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# Beneficial effects of gamma linolenic acid supplementation on nerve conduction velocity, Na+, K+ ATPase activity, and membrane fatty acid composition in sciatic nerve of diabetic rats

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*Metabolic and vascular abnormalities are implicated in the pathogenesis of diabetic neuropathy. Two principal metabolic defects are altered lipid metabolism resulting from the impairment of delta-6-desaturase, which converts linoleic acid (LA) into gamma linolenic acid (GLA), and reduced nerve Na+, K+ ATPase activity. This reduction may be caused by a lack of incorporation of (n-6) fatty acids in membrane phospholipids. Because this ubiquitous enzyme maintains the membrane electrical potential and allows repolarization, disturbances in its activity can alter the process of nerve conduction velocity (NCV). We studied the effects of supplementation with GLA (260 mg per day) on NCV, fatty acid phospholipid composition, and Na+, K+ ATPase activity in streptozotocin-diabetic rats. Six groups of 10 rats were studied. Two groups served as controls supplemented with GLA or sunflower oil (GLA free). Two groups with different durations of diabetes were studied: 6 weeks with no supplementation and 12 weeks supplemented with sunflower oil. To test the ability of GLA to prevent or reverse the effects of diabetes, two groups of diabetic rats were supplemented with GLA, one group for 12 weeks and one group for 6 weeks, starting 6 weeks after diabetes induction. Diabetes resulted in a 25% decrease in NCV ($P < 0.0001$), a 45% decrease in* Na+, K+ ATPase *activity ($P < 0.0001$), and an abnormal phospholipid fatty acid composition. GLA restored NCV both in the prevention and reversal studies and partially restored Na+, K+ ATPase activity in the preventive treatment group ($P < 0.0001$). These effects were accompanied by a modification of phospholipid fatty acid composition in nerve membranes. Overall, the results suggest that membrane fatty acid composition plays a direct role in NCV and confirm the beneficial effect of GLA supplementation in diabetic neuropathy.* (J. Nutr. Biochem. 10: 411–420, 1999) 

**Keywords**: diabetes; gamma linolenic acid; Na+, K+ ATPase; nerve conduction velocity; rats

## Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and by specific long-term microvascular and neurologic complications.[1] Various organs are affected, including kidney,[2] retina,[3] heart,[4,5] and peripheral nerve.[6]

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Diabetic neuropathy is a common complication of diabetes but the mechanisms underlying its pathogenesis are not fully understood.[7] In addition, the relative importance of metabolic and ischemic-hypoxic factors in diabetic neuropathy is debated. Fatty acid composition is changed in humans[8,9] and animals with diabetes.[10–12] Diabetes inhibits delta-6-desaturase,[10] which converts linoleic acid (LA) into gamma linolenic acid (GLA), the precursor of arachidonic acid and, ultimately, several vasoactive prostanoids. In experimental and clinical diabetes, GLA production is reduced.[13,14] Consequently, the levels of dihomo gamma linolenic acid (DGLA), which is a product of GLA elongation, and arachidonic acid[11,14] also are reduced, which results in a decreased production of the prostanoids, prostacyclin, and prostaglandins.[15–18]

Because these prostanoids are not stored in mammalian tissues and because the concentration of unesterified arachidonic acid in tissues in the basal state is close to zero, it is thought that the rate limiting step for their synthesis is the release of arachidonic acid from membrane phospholipids through the activation of phospholipase A2. These metabolites have several important pharmacologic and biological actions.[19–21] Defects in their synthesis may decrease nerve blood flow, leading to reduced nerve conduction velocity (NCV). In fact, prostacyclin and prostaglandins are known to increase blood flow,[21] particularly in nerves.[22] On the other hand, delta-6-desaturase inhibition makes the fatty acid composition of membrane phospholipids abnormal.

Only one study has investigated the effect of short duration alloxan diabetes on fatty acid composition in the sciatic nerve.[23] These changes in membrane structure may impair the Na+, K+ ATPase activity (EC 3.6.1.37); it is reduced in the sciatic nerve of diabetic animals[24–26] and in insulin-dependent diabetic patients.[6] In addition, Na+, K+ ATPase plays a central role in membrane repolarization and thus may influence NCV. A lack of GLA and derived metabolites because of delta-6-desaturase inhibition may be bypassed by dietary supplementation with a GLA-rich oil. Moreover, 4 to 5 weeks of GLA diet supplementation completely prevented the development of the motor NCV deficit but had unfavorable effects on nerve Na+, K+ ATPase activity in diabetic rats; inversely, no deficit in Na+, K+ ATPase activity was observed in untreated diabetic rats.[27]

To further investigate the ability of GLA treatment in reducing deficits in Na+, K+ ATPase activity and NCV induced by diabetes, we conducted two experiments in which diabetes lasted long enough to observe simultaneous decreases in NCV and Na+, K+ ATPase.[22,28] In a prevention study, GLA was fed after diabetes induction for 12 weeks. In a reversal study, GLA supplementation was started 6 weeks after diabetes induction and given for 6 weeks. The aim was to test whether GLA could prevent or reverse the nerve abnormalities in streptozotocin-diabetic rats.

## Methods and materials

### Animals

The study was done according to the guidelines of the French Department of Agriculture, Fishing, and Diet on the experimental use of laboratory animals with agreement no. A 13823.

**Table 1** Fatty acid composition of sunflower and di-linolein-mono-gamma-linolenate 45 oils

| | Sunflower oil (g/kg oil) | DLMG 45 oil (g/kg oil) |
|---|---|---|
| Linoleic acid | 648 | 623 |
| Gamma linolenic acid | ND | 245 |
| Oleic acid | 228 | 19 |

ND–not detectable.

Sixty male Sprague-Dawley rats (Iffa Credo, L'Arbresle, France) weighing approximately 190 g were entered in the experiments after acclimatization for 1 week. Their body weight at the beginning of the study averaged 232 ± 12 g, and they were separated into six age-matched groups ($n$ = 10). In the four diabetic groups, diabetes was induced by injecting into a tail vein 65 mg/kg of streptozotocin (Sigma Chemical Co., St. Louis, MO USA) freshly dissolved in citrate sodium buffer 0.01 mol/L, pH 5.5. Rats from the two control groups were injected with buffer only. All diabetic rats were maintained in the hyperglycemic state without insulin. In the six groups, blood samples were collected from the tip of the tail after the initial blood volume was discarded to avoid contamination of the sample with non-blood fluid compartments, and blood glucose was measured with a reagent strip (Reflolux, Boehringer Mannheim, Mannheim, Germany). Rats were given free access to normal rat chow (A04, UAR, Epinay sur Orge, France) and water.

Gavage was started on the day of streptozotocin or buffer administration. Two oils were chosen for supplementation: di-linolein-mono-gamma-linolenate (DLMG 45) containing GLA and sunflower oil because it contains no GLA (Scotia Pharmaceuticals, Guildford, Surrey, UK) (*Table 1*). All the groups treated with DLMG 45 received 260 mg of GLA (approximately 1 mL of DMLG 45) by gavage in a single daily dose, as previously described.[29] The same volume of sunflower oil was given to the other groups to avoid differences in fat intake; sunflower oil and DLMG have similar caloric value. The oil was administered daily at 9:00 AM. No differences were observed in dietary intake between groups supplemented with the two oils.

The two control groups were treated with sunflower oil or GLA for 12 weeks. Diabetic rats were divided into four groups (1) A 6-week diabetic group without treatment allowed us to determine whether neuropathy occurred after this duration of diabetes to study the reversal effect of GLA. (2) A 12-week diabetic group was treated with sunflower oil for 12 weeks. (3) A reversal GLA diabetic group received no gavage for 6 weeks and then was treated with GLA for the next 6 weeks. (4) A prevention GLA diabetic group was treated with GLA for 12 weeks (*Figure 1*).

### Measurement of NCV

After 6 weeks for the 6-week diabetic group or 12 weeks for the other groups, NCV was recorded in a temperature-controlled environment from the left sciatic tibial nerve of rats under ether anesthesia by a noninvasive method adapted from Stevens et al.[30] The rectal temperature was maintained at 36 to 37°C with a heating lamp and pad. The left sciatic nerve was stimulated proximally at the sciatic notch and distally at the ankle via bipolar electrodes with supramaximal stimuli (6 mA) using a 0.3-ms rectangular pulse from a stimulator at 10 Hz on Neuropack 2 EMG (Nihon Kohden, Claye-Souilly, France). The muscle action potential was recorded from the first interosseous muscle of the left hindlimb by unipolar pin electrodes. The latencies were measured from the stimulus artifact to the onset of the negative M-wave

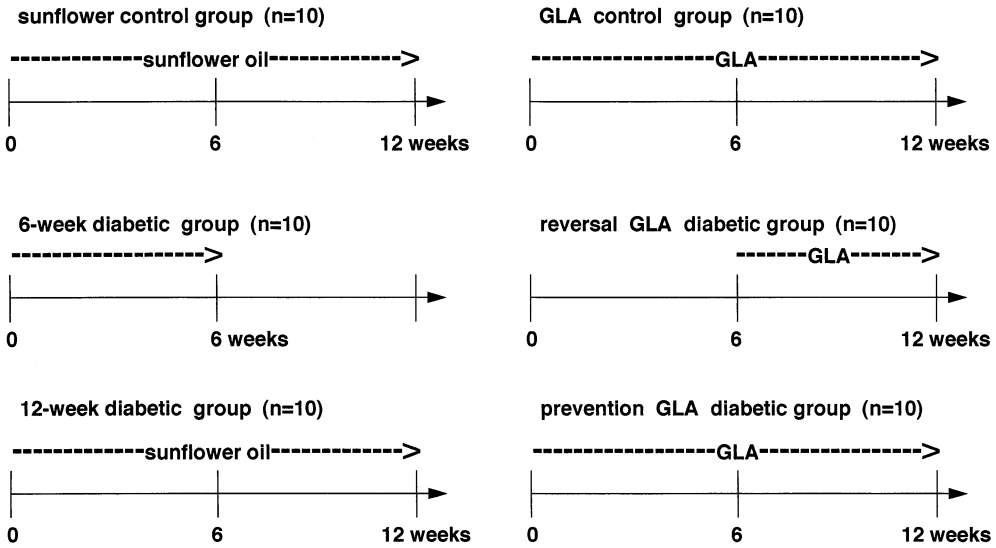


**Figure 1** Study design. Six groups of rats were studied: two control groups fed either sunflower oil or gamma linolenic acid (GLA); one 6-week diabetic group without supplementation and one diabetic group supplemented with GLA after 6 weeks of diabetes and during the following 6 weeks to determine whether GLA has reversal effects; one 12-week diabetic group supplemented with sunflower oil and one diabetic group supplemented with GLA for 12 weeks to assess the preventive effect of GLA.

deflection. The NCV (in meters by seconds) was calculated as the ratio of the distance between the two sites of stimulation in millimeters divided by the difference between proximal and distal latencies in milliseconds.

### *Tissue preparation*

One day after NCV was measured, the animals were anesthetized with ethyl ether. An incision was made and segments of the sciatic nerves from the spine to the peroneal bifurcation were dissected. After removal of adherent muscle tissue, the segments were measured, weighed, and rinsed in ice-cold saline. For Na+, K+ ATPase activity measurement, the nerve specimen was chopped and homogenized at 4°C by a Potter homogenizer (94348 electronic, Heidolph, Kelheim, Germany) with three bursts not exceeding 15 seconds in 2 mL of ice-cold Tris buffer 0.011 mol/L (pH 7.4). During this operation, the sample was cooled in ice. The resulting homogenate was passed through a paper filter (600F4252, Fioroni, La Chapelle St Mesmin, France) to remove debris.

### *Measurement of Na+, K+ ATPase activity*

Na+, K+ ATPase activity was calculated by spectrophotometric determination of inorganic phosphate released from adenosine triphosphate (ATP), with or without ouabain, which is a specific enzyme inhibitor, by a method adapted from Rahmani-Jourdheuil et al.[31] and Kim et al.,[32] as previously described.[33] Briefly, membrane samples (29.4 ± 0.7 μg) were pre-incubated for 10 minutes at 37°C in a medium containing 92 mmol/L Tris-HCl (pH 7.4), 100 mmol/L NaCl, 20 mmol/L KCl, 5 mmol/L MgSO4 7H2O, and 1 mmol/L EDTA. These assays were done with or without 1 mmol/L ouabain (O-3125, Sigma Chemical Co.), a specific inhibitor of Na+, K+ ATPase. After incubation with 4 mmol/L vanadate-free ATP (A-5394, Sigma Chemical Co.) at 37°C for 10 minutes, the reaction was stopped by the addition of ice-cold trichloroacetic acid at a final concentration of 5%. After centrifugation at 4°C and 5,500 g for 10 minutes, the amount of inorganic phosphate in the supernatant was determined according to the method of Hurst.[34] Na+, K+ ATPase activity was calculated as the difference between inorganic phosphate released per milligram of protein per hour with or without ouabain. Protein amounts were determined by the Bio-Rad protein assay (Laboratories GmbH, Munich, Germany). All assays were done in triplicate, and blanks for substrate, membrane, and incubation time were routinely included to compensate for endogenous phosphate and non-enzyme-related breakdown of ATP. The coefficient of variation under these experimental conditions was 8%.

### *Determination of fatty acid phospholipid composition*

Fatty acids were analyzed as methyl esters by gas chromatography on a Perkin Elmer model Autosystem XL with a fused silica capillary column (30 m × 0.22 mm inner diameter) BPX 70 (SGE, Villeneuve Saint Georges, France) equipped with a flame ionization detector and using hydrogen as carrier gas. The temperature program ranged from 160°C to 205°C at 1°C/min. Peak areas from the resulting chromatogram were measured with a Perkin Elmer 1022 S integrator. After extraction of lipids,[35] fatty acid methyl esters were prepared according to the methanolysis method.[36]

### *Statistical analysis*

Results are expressed as mean ± SEM. Before assessing the different variables using one-way analysis of variance (ANOVA) or a nonparametric Kruskal-Wallis test, we did a Kolmogorov-Smirnov test for normality and a Bartlett test for homogeneous variance for each group. One-way ANOVA was used to analyze body weight, plasma glucose, and treatment effects on NCV and Na+, K+ ATPase activity. Differences between groups were identified by the Bonferroni-Dunn test. *P*-values of less than 0.0033 were considered significant. Fatty acid composition was investigated by Kruskal-Wallis test. Differences between groups were identified by the Mann-Whitney U test and were considered significant at a *P*-value of less than 0.0033. This *P*-value was determined by dividing the *P*-value = 0.05 by 15, the number of comparisons between the six groups, as in the very conservative

**Table 2** Comparison of body weight and plasma glucose levels

| Diet group | Body weight (g) | Plasma glucose (mmol/L) |
|---|---|---|
| Sunflower control | 519 ± 18 | 4.7 ± 0.1 |
| GLA control | 499 ± 13 | 5.1 ± 0.1 |
| 6-Week diabetes | 279 ± 10[a] | 25.2 ± 0.8[a] |
| 12-Week diabetes | 325 ± 19[a] | 26.3 ± 0.7[a] |
| Reversal GLA diabetes | 333 ± 16[a] | 26.3 ± 1.1[a] |
| Prevention GLA diabetes | 334 ± 23[a] | 25.2 ± 2.0[a] |

Note: See *Figure 1* for a detailed description of groups. Values are means ± SEM, $n = 10$. Significance was determined by analysis of variance and the differences between groups were assessed by the Bonferroni-Dunn test. [a] $P < 0.0001$ vs. sunflower and gamma linolenic acid (GLA) control.

Bonferroni-Dunn test.[28] All analyses were done by STATVIEW® software (Abacus Concepts, Berkeley, CA USA).

## Results

### *Metabolic characteristics*

*Table 2* summarizes the metabolic data in all groups. Relative to the two control groups, the plasma glucose levels of the four diabetic groups were increased approximately 500% ($P < 0.0001$) whereas body weight was markedly reduced by approximately 35% ($P < 0.0001$ ). Treatment of diabetic rats with GLA supplementation was unable to restore these two parameters.

### *Sciatic NCV*

Relative to the two control groups, NCV was approximately 25% lower after 6 weeks of diabetes and remained unchanged at 12 weeks ($P < 0.0001$). GLA supplementation not only prevented abnormal NCV in diabetic rats but also reversed their diabetes-induced decrease in NCV ($P < 0.0001$; *Figure 2*).

### *Sciatic nerve Na+, K+ ATPase activity*

Relative to the two control groups, Na+, K+ ATPase activity was dramatically decreased by approximately 40% with both 6 and 12 weeks diabetes ($P < 0.0001$). GLA treatment partially prevented this loss of activity ($P < 0.0001$), but once the abnormality was established, GLA treatment had no effect (*Figure 3*).

### *Membrane phospholipid fatty acid composition of sciatic nerve*

The fatty acids were the following: GLA, myristic acid as C14:0, palmitic acid as C16:0, stearic acid as C18:0, oleic acid as C18:1 (n-9), eicosenoic acid as C20:1 (n-9), and docosatetraenoic acid as C22:4 (n-6). Table 3 shows the results of the phospholipid fatty acid analysis of the sciatic nerve homogenate. In the control rats and the diabetic rats supplemented with GLA, GLA concentration in membrane phospholipids increased significantly ($P < 0.0033$); GLA was not detected in membranes of control or diabetic rats treated with sunflower oil (*Figure 4*).

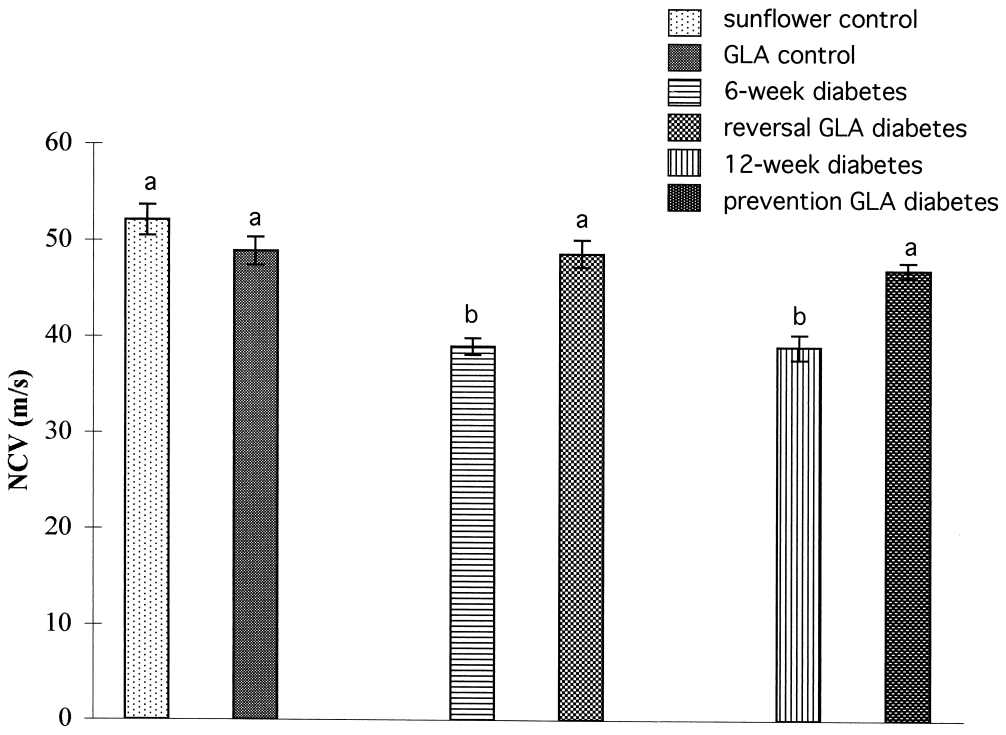


**Figure 2** Nerve conduction velocity (NCV). Diabetes affects nerve conduction velocity in rats. See *Figure 1* for a detailed description of groups. NCV was determined by a noninvasive method with microelectrodes. Values are means ± SEM, $n = 10$. Bars not bearing the same superscript are significantly different (Bonferroni-Dunn test, $P < 0.0001$). GLA, gamma linolenic acid.

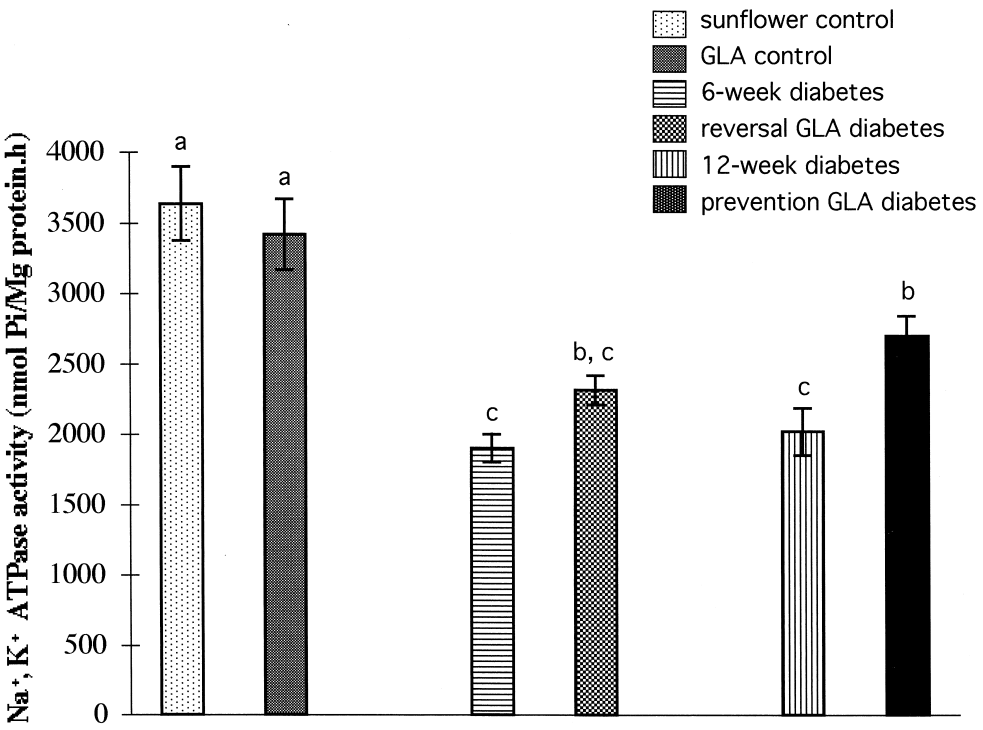


**Figure 3** Na+,K+ ATPase activity in sciatic nerve homogenate. Diabetes affects Na+,K+ ATPase activity in rats. See *Figure 1* for a detailed description of groups. Na+,K+ ATPase activity was calculated by a spectrophotometric method. Values are means ± SEM, $n = 10$. Bars not bearing at least one same superscript are significantly different (Bonferroni-Dunn test, $P < 0.0001$). GLA, gamma linolenic acid.

**Table 3** Phospholipid fatty acid composition of sciatic nerve homogenate (g/100 g fatty acid methyl esters)

| Fatty acid | Sunflower control | GLA control | 6-Week diabetes | 12-week diabetes | Reversal GLA diabetes | Prevention GLA diabetes |
|---|---|---|---|---|---|---|
| 14:0 | 3.0 ± 0.6$^{a}$ | 2.8 ± 0.5$^{a}$ | 1.9 ± 0.4$^{a}$ | 3.2 ± 0.8$^{a}$ | ND$^{b}$ | ND$^{b}$ |
| 16:0 | 21.8 ± 1.2$^{b}$ | 22.6 ± 1.1$^{b}$ | 22.1 ± 0.8$^{b}$ | 21.8 ± 0.9$^{b}$ | 24.9 ± 1.0$^{a,b}$ | 27.3 ± 1.6$^{a}$ |
| 16:1 | 2.0 ± 0.4 | 3.3 ± 0.5 | 1.8 ± 0.2 | 2.3 ± 0.6 | 1.9 ± 0.3 | 2.6 ± 0.2 |
| 18:0 | 10.6 ± 0.8$^{a}$ | 8.4 ± 0.4$^{b}$ | 11.6 ± 0.3$^{a}$ | 11.3 ± 0.3$^{a}$ | 10.9 ± 0.5$^{a}$ | 10.1 ± 0.8$^{a,b}$ |
| 18:1 | 43.4 ± 2.3$^{a,b}$ | 34.5 ± 1.8$^{c}$ | 47.0 ± 0.8$^{a}$ | 41.6 ± 2.2$^{b,c}$ | 39.0 ± 1.5$^{b,c}$ | 37.6 ± 2.5$^{b,c}$ |
| 18:2(n-6) | 10.1 ± 2.7$^{a,b}$ | 18.3 ± 2.9$^{a}$ | 4.1 ± 0.8$^{b}$ | 9.0 ± 1.8$^{a,b}$ | 12.5 ± 2.6$^{a}$ | 11.3 ± 4.2$^{a,b}$ |
| 18:3(n-3) | 0.2 ± 0.1$^{b,c}$ | 1.0 ± 0.3$^{a}$ | ND$^{b}$ | 0.1 ± 0.1$^{b,c}$ | 0.4 ± 0.1$^{a,c}$ | 0.2 ± 0.1$^{b,c}$ |
| 18:3(n-6) | ND$^{b}$ | 0.7 ± 0.1$^{a}$ | ND$^{b}$ | ND$^{b}$ | 0.6 ± 0.2$^{a}$ | 0.4 ± 0.2$^{a}$ |
| 20:0 | 1.2 ± 0.3$^{a,b}$ | 1.1 ± 0.1$^{b}$ | 1.9 ± 0.3$^{a}$ | 1.7 ± 0.1$^{a}$ | 1.4 ± 0.2$^{a,b}$ | 1.6 ± 0.2$^{a,b}$ |
| 20:1(n-9) | 1.2 ± 0.3$^{b}$ | 2.7 ± 0.5$^{a,c}$ | 3.0 ± 0.6$^{a,c}$ | 1.9 ± 0.3$^{b,c}$ | 3.6 ± 0.3$^{a}$ | 3.8 ± 0.5$^{a}$ |
| 20:3(n-6) | 0.4 ± 0.2 | 0.5 ± 0.2 | 0.3 ± 0.2 | 0.4 ± 0.1 | 0.8 ± 0.2 | 0.5 ± 0.2 |
| 20:4(n-6) | 3.6 ± 0.4$^{a,c}$ | 2.6 ± 0.4$^{b,c}$ | 4.0 ± 0.1$^{a}$ | 3.1 ± 0.3$^{b,c}$ | 2.6 ± 0.4$^{b,c}$ | 3.0 ± 0.3$^{b,c}$ |
| 22:4(n-6) | 0.7 ± 0.2 | 0.7 ± 0.2 | 0.6 ± 0.2 | 0.9 ± 0.2 | 0.3 ± 0.2 | 0.8 ± 0.3 |
| 24:1(n-9) | 0.5 ± 0.2 | 0.2 ± 0.1 | 0.4 ± 0.2 | 0.7 ± 0.2 | 0.3 ± 0.2 | 0.3 ± 0.2 |
| 22:6(n-3) | 1.0 ± 0.2$^{b,c}$ | 0.4 ± 0.2$^{b}$ | 1.3 ± 0.5$^{a,c}$ | 1.1 ± 0.4$^{b}$ | 0.8 ± 0.3$^{b,c}$ | 0.5 ± 0.2$^{b}$ |
| ΣSFA | 36.6 ± 1.5 | 34.9 ± 1.4 | 37.5 ± 0.7 | 38.0 ± 1.4 | 37.2 ± 1.3 | 39.0 ± 2.2 |
| ΣMUFA | 47.3 ± 2.0$^{a,c}$ | 40.9 ± 2.4$^{b,c}$ | 52.2 ± 1.0$^{a}$ | 47.3 ± 1.7$^{a,c}$ | 44.8 ± 1.8$^{b,c}$ | 44.3 ± 2.6$^{b,c}$ |
| ΣPUFA | 16.1 ± 2.2$^{b}$ | 24.2 ± 3.0$^{a}$ | 10.3 ± 0.8$^{b}$ | 14.7 ± 1.6$^{b}$ | 18.0 ± 2.3$^{a,b}$ | 16.7 ± 4.1$^{a,b}$ |
| Σ(n-3) | 1.3 ± 0.3 | 1.4 ± 0.3 | 1.3 ± 0.5 | 1.3 ± 0.4 | 1.2 ± 0.3 | 0.7 ± 0.3 |
| Σ(n-6) | 14.8 ± 2.2$^{b}$ | 22.8 ± 2.7$^{a}$ | 9.0 ± 0.7$^{c}$ | 13.4 ± 1.7$^{b,c}$ | 16.8 ± 2.3$^{a,b}$ | 15.9 ± 4.0$^{a,b}$ |

Note: See *Figure 1* for a detailed description of groups. Values are means ± SEM, $n = 10$; 20:5(n-3) and 22:1(n-9) with low values do not appear on this table. Statistical analysis of fatty acid composition was done by Kruskal-Wallis test. Differences between groups were identified by the Mann-Whitney U test and were considered significant for a $P$-value lower than 0.0033. Values in the same lane not bearing at least one same superscript letter were significantly different.
GLA–gamma linolenic acid. MUFA–monounsaturated fatty acids. ND–not detectable. PUFA–polyunsaturated fatty acids. SFA–saturated fatty acids. Σ–sum.

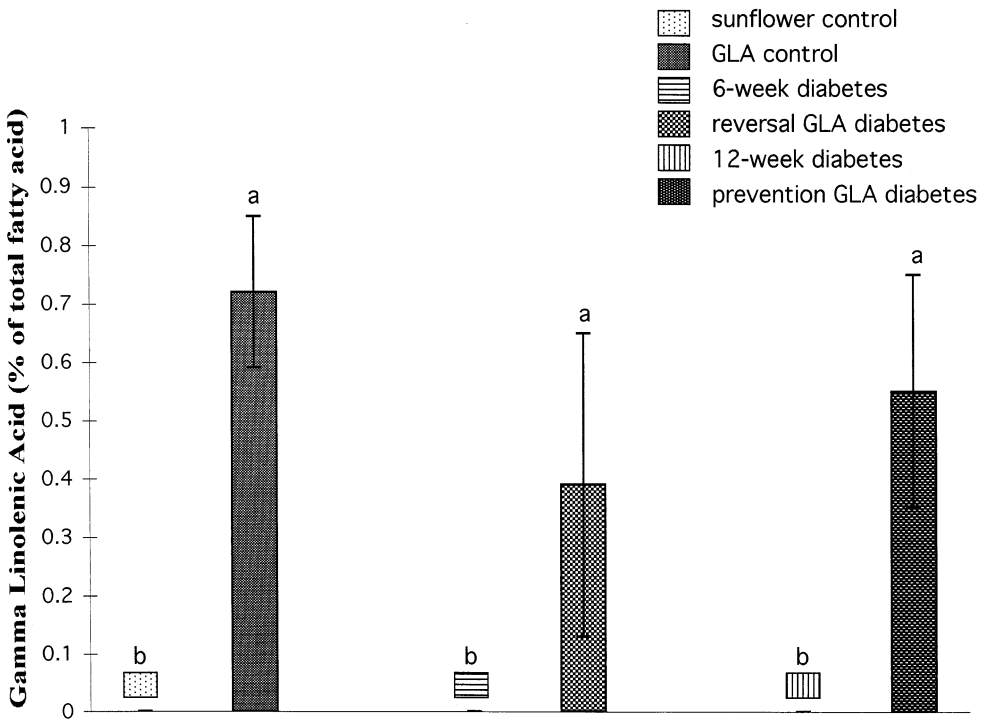


**Figure 4** Gamma linolenic acid (GLA) incorporation into sciatic nerve membrane. GLA supplementation increases its concentration in this membrane. See *Figure 1* for a detailed description of groups. After extraction of free acids, fatty acids were prepared according to the methanolysis method. Values are means ± SEM, n = 10. Bars not bearing at least one same superscript are significantly different (Mann-Whitney U test, $P < 0.0033$).

Fatty acid composition was altered in the 6-week diabetic animals. The proportions of (n-6) were significantly decreased ($P < 0.0033$; *Figure 5*), those in C20:1 (n-9) were increased ($P < 0.0033$), and those in LA tended to increase ($P < 0.030$). At 12 weeks, the proportions of each fatty acid had not changed.

In control rats, GLA supplementation changed the proportion of fatty acids; it increased polyunsaturated, particularly (n-6) fatty acids ($P < 0.0033$) and decreased C18:0 and C18:1 (n-9) ($P < 0.0033$).

In diabetic rats with GLA supplementation, the proportion in (n-6) fatty acids was restored to the normal range at 12 weeks (i.e., that of sunflower oil control; *Figure 5*). C14:0 was no longer detected in the membranes, and C20:1 (n-9) increased significantly ($P < 0.0033$) in GLA-treated diabetic animals, whereas C16:0 increased in the prevention GLA group ($P < 0.0033$).

### *Correlations between Na+, K+ ATPase activity, NCV, and phospholipid fatty acid composition*

The correlation between Na+, K+ ATPase activity and NCV in all groups was highly correlated with $r = 0.60$ and $P < 0.0001$ (*Figure 6*).

Na+, K+ ATPase activity was correlated with C22:4 (n-6) ($r = 0.42$, $P < 0.033$), less correlated with (n-6) fatty acids ($r = 0.29$, $P < 0.046$), and nearly correlated with polyunsaturated fatty acids ($r = 0.28$, $P < 0.053$).

NCV correlated in decreasing order with polyunsaturated fatty acids ($r = 0.36$, $P < 0.012$), (n-6) fatty acids ($r = 0.35$, $P < 0.014$), monounsaturated fatty acids ($r = -0.33$, $P < 0.020$), 18:2 (n-6) fatty acids ($r = 0.33$, $P < 0.020$), and (n-9) fatty acids ($r = -0.30$, $P < 0.036$).

## Discussion

We measured diabetes-induced abnormalities on NCV, Na+, K+ ATPase activity, and phospholipid fatty acid composition in the sciatic nerve of rats to investigate possible relationships between these variables. We also ascertained that GLA supplementation can prevent and reverse these abnormalities. Our study showed that supplementation with GLA completely prevented and restored the slowing of NCV in 6- and 12-week diabetic rats. It also demonstrated that GLA, administered preventively, partially prevented the decline of sciatic nerve Na+, K+ ATPase activity in diabetic rats. At 6 weeks, diabetes reduced the incorporation of (n-6) fatty acids in sciatic nerve membrane; in contrast, GLA-supplemented diabetic animals had normal proportions of (n-6) fatty acids. Moreover NCV and Na+, K+ ATPase activity were strongly correlated, whereas (n-6) fatty acids were only moderately correlated with NCV and Na+, K+ ATPase activity.

The development of diabetic neuropathy is associated with several metabolic and vascular defects that affect the peripheral nerve.[7,37] Among the affected pathways, delta-6-desaturase, which regulates the first step of conversion of LA into GLA, is rate limiting.[19] This step is defective in diabetic patients and experimental diabetic animals, leading

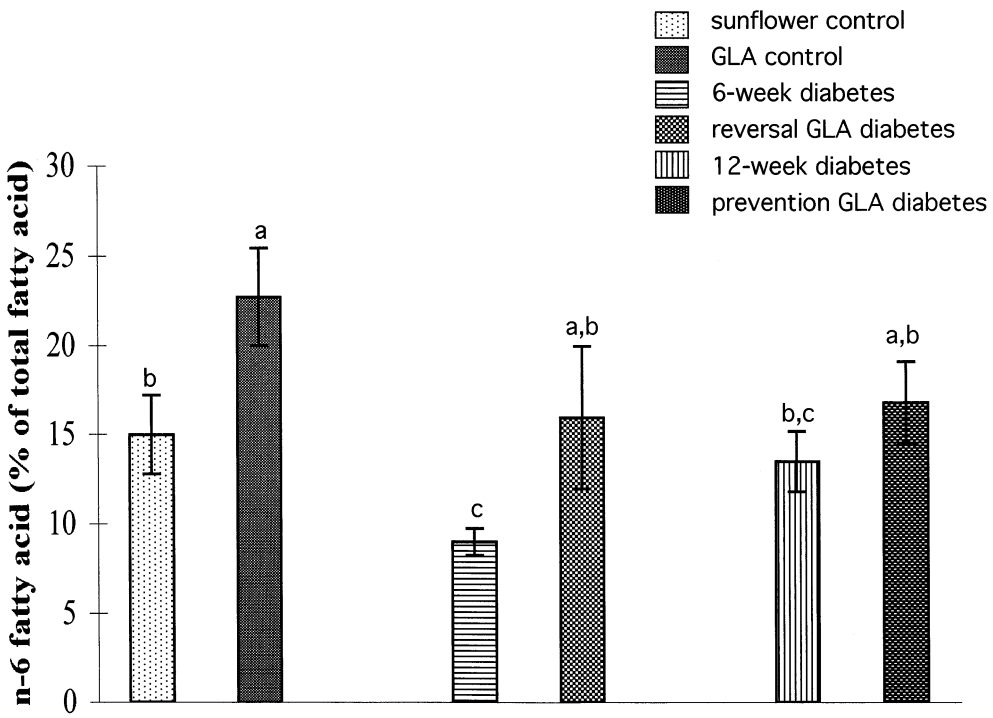


**Figure 5** (n-6) Fatty acid incorporation in sciatic nerve membrane. Diabetes induces changes in (n-6) polyunsaturated fatty acid incorporation. See *Figure 1* for a detailed description of groups. After extraction of free acids, fatty acids were prepared according to the methanolysis method. The (n-6) fatty acids are 18:2(n-6), 18:3(n-6), 20:4(n-6), and 22:4(n-6). Values are means ± SEM, n = 10. Bars not bearing at least one same superscript are significantly different (Mann-Whitney U test, $P < 0.0033$). GLA, gamma linolenic acid.

to a substantial reduction in the availability of subsequent metabolites to the tissues.[38,39] Through its various derivatives, LA, which is the immediate precursor of GLA, has at least three important roles in relation to the peripheral nerves. First, through the eicosanoid metabolites, it exerts vascular effects, regulating the balance of platelet aggregation and promoting vasodilatation, particularly via prostacyclin, thus maintaining healthy blood flow in the small

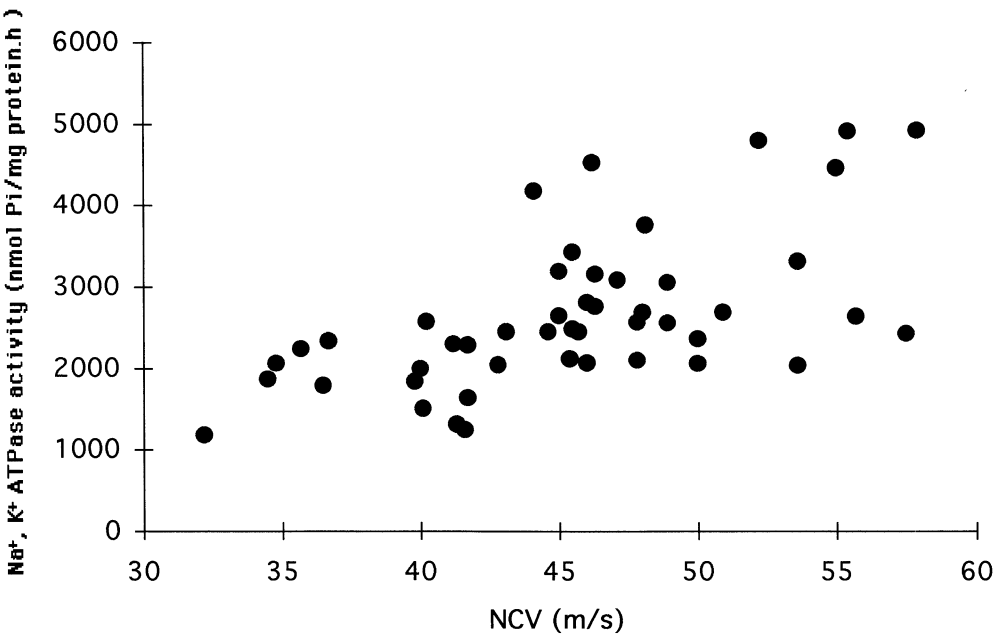


**Figure 6** Correlation between nerve conduction velocity (NCV) and Na+,K+ ATPase activity. NCV and Na+,K+ ATPase activity were determined in the same sciatic nerve. Correlation was positive and significant ($r = 0.60$, $P < 0.0001$).

neural blood vessels.[40] Second, the longer chain polyunsaturated fatty acid metabolites, notably arachidonic acid, form key structural components of the axonal membrane.[41] Therefore, these metabolites are likely to modulate several membrane functions, including fluidity, and therefore the behavior of several membrane-associated enzymes and receptors; thus, these metabolites would contribute to the electrical properties of the axon and its myelin sheath.[14] Third, the metabolites are important in various cell signaling systems, including the nitric oxide system and the phosphatidyl-inositol/diacylglycerol cycle.

The effect of diabetes on NCV is well documented. NCV data for the two diabetic groups, which differed only in the duration of diabetes, were in agreement with a previous study.[42] Both prevention and reversal modes of treatment with GLA corrected the deficits in NCV. This finding is consistent with other studies.[29,43,44]

A previous study showed that dietary supplementation with GLA had an inhibitory effect in 4- to 5-week streptozotocin-diabetic rats but reported that diabetes had no effect on Na+, K+ ATPase activity.[27] Other studies, however, have reported that diabetes induces a decrease in nerve Na+, K+ ATPase activity.[45–48] In our study, the decrease in sciatic nerve Na+, K+ ATPase activity in diabetic rats was significant at 6 weeks and remained unchanged at 12 weeks. Reversal treatment with GLA for 6 weeks did not reverse the decrease that had been established in Na+, K+ ATPase activity, whereas prevention treatment with GLA partially prevented this decrease. We show for the first time that long-term supplementation with GLA has a positive effect on sciatic nerve Na+, K+ ATP activity. Although this activity is not the only parameter involved in NCV, they were strongly correlated.

Our findings for 6-week diabetes agree with those of another study of phospholipid fatty acid composition.[23] GLA was not detected in rats not treated with GLA. This is not surprising because in the rat, GLA is very rapidly elongated to DGLA and then desaturated to arachidonic acid. However, the sum of (n-6) fatty acid concentrations shows that these concentrations are depleted in 6-week diabetes but not in 12-week diabetes, probably because the sciatic nerve adapts to preserve its membrane integrity. This adaptation may take place to the detriment of the availability of vasoactive prostanoid and leukotriene derivatives.[49] The parallel restoration of NCV and (n-6) fatty acids incorporation into the sciatic nerve because of GLA supplementation suggests that phospholipid fatty acid composition is directly related to nerve conduction, whereas Na+, K+ ATPase activity is only partially related. NCV restoration in GLA-supplemented diabetic animals may be attributed to a normalization of vasoactive prostanoid production and to a related improvement in nitric oxide synthesis, as shown in other studies.[49–55] Treatment of diabetic rats with vasoactive prostanoid analogs improves NCV and endoneurial blood flow[56,57] and also improves Na+, K+ ATPase activity.[22] However, a recent study suggested that prostanoids resulting from the action of cyclooxygenase were unlikely to mediate the effects of GLA supplementation because a diet rich in aspirin, which is an inhibitor of cyclooxygenase, enhanced the effects of GLA supplementation on NCV.[58] The positive effect of GLA on NCV may be related to production of lipoxins, which are derived from leukotrienes that result from the action of lipoxygenase on arachidonic acid. These substances are known to elicit vasodilatation and increase blood flow.[21] An alternative mechanism would be incorporation of metabolites derived from GLA into membrane phospholipids, thus restoring normal membrane properties and conductance.

The correlation between Na+, K+ ATPase activity and polyunsaturated fatty acids, especially (n-6) fatty acids, confirms the influence of phospholipid fatty acid in enzyme activity. In addition, the correlation between NCV and polyunsaturated and monounsaturated fatty acids suggests that fatty acids play a direct role in nerve conductance.

We observed that diabetes induced a similar decrease in NCV and Na+, K+ ATPase activity after 6 weeks, which remained unchanged at 12 weeks, whereas the abnormal (n-6) fatty acid composition in the sciatic nerve observed after 6 weeks of diabetes seemed to correct spontaneously in the next 6 weeks. Thus, GLA supplementation can prevent or reverse diabetes-induced abnormalities in the sciatic nerve and may have a role in treating diabetic neuropathy.

In conclusion, GLA supplementation had a beneficial effect in both preventing and reversing abnormalities in NCV. This beneficial effect was associated with a normalization of (n-6) fatty acids incorporation into phospholipid membranes and a partial restoration of Na+, K+ ATPase activity, suggesting that GLA supplementation improves NCV via fatty acid composition modulation and moderately improves Na+, K+ ATPase activity.

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