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Alteration of brain and liver microsomal polyunsaturated fatty acids following dietary vitamin E deficiency

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The effects of dietary vitamin E deficiency on fatty acid composition of brain and liver microsomes were studied in rats fed a vitamin E-deficient diet for 9 weeks. In brain microsomes, vitamin E deficiency resulted in a significant decrease in palmitic acid and total saturated fatty acids. Cervonic acid was increased. In contrast, no marked changes were observed in the levels of (n-6) polyunsaturated fatty acid (PUFA). In liver microsomes, vitamin E deficiency resulted in significant alterations in fatty acid composition: higher amounts of stearic acid and total saturated fatty acids, lower amounts of mono-unsaturated fatty acids, linoleic and dihomo gamma linoleic acids. In contrast, arachidonic acid was not altered. The overall decrease in the amounts of (n-6) PUFA was compensated by an increase in the level of (n-3) PUFA. It is concluded that vitamin E may alter the enzymatic activities of chain elongation-desaturation and the relationship between vitamin E and PUFA in brain and liver microsomes.

Many studies have established that lipid peroxidation may be involved in a number of pathological processes induced by exposure to xenobiotic compounds, aging, ischemia and radiation [17, 26] and also during normal metabolic processes [16]. Brain membranes contain relatively large amounts of polyunsaturated fatty acids and thus are highly susceptible to peroxidative damage, leading to alterations in membrane function and enzyme activities [10]. All living cells exposed to free radicals are protected by antioxidant enzymes including superoxide dismutase, glutathione peroxidase and catalase and by scavengers of free radicals such as vitamin E, vitamin C and thiol-containing compounds. It is known that vitamin E is very important for the maintenance of normal neurological structure and function in humans [22, 25, 28] as well as in experimental animals [27]. In membranes, one of the principal biological defenses against lipid peroxidation is vitamin E. Its most important role is to protect polyunsaturated fatty acids (PUFA) against deleterious effects of free radicals. In addition, specific effects of α-tocopherol that do not involve its antioxidant properties and act upon the architecture of membranes by controlling their lipids profile have been suggested [5, 11, 13]. It has been reported by several investigators that vitamin E affects contradictorily the fatty acid profile of the rat tissues. In tocopherol-deficient rats, Berhnard et al. [1] reported an increased synthesis of hepatic arachidonate. In antioxidant-deficient rats, Bieri and Andrews [2] have shown a net decrease in docosapentaenoic acid and a slight increase in arachidonic acid in the testes. In addition, Lee and Barnes [20] and Farnsworth et al. [14] reported, respectively, during vitamin E deficiency, no significant changes of PUFA in brain and other tissues and variable changes in retinal tissues. Finally, Buttriss and Diplock [5] have shown a decrease in PUFA of liver microsomes and mitochondria from rats fed a vitamin E-deficient diet and an increase in PUFA of liver microsomes from rats fed a vitamin E- and selenium-deficient diet. However, in their study, only certain fatty acids were examined and brain membranes were omitted.

In general, vitamin E deficiency studies have been performed on weanling animals [8, 29, 30] In this work, vitamin E deficiency was started with pregnant female rats to obtain severe depletion especially for the brain [7]. The present experiments were designed to determine whether early dietary vitamin E deficiency affects the fatty acid content of brain and liver microsomes.

Female Sprague–Dawley rats were purchased from Iffa Credo (l'Arbresle France) and were fed a standard laboratory diet. From the 14th day of gestation, breeding female rats were divided into two groups. One was maintained on a synthetic diet containing 5% corn oil and 50 ppm of vitamin E, the other group was fed a diet containing 5% corn oil without vitamin E. Fatty acid analysis of

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the non-deficient and the deficient diets did not show any significant differences. Animals were maintained under standardized conditions of light (07.00–19.00 h), temperature 22°C ± 1, humidity (70%) and received water ad libitum. Rats were maintained on these diets during gestation and suckling and pups were fed the corresponding diets for 9 weeks. For microsome isolation, brain and liver were homogenized in 0.32 M sucrose (5 ml/g). The homogenate was spun down at 12,500 × *g* for 20 min to eliminate tissue debris, mitochondria, nuclei and other particles such as myelin and nerve endings for brain. The resulting supernatant was centrifuged at 100,000 × *g* for 1 h to sediment the microsomes. The pellet was collected and lyophilized. Vitamin E was determined by HPLC according to Katsui's method [19] with slight modifications [4, 9] on small portions of brain and liver set aside before preparation of microsomes.

Lipids were extracted from microsomes using chloroform/methanol 2:1. Heptadecanoic acid was added as an internal standard. The lipids were dissolved in 2 ml of methanol/cyclohexane 4:1 and transesterified according to the method of Lepage and Roy [21]. The methyl esters were analysed on a Delsi gas chromatograph equipped with a flame ionisation detector and a silica capillary column (length 30 m, internal diameter 0.32 mm, stationary phase Omegawax Supelco-France). Helium was used as the carrier gas. The oven, injector, and detector temperatures were maintained respectively at 200°, 230° and 250°C. Peaks were identidied by comparison with known standards. Areas were calculated with a Merck-Hitachi integrator and fatty acid concentrations were reported as percent of total microsome fatty acid concentrations. Statistical analysis was performed using Student's *t*-test.

In brain and liver, vitamin E concentrations were, respectively, 17.6 ± 0.1 and 30.9 ± 1.3 μg/g in control animals and 4.3 ± 0.5 and 0.8 ± 0.2 μg/g in deficient animals. This depletion was more severe than that reported previously [4] because in this study the vitamin E-deficient diet was started after 2 weeks of gestation.

Differences in fatty acid composition of brain and liver microsomes between control and vitamin E-deficient rats are presented in Table I. Cervonic acid (22:6n-3) increased in both brain and liver by 26% and 43%, respectively, whereas 22:5n-3 was not significantly altered, but its concentration was extremely low. These results were rather unexpected as we have previously found that there is no relationship between (n-3) polyunsaturated fatty acids and vitamin E content during development and aging, at least in the sciatic nerve [9]. Two diseases produced by vitamin E deficiency in laboratory animals, i.e. chicken encephalomalacia and muscular dystrophy, are less severe when the diet was supplemented with vitamin

TABLE I

ALTERATION OF BRAIN AND LIVER MICROSOMAL TOTAL FATTY ACIDS (MOL %) FOLLOWING DIETARY NUTRITIONAL VITAMIN E DEFICIENCY

Values are expressed as means ± S.D. of five animals. Values bearing letters a and b are respectively significantly different from control group at $P < 0.01$ and $P < 0.05$.

	Liver microsomes		Brain microsomes	
Fatty acids	Normal diet	Vit E −	Normal diet	Vit E −
14:0	0.16 ± 0.05	0.12 ± 0.02	0.13 + 0.01	0.11 ± 0.01
16:0	18.22 ± 0.54	18.43 ± 0.71	29.28 ± 0.41	26.38 ± 1.39[a]
18:0	23.07 ± 0.90	26.52 ± 1.18[a]	18.30 ± 0.45	18.47 ± 0.55
Σ Saturated	41.45 ± 0.73	45.07 ± 0.79[a]	47.71 ± 0.39	44.95 ± 0.91[a]
16:1(n-9)	0.07 ± 0.01	0.04 ± 0.01[a]	0.18 ± 0.01	0.15 ± 0.01[a]
16:1(n-7)	1.00 ± 0.05	0.78 ± 0.09[a]	0.41 ± 0.02	0.39 ± 0.06
18:1(n-9)	5.71 ± 0.13	4.98 ± 0.40[a]	13.95 ± 0.10	13.74 ± 0.36
18:1(n-7)	3.88 ± 0.34	1.80 ± 0.55[a]	4.00 ± 0.08	3.91 ± 0.02[b]
Σ Mufa	10.65 ± 0.47	7.61 ± 0.83[a]	18.54 ± 0.16	18.18 ± 0.34
18:2(n-6)	11.90 ± 1.18	9.28 ± 0.86[a]	0.49 ± 0.06	0.41 ± 0.03[b]
20:3(n-6)	0.78 ± 0.09	0.29 ± 0.09[a]	0.15 ± 0.01	0.13 ± 0.01
20:4(n-6)	30.29 ± 1.14	30.90 ± 0.37	15.30 ± 0.27	15.25 ± 0.52
22:4(n-6)	0.40 ± 0.04	0.46 ± 0.04	2.39 ± 0.16	2.71 ± 0.16[b]
22:5(n-6)	2.22 ± 0.50	3.15 ± 0.53[b]	3.83 ± 0.30	3.69 ± 0.15
Σ (n-6)	44.08 ± 0.10	45.58 ± 0.59[a]	22.16 ± 0.39	22.19 ± 0.74
22:5(n-3)	0.23 ± 0.03	0.21 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
22:6(n-3)	2.08 ± 0.17	2.98 ± 0.21[a]	11.53 ± 0.71	14.64 ± 0.22[a]
Σ (n-3)	2.31 ± 0.18	3.18 ± 0.21	11.59 ± 0.71	14.68 ± 0.22[a]
(n-6)/(n-3)	19.86 ± 1.53	13.88 ± 0.96[a]	1.92 ± 0.14	1.51 ± 0.06[a]
(n-6) + (n-3)	47.89 ± 0.72	47.27 ± 0.23	33.75 ± 0.40	36.87 ± 0.76[a]

E-deficient oils rich in α-linolenic or cervonic acid [6]. It is speculated that the cervonic acid increases in order to compensate the effects of the diet deficient in vitamin E and rich in (n-6) fatty acids. As suggested by Witting [31], during vitamin E deficiency the fatty acids are peroxidized but this process is largely compensated by a homeostatic mechanism that increases the turnover of PUFA. Indeed, in rats depleted in vitamin E and selenium, Buttriss and Diplock [5], have found a marked rise in (n-3) and (n-6) PUFA. Additionally, in ethanol intoxication peroxidative damage has been induced by free radicals and a simultaneous increase in PUFA observed in rat liver [24].

It has been suggested by Infante that vitamin E may control the desaturase [18]. We have recently shown [12] that high concentrations of vitamin E (up to 20 fold the control level) increases delta-6 desaturase activity in brain microsomes (2 fold) and decreases its activity in liver (1.2 fold). This results previously described are not

in agreement with the present data. This apparent discrepancy could be explained by the differences in experimental procedures. Firstly, the delta-6 desaturase activity was determined in vitro with very young rats (6 days old) because its activity is maximum at this age. Secondly, the vitamin E concentrations used in this experiment far exceed those found in the membranes. Nevertheless it could also be speculated that vitamin E alters the activity of delta-5 or delta-4 desaturase.

Table I also shows that in brain microsomes total saturated fatty acids were slightly decreased (6%) 16:0 being affected and 18:0 not changed. In contrast, in liver microsomes, total saturated fatty acids were slightly increased (8%) 16:0 being normal and 18:0 being slightly increased. Mono-unsaturated fatty acids were not affected in brain microsomes except 16:1 (n-9) which was slightly decreased. In contrast, mono-unsaturated fatty acids (both (n-9) and (n-7) series) were markedly altered in liver microsomes by the vitamin E deficiency. 18:1(n-9) was reduced by 13% and 18:1(n-7) by 64%. It has been shown that delta-9 desaturase activity is decreased both by vitamin E-deficient diet and by vitamin E-supplemented diet [23] explaining our results in the liver. Feeding rats a vitamin E-deficient diet for 9 weeks reduced brain and liver vitamin E content, respectively, by 76% and by 97.4%. It is speculated that the decrease in brain vitamin E concentation is not sufficient to affect delta-9 desaturase as occurs in the liver.

It is generally accepted that there is a close relationship between (n-6) fatty acids and vitamin E [9, 11]. However, in this study, vitamin E deficiency only slightly altered (n-6) fatty acids. In brain microsomes, 18:2 (n-6) were very slightly decreased and 22:4(n-6) was increased by 13%, other (n-6) fatty acids were not affected. In liver microsomes, 18:2(n-6) and 20:3(n-6) were decreased by, respectively, 22% and 63%. After dietary manipulation (α-linolenic acid deficiency or excess linoleic acid), a replacement of 22:6(n-3) by 22:5(n-6) has been reported [3, 15]. In this study, as vitamin E deficiency increased levels of 22:6(n-3) it would have been expected that 22:5(n-6) would decrease in the same proportion.

In conclusion, this study shows that early vitamin E deficiency for 9 weeks changes the concentration of some fatty acids of liver and brain microsomes. This suggests a metabolic role of vitamin E via many different mechanisms: protection against peroxidation, structural modifications, and direct or indirect control of fatty acid metabolism.

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