Acetyl-L-Carnitine Deficiency as a Cause of Altered Nerve *myo*-Inositol Content, Na,K-ATPase Activity, and Motor Conduction Velocity in the Streptozotocin-Diabetic Rat

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Defective metabolism of long-chain fatty acids and/or their accumulation in nerve may impair nerve function in diabetes by altering plasma or mitochondrial membrane integrity and perturbing intracellular metabolism and energy production. Carnitine and its acetylated derivatives such as acetyl-L-carnitine (ALC) promote fatty acid β-oxidation in liver and prevent motor nerve conduction velocity (MNCV) slowing in diabetic rats. Neither the presence nor the possible implications of putative ALC deficiency have been definitively established in diabetic nerve. This study explored sciatic nerve ALC levels and the dose-dependent effects of ALC replacement on sciatic nerve metabolites, Na,K-ATPase, and MNCV after 2 and 4 weeks of streptozotocin-induced diabetes (STZ-D) in the rat. ALC treatment that increased nerve ALC levels delayed (to 4 weeks) but did not prevent nerve *myo*-inositol (MI) depletion, but prevented MNCV slowing and decreased ouabain-sensitive (but not -insensitive) ATPase activity in a dose-dependent fashion. However, ouabain-sensitive ATPase activity was also corrected by subtherapeutic doses of ALC that did not increase nerve ALC or affect MNCV. These data implicate nerve ALC depletion in diabetes as a factor contributing to alterations in nerve intermediary and energy metabolism and impulse conduction in diabetes, but suggest that these alterations may be differentially affected by various degrees of ALC depletion.

ALTHOUGH THE CAUSE of diabetic neuropathy remains unknown, alterations in fatty acid metabolism, including the well-described block in the conversion of γ-linoleic acid to γ-linolenic acid, have been invoked.[1,2] Accumulation of long-chain fatty acid esters in the nerve may perturb cellular metabolism and membrane function,[3,4] potentially by mechanism(s) involving alterations in nerve metabolites, protein kinase C (PKC), and/or Na,K-ATPase activity.[5-8] Perturbations in nerve Na,K-ATPase have emerged as a possible common denominator for altered energy metabolism,[7,9] nerve conduction slowing, and structural abnormalities in acute experimental diabetes, based on direct measurement of reduced nerve Na,K-ATPase activity in animals[10-12] and its correction by treatment with *myo*-inositol (MI),[7,12] aldose reductase inhibitors,[13,14] or prostacyclin analogs.[15] Moreover, pharmacological impairment of nerve Na,K-ATPase in nondiabetic rats reproduces diabetic nerve conduction slowing.[16,17] Fatty acid–mediated changes in PKC activation in diabetes[18-24] may impair Na,K-ATPase activity directly through secondary alterations in nerve metabolites including MI and taurine,[25] or by altering the synthesis of the endothelium-derived relaxing factor, nitric oxide, which has also been shown to regulate Na,K-ATPase both in vivo[16] and in vitro.[26]

Carnitine and its acetyl esters such as acetyl-L-carnitine (ALC) facilitate β-oxidation of nonesterified fatty acids in the liver for energy production.[27] ALC can prevent slowing of nerve conduction in rats with streptozotocin-induced diabetes (STZ-D),[28-31] as well as vascular dysfunction,[32] although the mechanisms remain obscure. Previous studies have not assessed the effects of experimental diabetes on sciatic nerve ALC levels, but have instead measured changes in total short-chain acyl carnitines.[31] Moreover, the functional and metabolic effects of ALC depletion have not been selectively evaluated, because the excessive ALC doses used also correct the depressed levels of free L-carnitine in diabetic nerve.[31] This study therefore evaluated in the STZ-D rat whether levels of sciatic nerve ALC are decreased, and explored the time- and dose-dependent effects of increasing nerve ALC levels on nerve metabolites, ouabain-sensitive Na,K-ATPase, and motor nerve conduction velocity (MNCV) to clarify their pathogenetic interrelationships.

MATERIALS AND METHODS

Animal Model

Barrier-sustained, cesarean-delivered male Wistar rats (200 to 300 g) were acclimatized for 1 week before being fasted overnight and rendered diabetic by an intraperitoneal (IP) injection of STZ 45 mg/kg (Upjohn, Kalamazoo, MI) in 0.2 mL 10-mmol/L citrate buffer, pH 5.5. Diabetes was defined as nonfasting plasma glucose greater than 16.7 mmol/L in tail vein blood (One Touch II; Lifescan, Milpitas, CA) 48 hours after STZ injection. Animals were subsequently maintained in individual air-filtered metabolic cages with ad libitum access to water, and were fed as a standardized synthetic diet (ICN Biomedicals, Cleveland, OH).

To examine the acute effects of ALC replacement on nerve osmolyte levels and MNCV, effects of IP ALC supplementation were studied after 14 days of STZ-D, a time point when MNCV slowing is observed together with depletion of nerve MI levels, without a measurable change in sciatic nerve ouabain-sensitive Na,K-ATPase activity. The experimental groups consisted of (1) nondiabetic (ND) control rats (n = 10); (2) ND ALC-treated rats given ALC 50 mg/kg by daily IP injection of an aqueous solution (n = 10); (3) STZ-D control rats (n = 11); and (4) STZ-D ALC-treated rats given 50 mg/kg ALC (n = 10; 50 mg/kg ALC IP was shown to restore nerve ALC levels to normal in STZ-D rats). On

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day 14, STZ-D was reconfirmed and MNCV was measured. The rats were killed 4 hours after ALC dosing, and the left and right sciatic nerves were rapidly excised and cleaned for further biochemical measurements.[33]

Impairment of the ouabain-inhibitable Na,K-ATPase is observed after 4 weeks of STZ-D. Given that ALC is easily soluble and stable in aqueous solution for up to 24 hours and having defined the replacement dose of ALC by IP injection to be 50 mg/kg, the dose-dependent effects of increasing nerve ALC by gavage on nerve metabolites and ouabain-sensitive nerve Na/K-ATPase activity were evaluated. Animals were randomized to the following experimental groups: (1) ND control rats (n = 22); (2) diabetic control rats (n = 17); (3) diabetic rats given 5 mg/kg/d ALC by gavage (n = 16); (4) diabetic rats given 25 mg/kg/d ALC by gavage (n = 14); (5) diabetic rats given 50 mg/kg/d ALC by gavage (n = 17); and (6) diabetic rats given 100 mg/kg/d ALC by gavage (n = 17). To ensure the stability of ALC in aqueous solution and lack of deacetylation to L-carnitine, in all studies the solution of ALC was administered to animals immediately after being prepared. After 4 weeks, blood glucose levels and MNCV were measured. The rats were killed 4 hours after ALC dosing, and the sciatic nerves were rapidly excised and cleaned for biochemical measurements. In all studies, investigators were unaware of treatment-group assignments.

Sciatic-Tibial MNCV

MNCV was measured on days 14 and 28 of the experimental protocols. Rats were lightly anesthetized by inhalation of Metofane (Pitman-Moore, Mundelain, IL). The left sciatic nerve was stimulated proximally at the sciatic notch and distally at the ankle via bipolar electrodes with supramaximal stimuli (8V) at 20 Hz.[34] Latencies of the compound muscle action potentials were recorded via bipolar electrodes from the first interosseous muscle of the hindpaw and measured from the stimulus artifact to the onset of the negative M-wave deflection. MNCV was calculated by subtracting the distal latency from the proximal latency, and the result was divided into the distance between the stimulating and recording electrode. Body temperature was monitored by rectal probe and maintained at 37°C with a warming pad. Hindlimb skin temperature was also monitored by a thermistor and maintained between 36° and 38°C by radiant heat.

Sciatic Nerve ALC and L-Carnitine

Sciatic nerve ALC and free L-carnitine were determined by liquid secondary ion-tandem mass spectrometry as previously described.[35] In brief, a cytosolic extract of ALC and L-carnitine were obtained by homogenizing the sciatic nerve in 2 mL buffer (cold water acidified with acetic acid to final pH 3 to 4) supplemented with $^2H_3$-acetyl-L-carnitine (40 mL 0.1-nmol/mL stock solution) and $^2H_3$-L-carnitine (40 mL 0.5-nmol/mL stock solution) as internal standards. The particulate fraction was collected by centrifugation and extraction with methanol. After resuspension in 4 mL methanol, the pellet was refrigerated at 4°C overnight and centrifuged at 1,200 × *g* for 20 minutes at 4°C. The methanolic supernatant was collected, dried, and reconstituted with the cytosolic extract. ALC and free L-carnitine were determined as butyl ester derivatives and normalized to protein.[36] In preliminary studies in which ALC content was measured in sciatic nerves from ND rats, ALC levels were shown to decline rapidly once the nerve was removed from the animal. Therefore, for measurement of ALC, nerves were frozen in liquid N_2 within 30 seconds of removal from anesthetized rats.

Sciatic Nerve MI, Taurine, Sorbitol, and Fructose

Sciatic nerve MI, sorbitol, and fructose were determined as previously described by gas-liquid chromatography of aldonitrile acetate derivatives from lyophilized aliquots of protein-free filtrates of sciatic nerves homogenized in 5% (wt/vol) trichloroacetic acid with α-D-methyl mannopyranoside as an internal standard.[33] Samples were analyzed using a Varian 3700 gas-liquid chromatograph (Varian, Sunnyvale, CA) equipped with a Varian 8100 autosampler, a 30-m × 0.25-mm (ID) SP-2100 fused silica capillary column (0.25-μm film thickness), a single flame-ionization detector, and a Varian Star Workstation integrator. Standard curves were generated daily, and the recovery-corrected values were expressed as nanomoles per milligram wet weight of tissue.

Nerve taurine was determined by reversed-phase high-performance liquid chromatography after precolumn derivatization with OPA (*o*-phthalaldehyde).[37] In brief, 5 mg sciatic nerve was homogenized in 1 mL 6% trichloroacetic acid and centrifuged at 4,000 × *g* for 10 minutes. The supernatants were purified on washed dual-bed, ion-exchange columns (2.5 cm AG 1-X8 100-200 mesh in the chloride form over 2.5 cm AG 50W-X8 200/400 mesh in the hydrogen form; Bio-Rad, Richmond, CA) by elution with 2 mL water and lyophilized. Samples and standards were dissolved in 100 μL water before analysis on a Waters system (Waters Chromatography Division, Millipore, Milford, MA) equipped with a model 501 pump, 717 autosampler, 3.9 × 150-mm Nova-Pak C18 column, and model 470 scanning fluorescence detector. Isocratic elution was performed at a flow rate of 2 mL/min using 43% solvent A (0.05 mol/L NaH_2PO_4, pH 5.3, plus 5 mol/L NaOH) combined with 57% solvent B (0.05 mol/L NaH_2PO_4 in 75% methanol/water). Glutamine, added after ion-exchange chromatography, was used as the internal standard. Standard curves were linear over the concentration range in nerve samples, and recovery of taurine was greater than 90%.

Sciatic Nerve Na,K-ATPase

Samples of rat sciatic nerve were homogenized on ice in 2 mL 0.2-mol/L sucrose plus 0.02-mol/L Tris-HCl, pH 7.5, by three 10-second bursts with a Polytron model PT 10-35 (Brinkman Instruments, Westbury, NY). Aliquots of homogenate were assayed enzymatically for total ATPase activity in 1 mL reaction mixture containing 100 mmol/L NaCl, 10 mmol/L KCl, 2.5 mmol/L $MgCl_2$, 1 mmol/L Tris-ATP, 1 mmol/L phosphoenolpyruvate, 30 mmol/L imidazole-HCl buffer, pH 7.3, 0.15 mmol/L NADH, 50 μg lactate dehydrogenase, and 30 μg pyruvate kinase. After an initial stabilization period, activity was monitored spectrophotometrically at 340 nm for at least 15 minutes; 20 μL 25-mmol/L ouabain was added and mixed, and activity was read for at least another 15 minutes. Ouabain-inhibitable Na,K-ATPase activity is defined as the difference in activity before and after addition of ouabain and is expressed as micromoles ADP formed per gram wet weight per hour.

Statistical Analysis

The data are expressed as the mean ± SEM. Differences among experimental groups were determined by ANOVA, and the significance of differences between these groups was assessed by the Student-Neuman-Keuls multiple-range test. Significance was defined as alpha = .05.

RESULTS

Effect of STZ-D and IP ALC Replacement on Nerve ALC and L-Carnitine Levels

Results of preliminary studies examining the effects of STZ-D and IP ALC administration on sciatic nerve ALC content are shown in Fig 1. STZ-D produced a 31% ($P < .05$) reduction in the ALC content of sciatic nerve. ALC administration by daily IP injection increased ALC content, with a dose of 50 mg/kg/d restoring normal nerve ALC content in STZ-D rats. Sciatic nerve free L-carnitine was reduced by 39% (7.1 ± 0.4 *v* 4.3 ± 0.5 nmol/mg protein, $P < .05$) in STZ-D rats, but, unlike ALC, it was not significantly increased by treatment with 50 mg/kg/d ALC (5.6 ± 0.4 nmol/mg protein, NS *v* untreated STZ-D rats and ND rats). This dose was therefore used in the subsequent experimental protocols to study the effects of nerve ALC replacement on nerve metabolites and MNCV.

Acute Effects of Nerve ALC Replacement on Nerve Metabolites and MNCV

Table 1 shows body weight and plasma glucose at baseline and 14 days in each of the experimental groups. Baseline body weight was similar in all groups. Plasma glucose values were greater than 16.7 mmol/L in all STZ-D rats. At day 14, STZ-D had produced a 20% to 22% decrease in body weight and a 3.7-fold increase in plasma glucose compared with day 14 values in ND controls. ALC treatment did not alter body weight or plasma glucose in either STZ-D or ND rats.

Effects of diabetes and ALC on MNCV. Baseline MNCV did not differ among experimental groups (Table 2). ALC did not affect MNCV at 14 days in ND rats. In untreated STZ-D rats, MNCV slowed from baseline by 20.7% ($P < .05$) at 14 days. ALC replacement of STZ-D rats preserved MNCV at 14 days at a significantly higher level than in untreated STZ-D rats and not different from that in ND control rats. Therefore, MNCV slowing in STZ-D rats was completely prevented by replacing nerve ALC.

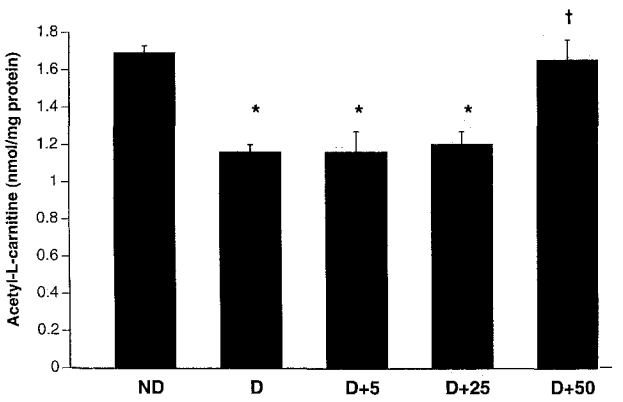

Fig 1. Effects of diabetes and ALC administration (IP) on sciatic nerve ALC content. Data are the mean ± SEM. ND, nondiabetic; D, untreated STZ-D; D + 5, STZ-D treated with 5 mg/kg/d ALC; D + 25, STZ-D treated with 25 mg/kg/d ALC; D + 50, STZ-D treated with 50 mg/kg/d ALC. *$P < .05$ *v* control ND. †$P < .05$ *v* D.

Table 1. Acute (14 days) Effects of Diabetes and IP ALC Replacement on Body Weight and Plasma Glucose in the Experimental Animal Groups

Parameter	ND (n = 10)	ND + ALC (n = 10)	STZ-D (n = 11)	STZ-D + ALC (n = 10)
Weight (g)				
Baseline	259 ± 4	259 ± 9	252 ± 4	253 ± 6
Day 14	328 ± 6	321 ± 8	256 ± 7	261 ± 7
Glucose (mmol/L)				
Day 3	6.8 ± 0.2	6.7 ± 0.2	25.8 ± 1.1*	26.7 ± 1.2*
Day 14	7.3 ± 0.5	7.2 ± 0.3	26.9 ± 2.4*	26.9 ± 2.1*

NOTE. Data are the mean ± SEM.
*$P < .01$ *v* ND control.

Effects of STZ-D and nerve ALC replacement on nerve osmolyte levels. Figure 2 shows the effect of ALC replacement on nerve osmolyte levels in ND and STZ-D rats. STZ-D rats showed a 45% decrease in nerve MI levels ($P < .01$) after 14 days, without a concomitant decrease in composite nerve ouabain-sensitive ATPase activity (data not shown). Nerve taurine decreased by 25%, but this difference failed to achieve statistical significance. ALC had no significant effect on nerve MI or taurine in ND rats, but selectively increased nerve MI by 71% ($P < .01$) in STZ-D rats to levels not statistically different from the levels in ND rats. ALC replacement did not affect either nerve sorbitol or taurine levels or ouabain-sensitive ATPase activity (data not shown) in STZ-D rats. Nerve sorbitol levels were similar in ND controls and ALC-treated ND rats, and were increased 4.5-fold and 5.4-fold ($P < .01$) in the respective STZ-D experimental groups. Nerve fructose levels were also similar in both untreated and ALC-treated ND rats and were increased 4.6-fold and 4.5-fold ($P < .01$) in the corresponding STZ-D groups. Thus, ALC replacement did not affect nerve osmolyte levels in ND rats, but selectively prevented MI depletion in the STZ-D group at a time when MNCV slowing was unassociated with a detectable decrease in composite ouabain-sensitive ATPase activity.

Dose-Dependent Effects of ALC Treatment by Gavage on Nerve Na,K-ATPase Activity, MNCV, and Nerve Metabolites After 4 Weeks of Diabetes

Effects of STZ-D and ALC on plasma glucose and body weight. Table 3 shows body weight and plasma glucose at baseline and after 4 weeks in each of the experimental groups. Baseline body weights were similar in all groups. Plasma glucose values were greater than 16.7 mmol/L in all

Table 2. Change in Sciatic MNCV (m/s) at Baseline (day 0), and After 14 Days (study end) in ND and STZ-D Rats and the Effect of IP ALC Replacement

Group	Baseline	Day 14
ND	53.4 ± 2.4	54.4 ± 1.5
ND + ALC	53.6 ± 2.1	54.0 ± 2.7
STZ-D	55.5 ± 0.9	44.0 ± 2.2*
STZ-D + ALC	55.7 ± 1.7	53.0 ± 2.8†

*$P < .05$ *v* ND.
†$P < .05$ *v* STZ-D.

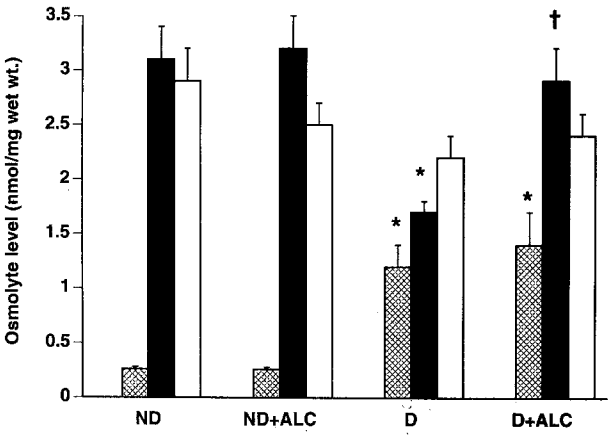

Fig 2. Effect of nerve ALC replacement (IP) after 2 weeks on nerve MI, taurine, and sorbitol levels in control and STZ-D rats. Sciatic nerves were removed at the end of the study and immediately frozen in liquid nitrogen. Nerve sorbitol (▨) and MI (■) levels were determined by gas chromatography as acetate derivatives, and taurine (□) by high-performance liquid chromatography. Data are the mean ± SEM. *$P < .01$ *v* control ND. †$P < .01$ *v* D.

STZ-D rats. After 4 weeks, STZ-D limited body weight gain to 9% over basal levels, significantly less than the ND control animals, and increased plasma glucose 5.4-fold compared with ND controls. ALC treatment did not alter body weight or plasma glucose level.

Effects of ALC on nerve ALC and free L-carnitine levels. Sciatic nerve ALC and free L-carnitine were decreased by 46% and 47% ($P < .05$), respectively, after 4 weeks of STZ-D (Table 4). ALC administration by daily gavage increased ALC content in a dose-dependent fashion, with doses of 50 and 100 mg/kg/d significantly increasing nerve ALC content by 34% and 48% ($P < .05$), respectively, compared with levels in untreated STZ-D rats. However, in the 100-mg/kg/d treatment group, levels of nerve ALC remained 20% ($P < .05$) lower than in ND control animals. Unlike nerve ALC, sciatic nerve free L-carnitine was not significantly increased by any dose of ALC.

Effects of STZ-D and ALC on MNCV. The dose-response effects of ALC on sciatic nerve MNCV are shown in Fig 3. ALC at the smallest dose of 5 mg/kg/d had no effect on MNCV. Partial correction of MNCV was observed at 25 mg/kg/d, as MNCV was 10.3% ($P < .05$) faster than in untreated STZ-D rats but still significantly slower than in ND controls ($P < .05$). At doses of 50 mg/kg/d and greater, complete prevention of MNCV slowing was observed.

Table 3. Effects of 4 Weeks of ALC (5-100 mg/kg) by Gavage and STZ-D on Body Weight and Blood Glucose

Group	Weight (g)		Blood Glucose (mmol/L)	
	Start	End	Start	End
ND	319 ± 3	441 ± 7	3.9 ± 0.1	4.1 ± 0.2
STZ-D	322 ± 3	351 ± 7*	19.9 ± 0.9*	22.2 ± 1.3*
STZ-D + 5 mg/kg ALC	321 ± 3	337 ± 8*	21.8 ± 0.8*	21.1 ± 1.0*
STZ-D + 25 mg/kg ALC	317 ± 6	335 ± 10*	19.4 ± 1.1*	21.3 ± 1.0*
STZ-D + 50 mg/kg ALC	321 ± 4	341 ± 8*	18.9 ± 1.2*	22.2 ± 0.9*
STZ-D + 100 mg/kg ALC	322 ± 5	325 ± 9*	19.4 ± 0.9*	23.0 ± 1.0*

NOTE. Data are the mean ± SEM.
*$P < .05$ *v* control.

Table 4. Effects of 4 Weeks of ALC (5-100 mg/kg) by Gavage and STZ-D on Sciatic Nerve ALC and Free L-Carnitine Levels (nmol/g wet weight)

Group	ALC	Free L-Carnitine
ND	54 ± 1.2	148 ± 9
STZ-D	29 ± 2*	79 ± 23*
STZ-D + 5 mg/kg ALC	32 ± 4*	79 ± 5*
STZ-D + 25 mg/kg ALC	36 ± 2*	86 ± 14*
STZ-D + 50 mg/kg ALC	39 ± 4*†	89 ± 18*
STZ-D + 100 mg/kg ALC	43 ± 3*†	82 ± 4*

NOTE. Data are the mean ± SEM.
*$P < .05$ *v* ND.
†$P < .05$ *v* STZ-D.

Effects of ALC on ouabain-sensitive and -insensitive Na,K-ATPase. The dose-dependent effects of ALC on ouabain-sensitive Na,K-ATPase activity are shown in Fig 4. After 4 weeks of STZ-D, there was a 28% ($P < .05$) decrease in ouabain-sensitive Na,K-ATPase activity. This decrease was selective for the ouabain-sensitive ATPase, as no change was measured in ouabain-insensitive ATPase activity (Table 5). ALC at the smallest dose of 5 mg/kg/d was found to increase ouabain-sensitive ATPase activity by 22%, to levels not significantly different from those in ND animals. Further increases in the dose of ALC produced a slight but statistically insignificant further increase in ATPase activity. Therefore, ALC was found to correct the ouabain-sensitive Na,K-ATPase at 5 mg/kg/d, a dose that had no observable effect on MNCV.

Effects of STZ-D and ALC on sciatic nerve osmolyte levels. Figure 5 shows the effect of 5 to 100 mg ALC on nerve MI and sorbitol levels in STZ-D rats. STZ-D rats showed a 31% decrease in nerve MI levels ($P < .01$) after 4 weeks. ALC treatment at any dose did not affect nerve MI levels in STZ-D rats, and indeed at doses above 5 mg/kg, there was a small (19%) but statistically insignificant decrease of MI

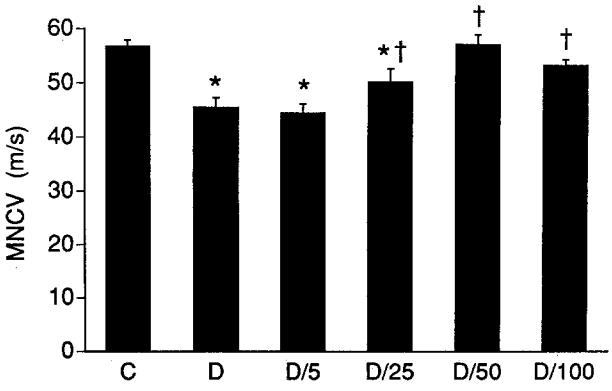

Fig 3. Dose-response effects of 5 to 100 mg/kg ALC (by gavage) on sciatic MNCV. Data are the mean ± SEM. *$P < .01$ *v* control ND (C). †$P < .01$ *v* D.

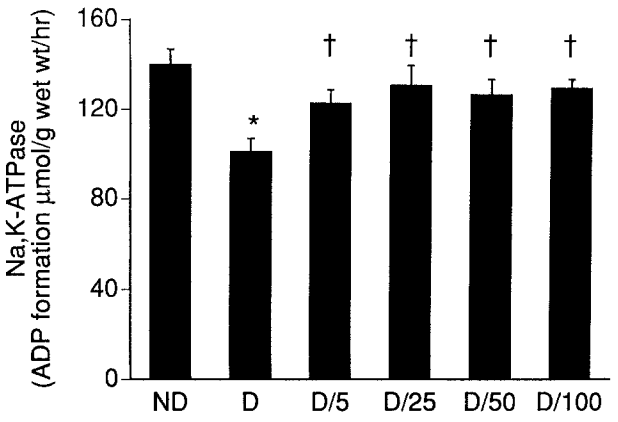

Fig 4. Dose-response effects of 5 to 100 mg/kg ALC (by gavage) on sciatic nerve ouabain-inhibitable Na,K-ATPase activity. Data are the mean ± SEM. *$P < .01$ *v* control ND. †$P < .01$ *v* D.

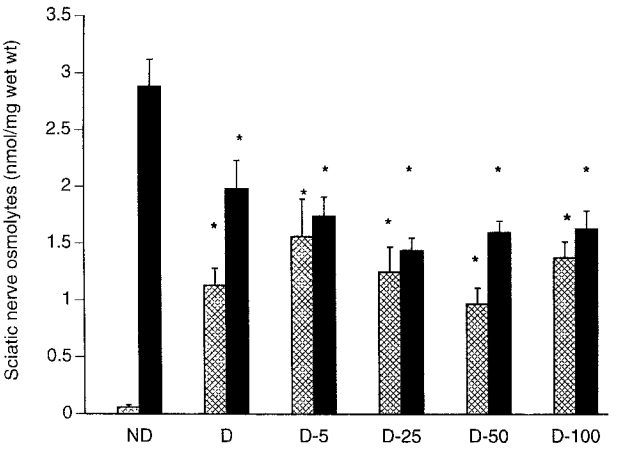

Fig 5. Dose-response effects of 5 to 100 mg/kg ALC (by gavage) on sciatic nerve osmolyte levels after 4 weeks of diabetes. (▨) Nerve sorbitol; (■) MI. Data are the mean ± SEM. *$P < .01$ *v* control ND.

levels. Levels of the β-amino acid taurine were also unaffected by ALC (2.0 ± 0.1 *v* 1.9 ± 0.2 nmol/mg wet weight, untreated *v* ALC-treated STZ-D rats, respectively). Nerve glucose, sorbitol, and fructose levels were increased in all STZ-D experimental groups and were unaffected by ALC. Thus, after 4 weeks of diabetes, ALC did not affect the levels of polyol pathway products or of nerve osmolytes, but nevertheless normalized nerve ouabain-sensitive ATPase activity and MNCV.

DISCUSSION

Accumulating evidence that ALC administration prevents the slowing of MNCV in STZ-D rats[28-31] typically involved doses of ALC exceeding that needed to selectively replace nerve ALC levels. The fact that depressed levels of free L-carnitine were also corrected[31] precludes a definitive assessment of the role of ALC depletion in experimental neuropathy. This study demonstrates that levels of ALC were depleted in the sciatic nerve of 2-week STZ-D rats, and that ALC treatment that prevented MNCV slowing and replaced nerve ALC levels also normalized nerve MI (but not taurine or sorbitol) levels. At 2 weeks, composite nerve ouabain-sensitive ATPase activity was not depressed (data not shown). After 4 weeks, sciatic nerve composite ouabain-sensitive ATPase activity was depressed, and increasing nerve ALC (but not L-carnitine) prevented this impairment without affecting ouabain-insensitive ATPase activity,[31] although nerve MI remained depleted despite ALC treatment that corrected MNCV. These data are consistent with the hypothesis that sciatic nerve ALC depletion may differentially impair membrane processes such as sodium-dependent or -independent MI transport and Na,K-ATPase, but the relationship between these impairments and their relationships to impaired nerve function are more complex temporally and mechanistically than previously postulated. Moreover, although these data are qualitatively consistent with a cause-effect relationship between defects in composite ouabain-sensitive ATPase and MNCV in 4-week STZ-D rats, correction of composite ouabain-sensitive ATPase activity is achieved at doses of ALC that fail to correct MNCV (and fail to completely replace nerve ALC levels). These data suggest that cell- or fiber-specific differences in Na,K-ATPase subfractions or in their ouabain and/or ALC sensitivity may contribute to the discrepant responses observed. Alternatively, as a result of altered nerve Na permeability[38,39] or other membrane defects in STZ-D, the magnitude of composite Na,K-ATPase correction achieved by ALC repletion in and of itself, may be insufficient to sustain normal MNCV. In either case, ALC depletion, which is associated with nerve MI depletion at 2 weeks and Na,K-ATPase impairment at 4 weeks, slows MNCV, since increasing nerve ALC preserved MNCV at both time points. (In a similar fashion, MI repletion corrects MNCV at both time points, as well.[40]) Unfortunately, the measurement of maximal MNCV currently used in most existing studies of diabetic rats measures it only in the most rapidly conducting large myelinated nerve fibers, yielding a grossly incomplete picture of the intactness of nerve electrophysiology at the nerve fiber level. More detailed electrophysiological analysis examining subsets of nerve fibers under various types of single and repetitive stimuli may be required to fully understand the implications (or lack thereof) of these time- and dose-dependent discrepancies in MNCV and nerve Na,K-ATPase activity in diabetic rats.

The biochemical mechanism by which nerve ALC repletion improves MNCV in diabetes is unknown. Acute STZ-D depletes nerve ALC and L-carnitine by an unknown

Table 5. Effect of STZ-D and ALC (5-100 mg/kg) by Gavage on Sciatic Nerve Ouabain-Insensitive Na,K-ATPase Activity After 4 Weeks of STZ-D

Group	Ouabain-Insensitive ATPase Activity (μmol ADP formed/g wet weight/h)
ND	93 ± 6
STZ-D	86 ± 7
STZ-D 5 mg/kg ALC	105 ± 7
STZ-D 25 mg/kg ALC	95 ± 8
STZ-D 50 mg/kg ALC	98 ± 6
STZ-D 100 mg/kg ALC	92 ± 7

NOTE. Data are the mean ± SEM.

mechanism, and ALC repletion normalizes MNCV in a dose-dependent fashion, initially with and then without accompanying preservation of nerve MI levels. ALC depletion could logically introduce two broad and possibly (but not necessarily) related types of metabolic defects, one primarily involving tissue bioenergetics[9,21,39,41,42] and the other involving altered signal transduction,[3-9] both of which could potentially reflect abnormal fatty acid metabolism and/or composition in the membrane lipid bilayer, and both of which could affect the membrane-bound, protein kinase–regulated sodium-dependent MI transporter[43] and Na,K-ATPase.[7]

Alterations in fatty acid metabolism in diabetes[1,2] have been invoked in the pathogenesis of diabetic neuropathy. Long-chain fatty acids and fatty acid esters accumulate in peripheral nerve in experimental diabetes[2,4,33] and may perturb membrane stability and function[4] potentially by mechanism(s) involving alterations in PKC and/or Na,K-ATPase activity.[4-8] Specific fatty acids (eg, arachidonate) directly activate some isoforms of PKC,[22] and long-chain fatty acid esters may inhibit PKC activity under some circumstances.[23,24] ALC promotes β-oxidation of fatty acids in liver,[27,44] and its replacement may therefore ameliorate defective fatty acid metabolism in diabetic nerve, thereby potentially reversing defects in phosphoinositide-mediated signal transduction, improving net tissue energy production, and also correcting membrane fatty acid composition.

Decreased Na,K-ATPase activity may reflect altered energy metabolism[7,9] and mediate the nerve conduction slowing and paranodal swelling observed in acute experimental diabetes.[7,10-14] Voltage clamp studies document decreased resting axolemmal membrane potential and a fourfold to fivefold increase in intra-axonal Na^+ content.[38,39] Moreover, this ATP-dependent Na,K-antiporter maintains the Na gradient necessary for transmembrane transport of MI and taurine. The Na,K-ATPase is a heterodimer consisting of an α-subunit (M_r 112 kd) that contains the catalytic subunit and the ATP- and ouabain-binding site[45-47] and is the substrate for protein kinases,[42] and a β-subunit (M_r 35 kd) that may be important for membrane binding.[48] Three different isoforms of the α-subunit have been identified (α1, α2, α3),[18,49,50] with α1 predominating in peripheral nerve and the Schwann cell.[18,51]

The effects of ALC on nerve Na,K-ATPase activity may be mediated by changes in PKC activity. Alterations in PKC activity have been proposed to mediate the impairment of ATPase activity observed by many[7,10-12,14-16,52] but not all[53] investigators in the diabetic rodent. Recently, PKC activation was shown to be without effect on the phosphorylation state of the α-subunit in nerves from ND rats,[18] whereas in the STZ-D rat, increased α-subunit ^{32}P-labeling was observed with PKC activation, suggesting that in this model, tonic endogenous PKC-mediated Na,K-ATPase phosphorylation exists that is diminished by diabetes and may potentially be sensitive to ALC.

The effects of increasing nerve ALC on nerve MI levels in the STZ-D rat appear to be transient. After 14 days of diabetes, ALC replacement prevents MI depletion; however, by 4 weeks, MI depletion is observed despite preservation of both Na,K-ATPase and MNCV. The lack of effect of ALC on nerve MI after 4 weeks may reflect that complete nerve ALC replacement was not achieved with the largest dose of ALC given by gavage. However, no incremental increases in nerve MI were observed in response to increasing ALC dose, and this lack of effect of ALC on nerve MI levels in longer-term diabetic rats is in agreement with other reports in more chronic models of experimental diabetic neuropathy that found no effect of ALC on nerve MI levels after 6 weeks of diabetes.[31] The reasons for this transient effect are not clear, but our observations serve to eliminate some potential mechanisms thought to mediate acute nerve MI depletion. For example, acute nerve MI depletion appears not to be primarily osmotically mediated, since MI levels changed independently of other nerve osmolytes. Impaired nerve ATPase activity also appears not to be the principal mediator of MI levels, as MI remained depleted despite correction of ATPase activity. Conversely, it remains possible that the protective effect of nerve ALC repletion on Na,K-ATPase could have been mediated by the prior transient prevention of MI depletion, since the latter also corrects impaired ATPase activity.[7,12]

Acute changes in kinase activity in the nerve may play a key role in regulating nerve MI levels. PKC regulates Na-dependent and -independent MI transport,[25,43] and so in diabetes, ALC-sensitive changes in PKC activity or altered nerve energy balance may mediate the major component of the rapid depletion of MI observed in diabetic rodents.[16,20,21] Moreover, preservation of sciatic MNCV at 4 weeks despite MI depletion may indicate that nerve ALC repletion can directly restore to normal the defects in nerve lipid and phosphoinositol metabolism, which have been proposed to be corrected by MI treatment.[7,20,21,54]

This study reports a dose-dependent discrepancy between correction of nerve ouabain-sensitive Na,K-ATPase and sciatic MNCV. The discordance at the lowest dose used in this study may reflect the differences in fiber type and/or in the α-isoform of the ATPase (or phosphorylation of its regulatory domain) that principally contribute to these measurements. For example, MNCV is determined by rapidly conducting large myelinated fibers, in which significant concentrations of ATPase enzyme may be limited to the paranodal Schwann cell processes and the nodal axolemma.[38,39,55,56] In these fibers (and their Schwann cells[55]), the predominant isoform may be α1,[18,57] which in rodents is highly ouabain-resistant.[56-58] In contrast, much of the measured composite ouabain-inhibitable Na,K-ATPase activity in whole sciatic nerve may reside in the ouabain-sensitive α2- and α3-isoforms[18,56,57] of the unmyelinated nerve fibers,[58] which contribute little to the measured MNCV. The correction of composite ATPase activity by partial nerve ALC replacement may reflect a pool of the enzyme residing in unmyelinated sensory fibers, which exhibit a delayed decrease in ATPase activity but are highly ALC-sensitive, thus contrasting with the potentially less ALC-sensitive or less ALC-accessable pool in large myelinated fibers. Alterna-

tively, composite ATPase correction may be necessary but not sufficient for the MNCV response, and other dose-dependent effects of ALC are required in addition.

In summary, these studies are consistent with the hypothesis that depletion of nerve ALC in the STZ-D rat results in sequential metabolic abnormalities involving MI and Na,K-ATPase that are associated with persistent slowing of MNCV. Detailed fiber-specific functional and biochemical studies are required to further explore the role of ALC in the maintenance of normal nerve function.

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