Prevention of nerve conduction deficit in diabetic rats by polyunsaturated fatty acids1–3

Richard J Head, Peter L McLennan, Daniel Raederstorff, Reto Muggli, Sharon L Burnard, and Edward J McMurchie

ABSTRACT The influence of diets containing γ-linolenic acid (GLA; 18:3n–6) on sciatic nerve conduction velocity (NCV) was determined in diabetic rats. NCV was lower in diabetic rats fed diets supplemented with olive oil or sunflower seed oil than in nondiabetic rats; rats supplemented with GLA during a 5-wk diabetic period, however, did not exhibit significantly lower NCV. The mean proportion of the phospholipid fatty acid linoleic acid (18:2n–6) was higher in the sciatic nerves of diabetic rats than in the nondiabetic groups irrespective of dietary lipid treatment. Additionally, the proportion of linoleic acid was higher in the diabetic rats fed sunflower oil than in all other groups. Dietary GLA supplementation did not significantly influence the fatty acid composition of nerve membrane phospholipids and there was no obvious correlation between the fatty acid composition of nerve membrane phospholipids and NCV. The content of fructose and glucose in sciatic nerves was higher, whereas that of myo-inositol was lower, in diabetic rats than in nondiabetic rats; however, this was not significantly influenced by dietary GLA. GLA administration did not significantly influence Na+–K+–exchanging ATPase or ouabain binding activity in sciatic nerve preparations, both of which remained nonsignificantly different in the diabetic and nondiabetic groups. The results suggest that dietary GLA can prevent the deficit in NCV induced by diabetes and that this effect is independent of the nerve phospholipid fatty acid profile, sugar and polyol content, Na+–K+–exchanging ATPase activity, and ouabain binding. GLA may prevent the deficit in NCV indirectly, possibly by its role as a precursor of vasodilatory prostaglandins. These results confirm that GLA is the active component of evening primrose oil. Am J Clin Nutr 2000;71(suppl):386S–92S.

KEY WORDS Diabetic neuropathy, streptozotocin, γ-linolenic acid, nerve conduction velocity, sciatic nerve polyols, Na+–K+–exchanging ATPase, ouabain binding, fatty acids, evening primrose oil, rats

INTRODUCTION

The impairment of nerve function is a well-established early manifestation of diabetes both clinically (1) and in experimental animal models (2). Impaired nerve function is characterized by a slowed conduction velocity, a lower action potential amplitude, and a lower rate of axonal transport. Diabetes-induced alterations in various biochemical processes have been proposed to underlie the development of diabetic neuropathy. For example, altered peripheral nerve polyol metabolism, altered Na+–K+–exchanging ATPase activity, and depletion of myo-inositol have been implicated in the pathogenesis of diabetic neuropathy (3). Abnormal fatty acid metabolism, particularly the lower ratio of desaturation of linoleic acid (LA; 18:2n–6) to γ-linolenic acid (GLA; 18:3n–6), has been reported in human and animal studies (4, 5). Rats made diabetic by streptozotocin treatment and who were fed evening primrose oil in their diets were reported to have normal rates of nerve conduction, or some restoration of nerve conduction deficits, without concomitant restoration of hyperglycemia or glycosuria (6–8). It has been proposed that evening primrose oil supplementation restores the diabetes-induced reduction in nerve arachidonic acid (20:4n–6) content by providing a source of GLA, thereby bypassing an intermediate step in fatty acid metabolism that is inhibited by the lower Δ6-desaturase (linoleoyl-CoA desaturase) activity reported in diabetes (9). The involvement of vascular factors such as vasocostriction, reduced nerve blood flow, and hypoxia have also been proposed to underlie the development of diabetic neuropathy (10–12). Furthermore, evidence exists that the ratio of prostaglandin to thromboxane A2 may be disrupted in favor of vasoconstriction in diabetes; this, together with increased platelet aggregation, suggests that vascular effects may be important in the development of diabetic neuropathy (13, 14).

The aim of this study was to evaluate the effectiveness of pure GLA (as the postulated active component of evening primrose oil) when administered in the diet in preventing the deficit in nerve conduction velocity (NCV) induced by diabetes. We also investigated the relation between diabetes, dietary GLA administration, sciatic NCV, neuronal phospholipid fatty acid composition, and Na+–K+–exchanging ATPase activity, polyol formation, and neuronal ouabain binding. By examining the above, we aimed to determine whether purified GLA was responsible for preventing the diabetes-induced deficit in NCV and whether this...
prevention was associated with the reversal of one or more key biochemical processes reported to be disturbed in diabetes.

**MATERIALS AND METHODS**

**Induction of diabetes**

Handling and use of animals complied with Australian codes of practice for the care and use of animals for scientific purposes and were approved by our institutional animal experimentation ethics committee. Male Hooded Wistar rats were rendered diabetic at 9–10 wk of age by the intraperitoneal injection of streptozotocin (Sigma, Sydney, Australia) in 20 mmol sodium citrate buffer/L, pH 4.5, at a dose of 50 mg/kg body wt. Diabetes was verified by testing for glycosuria >30 mg/L with Diastix (Bayer Diagnostics, Mulgrave, Australia) on a drop of rat urine expressed 48 h postinduction. The age-matched, nondiabetic groups were administered 20 mmol sodium citrate buffer/L alone. Blood samples for the measurement of plasma glucose (Unimate 5 glucose assay kit; Roche, Basel, Switzerland) were taken when the animals were killed.

**Diets**

Rats were fed a standard stock diet (Ridley Agrimachineryproducts, Murray Bridge, South Australia) (15) from the time they were weaned until they were 9 wk old, when diabetes was induced and an experimental diet was introduced. Rats were fed the experimental diets from 9 to 14 wk of age; the experimental diets contained either 0.5% (by wt) GLA (95% pure; F Hoffmann-La Roche, Basel, Switzerland) plus 5.5% olive oil (GLA diet), 6% olive oil (OO diet), or 6% sunflower seed oil (SSO diet) as the sole source of fatty acids. Diets also contained 18% protein, 35% starch, and 32.5% sucrose, plus amino acids, choline chloride, methyl cellulose, salts, vitamins, and water. The individual constituents of the experimental diets were described previously (16, 17) with the following exception: our study focused on the fatty acid composition of the experimental diets reported to be disturbed in diabetes.

**Motor nerve conduction velocity**

Rats were anesthetized by intraperitoneal injection of 60 mg pentobarbitone sodium/kg body wt (Ceva Chemicals, Hornsby, Australia) and maintained at 37°C on a heated table. Stimulation and recording of the sciatic nerve trunk were done by using platinum electrodes essentially as described previously (11). The sciatic nerve in the right leg was exposed at 3 sites. The 2 proximal sites, the first located near the sciatic notch and the second ≈15 mm distal, were used for stimulating and the third site at the distal end of the tibial nerve, located above the foot, was used for recording. The nerve was bathed in warmed paraffin oil and maintained at 37°C under radiant heat. The sciatic nerve was stimulated by constant voltage pulses delivered directly to the nerve at 20% above threshold for the myelinated A fibers. The compound action potentials were fed through an analog-to-digital converter into a computer for display, analysis, and storage by using a data acquisition board (AT-MIO-16) and the program LABVIEW (both from National Instruments, Austin, TX). Action potentials from both sites were averaged 6–8 times. Conduction velocity was calculated from the difference in latencies of the action potentials and the nerve length separating the 2 cathode stimulation points.

**Nerve dissection**

Rats were anaesthetized and their sciatic nerves were surgically exposed. Two ligatures were applied to the nerve before removal, one at the sciatic notch and the other ≈3 cm distal to the first. Endoneurial preparations were derived from the tibial division of the sciatic nerve as described previously (18). After it was removed from the animal, the ligated nerve was placed in 2 mL Krebs-Ringer bicarbonate buffer, pH 7.4, containing 14 mg collagenase type 1A (Sigma), 20 mmol glucose/L, and 4.5% (wt:vol) defatted bovine serum albumin equilibrated with 5% carbon dioxide and 95% oxygen at 37°C in a shaking water bath. After 12 min of digestion, the nerve segment was rinsed for 2 min in 2 mL of a similar medium containing aprotinin (200 kallikrein inhibitor units; Sigma) in the absence of collagenase. The nerve was then transferred to a petri dish containing Krebs-Ringer bicarbonate buffer (pH 7.4) containing 5 mmol glucose/L, 4.5% (wt:vol) defatted bovine serum albumin, and 0.5 mmol myo-inositol/L. The major fascicle of the tibial division of the sciatic nerve was then separated and the perineurial membrane was removed, leaving the endoneurial preparation free of secondary nerve branches. The endoneurial sample was blotted and either frozen for assay of Na+-K+-exchanging ATPase activity or processed immediately for [3H]ouabain binding as follows: the endoneurial sample was incubated in buffer containing 250 mmol sucrose/L, 3 mmol MgSO4/L, 1 mmol sodium orthovanadate/L, and 10 mmol tris/L, pH 7.35 (vanadate buffer), for 10 min on ice. The sample was then removed, finely chopped, and homogenized in 2 mL vanadate buffer, first with a homogenizer (Polytron type 10–35; Kinematica GmbH, Kriens/Luzern, Switzerland) for 15 s and then by 5 strokes in a tissue grinder (Tenbroeck; Wheaton Scientific, Downey, CA). The homogenate was frozen in liquid nitrogen and stored at −80°C until [3H]ouabain binding assays were performed.

**TABLE 1**

Fatty acid analysis of the experimental diets

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>OO group</th>
<th>SSO group</th>
<th>GLA group</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>0.2</td>
<td>0.2</td>
<td>TR</td>
</tr>
<tr>
<td>16:0</td>
<td>16.8</td>
<td>6.7</td>
<td>13.3</td>
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<td>16:1</td>
<td>1.3</td>
<td>0.0</td>
<td>1.4</td>
</tr>
<tr>
<td>18:0</td>
<td>3.1</td>
<td>4.7</td>
<td>2.8</td>
</tr>
<tr>
<td>18:1</td>
<td>63.0</td>
<td>22.2</td>
<td>57.2</td>
</tr>
<tr>
<td>18:2</td>
<td>14.1</td>
<td>63.7</td>
<td>14.9</td>
</tr>
<tr>
<td>18:3n–6</td>
<td>0.0</td>
<td>0.1</td>
<td>8.7</td>
</tr>
<tr>
<td>18:3n–3</td>
<td>0.6</td>
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<td>0.6</td>
</tr>
<tr>
<td>20:0</td>
<td>0.5</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>20:1</td>
<td>0.3</td>
<td>TR</td>
<td>0.3</td>
</tr>
<tr>
<td>22:0</td>
<td>0.2</td>
<td>0.9</td>
<td>TR</td>
</tr>
<tr>
<td>22:1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>24:0</td>
<td>TR</td>
<td>0.3</td>
<td>TR</td>
</tr>
<tr>
<td>24:1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

n = 2 analyses for each diet. OO, diet containing 6% olive oil; SSO, diet containing 6% sunflower seed oil; GLA, diet containing 0.5% purified γ-linolenic acid (18:3n–6) plus 5.5% olive oil; TR, trace.
Sucrose density gradient fractionation of axolemmal membranes

Fractions used to measure Na⁺-K⁺-exchanging ATPase activity were from an endoneural preparation derived from a single sciatic nerve, prepared as described above. Each preparation was homogenized on ice in 2 mL of 10 mmol tris/L, pH 7.5 (tris buffer), containing 500 mmol sucrose/L and 150 mmol NaCl/L with a homogenizer for 15 s, followed by 5 strokes in a tissue grinder. A portion of the homogenate was stored at −80°C for measurement of Na⁺-K⁺-exchanging ATPase activity (homogenate fraction). To the remainder, 4.3 mL of the above buffer was added and this suspension was layered onto the top of 5.2 mL tris buffer containing 1.2 mol sucrose/L in an SW-41 Ultra-Clear Centrifuge Tube (Beckman, Palo Alto, CA). This was covered with 2.5 mL tris buffer containing 250 mmol sucrose/L, resulting in the formation of a 3-layer, discontinuous sucrose density gradient. After centrifugation at 140 000 × g for 60 min at 5°C with a swinging bucket SW-41 rotor in a L8–80 ultracentrifuge (Beckman), particulate layers appeared at the 500 mmol/L to 1.2 mol/L and the 250–500 mmol/L sucrose interfaces. The layer at the 250–500 mol/L sucrose interface, which was enriched with Na⁺-K⁺-exchanging ATPase activity as described by Kim et al (19), was transferred to centrifuge tubes. These tubes were filled with tris buffer containing 250 mmol sucrose/L and centrifuged at 150 000 × g for 30 min at 5°C in a Ti60 rotor (Beckman). The pellet was resuspended in 250 mL of 30 mmol tris/L containing 250 mmol sucrose/L, 100 mmol NaCl/L, and 2.5 mmol MgCl₂/L, pH 7.5, by 5 strokes of a tissue grinder. The sucrose density gradient fraction was stored at −80°C.

Measurement of Na⁺-K⁺-exchanging ATPase

To determine ouabain-sensitive Na⁺-K⁺-exchanging ATPase activity, portions of the homogenate and the sucrose density gradient fraction [at final protein concentrations as determined by the Lowry assay (20) of ~30–80 and 20–60 mg/L, respectively] were preincubated in 30 mmol imidazole buffer/L, pH 7.3, containing (at final concentrations) 250 mmol sucrose/L, 1 mmol phosphoenolpyruvate/L, 0.6 mmol NADH/L, 100 mmol NaCl/L, 10 mmol KCℓ/L, 2.5 mmol MgCl₂/L, 14.9 × 10⁻³ U lactate dehydrogenase/L (Sigma), and 23 × 10⁻³ U pyruvate kinase/L (Sigma) in a final volume of 0.5 mL and in the presence or absence of 2 mmol ouabain/L. The assay was essentially as described by Kim et al (19) and Lattimer et al (21), with the rate of ADP production determined by using the double-enzyme-linked reaction catalyzed by lactate dehydrogenase and pyruvate kinase and by continuously recording the spectrophotometric conversion of NADH to NAD⁺ by the change in absorbance at 340 nm. The reaction was initiated by the addition of disodium ATP (final concentration of 1 mmol/L) and was allowed to proceed for 20 min at 37°C before the actual rate was measured over the next 20 min with a spectrophotometer (model 250; Gilford Instrument Laboratories, Oberlin, OH). Total ATPase activity was defined as the linear rate of change of NADH absorbance expressed as the equimolar amount of ATP converted to ADP per hour per milligram protein. Ouabain-sensitive ATPase activity was taken as the difference between the rate in the absence or presence of 2 mmol ouabain/L.

[³H]Ouabain binding assay

Thawed homogenates were preincubated in vanadate buffer for 1 h at 37°C with aprotinin (200 kallikrein inhibitor units) either in the presence or absence of excess unlabelled ouabain (2 mmol/L). [³H]Ouabain (Dupont, Sydney, Australia) binding was initiated by the addition of either 50 or 100 mmol [³H]ouabain/L (final concentration: 899 and 1798 GBq/mL, respectively). The homogenates were then incubated at 37°C in a final volume of 360 μL with the homogenate protein concentration adjusted to between 0.2 and 0.7 g protein/L. Duplicate 75-μL aliquots were taken at the start of the reaction and after 2 h and subjected to rapid filtration with a Millipore (Bedford, MA) filtration apparatus and were then washed 3 times with 4 mL ice-cold vanadate buffer. The 2 concentrations of ouabain were chosen on the basis of initial experiments in which the dissociation constant for ouabain binding to sciatic nerve homogenates was determined to be 45 mmol/L in nondiabetic rats. This value compares well with previous reports for rat sciatic nerve (22). Specific binding of [³H]ouabain to sciatic nerves homogenates was 91% and 89% of total [³H]ouabain binding when 50 and 100 mmol [³H]ouabain/L were added, respectively.

Sugar and polyol analysis

Sciatic nerves were removed from anaesthetized diabetic and nondiabetic animals and washed in 0.9% (w:v) NaCl. The major fascicle of the tibial division of the sciatic nerve was separated and the perineurial membrane removed, leaving the endoneurium. The sample was blotted and frozen immediately in liquid nitrogen. Sugars and polyols were extracted according to the method of Tomlinson et al (23) and processed as described previously by us (11). Essentially, the deproteinized sample was centrifuged and the supernate was freeze-dried. A mixture of standards (D-glucose, D-fructose, D-mannose, D-sorbitol, myo-inositol, and α-D-methylmannoside) was treated similarly. The lyophilized supernate was silylated for 24 h at room temperature and water was added. The silylated sugars and polyols were extracted with cyclohexane and dried under nitrogen, reconstituted in a small volume of cyclohexane, and assayed by gas-liquid chromatography with a 25-m BP1 capillary column (Scientific Glass Engineering, Victoria, Australia) in a gas chromatograph (model 6500; Dani, Monza, Italy) fitted with a flame ionization detector. The temperature was maintained at 140°C for the first 12 min and then raised at a rate of 5°C/min for 8 min.

Fatty acid analysis and protein determination

The phospholipid fatty acid content of the sciatic nerves and the total fatty acid content of the experimental diets were analyzed as we described previously (11). The nerves were powdered in liquid nitrogen and extracted by the method of Bligh and Dyer (24). Phospholipids were separated from the other lipid classes by thin-layer chromatography as described (11). The total phospholipid fraction and the dietary fatty acids were transesterified and fatty acid methyl esters were analyzed by capillary gas chromatography on a BPX70 capillary column (Scientific Glass Engineering, Melbourne, Australia) as described previously (11). A standard mixture of fatty acid methyl esters was used to establish the response factors for the components identified (11). Protein assays were carried out by using the method of Lowry et al (20).

Statistical analysis

Data are expressed as means ± SEMs. For comparisons between 2 experimental groups, the significance of the difference between the means was calculated. For analysis of initial and final body weights, a one-way analysis of variance (ANOVA) was combined with an analysis of covariance with initial weight as the covariate. For analysis of plasma glucose concentrations, nerve
Tabled changes in body weight of nondiabetic and diabetic rats after 5 wk of dietary lipid supplementation.

<table>
<thead>
<tr>
<th></th>
<th>Body weight</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>OO group</td>
<td>SSO group</td>
<td>GLA group</td>
<td></td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td></td>
<td>304 ± 7.8</td>
<td>300 ± 5.9</td>
<td>290 ± 5.9</td>
<td>296 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>439 ± 8.8</td>
<td>417 ± 8.5</td>
<td>417 ± 8.5</td>
<td>429 ± 6.3</td>
</tr>
<tr>
<td>Diabetic</td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td></td>
<td>304 ± 10.2</td>
<td>275 ± 11.9</td>
<td>272 ± 6.4</td>
<td>265 ± 6.1</td>
</tr>
<tr>
<td></td>
<td>375 ± 14.2</td>
<td>267 ± 6.9</td>
<td>279 ± 8.7</td>
<td>267 ± 6.9</td>
</tr>
</tbody>
</table>

1SEM, n in brackets. Initial body weight is at 9 wk of age; final body weight is at 14 wk of age. OO, diet containing 6% olive oil; SSO, diet containing 6% sunflower seed oil; GLA, diet containing 0.5% purified γ-linolenic acid plus 5.5% olive oil. Analysis of covariance with initial weight as the covariate showed that initial weight was correlated with final weight (P < 0.0001) and the effect of diabetes on body weight was significant (P < 0.0001). The interaction between diet and diabetic state was not significant. 2Significantly different from OO group, P < 0.05 (one-way ANOVA, Tukey test).

Results

Body weight and plasma glucose

Mean initial and final body weights of the animals are given in Table 2. The final body weight of the diabetic OO group was 37% lower than that of the OO nondiabetic group, whereas the final body weights of the SSO and GLA diabetic groups were 36% and 38% lower than those of the respective nondiabetic groups. Mean plasma glucose concentrations were higher in the diabetic groups than in the nondiabetic groups and this was independent of dietary lipid supplementation (Table 3).

Nerve conduction velocity

After 5 wk of dietary lipid supplementation concurrent with diabetes, sciatic NCV was measured. Mean NCV in the OO, SSO, and GLA diabetic groups was 28%, 38%, and 16% lower than that in the respective nondiabetic groups. A one-sided two-way ANOVA, used to examine whether the effect of diabetes was less when the rats were fed the GLA diet, showed that the difference in NCV between the diabetic and nondiabetic GLA groups was significantly less than the average difference in the 2 other dietary groups (Table 4). In addition, a one-way ANOVA showed no significant differences with dietary treatment among the nondiabetic groups and a significant difference among the diabetic groups.

Neuronal Na+-K+-exchanging ATPase activity

Preliminary experiments were undertaken to determine the degree of enrichment of Na+-K+-exchanging ATPase activity and ouabain sensitivity after the sucrose density gradient purification step by comparing the ATPase activity of the homogenate and sucrose density gradient fractions from the endoneurial preparations. The proportion of ouabain-sensitive ATPase activity rose from a mean value of 16% in the homogenate fractions to ~33% in the sucrose density gradient fractions, and the mean specific activity was 2-fold higher in the sucrose density gradient fractions than in the homogenate fractions. No significant differences in total or ouabain-sensitive ATPase activity were observed in either the homogenate or sucrose density gradient fractions as a result of the dietary lipid treatment or the diabetic state (not shown).

Neuronal [3H]ouabain binding

Specific [3H]ouabain binding activity was not significantly different at either concentration of ouabain as a result of the dietary lipid treatment or the diabetic state (not shown).

Neuronal sugar and polyol content

Concentrations of mannose, fructose, glucose, sorbitol, and myo-inositol in the sciatic nerves of nondiabetic and diabetic rats fed the 3 diets are shown in Table 5. For both the nondiabetic and diabetic groups, differences in mean sugar and polyol contents between the 3 diets were not significant, indicating that dietary lipid supplementation did not influence neuronal sugar or polyol concentrations.

However, differences were evident in the mean fructose, glucose, and myo-inositol contents during the diabetic state. The mean sciatic nerve fructose and glucose concentrations were significantly higher, whereas the mean myo-inositol concentration was significantly lower, in the diabetic groups than in the nondiabetic groups.

Neuronal phospholipid fatty acid composition

Sciatic nerve phospholipid fatty acid compositions are shown in Table 6; the overall fatty acid distribution was comparable with data we published previously (11). The mean proportion of linoleic acid was higher in the diabetic groups, particularly those fed the SSO diet, resulting in a significantly higher proportion of

<table>
<thead>
<tr>
<th>Plasma glucose</th>
<th>OO group</th>
<th>SSO group</th>
<th>GLA group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiabetic</td>
<td>14 ± 0.6</td>
<td>16 ± 0.7</td>
<td>14 ± 0.7</td>
</tr>
<tr>
<td>Diabetic</td>
<td>39 ± 1.5</td>
<td>45 ± 4.6</td>
<td>41 ± 1.6</td>
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</table>

1SEM, n = 6. OO, diet containing 6% olive oil; SSO, diet containing 6% sunflower seed oil; GLA, diet containing 0.5% purified γ-linolenic acid plus 5.5% olive oil. There was a significant effect of diabetic state on plasma glucose concentrations, P < 0.0001 (two-way ANOVA), but no significant effect of diet and no significant interaction between diet and diabetic state.
cant differences in the extent of specific $[3\text{H}]$ouabain binding to the tissue associated with diabetes (2, 19). This finding suggests that lower myo and sugar contents. Altered polyol metabolism and an associated –

**TABLE 4**

Sciatic nerve conduction velocity after 5 wk of dietary lipid supplementation in nondiabetic and diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>OO group</th>
<th>SSO group</th>
<th>GLA group$^2$</th>
</tr>
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<tbody>
<tr>
<td>Nerve conduction velocity (m/s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>46.9 ± 2.2 [14]</td>
<td>53.0 ± 2.1 [14]</td>
<td>48.9 ± 3.8 [14]</td>
</tr>
</tbody>
</table>

$^1\bar{x} \pm \text{SEM}; \bar{n}$ in brackets. OO, diet containing 6% olive oil; SSO, diet containing 6% sunflower seed oil; GLA, diet containing 0.5% purified $\gamma$-linolenic acid plus 5.5% olive oil.

$^2$The effect of diabetes was significantly less in rats fed the GLA diet than in those fed the OO or SSO diet; $P < 0.05$ (one-sided, two-way ANOVA).

$^3$Significantly different from the OO and SSO groups, $P < 0.05$ (one-way ANOVA, Tukey test).

total $n-6$ fatty acids in the diabetic groups. The mean proportion of GLA in the membrane phospholipids was not significantly different between groups and was not significantly influenced by either diet or the diabetic state.

**DISCUSSION**

The key finding of the present study was that the incorporation of purified GLA into an experimental diet prevented a diabetes-induced deficit in NCV. This protection occurred independently of any obvious alteration in the biochemical indexes measured, which included neuronal phospholipid fatty acid composition, Na$^+\text{-K}^+$-exchanging ATPase activity, ouabain binding, and polyol and sugar contents. Altered polyol metabolism and an associated decrease in neuronal myo-inositol content were reported to be associated with diabetes (2, 19). This finding suggests that lower tissue myo-inositol concentrations may result in abnormal neuronal Na$^+\text{-K}^+$-exchanging ATPase activity through a reduction in the formation of diacylglycerols and inositol trisphosphate and incomplete activation of the sodium pump by activated protein kinase C (3, 21, 25). Lower Na$^+\text{-K}^+$-exchanging ATPase activity may underpin the depressed NCV in diabetes (21). In addition, inhibition of aldose reductase has been reported to restore neuronal polyol metabolite content (principally the myo-inositol) and the nerve conduction deficit in diabetes (2). In the present study, however, although endoneurial concentrations of fructose and glucose were significantly higher and myo-inositol concentrations were significantly lower in diabetic rats than in nondiabetic rats, concentrations were not restored to normal by dietary GLA administration. This finding suggests that GLA was not mediating its beneficial effect on NCV by altering the metabolic changes associated with neuronal polyol metabolism. This confirms earlier findings of Tomlinson et al (26).

Endoneurium-associated Na$^+\text{-K}^+$-exchanging ATPase activity was measured in both a homogenate fraction and a purified fraction obtained by sucrose density gradient centrifugation. The purification step significantly increased the ouabain-sensitive component of the total ATPase activity in terms of both specific activity and ouabain sensitivity. Despite this purification step, however, there were no significant effects of diet or diabetic state on mean Na$^+\text{-K}^+$-exchanging ATPase activity. Furthermore, we detected no significant differences in the extent of specific $[3\text{H}]$ouabain binding to the endoneurial preparation, indicating that neither diabetic state nor diet was influencing the mean number of ouabain binding (sodium pump) sites. This finding implies that changes in either Na$^+\text{-K}^+$-exchanging ATPase activity or number were not determinants of the depressed NCV or its prevention by GLA. Lockett and Tomlinson (7) also reported a negligible effect of diabetes and evening-prime-oil supplementation on neuronal Na$^+\text{-K}^+$-exchanging ATPase activity in rats.

Although numerous studies have associated evening primrose oil (and by inference GLA) feeding with improved nerve function in diabetes, there are apparently no reports of effects on nerve fatty acid composition in diabetic or nondiabetic animals. As expected, the mean proportions of LA were higher in the nerve phospholipid fatty acids of diabetic animals than in those of nondiabetic animals [a result we reported previously (11)], and this was exacerbated by supplementation with the LA-rich SSO diet. However, although a metabolic defect in the desaturation of LA to GLA is well established in diabetes (4) and the expected higher proportion of LA was observed in the diabetic animals in this study, the concomitant lower proportion of arachidonic acid (20:4n–6) and other n–6 metabolites was not observed. Furthermore, dietary GLA supplementation did not significantly alter the phospholipid profile. Failure of dietary GLA to increase the proportion of sciatic nerve phospholipid GLA was also reported previously by us (11). This partly confirms previous reports that sciatic nerve (27) and brain (28) conserve arachidonic acid in diabetes whereas other tissues including liver, heart, kidney, spleen, plasma, and aorta display the expected relatively higher proportion of LA and lower proportion of arachidonic acid (28, 29). In this study there were no significant correlations between individual fatty acids or groups of fatty acids and NCV. It is therefore difficult to reconcile the effects of either diabetes or GLA on nerve function with their effects on nerve fatty acid composition.

As mentioned previously, vascular prostanoid ratios and responses are altered in the diabetic state in favor of greater vasoconstrictor status [reviewed by Öztürk et al (30)]. Fur-

**TABLE 5**

Sciatic nerve endonurial sugar and polyol contents after 5 wk of dietary lipid supplementation in nondiabetic and diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>OO group</th>
<th>SSO group</th>
<th>GLA group$^3$</th>
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<tbody>
<tr>
<td>Mannose</td>
<td>0.23 ± 0.07 [6]</td>
<td>0.50 ± 0.32 [6]</td>
<td>0.34 ± 0.14 [6]</td>
</tr>
<tr>
<td>Fructose</td>
<td>1.41 ± 0.36 [6]</td>
<td>1.15 ± 0.28 [5]</td>
<td>1.38 ± 0.19 [5]</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.25 ± 0.60 [6]</td>
<td>1.53 ± 0.40 [5]</td>
<td>2.26 ± 0.46 [5]</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>0.54 ± 0.36 [6]</td>
<td>0.31 ± 0.09 [6]</td>
<td>0.27 ± 0.06 [4]</td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>3.33 ± 0.79 [6]</td>
<td>3.04 ± 0.64 [6]</td>
<td>2.95 ± 0.68 [6]</td>
</tr>
</tbody>
</table>

$^3$Significantly different from the OO and SSO groups, $P < 0.05$ (one-way ANOVA, Tukey test).
thermore, the production of vasodilatory prostacyclin is lower in diabetes (31–34). In association with the restoration of NCV, evening primrose oil and GLA improve neurovascular blood flow, which is impaired in diabetes (8, 26). In addition, evening primrose oil can attenuate the diabetes-induced reduction in prostacyclin production in numerous tissues (35) and its restorative effects on nerve conduction and neurovascular blood flow are abolished by inhibition of cyclooxygenase (36). Other treatments unrelated to fatty acids or their metabolism have also been found to simultaneously restore nerve conduction and improve neurovascular blood flow (10, 14). Together, these findings suggest that GLA restores or prevents the development of impaired nerve conduction in diabetes by improving neurovascular blood flow, perhaps through eicosanoid mechanisms.

We have established that dietary administration of purified GLA can prevent the deficit in NCV induced by diabetes, confirming GLA as the active component of evening primrose oil. There was no significant association between this prevention of the NCV deficit with nerve fatty acid composition, changes in neuronal sugar and polyol contents, Na⁺-K⁺-exchanging ATPase activity, or ouabain binding, supporting the suggestion that GLA acts through indirect mechanisms, probably by improving neuronal blood supply, conceivably involving a shift in prostanooid metabolism.

We acknowledge the skilled assistance of W. Leifert, G. Patten, M. Adams, J. Dallimore, and T. Bridle, and the dedicated care of animals by J. Greenfield. We also acknowledge R. Correll and P. Clifton for consultation on the statistical treatment of the data.

REFERENCES