sorbinil-supplemented diets were started the day after diabetes was induced. The diets were prepared as described above. For all groups, food and water were provided ad libitum during the 6-wk experimental period.

Bound and free serum L-fucose levels. Protein associated and free L-fucose levels in serum were determined by modifications of the methods described by Djurdjic and Mandic (32) and Cohenford et al. (33), respectively. Blood was collected from anesthetized rats by cardiac puncture before death of the animals for tissue collection. The sampled blood was allowed to clot, and serum was obtained after centrifugation. For bound L-fucose determination, 0.2 ml of serum was diluted with 1 ml of 1 M NaCl, 5 ml of ethanol was added, and the samples were vortexed and centrifuged for 5 min at 1000 *g*. The pellet was resuspended in 1 ml of water to which 5 ml of a 6:1 mixture of H_2SO_4-H_2O was added. The samples were then heated in a boiling water bath for 4 min followed by the addition of 1.0 ml of CPS reagent (1.0% L-cysteine hydrochloride and 0.075% of phenol in water). The samples were allowed to cool on ice for 60 min, and the absorbance was measured at 398 nm and compared with a standard curve prepared similarly using a concentration range of 0–600 nmol L-fucose. Unbound L-fucose levels were determined by diluting 0.5 ml of serum with 1.5 ml of Tris-HCl buffer at pH 8.5. The samples were centrifuged in a Centricon 10 microconcentrator (Amicon, Beverly, MA), and the filtrate was divided into two 0.6-ml aliquots and lyophilized overnight. One sample was used for background and was resuspended in 1.0 ml of Tris-buffer and 0.5 ml of NAD (10 mM); the other sample was resuspended in 0.5 ml of Tris-buffer, 0.5 ml of NAD, and 0.5 ml of L-fucose dehydrogenase (1 U/5 ml from porcine liver, Sigma). Both samples were incubated for 50 min at room temperature, and 1.5 ml of a neocuproine-copper reagent (76 mg $CuSO_4$ and 197 mg neocuproine-HCl in a 0.2 M sodium acetate buffer, pH 4.7) was added. The absorbance was measured at 455 nm. The concentration of L-fucose was determined by comparison to a standard curve prepared similarly using a concentration of 0–400 nmol L-fucose.

MNCV. MNCV was determined using a noninvasive procedure in the sciatic-posterior tibial conducting system in a temperature-controlled environment as described previously (6,34). Rats were anesthetized with methoxyflurane. The left sciatic nerve was stimulated first at the sciatic notch and then at the Achilles tendon (35). Stimulation consisted of single 0.2-ms supra maximal (8 V) pulses through a bipolar electrode. The evoked potentials were recorded from the first interosseous muscle with a unipolar platinum electrode and displayed on a digital storage oscilloscope (model 54600A, Hewlett Packard, Rolling Meadows, IL). MNCV was calculated by subtracting the distal from the proximal latency measured in milliseconds from stimulus artifact of the takeoff of the evoked potential, and the difference was divided into the distance between the two stimulating electrodes measured in millimeters using a Vernier caliper. The MNCV was reported in meters per second.

Na^+-K^+-ATPase activity. Total and ouabain-inhibited ATPase activities were measured in crude homogenates of sciatic nerve (10). Sciatic nerves were desheathed and homogenized in a ground glass homogenizer at 4°C in 1 ml of 0.2 M sucrose with 0.02 M Tris-HCl buffer, pH 7.5. The samples were then centrifuged at 100 *g* for 10 min at 4°C. An aliquot of the supernatant (50 μl) was added to two cuvettes containing 100 mM NaCl, 10 mM KCl, 2.5 mM $MgCl_2$, 2 mM EGTA, 1 mM Tris-ATP, 1 mM 3-(cyclohexylammonium)phosphoenolpyruvate, 30 mM imidazole-HCl buffer (pH 7.3), 0.15 mM NADH, 50 μg lactate dehydrogenase, and 30 μg pyruvate kinase with

TABLE 1
Body weight, plasma glucose levels, serum L-fucose levels, and dietary intake in diabetic and L-fucose–fed rats

Animal group	*n*	Beginning body weight (g)	Final body weight (g)	Glucose (mM)	L-Fucose (mg/dl)		Dietary intake g · day^{-1} · kg^{-1} rat
					Free	Bound	
Control	15	294.0 ± 4.0	395.3 ± 6.1*	6.9 ± 0.4	1.75 ± 0.20	23.3 ± 7.8	68.5 ± 1.7
10% L-fucose	8	281.0 ± 2.8	323.6 ± 6.4*	7.2 ± 0.4	3.29 ± 0.29†	37.7 ± 10.7	68.1 ± 0.6
20% L-fucose	15	288.1 ± 4.7	279.7 ± 7.9	7.5 ± 0.5	4.59 ± 0.24†	33.3 ± 15.7	63.9 ± 2.5
10% L-fucose plus 1% *myo*-inositol	3	277.0 ± 6.0	363.0 ± 10.0*‡	5.9 ± 0.3	2.96 ± 0.01†	33.8 ± 10.4	63.4 ± 2.7
20% L-fucose plus 1% *myo*-inositol	15	293.1 ± 4.3	280.0 ± 8.2	7.4 ± 0.4	5.60 ± 0.86†	38.2 ± 3.2	65.5 ± 2.6
Diabetic untreated	6	265.5 ± 4.5	290.1 ± 19.1	27.8 ± 1.4†	2.13 ± 0.07	48.0 ± 8.1†	Not determined
Diabetic plus 1% *myo*-inositol	5	267.2 ± 4.6	274.2 ± 15.8	26.7 ± 0.7†	2.15 ± 0.19	43.7 ± 7.4	Not determined
Diabetic plus sorbinil	6	269.2 ± 2.5	281.8 ± 17.2	27.9 ± 2.1†	2.07 ± 0.10	39.1 ± 6.9	Not determined

Data are means ± SE; the experimental period lasted for 6 wk. Data for dietary intake was taken in 3 consecutive weekly intervals between wk 4 and 6.
*$P < 0.02$ vs. beginning body weight.
†$P < 0.005$ vs. control.
‡$P < 0.005$ vs. 10% L-fucose final body weight.

or without 1 mM ouabain to inhibit the ouabain-sensitive Na^+-K^+-ATPase fraction. Preliminary investigation indicates that this concentration of ouabain produced maximal inhibition with a resulting ATPase activity similar to that in buffer void of Na^+. After a 20-min stabilization period, the oxidation of NADH was recorded over a 30-min period. The activity was expressed as μmol ADP per gram wet weight per hour.

Tissue *myo*-inositol, sorbitol, and L-fucose levels. After MNCV measurements and while still under anesthesia, a blood sample was collected via cardiac puncture. The animals were killed by opening the diaphragm and severing the aorta. The right sciatic nerve was obtained, desheathed, and weighed for determination of Na^+-K^+-ATPase activity as described above and for determination of tissue *myo*-inositol, sorbitol, and L-fucose levels (35). Nerve samples were boiled for 10 min in water containing α-D-methylmannopyranoside as an internal standard and deproteinized with 0.5 ml of 0.19 M $Ba(OH)_2$ and 0.19 M $ZnSO_4$. After centrifugation, the supernatant was collected, frozen, and lyophilized. The samples were derivatized as described previously and the intracellular contents determined by gas-liquid chromatography as described previously (30). The data were expressed as nanomole per milligram wet weight. Serum *myo*-inositol concentration was determined by treating a 0.2-ml aliquot as described above.

Statistical analysis. Data are presented as mean ± SE, and the significance of differences was calculated by ANOVA and Student's *t* test.

RESULTS

Effect of L-fucose diet on body weight and serum L-fucose content. Data in Table 1 show the plasma glucose levels, serum free and bound L-fucose levels, body weights, and food consumption for each group of rats in the study. Blood glucose levels were increased fourfold in the diabetic groups. The blood glucose levels were normal in L-fucose–fed rats. The diabetic rats and rats fed a diet containing 20% L-fucose failed to gain weight or lost ~5% of their body weight, respectively, over the 6-wk study. Rats fed a diet containing 10% L-fucose gained 60% less weight than control rats. However, when 1% *myo*-inositol was added to the 10% L-fucose diet, weight gain improved to 85% of control values. An improvement in weight gain was not observed when 1% *myo*-inositol was added to the 20% L-fucose diet. All rats on the L-fucose–supplemented diet consumed a similar amount of food based on body weight. The amount of chow consumed by diabetic rats was not determined. The free L-fucose content in serum was significantly increased in rats fed a diet containing 10 or 20% L-fucose. The serum content of free L-fucose in diabetic rats was increased by ~20%, but the differences were not statistically significant. In another separate study with more severely diabetic rats (65 mg/kg STZ), the serum free L-fucose content was increased from 1.80 ± 0.06 to 2.54 ± 0.10 mg/dl ($P < 0.05$, $n = 6$) after a 3-wk period. Bound L-fucose levels in serum were increased 40–65% in L-fucose–fed rats. However, this change was not statistically significant. Serum bound L-fucose levels were significantly increased in untreated diabetic rats. In contrast, bound L-fucose levels in serum were slightly less in diabetic rats treated with *myo*-inositol or sorbinil compared with untreated diabetic rats and were not statistically different from controls even though the levels were 70–80% greater than control values.

Na^+-K^+-ATPase activity. Feeding rats a diet containing 10 or 20% L-fucose for a 6-wk period caused a significant decrease in composite and ouabain-sensitive Na^+-K^+-ATPase activity (Table 2). The activity of Na^+-K^+-ATPase was significantly improved when 1% *myo*-inositol was added to the L-fucose diets compared with rats fed diets containing L-fucose alone. The decrease in Na^+-K^+-ATPase activity in the sciatic nerve from rats fed an L-fucose diet was similar to the decrease observed in untreated diabetic rats. As previously reported by other investigators and confirmed in these studies, treatment of

TABLE 2
Sciatic nerve composite and ouabain-inhibited Na^+-K^+-ATPase activity and MNCV in diabetic and L-fucose–fed rats

Animal group	*n*	ATPase (μmol ADP · g wet wt^{-1} · h^{-1})		MNCV (m/s)
		Composite	Ouabain sensitive	
Control	10	490.0 ± 41.0	246.3 ± 17.9	49.5 ± 2.3
10% L-fucose	7	297.3 ± 22.4*†	144.0 ± 6.2*†	35.1 ± 4.7*†
20% L-fucose	10	326.5 ± 10.4*†	159.9 ± 9.9†	31.1 ± 3.3*†
10% L-fucose plus 1% *myo*-inositol	3	408.4 ± 24.1	225.6 ± 10.9	44.9 ± 1.2
20% L-fucose plus 1% *myo*-inositol	10	432.6 ± 21.6	216.6 ± 11.7	45.7 ± 3.7
Diabetic untreated	6	342.2 ± 24.7*‡	174.5 ± 11.4*‡	37.8 ± 2.8*‡
Diabetic plus 1% *myo*-inositol	5	471.9 ± 31.0	254.8 ± 15.2	45.9 ± 4.1
Diabetic plus sorbinil	6	454.0 ± 28.1	227.1 ± 17.1	44.7 ± 1.3

Data are means ± SE. Na^+-K^+-ATPase activity was determined in sciatic nerves of diabetic rats or rats fed a normal or L-fucose–containing diet. MNCV was also determined in these animals. Composite ATPase activity represents total activity, and ouabain-sensitive ATPase activity denotes the portion of total activity inhibited by 1 mM ouabain.
*$P < 0.01$ vs. control.
†$P < 0.01$ vs. respective *myo*-inositol–supplemented L-fucose diet.
‡$P < 0.05$ vs. *myo*-inositol– or sorbinil–supplemented diets for diabetic rats.

STZ-induced diabetic rats with sorbinil or *myo*-inositol significantly prevented the Na^+-K^+-ATPase defect (10,36).

MNCV. The data in Table 2 show that MNCV was significantly slowed in rats fed a diet containing 10 or 20% L-fucose after a 6-wk period. *myo*-Inositol supplementation of diets containing L-fucose significantly improved MNCV compared with rats maintained on L-fucose diets alone. MNCV slowing was detected after only 3 wk on the L-fucose diets. The values were 45.7 ± 2.1, 37.5 ± 4.2, and 36.1 ± 3.4* m/s for control, 10% L-fucose, and 20% L-fucose, respectively ($n = 4$, *$P < 0.05$ vs. control). The slowing of MNCV in L-fucose–fed rats was similar to untreated diabetic rats. As previously reported by other investigators, MNCV slowing was significantly prevented by treating STZ-induced diabetic rats with sorbinil or *myo*-inositol (6,9).

Sciatic nerve *myo*-inositol content. The *myo*-inositol content of the sciatic nerve was significantly decreased in rats fed a diet containing 10 or 20% L-fucose and was restored to normal values when the L-fucose diets were supplemented with 1% *myo*-inositol (Table 3). The sciatic nerve levels of glucose, sorbitol, fructose, and L-fucose were not effected by feeding rats L-fucose–supplemented diets. The observation that polyols do not accumulate in sciatic nerve of rats fed L-fucose diets agrees with our previous studies in cultured neural and endothelial cells. Analysis of cultured mammalian cells grown in medium containing 10–30 mM L-fucose indicated that L-fucose is not converted to a polyol and is not likely to be a substrate for aldose reductase (30,31). The decrease in *myo*-inositol levels in sciatic nerve from untreated diabetic rats is greater than that observed in rats fed a diet containing 10 or 20% L-fucose. As expected, sorbitol

TABLE 3
Sciatic nerve intracellular levels of glucose, fructose, sorbitol, *myo*-inositol, and L-fucose in diabetic and L-fucose–fed rats

Animal Group	*n*	Determination (nmol/mg wet wt)				
		Glucose	Fructose	Sorbitol	*myo*-Inositol	L-Fucose
Control	15	4.7 ± 0.9	2.0 ± 0.3	0.6 ± 0.4	11.4 ± 0.8	0.19 ± 0.06
10% L-fucose	7	5.1 ± 0.8	1.6 ± 0.2	0.7 ± 0.3	8.2 ± 0.7*†	0.24 ± 0.06
20% L-fucose	15	5.2 ± 1.0	1.9 ± 0.5	0.3 ± 0.2	8.0 ± 0.3*†	0.35 ± 0.08
10% L-fucose plus 1% *myo*-inositol	3	6.1 ± 0.5	1.6 ± 0.4	0.6 ± 0.3	11.6 ± 0.2	0.31 ± 0.10
20% L-fucose plus 1% *myo*-inositol	15	3.2 ± 0.3	0.9 ± 0.2	0.5 ± 0.2	11.5 ± 0.9	0.25 ± 0.05
Diabetic untreated	6	25.5 ± 3.1*	9.3 ± 1.1*‡	3.8 ± 1.1*‡	6.8 ± 1.4*§	0.13 ± 0.04
Diabetic plus 1% *myo*-inositol	5	29.7 ± 3.4*	8.0 ± 0.6*	3.7 ± 0.8*	12.6 ± 2.5	0.10 ± 0.02
Diabetic plus sorbinil	6	28.7 ± 2.1*	1.2 ± 0.2	0.3 ± 0.2	10.1 ± 0.6	0.17 ± 0.02

Data are means ± SE.
*$P < 0.01$ vs. control.
†$P < 0.0025$ vs. respective *myo*-inositol–supplemented L-fucose diet.
‡$P < 0.01$ vs. sorbinil–supplemented diet for diabetic rats.
§$P < 0.05$ vs. sorbinil- or *myo*-inositol–supplemented diets for diabetic rats.

levels were increased in diabetic sciatic nerve. Treatment of diabetic rats with sorbinil prevented the increase in sorbitol levels and maintained nerve *myo*-inositol at near control levels. Diabetic rats receiving *myo*-inositol supplemented diet also maintained normal nerve *myo*-inositol levels but showed increased sorbitol levels.

Serum *myo*-inositol concentration of rats fed a 10 or 20% L-fucose diet was similar to rats fed a normal diet. The concentration of *myo*-inositol in serum of rats fed a normal, 10% L-fucose, or 20% L-fucose diet was 67.7 ± 4.4, 69.6 ± 11.6, or 68.0 ± 10.9 μM, respectively ($n = 7$). When 1% *myo*-inositol was added to the 20% L-fucose diet, the serum *myo*-inositol concentration was significantly increased to 441.1 ± 111.7 μM ($P < 0.01$).

DISCUSSION

The pathophysiological sequence resulting in clinical diabetic neuropathy has eluded investigators for many years. Metabolic abnormalities may be responsible for the initial defects in nerve function, and these defects may precede and contribute to the characteristic structural changes in chronic diabetic neuropathy (11).

Hyperglycemia-induced activation of aldose reductase, leading to sorbitol accumulation and a subsequent decrease in *myo*-inositol metabolism and content, has been proclaimed to be a primary factor in the development of the acute defects in diabetic neuropathy (2,11). This hypothesis is widely supported by studies from diabetic animal models that have shown that blocking aldose reductase activity or dietary replenishment of *myo*-inositol levels prevents the initial defects associated with diabetic neuropathy (6,7,9,10,13–15,35–37). Despite this convincing evidence, this hypothesis remains controversial (38). Short-term treatment of patients with chronic diabetes with ARIs has not consistently provided clinical improvement (3). Although long-term interventions with these compounds have demonstrated functional and structural improvements (39,40).

It is not known to what extent sorbitol accumulation or *myo*-inositol deficiency contributes to the development of neural defects. Our studies aimed to determine if *myo*-inositol deficiency induced under normal glycemic conditions could cause defects in neural activity comparable with those seen in diabetic neuropathy and to ascertain if L-fucose, a monosaccharide that is present in low concentration in normal serum but increased in diabetes, may be a factor in diabetic neuropathy (29).

Rats fed a diet containing 10 or 20% L-fucose developed defects in Na^+-K^+-ATPase activity and MNCV similar to those seen in diabetic rats (6,7,9,10,13–15,35,36). These defects are accompanied by a decrease in *myo*-inositol content in the sciatic nerve in both diabetic and L-fucose–fed rats. Dietary replenishment of nerve *myo*-inositol levels restored Na^+-K^+-ATPase activity and MNCV without improving weight gain in rats fed a diet containing 20% L-fucose. This latter observation suggests that the effect of L-fucose on nerve function was not the result of impaired growth but most likely because of *myo*-inositol deficiency. In diabetic rats, Na^+-K^+-ATPase activity and MNCV were restored by sorbinil treatment or dietary *myo*-inositol supplementation, and both regimens restored sciatic nerve *myo*-inositol content.

L-Fucose did not accumulate in sciatic nerve of L-fucose–fed rats suggesting that *myo*-inositol depletion in sciatic nerve was not directly the result of L-fucose accumulation. Furthermore, analysis by gas chromatography revealed that L-fucose was not converted into a polyol in sciatic nerve. This is consistent with previous findings from cultured neural and endothelial cells that showed that L-fucose is not likely to provide a substrate for aldose reductase (30,31). In addition, the decrease in sciatic nerve *myo*-inositol levels in L-fucose–fed rats was not caused by a decrease in circulatory *myo*-inositol levels. Therefore, L-fucose is likely to cause a decrease in *myo*-inositol content in nerve tissue secondary to inhibition of the *myo*-inositol transporter and thereby causing a decrease in Na^+-K^+-ATPase activity and possibly altering other neural cell functions by disrupting phosphoinositide metabolism (13,18,25). Nerve *myo*-inositol levels were restored in rats fed a diet containing 10 or 20% L-fucose by adding 1% *myo*-inositol to the diet. This probably occurred because of the sixfold increase in serum *myo*-inositol levels that was probably sufficient to overcome the inhibition of *myo*-inositol transport caused by L-fucose.

As previously mentioned, plasma L-fucose levels have been found to be increased in patients with diabetes in both the free and the protein-bound form (29,41). In these studies, free levels of serum L-fucose were increased significantly in the L-fucose–fed rats. In diabetic rats, serum L-fucose levels were only increased by ~20%, and this change was not statistically significant. In a previous unrelated study, we found that L-fucose was significantly increased in serum from a more severely diabetic set of rats after a 3-wk period by ~40% compared with the more moderately diabetic rats used in these studies. Whether the STZ-induced diabetic rat is a good model for examining changes in serum L-fucose levels and metabolism is unknown and will require additional investigation (42). In support of this model, bound L-fucose levels were significantly increased in the untreated diabetic rats as they are in humans (41; unpublished observations). In diabetic rats treated with 1% *myo*-inositol or sorbinil the level of bound L-fucose was decreased compared with untreated diabetic rats but increased nonsignificantly above control values. Surprisingly, bound L-fucose levels were not significantly increased in L-fucose–fed rats. This result may be a difference in the metabolism of L-fucose by the liver or other tissues in diabetic versus L-fucose–fed rats. In diabetes, three serum proteins (haptoglobin, α_1-acid glycoprotein, and α_1-antitrypsin) synthesized in the liver are mainly responsible for the increase in bound L-fucose levels (41). The metabolism and synthesis of these proteins may be altered in diabetes leading to changes in serum L-fucose content (41). In human diabetic sera, both protein-bound and free L-fucose levels are significantly increased by ≥50% (29). Radhakrishnamurthy et al. (29) have reported free L-fucose levels in human diabetic sera close to 3 mg/100 ml. We have obtained similar values for free L-fucose levels in sera from 34

diabetic patients using an enzyme assay (unpublished observations). Whether increased serum L-fucose levels contribute to the development of diabetic complications remains to be determined. However, note that serum free L-fucose levels in rats fed a diet containing 10% L-fucose are similar to levels observed in diabetic patients.

In summary, our studies in L-fucose–fed rats lend support to the hypothesis that *myo*-inositol deficiency may contribute to neural dysfunction in acute diabetic neuropathy.

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