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ABSTRACT

Aims. - To explore exposure to 22 persistent organic pollutants (POPs) and incident type 2 diabetes in a population-based, prospective cohort.

Methods. - This case-cohort study on 753 participants without type 2 diabetes at baseline, was followed-up over nine years, as part of the French D.E.S.I.R. cohort. We measured 22 POPs in fasting serum at baseline. The associations between baseline POP concentrations, pre-adjusted for lipids, BMI, age and sex, with incident type 2 diabetes, were assessed using Prentice-weighted Cox regression models (time scale: age), adjusted for traditional confounding factors. POPs were also modelled summed in functional groups: polychlorinated biphenyls (∑PCB) and organochlorines (∑OC) and also individually, after log-transformation, in adjusted Cox models.

Results. - There were 200 incident diabetes cases over nine years. Pre-adjusted POP concentrations were not related to diabetes risk for any of the 22 POPs examined. For the PCBs, hazard ratios (HRs) per interquartile range of the pre-adjusted POPs, ranged from 0.87 (95% CI: 0.64,1.19) to 1.22 (0.93,1.59,). For (*p, p'-DDE)* and (*p, p'-DDT)*, the HRs were 1.09 (0.83,1.43) and 0.89 (0.70,1.13), respectively. The HRs for PeCB, HCB, *β*-HCH, γ–HCH, Oxychlordane, Trans-nonachlor were 0.98 (0.85,1.13), 1.06 (0.84,1.33), 1.22 (0.93,1.59), 1.13 (0.89,1.42), 1.00 (0.76,1.31), 0.86 (0.66,1.43), respectively. HRs for ∑PCB and ∑OC did not differ significantly from one.

Conclusion. - We did not observe any relations between exposure to POPs and diabetes in this population-based cohort. These results do not support causal inferences reported in previous studies linking serum POP concentrations and diabetes risk.

KEY WORDS:

Diabetes mellitus, Incidence, Organochlorine insecticides, Pesticides, Persistent organic pollutants, Polybrominated biphenyls

Introduction

Diabetes is one of the most challenging health problems of the 20th century, with large and rapid increases in prevalence observed across almost all developed and developing countries [1]. Traditional risk factors (obesity, unhealthy diet and lack of physical activity) are likely to be key contributors, however, they are unlikely to be the sole reasons for increases in diabetes prevalence [2]. In recent years, there has been increased attention on environmental risk factors which may be contributing to the epidemic. Endocrine and metabolic disruptors are a broad group of compounds which have been implicated in the development of type 2 diabetes [3]. One class of these compounds is the persistent organic pollutants (POPs) which are ubiquitous in our environment, due to their lipophily. Some POPs are intentionally made and others are by-products; they comprise a group of diverse substances and include organochlorine insecticides such as dieldrin, dichlorodiphenyltrichloroethane (*p, p'* DDT) and its main metabolite in humans, dichlorodiphenyldichloroethylene (*p, p'* DDE), as well as several industrial chemical products or by-products including polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs, *e.g.*, 2.3.7.8-tetrachlorodibenzo-*p*-dioxin, or TCDD), hexachlorobenzene (HCB), polybrominated biphenyls (PBBs), polybrominated diphenylethers (PBDEs) [4]. These compounds are resistant to biodegradation and persist in the environment for long periods of time. Humans are predominantly exposed through the consumption of contaminated food, mainly meat, fish, and dairy products even at low doses, although some exposure also exists through air and water [4].

Most of the studies linking POPs to diabetes in humans are cross-sectional in design. A few cohort studies have measured exposure to POPs and diabetes in a prospective setting, but only a few of these are population-based and many have small numbers of diabetes cases (a few tens). Furthermore, the results from these studies are mixed and no single POP predicts diabetes consistently. The only study which has large numbers, is the Nurses' Health study (NHS). Using two independent, nested case-control studies from within the NHS, Wu et al. showed that after multivariable adjustment, plasma HCB concentration was positively associated with incident type 2 diabetes [pooled OR 3.59 (95% CI 1.49,8.64), comparing the third terile with the first tertile [5]. Further work published more recently, using a more contemporary sample within the NHS population and with new measurements of a broader set of POPs, showed that a score,

summing the concentrations of five dioxin-like PCBs congeners (DL-PCBs), was an independent risk factor for diabetes with an OR of 1.78 [1.14,2.76], comparing the third tertile with the first tertile [6]. POPs have been linked to diabetes in two studies from Sweden. In a case-control study of women aged 50–59 years, after a follow-up of 6 years, (*p, p*'-DDE) was related to an increasing risk of diabetes [4th quartile versus 1st, OR 5.5 (1.2,25)] [7]. In a more recent study, the prospective investigation of the Vasculature in Uppsala Seniors (PIVUS) study, the ORs for type 2 diabetes according to quintiles of a sum of ranks of PCB measures (vs. the lowest quintile) were 4.5 (0.9,23.5), 5.1 (1.0,26.0), 8.8 (1.8,42.7) and 7.5 (1.4,38.8) (p_{trend} <0.01) after adjustment for known risk factors [8]. There were also significant relationships with the organochlorines. However, with only 36 cases of diabetes, the confidence intervals around the odds ratios was large, leading to some uncertainty relating to the effect size [8].

POPs are strongly associated with lipids and obesity [9]. Disentangling the mediating and potentially confounding effects of lipids and body mass index (BMI) in regard to lipophilic environmental compounds has presented some challenges [10, 11]. While the ideal methodology would be to assess POPs in fat tissue rather than serum, this is difficult to do in large numbers within the general population. Recently, new statistical methods have been developed which may overcome some of these challenges [10, 12, 13]. These include an approach [10, 12] which pre-adjusts the environmental compounds for factors such as lipids, BMI, age, sex, followed by modelling with confounding factors. Here, we examine the prospective relationship between POP concentrations with incident type 2 diabetes, in a population-based cohort in France, using this novel approach, coupled with conventional analytic approaches, to investigate the relationship between POPs and incident type 2 diabetes over 9 years of follow-up.

Material and methods

Source population

D.E.S.I.R. is a prospective, population-based cohort study that aimed to explore the development of the insulin resistance syndrome and type 2 diabetes [14]. Between 1994 and 1996, the D.E.S.I.R. study recruited 5212 men and women aged 30–65 years, from volunteers who were offered four free health examinations over nine years, by the French Health Insurance System in 10 health examination centers in central-western France. Participants attended a

baseline visit and a range of biological and clinical tests were conducted. These examinations were repeated at 3-, 6-, and 9-year visits. The procedures and methods undertaken at each follow-up have been previously published [14]. The study protocol was approved by French Ethics Committees and written informed consent was obtained from all participants.

Study population

We conducted a case-cohort study nested within the French D.E.S.I.R. cohort. Participants were recruited between 1994-1996 and followed for 9 years. All participants with blood glucose and BMI data at baseline and 9-year visits were eligible for this study (n=3409). We randomly selected 600 (11.5%) participants at baseline from all participants, consistent with sampling fractions of other case-cohort studies [15]. We refer to this as the subcohort hereafter. Incident type 2 diabetes was defined by treatment with glucose-lowering agents or by a fasting plasma glucose above 7·0 mM or by HbA_{1c} above 6·5% at any of the three 3-yearly health examinations after inclusion in the study. After excluding participants with prevalent type 2 diabetes or uncertain diabetes status at baseline, and one missing serum for assays, the final study population included 583 subcohort members and 200 incident cases of type 2 diabetes, 30 of whom were in the subcohort. The sampling strategy is shown in Fig. 1.

Covariates

Blood samples were collected after at least 12 h of fasting at each of the four examinations. All samples were stored at -80 °C. Blood samples were analyzed for glucose and lipids as previously described and weight, height and waist circumference were measured [14]. Blood pressure was measured in duplicate using a mercury sphygmomanometer in a supine position, and mean values were calculated. Information on family history of diabetes, treatment for diabetes, hypertension, elevated lipids was collected during an interview with a physician. Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or ongoing blood pressure-lowering treatment. A self-administered questionnaire provided data on education level, living with a partner, smoking status, diet, and physical activity (at home, at work and during sporting activities). Physical activity was categorized as: sedentary, moderately active and active based on the frequency of participation in active sporting activities

(never, <1 once a week, 1–2 times a week, >2 times a week) and the intensity of usual physical activity at home and at work (low, moderate, important, intensive). The dietary intake (in Kcal per day) was calculated using the NAQA food consumption questionnaire, [16] describing usual food consumption, based on the composition of meals and snacks and the frequency of consumption of meat, fish, fried foods, butter, cheese, dairy products, bread, sugary desserts, sugar, soft drinks and alcoholic beverages.

Chemical analysis of persistent organic pollutants

We used the literature to guide us in the choice of POPs to study, according to their presence in appropriate concentrations is similar populations and whether they have been used historically, worldwide.

The methods used for the laboratory analysis of 22 POP in serum samples has been described previously[13, 17] and we provide the same detail below. The POP compounds measured were ten PCBs (PCBs -74, -99, -118, -138, -153, -156, -170, -180, -183, -187); nine pesticides or pesticide metabolites (pentachlorobenzene (PeCB), hexachlorobenzene (HCB), alpha-hexachlorocyclohexane (*α*-HCH), beta-hexachlorocyclohexane (β-HCH), gamma-hexachlorocyclohexane (γ-HCH), oxychlordane, *trans*-nonachlor, (*p, p'*-DDT), (*p, p'*-DDE)). Three brominated diphenyl ethers (BDE-47, BDE-99, BDE-153) were also were measured.

Pretreatment of the serum samples was as follows: ethanol and ^{13}C-labelled internal standards of each compound in toluene were added to samples (200 µL) in test tubes and mixed to precipitate the proteins and equilibrate internal standards. Dichloromethane-hexane (1:4) was added for extraction followed by activated silica to bind the sample water, ethanol, and precipitate. Samples were mixed, and layers were allowed to separate. The upper dichloromethane-hexane layer was poured to a solid phase extraction cartridge (SPE cartridge) containing from bottom to top 10% $AgNO_3$ impregnated silica and a mixture of Na_2SO_4 and silica. Elution of SPE-cartridges was performed with dichloromethane-hexane, and the eluate was concentrated to 15-20 µL for gas chromatography - triple quadrupole mass spectrometry (GC-MS/MS) analysis. The instrument used was an Agilent 7010 GC-MS/MS system (Wilmington, DE, USA), GC column DB-5MS UI (J&W Scientific, 20 m, ID 0.18 mm, 0.18

μm). Limits of quantification (LOQ) ranged from 5 pg/mL for PCB congeners and trans-nonachlor to 40 pg/mL for *p, p'*-DDE. Two blank samples and two control samples (NIST SRM 1958) were included in each batch of samples. Measured concentrations of POPs in SRM 1958 were 80–105% of the certified/reference concentrations. The coefficient of variation (CV-%) from SRM 1958 ($n = 18$) was below 3.6% for all compounds. The THL laboratory participates three times a year in AMAP interlaboratory comparisons (Ring Test for Persistent Organic Pollutants in human serum, National Institute of Public Health, Quebec, Canada). In AMAP ring tests for POPs, laboratory's results varied from 83 to 132% of the assigned values, depending on the compound.

Machine values were used in the analyses, including for POP concentrations below the level of quantification (LOQ). POPs that had more than 70% of values below the LOQ were not analyzed (α- HCH, BDE-49, BDE-99 and BD-153).

Statistical analysis

Descriptive data are presented as n (%), mean (standard deviation), median (25^{th} and 75^{th} percentiles), and comparisons tested by χ^2 or Student-t tests.

Hazards ratios for incident diabetes were modeled in age-adjusted (crude) and multivariable Prentice-weighted Cox regression models, adapted to the case-cohort design [18], allowing estimation of adjusted hazard ratios (HRs) associated with POPs for incident type 2 diabetes, with their 95% confidence intervals (CI).

Three analytical approaches were used to define the variables representing POP concentrations in serum.

Approach 1: Lipid, BMI, age, sex-adjusted POP levels

POPs are lipophilic compounds, and lipids, age, BMI and sex have been shown to be related to POPs [12, 13], and also to incident diabetes. It has been argued that it is difficult to disentangle the possible causal effects of lipophilic substances such as POPs with incident diabetes, independently of the concomitant confounding and mediating effects. Adjusting for lipids may not adequately remove the effect of confounding. To address this, alternative

methodological approaches have been developed, using 'pre-adjusted residuals'. Indeed, residuals of the linear regression model where a POP is explained by a set of variables correspond theoretically, if the model is correct, to the part of the POP's variability unexplained by these variables. Linear regression was used in the subcohort sample to model log-transformed POP concentrations with predictors: LDL-cholesterol, triglycerides (log-transformed), BMI, age, sex where all were assessed at baseline. Two-way interaction terms between all covariates were also included to model exposure at best, using a multiplicative variable. Residuals were estimated for the subcohort and then calculated for the cases, applying the model (with identical parameters) derived from the subcohort. We refer to these as 'pre-adjusted residuals' [12].

To allow easier interpretation of the results, the residuals were divided by the interquartile range (IQR) and the results are reported per one unit increase in the IQR of the residual, for each of the POPs. These pre-adjusted POP levels can be interpreted as those expected at baseline, if all study participants had the same LDL-cholesterol and triglyceride levels, BMI, age and sex.

Approach 2: Sum of PCBs, sum of organochlorine chemicals

Since populations are exposed to a variety of organic pollutants at the same time we also modelled POPs in functional groups according to the literature [19]. Modelling composite variables also decreases the number of separate analyses undertaken thereby reducing the potential for false positives.

POPs were grouped and summed according to two functional classes [19]:

a) the sum of PCBs ($\sum$PCBs) for each participant, adding concentrations of all ten PCBs as previously described [20];

b) the sum of organochlorine chemicals (OCs), ($\sum$OCs) adding concentrations of eight chemicals: PeCB, HCB, β- and γ -HCH, oxychlordane, *trans*-nonachlor, (*p, p'*-DDT) and (*p, p'*-DDE).

Both summed variables (units, ng/L) were log-transformed and modelled as continuous variables.

Approach 3: POPs as continuous variables without pre-adjustment

The logarithms of the POP were modelled per IQR.

The pre-adjusted POP residuals, the summed POPs and the log-transformed POP concentrations - all per IQR - were modeled in age-adjusted (crude) and multivariable Prentice-weighted Cox regression models, adapted to the case-cohort design [18]. We estimated the date of diabetes onset as the midpoint between the last visit without diabetes and the first visit with diabetes. Age was used as the timescale. Participants were followed-up until the earliest of diabetes onset, death or nine years after entry into the study. The proportional-hazards assumption was checked using Schoenfeld residuals and the assumption was not violated.

Multivariable models were adjusted for potential diabetes risk factors: sex (men/women), waist circumference (continuous), family history of diabetes (yes/no), smoking (Never, exsmoker, current), physical activity (sedentary, moderately active or active), education (no or partial primary school, secondary school, post secondary school), living with a partner (yes/no), HDL-cholesterol (continuous), triglycerides (continuous), hypertension (yes/no) and calorie intake (continuous).

Multiple fractional polynomials were used to determine the functional form, for each POP, with incident diabetes; none of the POPs significantly deviated from a linear relation.

As relationships between POPs and diabetes have been reported to vary by sex [21], we compared the likelihood of models with and without the sex interaction term; a P value of 0.2 was considered as suggestive of an interaction. Spearman correlation coefficients were calculated between the pre-adjusted POP residuals in the subcohort. All analyses used Stata/SE version 15.1. We adhered to reporting guidelines of case-cohort studies as per recommendations by Sharp et al. [15].

Results

Study population

Baseline characteristics of subcohort members, without the cases of incident diabetes, and all cases of incident type 2 diabetes, are presented in Table 1. In the subcohort without diabetes, the mean age (± SD) at inclusion was 48 ± 10 years and 54% were women. Sociodemographic characteristics (sex, age, education level) of individuals from the random subcohort did not differ substantially from those of other participants included in the D.E.S.I.R. cohort (data not shown). The mean age (SD) when type 2 diabetes was diagnosed was 52 ± 9 years. The incidence rate (95% CI) of type 2 diabetes was 5·5 (4·8–6·3) per 1000 person-years in the entire cohort. Compared to the subcohort without the diabetes cases, the incident cases of diabetes were significantly more likely to be men, smoke, have a family history of diabetes, have hypertension, be less likely to engage in intensive physical activity at baseline. They were also significantly older, had a higher mean BMI and waist circumference, lower mean HDL and higher median triglycerides levels, and consumed more calories than members of the subcohort without diabetes (Table 1).

Concentration of persistent organic pollutants

As more than 90% of the concentrations of α-HCB, BDE-47, BDE-99 and BDE-153 were below the LOQ, they were excluded from the analyses (data not shown in Table 2). For the people in the subcohort, the highest concentrations were found for (*p, p'*-DDE), PCB-153 and PCB-180 (median concentrations: 1962, 1480 and 1191 ng/mL, respectively) (Table 2). Among the PCBs, the median concentrations ranged from 139 ng/mL for PCB99 to 1480 ng/mL for PCB-153. The concentration of the remaining organochlorine pesticides ranged from 78 ng/mL for oxychlordane to 562 ng/mL for β-HCH. The medians of HCB, β-HCH, γ-HCH, oxychlordane, *trans*-nonachlor, (*p, p'*-DDE), (*p, p'*-DDT), PCB-99, PCB-118, PCB-138 and PCB-187 were significantly higher in the incident cases of diabetes versus the subcohort without cases. The Spearman correlation coefficients (*rho*) of the pre-adjusted POP are shown in Table S1 (Supplementary analyses).

Associations of persistent organic pollutant exposure with incident diabetes

The associations between POP exposure and the risk of developing type 2 diabetes using approaches 1, 2 and 3 are presented respectively, in Tables 3, 4 and 5.

Approach 1 pre-adjusted POP concentrations

In analyses where the POP were pre-adjusted for lipids, BMI, age and sex and then the residuals modelled by Cox regression, PCB180 was the only chemical associated with diabetes risk, with a hazards ratio of 0.77 (0.61,0.98). After additionally adjusted for confounding factors, no statistically significant relationships were observed (Table 3). There were no significant interactions with sex (data not shown).

Approach 2: Summed POP concentrations

In multivariable-adjusted analyses, the HRs for summed OCs was 1.00 (0.72,1.41) and 0.66 (0.35,1.23) for summed PCBs (Table 4). There were no significant interactions with sex (data not shown).

Approach 3 (modelling of log- transformed POP)

In age-adjusted analyses, HCB, *β*-HCH, *γ*- HCH, oxychlordane, *trans*-nonachlor and (*p, p'*-DD, were significantly associated with incident diabetes (Table 2). All of these relationships were attenuated and became non-significant after adjustment in the Cox models for diabetes risk factors (Table 5). There were no significant interactions with sex (data not shown).

Discussion

Using a population-based study from France, we observed that exposure to the 18 different POPs measured (with quantifiable serum levels) at baseline, did not independently increase or decrease the risk of incident diabetes over nine years, using three analytic approaches.

We observed the largest effect sizes in relation to diabetes risk with hexachlorobenzene, β-HCH and some PCBs although none of these were significant. This is broadly consistent with the literature. In the Nurses' Health study (NHS), both HCB and total PCBs were associated with incident type 2 diabetes, in agreement with a meta-analysis of prospective studies [5]; they are also consistent with a later study in the same NHS population [6]. In this study, Zong et al. [6] showed significant positive findings with HCB, β- HCH, *p, p'-DDE* and dioxin-like PCBS (DL-PCBs) and incident diabetes, in models with similar covariate adjustment to ours. In the PIVUS study, POPs were shown to independently predict diabetes risk for type 2 diabetes: the ORs (95% CI) according to quintiles of a sum of ranks of PCB measures (vs. the lowest quintile) were 4.5 (0.9,23.5), 5.1 (1.0,26.0), 8.8 (1.8,42.7) and 7.5 (1.4,38.8) (p_{trend} <0.01) after adjustment for known risk factors [8]. In a very recently published hospital based case- control study from China, OC exposure was positively associated with the prevalence of type 2 diabetes β-HCH and (*p, p'-DDE)* were associated with higher levels of fasting plasma glucose, even among those without diabetes.[22] It is noteworthy that this study modelled log transformed POPs as continuous variables expressed per wet-weight of lipids and the models, unlike ours, were minimally adjusted for age BMI and sex [22].

Inconsistencies in previous studies regarding congener-specific findings are well known in the POPs and diabetes literature. There are many possible factors at play which may explain these differences. These include small numbers of diabetes cases, use of different study designs, insufficient adjustment for confounding factors, differential background exposure status, lack of or inadequate lipid adjustment and differences in other population characteristics, that may affect POP retention in the body [4]. We employed a newly developed statistical approach to analyse the POPs which take into account that POP concentrations are related to obesity, lipids and other covariates [10, 13, 23]. It has been reported that pre-adjusting the POPs before modelling leads to more conservative relationships, which is consistent to what we observe. However, we do not fully attribute our null findings to this methodology as we employed two additional methods and

the results across the three methods were consistent with each other: all three methods failed to detect significant associations of POPs with incident diabetes, after adjustment for covariates.

Strengths and weaknesses

A key strength of our study is the use of a population-based, longitudinal sample with carefully collected covariate data, dedicated to the study of diabetes determinants. In addition, our thorough analytical approach, performing three methods, represents another strength. There are several reasons why we may have failed to show more significant findings, if such associations exist. The most compelling of these is statistical power. In the D.E.S.I.R study, as there were only 200 incident cases of diabetes accrued over the 9 years,s we cannot exclude the possibility that we were underpowered to observe significant effects. We can however exclude the possibility of low exposure levels as the reason for lack of significance, as the concentrations detected for the POPs in the D.E.S.I.R. study are broadly similar, if not slightly higher, to those reported in other European countries, as reported in the EPIC study [13]. Of note, the same laboratory was used to test the POP in both studies. In particular, concentrations measured in D.E.S.I.R. are closest to the concentrations observed in Denmark in 2007. Another key limitation of these analyses is that our finances only allowed us to assay POPs at baseline and we acknowledge that the measurement of POPs at other time points would have been very useful in understanding the relationship between POPs and diabetes risk over time. This is because serum concentrations of POPs are directly affected by weight changes, which are becoming more common in modern societies[4].

Given that the cost of assaying POPs is prohibitive, we chose to use a case-cohort design to test our hypothesis that POPs exposure increases the risk of diabetes. This design is an efficient and cost effective method for risk assessment, as sample testing is only performed for the subcohort and the cases, rather than for the full cohort. Further, statistical efficiency is comparable for the case-cohort and the full cohort analyses [18].

The link between POP exposure and diabetes

The mechanisms linking POPs (organochlorine pesticides and PCBs) exposure to diabetes have not been fully elucidated, but several mechanistic pathways have been postulated. POPs have been shown to bind to several receptors, including the aryl hydrocarbon receptor and several nuclear receptors such as the estrogen receptors alpha and beta or PPARs, that regulate glucose and lipid homeostasis [4]. POPs are known to have endocrine disrupting properties similar to that of bisphenol A and thus binding to such sites that rapidly activate different signaling cascades, alter the regulation of glucose and lipid metabolisms [3]. Less is known about whether POPs increase diabetes risk via damage to β cells or by increasing insulin resistance. In animal studies, POPs have been related to both increased insulin resistance and reduced insulin secretion,[24] although the human studies are less clear than the animal work in this area. For human studies, the evidence is more consistent with high serum concentrations of POPs being associated with decreased insulin secretion rather than increased insulin resistance [25]. It is also apparent that POPs can be associated with reduced insulin secretion at low concentrations (in the range of pmol/L) [26]. The reason why human studies do not support the observation that high serum POPs increase insulin resistance as shown in animal studies is puzzling, and could be because the high level of insulin seen in type 2 diabetes may mask any measurable effect of POPs on insulin resistance [27].

Conclusion

In this cohort of middle-aged men and women, overall, we did not observe associations between serum POP concentrations and incident diabetes, independently of traditional confounding factors, with any one of the three methods of statistical analysis used.

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Fig. 1. The sampling strategy of the study

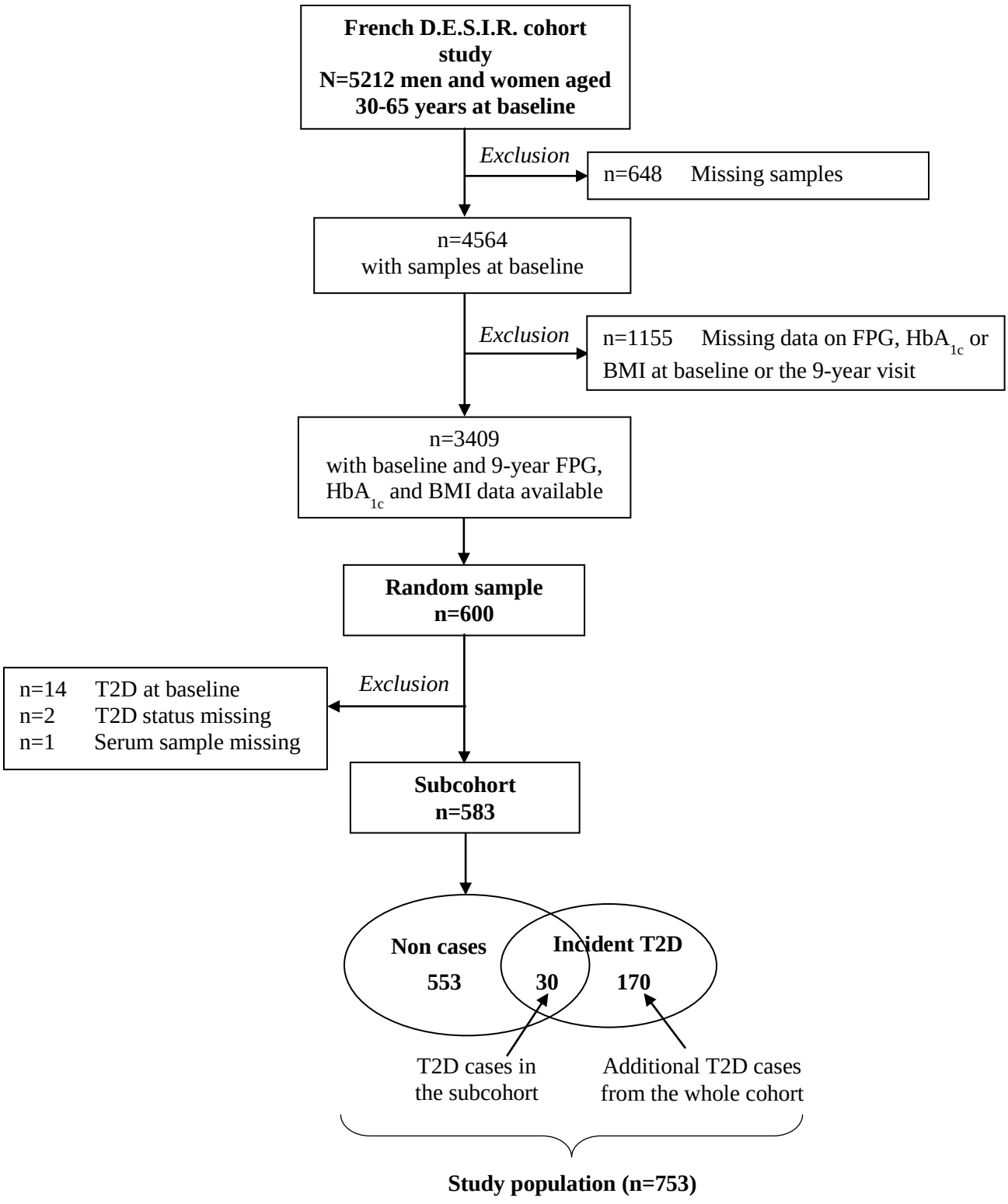

Table 1

Baseline characteristics of the French D.E.S.I.R. cohort, according to the case-cohort design (n=753)

	Subcohort, not including incident diabetes cases n=553	All incident diabetes cases n=200	*P* value
Age (years)	48 ± 10	51 ± 9	<0.001
Women	297 (54)	72 (36)	<0.001
Body mass index (Kg/m^2)	24.3 ± 3.2	27.3 ± 4.6	<0.001
Waist circumference (cm)			
Men	89 ± 9	97 ± 10	<0.001
Women	75 ± 8	88 ± 12	<0.001
Physical activity			0.002
Sedentary	122 (22)	73 (37)	
Moderatively active	297 (54)	98 (49)	
Active	133 (23)	29 (15)	
Smoking			<0.001
Never	298 (54)	82 (41)	
Former	162 (29)	60 (30)	
Current	93 (17)	58 (29)	
Education			0.963
No or partial primary school	80 (15)	30 (15)	
Secondary school	358 (64)	130 (65)	
Post-secondary school	115 (21)	40 (20)	
Diabetes in family	90 (16)	51 (26)	<0.001
Daily calorie intake (Kcal)	2101 ± 504	2250 ± 565	<0.001
Living with a partner	457 (83)	159 (80)	0.324
HDL cholesterol (mmol/l)	1.7 ± 0.4	1.5 ± 0.4	<0.001
Triglycerides (mmol/l)	0.9 (0.6, 1.3)	1.3 (1.0, 2.0)	<0.001
Hypertension	185 (33)	121 (61)	<0.001

Data are n (%), mean ± SD, median (25th, 75th percentile).

*Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure≥90 mmHg or treatment for hypertension.

Table 2

Concentrations of persistent organic pesticides (POPs) (ng/l) using medians (25^{th}, 75^{th} percentiles). The French D.E.S.I.R. study.

Persistent organic pollutants	All cases of incident diabetes n=200		Subcohort with diabetes cases n=583		Subcohort without diabetes cases n=553		Percentage < limit of quantification
PeCB	9.80	(7.46, 10.3)	10.1	(7.46, 13.3)	10.2	(7.57, 13.2)	49
HCB	354	(237, 545)	283	(197, 413)	272	(194, 403)*	0
β -HCH	811	(459, 1176)	562	(315, 879)	513	(301, 837)*	0
γ -HCH	18.4	(12.2, 30.5)	14.6	(9.81, 25.7)	14.7	(9.71, 25.7)*	61
Oxychlordane	89.6	(69.1, 130)	78.1	(58.7,104)	76.8	(52.3,104)*	0.9
Trans-nonachlor	72.0	(52.5, 95.0)	59.1	(42.1, 81.2)	57.4	(41.1, 79.6)*	0
p, p'-DDT	90.7	(65.6, 131)	78.0	(51.9 116)	74.6	(49.7 112)*	0.4
p, p'-DDE	2612	(1572, 4452)	1962	(1225, 3408)	1903	(1204, 3139)*	0.4
PCB-74	180	(118, 270)	158	(101, 237)	155	(104, 234)	0
PCB-99	167	(115, 228)	139	(90.4,195)	133	(90.4, 191)*	0
PCB-118	410	(265, 554)	305	(201, 458)	300	(200, 445)*	0
PCB-138	991	(757, 1238)	871	(693, 1136)	859	(683, 1124)*	0
PCB-153	1563	(1271, 1992)	1480	(1174, 1905)	1481	(1148, 1898)	0
PCB-156	192	(148, 247)	178	(137, 232)	181	(136, 231)	0
PCB-183	160	(125, 211)	149	(111, 195)	149	(109, 193)	0
PCB-187	323	(250, 442)	295	(211, 394)	290	(207, 390)*	0
PCB-170	528	(413, 670)	515	(405, 667)	518	(405, 663)	0
PCB-180	1187	(932, 1511)	1191	(921, 1478)	1198	(926, 1480)	0

Notes:

**P*-value below 5%, comparing median values between all cases of incident diabetes vs. subcohort without cases of incident diabetes

α-HCB, BDE-47, BDE-99, BDE-153 were also measured but, as more than 90% of samples were lower than the limit of quantification (LOQ), these compounds were excluded from further analyses.

Table 3

Hazard ratios (95% confidence intervals) for the relationship between pre-adjusted persistent organic pesticides (POPs) and 9 year incident diabetes (per one interquartile range (IQR) in the pre-adjusted (BMI, lipids, age and sex residuals of log of POPs). The French D.E.S.I.R study (n=753).

Persistent organic pollutants	Age-adjusted hazard ratios (95% CI)			Fully adjusted hazard ratios* (95% CI)		
PeCB	0.95	(0.84,	1.08)	0.98	(0.85,	1.13)
HCB	0.96	(0.80,	1.16)	1.06	(0.84,	1.33)
β- HCH	1.17	(0.95,	1.43)	1.22	(0.93,	1.59)
γ -HCH	1.19	(0.98,	1.45)	1.13	(0.89,	1.42)
Oxychlordane	0.95	(0.77,	1.18)	1.00	(0.76,	1.31)
Trans-nonachlor	0.83	(0.66,	1.04)	0.86	(0.66,	1.13)
p, p'-DDT	0.93	(0.77,	1.12)	0.89	(0.70,	1.13)
p, p'-DDE	1.06	(0.85,	1.32)	1.09	(0.83,	1.43)
PCB-74	0.94	(0.77,	1.15)	0.89	(0.70,	1.13)
PCB-99	1.04	(0.86,	1.26)	1.09	(0.83,	1.43)
PCB-118	0.99	(0.82,	1.19)	1.02	(0.80,	1.29)
PCB-138	0.96	(0.79,	1.17)	1.06	(0.85,	1.33)
PCB-153	0.90	(0.73,	1.11)	1.09	(0.88,	1.35)
PCB-156	0.84	(0.68,	1.05)	1.02	(0.80,	1.31)
PCB-170	0.80	(0.63,	1.01)	0.98	(0.75,	1.27)
PCB-180	0.77	(0.61,	0.98)	0.94	(0.71,	1.24)
PCB-183	0.93	(0.77,	1.13)	0.90	(0.66,	1.23)
PCB-187	0.83	(0.64,	1.06)	0.87	(0.64,	1.19)

Notes:

*Fully adjusted models were adjusted for age (time scale), sex, waist circumference, family history of diabetes, smoking, physical activity, education, living with a partner, HDL-cholesterol, triglycerides, hypertension and calorie intake.

Table 4

Hazard ratios (95% confidence intervals) for the relationship between summed persistent organic pesticides (POPs) and 9 year incident diabetes. The French D.E.S.I.R study (n=753).

Persistent organic pollutants	Adjusted hazard ratios (95% CI) for the summed POPs
	Total Population n=753
Sum of PCBs* (per log)	0.66 (0.35 –1.23)
Sum of OC POPs† (per log)	1.00 (0.72–1.41)

Notes:

Models adjusted for age (time scale), sex, waist circumference, family history of diabetes, smoking, physical activity, education, living with a partner, HDL-cholesterol, triglycerides, hypertension and calorie intake.

* Sum of PCB74, 99, 118, 138, 153, 156, 170, 180, 183, 187 concentrations.

† Sum of PeCB, HCB, β-HCH, γ- HCH, Trans-nonachlor, Oxychlordane, (*p, p'*-DDT) and (*p, p'*-DDE) concentrations.

Table 5

Hazard ratios (95% confidence intervals) for the relationship between persistent organic pollutants (POPs) (per interquartile range (IQR) in log-transformed POPs) and 9 year incident diabetes. The French D.E.S.I.R study (n=753).

Persistent organic pollutants	Age-adjusted hazard ratios (95% CI)			Multi-adjusted hazard ratios (95% CI)		
PeCB	0.93	(0.84,	1.04)	0.96	(0.85,	1.10)
HCB	1.66	(1.28,	2.14)	1.07	(0.75,	1.52)
β- HCH	1.25	(1.03,	1.53)	1.13	(0.88,	1.46)
γ –HCH	1.45	(1.16,	1.81)	0.88	(0.65,	1.19)
Oxychlordane	1.45	(1.14,	1.84)	0.74	(0.52,	1.04)
Trans-nonachlor	1.22	(1.02,	1.45)	0.94	(0.74,	1.19)
p, p'-DDT	1.25	(1.00,	1.56)	1.01	(0.76,	1.34)
p, p'-DDE	0.93	(0.74,	1.17)	0.83	(0.62,	1.11)
PCB-74	1.22	(0.97,	1.52)	0.93	(0.72,	1.20)
PCB-99	1.21	(0.94,	1.56)	0.94	(0.69,	1.27)
PCB-118	1.12	(0.91,	1.38)	0.88	(0.68,	1.13)
PCB-138	0.98	(0.78,	1.24)	0.82	(0.62,	1.10)
PCB-153	0.86	(0.66,	1.11)	0.76	(0.55,	1.06)
PCB-156	0.89	(0.69,	1.15)	0.76	(0.53,	1.10)
PCB-170	0.81	(0.63,	1.05)	0.75	(0.52,	1.08)
PCB-180	1.11	(0.89,	1.38)	0.92	(0.71,	1.21)
PCB-183	1.10	(0.85,	1.43)	0.87	(0.64,	1.18)
PCB-187	0.93	(0.84,	1.04)	0.96	(0.85,	1.10)

Notes: Models adjusted for age (time scale), sex, waist circumference, family history of diabetes, smoking, physical activity education, living with a partner, HDL- cholesterol, triglycerides, hypertension and calorie intake.

Supplementary Table S1

Spearman correlations coefficients between pre-adjusted Persistent organic pollutants in the subcohort population, n=583: the French D.E.S.I.R. Study. Statistically significant correlation coefficients are shown in bold text.

	PeCB	HCB	*β* -HCH	γ -HCH	Oxychlordane	Trans nonachlor	*p, p'*		PCB								
							DDT	DDE	74	99	118	153	138	156	187	183	180
PeCB	1																
HCB	0.39	1															
β -HCH	0.27	**0.71**	1														
γ -HCH	0.31	0.04	0.18	1													
Oxychlordane	0.29	0.68	0.69	0.08	1												
Trans-nonachlor	0.24	0.53	0.52	0.05	0.79	1											
p, p'-DDT	0.28	0.31	0.27	0.16	0.22	0.32	1										
p, p'-DDE	0.24	0.44	0.42	0.08	0.33	0.38	0.60	1									
PCB-74	0.28	0.61	0.57	0.07	0.58	0.47	0.28	0.41	1								
PCB-99	0.25	0.46	0.41	0.06	0.44	0.44	0.37	0.61	0.67	1							
PCB-118	0.24	0.48	0.45	0.03	0.43	0.44	0.33	0.43	**0.76**	**0.72**	1						
PCB-153	0.27	0.55	0.50	0.04	0.59	0.59	0.30	0.54	0.64	**0.79**	0.65	1					
PCB-138	0.25	0.53	0.50	0.04	0.56	0.56	0.34	0.60	0.64	**0.88**	0.68	**0.96**	1				
PCB-156	0.30	0.56	0.53	0.04	0.65	0.57	0.16	0.29	0.62	0.54	0.52	**0.86**	**0.77**	1			
PCB-187	0.22	0.42	0.35	0.04	0.43	0.57	0.30	0.41	0.49	0.61	0.53	**0.83**	**0.79**	**0.75**	1.		
PCB-183	0.24	0.49	0.41	0.04	0.46	0.51	0.36	0.59	0.54	**0.82**	0.58	**0.88**	**0.90**	0.63	**0.79**	1.	
PCB-180	0.22	0.45	0.42	0.04	0.55	0.54	0.16	0.29	0.46	0.47	0.41	**0.86**	**0.75**	**0.88**	**0.79**	**0.71**	1
PCB-170	0.23	0.49	0.45	0.05	0.58	0.55	0.18	0.29	0.49	0.49	0.42	**0.86**	**0.76**	**0.90**	**0.80**	**0.74**	**0.98**