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Suppression of oxidative stress in aging NZB/NZW mice: Effect of fish oil feeding on hepatic antioxidant status and guanidino compounds

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Abstract

Oxidative stress caused by excessive reactive species (RS) and lipid peroxidation is known to be casually linked to age-related inflammation. To test the hypothesis that fish oil (FO) intake has a beneficial effect on nephritis due to its suppressive action of oxidative stress and the enhancement of antioxidant defenses, we examined the effect of dietary FO on various oxidative stress-related parameters and guanidino compound (GC) levels using (NZB × NZW) F_1 (B/W) mice. These mice were fed diets supplemented with either 5% corn oil (control) or 5% FO. At 4 and 9 months of age, the hepatic oxidative status was estimated by assessing RS generation produced from xanthine oxidase, the prostaglandin pathway and lipid peroxidation. To evaluate the effect of FO on redox status, including antioxidant defenses, GSH and GSSG levels and antioxidant enzyme activities were measured. To correlate the extent of oxidative status with the nephritic condition, creatinine, guanidino acetic acid and arginine levels were measured. Results indicated that increased levels of lipid peroxidation, RS generation and xanthine oxidase activity with age were all significantly suppressed by FO feeding. Furthermore, reduced GSH levels, GSH/GSSG ratio and antioxidant enzyme activities in the FO-fed mice were effectively enhanced compared to the corn oil-fed mice. Among several GCs, the age-related increase of creatinine level was blunted by FO. Based on these results, we propose that dietary FO exerts beneficial effects in aged, nephritic mice by suppressing RS, superoxide and lipid peroxidation, and by maintaining a higher GSH/GSSG ratio and antioxidant enzyme activities.

Keywords: *Dietary fish oil, reactive species, lipid peroxidation, xanthine oxidase, antioxidant defenses, guanidino compound*

Abbreviations: *FO, fish oil; RS, reactive species; GC, guanidino compound; DCFH-DA, 2′,7′-dichlorofluorescein-diacetate; TBARS, thiobarbituric acid reactive substances; B/W, (NZB × NZW)F_1; GSH, glutathione; GSSG, oxidized glutathione*

Introduction

The evidence is clear that the health and disease status of both human and animals is responsive to various nutritional interventions. One such case is exemplified by the epidemiological evidence from an Eskimo population that shows the effect of fish oil (FO) consumption on cardiovascular and inflammatory diseases [1]. Further evidence from clinical settings have shown dietary FO to retard the progression of renal disease in patients [2].

It was already reported that the effect of FO on aging in normal mice [3,4]. However, for the study on the effect of FO on inflammation and oxidative stress-related aging process, we selected to use nephritic model, (NZB × NZW)F_1 (B/W). These experimental mice progressively develop an autoimmune disease that is similar to human systemic lupus erythematosus and die at 6–12 months of age from fatal lupus nephritis. B/W mice fed diets supplemented with ϖ-3 fatty acids-enriched FO show extended life spans

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possibly by retarding the onset of autoimmune disease [5]. Also, the precise mechanisms for protection against renal disease are yet to be established, recent evidence indicates a strong possibility that the anti-inflammatory action of FO might be an underlying mechanism [6].

Oxidative stress can occur when the production of reactive species (RS) overwhelms the antioxidant defense systems, which then leads to oxidative cellular injuries. A recent proposal of the molecular inflammation hypothesis of aging [7], linking the biological aging process to the age-related pathological process, highlights the involvement of RS and the importance of redox balance in the modulation of age-related inflammatory processes [8]. RS generation is intricately implicated in the inflammatory process, and xanthine oxidase is one of the major sources of intracellular superoxide radicals produced under normal physiological conditions and during aging [9]. In addition, increased prostaglandin biosynthesis catalyzed by cyclooxygenase is an important source of RS with age [7].

To fend off oxidative threats, organisms are armed with enzymatic and non-enzymatic defenses. Cellular enzymatic antioxidant defenses include superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-*S*-transferase to defend against oxidative stress [10]. Glutathione (GSH) is an example of an abundant and potent non-enzymatic biological antioxidant responsible for the maintenance of redox balance. Thus, a relatively high level of these anti-oxidative defenses in the liver can signify the animal's overall redox status.

The kidney and liver maintain close metabolic interactions, and damage to either organ can markedly disturb guanidino compound (GC) metabolism in both organs [11]. Hepatic and renal GCs are reliable markers for renal function because GC levels are modified during renal insufficiency [12]. GC levels are considered to be involved in oxidative stress [13] and can be improved by anti-oxidative therapy [14]. The urea cycle is known to operate actively in the liver of ureotelic animals; the liver contributes to arginine anabolism and catabolism; and arginine is a key amino acid involved in several metabolic pathways, including in the production of guanidino acetic acid and creatinine [15]. In previous work, we examined the modulation of GC levels and antioxidant defenses in the kidney of lupus nephritic B/W mice [6].

The important question we ask now is how FO feeding ameliorates uremic conditions, and strengthens the anti-oxidative defense system. To answer this question, in this work we propose the hypothesis that a beneficial effect of FO feeding is the suppression of age-related oxidative stress. To obtain further information, we measured total RS, cyclooxygenase-derived and xanthine oxidase-derived RS generation utilizing specific inhibitors (indomethacin and oxypurinol, respectively). In addition, we investigated xanthine oxidase activity, the redox status by GSH and oxidized glutathione (GSSG) levels and their ratio, and antioxidant scavenging enzymes, including superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-*S*-transferase activities. We also attempted to correlate levels of creatinine, guanidino acetic acid and arginine with the modulation of oxidative stress, including anti-oxidative defenses, in the liver through dietary FO feeding in B/W mice.

Materials and methods

Animals and dietary groups

Detailed information regarding animals has been reported [6]. Briefly, female B/W mice (5 mice /group) at 4 and 9 months of age were used for the study. A semi-purified diet supplementation was implemented throughout their life containing either 5% corn oil (control group) that has high levels of oleic acid (18:1 ω9), or 5% fish oil (FO; deodorized menhaden oil) with a high content of palmitic acid (16:0), eicosapentaenoic acid (20:5 ω3) and docosahexaenoic acid (22:6 ω3). The overall diet contained the following ingredients (wt/wt): Casein, 20%; dextrose, 33%; starch, 32%, oil 5%; fiber, 4.5%; DL-methionine, 0.3%; AIN salt mixture, 3.5%; AIN vitamin mixture, 1.5%; DL-methionine, 0.3%; and choline chloride, 0.2%.

Mice were divided into aged-matched groups and designated as follows: CO/Y, corn oil-fed young mice; CO/O, corn oil-fed old mice; FO/Y, FO-fed young mice; FO/O, FO-fed old mice. Fresh food was provided daily, and precautions were taken to avoid lipid peroxidation. Animals were killed by decapitation at 4 and 9 months of age; livers were collected and were snap frozen in liquid nitrogen and stored at −80°C until analysis.

All the experiments were repeated at least three times to ascertain the reproducibility of the results.

Tissue preparation

The liver was rinsed in ice-cold physiological saline and minced with scissors. To yield liver homogenates, tissues were homogenized in ice-cold homogenization solution (50 mM potassium phosphate buffer containing 1 mM EDTA, 1 mM *p*-aminobenzamidine, 1 μM pepstatin, pH 7.4).

Measurement of total RS generation

RS generation was measured by the method of Ali et al. [16]. In brief, 25 mM 2′,7′-dichlorofluorescein-diacetate (DCFH-DA) was added to homogenates, and after 30 min, changes in fluorescence were determined

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at an excitation wavelength of 486 nm and emission wavelength of 530 nm.

Assessment of lipid peroxidation

To determine lipid peroxidation, the thiobarbituric acid assay was used to estimate levels of thiobarbituric acid reactive substances (TBARS) [17]. In brief, sampling mixtures (40 μl of 8.1% SDS, 300 μl of 20% acetic acid, 200 μl of 1.2% thiobarbituric acid and 150 μl of homogenates) were combined in a microtube and incubated and heated for 30 min at 95°C. After cooling on ice, 300 μl of *n*-butanol was added and centrifuged for 10 min at 24°C. After centrifugation, the optical density of the supernatant was determined at a wavelength of 540 nm..

Determination of cyclooxygenase-derived RS

Liver homogenates were preincubated with 1 mM indomethacin for 10 min, and then RS generation was measured. The difference between treatments with indomethacin and without indomethacin was taken as the amount of cyclooxygenase-derived RS generation.

Measurement of superoxide production

Xanthine oxidase-derived superoxide production was estimated utilizing the DCFH-DA method in the presence of xanthine oxidase inhibitor. Briefly, liver homogenates were preincubated with 10 mM specific inhibitor of xanthine oxidase, oxypurinol for 1 min, and then supeoxide generation was assessed. The difference between treatments with oxypurinol and without oxypurinol was considered xanthine oxidase-derived superoxide generation.

Determination of xanthine oxidase activity

Xanthine oxidase activity was determined fluorometrically using a fluorescence microplate reader at an excitation wavelength of 360 nm and emission wavelength of 460 nm with 20 nm bandwidths [18]. Xanthine oxidase activity was calculated from the slope of the linear increase in fluorescence obtained during 2 min following the addition of the substrate, pterin (10 μM).

Measurement of GSH and GSSG: an oxidative stress marker

GSH and GSSG assays were carried out by the method of Pandey et al. [19]. In brief, 25% metaphosphoric acid-added liver homogenates were used for the assays. To assay for GSH, 1 mM EDTA-50 mM phosphate buffer (pH 8.0) was added to homogenates followed by *o*-phthalaldehyde. After 20 min at room temperature, the fluorescence was estimated at an excitation wavelength of 360 nm and emission wavelength of 460 nm. GSSG was assayed after preincubation with N-ethylmaleimide for 20 min and 0.1 M NaOH was substituted for 1 mM phosphate buffer.

Detection of antioxidant enzyme activities

Determination of superoxide dismutase and catalase activities. Superoxide dismutase activity was determined using the xanthine oxidase-cytochrome c system [20], and samples from the liver homogenates were used for the assay. Briefly, the reaction mixture consisted of 2.4 ml of 50 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM EDTA, 0.3 ml of 0.1 mM ferricytochrome *c*, 0.2 ml of 1% deoxycholate, 0.1 mM of 1.5 mM potassium cyanide, 0.3 ml of 0.5 mM xanthine and 20 μl appropriately diluted xanthine oxidase to produce a rate of reduction of ferricytochrome c at 550 nm of 0.025 absorbance unit/min at 25°C. One unit of superoxide dismutase is defined as the amount of superoxide dismutase required to inhibit the rate of cytochrome c reduction by 50%. Catalase activity was measured following the method described by Aebi [21], and liver homogenates were used in this assay. The reaction mixture (3 ml) consisted of 50 mM phosphate buffer (pH 7.0), 1 ml 30 mM H_2O_2 in phosphate buffer and catalase activity was expressed as μmoles of hydrogen peroxide (H_2O_2) reduced/min/mg protein, monitoring spectrophotometrically at 240 nm.

Determination of glutathione peroxidase, glutathione reductase and glutathione-S-transferase activities. Glutathione peroxidase activity was determined by monitoring the oxidation of NADPH at 340 nm according to the method of Tappel et al. [22] Liver homogenates were used. Glutathione peroxidase activity was measured as the amount of NADPH consumed per min per milligram of protein. Glutathione reductase activity was estimated according to the method described by Eklow et al.[23] Absorbance at 340 nm was recorded for 2 min. One unit of activity is defined as 1 nmole of NADPH oxidized. Glutathione-*S*-transferase activity was measured by the method of Habig et al.[24] The conjugate of GSH with 1-chloro-2,5-dinitrobenzene (CDNB) was measured at 340 nm spectrophotometrically.

GC levels

Liver homogenates were deproteinized by the addition of trichloroacetic acid (final concentration 10%). After centrifuging at 3000 rpm for 15 min, the supernatant was filtered through a 0.2-μm membrane filter, and the filtrate was analyzed. Arginine, creatinine and guanidino

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acetic acid levels were determined using a spectroscopic liquid chromatograph employing a step-gradient system according to the method of Higashidate et al.[25] A fluorescence spectrometer, model FP-210 (Japan Spectroscopic Co., Tokyo, Japan) was used (excitation 365 nm, emission 495 nm) to determine creatinine, arginine and guanidino acetic acid levels.

Protein assay

Protein assays were carried out at all stages of the analyzing procedure according to the method of Lowry et al.[26] All samples were assayed in duplicate.

Statistical analysis

All results are expressed as mean ± SEM and analyzed statistically using a repeated measures analysis of variance (ANOVA) procedure. Differences among groups at different ages were tested using a Student's t test and Duncan multiple range tests for multiple comparisons.

Results

Effects of age and FO feeding on RS, xanthine oxidase-derived superoxide and cyclooxygenase-derived RS generation

The amounts of total RS generation in the four experimental groups are shown in Figure 1(A). The generation of total RS in the CO/Y, CO/O, FO/Y and FO/O groups were 36.53 ± 1.34, 43.45 ± 1.72, 25.82 ± 1.26, 23.53 ± 1.23 (nmole DCF/min/mg protein), respectively. Total RS generation increased with age by 18.9% in the corn oil-fed mice, while in the FO/Y, FO/O groups, total RS generation was reduced 29.3%, 45.8%, compared to CO/Y, CO/O, respectively. Figure 1(B) shows superoxide production from xanthine oxidase in the CO/Y, CO/O, FO/Y, FO/O groups with values of 10.8 ± 1.12,

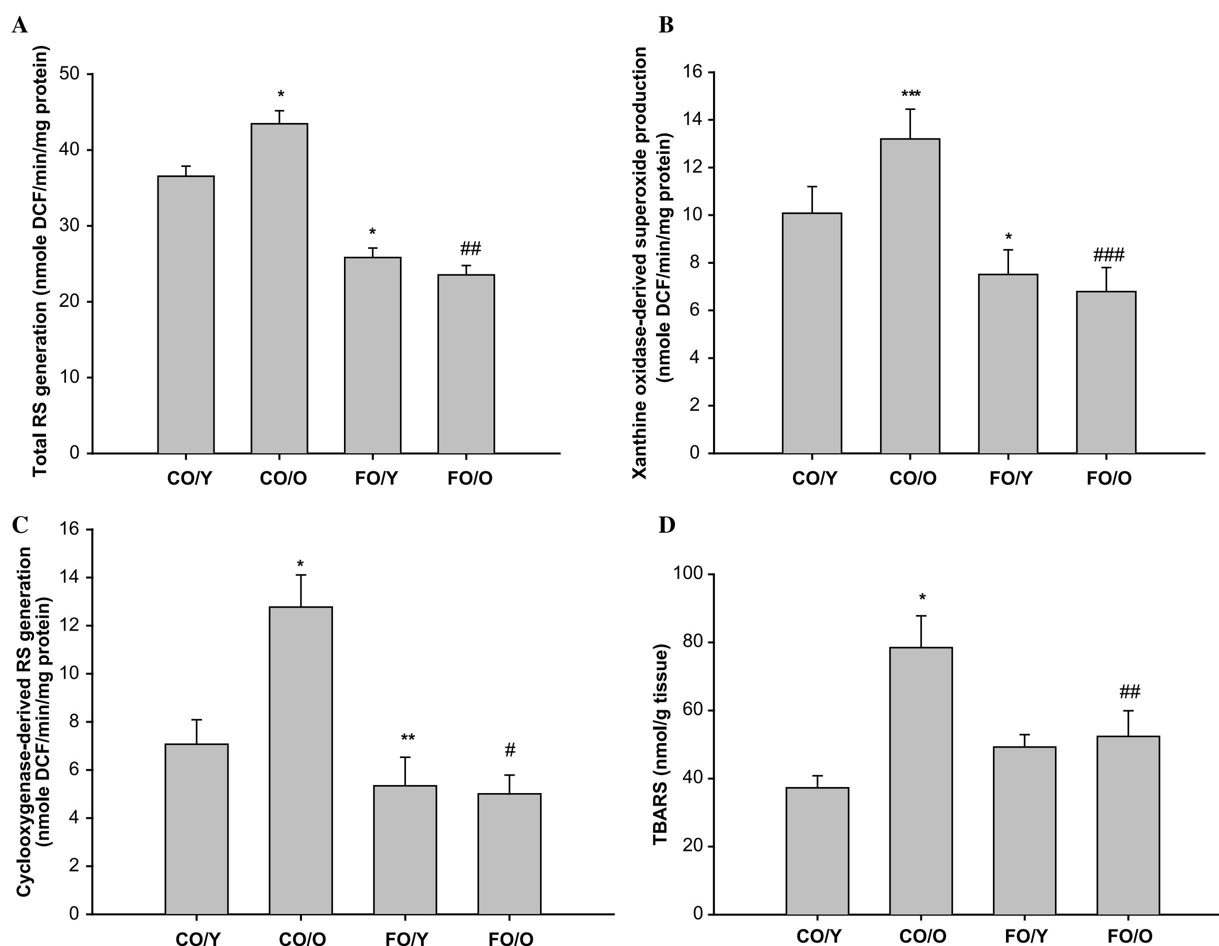

Figure 1. Effects of age and FO on the suppression of oxidative stress. (A) Changes in total RS generation by FO during aging. Results are expressed as the mean ± SEM. ($^{*}p < 0.05$ vs. CO/Y, $^{##}p < 0.01$ vs. CO/O) CO/Y, corn oil-fed young mice; CO/O, corn oil-fed old mice; FO/Y, FO-fed young mice; FO/O, FO-fed old mice. (B) Effects of age and FO on superoxide production. Results are expressed as the mean ± SEM. ($^{*}p < 0.05$ vs. CO/Y, $^{***}p < 0.001$ vs. CO/Y, $^{###}p < 0.001$ vs. CO/O) (C) Effects of age and FO on cyclooxgyenase-derived RS generation. Results are expressed as the mean ± SEM. ($^{*}p < 0.05$ vs. CO/Y, $^{**}p < 0.01$ vs. CO/Y, $^{#}p < 0.05$ vs. CO/O) (D) Effects of corn oil and FO feeding on hepatic TBARS levels. Results are expressed as the mean ± SEM. ($^{*}p < 0.05$ vs. CO/Y, $^{##}p < 0.01$ vs. CO/O).

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13.2 ± 1.25, 7.51 ± 1.03, 6.79 ± 1.01, respectively. Xanthine oxidase-derived superoxide production increased with age by 22.2% in corn oil-fed mice, while in the FO/Y, FO/O groups, xanthine oxidase-derived RS generation showed a significant decrease of 30.5 and 48.6%, compared to CO/Y, CO/O, respectively.

The values for cyclooxygenase-derived RS generation (Figure 1(C)) in the CO/Y, CO/O, FO/Y, FO/O groups were 7.07 ± 1.02, 12.77 ± 1.34, 5.34 ± 1.19, 5.01 ± 0.06 nmole DCF/min/mg protein, respectively. Corn oil-fed mice showed *two fold increase* of cyclooxygenase-derived RS generation during aging while FO reduced this generation by 24.6 and 60.8% in the FO/Y and FO/O groups, compared to the CO/Y and CO/O groups, respectively. Thus, as shown in Figure 1, the age-related increases of total RS, xanthine oxidase-derived superoxide and cyclooxygenase-derived RS generation observed in corn oil-fed control mice were effectively reduced by FO feeding.

Lipid peroxidation

As shown in Figure 1(D), TBARS levels increased during aging in the corn oil-fed groups and FO blunted this increase, indicating that age-related enhanced lipid peroxidation was effectively decreased by FO feeding.

Effects of age and FO on xanthine oxidase activity

As reported in Figure 2, xanthine oxidase activities in the CO/Y, CO/O, FO/Y and FO/O groups were 0.087 ± 0.009, 0.139 ± 0.017, 0.055 ± 0.009 and 0.042 ± 0.008 mU/mg protein, respectively. Xanthine oxidase activities were enhanced during aging, but were significantly reduced by FO feeding.

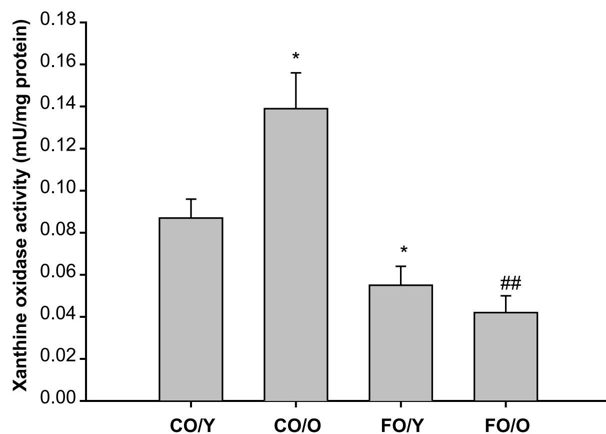

Figure 2. Effects of age and FO on xanthine oxidase activity. Results are expressed as the mean ± SEM. (* $p < 0.05$ vs. CO/Y, ## $p < 0.01$ vs. CO/O).

Changes in GSH and GSSG levels and the GSH/GSSG ratio

GSH and GSSG levels and their ratio are shown in Figure 3(A)–(C), respectively. The levels of GSH

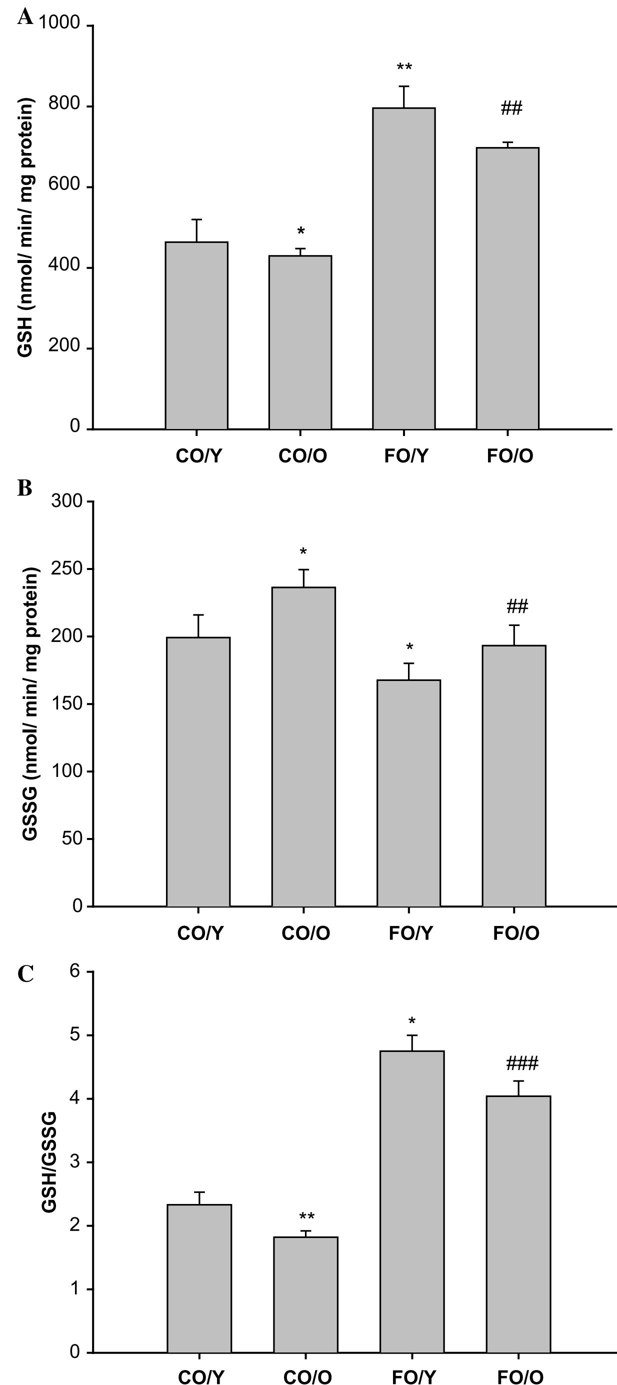

Figure 3. Effects of age and FO on oxidative stress markers. (A) Effects of age and FO on GSH levels. Results are expressed as the mean ± SEM. (* $p < 0.05$ vs. CO/Y, ** $p < 0.01$ vs. CO/Y, ## $p < 0.01$ vs. CO/O) DCF, dichloro fluorescein. (B) Effects of age and FO on GSSG levels. Results are expressed as the mean ± SEM. (* $p < 0.05$ vs. CO/Y, ## $p < 0.01$ vs. CO/O) (C) Effects of age and FO on the GSH/GSSG ratio. Results are expressed as the mean ± SEM. (* $p < 0.05$ vs. CO/Y, ** $p < 0.01$ vs. CO/Y, ### $p < 0.001$ vs. CO/O).

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were 463.51 ± 56.0, 429.50 ± 18.1, 795.90 ± 54.1, 697.33 ± 13.7 nmol/min/mg protein in CO/Y, CO/O, FO/Y, FO/O, respectively. The GSH level significantly decreased during aging; however, it was markedly increased (49.9, 62.4%) in the FO/Y and FO/O groups, compared to the CO/Y and CO/O groups. The levels of GSSG were 199.28 ± 16.7, 236.35 ± 13.2, 167.71 ± 12.4, 193.22 ± 15.1 nmol/min/mg protein in CO/Y, CO/O, FO/Y, FO/O, respectively. The GSSG level increased with age; however, the GSSG level was significantly reduced (16.5%) in the FO/O group compared to the CO/O group.

Figure 3(C) shows data on the ratio of reduced to oxidized glutathione (GSH/GSSG). The GSH/GSSG ratio showed a significant decrease with age, while it markedly increased, 103.9, 121.9% in FO/Y, FO/O groups, compared to the CO/Y and CO/O groups. Thus, reduced GSH levels and GSH/GSSG ratios with age were effectively increased by FO.

Superoxide dismutase activity

The effect of FO on changes in superoxide dismutase activity is shown in Figure 4(A). Superoxide dismutase activities were 0.208 ± 0.016, 0.182 ± 0.014, 0.458 ± 0.028, 0.443 ± 0.021 U/mg protein in the CO/Y, CO/O, FO/Y, FO/O groups, respectively. The decreased superoxide dismutase activities with age were markedly increased (120, 143%) in the FO/Y and FO/O groups compared to CO/Y and CO/O groups, respectively.

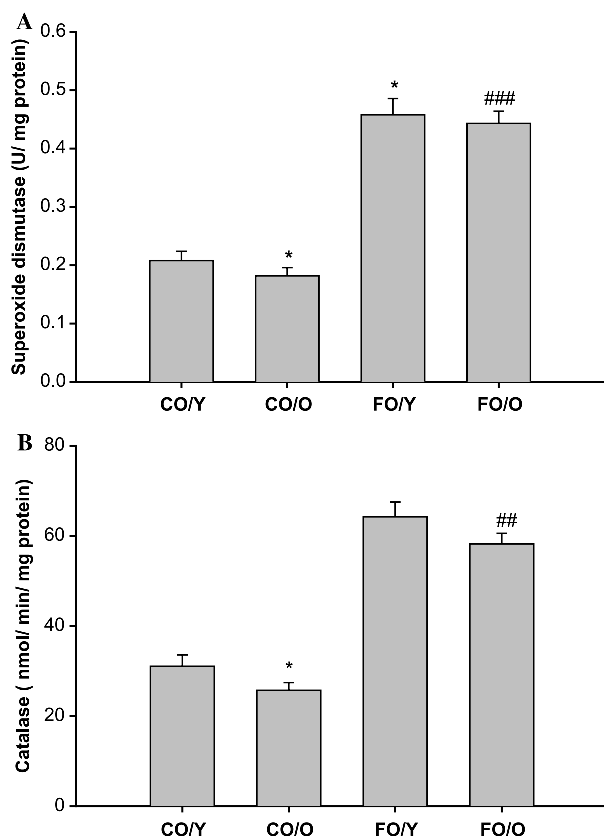

Figure 4. Modulation of antioxidant enzyme activity by FO with age. (A) Effects of age and FO on superoxide dismutase activity. Results are expressed as the mean ± SEM. (*$p < 0.05$ vs. CO/Y, ###$p < 0.001$ vs. CO/O) (B) Effects of age and FO on catalase activity. Results are expressed as the mean ± SEM. (*$p < 0.05$ vs. CO/Y, ##$p < 0.01$ vs. CO/O)

Catalase activity

The changes in catalase activity are shown in Figure 4(B). Catalase activities were 31.06 ± 2.52, 25.73 ± 1.73, 64.25 ± 3.25, 58.24 ± 2.33 mU/mg protein in the CO/Y, CO/O, FO/Y, FO/O groups, respectively. Catalase activity showed similar results to superoxide dismutase activity, with an age-related decrease that was significantly increased (106.9, 126.4%) in the FO/Y and FO/O groups, compared to the CO/Y and CO/O groups, respectively.

Glutathione peroxidase activity

The results from the glutathione peroxidase activity assay are shown in Figure 5(A). Glutathione peroxidase activities were 37.71 ± 1.37, 34.54 ± 0.56, 49.31 ± 1.23, 48.26 ± 1.43 mU/mg protein in the CO/Y, CO/O, FO/Y, FO/O groups, respectively. Glutathione peroxidase activity showed a slight but significant reduction with age, while the activity significantly increased (30.8, 39.7%) in the FO/Y and FO/O groups, as to CO/Y and CO/O, respectively.

Glutathione reductase activity

The changes in glutathione reductase activities are shown in Figure 5(B). Glutathione reductase activities were 45.91 ± 0.38, 37.62 ± 0.32, 59.34 ± 0.23, 55.32 ± 0.28 mU/mg protein in the CO/Y, CO/O, FO/Y and FO/O groups, respectively. Glutathione reductase activities showed a moderate decrease with age, while the activity significantly increased 20.5, 47.0%, in the FO/Y and FO/O groups compared to the CO/Y and CO/O groups, respectively.

Glutathione-S-transferase activity

Glutathione-*S*-transferase activities are posted in Figure 5(C). Glutathione-*S*- transferase activities were 110.25 ± 5.37, 100.40 ± 2.48, 162.23 ± 9.31, 158.43 ± 8.24 mU/mg protein in the CO/Y, CO/O, FO/Y and FO/O groups, respectively. Glutathione-*S*-transferase activity showed a slight reduction with age; whereas, it significantly increased 47.1, 57.8% in the FO/Y and FO/O groups, compared to the CO/Y and CO/O groups.

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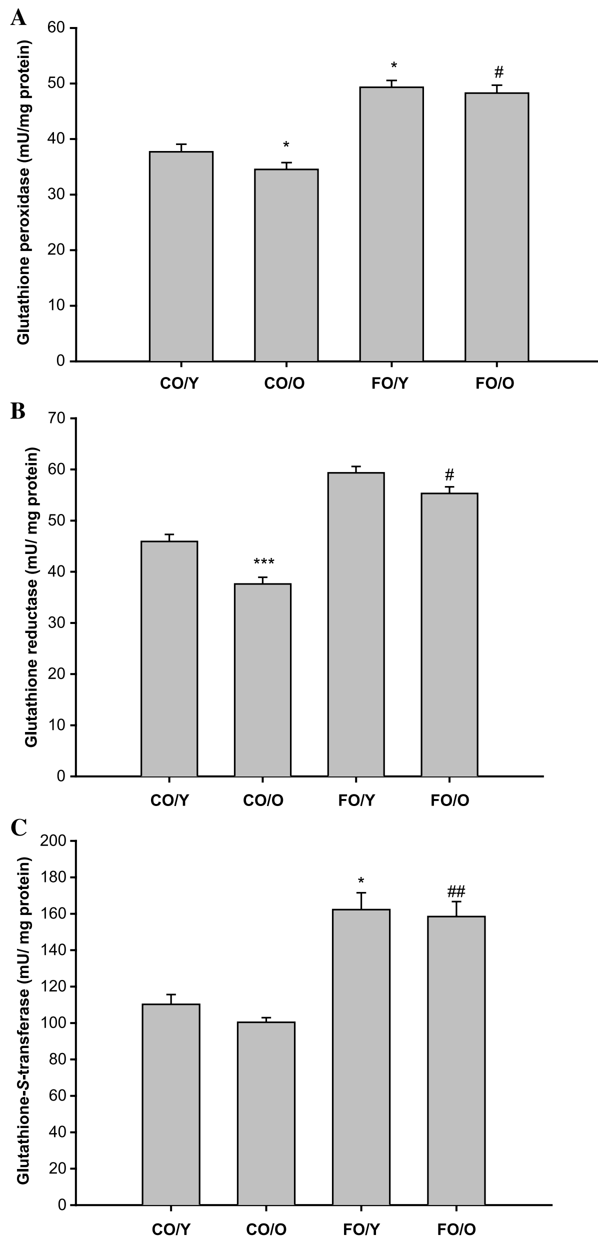

Figure 5. Effects of age and FO on antioxidant enzyme activities. (A) Effect of FO on glutathione peroxidase activity during aging. Results are expressed as the mean ± SEM. ($*p < 0.05$ vs. CO/Y, $^{\#}p < 0.05$ vs. CO/O) (B) Effect of FO on glutathione reductase activity during aging. Results are expressed as the mean ± SEM. ($***p < 0.001$ vs. CO/Y, $^{\#}p < 0.05$ vs. CO/O) (C) Effect FO on glutathione-*S*-transferase activity during aging. Results are expressed as the mean ± SEM. ($*p < 0.05$ vs. CO/Y, $^{\#\#}p < 0.01$ vs. CO/O).

Effect of dietary FO on creatinine levels with age

The changes in creatinine levels are posted in Table I. The creatinine levels showed a 54.5% increase in the corn oil-fed old mice; however, in the FO/Y and FO/O groups, the creatinine levels were decreased 58.9, 66.1%, compared to CO/Y and CO/O, respectively. Thus, the increased creatinine with age was effectively reduced by FO in both young and old mice.

Determination of arginine levels

The changes in arginine levels are shown in Table I. Arginine levels in the CO/Y, CO/O, FO/Y, FO/O groups were 0.60 ± 0.03, 0.31 ± 0.01, 0.57 ± 0.03, 0.54 ± 0.04 μg/mg protein, respectively. As shown in Table I, arginine levels in the corn oil-fed groups were reduced by 48.4% with age, while this decrease was blunted by FO. In the FO/O group, arginine levels markedly increased by 75.5% compared to the CO/O group. Thus, arginine levels were shown to reduce with age, while FO lessened the drop in old mice.

Effects of age and FO on guanidino acetic acid levels

The effects of FO on guanidino acetic acid levels are posted in Table I. The guanidino acetic acid levels showed a significant decrease (58.3%) with age in the corn oil-fed groups; but in the FO/O group, guanidino acetic acid levels were significantly increased (85.7%) compared to the CO/O group. Thus, FO increased guanidino acetic acid levels in old mice, and this beneficial effect was detected even in the FO/Y group.

Discussion

The major aim of this present study is to elucidate the beneficial action of dietary FO on the amelioration of renal insufficiency during aging. Oxidative stress in cells and tissues due to RS, like superoxide and lipid peroxidation, is known to cause irreversible damage [27]. In our mouse model, the redox status was disrupted due to the unregulated production of RS from the xanthine oxidase reaction, the cyclooxygenase-mediated prostaglandin pathway, and a weakened anti-oxidative defense system in the corn oil-fed mice during aging. The present study clearly showed FO feeding to restore the redox balance by the activation of key components of the cellular defense system, such as GSH, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase. In B/W mice, FO feeding seems to set up various strategies for an overall defense against oxidative stress.

Ceballos-Picot et al. previously reported that GSH (a major biological antioxidant) and the ratio of GSH and GSSG (an indicator of the magnitude of an oxidative stress insult) are closely correlated with renal insufficiency [28]. Our data showed that a reduced GSH/GSSG ratio, indicative of oxidative insult, was up-regulated by FO, thereby putatively attenuating renal insufficiency. Our findings that reduced GSH levels with age was effectively blunted by FO are consistent with findings on FO-fed Swiss-Webster mice [29].

FO-fed B/W mice are reported to higher levels of the renal antioxidant enzymes, namely catalase, glutathione peroxidase, superoxide dismutase-

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Table I. Modulation of hepatic guanidino levels by FO with age.

Group	Creatinine levels (μg/mg protein)	Arginine levels (μg/mg protein)	Guanidino acetic acid levels (μg/mg protein)
CO/Y	1.05 ± 0.15	0.60 ± 0.03	0.048 ± 0.003
CO/O	1.63 ± 0.13***	0.31 ± 0.01**	0.028 ± 0.001*
FO/Y	0.43 ± 0.11**	0.57 ± 0.03	0.050 ± 0.002**
FO/O	0.55 ± 0.08###	0.54 ± 0.04#	0.052 ± 0.003###

Results are expressed as mean ± SEM. Statistical significance: (*$p < 0.05$ vs. CO/Y, **$p < 0.01$ vs. CO/Y, ***$p < 0.001$ vs. CO/Y, #$p < 0.05$ vs. CO/O, ###$p < 0.001$ vs. CO/O).

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mRNAs compared to corn oil-fed mice [30]. In our study, superoxide dismutase, catalase, glutathione peroxidase activities significantly decreased with age in corn oil-fed mice, while their activities were increased by FO feeding. Similar results were reported on increased superoxide dismutase, catalase, and glutathione peroxidase activities by FO feeding compared to dietary corn oil and krill oil feeding, although the effect of age was not reported [31]. Current findings on the beneficial effects of FO feeding support our previous data showing that the age-related changes in redox status of rat serum [32] and the modulation of GSH and GSH-related antioxidant enzyme activities were reduced during the aging process in Fischer 344 rats [33]. Additionally, our data on the age-related increase of lipid peroxidation that was effectively attenuated by FO feeding in B/W mice are in agreement with the findings of Kakarla et al. [34] on increased lipid peroxidation and reduced antioxidant enzyme activity with age. It is important to note that our experimental conditions using life long feeding contrast with the short-term feeding of previous studies [35,36].

To clarify how FO feeding reduces lipid peroxidation, we attempted to estimate oxidative status. In the current study, we found that oxidative stress in the corn oil-fed mice was significantly increased with age as compared to the FO-fed mice. Our findings are consistent with other documented evidence that FO enriched with ϖ-3 fatty acids suppresses oxidative insult, as shown in the myocardium and coronary arteries of atherosclerotic monkeys [37]. The results of our present work also is in-line with an earlier report that FO-fed mice show lower TBARS and higher activities of superoxide dismutase, catalase, glutathione peroxidase, but without considering age, compared to corn oil-fed mice [38].

Among various intracellular sources of free radials, xanthine oxidase is one of the major superoxide production sources; and to our knowledge, the only data available at present are those showing that xanthine oxidase-induced RS have age-relate increases in rat kidney [39]. Our results clearly showed that FO supplementation suppresses xanthine oxidase-induced superoxide production, an important contributing factor for overall increased oxidative stress during aging.

Another way by which FO can reduce oxidative stress is by its ability to suppress the cyclooxygenase pathway, which is involved in RS generation (Figure 1(C)). Cycloooxygenase is a key pro-inflammatory enzyme that catalyzes prostaglandin synthesis eliciting RS generation [7]. RS are casually involved in inflammation, and FO's anti-inflammatory action is related to suppression of the production of pro-inflammatory cytokines, prostaglandin E_2, thromboxane B_2 [40], and the modulation of adhesion molecule expression. Our current data showing the suppression of cyclooxygenase-induced RS with age are consistent with findings reported on rat kidney [41].

A decrease in the radical scavenging enzymes, superoxide dismutase, catalase and glutathione peroxidase was reported to correlate to the formation of excessive radicals and the deterioration of defense mechanisms, thereby contributing to enhanced GC production [42]. In renal insufficiency, diet and aging are factors influencing the characteristic functional and morphologic modifications of the kidney and can greatly affect GC metabolism [43]. In addition, GC synthesis is also known to be influenced by the interaction of the liver [12]. Since the liver plays a central role in detoxification of various cytotoxic products, its normal function could be an important factor influencing changes in age-related GC metabolism caused by oxidative damage from increased oxidative stress with age.

In the present study, creatinine levels showing age-dependent increases suppressed by FO indicates that FO normalized the age-related modification of GC metabolism and maintained renal function. Creatinine, mostly synthesize in liver, increases during aging and might be due to age-related changes in catabolism and creatinine clearance. Guanidino acetic acid and arginine levels are reported to be strongly altered in uremic rats [44], and our findings in this study of increased creatinine and reduced guanidino acetic acid and arginine, which are indicators of the extent of the uremic condition, were similar. The important point is that FO feeding effectively modulated the severity of renal insufficiency. The reason for the difference may come from the fact the difference in fatty acids composition. As is enriched in $\omega 3$ fatty acids whereas the corn oil is enriched in $\omega 6$ fatty acids [45]. Especially, among various ω-3 fatty acids in FO,

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docosahexaenoic acid and eicosapentaenoic acid are expected as active component. In our recent work, we confirmed the beneficial effects of these two omega-3 fatty acids in isolated kidney cells (unpublished data).

In the past, some comments were made about FO's protective action possibly being related to its high vitamin E content [31]. However, based on the results from our present study, we suggest that the major reason the feeding of polyunsaturated fatty acids enriched FO showed beneficial effects (compared to corn oil feeding), was mainly due to its ability to sustain a well-maintained antioxidant defense system rather than the vitamin E content.

In summary, our present study demonstrated that FO feeding suppressed oxidative stress by reducing lipid peroxidation, RS generation and xanthine oxidase activity, and strengthened the antioxidant defense system such as GSH and antioxidant enzyme activities, and modulation of GC levels in the liver of aged B/W mice.

From the present results, we suggest that the suppression of oxidative stress and the enhancement of antioxidant defenses are likely plausible mechanisms underlying the protective effect of FO supplementation during aging in lupus nephritic mice.

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