PERSISTENT ORGANIC POLLUTANTS IN HUMAN PLASMA RELATED TO MEASURED INTAKE OF COD LIVER AND COD LIVER OIL

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Introduction

Plasma is the most common medium to determine levels of persistent organic pollutants (POPs) in humans and the levels found here are considered to reflect the body burden of POPs[1]. Haddad et al. supports this by showing that the adipose tissue: blood-partitioning coefficient is equal to the ratio of lipids in adipose tissue and blood[2]. A plasma sample is easy to obtain and the analyses is rapid. There are however problems with the variations of lipids in plasma following a meal, and whether this affects the levels of POPs in plasma significantly. Significant changes in PCB and lipid concentrations during pregnancy have also been reported[3]. Phillips et al. have shown that there are no significant differences between fasting and non-fasting samples when the levels of POPs are adjusted to the levels of lipids in plasma[4]. However, in a study by Kuwabara et al. it was found that digestion of PCB contaminated fish gave a significant increase after 5 hours in the levels of PCBs that was not related to a similar increase in the amount of lipids[5]. Bergman et al. claim that the lipid content in human plasma varies depending on the diet and how soon after the meal the blood sample is taken, and it is therefore more correct to express the concentration on a fresh-weight basis[6]. Another problem when lipid levels of POPs are being compared is the determination of lipids. The lipids are quantified either by gravimetric determination of the extractable organic material or by a summation of the different enzymatically determined lipid classes [4]. Of the different summation formulas the following is the preferred one; TL = 1.677(TC-FC) + FC + TG + PL [7,4,8]. Here TL is total lipids, TC is total cholesterol, FC is free cholesterol, TG is triglycerides and PL is phospholipids. Several studies are recommending the enzymatic summation method rather than the gravimetric method[8,9].

The aim of this study was to determine how much a lipid rich meal with a considerable amount of POPs affects the levels of these compounds in plasma and if the relative congener pattern in the food determines the observed changes. Further, any difference in levels of POPs between fasting and non-fasting samples was studied both on wet weight (ww.) and lipid weight (lw.) levels.

Materials and Methods

Study design

The 33 participants from the city Tromsø were served a traditional North Norwegian fish dish consisting of cod with liver, hard roe, fresh cod liver oil, and potatoes. The meal was prepared the traditional way as follows: Both the cod and the hard roe were boiled separately in water. The liver was boiled in small amounts of water and the oil from the boiled liver was served as fresh cod liver oil. Participants could eat as much as he or she wanted, but the amount of liver and cod liver oil eaten by each participant was weighed and registered (Table 1).

The meal was served between 6 and 7 p.m. on day 1. Blood samples were collected just before the meal, after 4 hours, 12 hours, and 5 days. The ‘12 hours’ and ‘5 days’ samples were taken in the

Table . Measured intake of cod liver, cod liver oil, *p,p'*-DDE and sum PCB

	Cod liver (g)	Liver oil (g)	*p,p'*-DDE mg	sum PCB mg
Average	116	22	6,3	23,9
Median	107	5,5	5,2	20,8
Min. - Max.	43 - 281	0 - 152	1,6 - 24	6,3 - 89

Table 2. Amount of lipids and percentage difference in levels of lipids in plasma

	0 hours (mg/dl)	4 hours (mg/dl)	12 hours (mg/dl)	5 days (mg/dl)	0-4 hours % difference (p-value)	4-12 hours % difference (p-value)	12 hrs- 5 d. % difference (p-value)	0-12 hours % difference (p-value)
average	680,8	753,2	611,4	653,1	10,9 (p<0.01)	-18,3 (p<0.01)	7,2 (p=0.084)	-9,8 (p<0.01)
geomean	669,2	739,9	602,1	644,3				
max	954,5	1040,2	820,1	854,1	35,6	-3,2	27,2	7,4
Min	464,4	460,9	434,7	474,0	-5,6	-33,5	-3,6	-27,5
Stdev	128,8	143,1	108,5	108,7	8,9	7,5	7,3	6,3

morning before breakfast, while the 0 and 4 hours blood samples were non-fasting.

Participants were asked to maintain their ordinary diet during the study period, except not to have any meals between lunch and the cod dish served on day 1. Body weight was measured on day 5. To estimate the participant's usual daily fish intake all participants answered a semi-quantitative food-frequency questionnaire.

Analytical procedures

The plasma samples were extracted according to method published by Furberg et al. [10].

The sample was further cleaned using a tandem florisil column manually packed with 1.5 g of florisil with 2 g of sodium sulphate on top. Hexane: dichloromethane (3:1) was used as eluent. The columns were pre-washed using 10 ml of eluent, before the sample was applied to the column and the POPs were eluted using 11 ml of eluent. The collected fraction was evaporated to 0.5 ml using a Zymark Turbovap 500 Closed Cell Concentrator (Hopkinton, USA), followed by a gentle flow of nitrogen for reduction to 100 ml. Gas chromatography (GC) was performed according to method and conditions published by Furberg et al.[10]. C-13 marked *p,p'*-DDE was included as internal standard in addition to the C-13 marked PCB congeners.

Due to missing data on free cholesterol the following summation formula was used for determining lipids in plasma; TL = 2.27 x TC + TG + 62.6 (mg/dl) [11]. Only 13 PCB congeners (table 3) and *p,p'*-DDE are included in this study to be sure that all levels were well above the limit of quantification.

The cod liver and cod liver oil was analysed with a slight modification of the method published by Kallenborn et al. [12].

Results and Discussion

The amount of cod liver and cod liver oil eaten by the participants is shown in table 2, together with the amounts of PCBs (sum of 11 congeners) and *p,p'*-DDE consumed.

Table 3. Average difference in levels of the different compounds between sampling hours

	0-4 hrs. % change (p-value*)		4-12 hrs. % change (p-value*)		12 hrs. - 5 d. % change		0-12 hrs. % change (p-value*) (p-value*)	
	ww	lw	ww	lw	ww	lw	ww	lw
p,p'-DDE	35	20	-40	-27	-12 (p=0.03)	-16	-20	-13
PCB 99	-2 (p=0.5)	-13	-21	-4 (p=0.06)	2 (p=0.8)	-3 (p=0.3)	-25	-18
PCB 101	-20	-28	-27	-10	-32	-36	-46	-41
PCB 118	-8 (p=0.09)	-18	-18	1 (p=0.8)	0 (p=0.5)	-5 (p=0.1)	-28	-22
PCB 138/163	10 (p=0.08)	-2 (p=0.1)	-17	1 (p=0.6)	-4 (p=0.2)	-10	-10 (p=0.02)	-2 (p=0.2)
PCB 149	-11 (p=0.04)	-19	-21	-2	-43	-47	-43	-37
PCB 153	8 (p=0.2)	-4 (p=0.03)	-18	0 (p=0.7)	0 (p=0.8)	-6 (p=0.1)	-12	-5 (p=0.02)
PCB 156	-1 (p=0.6)	-11 (p=0.02)	-16	2 (p=0.9)	12 (p=0.4)	5 (p=0.3)	-16	-9 (p=0.03)
PCB 170	-3 (p=0.06)	-13	-10 (p=0.03)	9 (p=0.3)	5 (p=0.9)	-2 (p=0.9)	-13	-6 (p=0.02)
PCB 180	-6 (p=0.04)	-16	-10 (p=0.02)	9 (p=0.08)	-3 (p=0.4)	-9 (p=0.1)	-15	-9
PCB 183	2 (p=0.4)	-12	-10 (p=0.05)	9 (p=0.4)	1 (p=0.7)	-5 (p=0.4)	-7 (p=0.06)	-5 (p=0.05)
PCB 187	1 (p=0.9)	-8	-11 (p=0.08)	6 (p=0.9)	4 (p=0.5)	-3 (p=0.2)	-10 (p=0.1)	-3 (p=0.2)
Sum PCB	2 (p=0.52)	-9	-16	2 (p=0.59)	-1 (p=0.47)	-7 (p=0.04)	-15	-8

*Where no p-value is indicated there is a significant difference ($p<0.01$).

For 22 of the plasma samples the lipids were determined both gravimetrically and using the summation formula. The levels using the summation formula are on average 15 % higher than the levels determined gravimetrically. The observed difference between the two methods is significant ($p=0.01$). This is comparable to the 20 % difference reported by Sjodin et al.[13]. All further calculations in this study are done using the levels of lipids determined from the summation formula. The average amount of lipids in the plasma samples is shown in table 2, together with the differences in levels. As expected the only non-significant change in the levels of lipids is observed from 12 hours to 5 days.

The average differences in levels of PCBs and *p,p'*-DDE together with the respective p-values are listed in table 3. Looking at DDE reveals that the level increased significantly going from 0 to the 4-hour sample, both on a ww. and lw. basis. As expected from the change in lipid levels the biggest change in ww. levels is appearing from 4 hours to 12 hours with an average drop in DDE ww. level of

41 %. For the lw. level the corresponding drop is 29 %. Both these differences in concentration are highly significant. The drop in level from 0 to 12 hours is also significant for both ww. and lw. levels.

For the PCB congeners the picture is more complex with smaller changes and some congeners having levels increasing and some dropping following the meal. These changes are also shown in table 3. Looking at the sum PCB values indicates small changes both on a ww. and a lw. basis but some changes are nevertheless significant. Going from 0 to 4 hour the lw. levels have a significant drop of 9 % whereas the ww. levels are unchanged. From 4 to 12 hours there is a significant drop in the ww. levels by 16 %. From 0 to 12 hours the differences observed for both ww. and lw. levels are significant. This clearly indicates that there are significant differences in levels of POPs between samples taken from fasting and non-fasting individuals even when the levels are related to the amount of lipids in plasma.

The three major PCB congeners in plasma, PCB153, 180 and 138 develop differently during the time period studied. The l.w. levels of PCB 180 drop significantly by 16 % going from 0 to 4 hours and by 9 % from 0 to 12 hours. The corresponding changes for PCB 138 and 153 are not significant. Compared to the level of PCB 138 and 153 the level of PCB 180 was much lower in the cod liver and cod liver oil. Thus implying that the changes observed in plasma depends on the relative congener pattern in the food.

No significant relationship was found between individual intake and corresponding changes in lipid levels and levels of the measured compounds.

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