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Does Consumption of Two Portions of Salmon Per Week Enhance the Antioxidant Defense System in Pregnant Women?

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Abstract

Salmon is a rich source of marine *n*-3 fatty acids, which may increase oxidative stress and, in turn, could affect the antioxidant defense system in blood plasma and erythrocytes of pregnant women. The Salmon in Pregnancy Study provided two meals of salmon per week to pregnant women from week 20 of gestation; the control group maintained their habitual diet low in oily fish. Higher selenium and retinol plasma concentrations were observed after dietary salmon supplementation. Besides, a concomitant increase in selenium and glutathione concentration as well as glutathione peroxidase and reductase activities were detected as pregnancy progressed. However, tocopherols, retinol, β-carotene, and coenzyme Q_{10} decreased in late pregnancy. Collectively, our findings lead to the hypothesis that increased farmed salmon intake may increase antioxidant defenses during pregnancy. Clinical trials identifier NCT00801502. *Antioxid. Redox Signal.* 16, 1401–1406.

Introduction

Reactive oxygen species (ROS) are generated during normal aerobic metabolism and increased levels are present during oxidative stress as a consequence of an imbalance between the formation and inactivation of these species. The antioxidant defense system (ADS) provides protection to avoid ROS-induced damage of cellular components. The capacity of this defense system is determined by a dynamic interaction between individual components, which include vitamins A, E, and β-carotene, coenzyme Q_{10} (CoQ_{10}), glutathione, and several antioxidant enzymes. The most important enzymatic antioxidants are superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx), with selenium as part of several selenium-proteins such as GPx.

Innovation

Marine *n*-3 fatty acids may increase oxidative stress. The Salmon in Pregnancy Study provided two meals of salmon per week to pregnant women from week 20 of gestation until parturition. An increase in antioxidant defenses was observed compared with a control group of pregnant women consuming their habitual diet.

Normal pregnancy is accompanied by a high metabolic demand and elevated requirements for tissue oxygen, which

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results in increased oxidative stress (8). If oxidative stress is a risk of pregnancy, then the mother's diet (specifically, the intakes of antioxidants, polyunsaturated fatty acids, and other unknown beneficial radical scavenging compounds) may play a role in modifying that process. The Salmon in Pregnancy Study (SiPS) is the first intervention trial with oily fish, rich in *n*-3 long chain-polyunsaturated fatty acids (*n*-3 LCPUFA), during pregnancy, and focuses on pregnant women whose offspring are at high risk of developing atopic diseases (6). Epidemiological data suggest that early exposure to oily fish (*e.g.*, during pregnancy or infancy) is associated with lower risk of atopy and allergic disorders in children (5). However, LCPUFAs are susceptible to peroxidation due to their high degree of unsaturation. Therefore, increased dietary consumption of *n*-3 LCPUFA might enhance oxidative stress with potential untoward effects during pregnancy. Thus, the hypothesis of the present study was that the consumption of two portions of salmon per week during gestation would increase oxidative stress and, in turn, would affect the ADS. Nonetheless, we have recently reported that increased salmon intake did not result into an increased oxidative stress in the same study population (by measurement of F_2-isoprostanes, 8-hydroxy-2'-deoxyguanosine, and lipid hydroperoxides) (4).

Indeed, the aim of this work, part of the SiPS, was to compare the ADS in plasma and erythrocytes of women with and without increased salmon consumption from week 20 of pregnancy. Particularly, we compared the concentration of plasma tocopherols, retinol, β-carotene, and CoQ_{10}, as well as glutathione and selenium, along with the activities of the key antioxidant enzymes CAT, SOD, GR, and GPx in erythrocytes.

Salmon Intake and Its Consequences for the ADS

As reported previously (6), the two groups did not differ in age, height, weight, or birth weight of offspring or with respect to skin prick test positivity (Supplementary Table S1; Supplementary Data are available online at www.liebertonline.com/ars). Further, pregnant women from SiPS increased their intakes of *n*-3 LCPUFA, enhanced their status of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and increased the status of EPA and DHA in their fetuses and neonates, after intake of two portions of salmon per week (6).

Erythrocyte antioxidant enzymatic activities and selenium and glutathione levels in pregnant women are shown in Table 1. Erythrocyte SOD, CAT, and GR activities did not differ between groups. However, GPx activity was significantly higher in the salmon group than in the control group ($p = 0.042$). Values in the salmon group were higher but not significantly different ($p = 0.409$, $p = 0.087$, and $p = 0.176$, at weeks 20, 34, and 38, respectively). When erythrocyte GPx activity at baseline (*i.e.*, week 20) was used as a covariate, GPx activity tended to be higher in the salmon group ($p = 0.063$). Unlike other dietary factors, intakes of oily fish and indeed of *n*-3 LCPUFA were by chance slightly, but significantly, different between groups at baseline (6). Erythrocyte selenium concentration was significantly higher in the salmon group than in the control group ($p < 0.001$). Comparison between groups at 20, 34, and 38 weeks of gestation showed significant differences for selenium, with values in the salmon group being higher ($p = 0.042$, $p < 0.001$, and $p = 0.018$, respectively). When week 20 selenium concentration was used as a covariate, significant differences between groups were retained ($p = 0.001$). Erythrocyte glutathione (reduce, oxidized, and total) concentrations were similar between groups. Erythrocyte GPx and GR activities along with selenium and glutathione (reduced, oxidized, and total) concentrations increased significantly during pregnancy (p ranging between < 0.001 and 0.029), while SOD and CAT activities did not change.

Plasma retinol, α- and γ-tocopherol, β-carotene, and CoQ_{10} levels in pregnant women are shown in Table 2. Plasma

Table 1. Erythrocyte Antioxidant Enzymatic Activities and Selenium and Glutathione Levels in Pregnant Women Consuming Their Habitual Diet (Control) or Consuming Salmon Twice Per Week (Salmon)

	Group						p-*Value*		
	Control (n = *54*)			*Salmon* (n = *54*)			*Source of variation*		
	20 weeks	*34 weeks*	*38 weeks*	*20 weeks*	*34 weeks*	*38 weeks*	*Group (G)*	*Time (T)*	*Interaction (G × T)*
CAT (nmol/L·g Hb)	2.96 ± 0.14	2.70 ± 0.12	2.85 ± 0.13	2.83 ± 0.11	2.99 ± 0.16	2.98 ± 0.13	0.345	0.904	0.156
SOD (U/mg Hb)	1.46 ± 0.09	1.42 ± 0.11	1.46 ± 0.13	1.38 ± 0.10	1.33 ± 0.10	1.43 ± 0.11	0.867	0.058	0.875
GR (U/g Hb)	2.70 ± 0.13	2.87 ± 0.14	3.30 ± 0.24	2.74 ± 0.13[a]	2.91 ± 0.12[a]	3.36 ± 0.23[b]	0.634	0.008	0.973
GPx (U/g Hb)	445 ± 21[a,b]	407 ± 21[a]	515 ± 34[b]	479 ± 19[a]	456 ± 26[a]	578 ± 34[b]	0.042*	< 0.001	0.740
Selenium (μg/kg RBC)	119 ± 5[a]	154 ± 5[b]	126 ± 4[a]	135 ± 6[a]	180 ± 6[b]	143 ± 6[a]	< 0.001*	< 0.001	0.118
GSH (mmol/L)	1.69 ± 0.03[a]	1.79 ± 0.04[b]	1.75 ± 0.04[a,b]	1.70 ± 0.03	1.78 ± 0.03	1.71 ± 0.05	0.640	0.029	0.409
GSSG (mmol/L)	0.16 ± 0.02[a]	0.18 ± 0.03[a]	0.23 ± 0.03[b]	0.20 ± 0.02	0.17 ± 0.02	0.22 ± 0.03	0.221	0.005	0.320
Total glutathione (mmol/L)	1.85 ± 0.03[a]	1.97 ± 0.04[b]	1.99 ± 0.04[b]	1.90 ± 0.03	1.95 ± 0.03	1.93 ± 0.04	0.882	0.007	0.057

Values are expressed as mean ± standard error of mean.

*Statistically significant differences ($p < 0.05$) between groups using a general linear model of variance. When GPx activity and selenium concentration at week 20 were used as a covariate, significant differences between groups were seen only for selenium ($p = 0.001$). Different letters denote statistical differences between time points within the study groups ($p < 0.05$).

CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; RBC, red blood cell; SOD, superoxide dismutase.

Table 2. Plasma Retinol, α- and γ-Tocopherol, β-Carotene, and Coenzyme Q_{10} Levels in Pregnant Women Consuming Their Habitual Diet (Control) or Consuming Salmon Twice Per Week (Salmon)

	Group						p-*Value*		
	Control (n = 54)			*Salmon* (n = 54)			*Source of variation*		
	20 week	*34 week*	*38 week*	*20 week*	*34 week*	*38 week*	*Group (G)*	*Time (T)*	*Interaction (G×T)*
α-tocopherol (*μM*)	29.66 ± 1.50	27.35 ± 2.15	26.20 ± 1.52	28.28 ± 1.47	26.38 ± 1.97	25.20 ± 1.50	0.725	0.178	0.254
γ-tocopherol (*μM*)	2.90 ± 0.19[a]	2.19 ± 0.23[b]	1.56 ± 0.20[b]	2.66 ± 0.17[a]	2.00 ± 0.23[a]	1.39 ± 0.13[b]	0.456	< 0.001	0.599
β-carotene (*μM*)	1.71 ± 0.15[a]	1.60 ± 0.15[a]	1.46 ± 0.14[b]	2.04 ± 0.14[a]	2.04 ± 0.15[a]	1.72 ± 0.16[b]	0.126	< 0.001	0.801
Retinol (*μM*)	1.06 ± 0.06[a]	0.85 ± 0.04[b]	0.86 ± 0.04[b]	1.20 ± 0.06[a]	1.06 ± 0.06[a,b]	1.07 ± 0.06[b]	0.002*	0.002	0.861
CoQ_{10} (*μM*)	0.44 ± 0.03[a]	0.21 ± 0.01[b]	0.25 ± 0.01[c]	0.42 ± 0.03[a]	0.21 ± 0.01[b]	0.25 ± 0.02[c]	0.471	< 0.001	0.353
$CoQ_{10}H_2$ (*μM*)	1.07 ± 0.06[a]	0.95 ± 0.11[a,b]	0.70 ± 0.08[b]	0.99 ± 0.07[a]	0.84 ± 0.11[a,b]	0.61 ± 0.06[b]	0.440	< 0.001	0.833
Total CoQ_{10} (*μM*)	1.52 ± 0.08[a]	1.18 ± 0.11[b]	0.95 ± 0.11[b]	1.42 ± 0.10[a]	1.06 ± 0.11[a,b]	0.87 ± 0.07[b]	0.447	< 0.001	0.741
α-tocopherol/total lipids (ng/mg)	2993 ± 158[a]	2630 ± 203[b]	2313 ± 123[b]	2985 ± 150[a]	2730 ± 209[a,b]	2288 ± 106[b]	0.781	0.004	0.169
γ-tocopherol/total lipids (ng/mg)	293 ± 20[a]	212 ± 23[b]	139 ± 19[b]	279 ± 16[a]	205 ± 23[a]	125 ± 11[b]	0.707	< 0.001	0.486
β-carotene/total lipids (ng/mg)	221 ± 20[a]	198 ± 18[a]	164 ± 17[b]	272 ± 18[a]	263 ± 20[a]	200 ± 18[b]	0.124	< 0.001	0.565
Retinol/total lipids (ng/mg)	72 ± 5[a]	56 ± 3[b]	51 ± 2[b]	86 ± 4[a]	72 ± 4[b]	67 ± 4[b]	0.001*	< 0.001	0.859
CoQ_{10}/total lipids (ng/mg)	92 ± 6[a]	41 ± 2[b]	44 ± 3[b]	91 ± 7[a]	43 ± 2[b]	47 ± 3[b]	0.961	< 0.001	0.412
$CoQ_{10}H_2$/total lipids (ng/mg)	219 ± 14[a]	186 ± 21[b]	127 ± 16[b]	209 ± 16[a]	175 ± 23[a]	114 ± 11[b]	0.652	< 0.001	0.645
Total CoQ_{10}/total lipids (ng/mg)	311 ± 19[a]	229 ± 22[b]	171 ± 17[b]	299 ± 21[a]	221 ± 24[a]	161 ± 13[b]	0.740	< 0.001	0.535

Values are expressed as mean ± standard error of mean.

*Statistically significant differences ($p < 0.05$) between groups using a general linear model of variance. When retinol at week 20 was used as a covariate, significant differences were retained ($p = 0.008$). Different letters denote statistical differences between time points within the study groups ($p < 0.05$).

CoQ_{10}, coenzyme Q_{10}; $CoQ_{10}H_2$, reduced coenzyme Q_{10}.

retinol was significantly higher in the salmon group than in the control group ($p = 0.002$). Significant differences were found between groups at weeks 34 and 38 for retinol, with higher values in the salmon group ($p = 0.032$ and 0.003, respectively). Significant differences between groups were retained when week 20 retinol was used as a covariate ($p = 0.008$). None of the other nonenzymatic antioxidants analyzed differed significantly between groups. During pregnancy, α- and γ-tocopherol, retinol, β-carotene, and CoQ_{10} (oxidized, reduced, and total) decreased significantly (p ranging from 0.002 to < 0.001).

No interactions were seen for any of the parameters determined when group and time effects were analyzed together.

Erythrocyte SOD, CAT, GR, and GPx activities, together with glutathione concentrations, remained similar after salmon supplementation, although activation of these protective systems may occur by LCPUFAs (1). A difference in selenium concentration was seen between groups at baseline, because oily fish intake was by chance slightly, but significantly, different between groups at week 20 (6). However, we also found significantly higher erythrocyte selenium concentrations in the salmon group in mid-pregnancy, reflecting an effect of salmon intake on selenium concentration. This elevated selenium plasma level might be an underlying factor to the increase observed for GPx activity in the salmon group, despite not being significant. Dietary selenium intake or supplementation has been related to higher antioxidant enzymatic activity and lower lipid peroxidation. In contrast, selenium deficiency leads to a decline in tissue levels of selenium-dependent antioxidant enzymes and thereby oxidative stress conditions develop (9). Several authors have indicated that supplementation with *n*-3 LCPUFA enhanced oxidative stress at dietary intakes higher than that contained in the fish supplied in our study (the equivalent of about 500 mg/day). In this way, Filaire *et al.* (3) observed that *n*-3 LCPUFA supplementation increased oxidative stress; however, it decreased when antioxidants were added in combination with *n*-3 LCPUFAs. In fact, as mentioned earlier, we did not find increased oxidative stress in our study population after salmon supplementation (4). Together with *n*-3 LCPUFA, oily fish supplies antioxidants, which may prevent increased oxidative stress after increased fish intake in pregnant women. In the present study, plasma vitamin E levels were similar in both control and salmon-supplemented groups. Since vegetable oils are the main source of vitamin E, the quantity of this vitamin provided by fish was relatively small. A similar behavior to vitamin E was found for β-carotene, without differences between groups. Beta-carotene is the precursor of retinol, essentially important for growth and development of cells and tissues. Plasma retinol concentration was significantly higher in the salmon group than in the control group despite that retinol concentration was also different between groups at baseline (6). The higher retinol plasma concentration in the salmon-supplemented group might be explained as a consequence of intake of salmon, since this fish is known to be rich in retinol. CoQ_{10} levels were not affected by salmon intake. Therefore, *n*-3 LCPUFA may have both pro-oxidant and antioxidant properties depending on experimental conditions, dosage, and the antioxidant contents of the background diet and of supplements provided.

Another important finding of this study was that erythrocyte GPx and GR activities along with glutathione and selenium concentration increased significantly in mid and late pregnancy. Besides, SOD and CAT activities were not different across the three time points studied. Information concerning the changes of these enzymes during normal pregnancy is conflicting and scarce. According to our results, it seems that GPx and GR enzymes have a major role in the elimination of blood peroxides generated during pregnancy. The enhanced maternal components of the ADS observed in our study population suggest a response of the antioxidant defenses to pregnancy-induced increases in oxidative stress (8). Our results also showed a significant decrease in all plasma nonenzymatic antioxidant concentrations beyond week 20 of pregnancy. This decline has been reported by others (7) and might be an indication of a higher level of oxidative stress toward the end of gestation as well as the increased maternal blood volume. The role of retinol and its metabolites in reproduction and embryonic development have been clearly established (2), and low cord and maternal serum retinol have been associated with poor vitamin A status, which in turn may affect fetal growth. The decline in plasma retinol levels during late pregnancy may reflect its enhanced transfer to and utilization by the fetus. Preservation of fetal retinol levels at the expense of a decline on the maternal side is of pivotal importance for appropriate pregnancy outcome and increased consumption of salmon could contribute to it.

Concluding Remarks and Future Directions

Under the conditions of the SiPS, our findings lead to the conclusion that increased consumption of salmon may raise selenium and retinol concentrations in pregnant women, with a concomitant increase in GPx and GR activities and selenium and glutathione concentrations, throughout pregnancy. This elevation in antioxidant defenses might be helpful to prevent and/or reduce additional oxidant stress during pregnancy. However, the influence of *n*-3 LCPUFA supplementation is still controversial and further research is needed to explore how fish and their *n*-3 PUFAs may affect oxidative stress.

Notes

Subjects and methodology

Subjects. The study design, the subjects, and their characteristics, aspects of their diet, and their compliance have been described in detail elsewhere (6) (Supplementary Table S1). The SiPS is a single-blind, randomized, controlled trial of increased consumption of farmed salmon by pregnant women from week 20 of gestation until the end of their pregnancy. Supplementary Figure S1 shows the progress of women through the study. In brief, a total of 123 pregnant women in the area of Princess Anne Hospital (Southampton, United Kingdom) were enrolled in the study. Inclusion criteria were age 18 to 40 years; < 19 weeks of gestation; healthy uncomplicated singleton pregnancy; baby at risk of atopy (one or more first-degree relatives of the baby affected by atopy, asthma, or allergy by self-report); consuming < 2

portions of oily fish per month excluding tinned tuna; and not using fish oil supplements currently or in the previous 3 months. All procedures were approved by the Southampton and South West Hampshire Research Ethics Committee (07/Q1704/43). The study was conducted according to the principles of the Declaration of Helsinki and all women gave written informed consent. The SiPS is registered at www.clinicaltrials.gov (NCT00801502).

Study design. Recruited women were randomly assigned to one of two groups; randomization was according to a random number table. Women in the control group ($n = 61$) were asked to continue their habitual diet, and women in the salmon group ($n = 62$) were asked to incorporate two portions of farmed-salmon (150 g/portion) into their diet per week from study entry (week 20) until they gave birth. Farmed salmon for use in the SiPS was raised using dietary ingredients selected to contain low levels of contaminants. Each 150 g salmon portion contained (on average) 30.5 g protein, 16.4 g fat, 0.57 g EPA, 0.35 g DPA, 1.16 g DHA, 3.56 g total *n*-3 PUFA, 4.1 mg α-tocopherol, 1.6 mg γ-tocopherol, 6 μg vitamin A, 14 μg vitamin D_3, and 43 μg selenium. Thus, two portions of salmon per week would typically provide 3.45 g EPA + DHA, 28 μg vitamin D_3, and 86 μg selenium. Contaminants contributed < 12.5% of the FAO/WHO provisional tolerable weekly intake for dioxin and dioxin-like polychlorinated biphenyls, < 11.5% for arsenic, < 0.00000008% for cadmium, 0.0000025% for mercury, and < 0.00000002% for lead.

Fifteen subjects were not able to complete the study for various reasons (delivery before appointment, cancelled because of feeling tiredness, busy, or some sort of injury), leaving a total of 54 subjects in the control group and 54 subjects in the salmon group. As reported previously (6), the two groups did not differ in age, height, weight, or birth weight of offspring or with respect to skin prick test positivity (Supplementary Table S1). No adverse events or negative health effects were observed or reported during this study.

Fasting maternal venous blood samples for the laboratory analyses were collected at week 20 of gestation, before the intervention started, at week 34 and at week 38. After centrifugation, aliquots of plasma and washed erythrocytes were frozen immediately and stored at −80°C until analyzed. Women also completed a 100-item food frequency questionnaire covering food intake over the preceding 12 weeks.

Analytical procedures

Determination of enzymatic antioxidant activities. Erythrocyte CAT, SOD, and GR activities were assayed spectrophotometrically and expressed as nmol/(L·g Hb), U/mg Hb, and U/g Hb, respectively. Erythrocyte GPx activity was determined spectrophotometrically by the coupled enzyme procedure with tert-butyl hydroperoxide as substrate and is expressed as U/g Hb. Hemoglobin concentration in the blood samples was determined spectrophotometrically by the colorimetric cyanmethemoglobin method, using Sigma Diagnostic reagents.

Determination of nonenzymatic antioxidant concentrations. Plasma concentrations of α- and γ-tocopherol, retinol, and CoQ_{10} were determined by high-pressure liquid chromatography coupled to an electrochemical detector (HPLC-EC), after extraction with 1-propanol. β-carotene was also determined after extraction with 1-propanol in a HPLC system attached to a multi-wavelength ultraviolet detector set at 450 nm. All compounds were identified by predetermining the retention times of individual standards. As all of them are lipid soluble, concentrations are given by ml of plasma and in relation to total plasma lipids.

Plasma triacylglycerols, phospholipids, and total cholesterol were measured spectrophotometrically using commercial enzymatic assay kits from Spinreact (Girona, Spain) (*Triglycerides*, Ref. 1001311, *Phospholipids*, Ref. 1001140; *Total cholesterol*, Ref. 1001091), according to the manufacturer's protocols, and the results were standardized using standard solutions for triacylglycerols, phospholipids, and total cholesterol calibration.

Erythrocyte selenium was determined by inductively coupled plasma mass spectrometry on an Agilent 7500 ICPMS. Selenium (^{78}Se) concentration in red blood cell samples was calculated using an external standard calibration. Frozen red blood cells (RBCs) were thawed, and aliquots of ~0.5 g were weighed. Selenium is expressed as μg Se/kg RBC.

Erythrocyte glultathione content was measured by HPLC with fluorescence detection at 420 nm, and is expressed as μmol/g Hb.

Statistical analysis

Results are given as mean ± standard error of mean. Conformity to a normal distribution was examined using the Kolmogorov-Smirnov test. It was calculated that a sample size of 50 women per group would have 93% power with a type I error of alpha < 0.05. Differences for each variable between treatment groups over time were evaluated using a general linear model of variance for repeated measures. *A posteriori* Bonferroni tests were performed to evaluate specific differences within groups between the considered gestational periods. When initial values were different, the statistical analyses were corrected using baseline values as a covariant. Mean comparisons for GPx, selenium, and retinol at each gestational period were determined by *a posteriori* Bonferroni tests. All statistical analyses were performed with the Statistical Package of Social Science (SPSS) 15.0 for Windows. *p*-values < 0.05 were considered statistically significant.

(A fully referenced methodology may be viewed as Supplementary Data online.)

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Abbreviations Used

ADS = antioxidant defense system
CAT = catalase
CoQ_{10} = coenzyme Q_{10}
DHA = docosahexaenoic acid
EPA = eicosapentaenoic acid
FFQ = food frequency questionnaire
GPx = glutathione peroxidase
GR = glutathione reductase
GSH = reduced glutathione
GSHt = total GSH
GSSG = oxidized glutathione
HPLC-EC = high-pressure liquid chromatography coupled to an electrochemical detector
H_2O_2 = hydrogen peroxide
MPA = metaphosphoric acid
n-3 LCPUFA = *n*-3 long chain-polyunsaturated fatty acid
NADPH = reduced nicotinamide adenine dinucleotide phosphate
OPA = *ortho*-phthalaldehyde
PCB = polychlorinated biphenyl
RBC = red blood cell
ROS = reactive oxygen species
SiPS = Salmon in Pregnancy Study
SOD = superoxide dismutase