EXPOSURE TO PERSISTENT ORGANOCHLORINE POLLUTANTS AND TYPE 2
DIABETES MELLITUS.

Running title: Exposure to persistent organochlorine pollutants and type 2 diabetes mellitus.

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Summary

Persistent organochlorine pollutants (POPs), such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), dichloro diphenyl trichloroethane (DDT) and its major metabolite 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p´-DDE) have been associated with type 2 diabetes mellitus (T2DM) in recent epidemiological studies. We have analyzed 2,2´,4,4´,5,5´-hexachlorobiphenyl (CB-153) and p,p´-DDE in 544 serum samples from Swedish women with a median age of 50 years. The participants were asked if they had diabetes and if so, what type of diabetes, years since diagnosis and what kind of treatment they had. Associations between exposure and T2DM were analyzed by logistic regression. Moreover, trends of T2DM prevalence were tested with Jonckheere-Terpstra´test.

Sixteen of the 544 women (3%) had diabetes, of which 15 were classified as T2DM. There was a significant association with T2DM for both CB-153 (an increase of 100 ng/g lipid corresponded to an Odds Ratio [OR] of 1.6, 95% confidence interval [CI] 1.0, 2.7) and p,p´-DDE (OR 1.3, 95% CI 1.1, 1.6). In addition, significant positive trends between quartiles of CB-153 and T2DM (p=0.004) and p,p´-DDE and T2DM (p=0.002) were observed.

The study shows an association between POP serum concentrations and an increased prevalence of T2DM.

Introduction

The incidence of type 2 diabetes mellitus (T2DM) is rapidly increasing world-wide. [1]
T2DM is characterized by peripheral resistance to insulin action and a relative deficiency
of insulin. The main factors identified as responsible for the disease are age, central
adiposity, lack of physical activity, and dietary glycemic load.[2] Moreover, a number of
genetic factors seem to be of importance for the pathogenesis.[2-3]

High and moderate body burdens of persistent organochlorine pollutants (POPs), such as
polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), dichloro
diphenyl trichloroethane (DDT) and its major metabolite 1,1-dichloro-2,2-bis (p-chlorophenyl)-
ethylene (p,p´-DDE), have been associated with T2DM in recent epidemiological studies. [4-10]
In Sweden the most important source of POP exposure is consumption of fatty fish from the
Baltic Sea. [11-12] That has been the rationale for using professional fishermen and their wives as
a study base for epidemiological evaluations of human health effects of POPs. Throughout
these studies, 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 of PCBs in serum
[13-14], the PCB derived dioxin-like effect[13] as well as with the total POP derived dioxin-like
effect.[15] Another relevant exposure biomarker is p,p´-DDE, which is present in relatively high
serum concentrations in subjects consuming fatty fish from the Baltic Sea.[16]

In a recent study, comprising Swedish fishermen from the east coast**, *i.e.*** by the Baltic
Sea and their wives, we found significantly higher levels of the major PCB congener CB-
153 in serum from diabetic men, as compared with non-diabetics but a more ambiguous
pattern with respect to p,p´-DDE.[17] In contrast, among the women there was an
association with p,p´-DDE but not with CB-153.

We have now performed a new, and almost completely independent, study of the association between T2DM prevalence and CB-153 and p,p'-DDE in serum among Swedish fishermen's wives. The aim of the present study was therefore to evaluate whether our previous finding of a positive association between POP and T2DM[17], could be confirmed.

Methods

Study population and interview

Altogether 1439 fishermen's wives from the Swedish east and west coasts, from previously established cohorts[18], born 1945 or later, were contacted for a fecundability study.[19] We collected blood samples from 544 women (38% participation rate), and in a telephone interview the participants were asked if they had diabetes mellitus and, if so, when it was diagnosed by a physician. Moreover, they were asked if they were using per oral antidiabetic drugs, insulin or were on diet. Information on current weight and height was also collected. Sixteen of the 544 women (3%) had diabetes. Two of the women had per oral antidiabetic drugs, four had insulin only and the remaining **ten** women were on diet treatment only. Among the four women with insulin treatment, one had a variant of type 1 diabetes (latent autoimmune diabetes in adult) diagnosis and the other three had T2DM. The focus of the present study is on the 15 women with T2DM and the 528 non-diabetic women.

There was an overlap of only 23 women between the present study and our previous study on POP and T2DM[17], and none of the overlapping women had T2DM.

The age distribution among the participants (median 50 years, range 29-59) did not differ from those who did not participate (median 50 years, range 27-58). 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.

Collection of blood samples

Blood samples were drawn from a cubital vein into 10 ml vacuum tubes for serum collection without additives (Becton Dickinson, Moylan, France). After cooling to room temperature the tubes were centrifuged at 4000 *g* for 15 min. Serum was transferred with ethanol rinsed Pasteur pipettes to ethanol rinsed brown glass bottles (Termometerfabriken, Gothenburg, Sweden). A piece of aluminium foil was placed on top of the bottles which were then sealed. Sera were stored at -80°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.[20-21] Levels of detection, coefficients of variation and participation in quality control programs have been described in detail elsewhere.[20]

Determination of serum lipids by enzymatic methods

Briefly, serum concentrations of triglycerides and cholesterol were determined by enzymatic methods using reagents from Roche Diagnostics (Mannheim, Germany). The inter-assay CVs for cholesterol and triglyceride determinations were 1.5-2.0 %. The average molecular weights of triglycerides were assumed to be 807. For cholesterol we used an average weight of 571, assuming that the proportion of free and esterified cholesterol in plasma was 1:2.[20] The total lipid concentration in serum (g/L) was calculated by the following equation[22]: Total = 1.13 + 1.31*(triglycerides + cholesterol).

Statistics

The effect **of** the exposure variables CB-153 and p,p´-DDE on diabetes risk was estimated using odds ratios (ORs) and 95 % confidence intervals (CIs) , obtained from logistic regressions. The exposure variables were treated as continuous variables and due to the high correlation between CB-153 and p,p´-DDE (r= 0.69) these variables were not included in the model simultaneously. We considered current age (as a continuous variable) and current Body Mass Index (BMI) (as a continuous variable) as potential confounders (table 1).

We used the method suggested by Greenland[23] for deciding which of the potential confounders should be included in the final multivariate linear regression models. Potential confounders were entered into bivariate and multivariate models if they changed the effect estimates by 10 % or more, and excluded if their exclusion changed the effect estimates by less than 5 %.

In addition, the exposure variables were categorized into four equally sized groups. Jonckheere-Terpstra´s test (StatXact Statistical Software) was applied in order to evaluate whether there were trends in the data with respect to prevalence of T2DM (table 2). We also tested if time elapsed since diagnosis of T2DM and the exposure variables and age, respectively, were correlated (Spearman's correlation test).

Results

CB-153 was significantly associated with T2DM, an increase of 100 ng/g lipid corresponded to an OR of 1.6, 95 % confidence interval [CI] 1.0, 2.7, p=0.05. When age was included in the model the association became weaker OR 1.4, 95 % CI 0.8-2.5, p=0.25. Regarding p,p´-DDE an increase of 100 ng/g lipid corresponded to an OR of 1.3, 95% CI 1.1, 1.5, p=0.004. Inclusion of age in the model did not change the association for p,p'-DDE. BMI did not fulfil the inclusion criteria for any of the models.

When the exposure variables were categorized into quartiles, significant positive trends were observed for CB-153 and T2DM (p=0.004) and p,p´-DDE and T2DM (p=0.002), (table 2).

Time elapsed since diagnosis of T2DM was not correlated with CB-153 (r_s= 0.10, p=0.70) or p,p´-DDE (r_s= -0.28, p=0.29).

Discussion

In the present study we found that women with high levels of POPs had a significant increased risk of having T2DM as compared to those with lower serum levels of POPs. The prevalence of 3% T2DM in the present study group with a median age of 50 years was in the same range as the self-reported prevalence of 1% in year 1999 for women 45-54 years of age, estimated from a population survey in Northern Sweden.[24] We had no access to medical records, but according to the information collected at the interview all reported cases of T2DM were well established.

Recent epidemiological evidence suggests a possible association between high body burdens of POPs and an increased risk of T2DM or modified glucose metabolism. Results from a population based study from Belgium indicated that T2DM patients had significantly higher serum levels of dioxins and PCBs than controls.[6] In a follow-up study of the Michigan PBB cohort, PCB serum levels measured in 1976 were risk factors for the incidence of adult-onset diabetes mellitus.[10] Longnecker et al.[8] reported that among men with background exposure to TCDD, higher serum levels were associated with increased prevalence of T2DM. An excess of T2DM has also been reported from a group of pesticide users, which had higher blood levels of DDT and p,p´-DDE compared to controls.[9] In our previous study on Swedish fishermen's wives the median serum levels of CB-153 was three time higher than those found in the present study.[17] The corresponding relation for p,p'-DDE was 2.5. In spite of the lower exposure range in the present study we found clear associations between CB-153 and p,p´-DDE, and T2DM. There are two reasons why the serum concentrations were lower in the present study. First, the previous study was restricted to fishermen's wives from the east coast, with an

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averagely higher POP exposure from the Baltic Sea fatty fish.[18] Second, the participants in the present study were as a median 11 years younger at sampling than those in the previous study. We have previously shown a clear birth cohort effect in this study base, with higher POP serum levels in older compared to younger women.[20]

Altered lipid metabolism [25], altered glucose transport [26-27] and alterations in the insulin signalling pathway [28] are mechanisms that provide biological plausibility to the association between dioxins and other POPs, and diabetes. T2DM in itself is also known to cause a dysregulation of fat metabolism, which in turn might influence the distribution and elimination of lipophilic POPs such as PCBs and dioxins.[20] Both *in vitro* and *in vivo* studies of mice, rats and guinea pigs exposed to TCDD, has been linked to drastic reductions in cellular glucose uptake [29], and in rabbits a lowering of the insulin production by the pancreas.[30] Further, mechanistic studies have indicated effects of TCDD on expression on genes implicated in T2DM.[31-34] There are no experimental data supporting that di-ortho PCB congeners such as CB-153, will have a diabetogenic effect by themselves, but CB-153 serves as a good proxy marker also for TCDD toxic equivalency (TEQ) and the total POP derived TEQ.

In the present study, we have only used two biomarkers for POP exposure: CB-153 and p,p´-DDE. The reason for this is that the GC-MS technique used at our laboratory does not allow analysis of *e.g.* dioxin-like PCBs. However, we believe that CB-153 is a sufficiently good proxy biomarker because it correlates very well with total PCB concentration in plasma and serum [13-14], and with TCDD, TEQ as well as with the total POP-derived TEQ.[15] The possibility of a reversed causality between POP exposure and T2DM poses a problem for interpretation of cross-sectional studies, including the present.

If, T2DM cause dysregulation in the lipid metabolism and it may influence distribution and elimination of lipophilic compounds such as dioxins and PCBs,[6] which could explain the associations found in the present study as well as in other cross-sectional studies. Moreover, if T2DM patients after diagnosis deliberately try to reduce their fat mass by dietary restrictions, this would result in increased POP concentrations in the remaining adipose tissue. Unfortunately, we have no information on weight reduction in the present study group. We considered current BMI as a potential confounder in the statistical analyses, but it had been preferable to adjust for change in BMI between time of diagnosis and time of blood sampling for POP analysis.

The hypothesis of a slower elimination of dioxins in T2DM patients was, however, not supported by a study on Vietnam veterans, in whom no difference in TCDD half-life was found between diabetic and non-diabetic patients.[35] Moreover, if T2DM slows down the excretion of POPs from the body, time elapsed since diagnosis would be positively correlated with CB-153 and p,p´-DDE in serum. However, no such correlations were observed in the present study. This speaks against that the findings in the present study were due to reversed causality. Thus, circumstantial evidence support that POP exposure might very well act as a risk factor for T2DM, but a longitudinal study is needed to obtain more conclusive evidence.

The previous[17] and the present cross-sectional studies of subjects relatively highly exposed to POPs comprise too few incident cases to evaluate a possible threshold effect for the association between POPs and T2DM. It might seem paradoxical for a hypothesized association that the body burdens of most POPs have been declining in the general population over the past decades whereas the incidence and prevalence of T2DM

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have increased quite dramatically in most industrialized countries during the same time period. However, the critical time window for POP exposure might be a long time before the T2DM is diagnosed. Thus, to what extent POP exposure may have contributed to the T2DM epidemic in the general population should be tested in large prospective studies.

Conclusion

In conclusion, this study shows an association between POP serum concentrations and an increased prevalence of T2DM.

Acknowledgements

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Table 1. Characteristics for 543 women from Sweden that participated in the study

	T2DM	
	No (n=528)	Yes (n=15)
	Mean, median (5th, 95th perc)	Mean, median (5th, 95th perc)
Exposure (ng/g lipid in serum)		
CB-153	98, 82 (30, 220)	130, 110 (56, 250)
p,p´-DDE	190, 140 (49, 500)	340, 240 (93, 970)
Age (years)		
Current	49, 50 (37, 57)	52, 53 (38, 57)
At diagnosis		43, 50 (12, 55)
Body Mass Index (kg/m^2)	25.5, 24.8 (20.3, 32.8)	27.9, 27.3 (21.4, 36.4)

| Time since diagnosis | 9, 5 |
| (years) | (0- 31) |

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Table 2. Prevalence of type 2 diabetes mellitus (T2DM) in relation to quartiles of lipid adjusted serum concentrations of 2,2´,4,4´,5,5´-hexachlorobiphenyl (CB-153) and dichloro-diphenyl-dichloro-ethane (p,p'-DDE), among 543 women from Sweden.

Exposure (ng/g lipid)	T2DM	p for
CB-153	Yes/No	trend [*]
< 58	1/135	
> 58-84	0/136	
> 84-118	7/128	
> 118	7/129	0.004
p,p´-DDE		
< 91	0/136	
> 91-144	2/134	
> 144-240	5/130	
> 240	8/128	0.002

* Jonckheere-Terpstra's test