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Oxidative stress and antioxidant status in mouse kidney: Effects of dietary lipid and vitamin E plus iron

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The purpose of this study was to determine the effects of dietary fat, vitamin E, and iron on oxidative damage and antioxidant status in kidneys of mice. Sixty 1-month-old male Swiss-Webster mice were fed a basal vitamin E-deficient diet that contained either 8% fish oil + 2% corn oil or 10% lard with or without 1 g all-rac-α-*tocopherol acetate or 0.74 g ferric citrate per kilogram of diet for 4 weeks. Significantly (*$P < 0.05$*) higher levels of lipid peroxidation products, thiobarbituric acid reactants (TBAR), and conjugated dienes were found in the kidneys of mice fed with fish oil compared with mice fed lard irrespective of vitamin E status. Mice maintained on a vitamin E-deficient diet had significantly higher renal levels of TBAR, but not conjugated dienes, than the supplemented group. Fish oil fed mice receiving vitamin E supplementation had lower levels of α-tocopherol than did mice in the lard fed group. Significantly higher levels of ascorbic acid were also found in the kidneys of mice fed with fish oil than were found in mice fed lard. The levels of protein carbonyls and glutathione (GSH), and activities of catalase, superoxide dismutase, selenium (Se)-GSH peroxidase, and non-Se-GSH peroxidase were not significantly altered by dietary fat or vitamin E. Dietary iron had no significant effect on any of the oxidative stress and antioxidant indices measured. The results obtained provide experimental evidence for the pro-oxidant effect of high fish oil intake in mouse kidney and suggest that dietary lipids play a key role in determining cellular susceptibility to oxidative stress.* (J. Nutr. Biochem. 10:674–678, 1999)

Keywords: vitamin E; fish oil; lard; iron; kidney; oxidative stress; antioxidants

Introduction

Polyunsaturated fatty acids (PUFA) are susceptible to oxidation, and the resulting products are toxic to the cell.[1] Cold water fish is a rich source of long-chain (n-3) PUFA, docosahexaenoic acid (22:6 n-3), and eicosapentaenoic acid (20:5 n-3). Increased intake of fish oil is associated with increased susceptibility of membranes to oxidation and an increased requirement for antioxidants. Vitamin E is the major lipid-soluble free radical chain-breaking antioxidant found in plasma, red cells, and tissues.[2,3] An increased requirement of vitamin E is suggested to counteract the pro-oxidant effect of high PUFA consumption.[4,5]

Iron is essential for maintaining proper cell functions; it is normally tightly controlled by transport and storage proteins. Iron overload, however, may result in deleterious reactions such as degradation of proteins and nucleic acids and peroxidation of PUFA.[6–8] Although the mechanism by which iron is involved in initiating or promoting oxidative damage is not entirely clear, ferrous iron can catalyze the formation of highly reactive hydroxyl radicals from hydrogen peroxide[9] and the decomposition of lipid hydroperoxides to form alkoxyl, peroxyl, and other radicals.[10] Chronic iron overload has been shown to increase hepatic lipid peroxidation products in rats.[11,12]

We have been interested in the interacting effect of

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dietary lipid, vitamin E, and iron on oxidative stress and antioxidant status. Although increased oxidative stress has been associated with high intake of fish oil and iron, relatively little experimental evidence concerning the interaction among fish oil, vitamin E, and iron is available. We have provided direct evidence for the pro-oxidant effects of vitamin E deprivation and high intake of fish oil and iron in the liver of mice maintained on a vitamin E-deficient diet.[13] The data presented here is a part of the same study, in which we investigated the effects of dietary lipid, vitamin E, and iron on oxidative stress and antioxidant status in the kidney.

Materials and methods

Chemicals

Mono- and di-basic sodium phosphate, hydrogen peroxide, hydrochloric acid, potassium chloride, high performance liquid chromatography (HPLC) grade methanol, chloroform, and hexane were purchased from Fisher Scientific (Cincinnati, OH USA) and EM Science (Gibbstown, NJ USA). Ethanol 95% was obtained from Midwest Grain Products of Illinois (Pekin, IL USA). Isobutyl alcohol was obtained from Mallinckrodt Chemist (St. Louis, MO USA). Dithionitrobenzoic acid, 1,1,3,3- tetramethoxypropane, 2-thiobarbituric acid, butylated hydroxytoluene, reduced nicotinamide adenine dinucleotide phosphate (NADPH), nicotinamide adenine dinucleotide (NAD), reduced glutathione (GSH), glutathione reductase, ethylenediaminetetraacetic acid (EDTA), sodium azide, Folin's reagent, copper sulfate, and sodium carbonate were purchased from Sigma Chemical Company (St. Louis, MO USA).

Diets and feeding regimen

Diets were purchased from Dyets (Bethlehem, PA USA). The basal diet (AIN-76) consisted of 20% vitamin-free casein, 10% fat, 15% corn starch, 45% sucrose, 5% cellulose, 0.3% D-L methionine, 0.2% choline bitartrate, 3.5% salt mix, and 1.0% vitamin mix (without vitamin E).[14] The concentration of lipid, vitamin E, and iron in each of the six diets and the fatty acid composition of lipid sources have been reported.[13] Diet 1 was a vitamin E-deficient diet containing 8% MaxEpa oil and 2% stripped corn oil. Diet 4 was a vitamin E-deficient diet containing 10% stripped lard. Diets 2 and 5 were the same as diets 1 and 4, respectively, with the addition of 1 g *all rac*-α-tocopherol acetate/kg diet. Diets 3 and 6 were the same as diets 1 and 4, respectively, with the addition of 735 mg ferric citrate/kg diet. The basal diet contained 210 mg ferric citrate/kg diet. The level of 1,000 mg *all rac*-α-tocopherol acetate/kg diet used in this study is based on the significantly lower tocopherol concentrations found in the plasma, liver, and kidney of fish oil-fed mice than those fed corn oil or coconut oil, even when the diet was supplemented with 500 mg/kg.[15] Sixty 1-month-old Swiss-Webster mice (Harlan Sprague Dawley, Indianapolis, IN USA) were randomly assigned to each of the six diets and allowed free access to feed. The experimental protocol was reviewed and approved by the University of Kentucky Institutional Animal Care and Use Committee.

Sample preparation

At the end of the 4-week feeding period, mice were anesthetized with pentabarbitol (100 mg/kg body weight). After blood withdrawal via heart puncture, kidneys were removed, blotted, and weighed, and a 10% tissue homogenate was prepared in 1.15% KCl in 0.05 M phosphate buffer, pH 7.4, using a tissumizer (Tekmar, Cincinnati, OH USA). Portions of the homogenate were processed immediately after homogenization for measuring the levels of oxidation products, thiobarbituric acid reactants (TBAR), conjugated dienes, and protein carbonyls, and levels of small molecular weight antioxidants, GSH, ascorbic acid, and vitamin E. Another portion of the homogenate was centrifuged at 9,000 × g for 20 minutes, and the supernatant fraction was immediately stored at −80°C before analysis of activities GSH peroxidase, superoxide dismutase (SOD), and catalase.

Oxidation products

The levels of the lipid peroxidation product TBAR, mainly malondialdehyde, were determined according to the modified method of Li and Chow[16] spectroflourometrically at 515 nm excitation and 550 nm emission following isobutyl alcohol extraction. 1,1,3,3-Tetraethoxypropane was used as the standard. The levels of conjugated dienes, another indicator of lipid oxidation, were measured spectrophotometrically.[17] The content of protein-bound carbonyls, which is used to assess protein oxidation, was determined spectrophotometrically at 375 nm by the 2,4-dinitrophenylhydrazine method of Levine et al.[18]

Antioxidant status

Non-protein sulfhydryls, mainly GSH, were measured spectrophotometrically after reacting with dinitrobenzoic acid.[19] Ascorbic acid was measured following reaction with 2,4-dinitrophenylhydrazine.[20] Vitamin E (α-tocopherol) was measured by HPLC procedure using the modified procedure of Hatam and Kayden[21] with fluorescence detection using 100% methanol as the mobile phase. Activities of enzymes were measured in the 9,000 × g supernatent fractions. Catalase activity was determined following hydrogen peroxide reduction.[22] The activity of GSH peroxidase was assayed using both cumene hydroperoxide (total activity) and hydrogen peroxide (Se-dependent form) as substrates by measuring the rate of NADPH oxidation.[23] The activity of non-Se-dependent GSH peroxidase was obtained by calculation. The activity of SOD was determined based on its inhibitory action on the superoxide-dependent reduction of ferricytochrome C by xanthine-xanthine oxidase.[24] The Folin reagent was used to measure protein concentration.[25] Total lipids were determined spectrophotometrically following oxidation with potassium dicromate and concentrated sulfuric acid.[26]

Analysis of data

Data obtained were analyzed using two-way analysis of variance to determine the significance ($P < 0.05$) of the effects (lipid, vitamin E/iron) and their interaction. When the F-test was significant, it was followed by Tukey's multiple comparison test. The Windows version of SYSTAT 5 statistical software (SYSTAT, Evanston, IL USA) was employed.

Results

The effects of dietary fats, vitamin E, and iron on the kidney levels of oxidation products TBAR, conjugated dienes, and protein carbonyls are shown in *Figure 1*. The kidneys of animal groups receiving fish oil had significantly ($P < 0.05$) higher levels of TBAR (*Figure 1A*) and conjugated dienes (*Figure 1B*), but not protein carbonyls (*Figure 1C*), than those receiving lard. Vitamin E supplementation significantly decreased the renal levels of TBAR. However, the fish oil-fed group receiving vitamin E had significantly

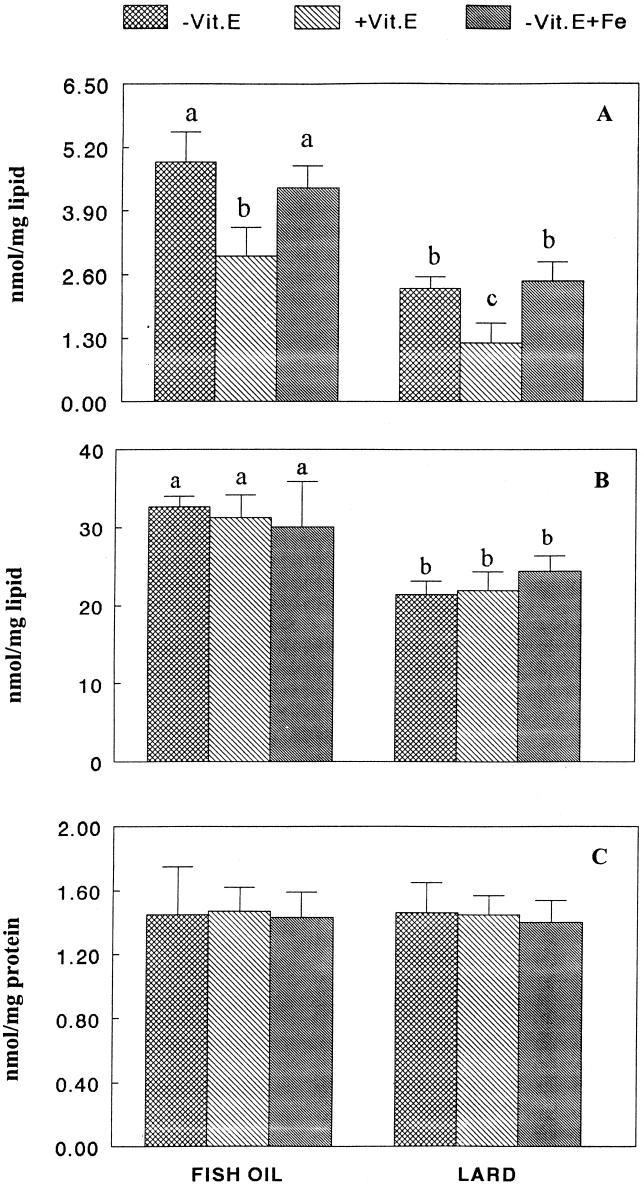

Figure 1 Effects of dietary lipids and vitamin E/iron on renal concentrations of *(A)* thiobarbituric acid reactants, *(B)* conjugated dienes, and *(C)* protein carbonyls in mice. Mice were fed the respective diets for 4 weeks. Bars represent means ± SD; $n = 10$. Those not sharing common letters are significantly different ($P < 0.05$). The basal vitamin E-deficient diet (-Vit.E) contained 0.21 g ferric citrate/kg diet; +Vit.E represents the basal diet supplemented with 1 g vitamin E acetate/kg diet; and -Vit.E+Fe represents the basal diet supplemented with 0.74 g ferric citrate/kg.

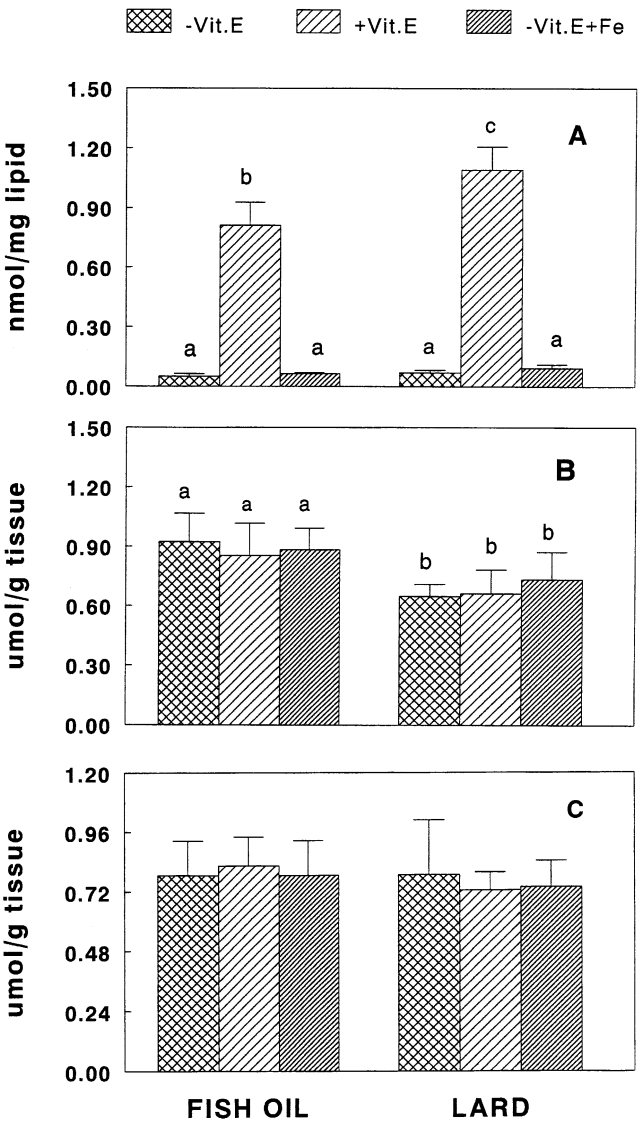

Figure 2 Effects of dietary lipids and vitamin E/iron on renal concentrations of *(A)* α-tocopherol, *(B)* ascorbic acid, and *(C)* glutathione. See *Figure 1* legend for more detail.

higher levels of TBAR than the lard-fed group supplemented with the vitamin. Dietary vitamin E had no significant effect on the levels of conjugated dienes (*Figure 1B*) or protein carbonyls (*Figure 1C*). Dietary iron had no significant effect on the renal levels of these oxidation products.

The effects of dietary fat, vitamin E, and iron on small molecular weight antioxidants are shown in *Figure 2*. As expected, vitamin E supplementation resulted in higher renal levels of α-tocopherol (*Figure 2A*). However, the levels of α-tocopherol were significantly lower in the kidneys of fish oil fed mice compared with mice fed lard. Dietary iron had no significant effect on the levels of α-tocopherol in animals receiving either fish oil or lard without vitamin E supplementation. The levels of ascorbic acid (*Figure 2B*) in the kidneys of fish oil-fed mice were significantly higher than those fed lard. Dietary vitamin E or iron had no significant effect on ascorbic acid levels in the two lipid groups. The levels of GSH (*Figure 2C*) were not affected by dietary fat, vitamin E, or iron. The effect of dietary lipid, vitamin E, and iron on the activities of important antioxidant enzymes is shown in *Table 1*. The renal activities of catalase, Se-GSH peroxidase, non-Se-GSH peroxidase, and SOD were not significantly affected by any of the treatments.

Table 1 Effect of dietary lipid, vitamin E, and iron on renal antioxidant enzyme activities

Enzyme	Fish oil			Lard		
	−Vit. E	+Vit. E	−Vit. E + iron	−Vit. E	+Vit. E	−Vit. E + iron
Superoxide dismutase (units/mg protein)	54.0 ± 2.5*	46.7 ± 2.1	48.7 ± 1.8	49.5 ± 1.8	57.4 ± 1.4	56.7 ± 2.1
Catalase (mmoles/min/mg protein)	0.65 ± 0.06	0.71 ± 0.07	0.72 ± 0.08	0.69 ± 0.10	0.70 ± 0.14	0.66 ± 0.03
Se-GSH peroxidase (μmoles/min/mg protein)	0.45 ± 0.03	0.59 ± 0.07	0.51 ± 0.03	0.49 ± 0.06	0.49 ± 0.03	0.48 ± 0.05
Non-Se-GSH peroxidase (μmoles/min/mg protein)	0.16 ± 0.01	0.13 ± 0.02	0.14 ± 0.04	0.14 ± 0.03	0.12 ± 0.04	0.13 ± 0.04

*Data are expressed as mean ± standard deviation (n = 10).
−Vit. E–vitamin E-deficient diet. +Vit. E–vitamin E-supplemented diet. GSH–reduced glutathione.

Discussion

It has been well-documented that susceptibility to lipid peroxidation is a function of fatty acid unsaturation and that increased intake of PUFA increases peroxidative tissue damage.[4,27–29] Iron overload has also been shown to increase the generation of lipid peroxidation products.[11,12,30] On the other hand, vitamin E supplementation has been shown to partially overcome increased susceptibility to peroxidative damage resulting from PUFA intake[15,29,31] or iron overload.[12,30,32] As we have previously reported, significantly higher levels of lipid peroxidation products, TBAR, and conjugated dienes, as well as protein oxidation product, protein carbonyls, were found in the livers of vitamin E-deficient mice fed with fish oil compared with the mice fed lard.[13]

In the current study, increased formation of lipid peroxidation products, TBAR, and conjugated dienes was found in the kidneys of fish oil-fed mice when compared with mice receiving lard, irrespective of the vitamin E status of the mice. In addition, the renal levels of α-tocopherol were significantly lower in vitamin E-supplemented mice receiving fish oil than those receiving lard. These changes in peroxidation products found in the kidney support the view that high intake of fish oil increases oxidative stress.[4,27–29]

Although dietary fat and vitamin E were found to alter the amounts of lipid peroxidation products generated in the kidney as in the liver, the magnitude of the differences observed was relatively smaller in the kidney than in the liver.[13] In addition, unlike the liver, dietary vitamin E and iron had no significant effect on the levels of conjugated dienes or protein carbonyls in the kidneys of fish oil-fed mice. These findings indicate that the liver was more profoundly affected than the kidney by dietary fat and vitamin E plus iron. The tissue-dependent difference in cellular sensitivity to dietary fat, vitamin E, and iron can be partly attributable to the fact that liver is the major organ for processing and storing nutrients and xenobiotics absorbed by the body. For example, hepatotoxicity is the most common finding in patients with iron overload because the liver is the major recipient of excess iron.[11]

Dietary iron has been shown to be similarly accumulated in liver and kidney of Fisher 344 rats fed ad libitum.[33] However, the increases of lipid peroxidation products were relatively higher in the liver than in the kidney. In addition, although iron-dextran injected rats had similar increases of iron in liver and kidney, significant declines in catalase and GSH peroxidase were found in the liver, but not in the kidney.[34] The findings suggest that factors such as vitamin E may be responsible for the differential effect between the liver and kidney on peroxidative damage observed. Compared with the liver,[13] the relatively smaller effect of dietary fish oil on oxidative products of kidney corroborated well with the relatively smaller differences in renal vitamin E levels observed. This finding is similar to the report of Meydani et al.,[15] which showed that hepatic vitamin E levels are more profoundly affected than those of the kidney by dietary vitamin E (30, 100, or 500 ppm) in 3- or 24-month-old C57BL/6Nia mice receiving coconut oil, corn oil, or fish oil for 6 weeks. In addition, subcutaneous vitamin E administration (20 mg/kg) has been shown to protect against high-dose (3,000−8,000 mg iron/kg diet), iron-induced hepatic damage and vitamin E depletion, as well as markedly reduce mortality rate, in mice without altering hepatic iron stores.[35] Thus, relatively smaller changes in vitamin E content by dietary lipids and vitamin E may play a role in the lesser effect on oxidative damage in the kidney compared with the liver.

Similar to liver and other organs, kidneys also possess a large number of antioxidant systems. Except for changes in ascorbic acid and vitamin E concentrations in the kidney, dietary lipids, vitamin E, and iron had no significant effect on the levels of GSH or activities of catalase, Se-GSH peroxidase, non-Se-GSH peroxidase, and SOD. Because the rodent species is capable of synthesizing ascorbic acid, higher levels of ascorbic acid in the kidneys of fish oil-fed mice may be due to an adaptive response to increased oxidative stress resulting from a higher intake of fish oil and iron.

The results obtained from this study demonstrate that formation of lipid peroxidation products was increased, whereas vitamin E content was decreased, in the kidneys of mice fed with fish oils compared with mice fed with lard, and that vitamin E supplementation reduced the amounts of TBAR generated. Unlike the liver, iron supplementation did not exhibit a pro-oxidant effect in the kidney. These data further support the view that dietary lipid plays a key role in determining cellular susceptibility to oxidative stress.

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