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The effects of vitamin E deficiency on rat skin

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SUMMARY

The effect of vitamin E deficiency on the levels of lipid peroxide and solubility of collagen was investigated in Wistar rats that were fed a vitamin E deficient diet for 3 and 6 months. The lipid peroxide content in the skin and subcutaneous tissue was markedly increased as compared with that of control rats on a normal diet. The lipid peroxide content in the tissues of rats deficient in vitamin E for 3 months, was greater than that found in those on the diet for 6 months.

The effect of UV irradiation on the lipid peroxide content in the skin of control rats was not significant, but there was a marked difference in lipid peroxide content in those animals on a deficient diet. The amount of insoluble collagen in the skin of rats on a vitamin E deficient diet for 6 months was increased and by more than in those animals on the diet for 3 months. The amount of soluble collagen in the rats deficient in vitamin E for 6 months was markedly reduced when compared with those on the diet for 3 months. These results suggest that vitamin E deficiency promotes the peroxidation of lipids and accelerates the cross-linking of collagen in the skin.

Vitamin E was first recognized as affecting fertility in rats.[1] It is a strong antioxidant and prevents cell damage caused by lipid peroxidation, and as an antioxidant, it acts as a free radical scavenger[2] protecting cells from oxidative damage. Thus, vitamin E must be considered as an important lipid antioxidant and is possibly involved in the inhibition of lipid peroxidation in the ageing process.[3] Very little information has been available on the effect of vitamin E on the skin though it has been demonstrated in rat skin that vitamin E deficiency causes an increase in soluble collagen,[4] but Blackett *et al.* found that in mice, the addition of vitamin E to the diet had no effect on the collagen content of the skin.[5] We have carried out studies on the production of lipid peroxide and the effect on skin collagen in rats fed on a diet deficient in vitamin E and compared with animals on a normal diet.

METHODS

Animals and diets

Two groups of five male weanling Wistar rats were raised on diets shown in Table 1. Both diets were in the form of powder and the vitamin E sufficient diet contained 23 mg dl-α-tocopherol acetate per 100 g. The stripped corn oil was purchased from Eisai Co. Ltd, (Tokyo), and the animals were maintained on these diets for up to 6 months.

TABLE 1. Component of vitamin E deficient diet and vitamin sufficient diet

	Vitamin E deficient diet	Vitamin E sufficient diet (control diet)
Cornstarch	36%	36
Vitamin free casein	25	25
α-starch	10	10
Cellulose powder	8	8
Minerals	6	6
Vitamins	2	2
Granulated sugar	5	5
Stripped corn oil	8	8
dl-α-tocopheryl acetate	—	23 mg/100 g

Haemolysis test

The determination of haemolysis with dialuric acid was carried out as described by Ikeda and Sugiyama.[6] Dialuric acid was purchased from Tokyo Kasei Kogyo Co. Ltd, (Tokyo) and its solution was prepared immediately before use.

Determination of vitamin E content

The vitamin E (α-tocopherol) content in the tissues was determined by the modified method of Katsui as follows.[7] After 1 ml of serum was shaken with 1 ml distilled water, the solution was mixed with 2 ml ethanol and then 10 ml hexane was added. The mixture was vigorously shaken with 10 ml *n*-hexane. After centrifugation (1,000 ***g*** for 5 min), *n*-hexane extracted fraction (8 ml) was evaporated and then an accurate volume of methanol was added to the residue. The other tissues were homogenized with 6-fold distilled water and then methanol was added. The mixture was vigorously shaken with *n*-hexane. After centrifugation (2,000 ***g*** for 10 min), the *n*-hexane extracted fraction was evaporated and an accurate volume of methanol was added to the residue. The methanol solution was analyzed using high pressure liquid chromatography (HPLC). Vitamin E was detected with UV detector at 280 nm. The analysis was carried out by HPLC with 6000 pump and the U6K injector of Waters Assoc. (Tokyo). The column used was Radial-Pak A (silica ODC) of Japan Waters Limited.

Determination of lipid peroxide

The lipid peroxide content of skin and subcutaneous tissue was determined by the TBA method as described by Yagi *et al.*[8] Skin and subcutaneous tissue were washed after removal in ice cold buffer, then minced and homogenized in the cold with 0·15 M KCl with 10 mM Tris buffer. The homogenate was then centrifuged at 2,000 ***g*** for 10 min and to 2 ml of supernatant, 2 ml of 7% sodium dodecyl sulphate, 1 ml of 0·67% thiobarbituric acid, and 2 ml of 50% acetic acid were added. The mixture was heated at 95°C for 1 h, and the red chromophore, a reaction product of lipid peroxide with thiobarbituric acid, was extracted using a mixture of butanol and pyridine. Following centrifugation, the red chromophore in the butanol–pyridine fraction phase was then measured at 532·5 nm.

UV irradiation

Rats raised on a vitamin E deficient and on control diets for 3 months and 6 months were irradiated with a bank of six 40w Toshiba fluorescent lamps for 2·5 h. The irradiated backs of the rats were 30 cm from the light source. At this distance the irradiance, as measured by a UV radiometer (Eisai, Co Ltd.), was 0·578 mW/cm^2. After exposure, the rats in each group were killed and content of lipid peroxide in the skin and subcutaneous tissue determined.

Determination of collagen

After removal of the subcutaneous tissue, the skin was minced and homogenized at 4°C with 20 mM Tris-HCl and 0·45 M NaCl at pH 7·4. The soluble collagen was removed by incubating the homogenate for 24 h at 4°C and then it was centrifuged at 12,500 ***g*** at 4°C for 30 min. The insoluble collagen was isolated by incubating the precipitate for 30 min at 90°C with 5% trichroloacetic acid and extracted in the supernatant after centrifuging at 2,000 ***g*** for 15 min. The soluble and insoluble collagen extracted from the tissue were assayed for hydroxyproline[9,10] and which reflected the collagen content.

RESULTS

Haemolysis

Haemolysis started to increase within 5 days after starting on the vitamin E deficient diet and reached up to 80% haemolysis after 10 days and 100% haemolysis within a month. No haemolysis was observed in the control rats during this period. A small amount of vitamin E was still detectable in the serum of the animal even after a month on the vitamin E deficient diet.

Body weight

There were no significant difference in the growth of the animals in both groups.

Content of vitamin E

The vitamin E (α-tocopherol) contents in the skin and the other tissues are shown in Table 2. In the control group of rats, the vitamin E content ranged from 0·4 to 10 μg/g depending on the tissue. In the vitamin E deficient rats, a small amount of vitamin E was detectable in the serum and liver, but in the skin and subcutaneous tissue none was detected. In rats fed on the diet deficient in vitamin E for 6 months, no vitamin E was detected in their tissues.

TABLE 2. Content of vitamin E (α-tocoperol) in the various tissues

	3 month		6 month	
	Control	Vitamin E deficient	Control	Vitamin E deficient
Serum (μg/ml)	7·72 + 0·55	0·20 + 0·02	1·26 + 0·08	N.D.
Liver (μg/g)	10·68 + 2·07	0·22 + 0·01	2·49 + 1·14	N.D.
Skin (μg/g)	0·39 + 0·02	N.D.	0·55 + 0·04	N.D.
Subcutaneous tissue (μg/g)	0·98 + 0·05	N.D.	0·48 + 0·04	N.D.

The values are expressed as μg/g tissue; mean + SD of five rats determinations. N.D., not detected.

TABLE 3. Content of lipid peroxide in the skin and subcutaneous tissue

Tissue		Non UV-irradiated		UV-irradiated skin	
		3 month	6 month	3 month	6 month
Skin	Control	1·97 + 0·05	2·15 + 0·06	2·30 + 0·14	2·84 + 0·17
	Vitamin E deficient	3·10* + 0·05	3·19*† + 0·07	3·85 + 0·12	4·05 + 0·16
Subcutaneous tissue	Control	0·80 + 0·13	1·00 + 0·03	0·77 + 0·05	1·07 + 0·07
	Vitamin E deficient	1·68 + 0·21	2·25*† + 0·83	2·57* + 0·28	3·47*† + 0·17

The values are expressed as nmol/mg protein; mean + SD of five rats determinations.
* < 0·001 in comparison to each normal group.
† < 0·05 in comparison to 3 and 6 months with vitamin E deficient rats.

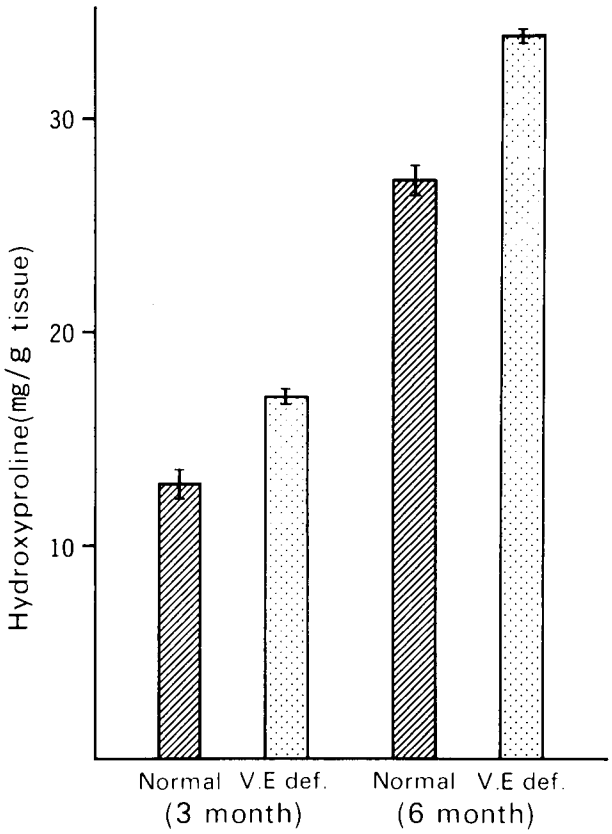

FIGURE 1. Content of insoluble collagen in the skin. ▨, rats fed with vitamin E sufficient diet; ▩, rats fed with vitamin E deficient diet.

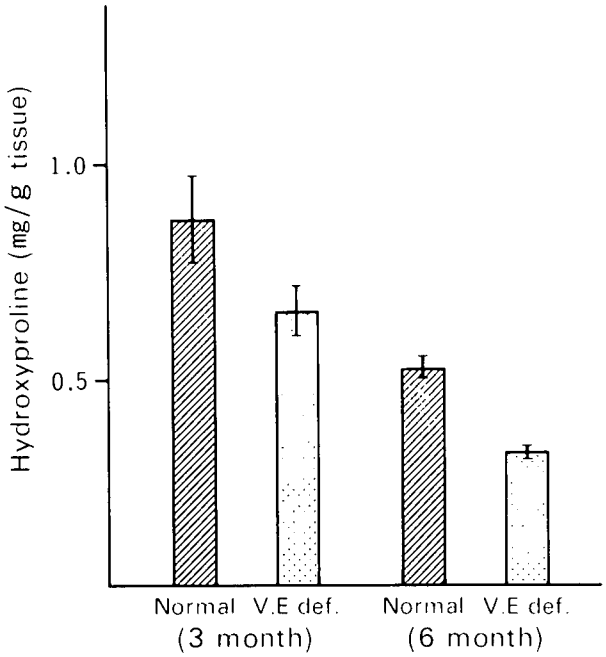

FIGURE 2. Content of soluble collagen in the skin. ▨, rats fed with vitamin E sufficient diet; ▩, rats fed with vitamin E deficient diet.

Lipid peroxide

The levels of lipid peroxide in the skin and subcutaneous tissue of vitamin E deficient rats are shown in Table 3. The vitamin E deficient rats showed high levels of lipid peroxide in both the skin and subcutaneous tissue when compared with that of controls. These differences were statistically significant ($P < 0{\cdot}001$). The difference in the lipid peroxide contents in the tissues between 3 and 6 months was greater ($P < 0{\cdot}05$) in the vitamin E deficient rats than the control animals.

The effect of UV irradiation was studied and in the controls there was no effect, but in vitamin E deficient rats the lipid peroxide contents of the skin after UV irradiation showed a significant increase. There was even more of an increase in these animals on vitamin E deficient diet for 6 months.

Collagen

Rats fed with the vitamin E deficient diet for 3 months showed a marked increase in the amount of insoluble collagen (Fig. 1). Those rats on a deficient diet for 6 months showed a greater increase in insoluble collagen as compared with the controls. Rats fed on a vitamin E deficient diet for 3 and 6 months showed a marked decrease in the amount of soluble collagen (Fig. 2).

DISCUSSION

Although a number of biochemical abnormalities were found in rats fed on a vitamin E deficient diet, no significant difference was found in the weight of the internal organs compared to controls. An increase in haemolysis was observed after only 10 days on a vitamin E deficient diet. This suggests that even a slight lack of vitamin E makes the erythrocyte membrane liable to

oxidative damage. A decrease in the vitamin E content of the skin and subcutaneous tissue occurred within 3 months and resulted in increased lipid peroxidation. This suggests its important role as an antioxidant and it is probably lost quicker in the skin than in the other tissues.

Currently there is much interest in the ageing of skin and the effect of UV light in this process. In vitamin E deficient rats exposed to UV light, there was a marked increase in the level of lipid peroxide in both the skin and subcutaneous tissue as compared with control rats similarly treated. Lipid peroxides are oxidation substances of unsaturated fatty acids[11] and are produced by a number of factors, particularly UV light. A synthetic antioxidant was found to improve vitamin E deficiency[12] and this suggests that the physiological role of vitamin E is closely related to its antioxidant activity.[13] The amount of lipid peroxides in the serum of patients with facial hypermelanosis,[14] and atopic dermatitis was reported to be higher than in healthy subjects and there may be some relationship between lipid peroxides and skin diseases. The application of synthetic lipid peroxides on guinea-pig skin produced histological changes with thickening and oedema of the epidermis.[15] Our data suggest that vitamin E inhibits the production of peroxides and protects the skin from damage. Rats raised on a vitamin E deficient diet were found to have a greater percentage of soluble collagen in their skin.[4] It was suggested that there was a defect in the formation of intermolecular and intramolecular cross-linkages in the collagen. However, our studies have shown that, in rats on a vitamin E deficient diet, the soluble collagen is markedly decreased when compared with control animals. The difference in these results may be due to a different extraction method of soluble collagen. It is generally observed that with age the amount of soluble collagen becomes markedly decreased and insoluble collagen increased.[16] We suggest that vitamin E in the skin reduces the increase in the amount of insoluble collagen by inhibiting the formation of peroxides. The changes in the skin produced by a lack of vitamin E in the rat resemble those seen in ageing, and vitamin E may have a part to play in preventing ageing of the skin.

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