The Salmon in Pregnancy Study: study design, subject characteristics, maternal fish and marine n–3 fatty acid intake, and marine n–3 fatty acid status in maternal and umbilical cord blood[1–4]

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ABSTRACT

Background: Oily fish provides marine n−3 (omega-3) fatty acids that are considered to be important in the growth, development, and health of the fetus and newborn infant.

Objectives: The objectives were to increase salmon consumption among pregnant women and to determine the effect on maternal and umbilical cord plasma marine n−3 fatty acid content.

Design: Women (*n* = 123) with low habitual consumption of oily fish were randomly assigned to continue their habitual diet or were provided with 2 portions of farmed salmon/wk to include in their diet from week 20 of pregnancy until delivery.

Results: Median weekly consumption frequency of study salmon in the salmon group was 1.94 portions, and total fish consumption frequency was 2.11 portions/wk in the salmon group and 0.47 portions/wk in the control group ($P < 0.001$). Intakes of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from the diet, from seafood, and from oily fish were higher in the salmon group (all $P < 0.001$). Percentages of EPA and DHA in plasma phosphatidylcholine decreased during pregnancy in the control group (P for trend = 0.029 and 0.008, respectively), whereas they increased in the salmon group (P for trend for both < 0.001). EPA and DHA percentages were higher in maternal plasma phosphatidylcholine at weeks 34 and 38 of pregnancy and in umbilical cord plasma phosphatidylcholine in the salmon group ($P < 0.001$ for all).

Conclusion: If pregnant women, who do not regularly eat oily fish, eat 2 portions of salmon/wk, they will increase their intake of EPA and DHA, achieving the recommended minimum intake; and they will increase their and their fetus' status of EPA and DHA. This trial was registered at clinicaltrials.gov as NCT00801502. *Am J Clin Nutr* 2011;94(suppl):1986S–92S.

INTRODUCTION

Seafoods, especially oily fish, are rich sources of the longer-chain n−3 polyunsaturated fatty acids (LC n−3 PUFAs) eicosapentaenoic acid (EPA, 20:5n−3) and docosahexaenoic acid (DHA, 22:6n−3). Examples of oily fish are salmon, sardines, pilchards, mackerel, herring, trout, and fresh tuna. The average content of EPA plus DHA in an adult portion of oily fish is between 1 and 3.5 g, depending mainly on the type of fish (1, 2).

The UK government recommends that all adults consume ≥2 portions of fish/wk, at least one of which should be oily (2); this has been translated into a minimum recommended intake of 450 mg EPA plus DHA/d for adults (2). The guideline range for oily fish intake among pregnant women in the United Kingdom is 1 or 2 portions/wk, with the upper limit being imposed because of concern about contaminants in some species of fish (2). Others have recommended an intake of 200 mg DHA/d for pregnant and lactating women (3). The European Food Safety Authority recently recommended 250 mg EPA plus DHA/d for healthy adults, with an additional 100–200 mg DHA/d for pregnant women (4). The Food and Agriculture Organization/World Health Organization (FAO/WHO) recommends that pregnant women consume

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[3] Supported by the European Commission under Framework 6: Sustainable aquafeeds to maximize the health benefits of farmed fish for consumers (Aquamax; FOOD-CT-2006-16249).

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a minimum of 300 mg EPA plus DHA/d, of which ≥200 mg should be DHA (5).

The UK National Diet and Nutrition Survey reports low consumption of oily fish by adults (6), including women (7), and a correspondingly low intake of LC n−3 PUFAs (2). It is recognized that provision of DHA to the fetus and neonate is important for growth and development, especially of the visual and central nervous systems (1, 2, 8). EPA and DHA may also be important for optimal development and functioning of the vascular, cardiac, and immune systems (9–12). Thus, a higher consumption of fish, especially oily fish, in pregnancy may be linked to better health outcomes in the offspring in infancy and childhood; and the protective effect of oily fish is linked to the nutrients that it contains, particularly LC n−3 PUFAs (2). Possible protective effects of early LC n−3 PUFA exposure have been examined in supplementation studies with fish oil (13–15). However, to the best of our knowledge there are no published intervention trials with fish during pregnancy.

The Salmon in Pregnancy Study (SiPS) is a randomized controlled trial of oily fish (in this case farmed salmon) in pregnant women. The SiPS specifically focuses on pregnant women whose offspring are at high risk of developing atopic disease, with one aim being to identify whether there is an effect on atopy outcomes in the offspring. The aim of this article is to describe the design of the SiPS, the characteristics of the included women, the composition of the salmon used in the intervention, the outcomes that will be reported from the study, the intake of fish and the status of EPA and DHA in maternal plasma and in umbilical cord plasma, and selected pregnancy outcomes in the 2 groups.

STUDY DESIGN AND METHODS USED

Study design and characteristics of the included subjects

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. All procedures were approved by the Southampton and South West Hampshire Research Ethics Committee (approval no. 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 (clinical trials identifier NCT00801502).

A randomly selected sample of pregnant women (n = 689) within the catchment area of the Princess Anne Hospital, Southampton University Hospitals NHS Trust, Southampton, United Kingdom, received general information about the SiPS when they received their postal invitations to attend the hospital for a routine ultrasound appointment to occur at approximately week 12 of gestation. Women who expressed an interest in the SiPS (n = 383) provided written informed consent at their week 12 appointment to enable screening for habitual consumption of fish and family history of atopy, allergy, or asthma (the primary outcome of the SiPS relates to atopy and its manifestations in infants born to women enrolled in the SiPS). Those women who reported low habitual consumption of oily fish (<2 portions/mo excluding canned tuna) and a family history of atopy, allergy, or asthma (one or more first-degree relatives of the infant affected by atopy, asthma, or allergy) received a detailed information sheet about the SiPS. Those who remained interested (n = 156) were further screened for inclusion into the SiPS. Inclusion criteria were as follows: age 18–40 y; <19 wk gestation; healthy, uncomplicated singleton pregnancy; infant 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/mo (excluding canned tuna); not using fish-oil supplements currently or in the previous 3 mo. Exclusion criteria were as follows: age <18 or >40 y; >19 wk gestation; no first-degree relatives of the infant affected by atopy, asthma, or allergy; consuming >2 portions of oily fish/mo (excluding canned tuna); use of fish-oil supplements within previous 3 mo; participation in another research study; known diabetic; or presence of any autoimmune disease, learning disability, terminal illness, or mental health problems. Women fulfilling the inclusion and exclusion criteria were recruited into the SiPS at their routine ultrasound clinic at week 19/20 (hereafter referred to as week 20) of gestation if they remained interested in the study. Women attended this clinic in a fasted state and provided written informed consent to enable their participation in SiPS. Ultimately, 123 women were recruited (**Figure 1**). At the clinic, women provided a blood sample (added to heparin) and a urine sample and completed a 100-item food-frequency questionnaire (FFQ) covering food intake over the preceding 12 wk (16–20).

The recruited women were randomly assigned to 1 of 2 groups; random assignment was according to a random number table. Women in one group (n = 61), referred to as the control group, were asked to continue their habitual diets; these women received the information sheet that described the possible health benefits of consuming oily fish during pregnancy and the government recommendation that pregnant women consume 1 or 2 oily fish meals/wk (2). They also received a cookbook providing recipes for healthy eating during pregnancy. Women in the salmon group (n = 62) were asked to incorporate 2 portions of salmon into their diet per week from study entry until they gave birth. They also received a cookbook that provided recipes for preparing and cooking salmon. The salmon (*see* below) was delivered to the homes of these women in individual frozen and vacuum-packed portions (150 g) on a monthly basis; sufficient portions were provided for each woman and her partner. Women in both groups received a diary in which to record any seafood consumed during the course of the study and the nature of its preparation and cooking.

The women attended a clinic at week 32–34 of gestation (hereafter referred to as week 34) and at week 38 of gestation while in a fasted state. At these clinics, the women provided a blood sample (added to heparin) and a urine sample; at week 34 they again completed the FFQ, and at week 38 they provided a fecal sample. Umbilical cord blood was collected and added to heparin at the births of their infants. Umbilical cord tissue and placental tissue were collected and stored frozen. The progress of women through the study is shown in Figure 1. The characteristics of the women and their pregnancies are provided in **Table 1**. There were no differences between the 2 groups with respect to age, height, and weight at study entry or with respect to skin-prick test positivity.

Women provided samples of breast milk at days 1, 5, 14, and 28 after giving birth, and infant feces were collected at days 7, 14, 28, and 84. Infant body composition was determined by dual-energy X-ray absorptiometry ≤14 d of birth. Women completed a FFQ at 3 mo after parturition. At the infant's age of 6 mo, infants, their mothers, and, where possible, their fathers attended

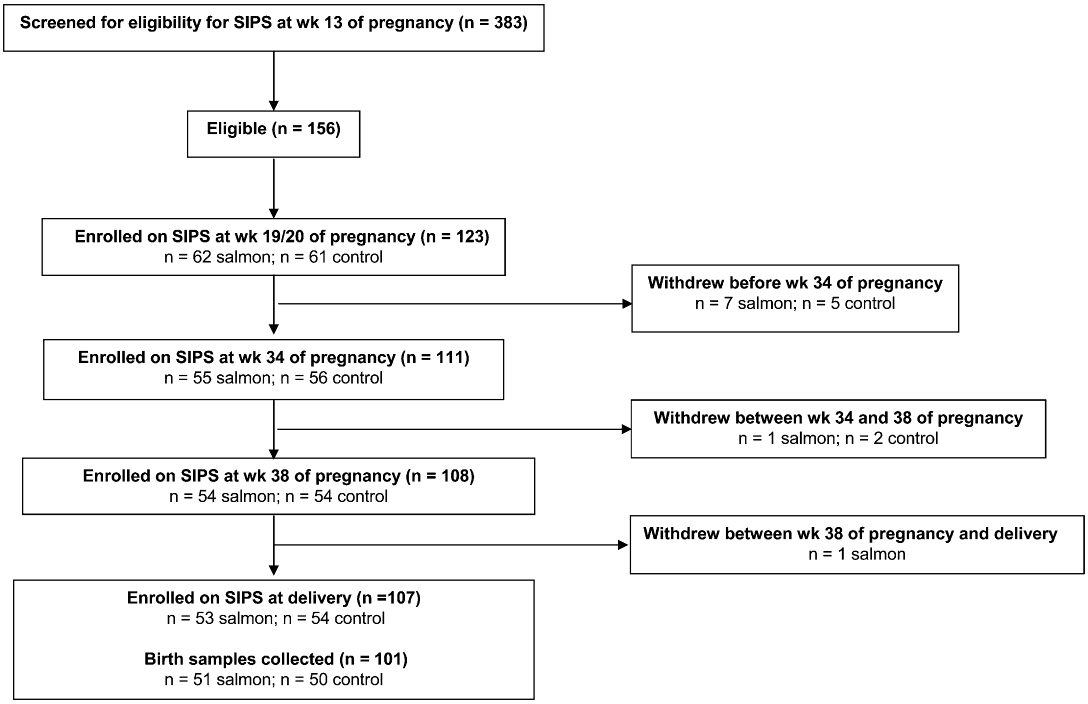

FIGURE 1. CONSORT (Consolidated Standards of Reporting Trials) diagram showing the flow of subjects through the study. SIPS, Salmon in Pregnancy Study.

a clinic at the Wellcome Trust Clinical Research Facility, Southampton General Hospital. At this visit, a FFQ (21) was completed for the infants (n = 86) who then underwent skin-prick testing for a range of common allergens (house dust mite, egg, cow milk protein, salmon, common grasses, common trees, cat, dog, common fungi, penicillin) and assessment for atopic dermatitis and its severity (22). A small blood sample (<5 mL) was collected from the infants and added to heparin. Mothers (n = 86) and fathers (n = 75) also underwent skin-prick testing.

Salmon

Salmon for use in the SiPS were raised at Skretting Aquaculture Research Centre, Stavanger, Norway. Their diet contained: fish meal (298.6 g/kg), soya concentrate (141.3 g/kg), maize gluten (99.5 g/kg), wheat gluten (29.9 g/kg), wheat (120.1 g/kg), southern hemisphere fish oil (132.1 g/kg), rapeseed oil (132.1 g/kg), flaxseed oil (33.5 g/kg), DL-methionine (0.32 g/kg), L-lysine (0.9 g/kg), monocalcium phosphate (5.64 g/kg), and vitamin and mineral mix (6.25 g/kg). The dietary ingredients used were selected to be low in contaminants. Salmon were farmed until they averaged 4 kg in weight and were then killed; a total of 2280 kg (gutted) of salmon were produced. They were then fileted into 150-g portions and the filets frozen individually in vacuum-sealed bags in Norway, from where they were shipped to Southampton and stored at −30°C until delivery to the women. Women then stored the filets in their home freezer until the day of cooking. Detailed nutrient and contaminant content of sample filets was determined at the National Institute of Nutrition and Seafood Research, Bergen, Norway. 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 (22:5n−3), 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 (sum of all retinols), 14 μg vitamin D_3, and 43 μg selenium; variance in content of all nutrients among several analyzed portions was <5% for protein and fat; <10% for individual fatty acids, α-tocopherol, and γ-tocopherol; and <20% for vitamin A, vitamin D_3, and selenium. Thus, 2 portions of salmon/wk would typically provide 3.45 g EPA +DHA, 28 μg vitamin D_3, and 86 μg selenium. For comparison,

TABLE 1
Characteristics of the women enrolled in the Salmon in Pregnancy Study and of their pregnancies[1]

	Control		Salmon	
	n	Values	*n*	Values
Age (y)	61	28.4 ± 0.6	62	29.5 ± 0.5
Height (cm)	61	165.6 ± 0.9	62	165.4 ± 0.8
Weight (kg)	61	71.3 ± 2.0	62	67.5 ± 1.6
First pregnancy (*n*)	61	23	62	27
Skin-prick test positive (*n*)[2]	38	21	48	31
Duration of pregnancy (d)	54	277 ± 2	53	282 ± 1
Mode of delivery (*n*)	54	—	53	—
Normal vaginal	35	—	35	—
Elective section	2	—	3	—
Emergency section	7	—	5	—
Instrumental	10	—	10	—
Infants				
Birth weight (g)	54	3425 ± 82	53	3449 ± 72
Head circumference at birth (cm)	54	34.7 ± 0.2	53	34.5 ± 0.2
Apgar score at 1 min	54	8.5 ± 0.2	53	8.5 ± 0.2
Apgar score at 5 min	54	9.1 ± 0.1	53	9.1 ± 0.1

[1] All values are means ± SEMs unless otherwise indicated. There were no significant differences between groups.

[2] Not all women agreed to be skin prick tested: in the Control group, 38 women were tested and 21 were positive, whereas in the Salmon group, 48 women were tested and 31 were positive.

average weekly intakes among typical (nonpregnant) women in the United Kingdom aged 20–40 y are as follows: <1.4 g EPA +DHA (2), and probably much less than this (23); 18.9 μg vitamin D (7); and 273 μg selenium (24). The content of contaminants (per 150-g salmon portion) was as follows: 52.5 pg dioxins and dioxin-like polychlorinated biphenyls (PCBs), 0.492 μg total dichlorodipheyl trichloroethanes, 51 μg arsenic, 0.15 μg cadmium, 3.45 μg mercury, and 0.15 μg lead. In comparison, wild salmon from the Pacific Ocean have been reported to have concentrations of 0.1–0.4 pg dioxin and dioxin-like PCBs/g (25), which would equate to 15–60 pg/150-g portion, whereas wild salmon from the contaminated Baltic Sea had concentrations of 3.3–17 pg dioxins/g (26), which would equate to 495–2550 pg/150-g portion. In one study, the dioxin and dioxin-like PCB content of salmon farmed on commercial fish oil was 0.9–2.9 pg/g (25), which would equate to 135–435 pg/150-g portion. The contaminants in the study salmon used in the SiPS provided <12.5% of the FAO/WHO provisional tolerable weekly intake for dioxin and dioxin-like PCBs, <11.5% for arsenic, <0.00000008% for cadmium, 0.0000025% for mercury, and <0.00000002% for lead.

FFQ

The FFQ used to assess the diet of the women was a modification of one validated for use in pregnant women in Southampton (16–20) and included questions of consumption frequency of different types of fish: "oily fish," "non-oily fish" (including canned tuna), "fish fingers and fish dishes," and "shellfish." Categories of consumption frequency were as follows: "more than once a day," "once a day," "3–6 times per week," "1–2 times per week," "once a fortnight," "once a month," "once every 2–3 mo," and "never."

Laboratory analyses

The laboratory analyses to be conducted as part of the SiPS and the center responsible for those measurements are listed in Supplemental Table 1 under "Supplemental data" in the online issue.

Plasma phosphatidylcholine fatty acid composition

Maternal and umbilical cord blood was collected and added to heparin, and plasma was prepared by centrifugation and stored at −80°C until analysis. Total lipid was extracted with chloroform/methanol (2:1 vol/vol); butylated hydroxytoluene was added to the extraction as antioxidant. Phosphatidylcholine, which typically contributes ≈75% of plasma phospholipid, was isolated by solid phase extraction on aminopropylsilica cartridges. Plasma phosphatidylcholine fatty acid methyl esters were generated by reaction with methanol containing 2% (vol/vol) sulphuric acid at 50°C for 2 h. Fatty acid methyl esters were separated by chromatography on a BPX-70 column (30 m × 220 μm; film thickness: 0.25 μm; SGE Europe, Milton Keynes, United Kingdom) fitted to a Hewlett-Packard HP6890 gas chromatograph (Hewlett-Packard, Avondale, PA) and run under standard conditions described elsewhere (27). Fatty acid methyl esters were identified by comparison with retention times of standards run previously and they were quantified by using ChemStation software (Hewlett-Packard).

Sample size, data presentation, and statistical analysis

The SiPS was powered according to an anticipated increase in maternal plasma phosphatidylcholine EPA content and an anticipated reduction in sensitization to egg in the infants at 6 mo of age; the latter outcome is not reported here. It was calculated that a sample size of 50 women/group would have 93% power to detect a 50% higher plasma phosphatidylcholine EPA content in the salmon group than in the control group (ie, EPA as 0.75% of fatty acids compared with 0.5% of fatty acids).

Data are presented as means ± SEMs or as medians and interquartile range as appropriate. Data for seafood and LC n−3 PUFA intake were compared between groups by Mann-Whitney *U* test. Data for maternal plasma phosphatidylcholine EPA and DHA were compared over time (weeks 20, 34, and 38) and between groups by 2-factor repeated-measures analysis of variance (time and treatment group as factors). Pairwise comparisons between groups for maternal plasma phosphatidylcholine EPA and DHA at a particular time point and between groups for umbilical cord plasma phosphatidylcholine EPA and DHA were made by using unpaired Student's *t* test. All statistical tests were performed by using SPSS version 15.0 (SPSS Inc, Chicago, IL), and in all cases a value of $P < 0.05$ was taken to indicate significance.

RESULTS

Intake of fish, EPA, and DHA

The FFQ at week 20 confirmed the women's self-reported low intake of oily fish. The 34-wk FFQ showed that all women in the salmon group reported having oily fish 1–2 times/wk, which was interpreted as 1.5 times/wk (**Table 2**). Thus, at 34 wk gestation, the salmon group consumed oily fish significantly more frequently than did the control group (medians: 1.5 and 0 times/wk for salmon and control groups, respectively; $P < 0.001$) (Table 2). The change in oily fish intake from week 20 to week 34 of gestation was significantly different between the 2 groups (−0.04 and 1.21 times/wk for the control and salmon groups, respectively; $P < 0.001$). At 34 wk gestation, intakes of EPA, DHA, and EPA plus DHA from the whole diet, from seafood, and from oily fish were significantly higher in the salmon group than in the control group (all $P < 0.001$) (**Table 3**). According to data derived from the FFQ, the mean daily intake of EPA plus DHA from total fish increased by 270 mg in the salmon group and decreased by 12 mg in the control group ($P < 0.001$).

The fish diaries showed that the median weekly consumption frequency of study salmon in the salmon group was 1.94 portions, whereas the median weekly oily fish consumption frequency in the control group was 0 portions ($P < 0.001$). Total fish consumption frequency was 2.11 portions/wk for the salmon group and 0.47 portions/wk for the control group ($P < 0.001$). The fish diaries showed that 80% of the volunteers in the salmon group consumed 2 (or more) portions of study salmon/wk for 81% of the weeks during intervention. Also, 24.5% of the volunteers in the salmon group consumed ≥2 portions of study salmon/wk for every week from week 21 to week 38 of gestation. Fish consumption data from the fish diaries was used to calculate daily intakes of EPA and DHA: median daily intakes from total fish were 162 mg EPA, 326 mg DHA, and 491 mg EPA plus DHA for the salmon group; and 10 mg EPA, 16 mg DHA, and 24 mg EPA plus DHA for the control group during the intervention (all $P < 0.001$).

Pregnancy outcomes

The duration of pregnancy was an average of 5 d longer in the salmon group, but this was not significantly different from the

TABLE 2
Weekly consumption frequency of seafood in the control and salmon groups during the study period[1]

	Data from FFQ at week 34			Data from fish diaries averaged over weeks 21–38		
Seafood group	Control (n = 54)	Salmon (n = 55)	P	Control (n = 47)	Salmon (n = 49)	P
Non-oily fish	0.25 (0.08, 1.5)	0.5 (0.25, 1.5)	0.204	0.33 (0.06, 0.56)	0.17 (0.06, 0.33)	0.036
Fish fingers and fish dishes	0.18 (0, 0.5)	0.1 (0, 0.25)	0.866	0 (0, 0.11)	0 (0, 0)	0.002
Oily fish	0 (0, 0.14)	1.5 (1.5, 1.5)	<0.001	0 (0, 0.07)	1.94 (1.87, 2.00)	<0.001
Shellfish	0 (0, 0)	0 (0, 0.1)	0.076	0 (0, 0)	0 (0, 0)	0.507

[1] All values are median numbers of times per week; interquartile ranges in parentheses. FFQ, food-frequency questionnaire. P values were derived from a Mann-Whitney U test comparing the 2 groups.

duration in the control group (Table 1). Mode of delivery, infant birth weight, infant head circumference at birth, and infant well-being immediately after birth, as judged by Apgar score, were not different between groups (Table 1).

Plasma phosphatidylcholine EPA and DHA

There was a significant effect of treatment group and a significant group × time interaction on the proportion of both EPA and DHA in maternal plasma phosphatidylcholine ($P < 0.001$ for all). The percentages of EPA and DHA in plasma phosphatidylcholine decreased during pregnancy in the control group (P for trend = 0.029 and 0.008, respectively), whereas they increased in the salmon group (P for trend < 0.001 for both) (**Figure 2**). EPA and DHA in maternal plasma phosphatidylcholine were different between the groups at weeks 34 and 38 of pregnancy ($P < 0.001$ for all; Figure 2). Changes in the percentages of EPA and DHA in plasma phosphatidylcholine between weeks 20 and 34 and weeks 20 and 38 of gestation were significantly different between groups ($P < 0.001$ for all).

The percentages of both EPA and DHA in umbilical cord plasma phosphatidylcholine were significantly higher in the salmon group than in the control group (EPA: 0.32 ± 0.2 and 0.63 ± 0.04 for the control and salmon groups, respectively; $P < 0.001$; DHA: 6.4 ± 0.2 and 7.4 ± 0.2 for the control and salmon groups, respectively; $P = 0.001$).

DISCUSSION

The SiPS aimed to increase the intake of salmon by pregnant women who do not normally consume oily fish with the aim of increasing their intake of LC n−3 PUFAs (and other key nutrients) to enhance their status of LC n−3 PUFAs and that of their fetus and neonate. It is hypothesized that the increased exposure to LC n−3 PUFAs would have effects on the maternal and fetal immune systems, as reported by others after early exposure to LC n−3 PUFAs from fish oil (13–15, 28), and reduce the risk of atopy in infancy (14). The salmon used was produced specifically for use in this study by aquaculture and provided 1.8 g EPA plus DHA, 2.08 g LC n−3 PUFAs, and 3.56 g total n−3 PUFAs/portion. Importantly, the ingredients used for the salmon feed were selected to be low in contaminants, so that as well as being rich in LC n−3 PUFAs, the salmon used here was very low in the full range of possible contaminants.

It was calculated that if the women in the salmon group consumed the desired 2 portions/wk they would take in 3.6 g EPA plus DHA from this source each week; on the basis of estimates of intake of EPA plus DHA among adults in the United KIngdom, this would likely increase the women's intake of EPA plus DHA by ≥18-fold and possibly more than this. Indeed, using the data available from the FFQ and the fish diaries, intake of EPA plus DHA was ≈20-fold higher in the salmon group than in the control group during intervention. It is evident from these data that consumption of 2 portions of oily fish/wk, with the EPA and DHA content of the salmon used here, will allow pregnant women to achieve the current recommendations for intake of LC n−3 PUFAs (2–5).

Previous studies have reported that high fish or LC n−3 PUFA intake is associated with longer duration of pregnancy (29–31). Here, pregnancy was ≈5 d longer in the salmon group than in the control group, which fits with differences previously reported. However, the difference was not significant, probably due to the small sample size, which limits power to detect this difference as significant. Other key pregnancy outcomes such as birth weight, infant head circumference at birth, and infant health immediately after birth were not different between groups.

TABLE 3
Intakes of long-chain n−3 polyunsaturated fatty acids from the total diet, seafood, and oily fish in the control and salmon groups calculated by using a food-frequency questionnaire at week 34 of gestation[1]

	Total diet			Seafood			Oily fish		
	Control	Salmon	P	Control	Salmon	P	Control	Salmon	P
EPA	12 (3, 35)	134 (128, 146)	<0.001	12 (3, 35)	133 (128, 146)	<0.001	0 (0, 15)	122 (122, 122)	<0.001
DHA	20 (6, 67)	269 (261, 298)	<0.001	20 (5, 63)	267 (259, 298)	<0.001	0 (0, 27)	249 (249, 249)	<0.001
EPA+DHA	30 (9, 103)	403 (309, 444)	<0.001	30 (8, 97)	403 (387, 444)	<0.001	0 (0, 41)	371 (371, 371)	<0.001

[1] All values are median milligrams per day; interquartile ranges in parentheses. n = 54 and 55 in the control and salmon groups, respectively. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. P values were derived from a Mann-Whitney U test comparing the 2 groups.

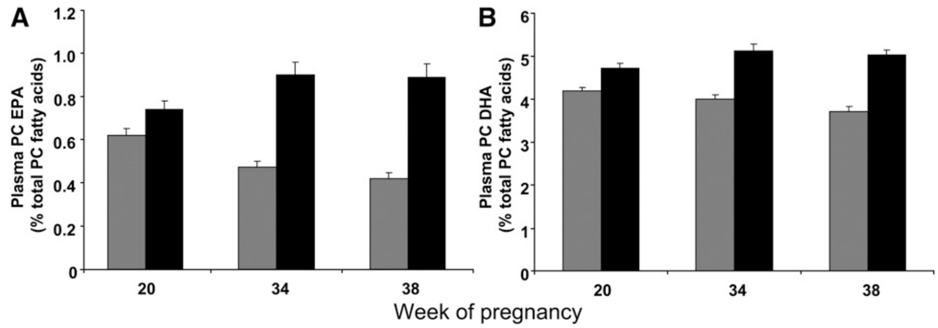

FIGURE 2. Mean (±SEM) concentrations of maternal blood plasma phosphatidylcholine (PC) eicosapentaenoic acid (EPA) (A) and docosahexaenoic acid (DHA) (B) in the control and salmon groups. Values are shown in g/100 g total fatty acids. Control group, gray bars; salmon group, black bars. Two-factor repeated-measures ANOVA indicated a significant effect of group ($P < 0.001$) but not of time and a significant group × time interaction ($P < 0.001$) for EPA and significant effects of group ($P < 0.001$) and time ($P = 0.003$) and a significant group × time interaction ($P < 0.001$) for DHA.

The decline in the percentage of EPA and DHA in plasma phosphatidylcholine over the course of pregnancy in the control group is entirely consistent with data reported previously (32–34), and indeed the percentage values for EPA and DHA reported here are very similar to those reported previously for plasma phospholipids during pregnancy in Dutch, Belgian, and German women (32–35). In contrast to the observations in the control group, not only did EPA and DHA not decline in plasma phosphatidylcholine in the salmon group but they increased. At 34 and 38 wk of pregnancy, the percentage of EPA in plasma phosphatidylcholine in the salmon group was ≈100% higher than in the control group, whereas DHA was ≈25% higher. Plasma phosphatidylcholine from umbilical cord blood showed higher EPA and DHA in the salmon group, indicating enhanced transfer of these functionally important fatty acids to the fetus, presumably as a result of the higher concentration in the maternal circulation. The higher percentage of EPA and DHA in the fetal circulation in the salmon group may result in better visual, neural, and immune development in those infants. The percentages of other fatty acids, including arachidonic acid, and the fatty acid composition of other plasma lipids and of erythrocytes and leukocytes in maternal and umbilical cord blood will be reported in future publications, as will immune and clinical outcomes in the infants.

In conclusion, this study shows that if pregnant women who do not normally consume oily fish eat salmon twice per week they will significantly increase their intake of LC n−3 PUFAs; they will meet current dietary recommendations for DHA and LC n−3 PUFAs; they will enhance their status of EPA and DHA, preventing the pregnancy-associated decline in these key fatty acids; and they will increase the status of EPA and DHA in their fetus and neonate.

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The authors' responsibilities were as follows—EAM, HMI, KMG, and PCC: were responsible for designing the study and had overall responsibility for all aspects of the study; GR: was responsible for producing the study salmon; L-SK, MV, and NDD: recruited and screened volunteers, carried out the intervention, and collected the blood samples and anthropometric measurements, questionnaire, and compliance data; L-SK and MV: carried out dietary analysis under the supervision of SMR; L-SK, MV, PSN, HU, AR, M-CF, BL, JH, EG, JO, MDM, and CMA: carried out the laboratory analysis (most of which is not reported here), supervised by EAM, PY, M-PV, LF, SB, AG, and PCC; EAM, L-SK, MV, and PCC: conducted the statistical analysis of the data reported here; PCC: wrote the draft of the manuscript; and all authors: contributed to and approved the final version of the manuscript. GR is employed by a company that produces feed for farmed salmon. None of the other authors had any personal or financial conflicts of interest.

REFERENCES

1. British Nutrition Foundation. Briefing paper: n−3 fatty acids and health. London, United Kingdom: British Nutrition Foundation, 1999.
2. Scientific Advisory Committee on Nutrition & Committee on Toxicity. Advice on fish consumption: benefits and risks. London, United Kingdom: The Stationary Office, 2004.
3. Cetin I, Koletzko B. Long-chain omega-3 fatty acid supply in pregnancy and lactation. Curr Opin Clin Nutr Metab Care 2008;11:297–302.
4. EFSA Panel on Dietetic Products, Nutrition, and Allergies. Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA J 2010;8:1461.
5. Joint FAO/WHO Expert Consultation on Fats and fatty acids in Human Nutrition. Fat and fatty acids in human nutrition: report of an expert consultation. Rome, Italy: Food and Agriculture Organization, 2010.
6. Department of Health and Food Standards Agency. National Diet and Nutrition Survey: adults aged 19 to 64 years. London, United Kingdom: The Stationary Office, 2004.
7. Department of Health and Food Standards Agency. National Diet and Nutrition Survey: NDNS headline results from year 1 of the Rolling programme (2008/2009). London, United Kingdom: The Stationary Office, 2009.
8. Innis SM. Dietary omega 3 fatty acids and the developing brain. Brain Res 2008;1237:35–43.
9. Calder PC, Krauss-Etschmann S, de Jong EC, et al. Workshop report: early nutrition and immunity—progress and perspectives. Br J Nutr 2006;96:774–90.
10. Kremmyda LS, Vlachava M, Noakes PS, et al. Atopy risk in infants and children in relation to early exposure to fish, oily fish, or long-chain omega-3 fatty acids: a systematic review. Clin Rev Allergy Immunol 2009;Dec 9 (Epub ahead of print; DOI:10.1007/S12016-009-8186-2).
11. Prescott SL, Dunstan JA. Prenatal fatty acid status and immune development: the pathways and the evidence. Lipids 2007;42:801–10.
12. Calder PC, Kremmyda LS, Vlachava M, et al. Is there a role for fatty acids in early life programming of the immune system? Proc Nutr Soc 2010;69:373–80.
13. Dunstan JA, Mori TA, Bardent A, et al. Maternal fish oil supplementation in pregnancy reduces interleukin-13 levels in cord blood of infants at high risk of atopy. Clin Exp Allergy 2003;33:442–8.
14. Dunstan JA, Mori TA, Barden A, et al. Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: a randomized, controlled trial. J Allergy Clin Immunol 2003;112:1178–84.
15. Denburg JA, Hatfield HM, Cyr MM, et al. Fish oil supplementation in pregnancy modifies neonatal progenitors at birth in infants at risk of atopy. Pediatr Res 2005;57:276–81.

16. Crozier SR, Inskip HM, Godfrey KM, Robinson SM. Dietary patterns in pregnant women: a comparison of food-frequency questionnaires and 4 d prospective diaries. Br J Nutr 2008;99:869–75.
17. Borland SE, Robinson S, Crozier, SR, Inskip HM; SWS Study Group. Stability of dietary patterns in young women over a 2-year period. Eur J Clin Nutr 2008;62:119–26.
18. Crozier SR, Robinson SM, Borland SE, Inskip HM; SWS Study Group. Dietary patterns in the Southampton Women's Survey. Eur J Clin Nutr 2006;60:1391–9.
19. Robinson S, Godfrey K, Osmond C, Cox V, Barker D. Evaluation of a food frequency questionnaire used to assess nutrient intakes in pregnant women. Eur J Clin Nutr 1996;50:302–8.
20. Crozier SR, Robinson SM, Godfrey KM, Cooper C, Inskip HM. Women's dietary patterns change little from before to during pregnancy. J Nutr 2009;139:1956–63.
21. Marriott LD, Robinson SM, Poole J, et al. What do babies eat? Evaluation of a food frequency questionnaire to assess the diets of infants aged 6 months. Public Health Nutr 2008;11:751–6.
22. Consensus Report of the European Task Force on Atopic Dermatitis. Severity scoring of atopic dermatitis: the SCORAD index. Dermatology 1993;186:23–31.
23. Meyer BJ, Mann NJ, Lewis JL, Milligan GC, Sinclair AJ, Howe PRC. Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids. Lipids 2003;38:391–8.
24. Department of Health. Dietary Reference Values for food, energy, and nutrients for the United Kingdom. London, United Kingdom: The Stationary Office, 1991.
25. Hites RA, Foran JA, Carpenter DO, Hamilton MC, Knuth BA, Schwager SJ. Global assessment of organic contaminants in farmed salmon. Science 2004;303:226–9.
26. Isosaari P, Hallikainen A, Kiviranta H, et al. Polychlorinated dibenzo-p-dioxins, dibenzofurans, biphenyls, naphthalenes and polybrominated diphenyl ethers in the edible fish caught from the Baltic Sea and lakes in Finland. Environ Pollut 2006;141:213–25.
27. Thies F, Nebe-von-Caron G, Powell JR, Yaqoob P, Newsholme EA, Calder PC. Dietary supplementation with eicosapentaenoic acid, but not with other long chain n−3 or n−6 polyunsaturated fatty acids, decreases natural killer cell activity in healthy subjects aged > 55 years. Am J Clin Nutr 2001;73:539–48.
28. Krauss-Etschmann S, Hartl D, Rzehak P, et al; Nutraceuticals for Healthier Life Study Group. Decreased cord blood IL-4, IL-13, and CCR4 and increased TGF-beta levels after fish oil supplementation of pregnant women. J Allergy Clin Immunol 2008;121:464–70.
29. Olsen SF, Secher NJ. Low consumption of seafood in early pregnancy as a risk factor for preterm delivery: prospective cohort study. BMJ 2002;324:447–50.
30. Olsen SF, Østerdal ML, Salvig JD, et al. Duration of pregnancy in relation to seafood intake during early and mid pregnancy: prospective cohort. Eur J Epidemiol 2006;21:749–58.
31. Olsen SF, Sørensen JD, Secher NJ, et al. Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration. Lancet 1992;339:1003–7.
32. Al MDM, van Houwelingen AC, Kester ADM, Hasaart THM, De Jong AEP, Hornstra G. Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status. Br J Nutr 1995;74:55–68.
33. Otto SJ, Houwelingen AC, Antal M, et al. Maternal and neonatal essential fatty acid status in phospholipids: an international comparative study. Eur J Clin Nutr 1997;51:232–42.
34. De Vriese SR, Dhont M, Christophe AB. FA composition of cholesteryl esters and phospholipids in maternal plasma during pregnancy and at delivery and in cord plasma at birth. Lipids 2003;38:1–7.
35. Berghaus TM, Demmelmair H, Koletzko B. Fatty acid composition of lipid classes in maternal and cord plasma at birth. Eur J Pediatr 1998;157:763–8.