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Does Increased Intake of Salmon Increase Markers of Oxidative Stress in Pregnant Women? The Salmon in Pregnancy Study

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Abstract

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. Salmon is a rich source of marine n-3 fatty acids. Since marine n-3 fatty acids may increase oxidative stress, we investigated whether increased salmon consumption could affect markers of oxidative stress in mid and late pregnancy. Urinary 8-iso-prostaglandin $F_{2\alpha}$, urinary 8-hydroxy-2′-deoxyguanosine, and plasma lipid peroxide concentrations did not change from week 20 to 38 of pregnancy and were not altered by increased consumption of salmon. Thus, increased intake of salmon during pregnancy does not increase oxidative stress, as judged by the markers of oxidative damage to lipids and DNA measured herein. *Antioxid. Redox Signal.* 15, 2819–2823.

Introduction

Oxidative stress is considered to cause damage to macromolecules and cells and to contribute to various pathologies. Polyunsaturated fatty acids are susceptible to oxidative damage (7), and several markers of such damage have been described including F_2-isoprostanes and lipid peroxides (LPO). F_2-isoprostanes are a family of prostaglandin derivatives generated *in vivo* by free radical-induced peroxidation of arachidonic acid (6). One major F_2-isoprostane, 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$), is increased in several situations associated with oxidative stress, including atherosclerosis, diabetes, obesity, cigarette smoking, neurodegenerative diseases, and asthma, and is currently regarded as one of the most reliable biomarkers of *in vivo* oxidative stress (1, 8). The extent of lipid peroxidation can also be estimated by the amount of primary peroxidation products such as LPO. Another marker of oxidative stress, 8-hydroxy-2′-deoxyguanosine (8-OHdG), is produced by oxidation of the nucleoside deoxyguanosine and is subsequently excreted directly into the urine, and is a sensitive marker to study oxidative DNA damage.

Increased intake of n-3 long chain polyunsaturated fatty acids (n-3 LCPUFA), which are highly unsaturated, as dietary supplements has been reported to result in enhanced lipid peroxidation in healthy adults, in smokers, in type-2 diabetics, in myocardial infarction survivors, and in pregnant women. Oily fish, such as salmon, are a rich dietary source of n-3 LCPUFA, eicosapentaenoic acid (EPA, 20:5 n-3), and docosahexaenoic acid

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. No increase in markers of oxidative stress was seen compared with a control group of pregnant women consuming their habitual diet.

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(DHA, 22:6 n-3). Despite their potential to be nonenzymatically peroxidized, n-3 LCPUFA are associated with benefits to human health especially with regard to early visual and neural development and reducing cardiovascular morbidity and mortality (2). As a result of this there are recommendations to increase intake of n-3 LCPUFA especially from oily fish (9). In the United Kingdom it is recommended that all individuals consume at least one portion of oily fish per week and the guideline range for pregnant women is one or two portions per week (9). These recommendations are based mainly upon the provision of health promoting n-3 LCPUFA and do not consider the possible effect of increases in n-3 LCPUFA on oxidative damage that may not be beneficial to health.

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 (4). The Salmon in Pregnancy Study (SiPS) is the first intervention trial with oily fish during pregnancy and focuses on pregnant women whose offspring are at increased risk of developing atopic diseases (5). The SiPS provided an opportunity to investigate for the first time whether increased oily fish intake in pregnancy increases oxidative stress, as indicated by increased concentrations of 8-iso-$PGF_{2\alpha}$ and 8-OHdG in urine and of LPO in plasma.

Salmon Intake and Its Consequences for Oxidative Stress

Results for the different indicators of oxidative stress are shown in Table 1. Urinary concentrations of 8-iso-$PGF_{2\alpha}$ were not significantly altered during pregnancy. However, there was a group effect on urinary 8-iso-$PGF_{2\alpha}$ concentrations, which were significantly lower in the salmon group than in the control group ($p=0.021$). Comparison between the two groups at 20, 34, and 38 weeks showed a trend toward a difference, with values in the salmon group being lower ($p=0.05$, $p=0.164$, and $p=0.094$, at weeks 20, 34, and 38, respectively). When 8-iso-$PGF_{2\alpha}$ concentrations at baseline (*i.e.*, week 20) were used as a covariant, the group effect failed to remain significant ($p=0.350$). Thus, urinary 8-iso-$PGF_{2\alpha}$ did not change from week 20 to 38 of pregnancy and was not affected by increased intake of salmon. It is not clear why urinary 8-iso-$PGF_{2\alpha}$ concentrations were lower in the salmon group at week 20 of pregnancy: age and smoking habits were not different between groups (Table 2) and body mass index was not different, although it tended to be lower in the salmon group (Table 2). Although the women were randomized to the two groups, there may have been some small dietary difference between those groups. Plasma LPO and urinary 8-OHdG levels did not change during pregnancy and were not different between the two groups (Table 1).

Table 2. General Characteristics of Women at 20 Weeks of Pregnancy and Birthweights of Their Neonates According to Group (Control *vs.* Salmon)

	Control group (n = *61*)	*Salmon group* (n = *62*)
Pregnant women		
Age at entry (years)	28.4 ± 0.6	29.5 ± 0.5
Body height (cm)	165.6 ± 0.9	165.4 ± 0.8
Body weight (kg)	71.4 ± 2.0	67.4 ± 1.6
Body mass index (kg/m^2)	26.0 ± 0.6	24.7 ± 0.6
Smoking habits	*n*	*n*
Never	31	34
Past	21	24
Current	9	4
Offspring		
Birth weight (g)	3425 ± 82	3449 ± 72

It has been reported that F_2-isoprostanes are increased in pregnant women compared to nonpregnant women, suggesting that normal pregnancy may be a state of mild-oxidative stress (1). In a Japanese population, F_2-isoprostane levels were higher in plasma and urine in the third trimester of pregnancy compared to the nonpregnant state. Further, a progressive increase in urinary F_2-isoprostanes was seen throughout uncomplicated pregnancy in Swedish women. In a recent study, an increase of plasma total isoprostanes in the third trimester of pregnancy compared to nonpregnant control women was observed. Nevertheless, some other studies reported that F_2-isoprostane levels do not change significantly during pregnancy. Differences between studies may be due to the method used for determination of isoprostanes or related to the exact timing of sample collection during gestation. In the present study there was no increase in F_2-isoprostanes from week 20 of pregnancy. Thus, any effect of pregnancy on

Table 1. Lipid and DNA Oxidation Biomarkers in Pregnant Women at Different Time Points (Weeks 20, 34, and 38 of Gestation) and According to Group (Control *vs.* Salmon)

	Group						p-*Value*		
	Control			*Salmon*			*Source of variation*		
	20 weeks	*34 weeks*	*38 weeks*	*20 weeks*	*34 weeks*	*38 weeks*	*Group (G)*	*Time (T)*	*Interaction G×T*
Isoprostanes/creatinine (nmol/mmol)	0.86 ± 0.04	0.92 ± 0.05	1.05 ± 0.07	0.80 ± 0.04	0.90 ± 0.05	0.90 ± 0.05	0.021[a]	0.402	0.813
LPO (μmol/L)	1163 ± 61	1182 ± 66	1138 ± 87	979 ± 43	1065 ± 51	967 ± 57	0.120	0.441	0.443
8-OHdG/creatinine (ng/mg)	39.83 ± 2.92	46.83 ± 3.80	38.90 ± 3.95	45.82 ± 3.67	39.88 ± 2.90	37.43 ± 2.65	0.775	0.223	0.145

Values are expressed as mean ± standard error of mean.

[a]Statistically significant difference ($p<0.05$) between groups using a general linear model. When F_2-isoprostanes at week 20 were used as a covariant, no significant differences were seen between groups ($p=0.350$). $n=54$ for the salmon supplemented group; $n=54$ for the control group.

8-OHdG, 8-hydroxy-2′-deoxyguanosine; LPO, lipid peroxide.

increasing oxidative stress (as indicated by urinary 8-iso-$PGF_{2\alpha}$) may have occurred before week 20 of gestation.

Cross-sectional studies have reported higher levels of LPO and/or thiobarbituric acid reactive substances (TBARS) during pregnancy. Longitudinal studies have shown that levels of lipid hydroperoxides and/or TBARS increase with advancing gestational age. However, in the current study the levels of plasma LPO did not differ significantly between time points and were unaffected by eating salmon. Once again, this suggests that any effect of pregnancy on oxidative stress affecting lipids may be established by week 20 of pregnancy.

Hung *et al.* (3) measured urinary 8-OHdG in women with uncomplicated pregnancies and found increased levels with advancing gestation. However, in the current study there was no significant change in urinary 8-OHdG levels from week 20 of pregnancy.

Thus, the current study suggests that any effect of pregnancy on oxidative stress in healthy uncomplicated pregnancies is established by week 20 of gestation and that increased consumption of salmon beyond that time point, which is consistent with current United Kingdom advice, does not increase oxidative stress as detected by the three markers used herein.

In the current study eating salmon and thereby increasing intake of n-3 LCPUFA (by the equivalent of about 500 mg/day) during pregnancy was not associated with increased oxidative stress as indicated by urinary 8-iso-$PGF_{2\alpha}$ and plasma LPO concentrations. There was a lower urinary 8-iso-$PGF_{2\alpha}$ concentration in the salmon group, but this was due to this difference being apparent at study entry (*i.e.*, at week 20 of pregnancy). Studies of n-3 LCPUFA supplementation in nonpregnant adults have reported an increase in markers of lipid peroxidation such as plasma TBARS, and at least one study in pregnant women has also demonstrated this. However, the current study suggests that increasing n-3 LCPUFA intake from salmon in pregnant women does not raise a concern about increased oxidative stress, at least beyond week 20 of pregnancy. There are at least three reasons why n-3 LCPUFA from salmon twice per week does not increase the concentrations of markers of oxidative stress, whereas n-3 LCPUFA supplements can. The first reason is related to dose or intake level. In the SiPS the n-3 LCPUFA were provided from salmon on 2 days per week resulting in an equivalent daily intake of about 500 mg EPA plus DHA per day. Many studies using fish oil supplements have provided several grams of EPA plus DHA each day, providing a much greater amount of substrate for *in vivo* lipid peroxidation. Second, there may be an inherent difference between providing n-3 LCPUFA in a purified oil in capsules compared with providing them within a food matrix as was done in SiPS. Third, capsules have been given in studies usually without any change in background diet and so the increase in n-3 LCPUFA intake occurs without a change in intake of antioxidant nutrients, other than vitamin E, which is typically present in fish oil capsules. However in SiPS, the salmon provided a range of nutrients involved in antioxidant defenses (see Ref. 5), including α-tocopherol, γ-tocopherol, vitamin A, and selenium, making a significant contribution to increased intake of these nutrients. Thus, in addition to increased intake of n-3 LCPUFA, the salmon used in SiPS provided an increased intake of antioxidant nutrients, which might act to counter any effect of n-3 LCPUFA in increasing oxidative stress.

Concluding Remarks and Future Directions

Under the conditions of the SiPS, our observations lead to the conclusions that increases in oxidative stress do not occur beyond week 20 of pregnancy and that increased intake of oily fish from 20 weeks of gestation until the time of delivery, which is consistent with current United Kingdom advice, does not enhance oxidative stress as indicated by the three markers measured herein. Apart from the risk of atopy to their unborn child, the women studied in the SiPS were healthy and had uncomplicated pregnancies; the role of oxidative stress in more complicated pregnancies and the impact of increased oily fish intake in such situations requires future study. Further, the effect of oily fish from earlier in and before pregnancy requires further study.

(A fully referenced discussion may be viewed as Supplementary Data; Supplementary Data are available online at www.liebertonline.com/ars)

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 (5). 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. Exclusion criteria were age <18 or >40 years; >19 weeks of gestation; no first-degree relatives of the baby affected by atopy, asthma, or allergy; consuming >2 portions of oily fish (excluding tinned tuna) per month; use of fish oil supplements within previous 3 months; participation in another research study; known diabetic; presence of any auto-immune disease; learning disability; terminal illness; and mental healthy problems. 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. 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 were 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 docosapentaenoic acid, 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. The

contaminants provided <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 tired, busy, or some sort of injury), leaving a total of 54 subjects in the control group and 54 subjects in the salmon group. The two groups did not differ in age, height, weight, or birth weight of offspring as earlier reported (5) (Table 2).

Fasting blood and first morning urine samples were collected at weeks 20, 34, and 38 of gestation. Samples were stored frozen at −70°C until analysis.

Measurement of urinary 8-iso-PGF$_{2\alpha}$ concentrations

Urinary samples were analyzed for 8-iso-PGF$_{2\alpha}$ by a highly specific and validated radioimmunoassay as described previously. The cross-reactivity of the 8-iso-PGF$_{2\alpha}$ antibody with 15-keto-13, 14-dihydro-8-iso-PGF$_{2\alpha}$, 8-iso-PGF$_{2\beta}$, PGF$_{2\alpha}$, 15-keto-PGF$_{2\alpha}$, 15-keto-13, 14-dihydro-PGF$_{2\alpha}$, TXB$_2$, 11β-PGF$_{2\alpha}$, 9β-PGF$_{2\alpha}$, and 8-iso-PGF$_{3\alpha}$ was 1.7%, 9.8%, 1.1%, 0.01%, 0.01%, 0.1%, 0.03%, 1.8%, and 0.6%, respectively. The detection limit of the assay was 23 pmol/L. The urinary levels of 8-iso-PGF$_{2\alpha}$ were adjusted for urinary creatinine concentration.

Measurement of plasma LPO concentrations

A colorimetric commercial assay kit (Oxystat; Biomedica, Vienna, Austria) was used to determine the concentration of total LPO in EDTA-plasma samples. Briefly, the peroxide concentration was determined by reaction of the biological peroxides with peroxidase and a subsequent color-reaction using 3,3′,5,5′-tetramethylbenzidine as substrate. After addition of sulfuric acid as stop solution, the colored liquid was measured photometrically at 450 nm. A calibrator was used to calculate the concentration of circulating biological peroxides in the sample, with a detection limit of 7 μmol/L.

Measurement of urinary 8-OHdG concentrations

The level of urinary 8-OHdG was determined by a competitive enzyme-linked immunosorbent assay kit (JAICA, Fukuroi, Japan). In brief, 50 μl of primary monoclonal antibody and 50 μl of sample or standard were added to microtiter plates, which were precoated with 8-OHdG, incubated at 37°C for 1 h, and washed with 250 μl of phosphate-buffered saline (PBS). One hundred microliters of HRP-conjugated secondary antibody was then added to each well, incubated at 37°C for 1 h, and washed with 250 μl of PBS. One hundred microliters of enzyme substrate was then added to each well and allowed to react at room temperature for 15 min. The reaction was terminated with 100 μl of 1 N phosphoric acid. Absorbance of each well was read at 450 nm by a microplate reader. The determination range was 0.125–10 ng/ml. The concentration of 8-OHdG was adjusted to urinary levels of creatinine (expressed as ng/mg creatinine).

Measurement of urinary creatinine concentrations

Creatinine concentrations in urine samples were measured on a Konelab 20 instrument (Thermo Clinical Lab Systems, Thermo Electron Corporation, Vantaa, Finland).

Statistical analysis

Results are given as mean ± standard error of mean. Normal distribution of data was examined using the Kolmogorov–Smirnov test. Differences 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 between groups at each time of gestation. Percentages of smoking categories (never, past, and current smoking) between groups were compared using a Chi-square test. 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.

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Abbreviations Used

8-iso-PGF$_{2\alpha}$ = 8-iso-prostaglandin F$_{2\alpha}$
8-OHdG = 8-hydroxy-2′-deoxyguanosine
DHA = docosahexaenoic acid
EPA = eicosapentaenoic acid
LPO = lipid peroxides
n-3 LC-PUFA = n-3 long-chain polyunsaturated fatty acids
PBS = phosphate-buffered saline
SiPS = Salmon in Pregnancy Study
TBARS = thiobarbituric acid reactive substances

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