Fish Oil Supplementation Prevents Diabetes-Induced Nerve Conduction Velocity and Neuroanatomical Changes in Rats1,2

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ABSTRACT Diabetic neuropathy has been associated with a decrease in nerve conduction velocity, Na,K-ATPase activity and characteristic histological damage of the sciatic nerve. The aim of this study was to evaluate the potential effect of a dietary supplementation with fish oil ([n-3] fatty acids) on the sciatic nerve of diabetic rats. Diabetes was induced by intravenous streptozotocin injection. Diabetic animals (n = 20) were fed a nonpurified diet supplemented with either olive oil (DO) or fish oil (DM), and control animals (n = 10) were fed a nonpurified diet supplemented with olive oil at a daily dose of 0.5 g/kg by gavage for 8 wk. Nerves were characterized by their conduction velocity, morphometric analysis and membrane Na,K-ATPase activity. Nerve conduction velocity, as well as Na,K-ATPase activity, was improved by fish oil treatment. A correlation was found between these two variables (R = 0.999, P < 0.05). Moreover, a preventive effect of fish oil was observed on nerve histological damage [endoneurial edema, axonal degeneration (by 10–15%) with demyelination]. Moreover, the normal bimodal distribution of the internal diameter of myelinated fibers was absent in the DO group and was restored in the DM group. These data suggest that fish oil therapy may be effective in the prevention of diabetic neuropathy. J. Nutr. 129: 207–213, 1999.

KEY WORDS: • neuropathy • Na,K-ATPase • fish oil • neuroanatomy • rats

The development of experimental diabetic neuropathy is accompanied by the appearance of a variety of metabolic abnormalities in nerve that are generally believed to contribute significantly to the pathogenesis of this disorder. Prominent among these are changes in essential fatty acid metabolism which may be important in the aetiology of peripheral nerve dysfunction (Dines et al. 1993). Diabetic neuropathy is characterized by neuroanatomical changes and by decreased nerve conduction velocity (Clark and Lee 1995). A relationship has been demonstrated between structural lesions and slowed nerve conduction velocity in chronically diabetic animals (Sima et al. 1990 and 1993). The reduced nerve conduction velocity has been associated with a diminished activity of Na,K-ATPase (Das et al. 1976, Greene and Lattimer 1983 and 1984, Greene et al. 1984 and 1985, Raccah et al. 1994). This membrane-bound enzyme is very sensitive to altered membrane environment (Gerbi et al. 1993a, 1994 and 1997a). In a previous study, we demonstrated that fish oil supplementation, rich in (n-3) fatty acids, improved sciatic nerve activity of Na,K-ATPase isoenzymes and expression in diabetic rats (Gerbi et al. 1997b). The aim of this study was to extend our biochemical observation with functional and anatomical nerve variables. We have studied the effect of fish oil supplementation on nerve conduction velocity and neuroanatomical lesions in diabetic rats.

MATERIALS AND METHODS

Animals. Five-week-old male Sprague-Dawley (n = 30) rats weighing ~200 g were randomly divided into three groups of 10. In two groups, diabetes was induced by intravenous injection of streptozotocin (STZ) at a dose of 60 mg/kg (STZ, Sigma, L’Isle d’Abeau, Chesne, France) diluted immediately before injection in citric acid buffer (0.01 mol/L pH 5.5). In the control group only citric acid buffer was injected. One group of diabetic animals (DM) was fed a nonpurified diet, 3% of lipids, (A04, UAR, Villemoisson-sur-orge, France) supplemented with (n-3) fatty acid-enriched fish oil concentrate (MaxEPA, Pierre Fabre Santé, Castres, France) administered for 8 wk at a daily dose of 0.5 g/kg by gavage. This supplement is rich in eicosapentaenoic acid [20:5 (n-3)] and docosahexanoic acid [22:6 (n-3)]. The other group of diabetic rats (DO) was fed a nonpurified diet supplemented with olive oil at the same dose (Table 1). Diabetic rats were not treated with insulin. The nondiabetic control group (CO) was also fed a nonpurified diet supplemented with olive oil.

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nerves were removed, rapidly rinsed with ice-cold saline in for MNCV in meters per second (m/s).

The latencies were measured from the stimulator at 10 Hz on via bipolar electrodes with supramaximal stimuli (6 mA) done by was stimulated proximally at the sciatic notch and distally at the ankle was maintained at 36 –37°C with heating lamp and pad. The left sciatic nerve from the left sciatic tibial nerve in temperature-controlled environment under ether anaesthesia. The rectal temperature was main-

Fatty acid composition of the oil supplements

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>MaxEPA</th>
<th>Olive oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/100 mg oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>∑ SFA</td>
<td>25.8</td>
<td>10.5</td>
</tr>
<tr>
<td>14:0</td>
<td>7.3</td>
<td>—</td>
</tr>
<tr>
<td>16:0</td>
<td>15.3</td>
<td>10.5</td>
</tr>
<tr>
<td>18:0</td>
<td>3.2</td>
<td>—</td>
</tr>
<tr>
<td>∑ MUFA</td>
<td>20.2</td>
<td>72.1</td>
</tr>
<tr>
<td>16:1(n-9)</td>
<td>7.8</td>
<td>—</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>12.4</td>
<td>72.1</td>
</tr>
<tr>
<td>∑ PUFA (n-6)</td>
<td>2.2</td>
<td>—</td>
</tr>
<tr>
<td>18:4(n-6)</td>
<td>2.2</td>
<td>—</td>
</tr>
<tr>
<td>∑PUFA (n-3)</td>
<td>35.3</td>
<td>0.6</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>0.8</td>
<td>—</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>17</td>
<td>—</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>12.3</td>
<td>—</td>
</tr>
</tbody>
</table>

1 SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. The α-tocopherol content of the different oils is 1.75 mg/g.

Olive oil was chosen as the placebo because it does not contain (n-3) fatty acids. Water was given with free access to all groups. All animal treatments adhered strictly to all institutional and national ethical guidelines.

Blood samples were collected regularly from the tip of the tail, and blood glucose was measured with a reagent strip (Reflolux, Boehringer Mannheim, Mannheim Germany).

Fatty acid composition of the oil supplementation. Fatty acids were analyzed as methyl esters on a Varian Model 3300 gas chromatograph (Varian, les Ulis, France) equipped with a flame ionization detector using a vapour capillary column (25 m × 0.2 mm internal diameter). The temperature program was 150 –210°C at 1.5°C/min. Peak areas from the resulting chromatogram were measured with a Merk model D 2000 integrator (Merk, Nogent, France). Fatty acid methyl esters were prepared according to Hagenfeldt (1966). Nona-decanoic acid (C19:0) was added to the mixture before methylation as internal standard.

Measurement of nerve conduction velocity. After 8 wk motor nerve conduction velocity was recorded in 10 animals of each group from the left sciatic tibial nerve in temperature-controlled environ-

Body weights and plasma glucose concentrations in CO, DO, and DM rats after 8 wk

<table>
<thead>
<tr>
<th>Body weight</th>
<th>Plasma glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>g</td>
<td>mmol/L</td>
</tr>
<tr>
<td>CO</td>
<td>471.0 ± 11.9a</td>
</tr>
<tr>
<td>DO</td>
<td>216.5 ± 11.5b</td>
</tr>
<tr>
<td>DM</td>
<td>205.0 ± 11.2b</td>
</tr>
</tbody>
</table>

1 Data are group means ± SEM, n = 10. Values in a column not bearing a common superscript letter are significantly different, P < 0.05. CO, control; DO, diabetic animals fed diet supplemented with olive oil; DM, diabetic animals fed diet supplemented with fish oil.

RESULTS

Changes in body weight and blood glucose. Rats given STZ exhibited a marked hyperglycemia and no gain of body weight at the end of 8 wk (Table 2). Fish oil supplementation had no effect on blood glucose levels and body weights in diabetic rats.

Effects of STZ-induced diabetes and fish oil supplementation on the NCV. Diabetic rats had 28.2% lower NCV than controls (Fig. 1). Fish oil supplementation (group DM) re-

| TABLE 2 | |
oil. However, the NCV in DM groups was still significantly lower than the control group (Fig. 1).

Effects of STZ-induced diabetes and fish oil supplementation on Na,K-ATPase activity. The Na,K-ATPase activity in the DO group was 68% lower than in CO ($P < 0.05$, Fig. 1). The fish oil supplementation in the DM group partially prevented the loss of the enzymatic activity ($P < 0.05$).

A significant correlation ($r = 0.999$) was found between diabetes and fish oil related changes in NCV and Na,K-ATPase. These effects were associated with attenuated anatomical damage and preventive effect on the bimodal distribution of myelinated fiber diameters. The improved NCV was strongly correlated with Na,K-ATPase activity.

These results support the hypothesis that treatment with (n-3) fatty acids is beneficial for diabetic patients (Malasanos and Stacpool 1991). This hypothesis resulted from experimental and clinical data showing that (n-3) fatty acids could restore hyperglycemia-induced alterations, such as platelet aggregation (Colwell et al. 1983, Szirtes 1970), vasoconstriction (Boulanger et al. 1990, Shimokawa and Vanhoutte 1988), endothelial permeability chemotaxis erythrocyte deformability, blood viscosity and granulocyte/endothelium interactions and elevated plasma triglyceride level (review Malasano and Stacpool 1991, Simopoulos 1991).

DISCUSSION

Data obtained in this study indicate that (n-3) fatty acid supplementation at a dosage corresponding to currently applied treatment protocols used in humans partially ameliorates diabetes-associated changes in Na,K-ATPase activity and NCV. These effects were associated with attenuated anatomical damage and preventive effect on the bimodal distribution of myelinated fiber diameters. The improved NCV was strongly correlated with Na,K-ATPase activity.

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Moreover, evidence suggests that dietary fish oils rich in eicosapentaenoic (20:5) and docosahexaenoic (22:6) fatty acids may prevent or delay diabetic complications attributable to microvascular diseases (Jensen et al. 1989). While (n-3) fatty acids are recognized for their antiplatelet actions and effects on lipoprotein metabolism, there is increasing evidence that their vascular protective actions are expressed at the level of the vessel wall after incorporation into the phospholipids of cell membranes (Leaf 1990). This preventive action of fish oil has been observed in cardiomyopathy (Gerbi et al. 1997c) and neuropathy (Gerbi et al. 1997b). Indeed, it was documented that this treatment prevents the loss of activity and expression of Na,K-ATPase isoenzymes. Nevertheless, deleterious effects of fish oil supplementation have been documented in retinopathy (Hammes et al. 1996). In this study, we extend the biochemical study of the treatment of neuropathy by fish oil to functional and anatomical nerve parameters. This is the first time that beneficial effects of fish oil supplementation on the neuroanatomical lesions have been found, although amelioration of altered NCV has been previously suggested (Dines et al. 1993).

Our morphological study confirmed the histological damage induced by diabetes on the sciatic nerve fibers (Dick et al. 1994; Sima et al. 1988 and 1993): endoneurial edema and axonal degeneration with occasional secondary segmental demyelination. The quantitative study of nerve fibers showed clearly that myelin sheath was unaffected by diabetes. In this study, in spite of the lack of microvascular abnormality, the hypoxic hypothesis remains plausible. However among these histological damages, endoneurial edema could result from altered sodium cell gradient related to impairment of Na,K-ATPase activity. In contrast, although we hypothesize that the loss of activity of Na,K-ATPase was associated with the altered membrane environment, the decrease of protein expression (Gerbi et al. 1997b) could result from demyelination. Then the decrease in Na,K-ATPase activity could be early as the result of metabolic abnormality and late as the result of histological damage. Moreover, the decrease in NCV could result from alterations in Na,K-ATPase activity, the membrane environment and histological damage, particularly the loss of myelinated large fibers. Dietary fish oil supplementation for 8 wk almost completely prevented or delayed the histological damage induced by diabetes. These effects were independent of hyperglycemia, as this variable was not affected by the dietary treatment. We have previously shown that dietary fish oil supplementation prevented the membrane alteration of the (n-6) fatty acid incorporation, and the protein expression and activity of the Na,K-ATPase α1 and α3 isoenzymes (Gerbi et al. 1997b). These preventive effects on Na,K-ATPase and histological nerve damage could be associated with the improvement of NCV. The relationships between biochemical, functional and neuroanatomical variables remain unresolved, as is the mechanism by which fish oil treatment prevents or delays the histological damage.

We speculate that fish oil prevents the membrane alteration, and by this mechanism prevents the changes in Na,K-

FIGURE 3 Photomicrograph (×930) of cross section through sciatic nerve of diabetic (A) and DM (B) rats. Axonal degeneration is evident in the untreated diabetics, while the profile of the DM group is similar to that in nondiabetic COs.

FIGURE 4 Photomicrograph of teasing (×190) of segmental demyelination of a diabetic myelinated nerve fiber (A) and CO group (B).
FIGURE 5  Photomicrograph (×10,000) of cross section of sciatic nerve of diabetic (A) degenerative axonal changes in diabetic myelinated nerve fiber by electron microscopic study. (B) Axonal atrophy in diabetic nerve fiber by electron microscopic study. (C) Normal nerve fibers in CO group.
ATPase activity, histological damage and prevents partially the loss of NCV. A second hypothesis is that fish oil acts by a transcriptional effect on Na,K-ATPase gene which induces a prevention in functional and histological parameters.

In conclusion, this nutritional study shows the beneficial effect of fish oil supplementation on diabetes-induced abnormalities of the sciatic nerve and suggests a treatment for diabetic neuropathy.

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LITERATURE CITED


