Alterations in lipid peroxides in rat liver by dietary n-3 fatty acids: modulation of antioxidant enzymes by curcumin, eugenol, and vitamin E

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Male Wistar rats fed a diet containing 10 wt% coconut oil, 10 wt% groundnut oil, or 10 wt% codliver oil for 10 weeks showed significant differences in lipid peroxides, mixed function oxidases, and antioxidant enzymes in liver homogenates. Rats fed codliver oil diets contained 136% and 80% higher levels of lipid peroxides, 38% and 28% higher activities of NADPH cytochrome C reductase, 42% and 30% higher levels of cytochrome P_{450}, and 41% and 30% higher levels of cytochrome b_5 compared with those observed in rats fed the other two diets. However, superoxide dismutase activity was reduced by 38%, catalase by 47%, glutathione peroxidase by 22%, and glutathione transferase activity by 32% in rats fed codliver oil as compared with those fed coconut oil diets. Vitamin E levels were lowered by 72% in animals fed codliver oil diets as compared with those on coconut oil diets. Ascorbic acid and glutathione levels were not changed by dietary lipids. The dietary antioxidants, curcumin (1 wt%), eugenol (0.17 wt%), or vitamin E (200 mg/kg diet) significantly enhanced ($P < 0.05$) the activities of antioxidant enzymes; viz, superoxide dismutase, catalase, glutathione peroxidase, and glutathione transferase and lowered lipid peroxides in liver of animals fed coconut oil, groundnut oil, or codliver oil. Dietary vitamin E (200 mg/kg diet) enhanced hepatic stores of vitamin E while curcumin and eugenol had no appreciable influence on hepatic vitamin E levels. These antioxidants did not influence the levels of ascorbic acid and glutathione in liver homogenates. These studies indicate that dietary lipids and antioxidants modulate lipid peroxidation in rat liver by influencing antioxidant defense systems. (J. Nutr. Biochem. 5:181–188, 1994.)

Keywords: lipid peroxides; mixed function oxidase; antioxidant enzymes; dietary lipids; curcumin; eugenol; vitamin E

Introduction

Lipid peroxidation is a natural phenomenon in biological systems that is required for many useful functions, such as prostaglandin synthesis.[1] However, uncontrolled production of lipid peroxides may lead to pathological conditions like inflammatory and cardiovascular diseases.[2,3] Therefore, it is essential to regulate the factors that are involved in the initiation, propogation, and termination of lipid peroxidation to control lipid peroxidation to desirable levels. It is well established that the nature of dietary lipids and the unsaturation of membrane phospholipids influences lipid peroxidation in biological systems.[4–6] However, additional factors may also participate in regulating the lipid peroxidation of membrane unsaturated fatty acids. These include free radical chain breaking antioxidants like vitamin E;[7] initiators of lipid peroxidation like the mixed function oxidase system;[8,9] and inherent antioxidant defense systems such as superoxide dismutase, catalase, glutathione peroxidase, ascorbic acid, selenium, and glutathione.[10–14] Under the conditions of stress, these various regulating factors have to function optimally to control the lipid peroxidation to desirable levels. One of these demanding factors could be nutritional stress. However, the role of dietary lipids and antioxidants on these various factors that regulate lipid peroxidation has not received adequate attention. We have recently observed that spice principles, curcumin (of turmeric) and eugenol (of cloves) could effectively control the extent of lipid peroxidation.[15] However, these spice principles did not affect fatty acid unsaturation and vitamin E levels in hepatic tissues (unpublished results). Therefore it was rea-

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soned that spice principles may affect lipid peroxidation by altering the activities of hepatic mixed function oxidases and antioxidant enzyme systems. To assess this hypothesis, the effect of different dietary lipids and antioxidant supplements, viz, curcumin, eugenol, and vitamin E on the activities of mixed function oxidase system and antioxidant status of rat liver was investigated.

Methods and materials

Refined coconut oil (CNO) and groundnut oil (GNO) was purchased locally. Refined codliver oil (CLO) was a gift from Dr. Baldur Hjaltason, LYSI, Reykjavik, Iceland. Thiobarbituric acid, vitamin E (α-tocopherol), xanthine oxidase, glutathione (reduced and oxidized), glutathione reductase, cumene hydroperoxide, hydrogenperoxide, Flavin Adenine Dinucleotide, 1-chloro 2,4-dinitrobenzene, sodium deoxycholate, Tris base, *o*-phthalaldehyde, dithiotreitol, and N-ethylmaleimide were obtained from Sigma Chemical Co., St. Louis, MO USA. Curcumin (>99% purity) was obtained from Flavours and Essence Ltd., Mysore, India. Eugenol (>99% purity) was obtained from Fluka Chemical Company, Buchs, Switzerland. NADPH, Cytochrome C, xanthine, EDTA, and sulphosalicylic acid were purchased from Sisco Research Laboratory, Bombay, India. Potassium iodide was purchased from Sd. Fine Chemicals, Bombay, India. Cadmium acetate was obtained from BDH, Bombay, India. High performance liquid chromatography (HPLC) grade methanol was obtained from Merck, Bombay, India. All the other chemicals used were of analytical grade. All the solvents were distilled prior to use.

Animals

Male Wistar rats weighing 70 to 75 g were fed the purified diets for 10 weeks. Animals had free access to water. The food intake and the growth of the animals were monitored at regular intervals.

Diets

The basal composition of the purified diets are 60% sucrose, 20% casein, 1% AIN76 vitamin mix, 3.5% AIN76 mineral mix, 5% fiber, 0.2% choline chloride, and 0.3% methionine.[16] These diets were supplemented with 10 wt% CNO, 10 wt% GNO, or 10 wt% CLO. In a parallel set of experiments, these diets were additionally supplemented with 1 wt% curcumin, 0.17 wt% eugenol, or 200 mg vitamin E/kg diet. These diets were stored in small containers flushed with nitrogen and kept at 4° C.[17] Fresh diets were provided to animals daily. The peroxide values of dietary lipids measured in terms of thiobarbituric acid reactive substances were 2.3, 2.7, and 24.4 nmoles/g oil for CNO, GNO, and CLO diets, respectively.[4] There were no significant changes in these values over 24 hr at room temperature while these diets were made available to animals. The fatty acid composition of dietary lipids is summarized in *Table 1*.

Liver homogenates and microsomes

The rats fasted overnight were sacrificed by cardiac puncture. The liver was perfused with saline and homogenized in 10 volumes of 150 mmol/L KCl. The liver microsomes were prepared as described by Lokesh et al.[4] The washed microsomes were suspended either in 150 mmol/L KCl or other appropriate buffers as indicated.

Lipid peroxides

The lipid peroxides in liver homogenates and microsomes was measured by iodometric method using cumene hydroperoxide as reference standard.[18] The molar extinction coefficient of cumene hydroperoxide used for quantitation is 1.73×10^4 M^{-1}.

Mixed function oxidases

NADPH cytochrome C reductase was measured by the reduction of cytochrome C as described by Pederson et al.[19] Cytochrome P_{450} and Cytochrome b_5 levels were quantitated in microsomes by the method of Omura and Sato.[20]

Antioxidant enzymes

Superoxide dismutase activity was measured by the inhibition of cytochrome C reduction mediated via superoxide anions generated by xanthine-xanthine oxidase and monitored at 550 nm.[21] One unit of superoxide dismutase was defined as the amount required to inhibit the reduction of cytochrome C by 50%. Catalase activity was assayed according to the method of Aebi[22] by following the

Table 1 Fatty acid composition of dietary lipids

Fatty acid	Coconut oil diet	Groundnut oil diet mole/100 mole	Codliver oil diet
12:0	50.3	—	—
14:0	27.0	1.2	11.5
16:0	10.8	17.0	22.8
16:1	—	1.1	15.2
18:0	0.9	4.0	5.0
18:1	8.5	39.9	14.7
18:2 n-6	2.5	34.5	1.9
20:0	—	1.3	1.8
20:4	—	1.0	1.5
20:5 n-3	—	—	14.6
22:6 n-3	—	—	11.0
Saturated fatty acid	89.0	23.5	41.1
Monounsaturated fatty acid	8.5	41.0	29.9
Polyunsaturated fatty acid	2.5	35.5	29.0
Unsaturation index*	13.6	114.0	149.0

*Unsaturation index: Sum of mole% × no. of double bonds of individual fatty acids.
Addition of curcumin, eugenol, or vitamin E did not alter the fatty acid composition of dietary lipids.

decomposition of hydrogen peroxide at 240 nm. Glutathione peroxidase activity was determined by NADPH oxidation in a coupled reaction system consisting of cumene hydroperoxide and oxidized glutathione.[23] Glutathione transferase activity was measured with 1-chloro 2,4-dinitrobenzene (CDNB) as the substrate. The enzyme activity is expressed as μmoles of CDNB-GSH conjugate formed per minute per mg of protein.[24]

Liver glutathione (GSH) was estimated by the method of Neuschwander-Tetri and Roll.[25] The glutathione was derivatized with *o*-phthalaldehyde (OPT) and the GSH-OPT complex was quantitated by reverse phase HPLC and measured at 340 nm.

Ascorbic acid was estimated by measuring the formation of 2,4-dinitrophenyl hydrazine derivative of dehydroascorbic acid as described by Roe and Khetler.[26] Vitamin E (α-tocopherol) was estimated by HPLC method as described by Rushing et al.[27]

Protein was estimated by the method of Lowry et al. using Bovine serum albumin as reference standard.[28]

Statistics

Statistical significance of mean differences were computed by the general linear model of Student's *t* test.[29] Correlation coefficients were calculated to determine the relationship between lipid peroxides and activities of protective enzymes against lipid peroxidation.

Results

There were no significant differences in the diet consumption (15 g/day/rat), body weight gains, and liver weights of rats on different dietary lipids and antioxidants *(Table 2)*.

Lipid peroxides

The rats fed a diet containing CLO showed 136% and 80% higher levels of lipid peroxides in liver homogenates compared with those fed diets containing coconut oil and groundnut oil, respectively *(Table 3)*. Similarly, the lipid peroxides in liver microsomes from rats fed CLO were higher by 93% and 62%, respectively, as compared with those observed in rats fed CNO and GNO diets *(Table 3)*.

The addition of curcumin, eugenol, or 200 mg of vitamin E in the diets significantly reduced the lipid peroxides in liver *(Table 3)*. Thus, dietary curcumin lowered lipid peroxides by 22%, 33%, and 47% in liver homogenates and by 29%, 24%, and 34% in microsomes from rats fed CNO, GNO, and CLO, respectively. Eugenol in the diets also reduced lipid peroxide levels by 20%, 35%, and 49% in liver homogenates and by 27%, 28%, and 32% in microsomes of rats fed CNO, GNO, and CLO, respectively. Two hundred mg of vitamin E in the diets similarly reduced lipid peroxides by 27%, 32%, and 53% in homogenates and by 43%, 31%, and 36% in liver microsomes of rats fed CNO, GNO, and CLO, respectively.

Effect on mixed function oxidase system

The effect of different dietary lipids and antioxidants on the mixed function oxidases, viz, NADPH cytochrome C reductase, cytochrome P_{450}, and cytochrome b_5 was investigated. The activity of NADPH cytochrome C reductase was enhanced by 38% and 28% in liver microsomes of rats fed CLO compared with those fed CNO and GNO, respectively *(Table 4)*. Cytochrome P_{450} contents were higher by 42% and 30% in rats fed CLO compared with those fed CNO and GNO diets, respectively. Cytochrome b_5 levels were also enhanced by 41% and 30% in CLO-fed animals as compared with those fed CNO and GNO, respectively *(Table 4)*. These data indicate that animals fed a diet enriched in (n-3) polyunsaturated fatty acids enhances the levels of mixed function oxidases. However, rats fed saturated or (n-6) polyunsaturated fatty acid-containing diets showed no significant differences in NADPH cytochrome C reductase activity and cytochrome P_{450} levels.

Effect on antioxidant enzymes

The antioxidant enzymes play a significant role in controlling lipid peroxidation.[30] The effect of dietary lipids and antioxidants on the activities of superoxide dismutase, catalase, glutathione peroxidase, and glutathione transferase was determined in liver homogenates. Superoxide dismutase activity was lowered by 12% and 38% in rats fed GNO and CLO, respectively, as compared with those fed CNO diets *(Table 5)*. Catalase activity was lowered by 11% and 47% in rats fed GNO and CLO, respectively, as compared with those on CNO diets. Similarly, the levels of glutathione peroxidase were reduced only in animals fed CLO diets to an extent of 22% compared with those fed CNO and GNO diets. Glutathione S transferase activity was, however, re-

Table 2 Influence of different types of dietary lipids and antioxidants on body weight gain in 10 weeks and liver weight in rats

Dietary group	Body weight gain (g)	Liver weight (g)
Coconut oil (CNO)	175 ± 3.67	9.93 ± 0.31
CNO + Curcumin	173 ± 1.63	9.16 ± 0.16
CNO + Eugenol	175 ± 4.08	9.41 ± 0.22
CNO + Vitamin E	181 ± 4.90	9.86 ± 0.40
Groundnut oil (GNO)	173 ± 2.50	8.63 ± 0.26
GNO + Curcumin	173 ± 1.75	8.91 ± 0.37
GNO + Eugenol	177 ± 2.20	9.01 ± 0.40
GNO + Vitamin E	170 ± 3.06	9.15 ± 0.34
Codliver oil (CLO)	179 ± 5.30	9.22 ± 0.70
CLO + Curcumin	172 ± 1.83	9.43 ± 0.43
CLO + Eugenol	174 ± 3.87	10.15 ± 0.28
CLO + Vitamin E	180 ± 4.90	9.92 ± 0.32

Results are mean ± SEM, n = 6 rats/group.

Table 3 Lipid peroxide levels in liver homogenate and microsomes of rats fed different dietary lipids and antioxidants

Addition to diets	Homogenates (nmoles/mg protein)			Microsomes (nmoles/mg protein)		
	CNO	GNO	CLO	CNO	GNO	CLO
Nil (control)	1.37[a] ± 0.03	1.80[b] ± 0.024	3.24[c] ± 0.085	10.50[d] ± 0.38	12.50[d] ± 0.143	20.30[e] ± 0.55
Curcumin	1.07* ± 0.03	1.21* ± 0.024	1.72* ± 0.036	7.45* ± 0.18	9.45* ± 0.098	13.44* ± 0.28
Eugenol	1.09* ± 0.028	1.17* ± 0.033	1.66* ± 0.036	7.65* ± 0.32	9.05* ± 0.081	13.70* ± 0.053
Vitamin E	1.00* ± 0.036	1.23* ± 0.020	1.53* ± 0.057	5.93* ± 0.29	8.62* ± 0.16	12.98* ± 0.098

Values are mean ± SEM, n = 6 rats/group.
[a,b,c,d,e]Means followed by different letters in the same row are significantly different from each other ($P < 0.05$).
*Significantly different from their respective control values ($P < 0.05$).
CNO, Coconut oil; GNO, Groundnut oil; CLO, Codliver oil.

Table 4 Influence of dietary lipids and antioxidants on liver microsomal mixed function oxidase system

Additional supplements to diets	NADPH cytochrome C reductase*			Cytochrome P_{450}**			Cytochrome b_5**		
	CNO	GNO	CLO	CNO	GNO	CLO	CNO	GNO	CLO
Nil (control)	56.0[a] ± 2.28	60.3[a] ± 1.9	77.4[b] ± 4.5	0.45[c] ± 0.03	0.49[c] ± 0.024	0.64[d] ± 0.03	0.460[e] ± 0.016	0.500[f] ± 0.024	0.650[g] ± 0.020
Curcumin	53.7 ± 2.9	60.7 ± 2.45	78.1 ± 2.7	0.53 ± 0.02	0.54 ± 0.024	0.60 ± 0.02	0.537† ± 0.016	0.568† ± 0.024	0.640 ± 0.010
Eugenol	53.4 ± 4.5	55.0 ± 2.95	75.0 ± 2.62	0.48 ± 0.04	0.50 ± 0.057	0.59 ± 0.024	0.527† ± 0.018	0.550† ± 0.016	0.650 ± 0.020
Vitamin E	55.3 ± 2.85	52.6 ± 2.4	74.7 ± 1.4	0.50 ± 0.028	0.48 ± 0.022	0.60 ± 0.03	0.460 ± 0.015	0.520† ± 0.020	0.610 ± 0.020

*units/min/mg protein; ** nmoles/mg protein.
Values are Mean ± SEM; n = 6 rats/group.
[a,b,c,d,e,f,g]Means with different superscript in the same row are statistically significant at $P < 0.05$.
†Statistically significant at $P < 0.05$ from the respective control value.
CNO, coconut oil; GNO, groundnut oil; CLO, codliver oil.

Table 5 Influence of dietary lipids and antioxidants on hepatic antioxidant enzymes in rat liver homogenates

Additional supplementation to diets	Superoxide dismutase (units/min/mg protein)			Catalase (μmoles H_2O_2 decomposed/min/mg protein)		
	CNO	GNO	CLO	CNO	GNO	CLO
Nil (control)	123[a] ± 2.8	108[b] ± 2.0	76[c] ± 4.0	227[d] ± 3.7	202[e] ± 6.5	106[f] ± 5.0
Curcumin	143* ± 5.1	121* ± 2.9	98* ± 5.8	249* ± 5.7	230* ± 9.4	125* ± 6.0
Eugenol	145* ± 13.	123* ± 6.8	107* ± 1.2	254* ± 10.1	226* ± 2.1	121* ± 3.6
Vitamin E	148* ± 4.4	127* ± 5.7	110* ± 5.9	253* ± 4.2	231* ± 7.8	122* ± 3.1

Values are mean ± SEM, n = 4 rats/group.
[a,b,c,d,e,f,g,h,i,j]Means with different superscript in the same row are statistically significant at $P<0.05$.
*Statistically significant from their respective control values $P < 0.05$.
CNO, coconut oil; GNO, groundnut oil; CLO, codliver oil.

Table 5 (Cont'd)

Additional supplementation to diets	Glutathione peroxidase (m units/min/mg protein)			Glutathione S transferase (units/min/mg protein)		
	CNO	GNO	CLO	CNO	GNO	CLO
Nil (control)	79[g] ± 2.6	78[g] ± 3.5	62[h] ± 4.2	53[i] ± 6.6	36[j] ± 1.7	37[j] ± 2.1
Curcumin	115* ± 8.0	88* ± 1.0	76* ± 3.5	70* ± 3.6	47* ± 1.4	53* ± 3.7
Eugenol	108* ± 5.8	88* ± 1.4	74* ± 2.7	70* ± 0.8	49* ± 2.5	49* ± 2.6
Vitamin E	95* ± 5.8	89* ± 2.6	91* ± 4.4	72* ± 4.0	47* ± 5.1	53* ± 3.2

duced in both GNO and CLO fed groups by 32% compared with those fed CNO *(Table 5)*.

However, rats fed a diet containing the antioxidants curcumin, eugenol, or vitamin E showed significantly elevated levels of antioxidant enzymes as compared with the non-supplemented groups *(Table 5)*. The correlation coefficients obtained from linear regression of liver lipid peroxides and different antioxidant enzyme activities are shown in *Table 6*. The results indicate that the lowering of lipid peroxides in rats fed curcumin, eugenol, and vitamin E is partially mediated by enhancing the activities of enzymes that scavenge oxygen free radicals and by enzymes capable of removing lipid peroxy radicals.

Effect on vitamin E, glutathione, and ascorbic acid

Small molecules like vitamin E, glutathione, and ascorbic acid are known to influence lipid peroxide levels.[30] The levels of these compounds were monitored in the livers of rats fed different dietary lipids supplemented with various antioxidants. Vitamin E levels were significantly lowered in rats fed polyunsaturated lipids *(Table 7)*. Thus the levels of vitamin E in liver homogenates of rats fed GNO and CLO were lower by 37% and 72%, respectively, as compared with those observed in rats fed CNO. It should be pointed out here that the rats on different dietary lipids received similar levels of vitamin E in their control diet. Addition of curcumin or eugenol to these diets had only marginal effects on elevating vitamin E levels in codliver oil-fed animals. However, 200 mg of dietary vitamin E enhanced hepatic vitamin E levels in rats fed CNO, GNO, and CLO by 8.9, 18.6, and 26.8 fold, respectively *(Table 7)*. These studies indicate that dietary vitamin E can significantly enhance hepatic vitamin E stores irrespective of the type of dietary lipids fed to the rats.

Dietary polyunsaturated lipids and antioxidants had no effect on glutathione and ascorbic acid levels in liver homogenates. However, ascorbic acid levels were marginally elevated by antioxidants only in animals fed CNO *(Table 7)*.

Discussion

Our findings indicate that dietary polyunsaturated fatty acids enhance lipid peroxides in rat liver. Even though CLO diets contained higher levels of thiobarbituric acid reactive substances (TBARS) as compared with CNO and GNO diets, it is unlikely that this contributes to the differences in tissue levels of lipid peroxides observed.[31,32] The endogenous levels of TBARS in CNO and GNO diets were comparable, but the lipid peroxide levels were always higher in animals fed GNO diets. A similar observation was made with animals fed coconut oil and sunflower oil.[4] Bergan and Draper failed to detect lipid peroxides in liver after feeding rats 2 mg of 1-^{14}C methyl linoleate hydroperoxide.[31] Hammer and Wills similarly noticed that addition of 2,6-di-t-butyl-4 methyl phenol reduces the peroxide levels in herring-oil diets, but this has no effect on tissue lipid peroxide levels.[5] Bunyan

Table 6 Correlation coefficients (R) between liver lipid peroxides and liver antioxidant enzyme activities in rats fed different types of dietary lipids and antioxidants

Dietary group	Superoxide dismutase	Catalase	Glutathione peroxidase	Glutathione S transferase
Coconut oil (CNO)	−0.800	−0.608	−0.430	−0.516
CNO + Curcumin	−0.578	0.620	−0.460	−0.368
CNO + Eugenol	−0.605	0.400	−0.100	−0.294
CNO + Vitamin E	−0.330	−0.270	−0.990	−0.430
Groundnut oil (GNO)	−0.532	−0.487	0.393	−0.288
GNO + Curcumin	−0.860	−0.872	−0.880	−0.980
GNO + Eugenol	−0.875	−0.234	−0.476	−0.144
GNO + Vitamin E	−0.580	−0.400	−0.928	−0.840
Codliver oil (CLO)	−0.132	−0.057	−0.790	0.412
CLO + Curcumin	−0.572	−0.980	−0.083	−0.658
CLO + Eugenol	−0.700	0.574	−0.700	−0.400
CLO + Vitamin E	−0.770	−0.606	−0.820	−0.574

Table 7 Influence of dietary lipids and antioxidants on vitamin E, glutathione and ascorbic acid levels in rat liver homogenates

Additional supplementation to diet	Vitamin E (nmoles/mg protein)			Glutathione (nmoles/mg protein)			Ascorbic acid (nmoles/mg protein)		
	CNO	GNO	CLO	CNO	GNO	CLO	CNO	GNO	CLO
Nil (control)	0.062^{a} ± 0.003	0.039^{b} ± 0.0002	0.011^{c} ± 0.0003	90.9^{d} ± 7.5	93.9^{d} ± 3.5	91.3^{d} ± 1.6	20^{e} ± 0.5	20^{e} ± 2	17^{e} ± 2
Curcumin	0.075 ± 0.005	0.039 ± 0.001	0.014 ± 0.001	94.8 ± 6.1	106.5* ± 3.2	98.3* ± 3.1	30* ± 3	22 ± 3	17 ± 1.5
Eugenol	0.080 ± 0.007	0.053 ± 0.001	0.016* ± 0.0002	97.6 ± 2.4	104.0* ± 1.2	99.3* ± 1.9	27* ± 3	22 ± 2	18 ± 1.8
Vitamin E	0.554* ± 0.02	0.723* ± 0.011	0.30* ± 0.015	103.4 ± 4.3	109.6* ± 4.3	114.2* ± 7.5	28* ± 4	20 ± 1.0	16 ± 1.0

Values are Mean ± SEM; n = 4 rats/group.
^{a,b,c,d,e}Means with different superscript in the same row are statistically significant at $P < 0.05$.
*Statistically significant from their respective controls ($P < 0.05$).
CNO, coconut oil diet; GNO, groundnut oil diet; CLO, codliver oil diet.

et al. demonstrated that feeding codliver oil or maize oil with peroxide values ranging from 3 to 330 u equivalents/g did not result in the accumulation of peroxides in rat tissues.[32] Therefore the small differences in the dietary lipid peroxides do not reflect on the tissue level lipid peroxides.

Mixed function oxidases, viz, NADPH cytochrome C reductase, cytochrome P_{450}, and cytochrome b_5 have been implicated to play an important role in lipid peroxidation.[33] Our study demonstrated that mixed function oxidases are significantly affected by dietary lipids. Higher levels were seen in animals fed codliver oil (rich in (n-3) fatty acids) as compared with those observed in rats fed coconut oil (poor in (n-3) fatty acids).

Saito et al.[34] indicated that polyunsaturated fatty acids, particularly 18:2(n-6) and 18:3(n-3) enhance the mixed function oxidase system. Our studies indicate that although CNO and CLO diets contain similar amounts of 18:2(n-6), the mixed function oxidase system was higher in rats fed the codliver oil diet. GNO contains 13.8-fold higher levels of 18:2(n-6) as compared with CNO. However, there were no significant differences in mixed function oxidases in animals fed CNO and GNO, indicating that 18:2(n-6) may not be responsible for enhancing the activities of mixed function oxidases. Because CLO contains high levels of (n-3) fatty acids, it is likely that this fatty acid may be responsible for the increased mixed function oxidases observed in rats fed CLO. This is in agreement with studies of Saito et al.,[35] who demonstrated that diets containing soybean oil or sardine oil enhance mixed function oxidases. Therefore it is the nature of the polyunsaturated fatty acids, but not the unsaturation index, that may influence mixed function oxidases. The differences in the dietary lipid peroxides may not affect the microsomal enzyme activities. Swanson et al. noticed that feeding a diet that differed in malodialdehyde contents by 5.5 to 6 fold did not affect NADPH cytochrome C reductase activity in mouse sarcoplasmic reticulum.[36] Glucose -6-phosphatase activity in rat liver microsomes was affected by different oxidized products of lipids only at concentrations greater than 0.3 mM, which is several orders of magnitude higher than the values normally found in microsomal preparations when these products are generated in situ by inducing lipid oxidation.[37,38] These studies indicate that small amounts of lipid peroxides present either in the diet or in the microsomal preparation are unlikely to affect the enzyme activities and mixed function oxidases associated with microsomes. The addition of curcumin, eugenol, or vitamin E to diets had no effect on mixed function oxidases, indicating that the nature of dietary lipids, but not the antioxidants, exhibit a major influence on mixed function oxidases.

In addition to mixed function oxidases, the levels of antioxidant enzymes also play a significant role in controlling lipid peroxidation.[39] The dietary polyunsaturated fatty acids influenced the activities of these enzymes. In general, polyunsaturated lipids lowered the activities of superoxide dismutase, catalase, glutathione peroxidase, and glutathione S transferase. The influence of (n-3) fatty acids (codliver oil) in lowering these enzyme systems was much greater than that observed with (n-6) fatty acids (groundnut oil). The lowering of these enzyme activities may reflect on higher lipid peroxide levels observed in liver. These results are in agreement with those reported recently by L'Abbe et al.[40] for antioxidant enzymes from heart, aorta, and liver of rats fed menhaden-oil diets. Contrary to these results, catalase activity in peroxisomes and in colon mucosa is enhanced by feeding fish oils to rats.[41,42] However, in agreement with our observation, superoxide dismutase and glutathione peroxidase activity in colon mucosa was reduced in menhaden oil fed rats.[42]

Codliver oil feeding also significantly lowered vitamin E incorporation into hepatic tissues. Rats fed groundnut oil also had lower levels of vitamin E in liver as compared with those fed CNO diets. This indicates that polyunsaturated fatty acid feeding increases the requirement for vitamin E.[43,44] It should be pointed out that rats from all three dietary groups received similar levels of vitamin E in their diets. Therefore, either the absorption of vitamin E is lowered[45] or its utilization is increased during the feeding of polyunsaturated fatty acid-containing diets. Meydani et al.[46] have made similar observations regarding the utilization of vitamin E in woman volunteers and in animals fed polyunsaturated fatty acid-containing diets.[44]

Taken together, these studies indicate that codliver oil enhances mixed function oxidases, lowers protective antioxidant enzymes, and vitamin E. In addition, fish oil also enhances the levels of highly unsaturated fatty acids such as eicosapentaenoic and docosahexaenoic acids, which are vulnerable to the oxidation.[6] These combined effects of fish oil on fatty acids and enzyme systems may contribute to the higher levels of lipid peroxidation observed in codliver oil-fed animals. It is surprising to note that instead of enhancing the inherent defense systems to prevent the peroxidation when highly unsaturated lipids were fed, the rats lowered their ability to protect the unsaturated lipids from oxidation. This underscores the need for enhancing the supply of exogenous antioxidants when highly unsaturated lipids are fed.

In addition to preventing the free radical-mediated lipid peroxidation, the possibility of antioxidants themselves influencing the factors that regulate lipid oxidation was evaluated. It has been demonstrated earlier that vitamin E deficiency decreases the activity of mixed function oxidases.[47,48] However, in our studies, when vitamin E levels were enhanced from 50 to 200 mg in the diets, no appreciable change was observed in the mixed function oxidase system. Therefore, deficiency rather than the excess of vitamin E in the diet may have a profound effect on mixed function oxidases. The data reported by Saito et al.[34] and current investigation supports this concept.

Dietary vitamin E and spice principle antioxidants, curcumin and eugenol, showed beneficial effects by enhancing the activities of antioxidant enzyme systems such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione transferase. The induction of glutathione S-transferase activity by curcumin and eugenol has been reported in mice and rats.[49,50] Therefore, the enhancement in the activities of various antioxidant enzymes by dietary vitamin E, curcumin, and eugenol may partly explain the lowering of lipid peroxides in rats fed these antioxidants.

The dietary lipids and antioxidants failed to alter the levels of glutathione and ascorbic acid. Curcumin and eugenol also did not significantly affect vitamin E levels in microsomes. These antioxidants also did not affect fatty acid compositions of liver microsomes influenced by dietary lip-

ids (data not shown). Therefore, it appears that the effect of dietary curcumin and eugenol in lowering lipid peroxide is mediated by enhancing the antioxidant enzyme activities. The daily consumption of curcumin and eugenol show wide variations.[51] Based on the intake from various dietary supplements, Sambaiah et al.[52] calculated that the average daily intake of turmeric in India is 4 g/adult/day. Turmeric contains 2 to 5% curcumin.[53] Based on this value, a normal adult in India consumes 80 to 200 mg of curcumin per day. The daily per capita consumption for eugenol is estimated to be around 0.6 mg.[51] Eugenol is generally recognized as safe (GRAS) by the U.S. Food and Drug Administration.[51,54] Curcumin and eugenol were found to be non-toxic even at very high concentrations. Curcumin at a 2 wt % level in the diet and eugenol at a 1.25% level has no adverse side efects on growth, hematological parameters, and enzyme activities.[52,55] Therefore, spice principles can be taken at considerably higher doses for their medicinal value than normally consumed in the diet. We are further studying the implications of these findings on free radical mediated pathological conditions.

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References

1 Lands, W.E.M. and Byrnes, M.J. (1981). The influence of ambient peroxides on the conversion of 5.8,11,14,17 eicosopentaenoic acid to prostaglandins. *Prog. Lipid Res.* **20,** 287–290

2 Freeman, B.A. and Crapo, J.D. (1982). Biology of disease. Free radicals and tissue injury. *Lab. Invest.* **17,** 112–126

3 Steinberg, D., Parthasarathy, S., Crew, T.E., Khoo, J.C., and Witztum, J.L. (1989). Beyond cholesterol: modification of low density lipoproteins that increases its atherogenicity. *N. Engl. J. Med.* **320,** 915–924

4 Lokesh, B.R., Mathur, S.N., and Spector, A.A. (1981). Effect of fatty acid saturation on NADPH dependent lipid peroxidation in rat liver microsomes. *J. Lipid Res.* **22,** 905–915

5 Hammer, C.T. and Wills, E.D. (1978). The role of lipid components of the diet in the regulation of the fatty acid composition of the rat liver endoplasmic reticulum and lipid peroxidation. *Biochem. J.* **174,** 585–593

6 Garrido, A., Garrido, K., Guerra, R., and Valenzuala, A. (1989). Ingestion of high doses of fish oil increases the susceptibility of cellular membranes to the induction of oxidative stress. *Lipids* **24,** 833–835

7 McCay, P.B. (1985). Vitamin E: Interactions with free radicals and ascorbate. *Ann. Rev. Nutr.* **5,** 323–340

8 Miller, O.N. (1976). Nutrition and drug metabolism introduction. *Fed. Proc.* **35,** 2459

9 Anderson, K.E., Conney, A.H., and Kappas, A. (1982). Nutritional influences on chemical biotransformations in humans. *Nutr. Rev.* **40,** 161–171

10 Wills, E.D. (1985). The role of dietary components in oxidative stress in tissues: In *Oxidative stress,* (H. Seis, Ed.), p. 197–218. Academic Press, New York, NY USA

11 Machlin, Z.J. and Bendich, A. (1987). Free radical tissue damage: Protective role of antioxidant nutrients. *FASEB J.* **1,** 441–445

12 Leibovitz, B., Hu, M.L., and Tappel, A.L. (1990). Dietary supplements of vitamin E, B-carotene, coenzyme Q_{10} and Selenium protect tissue against lipid peroxidation in rat tissue slices. *J. Nutr.* **120,** 97–104

13 Hu, M.L. and Tappel, A.L. (1992). Glutathione and antioxidants protect microsomes against lipid peroxidation and enzyme inactivation. *Lipids* **27,** 42–45

14 Frei, B., England, L., and Ames, B.N. (1989). Ascorbate is an outstanding antioxidant in human blood plasma. *Proc. Natl. Acad. Sci. U.S.A.* **86,** 6377–6381

15 Pulla-Reddy, A.Ch. and Lokesh, B.R. (1992). Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. *Mol. Cell. Biochem.* **111,** 117–124

16 Report of the American Institute of nutrition. Ad hoc (1977). Committee on standards for nutritional studies. *J. Nutr.* **107,** 1340–1348

17 Lokesh, B.R., Black, M.J., German, B.J., and Kinsella, J.E. (1988). Docosahexaenoic acid and other dietary polyunsaturated fatty acids suppress Leukotriene synthesis by mouse peritoneal macrophages. *Lipids* **23,** 968–972

18 Buege, J.A. and Aust, S.D. (1978). Microsomal lipid peroxidation. *Methods Enzymol.* **52,** 302–310

19 Pederson, T.C., Buege, J.A., and Aust, S.D. (1973). Microsomal electron transport. The role of reduced nicotinamide adenine dinucleotide phosphate cytochrome C reductase in liver microsomal lipid peroxidation. *J. Biol. Chem.* **248,** 7134–7141

20 Omura, T. and Sato, R. (1964). The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.* **239,** 2370–2378

21 Flohe, L. and Otting, F. (1984). Superoxide dismutase assays. *Methods Enzymol.* **105,** 93–104

22 Aebi, H. (1984). Catalase in vitro. *Methods Enzymol.* **105,** 121–125

23 Tappel, A.L. (1978). Glutathione peroxidase and hydroperoxides. *Methods Enzymol.* **52,** 506–513

24 Holig, W.H., Pabst, M.J., and Jakoly, W.B. (1974). Glutathione-S-transferase. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* **249,** 7130–7139

25 Neuschwander-Tetri, B.A. and Roll, J.F. (1989). Glutathione measurement by high performance liquid chromatography separation and fluorometric detection of the glutathione-orthophthalaldehyde adduct. *Anal. Biochem.* **170,** 236–241

26 Roe, J.H. and Khetler, C.A. (1943). The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenyl hydrazine derivative of dehydro-ascorbic acid. *J. Biol. Chem.* **147,** 399–407

27 Rushing, G.L., Cooper, W.M., and Thompson, H.C. Jr. (1991). Simultaneous analysis of vitamin A and E in Rodent feed by high pressure liquid chromatography. *J. Agric. Food Chem.* **39,** 296–299

28 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275

29 Dowdy, S. and Wearden, S. (1983). *Statistics for research,* John Wiley and Sons, New York, NY USA

30 Harris, E.D. (1992). Regulation of antioxidant enzymes. *J. Nutr.* **122,** 625–626

31 Bergan, J.G. and Draper, H.H. (1970). Absorption and metabolism of 1-^{14}C methyl linoleate hydroperoxide. *Lipids* **5,** 976–982

32 Bunyan, J., Green, J., Murrell, E.A., Diplock, A.T., and Cawthorne, M.A. (1968). On the postulated peroxidation of unaturated lipids in the tissues of vitamin E deficient rats. *Br. J. Nutr.* **22,** 97–110

33 Kanner, J., German, J.B., and Kinsella, J.E. (1987). Initiation of lipid peroxidation in biological systems. *CRC Crit. Rev. Food Sci. Nutr.* **25,** 317–364

34 Saito, M., Shirao, M.K., Kobataka, Y., and Yamaguchi, M. (1990). Influence of different types and levels of dietary lipids on liver microsomal mixed function oxidase system in rats. *Ann. Nutr. Metabolism* **34,** 288–296

35 Saito, M., Oh-Hoghi, A., Kubota, M., Nishide, E., and Yamoguchi, M. (1990). Mixed function oxidases in response to different types of dietary lipids in rats. *Br. J. Nutr.* **63,** 249–257

36 Swanson, J.E., Lokesh, B.R., and Kinsella, J.E. (1989). Ca^{2+}-Mg^{2+} AT Pase of mouse cardiac sarcoplasmic reticulum is affected by membrane n-6 and n-3 polyunsaturated fatty acid content. *J. Nutr.* **119,** 364–372

37 Agerbo, P., Jorgensen, B.M., Jensen, B., Borresen, T., and Holmer, G. (1992). Enzyme inhibition by secondary lipid autoxidation products from fish oil. *J. Nutr. Biochem.* **3,** 549–553

38 Koster, J.F., Slee, R.G., Montfort, A., Lang, J., and Esterbauer, H. (1986). Comparison of the inactivation of microsomal glucose-6-phosphatase by in situ lipid peroxidation derived 4-hydroxynonenal and exogenous 4-hydroxynonenal. *Free Rad. Res. Commun.* **1,** 273–287

39 Allen, R.G. and Venkataraj, V.S. (1992). Oxidants and antioxidants in development and differentiation. *J. Nutr.* **122,** 631–635

40 L'Abbe, M.R., Trick, K.D., and Beare-Rogers, J.L. (1991). Dietary (n-3) fatty acids affect rat heart, liver and aorta protective enzyme activities and lipid peroxidation. *J. Nutr.* **121,** 1331–1340

41 Yamazaki, R.K., Shen, T., and Schade, G.B. (1987). A diet rich in (n-3) fatty acids increase peroxisomal B-oxidation activity and lowers plasma triacylglycerols without inhibiting glutathione dependent detoxication activities in the rat liver. *Biochim. Biophys. Acta* **920,** 62–67

42 Kuratko, C. and Pence, B.A. (1991). Changes in colonic antioxidant status in rats during long term feeding of different high fat diets. *J. Nutr.* **121,** 1562–1569

43 Hu, M.L., Frankel, E.M., Leibovitz, B.E., and Tappel, A.L. (1989). Effect of dietary lipids and vitamin E on in vitro lipid peroxidation in rat liver of kidney homogenates. *J. Nutr.* **119,** 1574–1582

44 Meydani, S.N., Shapiro, A.C., Meydani, M., Macanley, J.B., and Blumberg, J.B. (1987). Effect of age and dietary fat (fish, corn and coconut oil) on tocopherol status of C57BL/6 Nia Mice. *Lipids* **22,** 345–350

45 Leka, L.S., Remaly, K.M., Bizinkanstar, P.A., and Meydani, M. (1989). Effects of fish oil on intestinal absorption of vitamin E in the rat. *FASEB J.* **3,** A951 (abst)

46 Meydani, M., Natiello, F., Goldin, B., Free, N., Woods, M., Schaefar, E., Blumberg, J.B., and Gorbach, S.L. (1991). Effect of long-term fish oil supplementation on vitamin E status and lipid peroxidation in women. *J. Nutr.* **121,** 484–491

47 Rowe, L. and Wills, E.D. (1976). The effect of dietary lipids and vitamin E on lipid peroxide formation, Cytochrome P_{450} and oxidative demethylation in the endoplasmic reticulum. *Biochem. Pharmacol.* **25,** 175–179

48 Zonnoni, V.G. and Sato, P.H. (1976). The effect of certain vitamin deficiencies on hepatic drug metabolism. *Fed. Proc.* **35,** 2464–2469

49 Susan, M. and Rao, M.N.A. (1992). Induction of Glutathione S-transferase activity by curcumin in mice. *Arzneimittel Forschung. Drug Res.* **42,** 962–963

50 Yokota, H., Hashimoto, H., Motoya, M., and Yuosa, A. (1988). Enhancement of UDP Glucoronyl transferase, UDP Glucose dehydrogenase and Glutathione S-transferase activity in rat liver by dietary administration of Eugenol. *Biochem. Pharmcol.* **37,** 799–802

51 (1985). Evaluation of the carcinogenic risk of chemicals to humans. IARC monographs **36,** 75–97

52 Sambaiah, K., Ratankumar, S., Kamanne, V.S., Satyanarayana, M.N., and Rao, M.V.L. (1982). Influence of turmeric and curcumin on growth, blood constituents and serum enzymes in rats. *J. Food Sci. Technol.* **19,** 187–190

53 Govindarajan, V.S. (1980). Turmeric-Chemistry, technology and quality. *CRC Rev. Food Sci. Nutr.* **12,** 199–301

54 Opdyke, D.J. (1975). Monographs on fragrance raw materials: eugenol. *Fd. Cosmet. Toxicol.* **13,** 545–547

55 National Toxicology Programme (1983). Carcinogenesis studies of eugenol in F344/N rats and B6C3F mice (feed studies) NTP/TR No. 223, NIH NO. 84-1779. U.S. Department of Health and Human Services, Washington, DC USA