Fish oil and vitamin E supplementation in oxidative stress at rest and after physical exercise

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Sen, Chandan K., Mustafa Atalay, Jyrki Ågren, David E. Laaksonen, Sashwati Roy, and Osmo Hänninen. Fish oil and vitamin E supplementation in oxidative stress at rest and after physical exercise. *J. Appl. Physiol.* 83(1): 189–195, 1997.—Fish oil supplementation and physical exercise may induce oxidative stress. We tested the effects of 8 wk of α-tocopherol (vitamin E) and fish oil (FO) supplementation on resting and exercise-induced oxidative stress. Rats (n = 80) were divided into groups supplemented with FO, FO and vitamin E (FOVE), soy oil (SO), and SO and vitamin E (SOVE), and for FOVE and SOVE they were divided into corresponding exercise groups (FOVE-Ex and SOVE-Ex). Lipid peroxidation [thiobarbituric acid-reacting substances (TBARS)] was 33% higher in FO compared with SO in the liver, but oxidative protein damage (carbonyl levels) remained similar in both liver and red gastrocnemius muscle (RG). Vitamin E supplementation, compared with FO and SO, markedly decreased liver and RG TBARS, but liver TBARS remained 32% higher in FOVE vs. SOVE. Vitamin E also markedly decreased liver and RG protein carbonyl levels, although levels in FOVE and SOVE were similar. Exercise increased liver and RG TBARS and RG protein carbonyl levels markedly, with similar levels in FOVE-Ex and SOVE-Ex. FO increased lipid peroxidation but not protein oxidation in a tissue-specific manner. Vitamin E markedly decreased lipid peroxidation and protein oxidation in both FOVE and SOVE, although liver lipid peroxidation remained higher in FOVE. Despite higher levels of hepatic lipid peroxidation at rest in FOVE compared with SOVE, liver appeared to be relatively less susceptible to exercise-induced oxidative stress in FOVE.

polyunsaturated fatty acids; β-oxidation; lipid peroxidation; protein oxidation; antioxidant

OXIDATIVE STRESS has been increasingly implicated in atherosclerosis and numerous other diseases and conditions, including cancer, aging, and physical exercise (18, 35, 36). α-Tocopherol (vitamin E) is a major lipid-phase, free radical chain-breaking antioxidant in the body (26). In detoxifying free radicals, vitamin E itself becomes a less reactive free radical. Vitamin E can then be regenerated by antioxidants such as vitamin C and glutathione (35). Epidemiological studies have suggested a protective role of vitamin E in reducing overall and cardiovascular mortality (32), and experimental studies have demonstrated its efficacy in protecting against oxidative stress (16, 26). However, the role of vitamin E supplementation in slowing progression of atherosclerosis remains controversial (20, 39).

Fish oils (FO) also purportedly have a beneficial effect on cardiovascular mortality as shown by several epidemiological studies (21), presumably via hypotriglyceridemic effects, increased membrane fluidity, decreased platelet thromboxane production, and altered leukocyte function (34). Not all studies show beneficial effects, however (1). FO induce peroxisomal β-oxidation, in which fatty-acyl oxidation yields hydrogen peroxide (H_2O_2) as a normal by-product. Under normal conditions, up to 20% of cellular O_2 consumption has, in fact, been estimated to occur in the peroxisome (7). Furthermore, (n-3) fatty acids making up FO are highly polyunsaturated. Thus concerns have been raised regarding increased oxidative stress from FO intake (11, 24, 28).

Manyfold increases in red cell membrane (3) and heart tissue (8) vitamin E levels have been found with FO and vitamin E cosupplementation compared with supplementation with vitamin E and placebo oil. Such findings are in contrast to the observations in the liver, kidney, and plasma (8, 24). Although most studies examining the effect of FO and vitamin E cosupplementation have shown protection against oxidative stress induced by FO, oxidative stress has remained increased in vitamin E supplementation with FO relative to vitamin E supplementation with placebo oil (24).

Increased free radical production during even moderate exercise can overwhelm antioxidant defenses, resulting in oxidative tissue damage (24, 35). The beneficial effects of regular exercise on cardiovascular and overall mortality (30) may be decreased by exercise-induced oxidative stress. This may be of particular concern in groups predisposed to oxidative stress. Such groups include patients with diabetes and coronary artery disease and may also include individuals who consume large amounts of FO (24, 28, 33). Peroxisomal β-oxidation may be a primary cause of FO-induced oxidative stress at rest. Therefore, favorable effects of FO on leukocyte function and vitamin E bioavailability could potentially decrease susceptibility to exercise-induced oxidative stress, because the relative role of peroxisomal β-oxidation may decrease during exercise.

The effect of FO and vitamin E supplementation on exercise-induced oxidative stress has not been previously reported. The aims of this study were to assess the effect of FO and vitamin E supplementation compared with soy oil (SO; placebo) and vitamin E supplementation on physiological antioxidant defenses and resting and exercise-induced oxidative stress in rat liver, heart, and skeletal muscle. A primary aim was to assess whether FO cosupplementation with vitamin E could actually decrease exercise-induced oxidative stress compared with vitamin E supplementation with SO.

MATERIALS AND METHODS

Animals. Male outbred Wistar rats (National Laboratory Animal Center, Kuopio, Finland) 8 wk of age (n = 80) were divided into six groups: FO, FO and vitamin E (FOVE), SO, SO and vitamin E (SOVE), and the vitamin E-supplemented

rats were divided into exercise groups (FOVE-Ex and SOVE-Ex). Each group consisted of 12–14 rats. Animals had free access to standard rat chow (Finnewos Aqua OY, Turku, Finland) containing 5% fat and 63 mg/kg all-rac-α-tocopherol acetate (Table 1; information provided by the manufacturer). All rats were housed, four animals to a cage, at 22 ± 2°C room temperature with 10:14-h dark-light cycles. The study was approved by the University of Kuopio Animal Research Ethics Committee.

Supplementation. FO (Bio-Marin, Pharma Nord, Vojens, Denmark) was administered intragastrically 1 g/kg body wt per day to keep FO intake constant among the supplemented animals (see Table 2 for fatty acid composition of the oil supplements). The FO contained <10 meq/g peroxides, according to manufacturer quality control, with 6.0 mg/g free RRR-α-tocopherol added to protect against oxidation. SO (Bio-Marin placebo, Pharma Nord) with similar vitamin E content served as a control for FO supplementation. SO contains predominantly linoleic acid [18:2(n-6)] and oleic acid to a lesser degree. RRR-α-tocopherol (Bio-E-Vitamin, Pharma Nord) was given intragastrically at a daily dose of 500 mg (750 IU)/kg body wt. All supplementation was done 5 days/wk over an 8-wk period. At the end of the supplementation period, the rats weighed on average 410.7 ± 38.8 g, with no significant difference between groups. Rats consumed on average 26 g/day rat chow; average fat in the diet was ~6.1%, of which the oil supplements were ~23%. Therefore, free RRR-α-tocopherol administered with the FO and SO in the FO- and SO-only groups was 6.0 mg/kg body wt. In addition, all rats consumed, on average, 1.6 mg/day all-rac-α-tocopherol acetate present in the diet. Thus baseline vitamin E consumption in all rats was high. We chose to use high α-tocopherol in the basal diet because of the higher vitamin E requirements with diets high in polyunsaturated fatty acids (12).

Exercise. Rats in the exercise groups were acquainted to treadmill running during the eighth week. Food and supplementation were withheld 24 h before rats were killed. On the day of killing, the rats ran at 1.08 km/h at a 10° uphill grade for 10 min and then at 1.44 km/h at the same grade until exhaustion. Exhaustion was defined as loss of the righting reflex when the rats were placed in a supine position. After running to exhaustion, rats were immediately killed by decapitation and then exsanguinated.

Sample collection. Rats were matched between groups for day of killing. After animals were killed, the heart, liver, superficial vastus lateralis muscle (VL), and red gastrocnemius muscle (RG) were quickly dissected out, freed from adipose and connective tissues, frozen in liquid nitrogen, and stored at −70°C.

Fatty acid determinations. Tissue lipids were extracted by the method of Folch et al. (14) and methylated with 14% BF3 in methanol. The respective fatty acid methyl esters were analyzed by using gas chromatography (HP 5890 series II; Hewlett-Packard) equipped with a Hewlett-Packard FFAP capillary column.

Table 1. *Composition of basal diet rat chow*

Ingredient	%Total
Wheat	43.0
Barley	34.0
Wheat germ	5.0
Soy	5.0
Vitamins and minerals	4.5
Calcium powder	2.0
Monocalcium phosphate	1.0
Animal fat	0.9
Sodium chloride	0.5
Lysine	0.1
Other	4.5

Table 2. *Fatty acid composition (mol% concentration) of soy oil, fish oil, and supplements*

Soy Oil		Fish Oil	
Fatty acid composition	%	Fatty acid composition	%
$C_{14:0}$ myristic	0.1	$C_{14:0}$ myristic	0.4
$C_{16:0}$ palmitic	10.2	$C_{16:0}$ palmitic	1.4
$C_{16:1}$ palmitoleic	0.1	$C_{16:1}$ palmitoleic	2.8
		$C_{16:2}$ hexadecadienoic	0.3
$C_{17:0}$ margaric	0.1	$C_{17:0}$ heptadecanoic	0.4
		$C_{17:1}$ heptadecenoic	0.7
		$C_{17:2}$ heptadecadienoic	0.1
$C_{18:0}$ stearic	3.7	$C_{18:0}$ stearic	0.3
$C_{18:1}$ oleic	22.1	$C_{18:1}$ oleic	5.8
$C_{18:2}$ linoleic	53.6	$C_{18:2}$ linoleic	1.1
$C_{18:3}$ linolenic	8.9	$C_{18:3}$ linolenic	0.9
		$C_{18:4}$ octadecatetraenoic	5.4
		18-Isomers other	0.6
$C_{20:0}$ arachidic	0.3		
$C_{20:1}$ gadoleic	0.3	$C_{20:1}$ gadoleic	1.9
		$C_{20:3}$ icosatrienoic	1.8
		$C_{20:4}$ arachidonic	1.6
		$C_{20:5}$ eicosapentaenoic	34.9
$C_{22:0}$ behenic	0.3	$C_{22:0}$ behenic	0.7
		$C_{22:1}$ cetolecic	2.3
		$C_{22:4}$ docosatetraenoic	0.5
		$C_{22:5}$ docosapentaenoic	4.1
		$C_{22:6}$ docosahexanenoic	26.4
$C_{24:0}$ lignoceric	0.1		
$C_{24:1}$ tetracosenoic	0.1		
Other	0.1	Other	5.6

Vitamin E determinations. Tissue vitamin E levels were determined by high-performance liquid chromatography, where a series quaternary pump (HP 1050, Hewlett-Packard) was coupled to an ultraviolet detector (Hewlett-Packard). Samples extracted in hexane and dried under nitrogen gas were separated by using a LiChrosorb (Si60, 5 μM, Hibar, Merck, Germany) column and hexane:*t*-butyl methyl ether (95:5 vol/vol) as the mobile phase at a 1.5 ml/min flow rate (22). The vitamin E peak was detected at 292 nm.

Thiobarbituric acid-reacting substances (TBARS) determinations. Tissue homogenization for TBARS was carried out as described before (36). After homogenization, the samples were were reacted with thiobarbituric acid and assayed spectrophotometrically at 532 nm (36).

Protein carbonyl determinations. Tissue homogenizations and protein carbonyl determinations were carried out as described by Reznick and Packer (31), with modifications reported by Yan et al. (40). Tissue protein was extracted in a protease-inhibitor (0.5 μg/ml leupeptin, 0.7 μg/ml antipain, 0.5 μg/ml aprotinin, 40 μg/ml phenylmethylsulfonylfluoride, 1 mM EDTA)-treated 0.1 M phosphate buffer, pH 7.4. DNA was removed from samples with 1% streptomycin treatment. The sample was then treated with 1 mM 2,4-dinitrophenylhydrazine. The protein was washed in ethyl acetate-ethanol (1:1 vol/vol) and dissolved in 6 M guanidine hydrochloride, pH 2.3. Tissue protein carbonyl content was quantitated by scanning the samples from 320 to 410 nm in a spectrophotometer. The peak absorbance was used to calculate protein carbonyl content (extinction coefficient 22,000 $l \cdot mol^{-1} \cdot cm^{-1}$).

Using methods somewhat similar to this, Cao and Cutler (6) reported difficulties in reliable determination of protein carbonyl content in crude tissue extracts. After slightly

different treatment with 1% streptomycin and washing in ethyl acetate-ethanol, rat liver protein carbonyl concentration had a coefficient of variation of 150–200%. In the present study, the relatively low SE, large decrease of tissue protein carbonyl content in response to vitamin E supplementation, and large increase of skeletal muscle carbonyls in response to exercise suggest that tissue protein carbonyl measurements in this study were reliable.

Statistical analyses. Results are presented as means ± SE. Eicosapentaenoic acid and vitamin E data for all tissues, heart (n-3) and (n-6) fatty acid results, and liver protein carbonyl data were log transformed for statistical analyses. The effect of FO supplementation and vitamin E supplementation in the resting groups, and the effect of FO supplementation and exercise in the exercise groups and their corresponding resting groups, was assessed using two-way analysis of variance (ANOVA). Interaction of vitamin E and FO refers to a combined effect of FO and vitamin E different from the effects of vitamin E and FO separately. Student's unpaired *t*-test with Bonferroni's correction was used to further analyze differences between group pairs (i.e., FO-supplemented groups, vitamin E-supplemented groups, and exercise groups). Statistical significance was defined as $P < 0.05$.

RESULTS

Effect of FO supplementation and vitamin E supplementation with FO on tissue fatty acid composition. In all tissues, FO supplementation markedly increased 20- and 22-carbon (n-3) fatty acids, eicosapentaenoic acid in particular (four- to eightfold in all tissues measured, $P < 0.001$; summarized in Fig. 1). FO markedly increased the fatty acid unsaturation index (i.e., sum of the percent composition of individual fatty acids × the respective number of double bonds) in

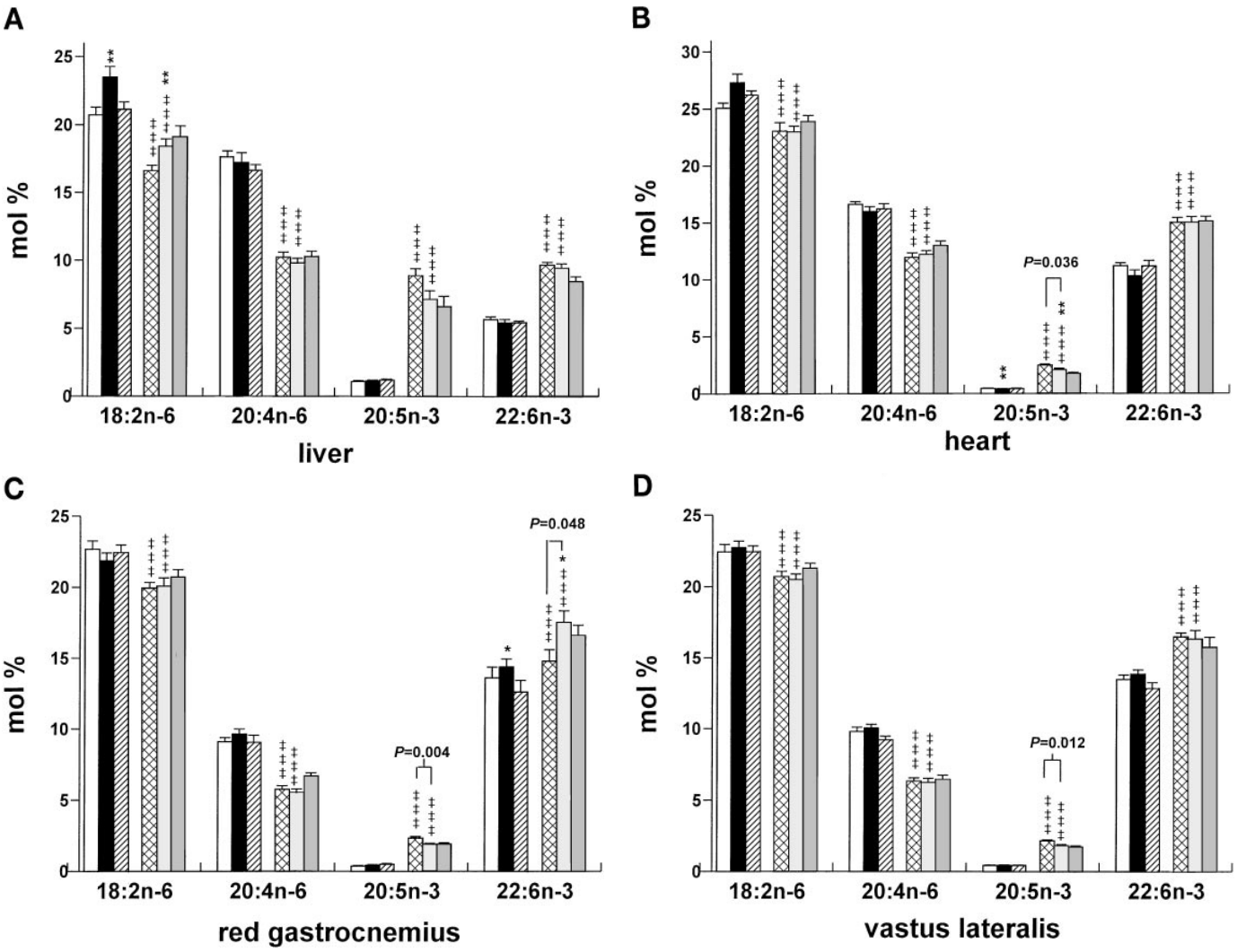

Fig. 1. Concentration of linoleic [18:2 (n-6)], arachidonic [20:4 (n-6)], eicosapentaenoic [20:5 (n-3)], and docosahexaenoic [22:6 (n-3)] fatty acids in liver (*A*), heart (*B*), red gastrocnemius (*C*), and superficial vastus lateralis (*D*) muscles in resting and exercised groups. SO, soy oil supplemented (open bars); SOVE, soy oil and vitamin E supplemented (solid bars); SOVE-EX, soy oil and vitamin E-supplemented animals exercised until exhaustion (hatched bars); FO, fish oil supplemented (crosshatched bars); FOVE, fish oil and vitamin E supplemented (light-gray bars); FOVE-EX, fish oil and vitamin E-supplemented animals exercised until exhaustion (dark-gray bars). Values are means ± SE. Effect of vitamin E supplementation, $*P < 0.05$; $**P < 0.01$. Effect of FO supplementation, ‡‡‡$P < 0.001$.

the liver, to a lesser degree in the heart and VL, and not at all in the RG (Table 3). FO supplementation also markedly increased the (n-3)/(n-6) ratio in all tissues. In contrast, vitamin E decreased the unsaturation index in all tissues except RG and decreased the (n-3)/(n-6) ratio in all tissues. Vitamin E supplementation had a small but significant lowering effect on eicosapentaenoic acid concentration in FOVE compared with FO (Fig. 1). Note that the interaction of FO and vitamin E was $P = 0.001–0.049$, except in VL ($P = 0.082$). Further analysis of the interaction by using Student's unpaired *t*-test with Bonferroni's correction showed a lower eicosapentaenoic acid concentration in FOVE vs. FO ($P = 0.004–0.036$; in liver, $P = 0.088$). The effect of vitamin E in lowering docosahexaenoic acid content was not significant except in the RG ($P = 0.02$), again seen mainly in FOVE vs. SOVE ($P = 0.02$).

A large decrease (25–40% in all tissues, $P < 0.001$) in arachidonic acid [20:4 (n-6)] was found in response to FO supplementation. FO very significantly decreased linoleic acid [18:2 (n-6)] concentrations relative to SO in all tissues ($P < 0.001$). Vitamin E supplementation elevated hepatic linoleic acid levels only (10–12%, $P = 0.002$).

Effect of exercise on tissue fatty acid composition. Only slight effects of exercise on fatty acid composition were seen in fatty acids that represented only a small percentage of the total fatty acid content (not shown). No effect of exercise on (n-3) or (n-6) fatty acid concentration was found, except for a small effect on docosahexaenoic acid in the liver (Fig. 1).

Effect of FO supplementation, vitamin E supplementation, and cosupplementation of FO with vitamin E on tissue vitamin E levels. FO supplementation lowered mean vitamin E levels somewhat in the RG and VL ($P = 0.021$ and 0.014, respectively; Fig. 2). There was no overall effect on vitamin E levels in other tissues. Vitamin E supplementation resulted in increases of ~21–53% in mean vitamin E levels in all tissues (Fig. 2), with relative increases generally greatest in those tissues low in vitamin E (e.g., skeletal muscle). Vitamin E levels in the liver were 20% higher in FOVE than SOVE (Student's unpaired *t*-test with Bonferroni's correction, $P = 0.002$).

Table 3. *Liver, heart, red gastrocnemius muscle, and superficial vastus lateralis muscle fatty acid unsaturation index and (n-3)/(n-6) ratio in groups at rest supplemented with soy oil, soy oil and vitamin E, fish oil, and fish oil and vitamin E*

Tissue	SO	SOVE	FO	FOVE
		Unsaturation index		
Liver	184 ± 5	185 ± 10*	225 ± 11†	212 ± 10*†‡
Heart	199 ± 6	194 ± 13	216 ± 9†	214 ± 10†
RG	178 ± 5	184 ± 4	180 ± 4	192 ± 5
VL	181 ± 10	184 ± 8	194 ± 9§	189 ± 17§
		(n-3)/(n-6)		
Liver	0.22 ± 0.03	0.26 ± 0.17	0.81 ± 0.15†	0.68 ± 0.13†
Heart	0.33 ± 0.07	0.30 ± 0.06	0.58 ± 0.09†	0.60 ± 0.07†
RG	0.51 ± 0.08	0.54 ± 0.06	0.86 ± 0.13†	0.78 ± 0.11†
VL	0.50 ± 0.04	0.50 ± 0.03	0.77 ± 0.08†	0.80 ± 0.06†

Values are means ± SD. SO, soy oil; SOVE, soy oil + vitamin E; FO, fish oil; FOVE, fish oil + vitamin E; RG, red gastrocnemius muscle; VL, vastus lateralus muscle. *Effect of vitamin E, $P = 0.033$; †effect of FO, $P < 0.001$; ‡FO vs. FOVE, $P = 0.014$ (Student's *t*-test with Bonferroni's correction); §effect of fish oil, $P = 0.006$.

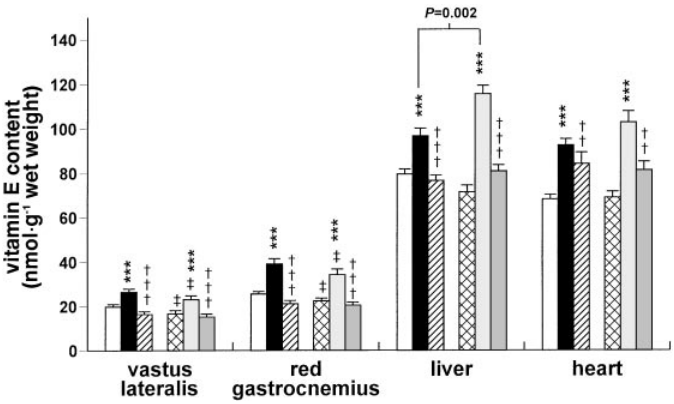

Fig. 2. Tissue α-tocopherol (vitamin E) content. Bars and groups as in Fig. 1. Values are means ± SE. Effect of vitamin E supplementation, ***$P < 0.001$. Effect of exercise, ††$P < 0.01$ and †††$P < 0.001$. Effect of FO supplementation, ‡$P < 0.05$.

Effect of exercise on tissue vitamin E levels. Exercise markedly decreased levels of vitamin E in all tissues in FOVE and SOVE, by ~10–20% in heart, 20–30% in liver, and 40% or more in the VL and RG compared with the resting groups (Fig. 2). There was no significant difference in the relative response to exercise between FOVE and SOVE.

Effect of FO supplementation, vitamin E supplementation, and cosupplementation on tissue lipid peroxidation levels. FO supplementation remarkably increased mean TBARS levels in the liver (by 32–33% relative to the corresponding SO-supplemented groups, $P = 0.002$; Fig. 3). FO had no significant effect in the RG or superficial VL. Vitamin E supplementation lowered mean TBARS levels by 18–19% in the liver ($P = 0.024$) and by 33% in the FO- and 23% in the SO-supplemented groups in the RG ($P = 0.002$). Liver TBARS levels were ~32% higher in FOVE than SOVE (Stu-

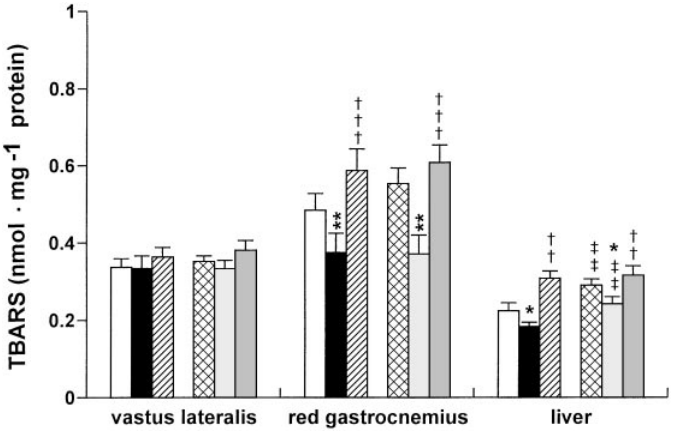

Fig. 3. Tissue thiobarbituric acid reacting substance (TBARS) levels. Bars and groups are as in Fig. 1. Effect of vitamin E supplementation, *$P < 0.05$ and **$P < 0.01$. Effect of exercise, ††$P < 0.01$ and †††$P < 0.001$. Effect of FO supplementation, ‡‡$P < 0.01$.

dent's *t*-test with Bonferroni's correction, $P = 0.050$). Neither FO nor vitamin E supplementation affected lipid peroxidation in the VL.

Effect of exercise on tissue lipid peroxidation. Exercise markedly elevated TBARS content in the liver (30% in FOVE-Ex group, 69% in SOVE-Ex; $P = 0.006$) and the RG (64% in FOVE-Ex, 57% in SOVE-Ex; $P < 0.001$) compared with the corresponding resting groups, with similar TBARS levels in the exercise groups (Fig. 3). No effect of exercise was seen in VL.

Effect of FO supplementation, vitamin E supplementation, and cosupplementation on tissue protein carbonyl levels. FO supplementation had no effect on protein carbonyl levels in the liver or RG (Fig. 4). In the VL, protein carbonyl levels were ~35% higher in FO than in the other groups ($P = 0.025$ for the effect of FO). Vitamin E supplementation led to a 33 and 20% decrease in mean protein carbonyl levels in the liver of FOVE and SOVE groups, respectively ($P = 0.001$). RG protein carbonyl content decreased by 41 and 56%, respectively, with vitamin E supplementation ($P = 0.002$). Vitamin E lowered VL protein carbonyl levels in FOVE only (interaction of FO and vitamin E, $P = 0.055$; further analysis of FOVE vs. FO with Student's unpaired *t*-test with Bonferroni's correction, $P = 0.050$).

Effect of exercise on tissue protein carbonyl levels. Protein carbonyl levels in the RG were roughly threefold greater in the exercise groups ($P < 0.001$ for the effect of exercise; Fig. 4). In the VL, exercise increased carbonyl content by 83 and 69% in FOVE-Ex and SOVE-Ex, respectively ($P < 0.001$). In the liver, the effect of exercise only tended to significance ($P = 0.096$); however, by using one-way ANOVA, protein carbonyl content was significantly higher in SOVE than in the other groups ($P = 0.05$).

DISCUSSION

FO supplementation induced lipid peroxidation in a tissue-specific manner but, with the exception of VL, did not cause protein oxidative damage. High-dose vitamin E cosupplementation decreased FO-induced lipid peroxidation, despite elevated lipid peroxidation in the liver compared with SOVE supplementation. Although FOVE supplementation increased lipid peroxidation at rest compared with SOVE, at least in the liver, FOVE appeared to decrease relative susceptibility to exercise-induced oxidative stress.

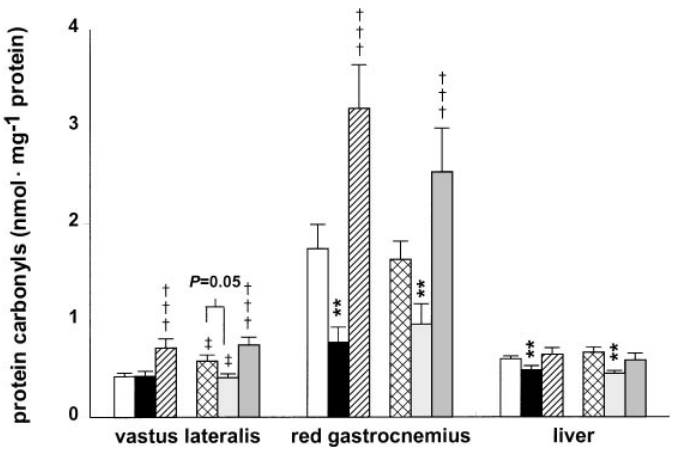

Fig. 4. Tissue protein carbonyl levels. Bars and groups are as in Fig. 1. Effect of vitamin E supplementation, $^{**}P < 0.01$. Effect of FO supplementation, $‡P < 0.05$. Effect of exercise, $†††P < 0.001$

FOVE supplementation at rest. FO-induced oxidative damage appeared to be largely limited to tissue lipids (except in the VL). Thus different mechanisms or factors possibly influenced oxidative damage to lipids and proteins. This is reinforced by our finding that although vitamin E feeding resulted in marked decreases of both tissue TBARS and protein carbonyl levels, there was no correlation between TBARS and protein carbonyl levels in any of the tissues. Protein oxidation has been suggested to be of greater significance in cell toxicity than lipid peroxidation or oxidative stress-associated vitamin E depletion (33). Findings of increased lipid peroxidation with FO supplementation have been previously reported in the liver (11, 24) and heart (24, 28).

At rest, hepatic TBARS levels were ~30% higher in FOVE compared with SOVE, despite somewhat higher vitamin E levels. At least in the liver, vitamin E supplementation seemed to be inadequate in controlling FO-induced oxidative stress, in agreement with other studies (24). The more marked induction of lipid peroxidation in the liver and incomplete compensation of vitamin E supplementation in FOVE may be caused by the many metabolic and detoxification functions of the liver, making this organ more sensitive to FO-induced oxidative stress.

Our findings are not consistent with the manyfold increase in vitamin E tissue levels induced by FO with or without vitamin E supplementation reported in some earlier studies (3, 8). In the present study, tissue levels of vitamin E increased only modestly, with a significant added effect of FO in the liver only. Leibovitz et al. (24) reported lack of significant changes in α-tocopherol content in either the liver or heart in 8-wk-old Sprague-Dawley rats with a diet supplemented with FO (10% of diet) and vitamin E (180 mg/kg diet), although at lower levels of vitamin E supplementation (35 mg/kg diet), liver α-tocopherol content was higher in the FO-supplemented group. Differences compared with our study may be because they used younger rats of a different strain (24). Berlin et al. (3) found manyfold increases in α-tocopherol content in human red cell membranes in response to FO supplementation. No other tissues were sampled, however, and relatively smaller amounts of FO supplements were used. Chautan et al. (8) found fourfold α-tocopherol increases in heart membranes but not in the liver, using the same rat strain as in the present study and a 4-wk diet rather high in basal vitamin E content (~200 mg/kg diet; 5.2 mg/day, on average, assuming similar levels of diet consumption) and fat content (17%). Differences in results may be caused in part by the much higher dietary fat content in their study. Vitamin E consumption does not seem to explain the discrepancy between our study and that by Chautan et al., since the total vitamin E received in the diet and with

the oil supplements in the FO and SO only groups in the present study did not differ greatly from their study.

Peroxisomal β-oxidation of 20- and 22-carbon (n-3) fatty acids may contribute to tissue lipid peroxidation (11). The (n-3)/(n-6) ratio in the tissues of the FO group was two- to fourfold higher in all tissues studied. FO supplementation is known to markedly induce peroxisome activity (10, 11, 38), resulting in increased formation of H_2O_2 as a by-product of β-oxidation (10). An increased (n-3)/(n-6) ratio markedly induced hepatic peroxisomal β-oxidation and lipid peroxidation in Wistar rats fed high-fat diets, even when the unsaturation index was kept nearly constant in part through compensatory supplementation of polyunsaturated (n-6) fatty acids (28).

FO-induced oxidative stress has also been attributed to the membrane incorporation of polyunsaturated fatty acids (24, 28) supplied through FO supplementation. FO were efficiently incorporated into tissues at the expense of membrane arachidonic and linoleic acids (37). An increased unsaturation index in the liver of FO-fed rats could in part explain increased lipid peroxidation in the liver. Vitamin E supplementation had significant effects on tissue fatty acid profiles, decreasing eicosapentaenoic acid concentration in all tissues and docosahexaenoic acid concentration in the RG and increasing linoleic acid concentration in the liver. These effects were primarily observed in the FO-fed group. Berlin et al. (3) found similar but even more striking effects of FOVE. They attributed the effects to more extended FO supplementation, because the cosupplementation was followed by further FO supplementation in their study (3), in contrast to the present study. Because larger vitamin E supplements were used in the present study than in most other studies not observing an effect of vitamin E supplementation on tissue fatty acid profiles (24), our observation could be related to the stabilizing effect of vitamin E in the membranes (27).

Exercise and FO and vitamin E supplementation. Exhaustive exercise markedly elevated TBARS and protein carbonyl levels in the RG and TBARS level to a lesser degree in the liver of both FOVE-Ex and SOVE-Ex. Despite higher resting levels of hepatic TBARS in FOVE, liver TBARS increased with exercise in FOVE-Ex by less than one-half that observed in SOVE-Ex, with no significant difference in absolute TBARS levels. Although two-way ANOVA showed no significant effect of FO or exercise on protein carbonyl content, with the use of one-way ANOVA, protein carbonyl content was significantly higher only in SOVE-Ex, suggesting increased exercise-induced oxidative stress in that group.

A more minor role of peroxisomal β-oxidation in total oxygen consumption during exercise could decrease susceptibility of skeletal muscle to exercise-induced oxidative stress in FO-supplemented groups. This, coupled with increased vitamin E content in the liver, could provide the FOVE group additional protection against oxidative stress during exercise. During exercise at moderate intensity, skeletal muscle mitochondrial fatty acid oxidation increases by approximately eightfold (9). Studies involving peroxisomal oxidation have focused on its role in the resting state (11, 28, 38). Meydani et al. (27) have shown, however, that in men not receiving FO supplements, docosahexaenoic and eicosapentaenoic acid levels relative to arachidonic acid remain constant in exercised muscle. Because membrane fatty acids undergo little change with exercise (15), peroxisomal fatty acid metabolism appears to remain largely unaffected during exercise.

The immunomodulatory effects of FO could also lessen the relative exercise-induced increase in oxidative stress. Exercise acutely induces an immune response, similar to inflammatory or ischemia-reperfusion reactions, in which reactive oxygen species (ROS) play a major role (2, 5). In long-lasting endurance events, this immune response may be an important secondary source of ROS (5, 19). Despite unfavorable effects at rest on lipid peroxidation (11, 24, 28), FO supplementation decreases the release of proinflammatory cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor-α (13); attenuates neutrophil function, leukotriene chemotactic activity (23), and neutrophil superoxide generation (17); and inhibits arachidonic acid metabolism (23), cyclooxygenase activity, and prostaglandin synthesis (29). All the above-mentioned mechanisms can directly or indirectly decrease ROS formation, perhaps more so in response to exhaustive exercise (17).

Unlike in the RG, no effect of exercise on TBARS was seen in the VL, which is mainly glycolytic. VL protein carbonyl levels, on the other hand, were significantly elevated in the exercise groups, although the relative increase was much less than in the RG. Exercise-induced oxidative stress has been shown to be highest in active, oxidative muscles (4, 35).

Exercise-induced depletion of vitamin E was seen in all tissues measured, especially in skeletal muscle. Loss of tissue vitamin E (probably because of enhanced consumption) in association with an acute pro-oxidant stressor (25), including exercise (4, 27), has been considered a marker of oxidative stress.

In summary, FO induced oxidative damage of lipids in a tissue-specific manner. FOVE supplementation decreased tissue oxidative stress, as measured by both TBARS and protein carbonyl levels, to below that seen in non-vitamin E-supplemented animals, but hepatic lipid peroxidation remained higher than in the SOVE-supplemented rats. Another novel finding was that FOVE supplementation appeared to offset the relative increase in exercise-induced oxidative stress in the liver compared with SOVE supplementation, despite a higher oxidative stress state at rest.

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