

# **A cross-sectional study of the association between persistent organochlorine pollutants and diabetes**

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**ABSTRACT**

**Background:** Experimental evidence supports the hypothesis that persistent organochlorine pollutants (POPs) may cause type 2 diabetes, whereas there is no fully convincing epidemiological evidence for such an association. In Sweden the most important source of POP exposure is fatty fish. We have assessed the association between serum levels of POPs and prevalence of diabetes in Swedish fishermen and their wives, with high consumption of fatty fish from the Baltic Sea.

**Methods:** We have in 196 men (median age 60 years) and 184 women (median age 64 years) analyzed 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis(p-chlorophenyl)-ethylene (p,p'-DDE) in serum with gas chromatography-mass spectrometry. The participants were asked if they had diabetes and, if so, since which year and about medication and diet. The Odds Ratios (OR) for diabetes with respect to dichotomized exposure variables were analyzed with logistic regression, adjusting for potential confounders. Moreover trends of diabetes prevalence with respect to trichotomized exposure variables were tested with Jonckheere-Terpstra's test.

**Results:** Six percent of the men and 5 % of the women had diabetes. There was a significant association between CB-153 and diabetes among the men (OR 12, 95% confidence intervals [CI] 1.5, 93). The OR was increased also for p,p'-DDE, but it was far from significant (OR 1.7, 95% CI 0.5, 6.4). Among the women the association between CB-153 and diabetes was close to unity, whereas a non-significant association was observed between p,p'-DDE and diabetes (OR 1.8, 95% CI 0.4, 8.0). There were among the men significant positive trends between CB-153 tertiles and diabetes ( $p=0.005$ ) and between p,p'-DDE and diabetes ( $p=0.04$ ). Among the women the pattern was very similar regarding p,p'-DDE exposure and diabetes ( $p=0.07$ ), whereas no such association was observed for the CB-153 exposure. Time elapsed since diagnosis of diabetes was among the men negatively correlated with CB-153 exposure ( $r_s=-0.59$ ,  $p=0.04$ ).

**Conclusions:** The study gives some support for that POP exposure might contribute to type 2 diabetes. Even if we cannot exclude the possibility of a reversed causality, the observed negative correlation between time elapsed since diabetes diagnosis and CB-153 level in serum, speaks for the hypothesis of POP as a risk factor.

## INTRODUCTION

It is widely believed that the increase in incidence of type 2 diabetes mellitus and obesity is the result of a complex interplay between genetic and environmental factors [1]. Type 2 diabetes is due to resistance to insulin action and a relative deficiency of insulin. Age, obesity, central adiposity, lack of physical activity and dietary glyceamic load are the main factors identified as responsible for the disease [2].

Experimental evidence has linked exposure to dioxin and other persistent organochlorine pollutants (POPs) to impaired glucose transport, leading to speculation that chronic low-level exposure to dioxins might be a risk factor for diabetes [3].

Previous epidemiological studies have recently been reviewed [3,4]. Several studies have linked high POP burdens to increased risks of diabetes or modified glucose metabolism. A major difficulty in these studies is, however, that exposure occurred sometimes many years before the epidemiologic study, which makes it difficult to determine whether the higher levels of dioxins and polychlorinated biphenyls (PCBs) in diabetics truly reflect a higher exposure to these pollutants, which in turn may contribute to diabetogenesis, or whether they are merely the consequence of diabetes-induced metabolic perturbations facilitating the accumulation of these pollutants. Thus, the possibility of a reversed causality cannot be excluded.

In Sweden, consumption of fatty fish from the Baltic Sea, off the Swedish east coast, is the single major exposure source for POP, and cohorts of professional fishermen and their families from the Swedish east coast have been found to constitute excellent study bases for epidemiological evaluations of human health effects of POP [5,6]. We have used 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) as a biomarker for POP exposure, because it correlates very well with both total concentration in serum of polychlorinated biphenyls (PCB) [7,8], with the PCB derived dioxin-like effect [8] as well as the total POP derived dioxin-like effect [9]. Another relevant exposure biomarker is the anti-androgenic compound

1,1-dichloro-2,2-bis(p-chlorophenyl)-ethylene (p,p'-DDE), which is the major metabolite of the insecticide DDT.

The aim of the present study was to assess the associations between biomarkers for POP and prevalence of diabetes in high exposure cohorts of middle-aged and elderly men and women. The results showed in men, but not in women, an association between the POP markers in serum and prevalence of diabetes.

## METHODS

### *Study population and interview*

Previously established cohorts of professional fishermen and their wives from the Swedish east coast [6,10] were linked to the Swedish Population Register. A postal questionnaire was sent in year 2000 to 1500 fishermen and 1291 fishermen's wives that were born between 1920 and 1954, living in Sweden and still alive at 31 December 1999 (28). There were 813 men (54 %) and 779 women (77 %) who responded to the questionnaire. Out of them 510 men and 596 women were positive to participate in future clinical studies. We invited a subset of them to a study mainly focused on bone mineral density [11], but also comprising other potential health effects of POP exposure. The aim was to include 200 men and 200 women in the study, and we consecutively contacted subjects by phone for agreements until enough subjects were recruited. Details of the recruitment process have been given elsewhere [11]. The final study groups comprised 196 men and 184 women.

The participants were interviewed, using a standardized questionnaire. The subjects were asked if they had diabetes and, if so, since which year. Moreover, they were asked if they had per oral antidiabetic drugs, insulin or were on diet. We measured current weight and height. In addition, they were asked about their weight at the age of 25 years. Descriptive data for the participants are shown in Table 1.

The 617 male non-participants had similar age distribution (median 62 years, range 49-84) as the participants (median 60, range 49-84). In addition, the BMI distributions were also very similar among the non-participants (median 26.5 kg/m<sup>2</sup>, range 17.1-39.9) and the participants (median 27.2, range 20.5-38.5). There was neither any difference between the 595 non-participating women and the participants with respect to age (median 65 years, range 49-84 *versus* median 64 years, range 49-83) or BMI (median 26.5 kg/m<sup>2</sup>, range 17.1-39.9 *versus* median 26.2 kg/m<sup>2</sup>, range 19.7-38.2).

The study was performed in accordance with the Declaration of Helsinki and approved by The Lund University Ethic's Committee. All participants provided written informed consents.

### *Blood sampling*

Venous blood samples were drawn between 8.00 and 10.00 A.M, after 12 hr fasting, into sterile Vacutainer glass tubes (BD Vacutainer, Plymouth, UK). Serum was separated by centrifugation (4000 rpm, 10 minutes) and transferred to glass bottles and special tubes. All serum samples were stored at  $-80^{\circ}\text{C}$  until analysis.

### *Determination of CB-153 and p, p'-DDE in serum*

The analyses were performed applying solid phase extraction using on-column degradation of the lipids and analysis by gas chromatography mass spectrometry as previously described [12-14]. Levels of detection, coefficients of variation and participation in quality control programs have been described in detail elsewhere [14].

### *Determination of serum lipids by enzymatic methods*

Serum concentrations of triglycerides and cholesterol were determined by enzymatic methods as described elsewhere [14]. The total lipid concentration in serum (g/L) was calculated by the following equations [15]:

Men:            Total =  $0.96 + 1.28 * (\text{triglycerides} + \text{cholesterols})$

Women:        Total =  $1.13 + 1.31 * (\text{triglycerides} + \text{cholesterols})$ .

### *Statistics*

Separate analyses were performed for men and women. The effect estimations (odds ratios, OR) between the exposure variables CB-153 and p,p'-DDE, respectively, and diabetes were

obtained from logistic regressions. The exposure variables were dichotomized into two equally sized groups. Due to the high correlation between CB-153 and p,p'-DDE (women  $r=0.68$ ; men  $r=0.64$ ) these variables were not included in the models simultaneously. As potential confounders we considered current age and BMI at 25 years of age (both as continuous variables). In addition, the exposure variables were categorized into three equal sized groups. For evaluation whether there were trends in the data with respect to prevalence of diabetes, Jonckheere-Terpstra test (StatXact Statistical Software) was applied. We did also test whether time elapsed since diagnosis of diabetes were correlated (Spearman's correlation test) with the exposure variables and age.

## RESULTS

Twelve of the 196 men (6 %) and 10 of the 184 women (5 %) had diabetes. Five of the male diabetics had per oral antidiabetic drugs, two had a combination of per oral drugs and insulin, one had insulin only, and the remaining four were only on a diet. The corresponding figures for the female diabetics were four, two, one and three, respectively.

When the exposure variables were dichotomized there was a significant association between CB-153 and diabetes among the men (OR 12, 95% confidence intervals [CI] 1.5, 93). The OR did not change more than marginally when age and BMI at 25 years of age were taken into account. The OR was increased also for p,p'-DDE among the men, but it was far from significant (adjusted OR 1.7, 95% CI 0.5, 6.4). Among the women the association between CB-153 and diabetes was close to unity, whereas a non-significant association was observed between p,p'-DDE and diabetes (adjusted OR 1.8, 95% CI 0.4, 8.0).

Using the exposure data categorized into tertiles there were among the men significant positive trends between CB-153 exposure and diabetes ( $p=0.005$ , Table 2) and between p,p'-DDE exposure and diabetes ( $p=0.04$ ). Among those in the highest tertile of CB-153 ( $>475$  ng/g lipid) the prevalence of diabetics was 14%. Among the women the pattern was very similar regarding p,p'-DDE exposure and diabetes ( $p=0.07$ ), whereas no such association was observed for the CB-153 exposure.

Time elapsed since diagnosis of diabetes was among the men negatively correlated with CB-153 exposure ( $r_s=-0.59$ ,  $p=0.04$ ), whereas it tended to be positively correlated among the women ( $r_s=0.53$ ,  $p=0.12$ ).

## DISCUSSION

The main finding of the present study was that male diabetics had significantly higher serum levels of both CB-153 and p,p'-DDE than healthy males. A similar trend, however, non-significant was seen also for p,p'-DDE and female diabetics. These results are in concordance with a number of previous epidemiological studies that have recently been reviewed. Except for some studies of chemical manufacturing workers, there is a rather consistent finding of a slight increase in diabetes type 2 incidence among subjects exhibiting elevated serum concentrations of dioxins and dioxin-like POPs (3,4). Since these reviews were published also a Belgian study have shown highly significant elevation of serum levels of dioxins, and PCBs among patients with type 2 diabetes [16]. Moreover, also DDE has been associated with diabetes. In a group of pesticide users and an unexposed group, Morgan et al [17] found that subjects with diabetes had higher blood levels of DDT and DDE.

The epidemiological findings have some biological plausibility as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in experimental studies decreases cellular glucose uptake [18]. Moreover, it has recently been hypothesized that dioxins and PCBs could promote diabetes by interaction with peroxisome proliferators-activated receptor- $\gamma$ , a ligand-activated transcription factor controlling lipid metabolism and homeostasis that is linked with diabetes [3,19].

An obvious caveat interpreting the epidemiological cross-sectional studies is to know the direction of the causality between POP exposure and type-2 diabetes. A reversed causality cannot be excluded, meaning that the disease affects the serum levels of POP. Type 2 diabetes is associated with a variety of metabolic changes, which quite conceivably could alter the metabolism of POPs. Diabetes can alter the pharmacokinetics of some drugs [20] and also the activity of cytochrome P450 [21]. Type 2 diabetes is also known to cause a dysregulation of fat metabolism, which in turn might influence the distribution and elimination of lipophilic compounds such as PCBs and dioxins [16]. If diabetics have a slower rate of excretion of

TCDD and other POPs, this could account for the observed associations with diabetes [4]. The possibility of a slower elimination of dioxins in diabetes was, however, not supported by a recent study on Vietnam veterans, in whom no difference in TCDD half-life was found between diabetic and non-diabetic patients [22]. Moreover, if diabetes would slow down the excretion of POPs from the body, time elapsed since diagnosis of diabetes should be expected to be positively correlated with CB-153 in serum. However, in the present study we instead found among the men a significant negative correlation between time since diabetes diagnosis and CB-153 in serum, which speaks against that the diabetes disease would slow down POP excretion.

In the present study we used self-reported diabetes, and had no access to medical records. However, considering the age distribution, time elapsed since diagnosis and that only one man and one woman had insulin as single therapy, we feel convinced that almost all of the patients had a type 2 diabetes. Moreover, the prevalence figures in the present study (6 % for men and 5 % for women), are well in concordance with what was observed in a recent Swedish population based study on similar age groups (about 7 % in men and about 5 % in women) [23].

The association between POP exposure and diabetes prevalence was stronger among the men than the women in the present study, but there was a tendency of association also for the women. We have no biological explanation for this difference, which might be a random finding considering the relatively small size of the study.

## **CONCLUSIONS**

This cross-sectional study gives some further support for the hypothesis that POP exposure might contribute to type 2 diabetes. Even if we cannot exclude the possibility of a reversed causality, the presently observed negative correlation between time elapsed since

diabetes diagnosis and CB-153 level in serum, speaks for the hypothesis of POP as a risk factor.

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## **COMPETING INTERESTS**

The author(s) declare that they have no competing interest.

## **AUTHORS CONTRIBUTIONS**

LH initiated the project. LR and ARH performed the statistical analyses. All authors participated in the design of the study and of writing the manuscript. All authors have read and approved the final manuscript.

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**Table 1.** Characteristics for the 196 men and 184 women from Sweden that participated in the study

	Male		Female	
	Diabetes		Diabetes	
	No (n=184)	Yes (n=12)	No (n=174)	Yes (n=10)
	Mean, median (5 <sup>th</sup> , 95 <sup>th</sup> perc)	Mean, median (5 <sup>th</sup> , 95 <sup>th</sup> perc)	Mean, median (5 <sup>th</sup> , 95 <sup>th</sup> perc)	Mean, median (5 <sup>th</sup> , 95 <sup>th</sup> perc)
Age (yr)				
Current	60, 59 (49, 75)	60, 60 (53, 67)	63, 61 (51, 77)	64, 64 (51, 74)
At diagnosis	-	52, 52 (30, 62)	-	55, 56 (45, 65)
Time since diagnosis (yr)	-	9, 5 (2, 26)	-	10, 10 (0, 27)
Body Mass Index (kg/m <sup>2</sup> )				
Current	28.6, 28.1 (23.5, 35.5)	29.4, 30.1 (22.4, 33.3)	27.9, 27.2 (21.3, 35.8)	30.4, 29.5 (26.6, 41.3)
At 25 years of age	23.4, 24.0 (20.0, 28.9)	26.0, 25.4 (21.3, 32.6)	22.0, 22.0 (17.9, 26.6)	23.0, 22.5 (19.7, 26.6)
Exposure (ng/g lipid)				
CB-153	430, 360 (110, 950)	670, 560 (360, 1600)	280, 240 (94, 620)	300, 230 (110, 810)
p,p'-DDE	800, 570 (110, 2100)	1100, 1100 (390, 2400)	800, 590 (100, 2300)	1600, 990 (300, 5300)

**Table 2** Prevalence of diabetes in relation to tertiles of lipid adjusted concentrations of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis(p-chlorophenyl)-ethylene (p,p'-DDE) in serum among 196 men and 184 women from Sweden.

Gender	Diabetes	p for
Exposure (ng/g lipid)	Yes/No	trend <sup>a</sup>
Male		
CB-153		
-290	0/64	
>290-475	4/61	0.005
>475	8/58	
p,p'-DDE		
-410	1/63	
>410-850	4/61	0.04
>850	7/60	
Female		
CB-153		
-180	3/57	
>180-475	4/57	0.94
>475	3/60	
p,p'-DDE		
-375	1/59	
>375-860	3/59	0.07
>860	6/56	

<sup>a</sup> Jonckheere-Terpstra test