Circulating contaminant and fatty acid levels in patients after a controlled diet of farmed salmon

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Introduction

A large number reports in our current literature indicate that fish, or fish oil supplements, can help reduce risk for death from coronary heart disease (CHD). Positive results have recently been published for a study involving a group of Norwegian CHD patients consuming farmed Atlantic salmon fillets1. The double blinded intervention study consisted of 58 CHD patients that were randomly allocated to 3 groups consuming 5 meals a week (700g fillet/week) for 6 weeks of preferentially fed Atlantic salmon. The Atlantic salmon were raised on fish feeds containing 100% fish oil (FO), 100% rapeseed oil (RO) or 50% of each (FO/RO), that resulted in fillets with high, intermediate and low levels of marine omega-3 polyunsaturated fatty acids (n-3 PUFAs). The patients consuming fillets from fish raised with fish oil feeds had significantly greater cardioprotective effects and serum concentrations of the n-3 PUFAs, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Converse to the known positive dietary benefits of eating fatty fish, some carcinogenic and cardiotoxic contaminants are also found in these fish. Advice regarding fish intake has been significantly complicated by reports that some fish species are burdened with potentially harmful levels of organohalogen contaminants such as polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs), dioxin-like polychlorinated biphenyls (DLPCBs), PCBs, and brominated flame retardants such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD). Data on short term fish consumption, with known levels of contaminants, and resulting circulating levels in humans are exceedingly rare. The study presented here examined these CHD patients for plasma exposure to selected contaminants after the intervention diets of farmed Atlantic salmon.

Materials and Methods

The study population and design has been described previously[1]. The tailormade Atlantic salmon fillets (collected in 2002) of the FO group were raised on 100% South-American fish oil (Denofa, Fredrikstad, Norway), the FO/RO Group on 50% South-American fish oil/50% rapeseed oil, and the RO group on 100% Rapeseed Oil (Oelmühle, Hamburg, Germany).

*Sample Analysis:*Farmed Atlantic salmon fillet samples (n=8, each group) were collected in 2002 and patient (n = 58) blood samples were drawn in 2003 in fasting condition at randomisation on day 0 and at the end of the study (6 weeks later). The fatty acid and lipid composition of fillets and patient serum were analysed as previously described[2]. For contaminant analysis, the plasma from each patient group was pooled into single samples to allow their detection. The methods for the determination of PCDD/Fs, PCBs, and brominated flame retardants used in this study are described elsewhere[3-5]. The results presented here are in accordance with World Health Organization (WHO) methods for the congener sum of 7 PCDDs, 10 PCDFs, and 12 DLPCBs. The ICES group of PCBs covers 7 congeners commonly found in the environment (PCB 28, 52, 101, 118, 138, 153, and 180).

Clean Up and Detection: The U.S. EPA standard mixtures (Cambridge Isotope Laboratories, Andover, MA, USA) for dioxin, furan and PCB congeners, PBDE 66 and 119 for PBDEs and HBCD were used as internal standards and added to 8 g freeze-dried homogenized fillet or to 20 mL of pooled plasma samples. Samples were then extracted with 100% hexane using an ASE 300 instrument (Dionex, Sunnyvale, CA, USA). The fillet and plasma extracts were purified by a Power-Prep (Fluid Management Systems, Inc. Watertown, MA, USA) procedure as described previously[6]. A mixture of two carbon-13 marked PCDD/Fs were added as recovery standards. The WHO indicated PCDD/Fs and DLPCBs were analysed with a high-resolution Thermo Finnigan MAT 95 HRGC/HRMS set at a resolution of greater then 10,000; a Thermo Finnigan Trace GC with MD 800 mass detector in EI mode for indicator PCBs; a Thermo Finnigan Trace GC coulpled to a DSQ mass detector in NCI mode for PBDEs and total HBCD. Accuracy and precision of the method was assessed by the simultaneous analysis of two certified reference materials (Wellington Laboratories Inc, Guelph, Ontario, Canada). Quality assurance of the results was demonstrated as the laboratory regularly participates in international inter-laboratory trials for these contaminants, in-house control samples were analysed together with the samples, and blank analyses were performed carrying out the entire analytical procedure omitting only the sample.

*Calculations and Statistical Analysis:*Concentrations with standard deviations for farmed salmon fillets were determined from fillets (n = 8) for each of the 3 treatment groups. Patient plasma concentrations and exposure during the treatment period were screened from a single pool of n = 20 patients for the FO group, n = 19 for the FO/RO group, and n = 19 for the RO treatment group. Estimations on plasma contaminant or serum fatty acid exposure during the treatment period were calculated non-compartmentally from a partial area under the concentration-time curve (AUC) determined by the trapezoidal rule from 0 to 6 weeks. The toxic equivalent (TEQ) values for dioxins and DLPCBs were calculated using current WHO toxic equivalency factors (TEFs) and the reported WHO-TEQ values for the fillets were calculated as upper bound-LOQ concentrations[7]. Two-sided paired t-tests for means was used where a p-value of < 0.05 was considered significant.

Results and Discussion

The levels of the selected organic contaminants found in the fillets used in this study were comparable to those typically found in farmed Atlantic salmon[8]. Table 1 shows the level of selected organohalogen contaminants found in the treatment fillets. A distinct concentration gradient was observed in the fillets for the organic contaminants PCDD/Fs, DLPCBs, indicator PCBs, and PBDEs. Estimations for the level of intake from the intervention diet for PCDD/Fs and DLPCBs in the FO group of patients were in the range of 13.1 - 16.8 pg WHO-TEQ/kg body weight (bw) per week (wk), which is comparable to the tolerable intake limit of 14 pg WHO-TEQ/kg bw/wk set by the European Commission Scientific Committee on Food (EC-SCF). For indicator PCBs and brominated flame retardants, the European Union has yet to

establish maximum limits in fish tissue, and neither the European Food Safety Authority (EFSA) or WHO have established limits on tolerable intake.

The results presented in Table 2 for human plasma show that baseline levels determined in patient plasma were in good agreement with concentrations and congener profiles found previously for Norwegians and members of the European community[9-11]. Also, the results presented here fit in well with the known pharmacokinetics of these contaminants in human blood, where the concentration-time profiles are complex, often exhibiting nonlinear (dose-dependent) kinetics.After 6 weeks on the intervention diets, patients in the FO and FO/RO treatment groups had substantially lower plasma concentrations and exposure to PCDD/Fs, DLPCBs, and PCBs than those in the RO group. Normalization of contaminant plasma concentrations with serum fatty acid and/or total lipids did not alter the trend in the results reported here on a whole-weight basis. Typically, the median half-lives for 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD) in human plasma are on the order of 7-9 years[12]. The 43% reduction in circulating plasma concentrations for dioxins and dioxin-like and indicator PCBs observed in this study with the FO fillet treated patients suggests a substantial induction in the clearance of these contaminants from plasma occurred in the FO patient group over the 6 week study period. For PCDD/Fs, DLPCBs, and PCBs, the liver and adipose tissues of both experimental animals and humans have been shown to rapidly sequester these compounds from plasma in a dose-dependent manner[13]. Of the examined PBDE congeners, there was an increase in PBDE 47 and 99 in serum for the FO group of patients, and a relative decrease of total PBDEs from baseline concentrations was found in patients receiving the FO/RO and RO treatments.

Figure 1 illustrates that circulating exposures to fatty acids EPA and DHA in the CHD patients over the 6 weeks of treatment were not significantly different between the groups consuming the FO and FO/RO fillets, while the fillet concentrations of EPA and DHA and contaminants were greater for the FO fillet compared to the FO/RO group. The estimated tolerable weekly intake for PCDD/Fs and DLPCBs was exceeded in some of these patients, and with the knowledge that these contaminants have similar toxicological targets and are present in combination with other contaminants in the fillet, the margin of safety to patients prescribed fish diets is likely to be substantially lower than that currently estimated. The results presented here suggest a reduction of marine oil ingredients in fish feeds would help reduce the level of several undesirable contaminants in farmed Atlantic salmon without decreasing the circulating exposure to beneficial EPA and DHA in cardiac patients.

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Table 1. Comparison of mean (SD) contaminant levels found in the treatment fillets. Atlantic salmon were raised feeds containing 100% fish oil (FO), 100% rapeseed oil (RO) or 50% of each (FO/RO).For dioxins (PCDD/Fs) and DLPCBs, levels are also expressed as the toxic equivalent (TEQ) as Upperbound-LOQ.

	FO Fillet	**FO/RO Fillet**	**RO Fillet**
Dioxins and DLPCBs (pg/g wet wt)			
PCDDs	0.21 (0.07) [a]	0.20 (0.19)	0.10 (0.05) [a]
PCDFs	1.88 (0.26) [a,b]	1.23 (0.35) [b,c]	0.62 (0.14) [a,c]
non-ortho PCBs	30.96 (4.27) [a,b]	16.81 (2.86) [b,c]	6.31 (1.36) [a,c]
mono-ortho PCBs	1399.31 (211.54) [a,b]	855.10 (113.10) [b,c]	428.82 (87.52) [a,c]
Sum	1432.36 (215.92) [a,b]	766.46 (322.50) [b,c]	435.85 (89.03) [a,c]
Sum pg WHO-TEQ/g wet wt	1.43 (0.18)	0.91 (0.10)	0.51 (0.07)
Estimated weekly intake*	13.1 – 16.8	8.5 – 10.6	4.6 – 6.1
Tolerable weekly intake**	14	14	14
Indicator PCBs (ng/g wet wt)			
PCB-28	0.16 (0.06) [a]	0.13 (0.03)	0.07 (0.02) [a]
PCB-52	0.57 (0.17) [a,b]	0.29 (0.18) [b,c]	0.15 (0.17) [a,c]
PCB-101	1.21 (0.24) [a,b]	0.79 (0.11) [b,c]	0.46 (0.11) [a,c]
PCB-118	1.22 (0.24) [a,b]	0.82 (0.10) [b,c]	0.54 (0.15) [a,c]
PCB-138	1.85 (0.33) [a,b]	1.26 (0.14) [b,c]	0.78 (0.20) [a,c]
PCB-153	1.89 (0.33) [a,b]	1.31 (0.15) [b,c]	0.83 (0.20) [a,c]
PCB-180	0.36 (0.08) [a,b]	0.25 (0.05) [b,c]	0.16 (0.04) [a,c]
Sum	7.25 (1.34) [a,b]	4.85 (0.64) [b,c]	3.00 (0.81) [a,c]
Flame Retardants (pg/g wet wt)			
PBDE-28	86.20 (13.95) [a,b]	58.05 (12.76) [b,c]	18.32 (5.13) [a,c]
PBDE-47	1488.80 (249.96) [a,b]	1110.27 (177.73) [b,c]	607.93 (110.56) [a,c]
PBDE-99	200.11 (32.29) [a]	154.05 (22.99)	102.77 (19.27) [a]
PBDE-100	232.26 (31.07) [a,b]	206.76 (29.55) [b,c]	179.65 (34.73) [a,c]
PBDE-153	34.87 (6.10) [a]	52.59 (18.13)	65.02 (27.28) [a]
PBDE-154	94.78 (13.64) [a,b]	65.63 (32.49) [b,c]	70.76 (19.91) [a,c]
PBDE-183	59.44 (nd)	77.85 (nd)	27.17 (nd)
HBCD	< 2000	nf	nf
Sum	2144.45 (327.23) [a,b]	1698.02 (243.40) [b,c]	1047.85 (193.52) [a,c]

of 67 kg; **European Commission 2000, *Opinion of the SCF on the Risk Assessment of Dioxins and Dioxin-like PCBs in Food*. Health and Consumer Protection Directorate-General, Scientific Committee on Food, November 2000.

Table 2. Comparison of contaminant levels in patient plasma. Patients consumed 700 g/week for 6 weeks of Atlantic salmon that were raised feeds containing 100% fish oil (FO), 100% rapeseed oil (RO) or 50% of each (FO/RO).

	Patients on FO fillet diet			Patients on FO/RO fillet diet			Patients on RO fillet diet		
Dioxins and DLPCBs	baseline pg/mL	6 weeks pg/mL	AUC_{0-6wk} pg*wk/mL	baseline pg/mL	6 weeks pg/mL	AUC_{0-6wk} pg*wk/mL	baseline pg/mL	6 weeks pg/mL	AUC_{0-6wk} pg*wk/mL
PCDDs	0.05	0.04	0.27	0.08	0.07	0.47	0.06	0.06	0.33
PCDFs	0.01	0.01	0.04	0.01	0.01	0.05	0.01	0.01	0.04
non-ortho PCBs	0.03	0.02	0.16	0.03	0.04	0.23	0.03	0.03	0.19
mono-ortho PCBs	11.42	6.53	53.86	11.33	13.25	73.74	12.14	11.71	71.57
Sum	11.52	6.59	54.32	11.46	13.37	74.49	12.24	11.81	72.13
Indicator	ng/mL	ng/mL	ng*wk/mL	ng/mL	ng/mL	g*wk/mL	ng/mL	ng/mL	ng*wk/mL
PCBs									
PCB-28	0.10	0.01	0.33	0.01	0.03	0.10	0.03	0.04	0.22
PCB-52	nf	nf	nd	nf	nf	nd	nf	nf	nf
PCB-101	0.02	0.02	0.10	0.02	0.02	0.12	0.02	0.02	0.10
PCB-118	0.12	0.08	0.58	0.16	0.12	0.85	0.11	0.10	0.62
PCB-138	0.57	0.34	2.72	0.70	0.53	3.68	0.53	0.49	3.06
PCB-153	0.69	0.40	3.26	0.74	0.65	4.19	0.64	0.60	3.71
PCB-180	0.39	0.22	1.84	0.51	0.40	2.73	0.40	0.38	2.35
Sum	1.88	1.06	8.83	2.14	1.75	11.67	1.73	1.62	10.07
Flame	pg/mL	pg/mL	pg*wk/mL	pg/mL	pg/mL	pg*wk/mL	pg/mL	pg/mL	pg*wk/mL
Retardants									
PBDE-28	2.04	2.76	14.40	nf	nf	nf	nf	nf	nf
PBDE-47	13.62	16.71	90.98	28.02	12.63	121.93	14.83	15.37	90.60
PBDE-99	5.90	10.77	50.03	11.76	8.05	59.43	11.70	8.93	61.88
PBDE-100	2.59	2.49	15.24	2.78	1.78	13.69	2.32	1.92	12.70
PBDE-153	2.85	2.07	14.76	3.30	5.87	27.52	3.25	3.30	19.66
PBDE-154	5.17	1.62	20.39	nf	2.93	nf	3.13	4.09	21.64
PBDE-183	nf	nf	nd	nf	nf	nf	nf	nf	nf
HBCD	nf	nf	nd	nf	nf	nf	nf	nf	nf
Sum	32.17	36.43	205.80	45.87	31.26	231.38	35.22	33.60	206.47

Figure 1. Concentrations of contaminants and fatty acids in the fillet, and resulting fatty acid exposure in CHD patient serum. Contaminant concentrations are the average sum results as shown in Table 1. The EPA and DHA levels in the fillet are g/100g wet weight, and exposure (AUC0-6wk) in serum as g*week/L.