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Tocotrienols–rich diet decreases advanced glycosylation end-products in non-diabetic rats and improves glycemic control in streptozotocin-induced diabetic rats

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Abstract

This study determined the effects of palm vitamin E (TRF) diet on the levels of blood glucose, glycated hemoglobin (gHb), serum advanced glycosylation end-products (AGE) and malondialdehyde (MDA) of diabetic Sprague-Dawley rats. The rats received either control (normal rat chow), TRF diet (normal chow fortified with TRF at 1g/kg) or Vitamin C diet (vitamin E-deficient but contained vitamin C at 45 g/kg). The animals were maintained on the respective diet for 4 weeks, made diabetic with streptozotocin (STZ), then followed-up for a further 8 weeks. At week-4, mean serum AGE levels of rats given TRF diet (0.7±0.3 units/ml) were significantly lower than those of control or Vitamin C diet rats (p£0.03). The levels increased after STZ and became comparable to the other groups. At week12, blood glucose (20.9±6.9mM) and gHb (10.0±1.6%) of rats on TRF diet remained significantly low compared to that of control or Vitamin C diet rats (p£0.03). MDA however, was not affected and remained comparable between groups throughout the study. This study showed that TRF may be a useful antioxidant; effectively prevented increase in AGE in normal rats, and caused decrease in blood glucose and gHb in diabetic rats. Further studies are needed to elucidate the mechanisms of action of TRF.

Key words: tocotrienols, streptozotocin, advaced glycosylation end-products, glycated hemoglobin.

INTRODUCTION

Oxidative stress has been implicated to be an important aetiological factor in the pathogenesis of diabetic complications. Hyperglycemia causes increased oxidative stress resulting in excessive production of free radicals.[1,2] Diabetes mellitus (DM) therefore, is associated with increased oxidative damage of various tissues and organs due to the accumulation of lipid peroxides and advanced glycosylation end-products (AGE).[3,4]

Antioxidants, such as vitamin C or E, are good scavengers of free radicals. Dietary supplementation of vitamin E, specifically, tocopherol, has been shown to be effective in reducing the levels of lipid peroxides or AGE in diabetic patients[5-8] and in streptozotocin (STZ)-induced diabetic rats.[1,9] Similar beneficial effects have also been reported for vitamin C; following supplementation, there was normalisation of vasodilatory response in patients with hypertension[10] and increased blood flow in type 1 and type 2 DM patients.[11,12]

The vitamin E extract of palm oil (TRF) is unique in that it consists of about 80% tocotrienols and 20% tocopherols.[13] In our earlier study, daily supplementation of 360 mg of palm vitamin E for 60 days was effective in reducing the serum malondialdehyde (MDA) levels of a group of poorly controlled Type 2 diabetes mellitus patients.[14] In view of this, we therefore undertook this study to further evaluate the effectiveness of TRF as an antioxidant. We subjected rats to a diet fortified with TRF and compared the serum levels of advanced glycosylation end-products (AGE), blood glucose, glycated hemoglobin (gHb) and MDA to that of rats fed with normal rat chow and vitamin-C rich diet, before and after STZ-induced diabetes.

MATERIALS AND METHODS

Male Sprague-Dawley rats of 2-3 months old and weighing between 250-300 g were used for the study. Three types of dietary preparations were used for the study; the control diet (normal rat chow), TRF diet (rat chow fortified with TRF at 1 g/kg weight) and Vit. C diet (special vitamin

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E free rat chow, but containing vitamin C at 45 g/kg weight). The latter was purchased from ICN Biomedicals, Inc., Costa Mesa, CA 92626. The control diet contained 0.022 and 0.039g/kg weight of a-tocopherol and vitamin C respectively.

The animals were divided into 3 main groups, each receiving the different diet. After 4 weeks, diabetes was induced with STZ, administered i.v. at a dose of 35 mg/kg body weight. Blood sugar was determined using a glucose reflectance meter (Glucometer 4, Bayer Diagnostics, Germany), at baseline and on the 3rd day after STZ administration to confirm diabetes. A blood sugar level of >9 mmol/L on at least 2 separate occasions was taken to be indicative of diabetes. The study was continued for another 8 weeks. Food and water were made available a d libiturn. The study protocol was approved by the Animal Care and Use Committee of the Institute.

The rats of all groups were sacrificed in batches at baseline and at week-4, -8 and -12 following initiation of the respective diet. Blood was collected in EDTA tubes and processed immediately. Plasma was aliquoted accordingly and stored frozen at -20°C until analysed. Glycated hemoglobin was determined using the IMx from Abbott Laboratories, USA, where interassay CV at 5.1, 10.0 and 16.7% were 4.1, 6.7 and 6.5% respectively. Serum MDA was determined by thiobarbituric acid method.[15] Intra-assay CV for MDA at 1.2 and 2.2 nmol/L were 6.8 and 9.7% respectively, and the corresponding inter-assay CV were 5.1 and 2.9% respectively. Serum AGE was measured by an in-house enzyme immunoassay developed in our laboratory.[16] We have also standardised the AGE unit for rat against normal rat serum using similar definition as proposed by Mitsuhashi *et. al.*[17] for AGE in human serum. The mean percent inhibition obtained for normal rat serum (n = 27) was 37 ± 5.5, and hence defined to be equivalent to one unit of AGE. The intra-assay CV at AGE levels of 1.0 and 1.45 units/ml were 5 and 6% respectively.

Serum tocotrienols and tocopherols were analysed by HPLC, using fluorescence detector (Model LC305, Bio-Rad Laboratories GmbH) at Ex 294 nm, Em 326 nm. The reversed phase column and the HPLC reagent kit for vitamin A/E analysis were also obtained from Bio-Rad Laboratories GmbH. The palm vitamin E and the standard used as reference were prepared and supplied by the Malaysian Palm Oil Board.

Statistical analysis

All results are presented as mean ± SD unless otherwise stated. Analysis of variance was used to detect differences among groups and Wilcoxon rank sum test was used to compare group means. A p value of less than 0.05 was considered to be significant.

RESULTS

The concentration of tocotrienols and tocopherols at baseline, week-4, -8 and -12 are shown in Table 1. Sera of rats fed with TRF diet contained both a-tocopherol and tocotrienols, but sera of rats on control diet contained only a-tocopherol. Total vitamin E levels of rats fed with TRF diet were significantly higher than that determined in rats given control diet ($p<0.0001$ at all points). There was no vitamin E isomers detected in samples collected from rats given Vit C diet.

The effect of the different diets on serum AGE levels is shown in Table 2. Significant decrease in AGE levels ($p<0.01$) were observed after 4 weeks of diet fortified with TRF. Mean serum AGE was significantly lower ($p = 0.03$) compared to the groups given control or Vit C diet. However, when the animals became diabetic, AGE increased significantly ($p<0.01$) and by week-12, the levels were comparable to the other groups.

The mean blood glucose and gHb levels measured at baseline, week-4, -8 and -12 following different dietary intakes are shown in Tables 3 and 4 respectively. There was no difference in the glucose and gHb levels between the 3 groups prior to diabetes induction. Although blood glucose became significantly elevated in all groups after STZ injection, the levels at week-8 and week-12 in rats fed with TRF-enriched diet remained significantly lower ($p£0.03$ and $p<0.01$ respectively). As expected, a similar trend was also observed for gHb. Increase in gHb was most significant in rats given the control or Vit C diet ($p=0.0001$ for both). On the other hand, increase in gHb in diabetic rats given TRF diet was less drastic; from 6.2% at week-4 to 8.8% at week-8 to 10% at week-12 ($p=0.03$). At week-12, the mean gHb level remained significantly lower than that of the control or Vit C group ($p = 0.04$). Neither diet nor hyperglycemia has any effect on the serum MDA levels, which remained within the range of 0.8 and 0.9 nmol/L throughout the study for all the three groups (Table 5).

TABLE 1 : Mean (BD) serum tocotrienols, a-tocopherol and total serum vitamin E levels in rats given control, TRF-enriched diet or vitamin-C rich diet at baseline, **week-**4, week-8 and week-12.

	n	Tocotrienols	a-Tocopherol	Total Vitamin E (ug/ml)
Control Diet				
Baseline	12	0	1.4 ± 0.3	1.4 ± 0.3
Week-4	12	0	1.7 ± 0.3	1.7 ± 0.3
Week-8	12	0	2.9 f 0.6	2.9 f 0.6
Week-12	12	0	2.1 f 0.5	2.1 f 0.5
TRF Diet				
Week-4	6	0.98 f0.3	4.3 f1.9	5.3 ± 0.7
Week-8	10	0.94 f0.3	4.6 ± 0.8	5.5 ± 0.4
Week-12	7	1.27 ± 0.6	5.8 ±1.8	7.1 ± 0.9

There was no detectable amount of vitamin E isomers present in rat sera given Vit. C diet.

TABLE 2: Mean (±SD) serum AGE levels in rats given control diet, TRF Diet or Vit. C Diet at week-4 prior to STZ and at week-8, week–12 after STZ.

	Serum AGE (units/ml)			
Type of diet	Week-0 (baseline)	Week-4	Week-8	Week-12
Control	1.0 ± 0.1 (n=14)	1.0 ± 0.3 (n=6)	1.1 ± 0.2 (n=12)$^{+}$	1.4 ± 0.5 (n=12)
TRF	1.0 ± 0.1 (n=14)	0.7 ± 0.3 (n=6)*$^{\#}$	1.4 ± 0.3 (n=10)$^{+}$	1.5 ± 0.4 (n=7)
Vit C	1.0 ± 0.1 (n=14)	1.0 ± 0.4 (n=13)	1.2 ± 0.3 (n=12)$^{+}$	1.5 ± 0.4 (n=12)

*p=0.01 versus baseline; $^{\#}$p=0.03 versus rats fed with control diet or Vit. C diet;
$^{+}$p<0.01 versus pre-diabetic group

TABLE 3: Mean (**±SD**) blood glucose levels in rats given control diet, TRF diet or Vit. C diet at week-4 prior to STZ and at week-8, week –12 after STZ.

	Blood glucose (mM)			
Type of diet	Week-0 (baseline)	Week-4	Week-8	Week-12
Control	7.2 ± 0.8 (n=9)	7.0 ± 0.7 (n=9)	27.0 ± 8.3 (n=6)	30.9 ± 4.8 (n=8)
TRF	7.1 ± 0.6 (n=15)	6.7 f 0.7 (n=15)	18.5 ± 6.4 (n=15)$^{\#+}$	20.9 ± 6.9 (n=15)*
Vit C	6.5 ± 1.0 (n=15)	7.0 ± 0.6 (n=15)	25.8 ± 8.1 (n=8)	22.2 f 9.4 (n=9)$^{=}$

*p<0.01, $^{\#}$p=0.02 versus respective control rats, $^{+}$p=0.03 versus rats given Vit. C diet;
$^{=}$p<0.05 versus control rats

TABLE 4: Mean (**±SD**) glycated hemoglobin in rats given control diet, TRF diet or Vit C diet at week-4 prior to STZ and at week-8, week–12 after STZ.

	Glycated hemoglobin (%)			
Type of diet	Week-0 (baseline)	Week-4	Week-8	Week-12
Control	4.8 ± 1.0 (n=12)	6.2 ± 2.3 (n=12)	10.6 f 2.4 (n=15)*	13.1 ± 3.4 (n=12)
TRF	4.8 f 1.0 (n=12)	6.2 ± 1.7 (n=6)	8.8 ± 2.4 (n=10)$^{+}$	10.0 ± 1.6 (n=7)$^{=}$
Vit C	4.8 ± 1.0 (n=12)	6.4 ± 2.4 (n=11)	10.7 ± 2.3 (n=12)*	13.0 ± 3.5 (n=12)

*p=0.0001, $^{+}$p=0.03 versus pre-diabetic group; $^{=}$p≤0.04 versus rats given control or Vit. C diet.

TABLE 5: Mean (BD) serum MDA in rats given control diet, TRF diet or Vit. C diet at week-4 prior to STZ and at week-8, week–12 after STZ.

	Serum MDA (nmol/L)			
Type of diet	Week-0 (baseline)	Week-4	Week-8	Week-12
Control	0.81 ± 0.1 (n=12)	0.85 ± 0.1 (n=11)	0.79 ± 0.2 (n=9)	0.89 ± 0.2 (n=9)
TRF	0.81 ± 0.1 (n=12)	0.84 ± 0.2 (n=6)	0.80 ± 0.2 (n=6)	0.86 ± 0.2 (n=5)
Vit C	0.81 ± 0.1 (n=12)	0.84 ± 0.3 (n=8)	0.78 ± 0.3 (n=10)	0.82 ± 0.1 (n=7)

DISCUSSION

The effectiveness of vitamin E supplementation in preventing or reducing diabetic complications has been demonstrated in several studies. D-a tocopherol was shown to act directly on the diacylglycerol-protein kinase C pathway, preventing glomerular dysfunctions[18] and normalizing the abnormal retinal blood flow[19] in diabetic rats. Vitamin E could also exert its protective effects indirectly; by improving insulin action,[20] and as antioxidant in many disease conditions associated with oxidative stress.[21-23] In this study, we showed that lower glucose and gHb levels were observed in diabetic rats fed with diet which have been fortified with higher concentrations of vitamin E, that is, 1 g of TRF for every kilogram of the normal rat chow. Our results imply that there exists a significant association between improved glycaemic control and the TRF diet. Although a more in-depth study is needed to elucidate the possible mechanisms involved, we postulated that TRF has effectively protected the p-cells from total damage by STZ and/or glucotoxicity. In the presence of residual functional islet cells, the blood glucose and gHb levels of rats fed with TRF diet were significantly lower than those on other diets. Our result is in agreement with an earlier study which showed that by pretreating rats with a-tocopherol before STZ administration, the severity of pancreatic damage could be significantly reduced.[24] Similar effects were also reported for soybean diet, postulated to be due to the presence of soybean trypsin inhibitor that promotes binding capacity of insulin receptor and arginine, a potent insulinotrophic agent.[25] This study also suggests that the amount of vitamin E present in the circulation or perhaps in the tissues during the chemical insult was an important factor. A mean serum a-tocopherol level of about 2 μg/ml seemed to be inadequate and improved glycaemic control was only observed when the circulating vitamin E levels were between 5–7 μg/ml, as in the TRF group. But whether our current observation is due to the synergistic effects of tocotrienols and a-tocopherol present in TRF needs further investigation.

Despite the fact that TRF diet caused lower blood glucose and gHb levels, the values measured at week-8 and week-12 were still within the diabetic range. As such, AGE levels of rats fed with TRF diet were comparable to that of the control and vitamin C groups. Interestingly however, we showed that 4 weeks of TRF diet could significantly reduce AGE levels in the non-diabetic rats; implying that the TRF was able to prevent the formation of AGE due to aging but not in the chronic hyperglycemic state.

In contrast to several other studies[9,14] that reported ascorbic acid as a more potent antioxidant than a-tocopherol or p-carotene, our results showed otherwise. There was no improvement in terms of AGE and glycaemic control, despite that the diet given to rats in the third group contained very high concentrations of vitamin C. We could not explain this present discrepancy but nevertheless agreed that future studies should include, amongst other things, measurement of vitamin C in the samples.

Abnormalities in lipid profile is one of the major contributing causes of vascular complications in diabetic patients.[2,26] Accumulation of oxidized and glycoxidized lipoproteins on vessel walls cause accelerated formation of atherosclerotic plaques.[27,28] Since these processes are associated with generation of free radicals, there have been a lot of interest and studies conducted on the use of antioxidants such as vitamin E to reduce the levels of lipid peroxides.[7,9,23] In this study however, neither TRF nor vitamin C has any effect on the MDA levels even after the rats became diabetic. This was rather unexpected as others have reported significant increase in MDA levels when rats became diabetic' or when diabetic rats were on vitamin E deficient diet.[9] One possibility is that the amount of vitamin E present in the control

diet was sufficient to suppress lipid peroxidation and fortifying the diet with TRF produced no additional effect. Similar reasoning may also be applicable to the group given the vitamin C-rich diet. The vitamin C probably was sufficient to inhibit further lipid peroxidation even after increased oxidative stress.

In conclusion, this study has demonstrated the potential use of palm oil vitamin E as an antioxidant. Further studies should be conducted to elucidate how TRF protects the pancreas. Dietary supplementation of this vitamin may be beneficial especially in high-risk individuals for TRF may provide protective effects on the pancreas and preserves the ability of the islet cells to secrete insulin.

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