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Potential Therapeutic Effect of Antioxidants in Experimental Diabetic Retina: A Comparison between Chronic Taurine and Vitamin E Plus Selenium Supplementations

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Although good glycaemic control can delay the development and progression of diabetic retinopathy, new therapies are needed to obtain a better control of this diabetic complication. Oxidative stress seems to be a contributing factor in diabetic retinal alterations, therefore, it has been suggested that antioxidants may be beneficial in reducing diabetic retinal changes. However, many questions are still open. In fact, it remains to be ascertained which antioxidants are the most active when they are chronically administered *in vivo* and their effective dosages. Therefore, we compared the effect of chronic taurine supplementations versus a mixture of vitamin E + selenium on biochemical retinal changes induced by diabetes at different stages of the disease. Briefly, streptozotocin (STZ) diabetic rats ware administered for 4 months following the dietary supplements: (a) 2% (w/w) taurine; (b) 5% (w/w) taurine; (c) 200 IU vitamin E + 8 mg selenium/kg diet (d) 500 IU vitamin E + 8 mg selenium/kg diet. In STZ diabetic rat in poor metabolic control (i.e. serum glucose >16.5 mmol/l), at 2, 4, 8, 16 weeks following the onset of diabetes, retinal conjugated dienes (CD) and lipid hydroperoxides (LP) were significantly and progressively increased, while sodium pump activity was gradually and significantly reduced. In taurine and vitamin E + selenium supplemented diabetic rats, glycaemia and body weight were not significantly different from those of non-supplemented diabetic animals. In diabetic rats, 2 and 5% taurine significantly decreased CD. This reduction is long lasting. Regarding CD, both vitamin E + selenium supplementations reduced CD only during the first 4 weeks of diabetes. Two percent taurine supplementation significantly lowered LP for the first 8 weeks of the disease while 5% taurine-induced-reduction lasted for the whole experimental time. A 200 IU vitamin E + 8 mg selenium supplementation did not significantly modify LP, while 500 IU vitamin E + 8 mg selenium significantly lowered them for the whole studied period. Finally, taurine preserved ATPase activity being more effective at 5% than 2%. Two hundred IU vitamin E + 8 mg selenium did not generally modify pump activity, while 500 IU vitamin E + 8 mg selenium partially prevented the decrease in pump activity. We conclude that taurine and vitamin E + selenium supplementations ameliorate biochemical retinal abnormalities caused by diabetes. These effects are dose- and time-dependent. Moreover, the effect of taurine on CD is longer lasting than that of vitamin E + selenium. In addition, taurine seems to better preserve ATPase activity in comparison with vitamin E + selenium. Finally, in diabetic animals a negative correlation is found between CD and LP on one side and Na^+K^+ATPase activity on the other; thus, lipid peroxidation and pump activity seem to be associated. The same inverse correlations are present in vitamin E + selenium supplemented diabetic rats, but are lost in taurine supplemented animals. Therefore, taurine effects may not be simply mediated by its antioxidant activity. Thus, chronical (4 months) taurine and vitamin E + selenium supplementations reduce biochemical retinal alterations in diabetic rat in poor metabolic control.

Keywords: Diabetic retinopathy; Lipid peroxidation; Na^+K^+ ATPase activity; Taurine; Vitamin E + selenium; Supplementation

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INTRODUCTION

Retinopathy is the earliest and most common complication of diabetes mellitus, affecting up to 90% of diabetic patients, progressing to blindness in about 5% of cases[1] and being the leading cause of acquired blindness among young adults in developed countries. Although, good glycaemic control can delay the development and progression of diabetic retinopathy[2] such metabolic control is often difficult to achieve and to maintain, and additional therapies need to be identified by which diabetic retinal alterations can be prevented and/or arrested. In this context, the correction of oxidative stress may have important implications in preventing diabetic retinal changes, since one of the consequences of chronic hyperglycemia is enhanced oxidative stress[3] and reactive oxygen species have been implicated in the pathogenesis of retinal diabetic alterations.[4] Streptozotocin (STZ) diabetic rats develop retinal alterations where the importance of oxidative stress has been clearly shown.[5]

In diabetes, defenses against oxygen free radicals are reduced[6] and antioxidants such as taurine, vitamin C, and uric acid are decreased[7–12] while, for other antioxidants such as vitamin E and gluthatione there are conflicting reports.[13–17] In this context, it is of relevance to determine which antioxidant might be supplemented to provide significant protection and whether their effects are long lasting.

Taurine and vitamin E scavenge different reactive oxygen species. Indeed, taurine is a potent scavenger of HClO,[18] being almost ineffective against other reactive oxygen species[19–20] while vitamin E is a well-known chain-branching antioxidant.[21] Moreover, they may exert their effects in different cellular compartments because taurine is mainly found in cytoplasm[18] while vitamin E is in membranes.[21]

Therefore, we compared *in vivo* the effect of chronic taurine supplementation versus vitamin E + selenium. Taurine has also been selected because it is decreased in retinal pigment epithelium[22] of STZ-diabetic rat and because its chronic (4 months) supplementation reduces retinal oxidative stress and improves sodium pump activity in experimental diabetes[23,24] in a dose- and time-dependent fashion. Furthermore, it decreases the release of VEGF in the retina of STZ-diabetic rats,[25] while vitamin E + selenium supplementation has been found to delay early renal diabetic lesions more actively than other antioxidants.[26] Therefore, we investigated whether both supplementations protect diabetic retina from lipid peroxidation and from the decrease in sodium pump activity[27] induced by diabetes. Insufficient enzymatic activity has been suggested as a contributing factor in the development of diabetic complications in excitable tissues.[28]

MATERIALS AND METHODS

Animals

Rats were housed in the Catholic University College of Medicine Animal Facility in accordance with the Guidelines of the American Physiology Society. Water and food were provided *ad libitum*. Eight week-old male Wistar rats were purchased from Harlan-Nossan (Milan, Italy). They were allowed to acclimatize for 1 week before starting the project. Finally, they were randomly assigned to different experimental groups. Riefer (Bolzano, Italy) supplied all diets.

Materials

STZ, ouabain and sodium pentobarbital were purchased from Sigma (St. Louis, MO, USA). All other reagents were of analytical grade and were obtained from Sigma (St. Louis, MO, USA).

Experimental Procedure

Diabetes was induced by a single (60 mg/kg) intraperitoneal injection of STZ. Two days later and subsequently at the times indicated in the figures, blood samples were taken from the tail vein for the determination of glucose, which was measured by glucose oxidase reagent strips (Lifescan, Milpits, CA, USA). Rats with glucose >16 mmol/l were considered diabetic. The experimental groups included diabetic rats fed either standard (which includes 80 mg/kg alpha-tocopherol but not selenium) or enriched diets. Four enriched diets were used: 2% (w/w) taurine, 5% (w/w) taurine, 200 IU vitamin E and 8 mg selenium/kg diet, and finally 500 IU vitamin E and 8 mg selenium/kg diet. Vitamin E was administered in the form of alpha-tocopherol. The duration of the study was 16 weeks. On the basis of average daily food consumption of rats, the daily dose of taurine ranged from 0.9 to 1.2 g/day and from 2.1 to 2.7/day for the group that received 2 and 5% taurine enriched diets, respectively. The daily mean doses of vitamin E were 8.4–14 and 17.1–29 IU/day according to the amount of supplemented vitamin E while selenium daily intake was 0.24–0.4 mg/day. Rat weight was noted. Supplementations were started after STZ injection and continued throughout.

On the day of sacrifice, rats were anesthetized with sodium pentobarbital (85 mg/kg intraperitoneally) and sacrificed (2, 4, 8, 16 weeks after the induction of diabetes) by cervical dislocation. Both retinas were rapidly dissected and immediately frozen in liquid nitrogen and stored at -80°C for no longer than 2 weeks after collections.

Biochemical Measurements

Total and ouabain-inhibited Na^+K^+ATPase activities were measured in the supernatant of retinal homogenates using the coupled assay of Norby[29] and they were expressed as μmolP/h/mg protein. LP and CD were measured in retinal lipids extracted as previously described.[30] The LP and CD content were determined with the FOX Version II assay for LP (FOX2)[31] and according to Prior,[32] respectively. Protein concentration was evaluated by the method of Bradford.[33] Each assay was performed in triplicate.

Statistical Analysis

Statistical analysis was performed using Statview software package (Abacus Concepts, Berkeley, CA). Values are given as means ± SD. Comparisons between groups were made using one-way ANOVA values corrected for multiple comparisons by the Fisher method. Significance was defined at $P < 0.05$. Correlations were calculated by Pearson's linear correlation coefficient (r).

RESULTS

As expected, STZ caused a stable increase in blood glucose from 5.7 ± 0.7 ($N = 12$) to 26.4 ± 2.3 mmol/l ($N = 8$, $P < 0.001$) after 4 months of diabetes, which was unaffected by taurine and vitamin E + selenium supplementations. In addition, supplementations did not influence the body weight of diabetic animals (Table I).

Throughout the study, retinal CD were significantly increased (Fig. 1). There was a tendency to raise with time although the differences among different experimental times were significant only during early stage of illness. For the whole duration of the disease, both taurine supplementations partially but significantly reduced CD elevation in diabetic animals, while vitamin E + selenium supplementations significantly lowered CD only in the early stage of disease. No significant difference has been found between low and high vitamin E supplementations.

Diabetes induced a significant and time-dependent increase in retinal LP (Fig. 2), which was significantly prevented by dietary taurine supplementations. In this regard, 5% taurine was more effective than 2% taurine. Vitamin E was able to reduce significantly retinal LP, but only at the dose of 500 IU. This effect of vitamin E was long lasting.

Retinal Na^+K^+ATPase activity was reduced by diabetes in a time-dependent manner (Fig. 3). After 4 months of disease, there was a marked reduction of about 71%. In diabetic rats supplemented with 2% taurine, pump activity was preserved, being higher than baseline for 4 weeks. Then, it remained significantly higher than in non-treated diabetic rats. In diabetic the rat supplemented with 5% taurine, ATPase activity was better preserved. In fact, during the first 2 months it was over baseline values. After 4 months of disease, as shown in Fig. 3, it was significantly higher in comparison with non-treated diabetic groups.

Vitamin E treatments partially reduced the decrease in pump activity induced by diabetes. However, the effect of 200 IU vitamin E + selenium was only seen up to 4 weeks. In contrast, the higher dose of vitamin E prevented the decrease in pump activity at all experimental times (Fig. 3).

In non-treated diabetic animals, an inverse correlation was found between CD and ATPase activity ($r = 0.3$; $P < 0.0001$) and between LP and ATPase activity ($r = 0.4$; $P < 0.0001$). These inverse correlations were lost in taurine supplemented animals while they were still present in the groups supplemented with vitamin E + selenium being $r = 0.57$; $P < 0.0001$ for CD and ATPase and $r = 0.51$; $P < 0.0001$ for LP and ATPase activity.

DISCUSSION

This is a chronic *in vivo* study that evaluates the time-course in lipid peroxidation and sodium pump activity in the diabetic retina, and compares the effects of two concentrations of two diverse antioxidants. As previously described,[23,24] this report confirms that diabetes induces a time-dependent increase in retinal lipid peroxidation and a time-dependent decrease in sodium pump activity. In diabetic rats in very poor metabolic control, the previous alterations are reduced by chronic dietary intake of taurine and vitamin E + selenium. The taurine effect is dose-dependent when LP and pump activity are considered while the dose dependence with vitamin E + selenium is clearly present only when LP is considered. Finally, the effects of taurine and vitamin E + selenium are not linked to a decrease in glycaemia as already shown.[23,24,34]

In experimental and clinical diabetes, taurine is significantly depleted.[7–9,22,35,36] As already suggested,[7,22] taurine intracellular depletion could promote tissue damage in diabetes. In this context, it is likely that taurine supplementation might counteract the decrease in taurine intra- and extra-cellular levels reducing the alterations induced by hyperglycaemia. According to this suggestion, in diabetic patients, taurine intake restores plasma and platelet taurine levels[7,8] decreasing platelet aggregation and in healthy rats, it does not affect retinal lipid peroxidation and pump activity.[23,24] In diabetes, as previously described in shorter studies[25,36] and

TABLE I Final body weight and blood glucose in control fed standard or taurine and vitamin E + Se supplemented rats

	D-rat	D-2% Tau	D-5% Tau	D-200 IU E + Se	D-500 IU E + Se
Body weight (g)	199 ± 14 (8)	231 ± 31 (5)	262 ± 34 (6)	206 ± 42 (7)	245 ± 34 (6)
Bood glucose (mmol/l)	26.4 ± 2.3 (8)	22.2 ± 1.0 (5)	22.5 ± 1.7 (6)	21.5 ± 2.1 (7)	24.6 ± 1.8 (6)

Diabetic rats (D-rat); diabetic rats supplemented with 2% taurine (D-2%Tau); diabetic rats supplemented with 5% taurine (D-5%Tau); diabetic rats supplemented with 200 IU vitamin E + 8 mg selenium/kg diet (D-200 IU + Se); diabetic rats supplemented with 500 IU vitamin E + 8 mg selenium/kg diet (D-500 IU + Se); Values are mean ± SD; in brackets the number of experiments.

in the very same experimental conditions,[23,24,36] taurine decreases lipid peroxidation. Previously, it has been shown that in an acellular model taurine is a very poor scavenger of free oxygen radicals.[19,20] However, it scavenges hypochlorite[18] and this could be important in retina, a tissue that contains hypochlorite-producing enzyme.[37]

It is well established[23,24,38] that Na^+K^+ATPase activity is dramatically decreased in the retina, in the nerve and the heart of diabetic animals. Here, we show a negative correlation between lipid peroxidation and pump activity in non-supplemented animals with the disease. In taurine supplemented diabetic rat, the inverse correlation between lipid

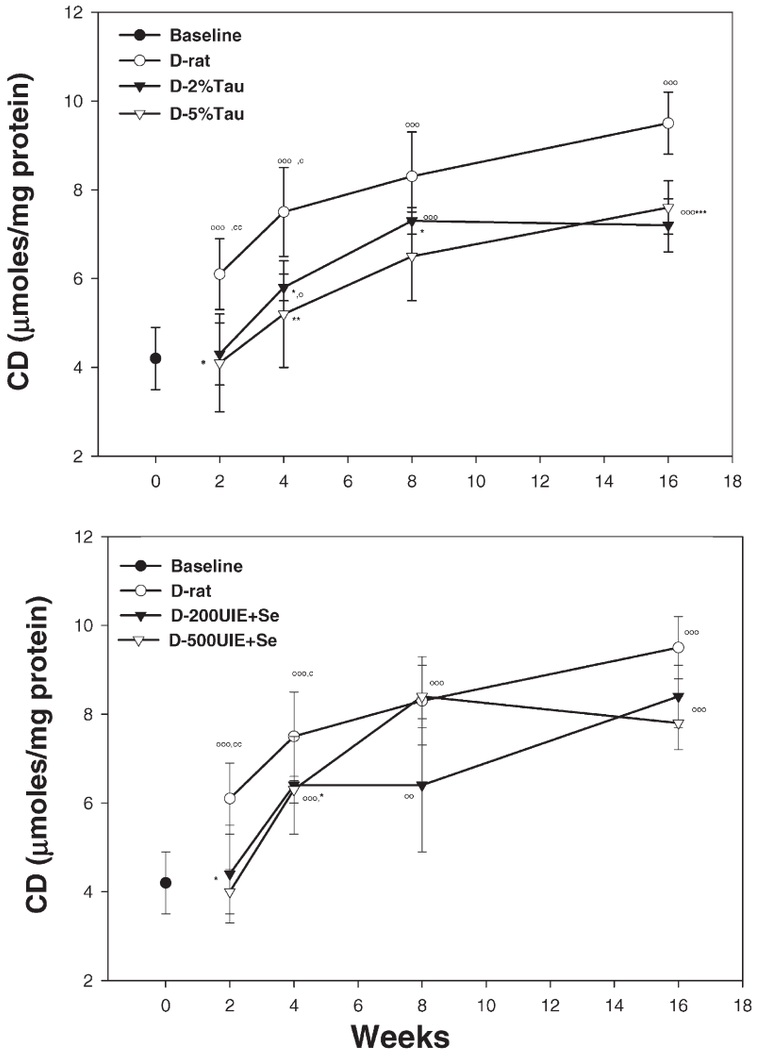

FIGURE 1 CD (µmol/mg protein) in the retina of diabetic rats fed standard or taurine or vitamin E + Se supplemented rats. Diabetic rats (D-rat); diabetic rats supplemented with 2% taurine (D-2%Tau); diabetic rats supplemented with 5% taurine (D-5%Tau); diabetic rats supplemented with 200 IU vitamin E + 8 mg selenium/kg diet (D-200 IU + Se); diabetic rats supplemented with 500 IU vitamin E + 8 mg selenium/kg diet (D-500 IU + Se); Values are mean ± SD of at least five experiments; versus diabetic rat $^{*}P < 0.05$, $^{**}0.05 < P < 0.01$, $^{***}P < 0.001$; versus baseline $^{o}0.05 < P < 0.01$; $^{oo}0.01 < P < 0.01$; $^{ooo}P < 0.001$; versus the previous values $^{c}0.5 < P < 0.01$; $^{cc}0.01 < P < 0.01$.

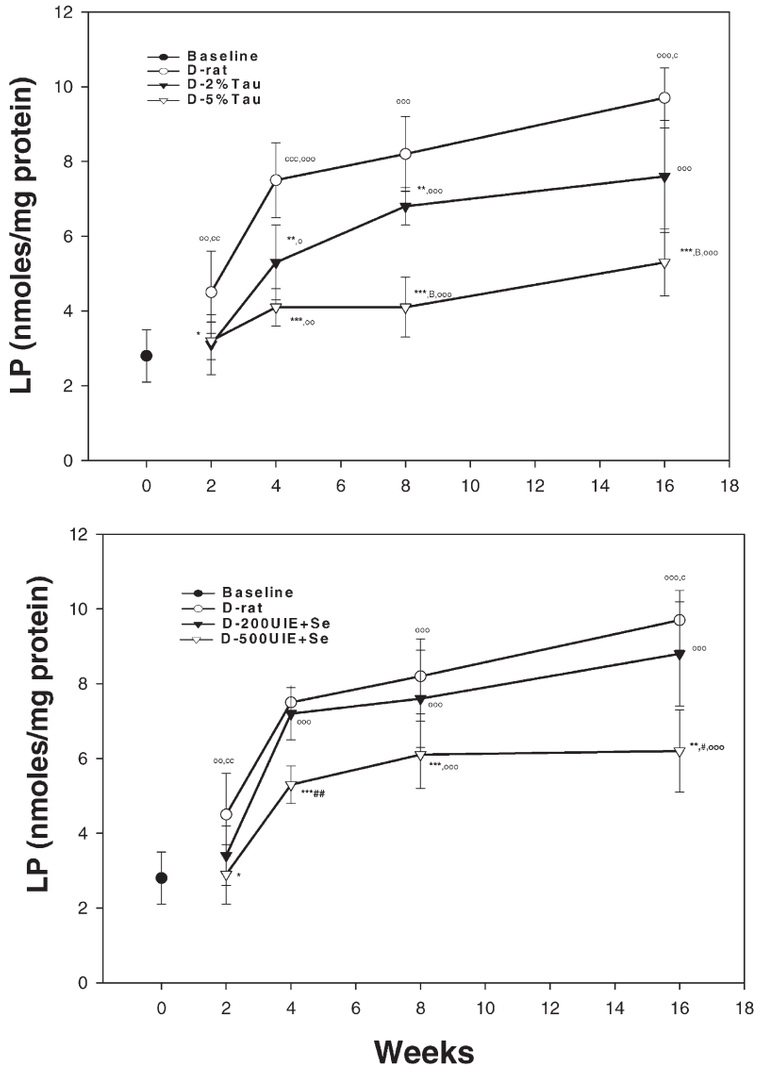

FIGURE 2 LP (nmol/mg protein) in the retina of diabetic rats fed standard or taurine or vitamin E + Se supplemented rats. Diabetic rats (D-rat); diabetic rats supplemented with 2% taurine (D-2%Tau); diabetic rats supplemented with 5% taurine (D-5%Tau); diabetic rats supplemented with 200 IU vitamin E + 8 mg selenium/kg diet (D-200 IU + Se); diabetic rats supplemented with 500 IU vitamin E + 8 mg selenium/kg diet (D-500 IU + Se); Values are mean ± SD of at least five experiments; versus diabetic rat $^{*}P < 0.05$, $^{**}0.05 < P < 0.01$, $^{***}P < 0.001$, $^{b}P < 0.001$ versus 2% taurine; $^{\#}P < 0.05$ versus D-200 IU + Se, $^{\#\#}0.01 < P < 0.001$; versus the previous values $^{c}0.5 < P < 0.01$; $^{cc}0.01 < P < 0.01$, $^{ccc}P < 0.001$.

peroxidation and pump activity is lost, strongly suggesting that the effect of taurine is not only linked to the reduction of lipid peroxidation. Indeed, taurine is very effective in preserving pump activity. Perhaps, it acts by scavenging hypochlorite, which is the most active oxidant in the reduction of pump activity.[39] The presence of a vicious circle between lipid peroxidation and enzymatic activity is likely. The inhibition of ATPase generates calcium overload, which in turn induces lipid peroxidation, which decreases ATPase activity. Taurine may interrupt the vicious circle either scavenging HClO[18] either modulating intracellular calcium.[18,40] Interestingly, in endothelial cells exposed to hyperglycemia, the amino acid reduces lipid peroxidation and calcium overload.[41] However, we cannot exclude other mechanisms such as the osmotic, membrane stabilizing and antihypoxic effects of taurine,[18,36] which could be important in explaining its beneficial effects on diabetic retina.

In normal rats, vitamin E + selenium does not affect retinal lipid peroxidation and pump activity (data not shown), suggesting that it specifically interferes with hyperglycaemia-induced alterations. In our experimental conditions, both doses of vitamin E reduce CD formations only during the early stages of disease, while LP formation, such as the impairment of pump activity, is significantly reduced only by 500 IU vitamin E + 8 mg selenium. Thus, the effect of vitamin E + selenium seems

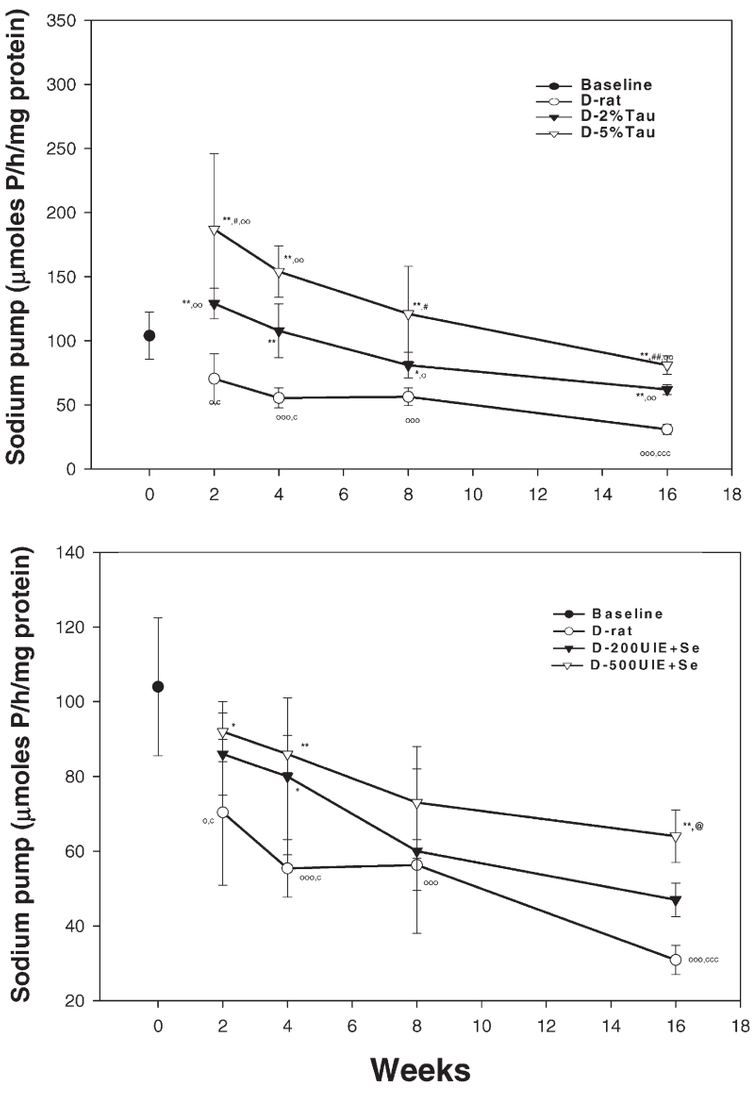

FIGURE 3 Na^+K^+ATPase (µmol P/h/mg protein) in the retina of diabetic rats fed standard or taurine or vitamin E + Se supplemented rats. Diabetic rats (D-rat); diabetic rats supplemented with 2% taurine (D-2%Tau); diabetic rats supplemented with 5% taurine (D-5%Tau); diabetic rats supplemented with 200 IU vitamin E + 8 mg selenium/kg diet (D-200 IU + Se); diabetic rats supplemented with 500 IU vitamin E + 8 mg selenium/kg diet (D-500IU + Se). Values are mean ± SD of at least five experiments; versus diabetic rat $^{*}0.05 < P < 0.01$, $^{**}P < 0.001$; versus 2% taurine $^{\#}0.05 < P < 0.01$, $^{\#\#}P < 0.001$; versus $^{@}$D-200 IU + Se $0.05 < P < 0.01$; versus baseline $^{o}0.05 < P < 0.01$, $^{oo}0.01P < 0.001$, $^{ooo}P < 0.001$; versus the previous values $^{c}0.5 < P < 0.01$, $^{ccc}P < 0.001$.

mainly due to vitamin E. Although selenium effect on diabetic retinopathy has not been extensively studied, it reduces retinal lesion induced by a high sucrose diet.[42] Moreover, selenium has been found to be decreased in the blood cells but not in the plasma of diabetic patients.[43] In vitamin E supplemented rats, parameters of lipid peroxidation and pump activity are inversely correlated, suggesting that the mixture effects are mainly linked to its antioxidant property. The findings concerning vitamin E plasma levels in diabetes are contradictory.[13–17] Small and short interventional trials suggest that vitamin E supplementations may be useful in diabetes,[44–50] while an observational study shows that high intake of vitamin E is associated with increased severity of retinopathy.[51] However, experimentally, D-alpha-tocopherol ameliorates retinal blood flow and prevents pericyte loss.[52] In addition, vitamin E reduces diacylglycerol levels and protein kinase C-beta activation in diabetic rats.[53] Both vitamin E + selenium supplementations exert their effect without influencing hyperglycaemia.

This is an *in vivo* study; therefore the amino acid and vitamin supplementations could act indirectly preserving the intracellular redox state and enhancing the antioxidant activity of other different molecules.[36,53] Moreover, the antioxidants may have effects on different metabolic pathways, which in turn affect retinal biochemistry. Both

supplementations may reduce platelet function[7,8,47] and micro thrombi are important in the development of retinopathy.[55]

It seems important to underlie that taurine effect at least on CD is long lasting in comparison with vitamin E + selenium and that taurine supplementations preserve more the activity of sodium pump.

In conclusion, both supplementations reduce retinal alterations in the absence of any significant effect on glycemia. This last point is relevant because the control of hyperglycemia is the most important way to control diabetic retinoapthy.[1,2] Thus, both supplementations may potentially provide additional risk reduction for the development of retinal diabetic alterations in addition to that obtained through intensive antidiabetic therapy alone. Moreover, both supplementations are safe,[18,56] inexpensive and readily available compounds. However, taurine activity on CD and pump activity is more long-lasting in comparison with vitamin E + selenium. Thus, further studies with taurine are encouraged also in view of the fact that it is decreased in diabetes. Obviously, the effect of antioxidants supplementation in diabetes should be studied carefully with reference to its ability to prevent or delay the development of long-term complications.

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