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Short communication

High rat food vitamin E content improves nerve function in streptozotocin-diabetic rats

P. Sytze van Dam [a,b,*], Bert Bravenboer [a], B. Sweder van Asbeck [b], Joannes J.M. Marx [b], Willem Hendrik Gispen [a]

[a] *Department of Medical Pharmacology, Rudolf Magnus Institute for Neurosciences, University Medical Center, P.O. Box 80040, 3508 TA Utrecht, Netherlands*

[b] *Department of Internal Medicine and Eijkman Winkler Institute, University Medical Center, Utrecht, Netherlands*

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Abstract

Antioxidants can improve nerve dysfunction in hyperglycaemic rats. We evaluated whether the standard supplementation of rat food with vitamin E (normally added for preservation purposes) or high-dose vitamin E treatment improves nerve conduction in maturing streptozotocin-diabetic rats, a model widely used to study diabetic neuropathy. Hyperglycaemic rats received food containing 25 mg/kg (non-supplemented), 70 mg/kg (standard food) or 12 g/kg (high-dose) vitamin E. Non-diabetic controls received non-supplemented food. Sciatic and tibial sensory and motor nerve conduction velocity were decreased in all diabetic animals. In comparison with standard feeding, the non-supplemented diabetic rats showed lower plasma vitamin E levels but no significant change in nerve conduction. High-dose treatment prevented nerve dysfunction by 50%, and led to attenuated endoneurial lipid peroxidation (measured as malondialdehyde). We conclude that high doses of vitamin E, but not standard vitamin E supplementation of rat food partially prevent nerve dysfunction in young adult streptozotocin-diabetic rats.

Keywords: Diabetic neuropathy; Streptozotocin; Oxidative stress; Vitamin E; Malondialdehyde; Nerve conduction

1. Introduction

Increased production of reactive oxygen species is associated with the development of neuropathy and other chronic complications of diabetes mellitus (van Dam et al., 1995; Baynes, 1991). Studies in the streptozotocin-diabetic rat have demonstrated that antioxidants can prevent experimental diabetic neuropathy (review van Dam and Bravenboer, 1997). This effect was sometimes associated with correction of reduced nerve microcirculation (Cameron et al., 1994; Nagamatsu et al., 1995) and protection against hyperglycaemia-induced neural morphological changes (Sagara et al., 1996). In adult diabetic rats supplemented with the naturally occurring free radical scavenger vitamin E (1000 mg/kg body weight), nerve conduction deficits were almost completely normalised, while lower doses had a partial beneficial effect (Cotter et al., 1995). Furthermore, vitamin E administration could normalise decreased nerve blood flow in maturing (Karasu et al., 1995) and adult (Cotter et al., 1995) diabetic rats. Recently, the possible benefit of vitamin E was also demonstrated in diabetic patients with neuropathy; vitamin E supplementation during six months improved some of the existing nerve conduction deficits (Tutüncü et al., 1998).

Streptozotocin-induced hyperglycaemia in maturing rats is a common experimental model to evaluate pathophysiology and therapeutic strategies for diabetic neuropathy. The defective development of peripheral nerve function is demonstrated by the absence of the normally observed increase in nerve conduction velocity during maturation, leading to a nerve conduction deficit of a similar magnitude as observed in adult streptozotocin-treated rats (Kappelle et al., 1993). Endoneurial vitamin E levels have been found to be elevated in normally fed maturing streptozo-

* Corresponding author. Department of Medical Pharmacology, Rudolf Magnus Institute for Neurosciences, University Medical Center, PO Box 80040, 3508 TA Utrecht, The Netherlands. Tel.: +31-30-250-6303; fax: +31-30-253-9032; E-mail: p.s.vandam@digd.azu.nl

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tocin-diabetic rats in comparison with non-diabetic controls (Nickander et al., 1994). As standard rat chow is generally supplemented with vitamin E to improve its tenability, and as maturing diabetic rats are hyperphagic, increased vitamin E intake can theoretically influence the outcome of studies in which antioxidant drugs or other therapeutical strategies are evaluated for their potential benefits in diabetic neuropathy.

The model of the maturing streptozotocin-diabetic rat has been used to evaluate the contribution of oxidative stress and vitamin E status to the pathogenesis of diabetic neuropathy (van Dam et al., 1996, 1998, 1999) and to demonstrate beneficial effects of antioxidants on nerve function (Sagara et al., 1996; Nagamatsu et al., 1995; Bravenboer et al., 1992). Love et al. (1997) demonstrated that administration of a daily supplement of α-tocopherol (1 g/kg body weight) during six weeks to young streptozotocin-diabetic rats could almost completely prevent nerve conduction deficits if the treatment was started immediately after onset of diabetes. No data are available on the effect of standard vitamin E supplementation of normal rat chow. Therefore, we performed the present study to evaluate the effects of the standard supplementation of vitamin E to rat chow on nerve conduction velocity and to compare this to a high vitamin E dose in maturing streptozotocin-diabetic rats. Furthermore, we measured plasma vitamin E levels and systemic and endoneurial lipid peroxidation, with the objective to assess the possible endoneurial and systemic effects of vitamin E status on alterations in nerve function.

2. Materials and methods

All animal procedures were approved by the University ethical committee for animal experiments. Male Wistar rats were housed under standard conditions. All animals received rat chow and water ad libitum. Diabetes was induced by streptozotocin 40 mg/kg i.v. (Serva, Heidelberg, Germany), and was confirmed after 48 h by measuring whole blood glucose levels (Medisense®, Amersfoort, The Netherlands).

Our standard rat food contains 70 mg/kg vitamin E, of which 45 mg/kg is artificially added by the manufacturer for preservation purposes. For this study, two special diets were prepared (Hope Farms, Woerden, The Netherlands): non-supplemented food, containing 25 mg/kg (naturally occurring) vitamin E, and food containing 12 g/kg vitamin E. The vitamin E supplements consisted of D-L-alpha-tocopheryl-acetate, which has a bioactivity of 67% of D-alpha-tocopherol. To avoid differences in toxic effects of streptozotocin, all animals were initially fed with non-supplemented food starting immediately after weanling. When the animals had reached a weight of 280 g, diabetes was induced. The diabetic animals were divided in three groups: a group continuing the non-supplemented food, a group starting a diet with standard rat food, and a group starting with rat chow containing 12 g/kg vitamin E (high-dose treatment group). Non-diabetic control animals continued the non-supplemented food. Food intake was measured weekly. Sciatic and tibial sensory and motor nerve conduction velocity were measured at week 0 (before streptozotocin injection), 4, 8 and 12. At the end of the experiment, plasma and sciatic nerve malondialdehyde, a parameter for lipid peroxidation, and plasma vitamin E were measured.

Sciatic motor and sensory nerve conduction were measured at the left hind paw as described previously (De Koning and Gispen, 1987). Tibial motor and sensory nerve conduction measurements were performed over the last 25 mm of the hind paw (measured from the ankle) using similar procedures. All measurements were performed by the same examiner under general anaesthesia (0.175 ml Hypnorm® containing 10 mg/ml fluanisone and 0.2 mg/ml fentanyl citrate, Janssen Pharmaceutica, Tilburg, The Netherlands). During the measurements, the body temperature of the animals was kept constant at 36–37°C.

The animals were killed by decapitation at the end of the study, and blood was subsequently collected in heparinised tubes, separated into plasma and red blood cells and processed immediately. Both sciatic nerves were located, removed, desheathed and stored in liquid nitrogen until homogenisation and further processing. Nerve homogenisation was performed on ice in a 50 mmol/l sodium phosphate buffer (pH 7.5) on the same day.

Malondialdehyde was measured as thiobarbituric acid reactive substance, according to Aust (Aust, 1985). Plasma vitamin E was measured with a standard high performance liquid chromatography technique (Lee et al., 1992). Protein concentrations in sciatic nerve homogenates were measured in a 96-well microtiter plate at 595 nm according to Bradford (Bradford, 1976). Haemoglobin concentrations in whole blood were assessed using hemiglobincyanide (Zijlstra and van Kampen, 1962).

2.1. Statistical analysis

All data are represented as mean ± S.E.M. Analysis of variance (ANOVA) with a post-hoc Bonferroni significance test was used to evaluate food intake and biochemical data. Inverse or logarithmic transformation was performed in case of non-equality of variances. Effects of diabetes or vitamin E intake on body weight and nerve conduction velocities were analysed using ANOVA for repeated measurements.

3. Results

All animals injected with streptozotocin became diabetic. Average food intake was increased in all diabetic animals in comparison with non-diabetic controls (Table 1, $P < 0.001$). No effects of vitamin E intake on mortality,

Table 1
Mean food intake, final weight, glucose, malondialdehyde and vitamin E per group of rats
Diabetic rats receiving rat chow containing 25 mg/kg (low), 70 mg/kg (standard) or 12 g/kg (high) vitamin E, and control animals receiving food containing 25 mg/kg vitamin E.
All data are mean ± S.E.M. See text for statistical methods.

	Diabetics low E $n = 10$	Diabetics standard E $n = 10$	Diabetics high E $n = 12$	Controls low E $n = 10$
Food intake (g/100 g body weight/day)	16.8 ± 0.8	14.8 ± 0.9	16.6 ± 0.5	6.6 ± 0.6[a]
Final weight (week 12) (g)	245 ± 15	221 ± 13	253 ± 9	494 ± 10[a]
Glucose (mmol/l)	25.1 ± 0.8	24.4 ± 0.6	25.2 ± 0.7	7.2 ± 0.2[a]
Plasma malondialdehyde (μmol/l)	1.21 ± 0.03	1.30 ± 0.06	1.26 ± 0.04	0.94 ± 0.03[a]
Nerve malondialdehyde (μmol/g protein)	4.57 ± 1.16	1.87 ± 0.24	1.26 ± 0.25[b]	2.85 ± 0.38
Plasma vitamin E (mg/l)	17.2 ± 3.3	35.4 ± 5.8	237.2 ± 18.4	6.9 ± 0.6[c]

[a] F (ANOVA) < 0.0001, $P < 0.001$ vs. diabetic groups.
[b] F (ANOVA) = 0.0017, $P < 0.01$ vs. diabetics (low E).
[c] F (ANOVA) < 0.0001, controls vs. diabetics (low E) $P < 0.01$, diabetics (low E) vs. (standard E) $P < 0.05$, (low E) and (standard E) vs. (high E) $P < 0.001$.

body weight or blood glucose were observed. The average daily vitamin E intake in the high vitamin E group (12 g/kg chow) was approximately 2 g/kg body weight. Diabetic animals with high vitamin E intake had significantly improved sciatic and tibial motor and sensory nerve conduction in comparison with diabetic animals with a normal diet or with vitamin E restriction (Fig. 1, $P < 0.01$). Although the animals with low vitamin E intake (25 mg/kg chow) showed somewhat reduced nerve conduction velocities in comparison with the normally fed diabetics, especially for sciatic sensory nerve conduction velocity at week 12, these differences were not significant.

Non-diabetic control animals had lower vitamin E levels than identically fed diabetics ($P < 0.05$). In the diabetic rats, plasma vitamin E levels reflected the vitamin E content of the diet (Table 1). We observed increased plasma malondialdehyde levels in all diabetic groups in comparison to controls, but between the three diabetic

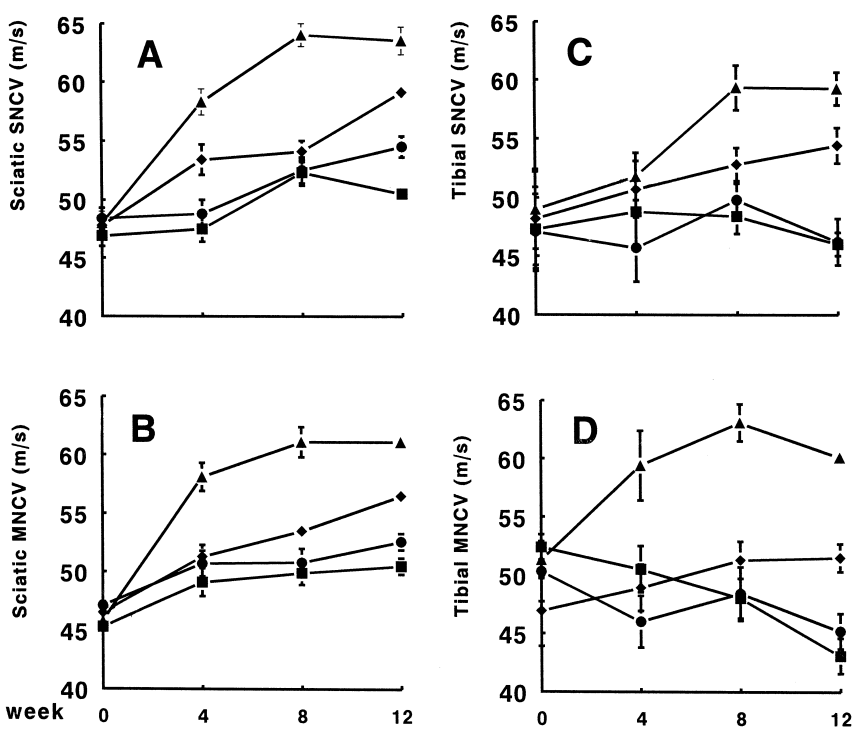

Fig. 1. (A) Sciatic sensory (SNCV) and (B) sciatic motor (MNCV) nerve conduction velocity, (C) tibial sensory and (D) tibial motor nerve conduction velocity in streptozotocin-diabetic rats receiving food containing vitamin E in different doses: 12 g/kg (– ♦ –), 70 mg/kg (– ● –) or 25 mg/kg (– ■ –), and non-diabetic controls receiving food containing 25 mg/kg vitamin E (– ▲ –). Sciatic and tibial sensory and motor nerve conduction velocities are reduced in all diabetic groups in comparison with controls ($P < 0.001$, analysis of variance for repeated measurements); the diabetic animals receiving 12 g/kg vitamin E had significantly higher sciatic and tibial sensory and motor nerve conduction velocities in comparison with the two other diabetic groups ($P < 0.01$). No significant differences between the other two diabetic groups were found. Values are expressed as mean ± S.E.M.

groups, no effects of vitamin E intake were observed. Sciatic nerve malondialdehyde levels in the diabetic animals with low vitamin E intake were not significantly different from identically fed controls. Within the three diabetic groups, a significant decrease in nerve malondialdehyde was observed in the 12 g/kg vitamin E treated group in comparison with the animals receiving the lowest (25 mg/kg) dose of vitamin E (Table 1, $P < 0.01$).

4. Discussion

The present data demonstrate that extremely high doses of vitamin E can partially prevent nerve conduction deficits in maturing hyperglycaemic rats. Positive effects of doses of vitamin E of 500 and 1000 mg/kg body weight on nerve conduction in streptozotocin-diabetes have been observed previously in maturing (Love et al., 1997) and adult (Cotter et al., 1995) rats. Lower vitamin E supplementation (190 mg/kg body weight) had no effect on nerve conduction, endoneurial glutathione or conjugated dienes when the treatment was started during weanling (Nickander et al., 1994). Interestingly, in the present study we found that while sciatic nerve malondialdehyde levels (reflecting endoneurial oxidative damage) were not significantly elevated in experimental diabetes, they could be lowered by high doses of vitamin E. In contrast, plasma malondialdehyde levels were not affected by vitamin E intake, whereas they were increased as a consequence of hyperglycaemia. Elevated nerve malondialdehyde levels have previously been demonstrated in non-diabetic vitamin E-deficient rats (van Dam et al., 1998). These findings suggest that, while endoneurial lipid peroxidation is not increased as a consequence of diabetes, nerve malondialdehyde concentration depends upon the vitamin E intake of the animals. The present malondialdehyde data indicate that, at least in young adult rats, if a direct effect of vitamin E on peripheral nervous tissue contributes to the observed effects on nerve conduction velocity in experimental diabetes, this effect is small in comparison with the contribution of alterations in nerve microcirculation, as demonstrated previously (Cotter et al., 1995; Karasu et al., 1995).

In comparison with previous studies, the present data show a relatively small correction of nerve conduction velocity. Love et al. (1997) observed a correction of between 80 and 85% in sciatic motor nerve conduction velocity and of approximately 70% in saphenous nerve sensory conduction velocity with the administration of vitamin E at a dose of 1 g/kg body weight to maturing diabetic rats. Cotter et al. (1995) using adult rats and the same dose of vitamin E, found a preventive effect of 80–90% on sciatic motor and saphenous sensory nerve conduction velocity. In the present study, we used a higher dose of vitamin E (approximately 2 g/kg body weight), but obtained a preventive effect of only 50–70%, depending on the nerve studied. This difference may be explained by a different mode of administration, as we used vitamin E supplemented food while in the aforementioned studies the treatment was given separately. As no plasma vitamin E levels were reported in these studies, the degree of gastro-intestinal resorption of this fat-soluble compound cannot be compared to our data. Differences in the content of the D-isomer of α-tocopherol could also explain the discrepancy, as the D-isomer is known to have the highest biological activity (Meydani, 1995). Furthermore, previous studies have focused on other branches of peripheral nerves using somewhat different techniques, which could explain the difference in treatment effect.

A second objective of the present study was to evaluate whether the usual addition of vitamin E supplements to rat chow influences the outcome of nerve conduction studies in young adult diabetic rats. In comparison with the non-supplemented food, standard food had no significant effects on nerve conduction velocity, in spite of increased plasma vitamin E levels. This implies that the relatively high vitamin E intake in hyperphagic diabetic rats using standard food (approximately five-fold increased in comparison to recommended daily intake) does not affect the outcome of studies on experimental diabetic neuropathy with a maximum duration of twelve weeks. Therefore, we conclude that there is no need to reduce the vitamin E content of standard food for optimal performance of further studies using this model. However, as we observed a tendency towards improvement of nerve conduction in the standard food group in comparison with the lowest vitamin E dose, especially for sciatic sensory nerve conduction velocity at week 12, vitamin E content of the food may be of importance in studies of longer duration.

Vitamin E is a lipophilic antioxidant, with the capacity to convert a lipid peroxyl radical into a lipid hydroperoxide, thus protecting against lipid peroxidation chain reactions (Burton, 1994). These characteristics indicate that the antioxidant effects of vitamin E may be concentrated in the cell membrane. Vitamin E deficiency is associated with central and peripheral nervous system deficits (Muller and Goss Sampson, 1990; Southam et al., 1991), but also with changes in vascular reactivity and reduced endothelium-dependent vascular relaxation (Rubino and Burnstock, 1994). Similar vascular changes, preventable by vitamin E, have also been observed in experimental diabetes (Keegan et al., 1995), while the combination of hyperglycaemia and vitamin E deficiency further decreases nerve conduction velocity (Nickander et al., 1994). Several other effects of vitamin E administration which may be beneficial in diabetes have been demonstrated. High doses of vitamin E can lead to increased insulin-mediated glucose disposal (Paolisso et al., 1994), normalisation of hyperglycaemia-induced attenuated protein kinase C activity in endothelial cells (Kunisaki et al., 1994), reduction of non-enzymatic protein glycation (Ceriello et al., 1991) and increased red blood cell glutathione (Costagliola et al., 1985). Furthermore, changes in

the cyclooxygenase pathway leading to reduced endothelium-dependent vascular relaxation have been observed in vitamin E-deficient rats (Davidge et al., 1993).

This large variety of experimental data suggests that various systemic effects occur after vitamin E administration; one or more of these mechanisms may contribute to the observed improvement in nerve blood flow and nerve function. It has been demonstrated that diabetes-induced changes in nerve blood flow can be reversed by vitamin E (Cotter et al., 1995; Karasu et al., 1995). Therefore, the protective effect of vitamin E on nerve function is suggestive for the possibility, that the high doses that were given have similar neurovascular effects as other antioxidants like probucol and *N*-acetylcysteine (Cameron et al., 1994; Cotter et al., 1995; Love et al., 1996).

In conclusion, the present experiments demonstrate that antioxidant treatment with very high doses of vitamin E can protect against nerve dysfunction in experimental diabetes, but that this protection may be somewhat smaller in the maturing diabetic rat than in adult rats. The relatively high vitamin E intake in our standard experimental conditions does not affect the outcome of our studies on experimental diabetic neuropathy, despite differences in plasma vitamin E levels and endoneurial lipid peroxidation. Although direct protective antioxidant effects on the nerve by vitamin E cannot be excluded, improvement of neurovascular supply is probably the principal mechanism explaining the effects of vitamin E in experimental diabetic neuropathy.

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