Vitamin E regulates changes in tissue antioxidants induced by fish oil and acute exercise

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ABSTRACT

ATALAY, M., D. E. LAAKSONEN, S. KHANNA, E. KALISTE-KORHONEN, O. HÄNNINEN, and C. K. SEN. Vitamin E regulates changes in tissue antioxidants induced by fish oil and acute exercise. *Med. Sci. Sports Exerc.*, Vol. 32, No. 3, pp. 601–607, 2000. **Purpose:** Prooxidant effects of fish oil supplementation could unfavorably affect the cardiovascular benefits of fish oil. We tested the effects of 8 wk vitamin E cosupplementation with fish oil on antioxidant defenses at rest and in response to exhaustive exercise in rats. **Methods:** Rats ($N = 80$) were divided into fish oil, fish oil and vitamin E (FOVE), soy oil, and soy oil and vitamin E (SOVE) supplemented groups. For the vitamin E supplemented rats, corresponding groups (FOVE-Ex and SOVE-Ex) performed an acute bout of exhaustive exercise after the supplementation period. **Results:** Fish oil supplementation increased the activity of catalase, glutathione peroxidase, and glutathione-S-transferase in the liver and red gastrocnemius (RG) muscle. Fish oil decreased liver total glutathione (TGSH) levels. Vitamin E supplementation decreased antioxidant enzyme activities to levels at or near those in SOVE in a tissue specific pattern. Vitamin E increased TGSH in liver, heart, and RG. Regression analysis showed TGSH to be a negative determinant of protein oxidative damage as measured by protein carbonyl levels in both liver and RG. Catalase activity was associated with liver lipid peroxidation as measured by thiobarbituric acid–reacting substances. The exercise-induced decrease in hepatic TGSH tended to be less in FOVE versus SOVE. Exhaustive exercise also modulated tissue antioxidant enzymes. **Conclusions:** Vitamin E supplementation markedly decreased fish oil induced antioxidant enzyme activities in all tissues. Sparing of glutathione may be an important mechanism by which vitamin E decreased tissue protein oxidative damage. **Key Words:** GLUTATHIONE, GLUTATHIONE DEPENDENT ENZYMES, CATALASE, LIPID PEROXIDATION, PROTEIN OXIDATION

Fish oil–induced oxidative stress could reduce the purported cardiovascular benefits of fish oil (17,23,28). Diets high in fish have not been conclusively shown to decrease cardiovascular morbidity or mortality (4). Although fish oil may decrease serum triglyceride levels, increase membrane fluidity, and decrease platelet thromboxane production, it may also cause oxidative stress. Oxidative stress is believed to play a major role in atherosclerosis (32) and many other diseases. Physical exercise also acutely induces oxidative stress (26,30).

The high degree of unsaturation of the (*n-3*) fatty acids making up fish oil may predispose to oxidative stress (17,23,28). Fish oils have also been shown to induce peroxisomal β-oxidation, in which fatty-acyl oxidation gives hydrogen peroxide as a normal byproduct (9). We recently showed in rats that, even with high dose supplementation of the lipophilic chain breaking antioxidant vitamin E with fish oil, liver lipid peroxidation as measured by thiobarbituric acid–reacting substances (TBARS) remained higher compared with supplementation of vitamin E with soy oil (28), in agreement with previous studies (17). Protein oxidative damage as measured by protein carbonyl levels in most of tissues examined was not affected by fish oil supplementation, although vitamin E strongly decreased protein carbonyl levels in all tissues measured (28).

As a compensatory mechanism against the prooxidant properties of fish oil, fish oil supplementation has also been shown to markedly induce activity of antioxidant enzymes such as glutathione peroxidase (GPX), glutathione-S-transferase (GST), and catalase (11,35), in addition to variable effects on glutathione (10). Glutathione is an ubiquitous cytosolic antioxidant tripeptide that regenerates vitamin E and ascorbate and protects against protein oxidative damage by maintaining protein thiols (22,34) in addition to direct free radical- and GPX-dependent hydrogen peroxide and peroxyl radical scavenging. GST catalyzes the reaction between the -SH group and potential alkylating agents, rendering them more water soluble and suitable for transport out of the cell (18). GST can also use peroxides as a substrate (3). Catalase is a hydrogen peroxide scavenging enzyme mainly localized to peroxisomes or microperoxisomes (21). These antioxidants and antioxidant enzymes

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work independently and in concert to protect against oxygen toxicity (27).

Many studies have shown that even moderate exercise may result in oxidative damage (2,28). Increased exercise-induced oxidative stress could reduce the beneficial effects of regular exercise (27). This may be particularly concerning to groups predisposed to oxidative stress, such as those who consume large amounts of fish or take fish oil supplements. We recently found that, despite higher hepatic lipid peroxidation at rest in fish oil and vitamin E supplemented (FOVE) rats compared with soy oil and vitamin E supplemented (SOVE) animals, FOVE rats tended to be relatively less susceptible to exercise-induced oxidative stress as measured by vitamin E depletion and protein carbonyl and TBARS formation (28). A possible mechanism for decreased susceptibility to exercise-induced oxidative stress may be because mitochondrial β-oxidation, hypothesized to be a primary source of exercise-induced oxidative stress, undergoes a marked increase during exercise, in apparent contrast to peroxisomal β-oxidation. Thus, the role of peroxisomal β-oxidation as a cause of oxidative stress appears to decrease during exercise relative to mitochondrial oxidation. Also, fish oil has potent antiinflammatory effects (8,13,16), which could contribute to decreased susceptibility to exercise-induced oxidative stress. The effect of fish oil and vitamin E supplementation on tissue antioxidant defenses during physical exercise has not been studied.

The aims of this study were 1) to assess the effect of fish oil supplementation alone and with vitamin E on physiological antioxidant defenses in liver, heart, and skeletal muscle at rest and after exhaustive exercise, and 2) to assess the determinants of previously reported (28) lipid and protein indices of oxidative stress in liver and skeletal muscle in an experimental rat model using soy oil, high in (*n-6*) fatty acid content, for comparison.

METHODS

Animals. Male outbred Wistar rats (National Laboratory Animal Center, Kuopio, Finland) 8 wk of age ($N = 80$) were divided into six groups: fish oil (FO), FOVE, soy oil (SO), and soy oil and vitamin E (SOVE) supplemented groups; and for the vitamin E supplemented rats, corresponding exercise groups (FOVE-Ex and SOVE-Ex) (28). Groups 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^{-1} all-rac-α-tocopherol acetate (nutritional content has been described in greater detail previously (28). All rats were housed four animals to a cage with aspen bedding (Tapvei OY, Kaavi, Finland) 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 and carried out in accordance with guidelines published by the American College of Sports Medicine.

Supplementation. Fish oil (Bio-Marin®, Pharma Nord, Vojens, Denmark) was administered intragastrically 1 g·kg^{-1} body weight·d^{-1} to keep fish oil intake constant among the supplemented animals (fatty acid composition of the fish oil supplements was 34.9% eicosapentaenoic acid and 26.4% docohexaenoic acid (for a more detailed description, see (28)). The fish oil contained under 10 meq·g^{-1} peroxides according to manufacturer quality control, with 6.0 mg·g^{-1} free RRR-α-tocopherol added to protect against oxidation. Soy oil (Bio-Marin® Placebo, Pharma Nord) with similar vitamin E contents served as a control for fish oil supplementation. Soy oil contains 53.6% linoleic acid (18:2(*n-6*)) and 22.1% oleic acid. RRR-α-tocopherol (Bio-E-Vitamin®, Pharma Nord) was supplemented intragastrically at 500 mg·kg^{-1} body weight·day^{-1}. All supplementation was done over an 8-wk period, 5 d·wk^{-1}. At the end of the supplementation period, the rats weighed an average of 411 ± 39 g, with no significant difference between groups. Rats consumed an average of 26 g·d^{-1} of rat chow, meaning that average overall fat consumption was approximately 6.6%, of which the oil supplements were about 24%. Free RRR-α-tocopherol administered with the fish and soy oil in the FO and SO groups was therefore 6.0 mg·kg^{-1} body weight. In addition, all rats consumed an average of 1.6 mg·d^{-1} 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 much 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 wk. At the end of the sample collection, the rats ran at 1.08 km·h^{-1} at a 10-degree uphill grade for 10 min and then at 1.44 km·h^{-1} at the same grade until exhaustion. Exhaustion was defined as loss of the righting reflex when placed in a supine position. Upon running to exhaustion, rats were immediately sacrificed.

Sample collection and preparation. Rats were matched between groups for day of euthanization. Food was withheld from all rats for 24 h before euthanization. After decapitation and exsanguination, the heart, liver, and the superficial vastus lateralis (VL) and red gastrocnemius (RG) muscles were quickly dissected out, placed in liquid nitrogen, and into storage at −70°C. Tissue homogenization for determinations of total glutathione (TGSH) levels, glutathione-related enzyme activities, and catalase activity were carried out as described before (6,29).

Assays. TGSH, selenium-GPX, glutathione reductase (GRD), and GST analyses were done spectrophotometrically from tissue as described before (6,29). Fatty acid composition and TBARS, protein carbonyls, and vitamin E content were determined according to methods published previously (28).

Tissue catalase activity was determined by monitoring the decrease in absorbance at 240 nm in the presence of 10 mmol hydrogen peroxide (1). One U of catalase activity was defined as the decomposition of 1 mol·min^{-1} of hydrogen peroxide at 25°C.

Statistical analysis. Results are presented as mean ± SE. The effect of fish oil supplementation and exercise was assessed using two-way ANOVA. Student's unpaired *t*-test with Bonferroni's correction was done in selected instances

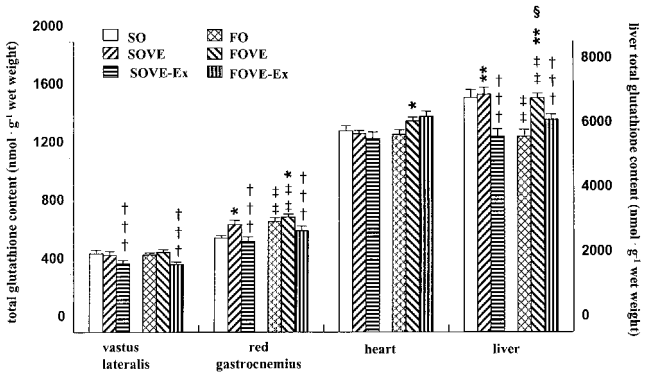

Figure 1—Tissue total glutathione levels (mean ± SE). SO, soy oil supplemented; FO, fish oil supplemented; SOVE, soy oil and vitamin E supplemented; FOVE, fish oil and vitamin E supplemented; SOVE-Ex, soy oil and vitamin E supplemented animals exercised until exhaustion; FOVE-Ex, fish oil vitamin E supplemented animals exercised until exhaustion. * Effect of vitamin E supplementation; † effect of exercise; ‡ effect of fish oil supplementation; § interaction between vitamin E and fish oil supplementation. *,†, ‡, §, $P < 0.05$; **,††, ‡‡, §§, $P < 0.01$; *,†††, ‡‡‡, §§§, $P < 0.001$.**

to further evaluate differences between group pairs. Interaction of vitamin E and fish oil refers to a combined effect of fish oil and vitamin E that is not the sum of the effects of vitamin E and fish oil separately. Effects of supplementation and interactions are mentioned only when statistically significant. Linear multiple regression analysis (backward step) was done to assess determinants of oxidative stress indices (28) in the liver and RG and VL. Statistical significance was defined as $P < 0.05$.

RESULTS

Effect of fish oil supplementation, vitamin E supplementation, and cosupplementation with vitamin E on mean TGSH levels and related enzymes at rest. Fish oil supplementation markedly decreased mean TGSH levels in the liver and increased TGSH in RG muscle (Fig. 1). Vitamin E supplementation had a statistically significant elevating effect on TGSH in the liver, heart, and RG ($P = 0.03–0.003$). The elevating effect of vitamin E was significantly greater in FOVE than SOVE in the liver ($P < 0.0001$) and tended to be greater in the heart ($P = 0.10$). There were no differences in mean tissue TGSH levels between FOVE and SOVE using Student's unpaired *t*-test with Bonferroni's correction.

Hepatic GPX activity increased 17% ($P = 0.005$) in response to fish oil supplementation, with smaller effects in the heart (Table 1). Fish oil tended to increase RG GPX activity ($P = 0.082$). Vitamin E supplementation lowered GPX activity in the heart ($P = 0.015$). In the liver, vitamin E lowered GPX activity only in the fish oil supplemented groups ($P = 0.040$). Fish oil supplementation remarkably induced GST activity in the liver and RG (both $P < 0.001$, Table 1) but not in the heart or VL muscle. Vitamin E supplementation lowered GST activity in the fish oil supplemented groups only ($P = 0.024$). Vitamin E supplementation increased GRD activity in the liver ($P = 0.004$) and tended to increase GRD in RG ($P = 0.062$) in the fish oil groups only (Table 1).

Effect of fish oil supplementation, vitamin E supplementation, and fish oil cosupplementation with vitamin E on catalase at rest. Catalase activity rose by 15% and 10% with fish oil supplementation in the liver ($P = 0.025$) and RG ($P = 0.007$; Table 1), respectively. Vitamin E supplementation decreased cardiac catalase activity in both cosupplementation groups and hepatic catalase activity in the fish oil group to levels similar to those in SOVE ($P = 0.01$).

TBARS correlations and multiple regression analysis for the groups at rest. Results for TBARS ($nmol \cdot mg^{-1}$ wet weight), fatty acid composition (individual fatty acids as mole-percent total fatty acid content), and α-tocopherol ($nmol \cdot g^{-1}$ wet weight) content in the liver and RG and VL muscles have been reported previously (28). Multiple backward regression analysis with α-tocopherol and TGSH levels, GPX, GRD, GST, and catalase activity and fish oil dietary consumption ($g \cdot kg^{-1}$ body weight) as explanatory variables showed catalase activity and fish oil consumption to be determinant of TBARS in the liver (Table 2). Substituting the liver (*n-3*)/(*n-6*) fatty acid ratio or the unsaturation index (i.e., sum of the percentage composition of individual fatty acids times the respective number of double bonds) (28) for fish oil consumption resulted in only minor differences (data not shown).

Multiple backward regression analysis with α-tocopherol, TGSH, GPX, GRD, GST, catalase, and fish oil consumption as explanatory variables showed GST to be the only variable determinant of TBARS levels in RG muscle (Table 2). Substitution of RG (*n-3*)/(*n-6*) ratio or unsaturation index (28) for fish oil consumption did not affect analyses. None of the above mentioned variables were determinant of TBARS levels in the VL.

Protein carbonyl correlation and multiple linear regression analysis. Protein carbonyl levels were determined in the liver, RG, and VL (28). Multiple backward regression analysis with α-tocopherol and TGSH levels, GPX, GRD, GST, and catalase activity and fish oil dietary consumption ($g \cdot kg^{-1}$ body weight) as explanatory variables showed TGSH and α-tocopherol content to be determinant of liver protein carbonyl levels at rest (Table 2). Multiple backward regression analysis with the same explanatory variables showed GST activity and TGSH to be determinant of RG protein carbonyl levels at rest, with α-tocopherol tending to significance ($\beta = -0.29$, $P = 0.11$) (Table 2). In the VL muscle, only vitamin E was predictive of protein carbonyl levels (Table 2). Exercise time to exhaustion was greater in FOVE-Ex than in SOVE-Ex (107.3 min ± 29.3 vs 78.2 min ± 8.5, $P = 0.017$).

Effect of exercise on mean total TGSH and related enzymes. Exercise decreased TGSH in the liver and RG and VL muscles by 9–18% ($P < 0.001$) compared with the corresponding resting groups (Fig. 1). Fish oil cosupplementation tended to decrease the lowering effect of exercise on TGSH (9% vs 18%) in the liver ($P = 0.093$ for the interaction).

TABLE 1. Tissue glutathione peroxidase (GPX), glutathione-S-transferase (GST), glutathione reductase (GR) and catalase activities (mean ± SE).

	SO	SOVE	SOVE-Ex	FO	FOVE	FOVE-Ex
GPX (nmol · min^{-1} · mg protein^{-1})						
VL	32.2 ± 1.9	33.3 ± 1.6	32.1 ± 1.1	35.5 ± 2.4	29.3 ± 2.1	32.1 ± 0.8
RG	44.1 ± 1.8	47.2 ± 2.8	48.5 ± 0.8	60 ± 2.3	47.4 ± 2.4	54.4 ± 4.7
heart	172.6 ± 3.4	164 ± 4.9*	188.7 ± 4.9††	189.1 ± 5.0‡	173.4 ± 5.7*‡	187.1 ± 4.6††
liver	345.8 ± 10.0	356.2 ± 8.4*	363.2 ± 17	405.6 ± 9.2‡‡	360.8 ± 15.2*‡‡§	348.2 ± 8.2
GST (nmol · min^{-1} · mg protein^{-1})						
VL	24.8 ± 0.6	24.1 ± 0.7	24.1 ± 0.5†	24.8 ± 0.7	26.2 ± 0.6	23.1 ± 0.6†¶
RG	32.9 ± 1.3	32.8 ± 0.9	37.6 ± 0.6†††	40.6 ± 1.4‡‡‡	36.1 ± 0.8*‡‡‡	33.9 ± 0.8†††¶
heart	66.6 ± 1.3	65.1 ± 1.3	68.2 ± 2	63.5 ± 1.3	65.2 ± 1.5	69.5 ± 2.1
liver	1049.5 ± 15.1	1093.2 ± 30.2	1263.9 ± 39.3††	1229.1 ± 34.7‡‡‡	1170.3 ± 30.2*‡‡‡	1235.2 ± 40.8††
GRD (nmol · min^{-1} · mg protein^{-1})						
VL	13.9 ± 0.8	14.4 ± 0.7	13.7 ± 0.5	13.7 ± 0.5	13.2 ± 0.8	14.3 ± 0.6
RG	18.2 ± 0.9	17.6 ± 0.9	20.3 ± 1.7	16.2 ± 0.9	21.4 ± 1.1	19.6 ± 2.2
heart	18.1 ± 0.7	18.1 ± 0.6	21.3 ± 1.1	17.8 ± 0.4	19.1 ± 0.7	18.0 ± 0.6
liver	83.2 ± 0.8	85.6 ± 7.6**	81.2 ± 1.6††	80.8 ± 3.2	95.6 ± 2.4**§	87.2 ± 1.6††‡‡‡
Catalase (μ · mg protein^{-1})						
VL	9.9 ± 0.4	10.4 ± 0.4	12.1 ± 0.5††	11.2 ± 0.5	10.4 ± 0.4	11.5 ± 0.5††
RG	11.7 ± 0.6	12.0 ± 0.7	11.6 ± 0.6†	13.5 ± 0.6‡‡	13.9 ± 0.7‡‡	11.6 ± 0.5†
heart	34.1 ± 0.7	30.3 ± 1.0**	33.2 ± 1.4†††	33.7 ± 1.3	31.4 ± 0.8**	36.4 ± 1.0†††
liver	1055.6 ± 31.2	1045.2 ± 31.2**	1008.8 ± 46.8	1211.6 ± 33.8‡	1047.8 ± 39.0‡**§	1094.6 ± 26.0

SO, soy oil supplemented; FO, fish oil supplemented; SOVE, soy oil and vitamin E supplemented; FOVE, fish oil and vitamin E supplemented; SOVE-Ex, soy oil and vitamin E supplemented animals exercised until exhaustion; FOVE-Ex, fish oil vitamin E supplemented animals exercised until exhaustion; VL, vastus lateralis muscle; RG, red gastrocnemius muscle.

* Effect of vitamin E supplementation, † effect of exercise, ‡ effect of fish oil supplementation, § interaction between vitamin E and fish oil supplementation, ¶ interaction between exercise and fish oil supplementation.

*, †, ‡, §, ¶ $P < 0.05$.

**, ††, ‡‡, §§, ¶¶ $P < 0.01$.

***, †††, ‡‡‡, §§§, ¶¶¶ $P < 0.001$.

Exercise increased GPX activity by 8–15% in the heart ($P < 0.005$) but had no significant effect in the other tissues. Exercise had a significant effect on GST activity in RG ($P < 0.001$, Table 1). The effects were complex, however, with higher preexercise but lower postexercise GST activity in the FOVE groups, and a decreasing effect of exercise in FOVE-Ex but an increasing effect in SOVE-Ex ($P = 0.059$ for the interaction). Exercise markedly induced GST activity in the liver ($P = 0.002$) and tended to induce activity in the heart ($P = 0.070$). The effects were also complex though less marked in the VL muscle, with changes similar to those seen in RG ($P = 0.015$ for the interaction). Exercise markedly decreased GRD activity to a similar degree in both fish oil and soy oil supplemented groups in the liver ($P = 0.003$, Table 1).

Effect of exercise on catalase activity. Exercise had a marked elevating effect on catalase activity in the heart ($P < 0.001$) and VL ($P = 0.004$) and a lowering effect on catalase activity in RG ($P = 0.033$, Table 1).

DISCUSSION

High-dose vitamin E cosupplementation with fish oil increased tissue TGSH and offset the marked, largely tissue-specific effects of fish oil on tissue antioxidant defenses. Differences among tissues in antioxidant defense response to fish oil is, in part, due to differences in metabolic activity and function among tissues reflected in pronounced differences in antioxidant levels and enzyme activities even in unsupplemented animals (15,30). Despite increased liver lipid peroxidation in fish oil supplemented groups at rest (28), supplementation of fish oil with vitamin E tended to decrease exercise-induced depletion of hepatic glutathione content, suggesting that vitamin E supplementation with fish oil decreased susceptibility to exercise-induced oxidative stress compared with vitamin E supplementation with soy oil.

Using multivariate linear regression, fish oil consumption and catalase activity were strong determinants of liver lipid

TABLE 2. Multiple backward step regression analysis of indices of oxidative stress in the liver and red gastrocnemius and vastus lateralis muscles.

Dependent Variables Oxidative Stress Indices	adjusted r^2	P	Determinant Variable(s)[a]	β	P
Liver protein carbonyls	0.32	< 0.001	TGSH	−0.38	0.002
			α-Tocopherol	−0.33	0.007
Liver TBARS	0.38	< 0.001	Fish oil consumption	0.40	0.002
			Catalase	0.43	0.001
RG protein carbonyls	0.13	0.015	GST	0.33	0.011
			TGSH	−0.31	0.031
RG TBARS	0.11	0.017	GST	0.33	0.017
VL protein carbonyls	0.09	0.018	α-Tocopherol	−0.33	0.018
VL TBARS	undefined				

TBARS, thiobarbituric acid reacting substances; RG, red gastrocnemius muscle; VL, vastus lateralis muscle; TGSH, total glutathione; GST, glutathione-S-transferase.

[a] Explanatory variables used in the regression analysis were tissue levels of protein carbonyls, TBARS μmol·mg^{-1}) and TGSH, activity of GST, GPX (glutathione peroxidase), GRD (glutathione reductase), and catalase and fish oil consumption (g·kg^{-1} body weight).

peroxidation at rest as measured by TBARS. Catalase has been shown to be a sensitive marker of fish oil–induced peroxisomal β-oxidation (9). Furthermore, the unsaturation index in the liver was not determinant of lipid peroxidation at all, whereas the (*n-3*)/(*n-6*) ratio was weakly predictive of lipid peroxidation levels. Thus, properties of fish oil such as induction of peroxisomal β-oxidation and consequent increased hydrogen peroxide production appear to be more important than the unsaturation index in inducing lipid peroxidation in the liver. Although supplementation of vitamin E with fish oil decreased liver catalase activity to levels similar to those in the SOVE group, lipid peroxidation remained elevated compared with SOVE (28).

In the liver, fish oil markedly depleted TGSH levels. Vitamin E supplementation increased TGSH levels mainly in the FOVE group, offsetting the lowering effect of fish oil. In regression analysis using measured antioxidant levels and antioxidant enzyme activities as explanatory variables, TGSH was determinant of liver protein carbonyl content in the groups at rest. Mechanisms by which vitamin E markedly decreased protein oxidative damage thus appear to be mediated not only directly but also via sparing of TGSH. The role of α-tocopherol in maintaining TGSH has been much less studied than the role of TGSH in preserving vitamin E levels. In isolated rat hepatic cells with Ca^{2+}-depletion–induced lipid peroxidation, α-tocopherol supplementation appeared to maintain intracellular TGSH indirectly, in part, by decreasing efflux of glutathione precursors (25).

Fish oil did not induce catalase activity in the heart. Lack of increased heart catalase activity in response to fish oil supplementation is in contrast to De Craemer et al. (9) and Vamecq et al. (33), who also showed increased myocardial peroxisomal induction with fish oil or increasing ratios of (*n-3*)/(*n-6*) fatty acids. A possible reason for the discrepancies may be because fish oil supplementation quantities were somewhat less in our study.

Fish oil supplementation did increase cardiac GPX activity, however. Although we did not measure indices of lipid peroxidation or protein oxidative damage in the heart, neither TGSH nor vitamin E depletion (28) occurred in the heart with fish oil, suggesting that fish oil–induced oxidative stress might be less than in the liver. Nevertheless, increased GPX activity in response to fish oil, also previously noted (8), may be a defense response to increased peroxisomal hydrogen peroxide production, increased lipid peroxidation, and other prooxidant effects of fish oil. These results cannot, however, be extrapolated directly to coronary artery disease because vascular tissue was not sampled. In contrast to GPX, no GST upregulation was found in the heart in response to fish oil. Vitamin E decreased heart GPX activity in both fish oil and soy oil groups.

Despite decreasing TGSH in the liver, fish oil increased TGSH levels in RG. Upregulation of TGSH in addition to other antioxidant enzyme activities may be a compensatory response in RG because protein and lipid indices of oxidative stress were not significantly affected by fish oil supplementation (28). As similarly observed in the liver, regression analysis showed that TGSH was a determinant of carbonyl levels in vitamin E supplemented animals in RG, suggesting that vitamin E may protect against protein oxidation by decreasing TGSH depletion. Vitamin E tended to increase GRD activity in RG in FOVE animals. Increased GRD activity may partially explain upregulation of TGSH levels in FOVE by increasing regeneration of glutathione from its oxidized form (31).

Induction of GST activity by fish oil is in agreement with previous studies (10,11,35). GST activity also appeared to play a role as a determinant of lipid and protein oxidation damage in RG muscle. The positive relation of GST activity to lipid and protein indices of oxidative stress suggest that its role as a determinant of oxidative stress is largely indirect and that upregulation is a reflection of the prooxidant effect of fish oil. Upregulation of catalase (9–11,33) and GPX activities (10,11,23) by fish oil is also in agreement with previous studies.

Neither fish oil nor vitamin E supplementation affected TGSH levels or antioxidant enzyme activity in VL. This is likely because VL is highly glycolytic, with little metabolic activity and consequent ROS production, especially at rest.

Effect of exercise. Maximal exercise time was longer in FOVE-Ex than SOVE-Ex. In trained cyclists, however, no benefit of fish oil supplementation on exercise performance was noted (24). Possible improved exercise performance in FOVE-Ex is likely to be mediated by factors other than oxidative stress or antioxidant defenses and is outside the scope of this article. In the liver, fish oil cosupplementation with vitamin E tended to spare the exercise-induced loss of TGSH. TGSH depletion with exercise occurred in both groups in all tissues except the heart. TGSH depletion in association with an acute prooxidant stressor, including exercise, is considered a marker of oxidative stress (25,29). Thus, despite the longer exercise time and consequent increased exposure to the prooxidant forces of exercise, vitamin E supplementation with fish oil appeared to attenuate the increase in oxidative stress occurring with exercise relative to soy oil and vitamin E supplementation. Exercise decreased liver GRD activity in both groups, although GRD activity was higher in FOVE and FOVE-Ex groups compared with corresponding SOVE and SOVE-Ex groups. These changes may have a positive effect on liver glutathione homeostasis, and may contribute to the smaller decrease in TGSH seen in FOVE versus SOVE.

In sharp contrast to mitochondrial β-oxidation, indirect evidence suggests that peroxisomal fatty acid β-oxidation remains largely unaffected during exercise (14,20). The substantial decrease in the relative contribution of peroxisomal β-oxidation during exercise coupled with fish oil–induced antioxidant enzyme upregulation in the resting state could offer additional protection against oxidative stress with exercise.

The immunomodulatory effect of fish oil may be another factor reducing the relative increase in oxidative stress with exercise. Exercise acutely induces an immune response similar to inflammatory reactions or ischemia-reperfusion (7), in which reactive oxygen species play a major role (5,19).

Eicosapentaenoic and docosahexaenoic acid have well-established antiinflammatory effects (8,13,16).

After exercise, heart GPX and catalase activity increased, possibly in response to exercise-induced oxidative stress. Exercise also increased catalase activity in VL but decreased activity in RG. Resting protein carbonyl and TBARS levels, and especially the exercise-induced increases, were much greater in RG than VL (28). High levels of oxidative stress may explain the decrease in catalase activity in RG because sufficient oxidative stress has been shown to inactivate catalase (21). GST changes in RG and VL were complex, decreasing slightly with exercise in FOVE and increasing somewhat with exercise in SOVE. Although not supported by TGSH, other enzyme and previously published TBARS and protein carbonyl results, a possible explanation is that GST may be inactivated in response to greater exercise-induced oxidative stress in FOVE-Ex.

Improved exercise performance in the fish oil and vitamin E supplemented rats requires confirmation and investigation of the possible mechanisms. Fish oil and vitamin E cosupplementation decreased the marked tissue-specific fish oil–induced increases in antioxidant enzyme activities to levels near those in the SOVE group. Vitamin E supplementation spared tissue TGSH levels, which may be an important mechanism by which vitamin E protects against protein oxidative damage. Less depletion of liver TGSH in FOVE versus SOVE is consistent with previously reported exercise-induced changes in TBARS and vitamin E content (28) suggesting decreased susceptibility to exercise-induced oxidative stress in FOVE despite increased levels at rest.

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