UiT The Arctic University of Norway

Faculty of Health Science

Department of Community Medicine

Persistent Organic Pollutants and Type 2 Diabetes Mellitus

Addressing causality with repeated measurements using novel study designs

Dolley Dixil Charles

A dissertation for the degree of Philosophiae Doctor

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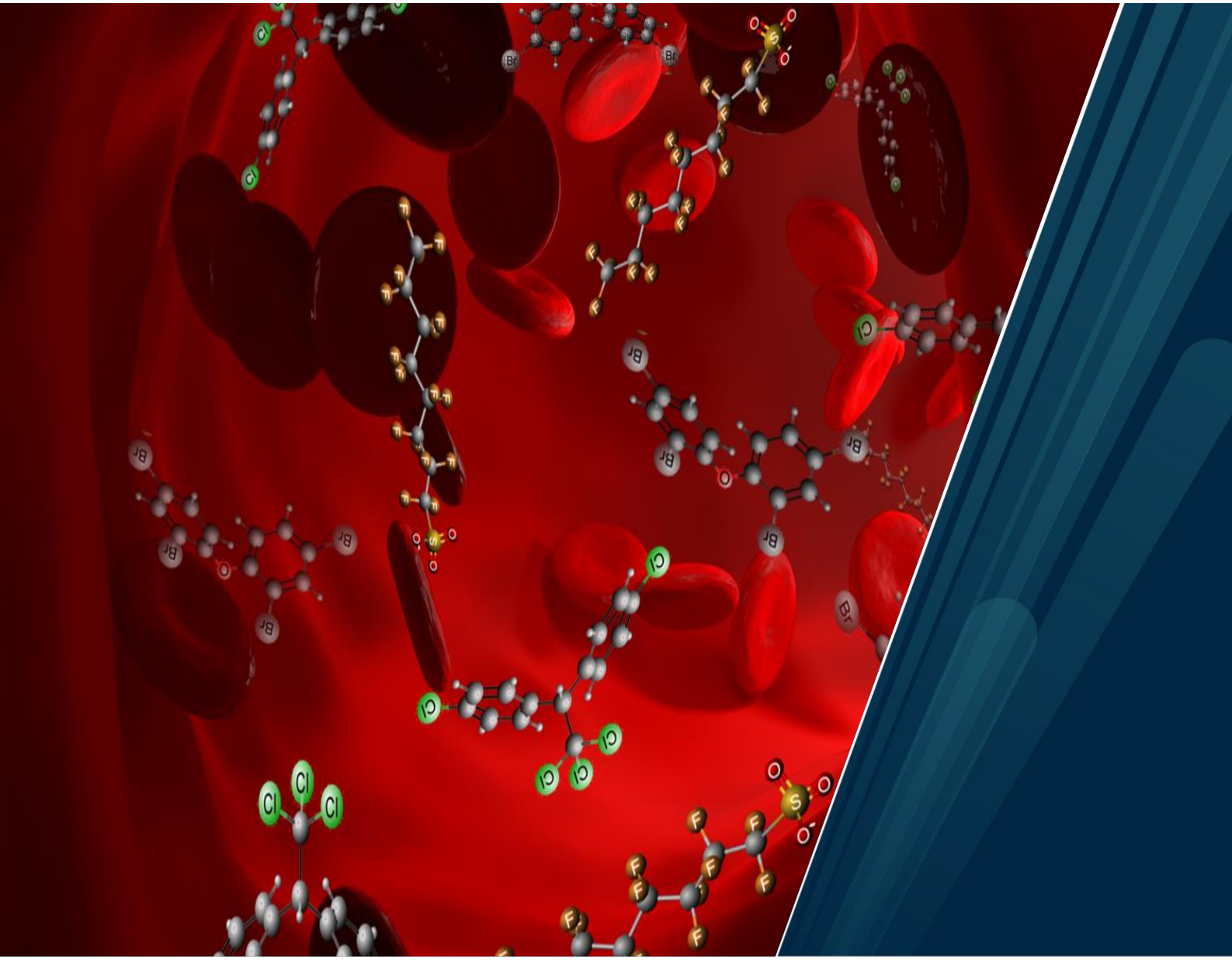

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By

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Summary

Background: Persistent Organic Pollutants (POPs) have been of interest to researchers as possible risk factors for Type 2 Diabetes Mellitus (T2DM). POPs are chemical substances that have been demonstrated to have negative impacts on the environment and biota as they are highly lipid soluble, persistent, and bioaccumulate in fatty tissues. Thus, several of the POPs have either been banned or are restricted in use. Risk factors related to T2DM have also been shown to influence human POP concentrations. Several previous studies have reported positive associations between single POP measurements, and prevalent and incident T2DM, although causality has not been established. Longitudinal studies with repeated pre-diagnostic POP measurements, which may help in addressing causality, are lacking.

Aim: The main aim of this thesis was to address causality in the POPs and T2DM relationship. Specifically, we investigated pre- and post-diagnostic associations between different classes of POPs (perfluoroalkyl acids [PFAAs], polychlorinated biphenyls [PCBs], organochlorine pesticides [OCPs] and polybrominated diphenyl ethers [PBDEs]), and T2DM using repeated measurements of POPs from the same individuals. We also compared the time trends of POPs within T2DM cases and controls to assess if T2DM status influences the body burden of POPs.

Methods: This thesis is based on studies using nested case-control study designs. Questionnaire data and blood samples from two different population-based studies were used to address the aims of this thesis. The Norwegian Women and Cancer study was used to investigate PFAAs-T2DM associations and time trends in PFAAs (2001-2005/06) with two repeated measurements. The Tromsø study was used to study the associations between o, PBDEs, and T2DM and their time trends (1986-2016) using three to five repeated POP measurements per individual.

Results: PFAAs and PBDEs were not associated with T2DM, while PCBs and OCPs were positively associated with T2DM, with strong associations only for *cis*-heptachlor epoxide, before and after diagnosis. Similar trends were observed for PFAAs and PBDEs within T2DM cases and controls, while the PCBs and OCPs declined slower in prospective cases compared to controls, and this trend continued in cases after T2DM diagnosis.

Conclusion: The results from this thesis do not support PCBs, OCPs, PBDEs, and PFAAs as being causal factors of T2DM but suggest that physiological changes related to T2DM may cause retention of some of the fat-soluble OCPs and PCBs already years before T2DM diagnosis leading to higher concentrations in prospective cases and thus positive associations with T2DM status.

List of papers

The thesis is based on the following papers.

1. Charles D, Berg V, Nøst TH, Huber S, Sandanger T, Rylander C. **Pre- and post-diagnostic blood profiles of perfluoroalkyl acids in type 2 diabetes cases and controls.** *Environ Int*. 2020;145.

2. Charles D, Berg V, Nøst TH, Bergdahl IA, Huber S, Ayotte P, Wilsgaard T, Averina M, Sandanger T, Rylander C. **Longitudinal changes in concentrations of persistent organic pollutants (1986-2016) and their associations with type 2 diabetes mellitus.** *Environ Res*. Mar 2022;204(Pt B):112129.

3. Charles D, Berg V, Nøst TH, Wilsgaard T, Bergdahl IA, Huber S, Ayotte P, Averina M, Sandanger T, Rylander C. **Polybrominated diphenyl ethers in type 2 diabetes mellitus cases and controls: repeated measurements prior to and after diagnosis** (submitted to International Journal of Hygiene and Environmental Health)

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Abbreviations

AhR	Aryl hydrocarbon receptor
AUC	Area under the curve
BDE	bromodiphenyl ether
BFR	brominated flame retardants
BLUP	best linear unbiased prediction
BMI	Body mass index
CI	confidence interval
CT	computer tomography
CVs	coefficients of variation
DAGs	directed acyclic graphs
DDE	dichlorodiphenyldichloroethylene
DDT	dichloro-diphenyl-trichloroethane
DEXA	dual Energy X-ray Absorptiometry
DM	diabetes mellitus
EDC	endocrine disrupting chemical
FFA	free fatty acid
GDM	gestational diabetes mellitus
GP	general practitioner
HCB	hexachlorobenzene
IQR	interquartile range
IR	insulin resistance
MDL	method detection limit
NOWAC study	Norwegian Women and Cancer study
OCPs	organochlorine pesticides
OR	odds ratio
p,p'-DDE	1,1-bis-4-chlorophenyl-2,2-dichloroethene
p,p'-DDT	1,1,1-trichloro-2,2-bis-4-chlorophenyl ethane
PBDEs	polybrominated diphenyl ethers
PCBs	polychlorinated biphenyls
PFDA	perfluorodecanoic acid
PFHpS	perfluoroheptane sulfonate

PFHxS	perfluorohexane sulfonate
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
PFUnDA	perfluoroundecanoic acid
PFAAs	perfluoroalkyl acids
POPs	persistent organic pollutants
PPV	positive predictive value
r_s	correlation coefficients
T	time point
T2DM	type 2 diabetes mellitus
WAT	white adipose tissue
β-HCH	beta-hexachlorocyclohexane

1 Introduction

Diabetes Mellitus (DM) refers to a group of chronic metabolic diseases. DM is characterized by elevated blood glucose levels (hyperglycemia) caused by insufficient insulin secretion, insulin resistance (IR), or a combination of both. Long-term complications of hyperglycemia include cardiovascular diseases, and damage to the eyes, kidneys, nerves, and blood vessels (1). There are two major types of DM. Type 1 Diabetes Mellitus (T1DM) occurs mostly in childhood due to insufficient insulin production by the pancreas. Type 2 Diabetes Mellitus (T2DM) is the most common type in adults, where the body is resistant to the insulin produced. In women, hyperglycemia, often first detected during pregnancy, is classified as gestational diabetes mellitus (GDM). Other less common types of diabetes include monogenic diabetes and secondary diabetes (1).

This thesis and the accompanying articles primarily focus on T2DM, specifically on the relationship between environmental pollutants, namely the perfluoroalkyl acids (PFAAs), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs), and T2DM.

1.1 Type 2 Diabetes Mellitus

1.1.1 Definition

T2DM, also known as non-insulin dependent or adult-onset diabetes, is a chronic condition characterized by hyperglycemia, IR, and a relative lack of insulin (2). With T2DM, the body does not use the insulin produced and thus is incapable of keeping blood glucose at normal levels (3).

1.1.2 History

The history of diabetes dates to 1500 B.C., when Egyptian physicians first recognized the symptoms of diabetes. Similar observations were made by Indian, Chinese, Greek, and Arab physicians. Around the fifth century B.C, the Indian physician Sushruta mentioned the sweet urine of the disease and termed it madhumeha (honey-like urine) and drew attention not only to urine having a sweet taste but also being sticky to touch and its ability to attract the ants. However, the term diabetes was introduced into the medical nomenclature by physician Aretaeus of Cappadocia. It arises from the Greek verb διαβαινω (diabaino= “passing through” refers to the great emptying of the urine) (4). Later, in 1675, Thomas Willis, an English

anatomist and physician was the first European medical writer who rediscovered the sweetness of urine and coined the term “mellitus” meaning “sweet” (4).

1.1.3 Epidemiology

T2DM makes up 90% of total DM cases. In 2021, the global prevalence of T2DM in adults (20-79 years of age) was 10.5% (5). The countries with the largest numbers of adults with diabetes in 2021 were China (140.9 million), India (74.2 million), and Pakistan (33 million). In 2021, the age-standardized prevalence (United Nations standard population, 2021) of T2DM in adults (20-79 years) was highest for Pakistan (30.8%), French Polynesia (25.2%), and Kuwait (24.9%) (Figure 1) (6). In several countries, an increasing trend in T2DM incidence was observed between 1960-2005. However, between 2006-2014, only a minority of populations showed a continued increase in the incidence of clinically diagnosed T2DM, while several populations showed stable or declining incidence trends (mainly in high-income countries) (7, 8). The prevalence of T2DM increases with age with the highest prevalence of 24% seen among 75–79-year-olds. This rising prevalence of diabetes is attributed to the aging of populations (6). Nearly 81% of the people with diabetes live in low- to middle-income countries (Figure 1) (5). The global health expenditure for diabetes was 966 billion USD in 2021 and is expected to reach one trillion USD by 2030 (6).

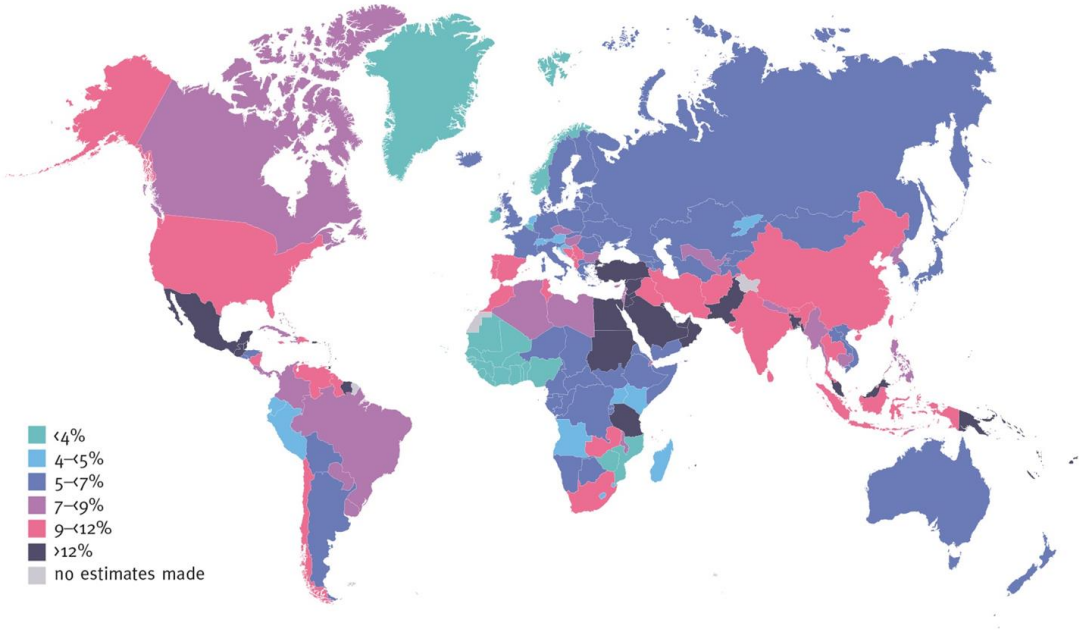

Figure 1. Estimated age-standardized prevalence of type 2 diabetes mellitus in adults (20-79 years) in 2021. *Source: Diabetes Atlas, 10th edition (6). Reprinted with permission from the International Diabetes Federation.*

In 2021, the prevalence of T2DM in Norway was 4.8% (age-standardized prevalence of 3.6%). Norway has a relatively low prevalence of T2DM compared to other countries, including Finland (9.7%), Sweden (6.8%), and Denmark (7.3%) (6). The incidence of T2DM in Norway showed a decreasing trend between 2009-2014, from 609 cases per 100,000 person-years in 2009 to 398 cases per 100,000 person-years in 2014, using data from the Norwegian Prescription Database, the Norwegian Patient Registry, and the primary care database (9). There are no other recent data about time trends in diabetes incidence in Norway (10). The European region has about 29.1 million (regional prevalence of 35.7%) people with undiagnosed diabetes (6). In Norway, the number of people with undiagnosed diabetes is estimated to be approximately 100,000, but the numbers are uncertain (11). Similar to other countries, T2DM in Norway increased with age, within certain ethnic groups, and was more common in people with lower education (12-15). In 2021, Norway ranked third among the countries with the highest yearly expenditure of an average of 11,779 USD/person with diabetes (20-79 years) (6).

1.1.4 Etiology and pathophysiology

For the body to function normally, blood glucose levels must be under control. This is accomplished by a complex system including various hormones and neuropeptides released from several key organs (brain, pancreas, liver, intestines), adipose, and muscle tissue. The pancreas is responsible for secreting the blood glucose-regulating hormones: insulin (produced by the β-cells) and glucagon (produced by the α-cells). Low blood glucose levels trigger the pancreas to secrete glucagon, which increases endogenous blood glucose levels through glycogenolysis (breakdown of glycogen occurring in the liver). Additionally, glucagon promotes renal and hepatic gluconeogenesis (the synthesis of glucose from non-carbohydrate precursors) to increase endogenous blood glucose levels. On the other hand, when blood glucose levels are high due to the intake of carbohydrate-rich foods, insulin is released to trigger insulin-dependent muscle and adipose tissues to take up glucose as well as to promote glycogenesis (the process of storing excess glucose) (Figure 2). Disturbances in the maintenance of glucose homeostasis lead to the development of T2DM (3).

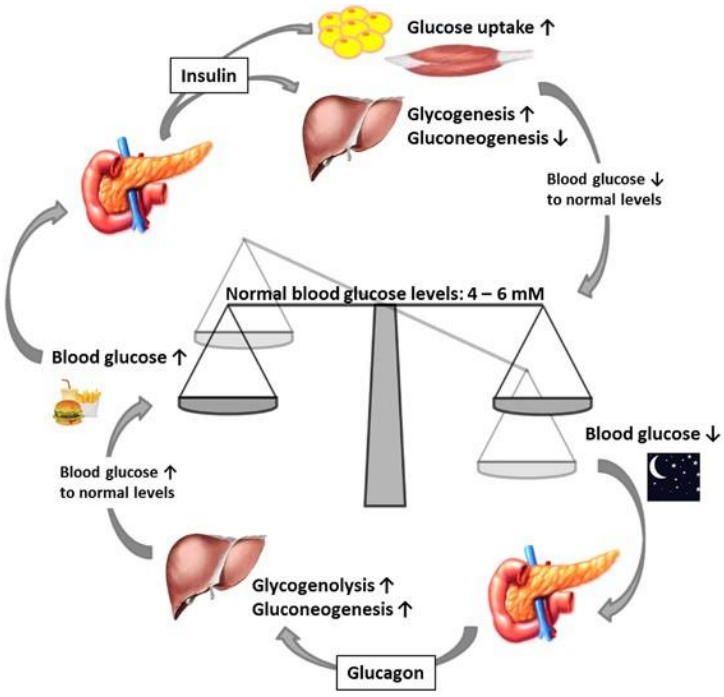

Figure 2. Schematic representation of glucose homeostasis.

Source: (3). Reprinted with permission from Springer Nature-the publisher under the license for Creative Commons Attribution-NonCommercial-No-Derivatives 4.0 International Licence.

T2DM is primarily the result of two interrelated problems (16):

i. Insulin resistance in the muscles, fat, and liver.
ii. Insufficient insulin production by the pancreas.

Briefly, unusually high blood glucose levels are caused by malfunctioning feedback loops between insulin secretion and insulin use. IR stimulates the liver to increase glucose production and less glucose uptake in muscle, liver, and adipose tissue. To maintain normal glucose levels, β-cells secrete more insulin. When insulin demands constantly increase and the β-cells are incapable of producing more insulin, this affects the body's ability to maintain normal glucose levels. Even if both processes happen early on and contribute to T2DM development, β-cell dysfunction is much more severe than IR. As β-cell dysfunction progresses, hyperglycemia intensifies, leading to the progression of T2DM (3, 17).

1.1.5 Risk factors for T2DM

T2DM is a multifactorial disease driven by a complex combination of genetic, metabolic, and environmental factors. Although there is strong evidence for non-modifiable risk factors of T2DM, such as, ethnicity, family history, and genetic predisposition (18), epidemiological studies show that T2DM can be prevented in many cases by improving modifiable risk factors like obesity, unhealthy diet, and physical activity. Obesity, indicated by body mass index (BMI) >30 kg/m^2, is the strongest, modifiable risk factor for T2DM (7, 19-22). Other possible risk factors that have received focus in recent years are stress, epigenetic changes, and various environmental contaminants like persistent organic pollutants (POPs) (23-26).

1.2 Persistent Organic Pollutants

POPs are intentionally or unintentionally produced organic chemical substances (27). They have been widely used for commercial purposes. These chemicals possess a combination of physical and chemical properties that allow them to resist degradation, are capable of long-range transport, and bioaccumulate in living organisms. Because of their ubiquity and lipid solubility, several of these POPs bioaccumulate in fatty tissues, with the highest concentrations found in species high up in the food chain, for example, in humans and meat-consuming mammals (27, 28). Although several of these substances proven to be useful in pest and disease control as well as in agriculture and industry, they have also demonstrated harmful effects on the environment and human health (27). Thus, several POPs have been banned or restricted through the Stockholm Convention. The list includes a wide range of

'legacy' contaminants like PCBs, OCPs, and dioxins, as well as new 'emerging' contaminants like some of the PFAAs and PBDEs (29).

1.2.1 Polychlorinated biphenyls and organochlorine pesticides

Historically, North America and Europe were the leading producers and consumers of PCBs. Production was the highest between the 1930s-1970s (29, 30). They were used widely in paint, electric capacitors, transformers and heat transfer fluids, lubricants, plasticizers, and hydraulic equipment (31). All PCBs are readily soluble in organic solvents, however, they have very low water solubilities (30). There are a total 209 PCB congeners. They are lipophilic, and their lipophilicity increases and volatility decreases with the increasing degree of chlorination (32). The half-lives of PCBs are estimated to be between 5-15 years, depending on the congener (33). PCB congeners 28, 52, 101, 138, 153, and 180 are those with the highest concentrations in the environment and in humans. They are commonly used as indicators of PCB exposure in research. PCB-153 is considered the main contributor to estimating PCB body burdens (34). Mechanisms of action within the human body depend on the chlorine substitution pattern of the congener (35).

OCPs were used extensively as pesticides and insecticides, for example, dichloro-diphenyl-trichloroethane (DDT), a well-known OCP, was developed in the 1940s to combat malaria, typhus, and other insect-borne human diseases. Other OCPs include the chlordanes that were produced for termite control, hexachlorobenzene (HCB), a fungicide, and hexachlorocyclohexane (HCH) used as an insecticide for cotton plants (36). Similar to PCBs, OCPs are highly persistent, with low water solubility, high lipid solubility, and low polarity (37). However, due to their ubiquitous nature and adverse effects on animal and human health, the production of most of these chemicals was stopped in developed countries in the late 1970s and 1980s. In 2004, the Stockholm Convention identified 12 POPs (the original 'dirty dozen') to be banned or restricted. These included several organochlorines like PCBs, OCPs (chlordanes, heptachlor, endosulfans, and DDT), polychlorinated dioxins, and furans. The only exception from the restricted use was the use of DDT for malaria control in some countries (38).

1.2.2 Polybrominated diphenyl ethers

Brominated flame retardants (BFR) are a group of chemicals used to reduce the flammability of consumer products and have been used in furniture, textiles, electronics, and building

materials (39). PBDEs are an important group of BFRs, which have been widely used from 1970 to 2005. They are structurally similar to PCBs but contain bromines instead of chlorines. There are a possible 209 congeners. However, the commercial PBDEs mixtures that actually exist are much fewer compared to PCBs (40). PBDEs are mainly composed of three commercial products (penta-, octa- and deca-BDE). The volatility of PBDE congeners decreases with increasing bromine atoms (41). Similar to PCBs, PBDEs are fat-soluble and hydrophobic. They have estimated half-lives between 1-12 years (42). Due to concerns about the effect of PBDEs on the environment and human health, penta- and octa-BDE were entirely phased out in 2009, followed by deca-BDE in 2017 after the Stockholm Convention classified them as POPs (43).

1.2.3 Perfluoroalkyl acids

Per- and polyfluoroalkyl substances (PFASs) are a class of organofluorine compounds used in industrial and commercial purposes for more than 50 years. PFASs can be water soluble and fat soluble depending on the compound, resist high temperatures and reduce friction (44). They are found in many products, such as carpets, impregnating and cleaning agents, food packaging materials, PFAS-treated textiles, cooking utensils, leather, firefighting foams, and ski waxes (45, 46). Perfluoroalkyl acids (PFAAs) are a subclass of PFASs, and perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are the most commonly detected PFAAs in the human body. PFAAs are amphiphilic in nature and have a chemical structure resembling fatty acids in living organisms. They bind to protein albumin in blood, liver, and eggs and do not accumulate in fat tissue (47). Also, they are able to bind and activate peroxisome proliferator-activated receptors (PPAR). These receptors are important for lipid metabolism, fatty acid storage, glucose metabolism, and regulation of energy homeostasis (48). The PFAAs are estimated to have half-lives between 3.5-8.5 years (49). Major manufacturers phased out the production of PFOS and PFOA in the early 2000s in response to growing international concern over the possibility that PFASs could persist in the environment indefinitely (50). In 2009, the Stockholm Convention classified some PFASs as POPs (51).

1.2.4 Exposure and time trends of POPs in humans

Humans are exposed to a wide range of POPs through many different routes. The exposure, retention, metabolism, and elimination of POPs in a person are affected by demographic, behavioral, and physiological factors (52). The main route of POPs exposure in humans is

diet, especially for fat-soluble POPs like PCBs, OCPs, and PBDEs, which bioaccumulate in animal food products (53-55). Other routes of exposure include drinking contaminated water, inhaling dust, and coming into direct contact with the chemicals (47). Additionally, POPs can be transferred from a mother to her infant through the placenta and breast milk (56).

In humans, lipophilic POPs bioaccumulate within the fat tissues, while non-lipophilic PFASs bind to proteins and accumulate in the kidneys, liver, and blood (57, 58). Although several POPs were banned in many countries some decades ago, human and animal exposures continue at low concentrations. Human blood concentrations of several PCB congeners and OCPs have shown decreasing trends from the 1080s to the early 2000s (59-61), including previous studies from the Tromsø study (one of the cohorts used in this thesis – Figure 3) (62). Among the PFAAs, PFOS and PFOA have shown decreasing trends since the production phase-out in 2002, while few other PFAAs showed increasing trends (63-66). Among PBDEs, both decreasing and increasing trends have been observed depending on the PBDE congener (67-69).

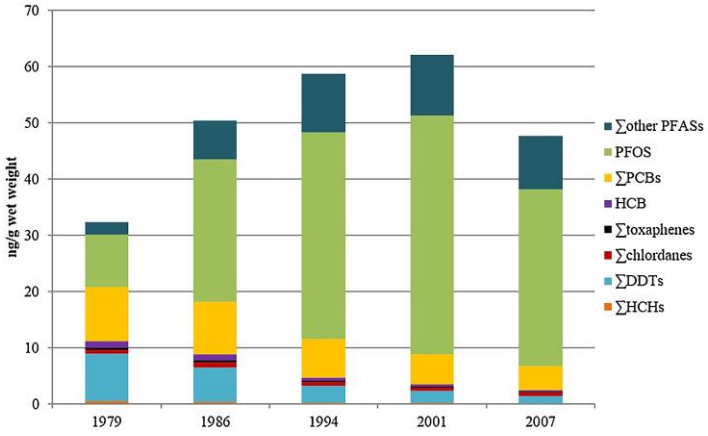

Figure 3. Wet- weight concentrations of PCBs, OCPs, and PFAAs from 1979 to 2007 for 53 men using repeated measurements in the Tromsø study. PFOS represents the sum of linear and branched forms. *Source: (62). Reprinted with permission from Elsevier- the publisher.*

Human blood concentrations of POPs were traditionally assumed to reflect lifetime accumulated exposure; however, studies have confirmed that reproductive history, birth year, blood lipids, weight change, BMI, and diet also influence intra-individual changes in POPs across the lifespan (59, 70-73). Levels of adiposity have a major influence on the circulating concentrations of POPs (52). Pharmacokinetic models show that during increasing POP

exposure (absorption phase), people with obesity are more likely to have lower concentrations of POPs compared to those without obesity due to the dilution effect. During decreasing exposure (elimination phase), the concentrations in people with obesity will surpass people without obesity as a result of the slower elimination of POPs from the former group (73-75). Varying changes in body weight and lipid levels also influence POP concentrations. Specifically, a decrease in body weight and an increase in lipid levels lead to a slower decline in the body burden of POPs (76, 77). Thus, individuals of the same age, with similar POP exposure, may experience different body burdens of POPs due to weight changes, lipid changes, and changes in levels of adiposity during etiologically relevant periods for T2DM. Older age groups are reported to have higher concentrations of 'legacy' POPs as they have a long period of cumulative exposure (59, 60), while PFAAs have shown mixed associations with age (65, 78). Among women, the POP concentrations in the body are further influenced by reproductive events: an increase in parity and lactation period have shown to be inversely associated with POP concentrations (60, 79). Not only do parity and breastfeeding by themselves influence the concentrations, but also the timing of giving birth with respect to POPs emission histories. For instance, predicted life course trajectories of women who were born and gave birth before peak emission showed a slower reduction in the body burden of PCB-153 compared to women who were born during or after peak emission and also gave birth much later (80). Thus, the exposure range and concentration levels in humans are not only influenced by the extent of industrialization, use of pesticides in agriculture, regulation of chemicals, and dietary patterns in a particular population but also influenced by intra-individual physiological changes over a person's lifespan.

1.3 Health effects of POPs

The bioaccumulation of POPs and their long half-lives in humans make them a major threat to human health. Some POPs are classified as endocrine-disrupting chemicals (EDCs) (81). An EDC is defined as "an exogenous chemical, or a mixture of chemicals, that can interfere with any aspect of hormone action" (82). Thus, they either imitate or block natural hormones and disrupt normal hormone homeostasis. For instance, DDT has been shown to have estrogen agonist activity and anti-androgenic properties, PCBs with both estrogenic and antiestrogenic effects as well as disrupting thyroid hormones, and DDE (Dichlorodiphenyldichloroethylene) with antiandrogenic activity (70).

In addition to endocrine disruption, POP exposure has also been associated with various health problems such as neurobehavioral, immunological, reproductive problems, cardiovascular diseases, and carcinogenic effects (38, 83). For instance, PCBs have been shown to be neurotoxic, PBDEs have been shown to have teratogenic, carcinogenic, and neurotoxic effects, and both PFASs and PBDEs have been reported to disrupt the thyroid hormone system (35). DM has also been associated with POPs and will be discussed below in section 1.3.1. However, the complete effect of human exposure to POPs is difficult to assess because, i) adverse effects develop latently and manifest at later ages compared to age or time of exposure, ii) POPs are expensive to measure, iii) studies require very large sample size, and iv) prospective studies with repeated measurements of POPs are required to correctly assess the risk of disease (84, 85).

1.3.1 Associations between POPs and T2DM

In 2016, 39% of adults (≥ 18 years of age) had overweight, and 13% had obesity globally. Among middle-aged men and women (45-59 years), the prevalence of overweight and obesity ranged between 5% in Africa to nearly 80% in Eastern Europe (86, 87). Although T2DM is closely related to obesity, it was earlier hypothesized that adiposity itself may not be a sufficient cause of T2DM and that environmental pollutants may also contribute to this global burden of disease. The earliest epidemiological studies on the POPs-T2DM association have linked dioxins, PCBs, furans, and OCPs to diabetes among populations exposed to high-level acute POP exposure, for instance, through industrial accidents (88, 89); occupational exposure (90, 91); and military personnel exposed to POPs during the Vietnam war (Air Force Health Study) (92). Similarly, PFOA was associated with T2DM among employees from a 3M company (93). However, the problem with these studies is that the POPs were estimated retrospectively by obtaining information on the period of employment, workplace, or address of residence on the date of the accident. If blood samples were measured, then the measurements were usually collected after disease onset. Reanalyzes of data from the Air Force Health Study questioned the earlier positive associations between dioxins and T2DM. They hypothesized that the earlier conclusions may have been a result of possible reverse causality, i.e., the possibility that the increased serum dioxin levels were due to the progression of T2DM (94). More recently, several longitudinal and cross-sectional studies in the general population reported positive, negative, and null associations between T2DM and the different classes of POPs (70). Although reviews of the POPs-T2DM association suggest

sufficient evidence for a positive association, causality has not been established, attributed to inconsistent results due to different study designs and methodologies, study populations, the distributional difference in POP mixtures among populations etc., (95-98). Longitudinal studies with repeated POP measurements in the same individuals that may address these issues have been lacking.

1.3.2 Obesogen hypothesis

The "obesogen hypothesis" proposes that chronic exposure to POPs leads to obesity, and thus, increasing the risk for T2DM (99). The hypothesis states that POPs may disturb appetite controls and promote hypertrophy and hyperplasia in adipocytes, inducing weight gain leading to obesity or altering adipocyte differentiation during development (100). The normal function of adipose tissue is to protect the body from free fatty acids (FFA), as FFA provokes oxidative stress. With obesity, there is an increase in lipolysis, causing the release of FFA. FFA causes lipotoxicity through oxidative stress but may also cause dysfunction of the insulin receptors and induce hyperglycemia (101). PCBs, OCPs, and PBDEs are stored in white adipose tissue (WAT), which has endocrine and immune functions. POPs may modify the actions of endogenous ligands of nuclear transcription factors and alter the metabolism, differentiation, and secretory function of adipose cells. These alterations include i) disruption of hormone (androgen, estrogen, and thyroid) function in WAT, ii) modifications in retinoic receptors' (retinoic acid, the active metabolite of vitamin A, binds to and activates retinoic acid receptors (RAR) and retinoid X receptors (RXR) which may have functions in regulating glucose and lipid metabolism) (102), and iii) interaction with transcription of PPAR (101).

Most of the studies on POPs and obesity are in vitro or animal studies, where chronic exposure to certain specific POPs was reported to have induced weight gain, as reviewed by Lee et al., (70). In epidemiological studies in humans, results have been more inconclusive, with mixed associations observed depending on the POP being studied. PCBs have shown weak evidence for having obesogenic effects in animal and human studies. DDT is the most studied pesticide for its possible obesogenic effects. Animal studies have reported that exposure to DDT/DDE leads to increased hepatic cholesterol, triacylglycerol, total lipids, and liver weight. Within humans, systematic reviews have indicated DDT as a "presumed" obesogen for humans (103). As for the other pesticides, a recent meta-analysis found no association between chlordanes, and adiposity (97), whereas both positive and no associations were observed for *p,p'*-DDE, β-HCH as reviewed by Xiao et al (104). Most of the evidence

for the PBDEs comes from rodent models which support PBDEs being obesogens, however, there are only limited and inconclusive results from human studies, where prenatal exposure to PBDEs showed limited associations to BMI in childhood attributed to differences in the PBDE studied, geographical location, and sex of the study participants (105). In vitro and animal studies indicate that PFOA and PFOS are obesogens, while there is no strong evidence for such effects in human studies. The most consistent adverse outcome reported in humans has been for gestational exposure to PFOA leading to low birth weight in the child (105). Thus, in summary, there is no conclusive evidence from human studies that POPs through inducing obesity lead to the development of T2DM.

1.3.3 Molecular mechanisms linking POPs to T2DM

POPs have been shown to disturb several key biological mechanisms in the body. Some POPs cause endocrine disruption of hormones like thyroid hormones, estrogen, androgen, and glucocorticoid homeostasis, which play important roles in glucose homeostasis and lipid metabolism (106). In vitro studies have linked POPs exposure (especially PCBs and OCPs) to impaired insulin responses and downregulation of genes that regulate lipid homeostasis (107). PCBs and dioxins may act by binding to the transcription factor aryl hydrocarbon receptor (AhR). AhR regulates gene expression when activated and is a coactivator of estrogen receptors. Thus, genes normally activated by estrogens are activated by AhR ligands (106).

Other proposed mechanisms include disruption of mitochondrial function and DNA methylation (108). Mitochondrial dysfunction may play a role in T2DM pathogenesis through the accumulation of diacylglycerol and other metabolites of fatty acid metabolism, a decline of mitochondrial DNA density, and increased production of reactive oxygen species (109). Accumulation of diacylglycerol within cells will suppress the insulin signaling pathways leading to insulin resistance. Animal studies have shown that chronic exposure to POPs in animals with obesity could cause mitochondrial dysfunction through AhR signaling pathway resulting in insulin resistance, visceral obesity, and glucose intolerance (108). Previous animal studies have demonstrated that early life exposure to EDCs may induce DNA hypermethylation and/or hypomethylation of several gene promoters and expression of miRNAs which may alter glucose metabolism and β-cell failure (110). Few human studies have also shown significant correlations between exposure to POPs and hypomethylation of DNA (111, 112). The current evidence on the metabolic effects of EDCs is primarily from in-

vitro and animal in vivo studies, and it remains a challenge to extrapolate animal results to effects on human health.

1.3.4 Complexity of the POPs-T2DM relationship and rationale of the study

"The Bradford Hill Criteria" has been widely used as the background framework in assessing causality, emphasizing nine criteria: the temporality of the relationship, its strength, specificity, experimental evidence, analogy, the presence of a plausible dose-response relationship, the consistency of findings in diverse studies, and coherence with other disciplinary findings and biomedical theory (113). Rather than considering them as absolute criteria, Hill suggested that these characteristics be considered as an aid to explore an association being studied and conclude whether or not it can be termed as causation. This is especially relevant for this thesis as all of Hill's criteria may not be relevant, however, some of these characteristics can be assessed in the POPs-T2DM associations. The POPs-T2DM relationship is exceedingly complex due to the accumulation of POPs in fatty tissues, decreasing time trends in human concentrations of many POPs, and the constant rise in the prevalence of T2DM. *First*, many of the physiological factors like obesity (BMI), blood lipids, weight changes, birth year, breastfeeding, and parity are not only risk factors for T2DM, but they also influence the metabolism/excretion of POP concentrations in the human body as discussed above in section 1.2.4. *Second*, it is not well understood which life stages are more sensitive to POP exposure in relation to T2DM development. Intrauterine exposure, early-life exposure, and exposure as adults have all been suggested as etiologically relevant periods (114, 115). *Third*, the majority of studies (prospective and cross-sectional) reporting positive associations have relied on a single blood sample. Although it is often assumed that a single measurement of POPs reflects long-term POP exposure, it does not necessarily reflect life-long exposure or past peak exposure to POPs which could be relevant for the pathogenesis of T2DM. Hence, prospective or nested case-control studies with only baseline information may fail to reveal if POPs have a role in the development of T2DM. This problem becomes more serious as the follow-up period gets longer due to the constant physiological dynamics in individuals at high risk for T2DM. Only two studies have assessed this association using two repeated samples (before and after T2DM diagnosis) from the same individuals, of which one of the studies is from our group (116, 117). Both studies found stronger associations between post-diagnostic POP concentrations and prevalent T2DM. *Fourth*, epidemiological evidence on POPs being obesogens and further contributing to the

development of T2DM in the adult population is extremely limited and inconclusive. Most evidence comes from cross-sectional studies or studies on prenatal exposure to POPs and the development of obesity in childhood (105). Previous studies of POPs and T2DM have indicated an interaction between BMI and POP concentrations, where the risk of T2DM is higher for people with obesity and high POP concentrations compared to people with obesity and low POP concentrations (118). *Fifth*, lifestyle changes prompted by T2DM diagnosis may also influence physiological factors like blood lipid levels and obesity and have been proposed to affect the associations between POPs and prevalent T2DM (116, 117, 119). Dyslipidemia is often seen in people with a high risk for T2DM due to elevated triglyceride concentrations in the blood. As many POPs are lipid-soluble, increased lipid levels may result in increased POP concentrations. After T2DM diagnosis, the use of glucose-lowering and lipid-lowering drugs lowers blood cholesterol levels (120), further complicating the relationship between POPs and T2DM. For instance, the use of metformin has been shown to decrease body weight, total cholesterol, triglyceride, and low-density lipoprotein levels (121). All these factors together emphasize the need for prospective studies with repeated POP measures to analyze whether the observed associations between POPs and T2DM reflect causality or are attributed to factors related to the disease itself. Further, such studies are expensive and require a large number of prospective samples. To disentangle these complex relationships and further the knowledge in this field, this PhD study was designed to assess the POPs-T2DM associations both prospectively and cross-sectionally using repeated measurements of POPs from the same individuals to shed light on causality. Moreover, repeated POP measurements may also shed light on how changes in physiological factors related to T2DM may influence intra-individual and inter-individual changes in POPs.

2 Aim of the thesis

This doctoral thesis aimed to address the hypothesis that POPs are causal factors of T2DM using novel study designs with repeated measures of POPs from before and after T2DM diagnosis. Specifically, we aimed to:

1. Examine the relationship between PCBs, OCPs, PBDEs, PFAAs, and T2DM prospectively and cross-sectionally in the same individuals.
2. Compare the time trends of PCBs, OCPs, PFAAs, and PBDEs between T2DM cases and controls.

3 Materials and Methods

3.1 Study Populations

This thesis is based on serum samples and data from two different population-based studies: the Norwegian Women and Cancer study (NOWAC study) and the Tromsø study (Figure 4).

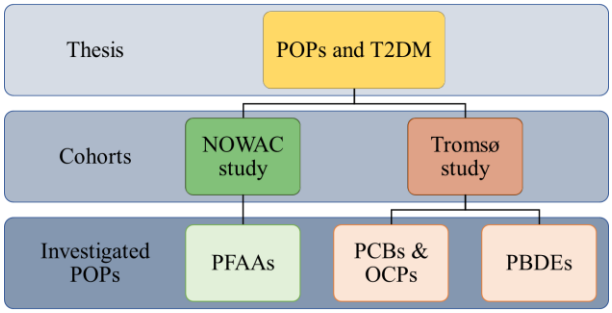

Figure 4. Overview of the cohorts and the POPs investigated in the respective cohorts.

3.1.1 Norwegian Women and Cancer Study (NOWAC) – PFAAs paper

The NOWAC study (Kvinner og Kreft studien in Norwegian) is a population-based, nationally representative prospective cohort study that started in 1991. A detailed description of this study, including the design, cohort profile, and data collection methods has been published previously (122). The NOWAC study has been previously validated for lifestyle characteristics, and there were no differences between initial responders and non-responders (123). Briefly, the NOWAC study was initiated to examine the association between oral contraceptive usage on the risk of breast cancer among ethnically Norwegian women. Later, the study expanded to include other risk factors and outcomes. A food frequency questionnaire (FFQ) was added in 1996-97. The cohort consists of over 170,000 women (30-70 years of age) who have answered between one and four extensive questionnaires on diet, lifestyle factors, medications, and self-reported diseases (122). Approximately, 50,000 participants have also donated blood samples, which are stored at -80º C, out of which a total of 7,849 women gave blood samples at two separate time points (T): time point 1 (T1) in 2001/02 and time point 2 (T2) in 2005/06. A detailed description of blood collection procedures has been reported elsewhere (124).

Study design for PFAAs paper using the NOWAC study:

For this paper, a longitudinal, 1:2 individually matched, nested case-control study design was used. Of the women who gave two blood samples, 53 had no T2DM at T1 (pre-diagnostic time-point) and a confirmed diagnosis of T2DM at T2 (post-diagnostic time-point). Seven cases were excluded because they had cancer or T1DM resulting in 46 T2DM cases. The 46 cases were then matched to two diabetes-free controls: control 1 was matched on birth year (+/-1 year) at T1 and year of blood collection at T2; control 2 was matched on birth year (+/-1 year) at T1, body mass index (BMI) (+/- 3 kg/m^2) at T2, and year of blood collection at T1 and T2. Since evidence shows that BMI is directly linked with both PCBs, OCP concentrations, and T2DM (a confounder), control group 2 was matched on BMI at T2 (as this study was designed initially to investigate the relationship between PCBs, OCPs, and T2DM). Two controls from control group 1 were excluded due to insufficient plasma, and five from control group 2 were excluded because matching was not possible. In total, there were forty-four matched pairs in case-control group 1 and forty-one matched pairs in case-control group 2 in the final sample (Figure 5).

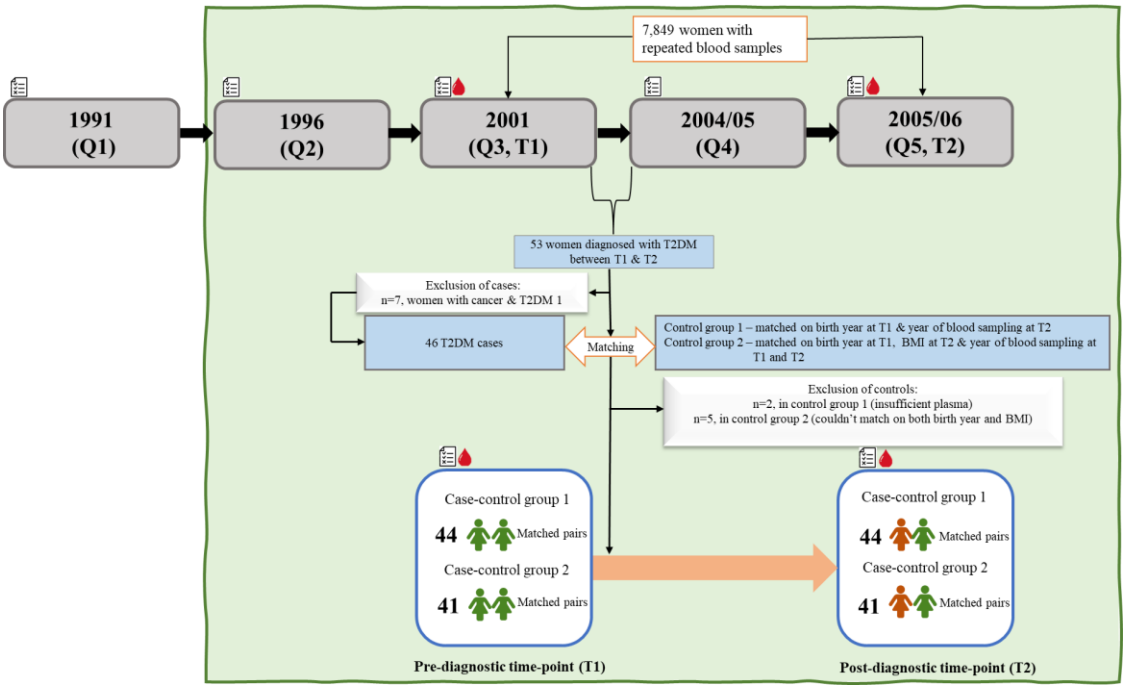

Figure 5. Study design and flow chart for study sample in PFAAs paper from NOWAC study. Grey boxes represent the years when the questionnaires (Q) and blood samples (T) were given the NOWAC study. The green box represents the questionnaires and blood samples used in the paper. The green figures within the blue boxes represent non-diabetic status, while the red figures represent diagnosed T2DM status.

3.1.2 Tromsø Study – PCBs, OCPs, and PBDEs papers

The Tromsø study is a population-based study initiated by the University of Tromsø. It was initially established in 1974 to investigate the increased mortality among men from cardiovascular diseases in northern Norway and has later expanded to include both genders and other chronic diseases. Since its initiation, seven surveys have been conducted, once every 7-8 years. The Tromsø cohort is the most extensive and widely participated population study in Norway, with 45,000 participants in one or more of the seven surveys. Over 15,000 participants have participated in three or more surveys. At each survey, the participants answered a questionnaire, gave a blood sample, and submitted to a thorough physical examination. Details of this study, including the design, cohort profile, enrollment methods, and data collection have been previously published (125). This thesis includes data from five Tromsø surveys, namely from Tromsø 3 to Tromsø 7, here referred to as time points (T), specifically, T1 to T5 in the two papers.

Study design for PCBs, OCPs, and PBDEs papers using the Tromsø study:

A longitudinal, nested case-control study design was used for the PCBs, OCPs, and PBDEs papers. We included T2DM cases and controls who had participated in between three and five of the Tromsø surveys in the study: 1986/87 (T1), 1994/95 (T2), 2001 (T3), 2007/08 (T4) and 2015/16 (T5). Seventy-six women and sixty-nine men who had participated in the Tromsø study were diagnosed with T2DM between 2001 (T3) and 2007/08 (T4). If the cases had also participated at T4 and T5, those questionnaire data and blood samples were included as well. An equal number of men and women who had at least participated in the same surveys as the cases were randomly selected as controls. Cases and controls were not individually matched. Participation in the different surveys is represented by four sample sets (Figure 6). The inclusion and exclusion criteria and study sample are also illustrated in Figure 6. Thus, the number of samples at each time-point was 255 at T1 and T3, 252 at T2, 120 at T4, and 108 at T5 adding up to 990 samples.

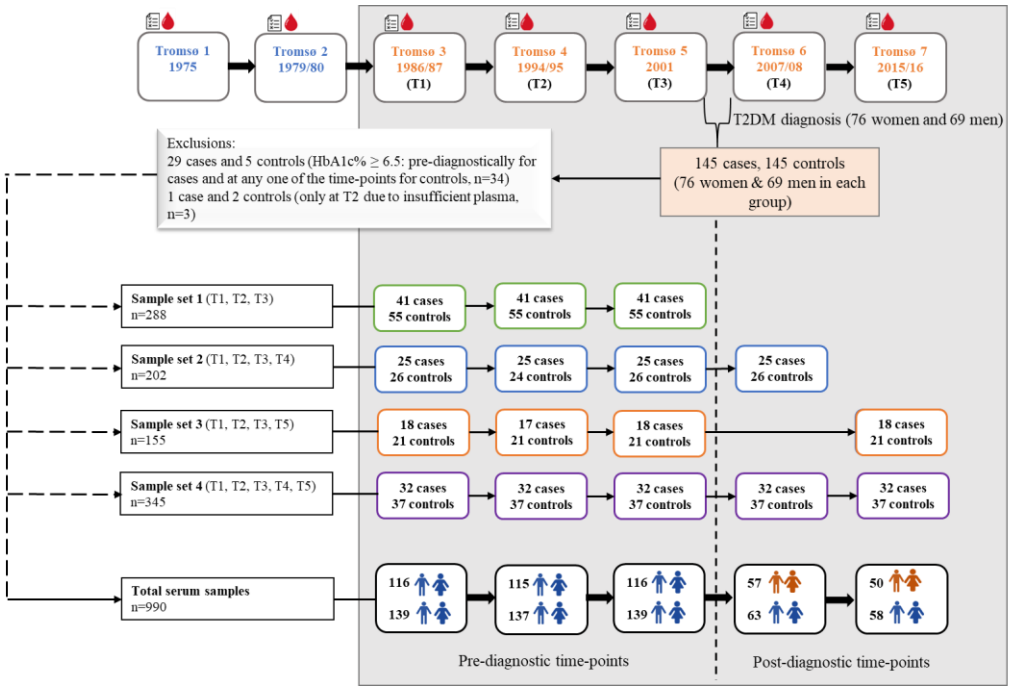

Figure 6. Study design and study sample for the papers (PCBs, OCPS, and PBDEs) from Tromsø study. The grey box represents the questionnaires and blood samples (T1-T5) and the flowchart for the different sample sets and the total study sample used in the two papers. The blue figures within the boxes represent non-diabetic status, while the red figures represent diagnosed T2DM status. The dashed line separates the pre-diagnostic time-points (T1-T3) from the post-diagnostic time-points (T4, T5).

3.2 Analytical Methods

The chemical analyses of PFAAs, PCBs, OCPs, and PBDEs and the lipid analyses were performed by the Department of Laboratory Medicine, University Hospital of North Norway.

3.2.1 PFAAs analyses

A total of 18 PFAAs were analyzed. The procedures for sample preparation, instrumental analysis, quantification, and quality control have been previously described in detail (126). A brief description of the analysis methods and a list of the PFAAs are presented in the paper. Briefly, an automated liquid handler was used to prepare the extracts. Waters Acquity ultra-high-pressure liquid chromatography system coupled to a Waters Xevo-TQ-S tandem mass-spectrometer was used for instrumental analysis. Four blank samples, four standard reference materials, and three bovine serum samples were prepared and analyzed together with the serum samples for quality assurance. All the quality controls were within acceptable limits

(within three times the standard deviation from the reference concentrations, together with a relative standard deviation of ≤ 15%) (126).

3.2.2 PCBs, OCPs, and PBDEs analyses

The method for POP analysis has been described in detail elsewhere (127). A brief description is also available in papers II & III. Briefly, Freedom Evo 200, a liquid handling station, was used for sample preparation. The serum samples were cleaned using automated solid-phase extraction. Gas chromatography coupled with tandem mass spectrometers was used for the instrumental analyses of PCBs, OCPs, and PBDEs. For quality assurance, four blank samples, four SRM samples, and three bovine serum samples were analyzed within each batch of serum samples. The measured PCBs, OCPs, and PBDEs had coefficients of variation (CVs) ranging between 4% and 26%, which is within previously established acceptable limits (127).

3.2.3 Lipids analyses

The Department of Laboratory Medicine, University Hospital of North Norway used a portion of the serum samples to analyze serum concentrations of triglycerides, total cholesterol, high-density lipoprotein, and low-density lipoprotein using coulometric methods on a Cobas® 8000 platform. Both the lab and the platform are accredited (128).

3.3 Identification of Type 2 Diabetes Mellitus cases

In the PFAAs paper, information on T2DM status was extracted from the self-reported NOWAC questionnaires. Participants in the NOWAC study were asked, "*Do you or have you had diabetes? Please answer yes/no. If yes, indicate the age of onset."* T2DM has previously been validated against doctors' confirmation/medical journals in the NOWAC study with a positive predictive value (PPV) of 97% (129). In the other two papers using the Tromsø study, T2DM was defined as a confirmed T2DM diagnosis recorded in a local diabetes registry and a HbA1c concentration above 6.5% measured at the time of blood sample. A high-performance liquid chromatography on a G8 analyzer (Tosoh Bioscience) was used to measure HbA1c% and the CVs were <3%.

3.4 Covariates

In all three papers, directed acyclic graphs (DAGs) were drawn based on previous literature to identify confounding factors to be included in the analyses.

The covariates included in the PFAAs paper were age (years), breastfeeding (months), and dietary factors (meat, fish, dairy, fruits, and vegetables [xg/day]). All the included covariates were extracted from NOWAC questionnaires at Q3 and Q5. If information was not available at the blood sampling time points, the previous questionnaire was used to acquire the missing information. Age, weight, and height were reported in all NOWAC questionnaires except at Q3. Information on parity and breastfeeding was only available at Q1 and Q2. Dietary factors were extracted from questionnaires Q2 and Q4 (Figure 5).

For the papers on PCBs, OCPs, and PBDEs, sex, age (years), weight change (kg), breastfeeding (months), parity, total lipids (g/L), physical activity (categorized into 0=inactive; 1= active) and BMI (kg/m^2) were the selected covariates to be included in the analyses. Since there was no conclusive evidence of POPs causing obesity, BMI was considered a confounder in our study. All covariates were either extracted directly or calculated from variables extracted from the Tromsø questionnaires at each time point. For instance, weight change was calculated for time points T2 to T5 as the difference between two adjacent weight measures (for example, weight change at T2 = [weight at T2] - [weight at T1]). As information for weight from the previous Tromsø survey (Tromsø 2) was unavailable, the weight change at T1 was set to zero. Cumulative breastfeeding was calculated at each time point by summing the reported number of months of breastfeeding for each child. Although lipid measurements were available at the different time points in the Tromsø surveys, the measurements from the lipid analysis in the serum were used.

3.5 Statistical Analysis

All statistical analyses were performed using STATA software, version 16 (StataCorp, 4905 Lakeway Drive, College Station, TX, USA).

3.5.1 Data handling

Only those PFAAs (ng/mL) with detection frequencies ≥90% at both time points were used in the analyses. A total of seven compounds (PFOA, perfluorononanoic acid [PFNA], perfluorodecanoic acid [PFDA], perfluoroundecanoic acid [PFUnDA], perfluorohexane sulfonate [PFHxS], perfluoroheptane sulfonate [PFHpS] and PFOS) were included. PFAA concentrations below the sample-specific method detection limit (MDL) were replaced by MDL/2. Linear and branched forms of PFHxS, PFHpS, and PFOS were summed and presented. Only those PCBs and OCPs with a detection frequency ≥70% were included in the

analyses, and concentrations below the sample-specific MDL were replaced by MDL/√2. The analyses included ∑PCBs (sum of PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187, and 194), 'dioxin-like PCBs' (∑DL-PCBs=sum of PCB 118 and 156), and 8 OCPs (beta-hexachlorocyclohexane [*β*-HCH], *trans*-nonachlor, *cis*-nonachlor, oxychlordane, *cis*-heptachlor epoxide, hexachlorobenzene [HCB], *p,p'*-DDT, and *p,p'*-DDE). A large proportion of the PBDE concentrations in the study sample were below the sample-specific MDL. Those concentrations <MDL were therefore replaced using distribution-based interval regression multiple imputation. A total of six different PBDE congeners (bromodiphenyl ethers [BDE] - 47, 99, 100, 153, 154, and 183) were included in the analyses. A detailed description of the multiple imputation is described in the PBDEs paper. The wet-weight concentrations (pg/mL) of PCBs, OCPs, and PBDEs were lipid-normalized (ng/g lipid) by dividing the wet-weight concentrations by the total lipid concentrations (g/L) according to the formula by Phillips et al.: Total lipids = 2.27*total cholesterol + triglycerides + 0.623 (g/L) (130).

3.5.2 Assessment of associations between POPs and T2DM

The association between pre-diagnostic PFAA concentrations and odds of developing T2DM at T1 were examined using multivariable conditional logistic regression. The results were presented as odds ratios (ORs) with 95% confidence intervals (CIs). ORs were estimated per 1 interquartile range (IQR) increase in PFAA concentrations and 50 ranks increase in ∑PFAAs among controls. All p-values were two-sided, and a 5% level of significance was used.

Logistic regression models were used to assess linear associations between PCB, OCPs, and PBDE concentrations (independent variable) and T2DM status (dependent variable) for the different time points. We have presented all results from the logistic regression models as ORs per 1-standard deviation (SD) increase in the POP measure in controls along with 95% CIs.

3.5.3 Assessment of time trends in POPs

We tested whether the longitudinal changes (δ) in PFAAs within individuals from T1 to T2 were significantly different from zero (T2 measurement – T1 measurement) using paired t-tests. Comparison of trends between cases and controls (longitudinal changes in cases – longitudinal changes in controls) were analyzed using one-sample t-tests. In order to control for confounding factors and identify factors associated with the longitudinal changes in

PFAAs, we performed linear regression models using the longitudinal changes in PFAA concentration from T1 to T2 as dependent variables.

The time trends in PCBs, OCPs, and PBDEs from T1 (1986/87) to T5 (2015/16) in cases and controls were assessed using multivariable linear mixed-effect models with a random intercept for individuals. Log-transformed POP concentrations were considered dependent variables. Among the independent variables, T2DM status and sex were considered to be constant over time, whereas time-indicator variables of each survey, weight change, parity, breastfeeding, total lipids, physical activity (only in the paper with PCBs and OCPs), and BMI were considered to be time-dependent. Age was considered a time-dependent variable in the PCBs and OCPs paper and a time-constant variable (age at T1) in the PBDEs paper. Interaction terms between T2DM status and time were included to assess whether the time trends were different in cases compared to controls. Interaction terms between sex and time were also included to assess whether the time trends for PCBs and OCPs were different between men and women. Predicted POP concentrations after adjusting for the above-mentioned covariates in the mixed models were plotted for T2DM cases and controls at each time-point in both papers.

3.5.4 Additional analyses

For the associations between PCBs, OCPs, and T2DM, the area under the curve (AUC) for the three pre-diagnostic measurements was calculated to quantify the cumulative exposure of POPs and used as independent variables in the logistic regression models. The pre-diagnostic POP concentrations were also modeled as a function of time, using linear mixed-effects models with random intercepts, random slopes, and unstructured covariance patterns. From the models, the best linear unbiased prediction (BLUP) of POP concentrations of each individual was extracted. The subject-specific predicted slope was then used as an independent variable in logistic regression models. The predicted subject-specific slope then represents a measure of each individual's pre-diagnostic time-trend in POP concentrations. The relationship between POPs and T2DM stratified by sex was also examined. Since many analyses were conducted for the PCBs and OCPs, we controlled for multiple comparisons and presented 99.5% CIs in addition to risk estimates, which corresponds to a Bonferroni correction for 10 tests.

The associations between PBDEs and T2DM were also assessed using PBDE concentrations divided into tertiles in the multivariable logistic regression. Furthermore, we compared the associations using the multiple imputation method with two other substitution methods for PBDE concentrations <MDL, i) dichotomization of the concentrations into <MDL and ≥MDL, and ii) substitute concentrations <MDL by MDL/√2.

3.6 Ethics

The NOWAC study was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate. The women in NOWAC were asked for informed consent in the NOWAC questionnaires for both the data in the questionnaires and the given blood samples. All data are stored and handled according to the permission given by the Norwegian Data Inspectorate. The part of the PhD project using the NOWAC study was approved by the Regional Committee for Medical Research Ethics (ref number: 2017/413/REK Nord).

The Tromsø study is performed in accordance with the 1964 Helsinki declaration and its later amendments. All participants provided written informed consent for the scientific use of data and link to health registries. The part of the PhD project using the Tromsø study was approved by the Regional Committee for Medical Research Ethics (ref number: 2015/1780/REK Nord).

4 Results

4.1 Study sample characteristics

The study sample from the Tromsø cohort consisted of 54% and 52% females among cases and controls, respectively. At T1, the mean age of cases and controls was 47.5±7.6 and 45.0±9.8 years, respectively. Cases had a higher BMI of 3.2 kg/m^2 and were ~7.9 kg heavier than controls. No differences in parity or breastfeeding were observed between female cases and controls. Cases had higher pre-diagnostic total lipids compared to controls but not in the post-diagnostic time points. Men had higher total lipids than women at T1 but not at the other time points. No differences in BMI were observed between men and women.

In the PFAAs paper, the mean age in the whole study sample at T1 was 52.0 years. The mean BMI at T1 was 26.9 kg/m^2. Cases were ~7 kgs heavier than both control groups. Cases also had higher meat intake compared to control group 2. Parity, breastfeeding, and other dietary factors were similar between the cases and both control groups. At T2, only BMI was significantly higher in cases compared to control group 1. No differences in demographic variables or dietary factors were observed between cases and control group 2.

4.2 Descriptive statistics for POPs

Among PCBs and OCPs, at T1, concentrations of ∑DL-PCBs, *cis*-heptachlor epoxide, and *p,p'*-DDT were higher in cases, while the other compounds were comparable between cases and controls. From T2 to T5, cases had higher mean concentrations of several OCPs compared to controls. Positive spearman correlations were observed between the different POPs at each time point with correlation coefficients (r_s) ranging between 0.09 and 0.98, strongest between *trans*-nonachlor, *cis*-nonachlor, and oxychlordane (r_s= over 0.85) at all time points, and the weakest between HCB and *p,p'*-DDT (r_s=0.09) at T5. Positive correlations were observed between BMI and POPs at the different time points, with the highest for cis-heptachlor epoxide at T2 (r_s=0.42) and the lowest for ∑PCBs at T3 (r_s=0.01). We observed higher mean concentrations of most POPs, except for HCB and *p,p'*-DDE in men in the pre-diagnostic time points compared to women. This difference was smaller in the post-diagnostic time points.

BDE-47 and BDE-153 were the most frequently detected (>65% and >42%, respectively), and BDE-154 and BDE-183 were the least detected compounds (<42% and <45%, respectively) at all five time points. Spearman correlations between PBDEs and the PCBs and OCPs showed the strongest negative correlation between *p,p'*-DDT and BDE-183 (r_s=-0.50) at T5 and the strongest positive correlation between ∑PCBs and BDE-47 (r_s=0.56) at T1. Cases and controls had similar PBDE concentrations at all time points except for BDE-99 at T1, and BDE-100 and BDE-183 at T4, for which controls had higher concentrations compared to cases.

∑PFOS and PFOA were the two most predominant PFAAs measured in cases and controls. At T1, no differences were observed in mean PFAA concentrations between cases and both control groups. At T2, cases had significantly lower PFOA concentrations compared to control group 2, whereas there were no differences across case-control pairs for any of the other PFAAs. Strong positive correlations were observed between the different PFAAs at both the time points, strongest between ∑PFOS and ∑PFHpS (r_s=0.92) at T1 and between PFUnDA and PFDA (r_s=0.90) at T2.

4.3 Associations between POPs and T2DM

PCBs and OCPs mainly demonstrated positive associations with T2DM although, with wide CIs, except *cis*-heptachlor epoxide (at T1, T2, T3, and T4), *p,p'*-DDT (at T2), and *cis*-nonachlor (at T3) which showed strong positive associations with T2DM. The observed associations were strongest for *cis*-nonachlor at T3 (OR=1.98, 95% CI: 1.27-3.08), also after adjusting for multiple testing. Using the cumulative exposure of POPs for the pre-diagnostic time points (measured as AUC) showed similar estimates as the logistic regression models done separately at each of the three pre-diagnostic time points. Only cis-heptachlor epoxide (OR= 1.75, CI: 1.29, 2.37) and *p,p'*-DDT (OR=1.46, CI:1.12, 1.91) were relatively strongly associated with T2DM also after controlling for multiple testing. In the BLUP models, the slope of an individual's pre-diagnostic time trend in PCB and OCP concentrations was positively associated with T2DM, indicating that increasing concentrations showed positive associations. However, wide 95% and 99.5% CIs indicated poor precision of the estimates. In the models stratified by sex, we observed strong associations for the same OCPs, but the associations were stronger in men for *cis*-nonachlor at T3, and *cis*-heptachlor epoxide and *p,p'*-DDT at T2.

BDE-47, 99, and 100 mainly demonstrated inverse associations with T2DM at the pre- and post-diagnostic time points, while BDE-153 and 154 showed positive associations. BDE-183 showed positive associations at T1 and T2 and negative associations from T3 to T5. We observed a strong positive association for BDE-154 at T5 (OR=1.65, 95% CI: 1.00, 2.71) and a strong inverse association for BDE-183 at T4 (OR=0.32, 95% CI: 0.15, 0.68), but not for any other PBDEs at any of the time points. The choice of substitution method for concentrations <MDL had a minor impact on the estimated associations between PBDEs and T2DM. The results were similar in the direction and strength of the associations for the different substitution methods.

Inverse associations were observed for case-control group 1 pre- and post-diagnostically for all PFAA concentrations. For case-control group 2, positive associations were observed for PFNA, PFDA, PFUnDA, PFHpS, and PFOS at the pre-diagnostic time point and for PFDA and PFUnDA at the post-diagnostic time point. However, the observed ORs had wide 95% CIs including 1, indicating low precision of the estimates.

4.4 Time trends in POPs

The overall time trends for PCBs and OCPs showed declining trends from T1 (1986/87) to T5 (2015/16). However, the declines were smaller in cases than in controls. In the pre-diagnostic time points, slower declines were observed for ∑DL-PCBs, ∑PCB*s*, *trans*-nonachlor, *cis*-nonachlor, oxychlordane, *cis*-heptachlor epoxide, *p,p'*-DDE. The slower declines were evident also after adjusting for multiple testing for *cis*-heptachlor epoxide, and *p,p'*-DDT (T1 to T2); and for ∑DL-PCBs, ∑PCB*s*, *trans*-nonachlor, *cis*-nonachlor, and *p,p'*-DDE (T1 to T3). In the post-diagnostic time points, slower declines in cases compared to controls were observed for ∑DL-PCBs, ∑PCB*s*, *trans*-nonachlor, *cis*-nonachlor, and *p,p'*-DDE from T1 to both post-diagnostic time points, also after adjusting for multiple testing. There were indications of sex differences in time trends of all POPs except for *trans*- and *cis*-nonachlor, oxychlordane, and *p,p'*-DDT, with women experiencing slower declines than men. In addition to T2DM status and time, sex, age, weight change, parity, total lipids, physical activity, and BMI showed to be important predictors of POP concentrations depending on the compound.

The PBDE congeners showed varying time trends from 1986 to 2016. Specifically, in the pre-diagnostic time points (T1 to T3), BDE-47, 99, 100, and 153 increased in concentrations, while BDE-154 and 183 decreased in concentrations. In the post-diagnostic time points,

BDE-47, 99, and 100 showed no consistent trends, BDE-153 increased, and BDE-154 and 183 decreased in concentrations. Similar time trends for all PBDE concentrations were observed for cases and controls during the study period except for BDE-183, which showed a faster decline in cases from T1 to T4 compared to controls. None of the other factors, including sex, age, weight change, parity, total lipids, breastfeeding, and BMI, were associated with time trends of PBDEs.

Within cases, PFOA and ∑PFOS concentrations decreased, while the other PFAA concentrations increased from T1 (2001) to T2 (2005/06). Only ∑PFHpS showed no changes from T1 to T2. Similar to cases, ∑PFOS decreased in both control groups, while PFNA, PFDA, and PFUnDA concentrations increased between T1 and T2. There were no changes in PFOA, ∑PFHxS, or ∑PFHpS concentrations. When comparing the cases and controls, T2DM status did not seem to influence the time trends in any of the PFAA compounds but were driven by age and dietary factors.

5 Discussion

5.1 Discussion of the main results

This thesis is unique to this research field as it explores POPs being causal factors for T2DM by using repeated POP measurements within the same individuals. We had three pre-diagnostic measurements (upto 15 years before T2DM diagnosis) and upto two post-diagnostic measurements for the PCBs, OCPs, and PBDEs. For the PFAAs, we had one pre- and one post-diagnostic measurement. Further, we assessed time trends of POPs within cases and controls before and after T2DM diagnosis to shed light on the chronological aspects of the development and progression of T2DM in relation to POP body burden.

In our study, PFAAs and PBDEs did not increase the risk for T2DM. Also, the trends in PFAAs and PBDEs were not influenced by T2DM status. In contrast, PCBs and OCPs were being retained within prospective cases compared to controls leading to higher concentrations within the same individuals (as seen with slower declines in concentrations within cases for PCBs, *trans*-nonachlor, *cis*-nonachlor, oxychlordane, *cis*-heptachlor epoxide, *p,p'*-DDE and *p,p'*-DDT). Some of the OCPs (*cis*-nonachlor, *cis*-heptachlor epoxide, and *p,p'*-DDT) showed strong positive pre-diagnostic associations with T2DM also after adjustments for multiple testing. *cis*-heptachlor epoxide was the only OCP strongly positively associated with T2DM before and after diagnosis. Similar concentrations of PCBs and several OCPs between cases and controls 15 years before diagnosis, but differences in time trends between the groups suggest that slower declines in prospective cases may be the reason for the observed positive associations. Thus, overall, the results do not support PCBs, OCPs, PBDEs, and PFAAs being causal factors for T2DM.

5.1.1 PCBs, OCPs, PBDEs, and T2DM

We observed declining trends for PCBs and OCPs from 1986 (T1) to 2016 (T5). At T1, although cases had higher BMI and were heavier than controls, we observed similar mean concentrations of ∑PCBs, several OCPs (*β*-HCH, *trans*-nonachlor, *cis*-nonachlor, oxychlordane, HCB, and *p,p'*-DDE), and PBDEs. Similar intra-individual changes in weight and BMI were observed in controls (~7 kgs and 2.5 BMI kg/m^2) and cases (8 kgs and 3 BMI kg/m^2) across the study period. As discussed in chapter 1, age, lipids, weight change, adiposity, and several other factors predict PCB and OCPs concentrations in the body (59, 76, 116, 131), and the half-lives of these compounds increase with increasing adiposity (73). The

slower declines in cases for PCBs and OCPs after adjusting for known confounders suggest that other factors or biological mechanisms, we have not accounted for in the study may affect time trends of POPs, creating apparent differences in trends between cases and controls. One possible explanation could be the decreased capacity of liver enzymes (mainly cytochrome P450) to metabolize these substances in people with obesity (132). We also observed slower declines in women compared to men for PCBs, *β*-HCH, *cis*-heptachlor epoxide, HCB, and *p,p'*-DDE. Although women usually have lower concentrations of POPs compared to men and have additional mechanisms of eliminating POPs from the body through childbirth and breastfeeding, most of the women included in our study had already given birth before T1. Thus, during the study period, huge declines in POP concentrations in women were not observed. Further, women, in general have higher body fat (about 10%) compared with men of a similar age and BMI (133, 134). However, why the trends were slower for PCBs and certain OCPs and not for others needs further investigation.

In our study, we observed strong pre-diagnostic positive associations between *cis*-nonachlor, *cis*-heptachlor epoxide, *p,p'*-DDT, and T2DM. Although this speaks to the "strength" criterion in addressing causality, because PCBs, OCPs, and T2DM share several physiological factors, there may be other contributors as those mentioned above that have not been considered that are the underlying cause for these associations. Most of the evidence for POPs and T2DM associations is based on systematic reviews and meta-analyses of studies using a single measurement of these compounds before T2DM diagnosis. This has been critically reviewed as a major disadvantage in correctly assessing the role of POPs in T2DM development (84), as the dynamics of adipose tissue continuously affect serum concentration of lipophilic POPs, and a single POP measurement may not represent life-long POPs exposure. For example, we observed a sudden increase in concentrations of *cis*-nonachlor in the cases from T2 to T3, and if we did not have previous *cis*-nonachlor measurements, one might conclude that *cis*-nonachlor increases the risk for T2DM using a single POP measurement. Our results showed consistently strong associations between *cis*-heptachlor epoxide and T2DM. But reflecting back to the mean concentrations of this compound, we observed higher mean concentrations for *cis*-heptachlor epoxide in cases compared to controls already at T1. Further, cases also demonstrated slower declines for *cis*-heptachlor epoxide in the pre-diagnostic time points. The three pre-diagnostic measurements preceded the clinical diagnosis of T2DM in cases. This helped us address “temporality” which is the most

important criterion for assessing the association with POPs as the exposure and T2DM as the outcome. However, T2DM is a slow-onset disease, and during the 15 years before T2DM was clinically diagnosed, cases may have gone through various changes like increased appetite, increase in weight, unbalanced blood pressure, and cholesterol levels but with normal glucose levels. Thus, these changes could have begun even before T1, already affecting *cis*-heptachlor epoxide elimination, and this is also a possible explanation for why cases had higher cis-heptachlor epoxide already at T1. In addition, there were no prior studies with repeated measurements to compare our findings to in order to evaluate consistency across studies for the relationship between *cis*-heptachlor epoxide and T2DM. Most evidence comes from cross-sectional or prospective studies with a single blood sample and conflicting results (26, 97). Therefore, we were unable to evaluate the "consistency" criterion of Hill's criteria. In general, clinical trials could definitely disentangle this complex relationship between POPs and T2DM, thereby addressing the "experimental evidence/biological plausibility" criterion in humans, but they are both unimaginable and impractical considering the adverse effects of POPs. One can argue that this criterion has been addressed in previous animal studies which have shown that animals exposed to PCBs and OCPs demonstrated diabetes-related changes, however, these studies are incompatible with the associations between POPs and increased T2DM incidence reported in epidemiological studies due to inconsistencies that may be attributed to physiological differences between rodents and humans in the development of T2DM, differences in exposure levels, duration of exposure etc. (26). Thus, taken together we can only conclude that the constantly increased serum *cis*-heptachlor epoxide concentrations due to slower declines in prospective cases may be the reason for the observed positive associations, and it would be hasty to conclude that *cis*-heptachlor epoxide causes T2DM based on our findings.

At the post-diagnostic time points, *cis*-heptachlor epoxide showed a strong positive association at one of the time points, while the other OCPs and PCBs demonstrated weak positive associations at both time points. Many previous cross-sectional studies have reported positive associations between different OCPs, PCBs, and T2DM (26). No conclusive evidence exists for cross-sectional associations with the except for a recent meta-analysis that reported oxychlordane, *trans*-nonachlor, and *cis*-heptachlor epoxide were associated with T2DM-related features like fasting and non-fasting blood glucose, oral glucose tolerance test, HbA1c%, insulin resistance assessed by Homeostatic Model Assessment of Insulin

Resistance, self-reported diabetes, anti-diabetic medication use, and medical records (97). The problem with cross-sectional studies is that with only a single POP measurement, it is impossible to know if the elevated POP concentrations were present before T2DM diagnosis or if it is a consequence of disease progression itself. In our study, the slower time trends in cases before T2DM diagnosis continued into the post-diagnostic time point (T4), at which *cis*-heptachlor epoxide demonstrated a strong association. Further, we observed that cases had a healthier lipid profile after diagnosis (135), but no changes in weight and BMI were observed. So, some of the T2DM-related physiological factors may have still influenced the slower elimination of the PCBs and OCPs after diagnosis. Also, fewer study participants in the two post-diagnostic time points and differences in those who participated across these time points may have influenced the associations. Therefore, we interpret our post-diagnostic associations cautiously.

There were no distinct time trends for PBDEs (1986-2016) in our study sample. PCBs and PBDEs are structurally similar, have several similar properties, and are metabolized by the same enzymes within the body (136). Thus, one would assume to observe similar associations and time trends for PCBs and PBDEs. However, the results for PCBs and PBDEs were contradictory to each other both in their associations (positive versus no associations respectively) with T2DM as well as their time trends (slower declines in PCBs for cases compared to controls and similar trends for PBDEs in cases and controls) in our study. One possible explanation for these differences could be that PBDEs and PCBs bioaccumulate in different tissues. Meironyte Guvenius et al.2001 demonstrated that, for some of the study participants, liver tissues had higher concentrations of PBDEs than adipose tissues within the same individuals (137). Thus, intraindividual differences in the bioaccumulation of PCBs and PBDEs may partly explain these differences. We also observed a varying range of correlations (r_s= -0.50 to 0.56) between PBDEs and PCBs, suggesting that these compounds may differ in key physical or chemical properties that make them behave differently within the body. Weak to moderate correlations between PCBs and PBDEs have also been reported in other studies (138). Although all the above factors may partly contribute to the differences in associations and time trends for PBDEs and PCBs, it does not explain fully what these specific properties are and what biological mechanisms are involved. Thus, our results show that findings for one class of POPs might not always be valid for another class of POPs, and the “analogy” criterion cannot be assessed in the POPs-T2DM associations.

5.1.2 PFAAs and T2DM

The general time trends in PFAAs (2001 to 2005/06) were as expected and similar to previous studies done in other population-based studies in adults (64, 65). T2DM status did not influence the PFAAs concentrations. Thus, it is unlikely that any changes related to T2DM onset affected PFAAs concentrations. The trends in our study sample were influenced by age and dietary changes for some PFAAs but were not consistent between the case-control groups. Also, no associations were observed between PFAAs and T2DM (before and after diagnosis) in our study. Although this is the first study with two repeated PFAAs measurements, our study results are also based on a small sample size which should be taken into consideration as we might have not had enough power to detect weak associations. But at the same time, a previous study with median PFAA concentrations comparable to our study and with a larger sample size has also produced similar results (139). Thus, our results may still be valid, but our interpretations are with caution. Background PFAS exposures in general populations have not demonstrated consistent positive associations with T2DM, but studies have linked PFAS exposure with increased glucose levels and insulin resistance in individuals with multiple risk factors for diabetes. However, this linkage was dependent on the specific PFAAs investigated and the sex of the participants in the study (140). Schillemans et al. 2021, recently published results from a population-based prospective case-control study where untargeted metabolomics was used to identify metabolites that were associated with PFAS exposures (individual PFASs and grouped PFASs). PFASs were correlated with 171 metabolites that were then investigated for associations with T2DM. 35 of the metabolites were also associated with T2DM. Principle Component Analysis of the 35 PFAS- and T2DM-related metabolite features showed two dominating patterns with opposite T2DM associations: the glycerophospholipid pattern associated with a decreased risk and the diacylglycerol pattern associated with an increased risk for T2DM (141). Their results suggest that PFAS may associate with two groups of lipids with opposite associations for T2DM. This may partly explain the convoluted results found for the PFAAs-T2DM associations in epidemiological studies. This was beyond the scope of the present study and thus, future studies with multiple repeated measurements of PFAAs along with relevant biomarkers over a longer study period may better assess these possible associations.

5.2 Methodological Considerations

5.2.1 Study design

A case-control study is a retrospective observational study usually designed to investigate associations between exposure and outcome. It is inexpensive to conduct and can assess multiple exposures as risk factors for the defined outcome. This is relevant for the present study as POPs are expensive to measure, and humans are usually exposed to a wide range of POPs together.

For the PFAAs paper, we used a 1:2 individually matched nested case-control study within the NOWAC study with two repeated measurements. It was part of a pilot study that initially intended to explore the relationship between lipophilic PCBs, OCPs, and T2DM. The PFAAs were only later included in the study. Since POP exposure is associated with birth year and sampling time (59), the cases in this study were matched to two different control groups: one matched on age and sampling time, and the other on age, sampling time, and BMI. Matching on certain variables (confounding factors) is usually done to ensure that cases and controls have the same unconditional distribution of matching factors. Other reasons could be that it is easier to choose controls from the same hospital or workplace, and it improves study efficiency by improving precision (which can reduce the variance and increase the power) (142, 143). NOWAC is a nationally representative cohort where women between the ages of 30-70 participated. As this pilot study was primarily designed to study associations between PCBs, OCPs, and T2DM, the implications of matching on BMI for the PFAAs were not taken into consideration. However, we later acknowledged that matching (on any of the confounders) is a bad idea as matching does not necessarily control for matching factors but may introduce bias (selection bias, discussed in 5.1.2) (144).

For the papers with PCBs, OCPs, and PBDEs, we used a longitudinal nested case-control study using the Tromsø cohort. We refrained from matching on any confounders in this study. In studies using participants from a huge cohort as the Tromsø study, cases and controls could have different age distributions if they are not matched. However, we had several reasons for not matching. In our study, we included cases who were diagnosed with T2DM during a specific time period (between T3 and T4). Since the inclusion criteria for the controls was that they should have at least participated in the same surveys as the cases, the controls automatically ended up with a similar age distribution. Also, the Tromsø cohort has a

minimum age criterion, so the controls will follow the cases (both for age and exposure time). Further, matching may have disadvantages, for example, if stratified analysis needs to be performed, then the case-control pairs need to be kept as a pair in the analyses. Additionally, as mentioned above, it may further introduce selection bias.

5.2.2 Selection bias

Selection bias is defined as "Bias in the estimated association or effect of an exposure on an outcome that arises from the procedures used to select individuals into the study or the analysis" (145). Participation is voluntary in NOWAC and Tromsø studies. Thus, potential bias due to differences in non-responders and responders is unavoidable. Further, as in any population-based study, there is always the possibility of self-selection bias. People who responded to the questionnaires decided and self-selected to be part of the studies. In general, participants attending population surveys are often healthier, have better socioeconomic status, and are usually younger (146-148). Validation studies for the NOWAC study revealed small differences between responders and non-responders for variables such as parity, breastfeeding, and education level (123). However, this may not be valid for all variables. As for the Tromsø study, in the first Tromsø survey, only men were invited to participate in order to study cardiovascular disease and the associated mortality in men, which was particularly high in northern Norway at the time. However, later surveys (including the ones used in this thesis) invited both men and women, and a wide range of health conditions are studied. From Tromsø 5 (T3 in this thesis) onwards, at each survey, participants from the previous survey have been invited to follow up, but new participants are invited as well in order to reduce the risk of selection bias. However, the response rate has consistently been higher among re-participants than new participants (149). Given that we have included repeated measures, it is probable that some individuals were more eager to participate in numerous repeat surveys, which could have caused a self-selection bias in this sample.

In a case-control study, the control population should represent the population from which the cases were selected (150). Figure 7 represents a simpler version of the DAG from the PFAAs paper, which is a matched case-control study. The arrow from T2DM to the selection of the control group is because, in a case-control study, disease status affects selection, denoting that having T2DM means the individual is selected into the study as a case. All other NOWAC participants were eligible controls, but the choice of controls was determined by the arrow from age to selection of control groups due to the matching. This indicated that, among the

controls, a participant's age will affect their selection into the study, on the condition that age affects T2DM (directly and indirectly).

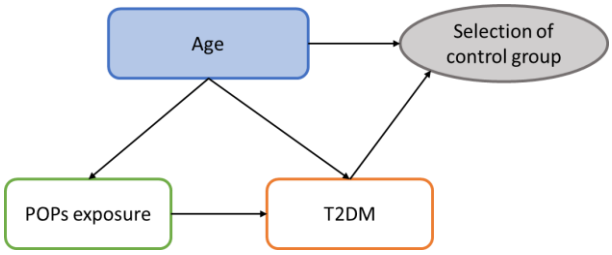

Figure 7. DAG representing case-control matching on a confounder (age) in the matched case-control study.

Matching on age has led to selection bias by opening the backdoor pathway going from POPs←age→selection←T2DM (Figure 7). Thus, adjustment for age is necessary. It can either be done by including age as a covariate in the analyses or do a conditional logistic regression (142). A conditional logistic regression may be the better choice when cases and controls are individually matched (as in our study) because an unconditional logistic regression in this situation will produce odds ratios that are the square of the true odds ratio estimate (142). Therefore, we performed conditional logistic regression to account for the selection bias caused by the matching of age, sampling time, and BMI. In the papers with PCBs, OCPs, and PBDEs, we did not match cases and controls. It was an inclusion criterion in the study design that controls participated at least in the same surveys as cases (but could have participated in more surveys), as otherwise, we could have had selection bias in the controls, that, they for instance, did not participate in more surveys due to severe illness or death. However, since this pre-requisite of participation in surveys ensured that the controls represented the source population, it is most unlikely that we have a selection bias among controls.

5.2.3 Confounding

In observational data, it is necessary to adequately address confounding as it is important for making valid causal inferences. According to DAG theory, a confounder is a common cause (an ancestor) of both the exposure and the outcome of interest. However, confounding is not always easy to recognize, and failing to adjust for a confounder can cause distorted associations. In all three papers, we drew DAGs based on previous literature to select which confounders to adjust for to obtain unbiased estimates. A DAG is a graphical representation

of the causal link between the exposure, outcome, and potential confounders. For the PFAAs-T2DM associations, we adjusted for confounders in two different ways, i) by matching for confounders in the study design or ii) by adjusting for them in the statistical analyses. For the PCBs, OCPs, and PBDEs, we adjusted for the confounders in the statistical analyses. DAGs are efficient tools to help decide which covariates to adjust to obtain unconfounded effect estimates (151). However, sufficient knowledge about the relevant underlying causal relationships between the exposure, outcome, and covariates is necessary to make the right decisions about confounding control. For instance, it is often challenging to differentiate between confounders and mediators due to the lack of information about the chronological ordering and latency of each variable in the data. While a confounder is a causal factor for both the exposure and outcome, a mediator is the effect of an exposure through which the exposure affects the outcome. A relevant example in this thesis would be the role of BMI (a measure of obesity) in the POPs-T2DM association. We provided two alternative models for the logistic regression in the papers on PCBs, OCPs, and PBDEs: model 1 with age and sex adjustments, and model 2 with additional adjustments for other confounders including BMI. Both models showed similar results for the associations. Even if the effect estimates of the two models did not change, we cannot conclude whether BMI is a confounder or a mediator based on these models. If BMI is in the causal pathway between POPs and T2DM, then adjusting for BMI (a mediator) will lead to biased results as it can reduce the relationship between the POPs and T2DM, i.e., only give the direct effect of POPs on T2DM and not the total effect (direct + indirect effects) on T2DM (Figure 8). To really address this issue, we would have to perform a mediation analysis, but this was beyond the scope of this thesis.

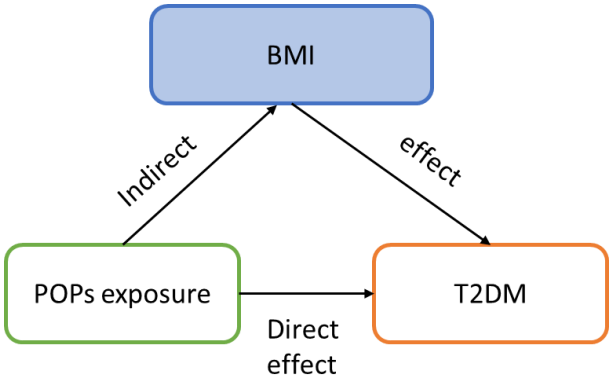

Figure 8. DAG illustrating the direct and indirect effects of exposure on outcome.

Residual confounding describes the situation when confounding is still present after adjustment for confounders in the design or the analysis. Incorrect adjustment or measurement errors for confounders, specifically continuous confounders, can result in considerable residual confounding if the association between that confounder and the outcome of interest is not linear. Stratifying or dichotomizing the confounder makes the adjustment for confounding easier; however, it will again result in inadequate control of confounding, thus, residual confounding (152). For instance, in the paper with PCBs and OCPs, according to the DAG, we needed to adjust for either physical activity or diet in the logistic regression models. In the Tromsø study, physical activity was measured using two different physical activity questionnaires at different surveys, namely, the Saltin-Grimby Physical Activity Level Scale and The Physical Activity Frequency, Intensity, and Duration questionnaires. We needed to harmonize the physical activity levels between the two questionnaires and create a new dichotomized variable (0=inactive; 1=active) for our study. This dichotomization may have led to the loss of information. But we chose to adjust for the physical activity variable as it had less missing, and the questionnaires were validated in the Tromsø study (153). Thus, although physical activity is adjusted for in the analysis, residual confounding due to the physical activity variable may still be present.

5.2.4 Effect modification

Some factors can also modify the effect of exposure. This occurs when the effect of one factor (e.g., time) on a certain outcome (e.g., POP concentrations) is different across the strata of a third factor (T2DM status). This phenomenon is called effect modification (154). For the PCBs, OCPs, and PBDEs, interaction terms for time and T2DM status were introduced to the mixed-effect models to assess if the time trends in these compounds varied between T2DM cases and controls over time. Our results showed that for several of the PCBs and OCPs, an effect modification was observed between T2DM status and time as slower declines in POP concentrations were observed in cases compared to controls for the same time period. For the PCBs and OCPs, we also introduced interaction terms between sex and time to observe differences in time trends across sex, and again we observed effect modification by sex. When effect modification by a factor is present, analysis of the combined groups (e.g., both sexes or cases and controls together) may be misleading (154). For instance, if we had not studied that T2DM status was an effect modifier, we would have concluded that all POPs declined over time in both groups and failed to observe the slower declines in T2DM cases. This may have led us to make different conclusions about the findings on the POPs-T2DM associations.

5.2.5 Information Bias

Information bias occurs as a result of measurement errors in the exposure, covariates, or outcome measure (155). Misclassification is a type of information bias that occurs when individuals or values are incorrectly assigned to a different category from where they belong. There are two types of misclassification: non-differential misclassification, which occurs when the probability of individuals being misclassified is equal between groups in the study, and differential misclassification, which occurs when the probability of being misclassified varies between groups in the study (145). Because data in the NOWAC and Tromsø studies are primarily collected through self-reported questionnaires, some degree of misclassification is to be expected in most variables in this thesis. This may have resulted in residual confounding. Information on covariates and diabetes status in the NOWAC questionnaires is self-reported. The question regarding diabetes in the NOWAC questionnaire makes no distinction between the different types of diabetes. Further, the T2DM status of the participants was not confirmed by blood tests as HbA1c% and glucose levels were not measured at the time of blood sampling in the NOWAC study, and this may have resulted in non-differential misclassification in the PFAAs paper. However, the self-reported T2DM was validated in a cross-sectional study (n=379) by re-contacting some of the study participants and confirming their T2DM status with their general practitioner (GP). The PPV of the questionnaire about T2DM status was 84% for confirmation by re-contacting the participants and 99% for the second confirmation from the GP. But the study could make no conclusions on subjects reporting not having diabetes if they actually did not have the disease (129). Since T2DM has established diagnostic criteria, it is doubtful that controls would have reported being T2DM-free if they had a confirmed T2DM diagnosis. T2DM status for the study participants from the Tromsø study was confirmed using a local diabetes registry and HbA1c% measured in the Tromsø surveys. Further, we excluded those controls with HbA1c measurements ≥ 6.5% at any of the five time points to ensure they were T2DM-free. Thus, we assume that it is unlikely that the study sample has misclassification in T2DM status. The POPs and lipid measurements included in this thesis were all measured at the Department of Laboratory Medicine, University Hospital of Northern Norway, using approved methods (126, 127), and meticulous laboratory quality control measures were undertaken to avoid systematic errors.

Reporting bias is another type of information bias where participants misreport information, for example, on lifestyle factors like level of physical activity, smoking status, alcohol consumption, and dietary factors, as well as on anthropometric measures. For example, it is common to underreport weight or BMI in self-reported questionnaires, and this was also seen in the NOWAC study. Self-reported BMI in the NOWAC showed a slight but statistically significant under-reporting of weight among women with overweight and obesity, with the greatest degree of under-reporting for those with obesity (156). Parity and breastfeeding variables and the FFQ in the NOWAC have been previously validated. There were no differences in the parity and breastfeeding variables between responders and non-responders. The FFQ, on the other hand, was good in ranking subjects for frequently consumed foods and macronutrients in terms of energy percentages but had weak ranking capacities for some micronutrients and rarely consumed foods (123, 157). In the Tromsø study, covariate information was also obtained from self-reported questionnaires. Height and weight were measured by health professionals, and systematic error due to incorrect or poor calibration of the instrument is still possible, however, unlikely. Since observational studies rely mostly on previously collected data, information bias is quite common. The best way to reduce such bias may be to collect data prospectively, but that may not always be possible. Also, using standardized methods and devices may help in reducing information bias. For instance, in our study, we used BMI as a proxy for body fatness, but the problem is that BMI does not necessarily reflect only body fatness but may also reflect muscle mass. Thus, including other better measures of obesity like waist circumference, Dual Energy X-ray Absorptiometry (DEXA), or computer tomography (CT) measurements would be more relevant and accurate. Additionally, cross-validating self-reported information using medical records or registries may be of benefit in reducing such bias.

5.2.6 Censored data and multiple imputation

One of the challenges when working with POPs is that they are usually left-censored data, i.e., in the study sample, there will be people with high concentrations (right-skewed), but a large proportion of people will also have concentrations below the MDL (also called non-detects). For example, figure 9 shows the distribution of BDE-47 at T1 (PBDEs paper). The data closer to zero on the x-axis (with no blue bins) is considered censored, i.e., there is incomplete information about those observations. This means that laboratory methods have

created a lower bound (MDL) below which BDE-47 levels could not be accurately reported. Thus, the true value of these observations lies between 0 and the MDL for that observation.

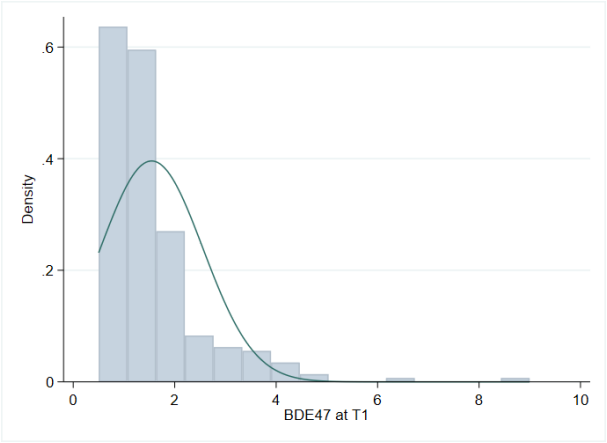

Figure 9. Data distribution for BDE-47 (pg/mL) at T1 in the Tromsø study.

There are different approaches to handling left-censored data. The most common and simpler methods to substitute are to either divide the <MDL concentrations by MDL/2 or MDL/√2. The problem with using single substitution methods is that they will result in impaired estimates of variances and covariances (158). For the PFAAs, we chose to include only those concentrations with ≥ 90% detection frequency, and the <MDL concentrations were divided by 2. PCBs and OCPs with a detection frequency of ≥70% at all five time points were included in the study. Those concentrations <MDL were substituted with MDL/√2. Hornung et al. suggested that for less skewed distributed data, the MDL/√2 substitution offers a better result, while for the more skewed distributions, the MDL/2 procedure is sufficient (159), although the bias caused by substitution increases with increasing censored observations. Further, these methods are adequate if the percentage of non-detects is <15% (160). Among the included OCPs, only cis-heptachlor epoxide had ≥15% non-detects (ranging between 10.9%-25.7%). The PBDEs had a high proportion of non-detects at all time points. With non-detects >15%, it is highly recommended to use distributional-based multiple imputation as they are robust to mild or moderate departures of the data from the assumed distributional shape (161). Further, this method is valid and feasible for handling longitudinal left-censored data (162). In the distributional-based multiple imputation approach, different datasets are created by replacing each <MDL concentration with several values (between 0 and the specific MDL). The different replaced values in the created datasets represent the uncertainty about which value to impute. Statistical analyses are then run using all the created datasets

and aggregated results are produced. Thus, for the PBDEs, we used multiple imputation with interval regression, where each non-detect was substituted with a value between 0 and the sample-specific MDL at that time point, and aggregated results from the 20 created datasets were presented in the results. Further, we also compared the PBDE-T2DM associations from the imputation method with other substitution methods like dichotomization(≤MDL/>MDL) and substituting with MDL/√2. The results from all three methods were similar in terms of direction and strength, and our overall conclusions on the associations remained the same. This suggests that using more common methods of substitution (MDL/2 or MDL/√2) may still be acceptable to use even if the POPs have a large percentage of non-detects, although it should be remembered that the uncertainty due to non-detectable values is unaccounted for in these methods.

5.2.7 Generalizability

The NOWAC cohort is a nationally representative cohort for corresponding age groups of the female population in Norway. Women in the target age groups were randomly selected using the population registry (123). However, the women invited to participate in the NOWAC study were ethnic Norwegians. So, that means that the results from this thesis can only be generalized to the Norwegian women population or populations with similar PFAAs concentrations and not to the entire population of Norway. The Tromsø study is representative of the Tromsø municipality in Northern Norway. Many factors relating to the POP concentrations like food traditions, geographical area, climatic conditions, etc., that may vary for the Tromsø population compared to Norway as a whole country. Thus, the results from papers using the Tromsø study may not be generalized to the entire population but might still be valid for populations with similar PCBs, OCPs, and PBDE concentrations. Although the mean POP concentrations may vary between populations and may not be generalizable, the findings on the POPs-T2DM associations and the time trends within T2DM cases and controls are relevant in the international context and generalizable to all general populations.

6 Conclusion

The results from this thesis show that certain OCPs (*cis*-nonachlor, *cis*-heptachlor epoxide, *p,p'*-DDT) demonstrated substantial positive associations with T2DM, and the same compounds also had slower declines in T2DM cases compared to controls. Together, this suggests that retention of these OCPs due to slower declines in prospective cases may lead to increased concentrations in the body, thereby resulting in positive associations between OCPs and T2DM. PCBs, PFAAs and PBDEs did not increase the risk for T2DM, and the time trends in PFAAs and PBDEs were similar between cases and controls, while PCBs declined slower in cases. Overall, the results from this thesis do not support POPs being causal factors of T2DM.

7 Future perspectives

Biomonitoring of POPs within humans and the environment are conducted regularly. However, studies investigating the POPs-T2DM associations do not focus on studying time trends of POPs in people at high risk of metabolic conditions like obesity and T2DM. Such studies may enhance our knowledge of the interindividual changes in POP concentrations over time and how they relate to the development and progression of the disease.

This thesis is the first to have studied repeated pre- and post-diagnostic associations between POPs and T2DM in adults. Further, the results on the POPs-T2DM associations contradict several of the previous studies that have reported increased odds for T2DM with a single blood sample before or after T2DM diagnosis. Since the measured POP concentrations are influenced by various factors, repeated measures are especially of great value as this may help understand if the observed positive associations may actually be due to disease progression bias. However, we have only addressed the associations and time trends in a certain time period (up to 15 years before T2DM diagnosis). There is still a lack of knowledge on how POPs exposure at different life stages (for example, utero exposure or exposure during puberty) may affect the development of diseases later in life.

This thesis could not explore the role of obesity (BMI) in the POPs and T2DM relationship, and longitudinal studies with large sample sizes using better measures of obesity (waist circumference, DEXA, CT) may shed light on this issue. Future studies should also include measures of lipids, enzymes, or other relevant biomarkers when studying POPs-T2DM associations, as they may play key roles in the metabolism of POPs and may differ in people who are at high risk for T2DM compared to those who are not.

PBDEs and PCBs showed differences in time trends and associations with T2DM, despite being structurally similar and lipophilic. This thesis showed that compounds with similar properties may have different associations with the same outcome. This may also be relevant to other health outcomes.

Thus, although our study may not have answered all the answers due to the complexity of the relationship between POPs, T2DM, and the associated physiological factors, this study is a step forward in this field and highlights the need to explore the chronological aspects of the complexity of this relationship in future studies.

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Paper I

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Pre- and post-diagnostic blood profiles of perfluoroalkyl acids in type 2 diabetes mellitus cases and controls

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ABSTRACT

Background: Studies exploring the associations between perfluoroalkyl acids (PFAAs) and type 2 diabetes mellitus (T2DM) are rather limited and have reported conflicting results. All studies to date, including prospective ones, have relied on a single blood sample to study this association. Similarly, studies investigating how T2DM status may influence the longitudinal changes in PFAA concentrations have not been previously performed. As PFAA concentrations in humans have changed considerably over the last two decades, and as individuals diagnosed with T2DM usually undergo lifestyle changes that could influence these concentrations, a single blood sample may not necessarily reflect the life-time exposure to PFAA concentrations. Hence, repeated measurements from the same individuals will extend our understanding of how PFAAs are associated with T2DM. The present study, therefore, aimed to explore associations between pre- and post-diagnostic PFAA blood profiles and T2DM and assess factors associated with longitudinal changes in PFAAs in T2DM cases and controls.

Methods: Questionnaire data and blood samples from women participating in the Norwegian Women and Cancer study were used to conduct a nested case-control study among 46 T2DM cases matched to 85 non-diabetic controls. PFAAs were measured in blood samples collected prior to (2001/02) and after (2005/6) T2DM diagnosis. We investigated the association between PFAAs and incident and prevalent T2DM using conditional logistic regression. We assessed the longitudinal changes in PFAA concentrations within and between matched cases and controls using t-tests and linear regression models.

Results: We observed no significant associations between pre-diagnostic PFAA concentrations and T2DM incidence. Similar results were observed for the post-diagnostic PFAA concentrations and T2DM prevalence. Decrease over time in PFAA concentrations were observed for PFOA and ΣPFOS concentrations, whereas increase over time were observed for PFNA, PFDA and PFUnDA concentrations. Longitudinal trends in PFAA concentrations among T2DM cases were similar to the changes observed in controls.

Conclusions: The study did not find evidence of association between PFAAs and incident or prevalent T2DM. The longitudinal changes in PFAAs concentrations were not influenced by T2DM status.

1. Introduction

The prevalence of type 2 diabetes mellitus (T2DM), a metabolic condition characterized by elevated blood glucose levels, has increased alarmingly worldwide and accounts for 90% of all diabetes (Saeedi et al., 2019). The estimated global prevalence of diabetes was 9.3% (463 million people) in 2019, and projections suggest that it will rise to 10.2% by 2030 and 10.9% by 2045 (Saeedi et al., 2019). Well-established risk factors of T2DM include older age, obesity, sedentary lifestyle, and genetic predisposition. Diet is considered a risk factor for T2DM, however, previous studies have shown that dietary factors associated with increased risk for T2DM are linked with other unhealthy lifestyle factors which showed highly significant associations with T2DM, such as physical inactivity and increased BMI (Aune et al., 2009; Bellou et al., 2018; Imamura et al., 2015). However, recent research has implied that other non-traditional factors like stress, epigenetic changes, and various environmental organic pollutants may also contribute to the increased prevalence of T2DM (Magliano et al., 2014; McAllister et al., 2009).

Legacy persistent organic pollutants, such as polychlorinated

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biphenyls (PCBs) and organochlorine pesticides (OCPs), are endocrine disrupting chemicals that may play a role in the development of metabolic conditions like T2DM (Alonso-Magdalena et al., 2011; Nadal et al., 2017; Taylor et al., 2013). However, evidence for emerging pollutants like perfluoroalkyl substances (PFASs) is rather limited.

PFASs are a class of organofluorine compounds that have been widely used in industrial and consumer products since the 1950s. Many PFASs are persistent and accumulate in the environment and in biota; today, the main route of human exposure to PFASs is through diet (Lin Pi et al., 2020; Vestergren et al., 2008; Vestergren and Cousins, 2009). Other sources of exposure include PFAS-treated clothing, food packaging materials, and cooking utensils, but also dust inhalation and skin absorption (Haug et al., 2011; Lau, 2015; Nadal et al., 2017). The most frequently detected of such compounds in human blood are perfluoroalkyl acids (PFAAs), a subclass of the PFAS family, in which perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are the most frequently studied. The elimination half-lives of PFAAs were estimated to be 3.5–8.5 years (Olsen et al., 2007a).

PFAAs are classified as endocrine disrupting chemicals (Lind and Lind, 2018). They have a chemical structure that resembles that of fatty acids, a feature that enables them to bind to and activate peroxisome proliferator-activated receptors. These receptors play an important role in lipid and glucose metabolism and in the regulation of energy homeostasis (Jiang et al., 2015; Lind et al., 2014; Wolf et al., 2008). Therefore, pharmaceutical drugs target these receptors for the treatment of dyslipidemia and T2DM. Since PFAAs bind to the same receptors, the effects that PFAAs may have on glucose metabolism are worth exploring (Lind et al., 2014). Other proposed mechanisms include effects of PFAAs on thyroid and steroid hormones which play a major role in adipocyte differentiation and energy storage which in turn may increase the risk of T2DM (Hatch et al., 2010). Animal studies have established no direct link between PFAA exposure and the pathogenesis of T2DM (Khalil et al., 2015). However, findings from both cross-sectional and prospective studies of PFAA concentrations in the blood of humans have reported inconsistent results: some studies have reported null (Cardenas et al., 2017; Karnes et al., 2014; Lind et al., 2014) or inverse associations (Donat-Vargas et al., 2019; MacNeil et al., 2009), and a few studies have reported positive associations (Christensen et al., 2016; He et al., 2018; Sun et al., 2018). Thus, the role of circulating PFAA concentrations in the development of T2DM is uncertain and needs to be further investigated with prospective studies. In addition, all studies to date have reported results from a single blood sample collected either prior or after T2DM diagnosis. As the general Norwegian population experienced a considerable increase in PFAA concentrations until the year 2001 (Nøst et al., 2014), followed by a significant decline, a single blood sample may not reflect lifetime exposure to PFAAs. Further, after T2DM diagnosis, many patients adopt lifestyle changes and are prescribed glucose-lowering drugs; both of these factors may affect the concentration of fat-soluble PCBs and OCPs (Tornevi et al., 2019). Little is known about whether and how these factors influence PFAA concentrations in T2DM patients. Therefore, the present study aimed to explore associations between pre- and post-diagnostic PFAA blood profiles and T2DM among T2DM cases and controls and explore the factors influencing the longitudinal trends in PFAAs concentrations using a longitudinal, nested case-control design.

2. Materials and methods

2.1. The Norwegian Women and Cancer study

The Norwegian Woman and Cancer (NOWAC) study, established in 1991, is an ongoing, population-based, prospective cohort study. The cohort is nationally representative and consists of over 170,000 women (30–70 years of age) who have answered between one and four extensive questionnaires on diet, lifestyle factors, medications, and self-reported diseases (Lund et al., 2008). Approximately, 50,000 participants have also given blood samples, which are stored at −80 °C, and completed a questionnaire about their use of medications at the time of blood collection. A total of 7849 women donated blood samples at two separate time points: time point 1 (T1) in 2001/02 and time point 2 (T2) in 2005/06. A detailed description of blood collection procedures has been reported elsewhere (Waaseth et al., 2008).

2.2. Study design and participants

This is a longitudinal, 1:2 individually-matched, nested case-control study. T2DM cases were defined as those reporting diabetes in the NOWAC questionnaire and/or reporting the use of diabetes medication at T2. This questionnaire information has been previously validated against medical journals/doctors confirmation in the NOWAC study (Rylander et al., 2014). Of the 7849 women who provided blood samples at T1 and T2, 53 were free of T2DM at T1 and reported T2DM at T2. Blood samples collected at T1 were then defined as pre-diagnostic samples (among cases) and those taken at T2 as post-diagnostic samples. Women with cancer, and those with an insufficient amount of plasma available were excluded, as were women with diabetes who were taking insulin (to ensure that no type 1 diabetes cases were included), leaving 46 T2DM cases in the analytical sample. Each T2DM case was then matched with two diabetes-free controls; control 1 was matched on birth year (± 1 year) at T1 and year of blood collection at T2; control 2 was matched on birth year (± 1 year) at T1, body mass index (BMI) (± 3 kg/m^2) at T2, and year of blood collection at T1 and T2. Our study is part of a larger study that intended to explore the relationship between lipophilic PCBs, OCPs, and hydrophilic PFAAs and T2DM. Since evidence shows that BMI is directly linked with both PCBs, OCP concentrations and T2DM (a confounder), we matched control group 2 on BMI at T2. Due to lack of sufficient plasma volume, two controls from control group 1 were excluded. In control group 2, only 41 available controls could be matched on both birth year and BMI. Thus, case-control group 1 consisted of 44 matched pairs and case-control group 2 consisted of 41 matched pairs.

2.3. Questionnaire data

Information on covariates was retrieved from NOWAC questionnaires. Each participant answered five questionnaires (Qs), in 1991 (Q1), 1998 (Q2), 2001/02 (Q3), 2004/05 (Q4), and 2005/06 (Q6). These questionnaires included detailed information on demographics, lifestyle factors, dietary factors, anthropometrics, health related questions, use of medications, and information on parity and total months of breastfeeding. Age, weight, and height were reported in all questionnaires except Q3 (filled out at T1). Information on breastfeeding and parity was only reported in Q1 and Q2.

2.4. Chemical analysis

A total of 18 PFAAs were analyzed at the Department of Laboratory Medicine, University Hospital of North Norway (Table A.1). The procedures for sample preparation, instrumental analysis, quantification, and quality control for PFASs have been previously described in detail (Huber and Brox, 2015). In short, an automated liquid handler (Tecan Freedom Evo 200, Männedorf, Switzerland) was used for the preparation of extracts, where 50 μL of plasma was applied. Instrumental analysis was conducted on a Waters Acquity ultra-high-pressure liquid chromatography system coupled to a Waters Xevo-TQ-S tandem mass-spectrometer (both Waters, Milford, MA, USA). Electrospray ionization in negative mode was applied for ionization of the analytes and multi-reaction monitoring mode for recording of the transitions. For quality assurance, four blank samples, four standard reference material (SRM) 1958 and SRM 1957 (NIST, Gaithersburg, MD, USA), and three bovine serum samples (Sigma Aldrich, Steinheim, Germany) were prepared and analyzed within each batch of 96 samples in order to control

background and carry-over. All the quality controls were within acceptable limits (within three times the standard deviation from the reference concentrations, together with a relative standard deviation of ≤15%), and all PFAA analyses were within the acceptable ranges (z-score of ≤1) of the international quality control program: the Arctic Monitoring and Assessment Ring Test for Persistent Organic Pollutants in Human Serum (organized by the Laboratoire de toxicologie, Institut National de Santé Publique du Quebec, Canada).

2.5. Statistical analysis

All statistical analyses were performed using STATA software, version 16 (StataCorp, 4905 Lakeway Drive, College Station, TX, USA). Only those PFAAs that had a detection frequency ≥90% were included in the statistical analyses. These included seven PFAA compounds – four perfluoroalkyl carboxylic acids: PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnDA); and three perfluoroalkyl sulfonic acids: perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS), and PFOS. PFAA concentrations below their individual method detection limits (MDL) were replaced by MDL divided by 2 (Table A.1). The linear and branched forms of PFHxS, PFHpS, and PFOS were summed and presented as ΣPFHxS, ΣPFHpS, and ΣPFOS, respectively. The individual PFAA compounds were ranked in the order of lowest to highest. The sum of the ranks of PFNA, PFOA, PFDA, PFUnDA, ΣPFHxS, ΣPFHpS, and ΣPFOS concentrations is presented as ΣPFAAs. Spearman correlations were performed to examine the linear relationship between different PFAAs at the two different time-points.

Descriptive statistics at T1 are presented as mean and standard deviation (SD) for demographic data and median (5th, 95th percentiles) for PFAA concentrations (ng/mL). We compared demographics and PFAA concentrations between cases and matched controls at T1 and T2 (mean in cases-mean in controls) using one-sample t-tests. We also tested whether the longitudinal changes (δ) within individuals from T1 to T2 were significantly different from zero (T2 measurement – T1 measurement) using paired t-tests. Comparison of trends between cases and controls (longitudinal changes in cases – longitudinal changes in controls) were analyzed using one-sample t-tests. Thus, we tested the null hypothesis that the longitudinal change in cases equaled the longitudinal change in controls. In order to control for confounding factors and identify factors associated with the longitudinal changes in PFAAs, we performed linear regression models using the longitudinal changes in PFAA concentration from T1 to T2 as dependent variables. Age at T1 centered around the mean age of 52, BMI at T1 centered around BMI = 25 kg/m^2, dietary changes (fish, meat, dairy, fruits and vegetables) and weight change from T1 to T2 were included as independent variables. We did not consider changes in parity and breastfeeding in the linear regression models as the participants were over 50 years of age at T1. BMI at T1 and weight change from T1 to T2 served as proxies for the matching on BMI at T2 for case-control group 2.

The association between pre-diagnostic PFAA concentrations and odds of T2DM at T1 were examined using multivariable conditional logistic regression. The covariates considered were age, breastfeeding and dietary factors (meat, fish, diary, fruit and vegetables). The selection of covariates was based on previous literature and drawing a directed acyclic graph (DAG) showing the assumed relations between PFAAs, T2DM, and the different covariates (Fig. 1). Weight and height at T1 were extracted from Q2. Since breastfeeding and parity were found to be highly correlated, only breastfeeding was considered for the logistic regression models. The covariates identified from the DAG was also included in the conditional logistic regression models exploring the associations between the post-diagnostic PFAA concentrations and prevalent T2DM. The results are presented as odds ratios (ORs) with 95% confidence intervals (CIs). ORs are estimated per 1 interquartile range (IQR) increase in PFAA concentrations and 50 ranks increase in ΣPFAAs. All p-values were two-sided, and a 5% level of significance was used.

3. Results

3.1. Study sample characteristics

At T1 (pre-diagnosis), the mean age and BMI for the whole study sample were 52.0 years and 26.9 kg/m^2, respectively. Cases had significantly higher weight than both control groups and higher meat intake compared to control group 2. However, there were no significant differences in parity, breastfeeding or the other dietary factors. At T2, only BMI was significantly higher in cases compared to control group 1. No differences in demographic variables or dietary factors were observed between cases and control group 2 (Table 1).

At both time points, ΣPFOS and PFOA were the two most prevalent PFAAs measured in both cases and controls. No significant differences were observed in mean PFOA, PFNA, PFDA, PFUnDA, ΣPFHxS, ΣPFHpS and ΣPFOS concentrations between cases and control groups at T1. At T2, cases had significantly lower PFOA concentrations compared to control group 2, whereas there were no differences across case-control pairs for any of the other PFAAs at T2 (Table 1). Strong positive correlations were observed between the different PFAAs at both the time-points with the correlation coefficient (r_s) ranging between 0.52–0.92 at T1, and 0.54–0.90 at T2 (Table A.2).

3.2. Longitudinal changes within cases and controls

Within cases, there were no significant changes in weight, BMI, parity, or breastfeeding from T1 to T2. Cases showed no mean difference in dietary factors from T1 to T2 (Table A.3). PFOA and ΣPFOS concentrations significantly decreased, whereas PFNA, PFDA, PFUnDA, and ΣPFHxS concentrations increased significantly from T1 to T2. ΣPFHpS showed no significant changes (Fig. 2, Table A.3). Both control groups increased significantly in weight, BMI, fruits and vegetables intake from T1 to T2, but there were no significant changes in parity, breastfeeding or fish intake. Reduced dairy intake in control group 1 and increased meat intake in control group 2 were observed at T2 (Table A.3). ΣPFOS decreased significantly in both control groups, and there were no significant changes in PFOA, ΣPFHxS, or ΣPFHpS concentrations. PFNA, PFDA, and PFUnDA concentrations increased significantly from T1 to T2 in both control groups (Fig. 2, Table A.3)

3.3. Longitudinal changes between cases and controls

Mean change in weight and BMI between T1 and T2 were lower for cases compared to both control groups; however, this change was only statistically significant for the comparison of BMI between cases and control group 2. The crude longitudinal changes in PFAA concentrations from T1 to T2 did not differ significantly between cases and control groups 1 or 2, except for ΣPFHpS, for which a significantly larger decline in concentration over time was observed among cases compared to both control groups (Fig. 2, Table A.4). However, after controlling for age and BMI at T1, changes in diet and body weight, T2DM status did not seem to influence the longitudinal trends in any of the PFAA compounds, but changes in PFAAs over time were rather driven by age and dietary factors (Table A.5).

3.4. Pre-diagnostic and post-diagnostic associations

After adjusting for relevant confounders, none of the PFAAs were significantly associated with increased or decreased odds of T2DM when measured at T1 (pre-diagnosis) or at T2 (post-diagnosis) (Tables 2 and 3). Inverse associations for pre-diagnostic concentrations of PFOA and ΣPFHpS were observed for both case control groups. PFNA, PFDA, PFUnDA, ΣPFHpS, ΣPFOS, and ΣPFAAs concentrations showed inverse

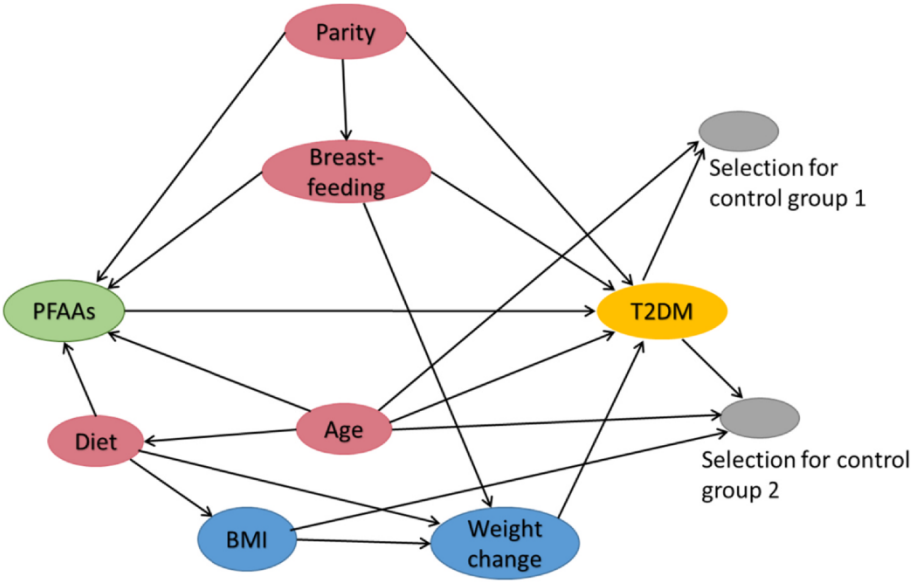

Fig. 1. Directed acyclic graph of the causal network between pre-diagnostic PFAAs and risk of type 2 diabetes mellitus. (The different colors in the DAG represent the following: green-exposure; yellow-outcome; pink-confounders; blue-mediators; grey-matching factors for control groups; arrows-direction of the pathways). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

associations for case-control group 1 and positive associations for case control group 2. However, none of the pre-diagnostic associations were statistically significant in the multivariable adjusted models for either of the case control groups. (Tables 2 and 3). Inverse and non-significant associations were observed between prevalent T2DM and post-diagnostic PFOA, PFNA, ΣPFHxS, ΣPFHpS, ΣPFOS, and ΣPFAA concentrations, and positive, non-significant associations for PFDA and PFUnDA in the multivariable adjusted models for control group 2 (Tables 2 and 3).

4. Discussion

In this study, none of the investigated PFAAs were significantly associated with increased odds of T2DM when measured prior to or after disease development. There were significant changes in PFAA concentrations from pre- to post-diagnosis within cases and controls; ΣPFOS concentrations decreased and PFNA, PFDA, and PFUnDA concentrations increased within cases and both control-groups, whereas PFOA concentrations decreased and ΣPFHxS increased significantly within cases. However, the longitudinal changes were not significantly different in cases versus controls and were mainly explained by differences in age and dietary intake after controlling for confounding factors. Thus, T2DM status did not influence the longitudinal changes in PFAAs.

Overall, our study results of no associations between pre-diagnostic PFAA concentrations and incident T2DM are in line with the findings from other prospective studies. For instance, the most recent, prospective, nested case-control study by Donat-Vargas et al. (2019) found inverse, mostly non-significant associations between pre-diagnostic PFAA concentrations (PFOA, PFDA, PFUnDA, PFHxS, and PFOS) and odds of T2DM in 124 case-controls pairs from the Swedish, prospective, population-based Västerbotten Intervention Programme cohort (Donat-Vargas et al., 2019). Another large prospective study conducted among residents of a community exposed to high levels of PFOA through drinking water (C8 Health project) presented a null association between PFOA and incident T2DM (n = 27,921; 814 cases). However, in that study, serum PFAA concentrations were only estimated and not measured (Karnes et al., 2014). A study conducted among overweight and obese individuals at high risk for T2DM (n = 957,204; T2DM cases) found no association between pre-diagnostic PFAA concentrations (PFNA, PFOA, ΣPFHxS, and ΣPFOS) and incident T2DM (Cardenas et al., 2017). In contrast to our results, positive, significant associations between pre-diagnostic PFOA concentrations (3rd vs. 1st tertile OR: 1.54, 95% CI: 1.04, 2.28), PFOS concentrations (3rd vs. 1st tertile OR: 1.62, 95% CI: 1.09, 2.41), and incident T2DM were reported by a recent, prospective, nested case-control study (n = 1586, 793 T2DM cases) conducted among women from the United States Nurses Health Study II. No association was observed between T2DM and PFNA, PFDA, or ΣPFHxS in that study. However, they reported PFAA concentrations (median PFOA and ΣPFOS concentrations of 7.36 ng/mL and 56.3 ng/mL, respectively) that were much higher than those we report in our study. Only the tertiles with the lowest concentrations (2.89 ng/mL for PFOA and 19.7 ng/ml for PFOS) were comparable to the concentrations in our sample (Sun et al., 2018). The different prospective studies vary in sample size, type of study participants (difference in gender, high-risk individuals, and general population or highly exposed individuals), median PFAA concentrations, year(s) of blood sampling, number of follow-up years, and statistical analyses. These differences make it difficult to extrapolate our findings to other prospective studies. For instance, evidence for increased risk of T2DM in general populations with high PFAA concentrations in the blood is still unclear. Thus, the dose–response relationship between different PFAA exposure concentrations and T2DM incidence in the general population needs further attention.

Similar to T1, at T2 (post-diagnosis), PFAA concentrations showed no associations with T2DM prevalence. In agreement with our results, previous studies that have examined this association also reported no associations between PFAAs and T2DM prevalence (He et al., 2018; Lind et al., 2014; MacNeil et al., 2009; Rylander et al., 2015). For instance, He et al. (2018) conducted a large, cross-sectional study of 7904 adults in the National Health and Nutrition Examination Survey in the United States. PFOA, PFNA, and PFHxS concentrations were slightly higher and PFOS was slightly lower in those samples compared to our study. They reported no association between serum PFOA and prevalent T2DM among women (n = 3948, OR: 1.47, 95% CI: 0.88, 2.46; P for trend = 0.737). However, a significant positive association was reported among men in the same study (n = 3956, OR: 2.66, 95% CI: 1.63, 4.35; P for trend = 0.001). The other PFAAs studied (PFNA, PFHxS, and PFOS) were not related to diabetes, regardless of gender (He et al., 2018). Other studies have also reported significant positive associations between certain PFAAs and prevalent T2DM in men (Christensen et al., 2016). Our study results are in agreement with previous studies that found no association between PFAAs and T2DM prevalence in women. The fact that other studies have observed significant associations in men indicates that there could be gender-dependent changes in PFAA concentrations after T2DM diagnosis.

Table 1
Demographic characteristics, dietary factors and PFAA concentrations of cases and controls and mean difference (Δ) between case-control pairs.

		T1 (pre-diagnosis)			T2 (post-diagnosis)		
		Mean (SD)	Median (5, 95 percentiles)	$\Delta Mean_{case\text{-}control}$ (95% CI)[e]	Mean (SD)	Median (5, 95 percentiles)	$\Delta Mean_{case\text{-}control}$ (95% CI)[e]
Case-control group 1							
Demographics							
Age (years)	Cases[a]	52.2 (4.93)	55.0 (45.0, 58.0)	0.25	56.1 (4.84)	58.5 (49.0, 61.0)	0.20
	Controls 1[b]	51.9 (4.92)	54.0 (44.0, 58.0)	(0.04, 0.46)	56.0 (4.90)	58.0 (48.0, 62.0)	(−0.02, 0.43)
Weight (kg)	Cases[a]	78.1 (16.8)	75.0 (60.0, 120)	6.30	79.8 (17.1)	77.0 (59.0, 110)	5.34
	Controls 1[b]	71.8 (14.2)	70.0 (53.0, 100)	(0.14, 12.4)	74.4 (15.9)	74.0 (55.0, 112)	(−0.82, 11.5)
BMI (kg/m^2)	Cases[a]	28.5 (5.50)	27.6 (21.0, 39.6)	2.67	29.2 (5.85)	28.3 (21.1, 40.6)	2.38
	Controls 1[b]	25.9 (4.52)	24.5 (20.4, 35.4)	(0.64, 4.70)	26.8 (5.04)	25.6 (20.7, 39.8)	(0.41, 4.36)
Parity	Cases[a]	2.32 (1.25)	2.00 (0.00, 4.00)	0.18	2.34 (1.24)	2.00 (0.00, 4.00)	0.18
	Controls 1[b]	2.14 (1.13)	2.00 (0.00, 4.00)	(−0.34, 0.71)	2.16 (1.11)	2.00 (0.00, 4.00)	(−0.33, 0.70)
Breastfeeding (months)	Cases[a]	12.3 (11.6)	10.0 (1.00, 36.0)	3.69	12.3 (11.6)	10.0 (1.00, 36.0)	3.69
	Controls 1[b]	8.65 (8.31)	7.00 (0.00, 24.0)	(−1.10, 8.46)	8.65 (8.31)	7.00 (0.00, 24.0)	(−4.83, 5.22)
Fish intake (g/day)	Cases	92 (54.2)	84 (17.3, 193)	−2.71	93 (47.2)	89 (33.4, 178)	−2.78
	Controls 1[b]	94 (50.8)	88 (24.4, 170)	(−21.6, 16.2)	95 (62.2)	75 (20.6, 228)	(−26.4, 20.9)
Meat intake (g/day)	Cases	122 (41.5)	126 (52.1, 177)	7.72	121 (32.7)	117 (71.9, 171)	17.3
	Controls 1[b]	114 (51.0)	104 (45.9, 202)	(−12.1, 27.5)	103 (49.2)	103 (40.1, 199)	(−1.22, 35.9)
Dairy intake (g/day)	Cases	266 (206)	240 (41.1, 674)	−21.8	230 (200)	228 (23.6, 578)	3.05
	Controls 1[b]	296 (223)	224 (30.4, 675)	(−111, 67.7)	232 (207)	154 (30.9, 609)	(−69.2, 75.3)
Fruits and vegetables intake (g/day)	Cases	360 (242)	298 (67.5, 895)	43.3	430 (362)	417 (130, 743)	30.7
	Controls 1[b]	322 (150)	286 (130, 614)	(−50.5, 137)	390 (176)	401 (150, 695)	(−62.1, 123)
PFAA compounds (ng/mL)							
PFOA	Cases[a]	2.52 (1.20)	2.32 (1.00, 4.13)	−0.27	2.35 (1.25)	2.10 (0.75, 4.54)	−0.30
	Controls 1[b]	2.79 (1.38)	2.41 (1.19, 4.90)	(−0.75 0.21)	2.66 (1.26)	2.52 (1.20, 5.59)	(−0.83, 0.22)
PFNA	Cases[a]	0.46 (0.25)	0.38 (0.18, 1.06)	−0.03	0.66 (0.37)	0.55 (0.26, 1.18)	−0.02
	Controls 1[b]	0.50 (0.25)	0.48 (0.27, 1.12)	(−0.12, 0.05)	0.69 (0.35)	0.60 (0.33, 1.45)	(−0.15, 0.11)
PFDA	Cases[a]	0.23 (0.14)	0.18 (0.07, 0.55)	−0.01	0.30 (0.18)	0.25 (0.12, 0.70)	−0.02
	Controls 1[b]	0.24 (0.14)	0.23 (0.12, 0.45)	(−0.06, 0.04)	0.33 (0.19)	0.28 (0.15, 0.72)	(−0.09, 0.04)
PFUnDA	Cases[a]	0.28 (0.17)	0.22 (0.11, 0.63)	−0.03	0.38 (0.27)	0.30 (0.12, 1.07)	−0.02
	Controls 1[b]	0.31 (0.21)	0.28 (0.15, 0.58)	(−0.10, 0.03)	0.40 (0.28)	0.33 (0.17, 1.10)	(−0.11, 0.06)
ΣPFHxS	Cases[a]	0.99 (0.64)	0.80 (0.36, 2.51)	−0.31	1.13 (0.77)	0.93 (0.33, 3.05)	−0.18
	Controls 1[b]	1.30 (1.31)	0.86 (0.39, 4.29)	(−0.75, 0.13)	1.30 (1.08)	1.05 (0.43, 3.85)	(−0.61, 0.26)
ΣPFHpS	Cases[a]	0.35 (0.17)	0.33 (0.15, 0.61)	−0.04	0.33 (0.18)	0.30 (0.12, 0.58)	−0.04
	Controls 1[b]	0.38 (0.33)	0.29 (0.13, 1.03)	(−0.14, 0.06)	0.37 (0.29)	0.29 (0.11, 0.96)	(−0.13, 0.06)
ΣPFOS	Cases[a]	22.2 (9.95)	20.1 (10.3, 37.9)	−2.99	18.5 (9.83)	16.0 (6.54, 32.0)	−3.20
	Controls 1[b]	25.2 (23.0)	19.0 (10.6, 70.2)	(−10.3, 4.36)	21.7 (20.0)	16.0 (6.09, 65.4)	(−9.69, 3.29)
Case-control group 2							
Demographics							
Age (years)	Cases[c]	52.0 (5.02)	55.0 (45.0, 58.0)	0.02	55.9 (4.89)	58.0 (49.0, 61.0)	−0.05
	Controls 2[d]	52.0 (4.42)	53.0 (46.0, 57.0)	(−0.51, 0.55)	56.0 (4.54)	57.0 (49.0, 61.0)	(−0.47, 0.37)
Weight (kg)	Cases[c]	77.3 (16.1)	75.0 (60.0, 105)	6.44	77.1 (15.9)	75.0 (58.0, 106)	2.63
	Controls 2[d]	70.9 (10.4)	70.0 (56.0, 85.0)	(1.99, 10.9)	74.4 (10.4)	74.0 (60.0, 92.0)	(−0.98, 6.25)
BMI (kg/m^2)	Cases[c]	28.2 (5.30)	27.6 (21.0, 36.6)	1.88	28.1 (5.33)	28.2 (20.7, 36.5)	0.47
	Controls 2[d]	26.3 (4.22)	25.3 (21.1, 31.8)	(0.59, 3.17)	27.7 (4.38)	26.0 (22.6, 36.7)	(−0.44, 1.38)
Parity	Cases[c]	2.15 (1.15)	2.00 (0.00, 4.00)	−0.22	2.17 (1.14)	2.00 (0.00, 4.00)	−0.20
	Controls 2[d]	2.37 (0.89)	2.00 (1.00, 3.00)	(−0.65, 0.21)	2.37 (0.89)	2.00 (1.00, 3.00)	(−0.63, 0.24)
Breastfeeding (months)	Cases[c]	12.0 (12.0)	8.00 (0.00, 36.0)	0.19	12.0 (12.0)	8.00 (0.00, 36.0)	0.19
	Controls 2[d]	12.3 (12.3)	9.00 (0.00, 30.5)	(−4.83, 5.22)	12.3 (12.3)	9.00 (0.00, 30.5)	(−4.83, 5.22)
Fish intake (g/day)	Cases	89 (51.0)	84 (17.3, 173)	−5.37	94 (48.8)	89 (33.4, 178)	−0.17
	Controls 2[d]	94 (48.4)	91 (32.2, 163)	(−28.2, 17.5)	94 (63.8)	73 (27.7, 224)	(−23.4, 23.1)
Meat intake (g/day)	Cases	123 (41.0)	124 (60.4, 177)	24.9	123 (33.1)	119 (71.9, 171)	4.83
	Controls 2[d]	98.0 (50.3)	87 (25.9, 165)	(2.09, 47.7)	118 (44.4)	115 (46.1, 190)	(−12.1, 21.8)
Dairy intake (g/day)	Cases	264 (208)	228 (41.1, 674)	−0.29	234 (210)	209 (23.6, 578)	19.8
	Controls 2[d]	264 (210)	222 (35.0, 608)	(−93.8, 93.2)	214 (181)	170 (33.9, 598)	(−71.3, 111)
Fruits and vegetables intake (g/day)	Cases	372 (245)	307 (67.5, 895)	68.3	444 (229)	422 (130, 826)	6.79
	Controls 2[d]	304 (132)	284 (122, 529)	(−15.8, 152)	437 (169)	384 (251, 695)	(−58.0, 71.6)
PFAA compounds (ng/mL)							
PFOA	Cases[c]	2.52 (1.22)	2.32 (0.99, 4.13)	−0.59	2.34 (1.24)	2.12 (0.75, 4.54)	−0.63
	Controls 2[d]	3.12 (1.69)	2.88 (1.30, 5.75)	(−1.21, 0.03)	2.98 (1.72)	2.64 (1.32, 7.10)	(−1.20, −0.07)
PFNA	Cases[c]	0.46 (0.26)	0.38 (0.18, 1.06)	−0.01	0.65 (0.37)	0.54 (0.26, 1.18)	−0.01
	Controls 2[d]	0.47 (0.23)	0.46 (0.18, 0.92)	(−0.12, 0.09)	0.66 (0.32)	0.61 (0.28, 1.23)	(−0.16, 0.13)
PFDA	Cases[c]	0.23 (0.14)	0.18 (0.07, 0.55)	0.001	0.31 (0.19)	0.25 (0.12, 0.70)	0.005
	Controls 2[d]	0.23 (0.10)	0.23 (0.10, 0.43)	(−0.05, 0.05)	0.30 (0.13)	0.28 (0.12, 0.61)	(−0.07, 0.08)
PFUnDA	Cases[c]	0.29 (0.18)	0.24 (0.11, 0.63)	−0.007	0.40 (0.28)	0.34 (0.13, 1.07)	0.04
	Controls 2[d]	0.30 (0.13)	0.30 (0.09, 0.55)	(−0.07, 0.06)	0.36 (0.17)	0.33 (0.09, 0.62)	(−0.06, 0.14)
ΣPFHxS	Cases[c]	1.03 (0.65)	0.91 (0.36, 2.51)	−0.30	1.15 (0.78)	0.95 (0.33, 3.05)	−0.28
	Controls 2[d]	1.33 (1.18)	1.08 (0.52, 4.14)	(−0.74, 0.14)	1.43 (1.16)	1.13 (0.54, 3.81)	(−0.73, 0.18)
ΣPFHpS	Cases[c]	0.35 (0.18)	0.33 (0.15, 0.61)	−0.01	0.33 (0.18)	0.29 (0.12, 0.58)	−0.02
	Controls 2[d]	0.36 (0.18)	0.34 (0.17, 0.61)	(−0.09, 0.06)	0.35 (0.18)	0.34 (0.13, 0.61)	(−0.09, 0.05)

(*continued on next page*)

Table 1 (*continued*)

		T1 (pre-diagnosis)			T2 (post-diagnosis)		
		Mean (SD)	Median (5, 95 percentiles)	ΔMean$_{case-control}$ (95% CI)[e]	Mean (SD)	Median (5, 95 percentiles)	ΔMean$_{case-control}$ (95% CI)[e]
ΣPFOS	Cases[c]	21.6 (10.1)	19.1 (10.3, 37.9)	−1.68	18.2 (9.97)	15.5 (6.54, 32.0)	−1.94
	Controls 2[d]	23.3 (12.5)	23.2 (8.65, 43.5)	(−6.27, 2.91)	20.1 (10.8)	18.6 (7.72, 34.1)	(−6.51, 2.63)

Abbreviations: PFAA, perfluoroalkyl acid; T1, time point 1 (2001/2); T2, time point 2 (2005/6); SD, standard deviation; CI: confidence interval; BMI, body mass index; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUnDA, perfluoroundecanoic acid; ΣPFOS, sum perfluorooctane sulfonate; ΣPFHxS, sum perfluorohexane sulfonate; ΣPFHpS, sum perfluoroheptane sulfonate.

[a] Case group 1, n = 44.

[b] Control group 1 matched on age at T1, n = 44.

[c] Case group 2, n = 41.

[d] Control group 2 matched on age at T1 and BMI at T2, n = 41.

[e] Mean difference between matched case-control pairs and 95% CI around the mean.

Taken together, the results at T1 and T2, combined with observations from previous studies, suggest no clear role of PFAAs in T2DM pathogenesis or its progression in general populations with low background exposure. However, there are no previous studies from other populations with a similar study design to which we can compare our findings.

Our study also examined the mean longitudinal changes in demographics and PFAA concentrations between the two blood measurements (~4 years) within the same individuals and across the cases and control groups. From T1 to T2, cases maintained a stable weight, whereas both control groups increased in mean body weight. This could be attributed to lifestyle changes adopted by cases after being diagnosed with T2DM. When comparing PFAA concentrations within the cases, PFOA and ΣPFOS concentrations significantly decreased from T1 to T2, whereas PFNA, PFDA, PFUnDA, and ΣPFHxS concentrations increased significantly. Similar to the trends in cases, ΣPFOS decreased significantly, and PFNA, PFDA, and PFUnDA concentrations increased significantly from T1 to T2 in both control groups. However, there were no significant changes in PFOA, ΣPFHxS, or ΣPFHpS concentrations. These findings are in line with previous studies of time trends conducted during the same time period that also included repeated measurements of PFAAs within the same individuals (Nøst et al., 2014; Olsen et al., 2007b, 2012; Toms et al., 2014). These studies reported declining PFOA and PFOS concentrations and increasing PFNA, PFDA, and PFUnDA concentrations (Fitz-Simon et al., 2013; Nøst et al., 2014; Olsen et al., 2007b, 2012; Toms et al., 2014). Thus, our study followed the temporal trends that could be expected for most PFAAs based on studies in other populations. The mean PFAA concentrations at both time points and the decline in PFOA and PFOS concentrations from T1 to T2 in our study were considerably lower than those in the studies mentioned above. One of the contributing factors could be the demographics of our study sample, which mainly included older women, among whom the decline in PFAA concentrations may be slower when compared to the younger women surveyed in previous studies.

When comparing longitudinal changes in PFAAs across cases and controls, no significant differences were observed for any of the PFAA compounds between the groups after controlling for confounding factors. Thus, cases and controls decreased or increased in a similar manner, which suggests that T2DM diagnosis, use of T2DM medication, and weight changes or BMI following T2DM diagnosis in cases have limited influence on PFAA concentrations. The longitudinal changes in PFAAs were rather influenced by age and changes in diet. No other prospective studies have been conducted among T2DM cases and controls with pre- and post-diagnostic PFAA measurements with which to compare the temporal changes observed in our study, suggesting the need for further longitudinal studies to explore intra-individual and inter-individual temporal changes in PFAAs between T2DM cases and controls, especially among individuals exposed to higher concentrations which would extend our understanding of how metabolic changes are related to PFAA concentrations.

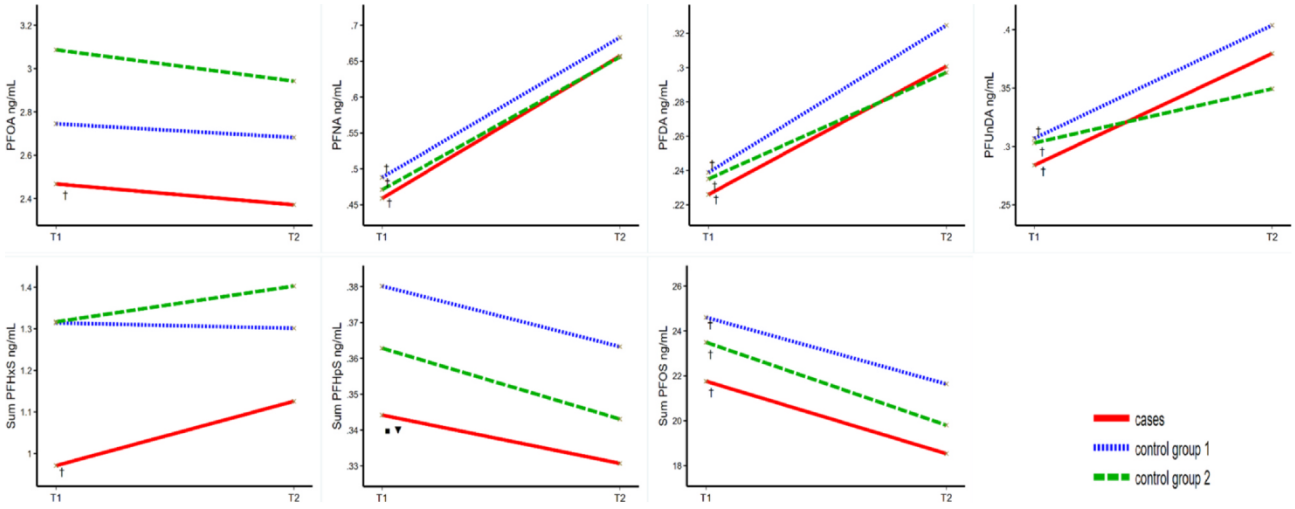

Fig. 2. Crude longitudinal changes (δ) in mean PFAA concentrations from T1 (2001/02, pre-diagnosis) to T2 (2005/06, post-diagnosis) in cases and control groups. †denotes significant change (paired *t*-test, $p < 0.05$) between T1 and T2 within the group; ■ denotes significant (one sample *t*-test, $p < 0.05$) difference in longitudinal change between cases and control group 1; ▼ denotes significant (one sample *t*-test, $p < 0.05$) difference in longitudinal change between cases and control group 2. Abbreviations: PFAA, perfluoroalkyl acid; T1, time point 1; T2, time point 2 PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUnDA, perfluoroundecanoic acid; PFOS, perfluorooctane sulfonate; PFHxS, perfluorohexane sulfonate; PFHpS perfluoroheptane sulfonate.

Table 2
Odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between a one-interquartile range increase in perfluoroalkyl acid concentrations, and 50 ranks increase for the sum of PFAAs compounds and type 2 diabetes mellitus in case-control group 1 (matched by age, n = 44).

	T1 (pre-diagnosis)		T2 (post-diagnosis)	
PFAA compounds(ng/mL)	Age adjusted OR (95% CI)	Multivariable adjusted OR (95% CI)	Age adjusted OR (95% CI)	Multivariable adjusted OR (95% CI)
PFOA	0.73 (0.43, 1.28)	0.65 (0.34, 1.26)	0.74 (0.44, 1.25)	0.67 (0.36, 1.26)
PFNA	0.83 (0.52, 1.32)	0.80 (0.47, 1.36)	0.91 (0.53, 1.55)	0.80 (0.43, 1.48)
PFDA	0.88 (0.57, 1.38)	0.89 (0.55, 1.44)	0.85 (0.56, 1.30)	0.85 (0.53, 1.36)
PFUnDA	0.82 (0.54, 1.23)	0.83 (0.54, 1.26)	0.88 (0.55, 1.39)	0.90 (0.54, 1.53)
ΣPFHxS	0.79 (0.56, 1.12)	0.72 (0.49, 1.05)	0.87 (0.63, 1.21)	0.80 (0.54, 1.20)
ΣPFHpS	0.88 (0.62, 1.24)	0.84 (0.57, 1.24)	0.87 (0.61, 1.25)	0.87 (0.56, 1.34)
ΣPFOS	0.88 (0.63, 1.22)	0.87 (0.60, 1.25)	0.84 (0.58, 1.20)	0.84 (0.55, 1.28)
ΣPFAAs	0.93 (0.79, 1.10)	0.91 (0.76, 1.10)	0.93 (0.79, 1.09)	0.89 (0.73, 1.08)

Abbreviations: T1, time 1 (2001/2); T2, time 2 (2005/6); PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUnDA, perfluoroundecanoic acid; ΣPFHxS, sum perfluorohexane sulfonate; ΣPFHpS, sum perfluoroheptane sulfonate; ΣPFOS, sum perfluorooctane sulfonate ΣPFAAs, sum perfluoroalkyl acids. [a]The models were adjusted for age, breastfeeding, fish, meat, dairy, fruits and vegetables intake.

This study is the first longitudinal study to measure plasma PFAA concentrations at two different time points, once prior to and once following T2DM diagnosis within the same individuals. This enabled us to examine the association between PFAAs and T2DM both prospectively and cross-sectionally within the same individuals. Further, we were also able to observe longitudinal changes in the different PFAA compounds intra-individually and between T2DM cases and controls, which is novel. Rigorous laboratory quality control procedures represent an additional strength of the present study. Potential confounders were either negated by matching for age, BMI, and year of blood collection, or adjusted for in the analyses. We used two different control groups: one matched on age and the other on age and BMI. Studies have shown that that elevated PFAAs concentrations are associated with weight gain/re-gain in obese individuals/individuals at high risk for T2DM undergoing a health intervention program (Cardenas et al., 2018; Liu et al., 2018). This may suggest that the results in our study show the indirect effect of PFAAs on T2DM masked by the matching on BMI for case-control group 2. However, previous studies from general populations (Barry et al., 2014; Blake et al., 2018; Lin et al., 2009; Nelson et al., 2010) have reported that PFAAs concentrations are not associated to BMI and our overall results also show that the ORs were similar for both case-control groups in the pre- and post-diagnostic associations. Therefore, we find it unlikely that this is a large bias in our study. Although, we do agree that matching by BMI is unnecessary in future studies. However, the potential for chance findings and bias from residual and unmeasured confounding is still possible. The conditional logistic regression models were adjusted for the selection bias introduced by the matching. However, this study is based on a small sample size, which limited the possibility to detect weak associations. The T2DM cases were chosen based on self-reported T2DM from questionnaires and were not confirmed by blood tests, as these blood samples were delivered several years before the present analyses. However, self-reported T2DM in the NOWAC study has been previously validated (Rylander et al., 2014). It should also be considered that the generalizability of this study may be limited to an older female Norwegian population.

This study adds to the evidence that neither pre- nor post-diagnostic measurements of PFAAs are associated with incident or prevalent T2DM, and that the temporal changes in PFAA concentrations are similar within T2DM cases and controls. Together, this suggests that cross-sectional studies of prevalent T2DM and PFAAs do not create biased results, as T2DM status, or post-diagnostic weight change merely influence longitudinal changes in PFAAs concentrations after diagnosis.

5. Conclusions

We observed no association between pre- or post-diagnostic PFAA concentrations and T2DM. The observed longitudinal changes in PFAA concentrations from pre- to post-T2DM diagnosis in cases were similar to the changes in controls.

Ethics approval and consent to participate

The NOWAC study has been approved by the Norwegian Data Inspectorate and the Regional Committee for Medical Research Ethics in Northern Norway. The present study was approved by the Regional Committee for Medical Research Ethics (REK, case number: 2015/1780). All participants provided written informed consent.

Table 3
Odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between a one-interquartile range increase in perfluoroalkyl acid concentrations, and 50 ranks increase for the sum of PFAAs compounds and type 2 diabetes mellitus in case-control group 2 (n = 41).

	T1 (pre-diagnosis)		T2 (post-diagnosis)	
PFAA compounds (ng/mL)	Age + BMI adjusted OR (95% CI)	Multivariable adjusted OR (95% CI)[a]	Age + BMI adjusted OR (95% CI)[a]	Multivariable adjusted OR (95% CI)[a]
PFOA	0.61 (0.35, 1.06)	0.73 (0.41, 1.32)	0.55 (0.31, 0.99)	0.57 (0.31, 1.04)
PFNA	0.94 (0.57, 1.57)	1.37 (0.69, 2.75)	0.97 (0.65, 1.43)	0.97 (0.64, 1.46)
PFDA	1.00 (0.62, 1.64)	1.52 (0.76, 3.07)	1.03 (0.70, 1.52)	1.03 (0.68, 1.54)
PFUnDA	0.93 (0.51, 1.70)	1.48 (0.64, 3.39)	1.21 (0.76, 1.92)	1.23 (0.75, 2.02)
ΣPFHxS	0.78 (0.53, 1.15)	0.70 (0.44, 1.09)	0.81 (0.58, 1.15)	0.76 (0.52, 1.11)
ΣPFHpS	0.92 (0.53, 1.59)	1.64 (0.71, 3.79)	0.87 (0.52, 1.44)	0.85 (0.50, 1.46)
ΣPFOS	0.81 (0.46, 1.42)	1.25 (0.57, 2.73)	0.79 (0.49, 1.28)	0.79 (0.48, 1.33)
ΣPFAAs	0.92 (0.79, 1.08)	1.00 (0.82, 1.24)	0.89 (0.75, 1.06)	0.88 (0.73, 1.06)

Abbreviations: T1, time 1 (2001/2); T2, time 2 (2005/6); PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUnDA, perfluoroundecanoic acid; ΣPFHxS, sum perfluorohexane sulfonate; ΣPFHpS, sum perfluoroheptane sulfonate; ΣPFOS, sum perfluorooctane sulfonate ΣPFAAs, sum perfluoroalkyl acids. [a]The models were adjusted for age, BMI, breastfeeding, fish, meat, dairy, fruits and vegetables intake.

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CRediT authorship contribution statement

Dolley Charles: Data curation, Formal analysis, Methodology, Visualization, Writing - original draft. **Vivian Berg:** Methodology, Visualization, Formal analysis, Validation, Writing - review & editing, Funding acquisition. **Therese H. Nøst:** Methodology, Visualization, Writing - review & editing. **Sandra Huber:** Formal analysis, Writing - review & editing. **Torkjel M. Sandanger:** Methodology, Visualization, Writing - review & editing. **Charlotta Rylander:** Conceptualization, Methodology, Supervision, Validation, Writing - review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.106095.

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Title: Pre- and post-diagnostic blood profiles of perfluoroalkyl acids in type 2 diabetes mellitus cases and controls
Supplementary Material

Table A.1. Detection frequencies and MDLs for all detected perfluoroalkyl acids in pre-diagnostic and post-diagnostic samples.

		T1 (pre-diagnosis)		T2 (post-diagnosis)	
Compound	**Abbreviation**	**Detection frequency (%)**	**MDL (min, max) (ng/mL)**	**Detection frequency (%)**	**MDL (min, max) (ng/mL)**
Perfluorohexanoic acid	PFHxA	11.8	0.005, 0.114	19.0	0.005, 0.021
Perfluorohepatanoic acid	PFHpA	62.7	0.004, 0.021	66.7	0.003, 0.024
Perfluorooctanoic acid	PFOA	100	0.004, 0.052	100	0.005, 0.052
Perfluorononanoic acid	PFNA	100	0.002, 0.011	100	0.001, 0.01
Perfluorodecanoic acid	PFDA	100	0.002, 0.017	100	0.003, 0.012
Perfluoroundecanoic acid	PFUnDA	100	0.002, 0.013	100	0.003, 0.014
Perfluorododecanoic acid	PFDoDA	70.6	0.002, 0.014	80.4	0.003, 0.014
Perfluorotridecanoic acid	PFTrDA	71.2	0.003, 0.023	86.9	0.003, 0.019
Perfluorotetradecanoic acid	PFTeDA	3.2	0.005, 0.066	12.4	0.005, 0.095
Perfluorobutane sulfonate	PFBS	2.6	0.001, 0.007	11.8	0.001, 0.008
Perfluoropentane sulfonate	PFPS	7.8	0.001, 0.011	15.7	0.001, 0.019
Sum perfluorohexane sulfonate	∑ PFHxS	100	0.002, 0.037	100	0.002, 0.021
Sum perfluoroheptane sulfonate	∑ PFHpS	100	0.001, 0.01	100	0.001, 0.011
Sum perfluorooctan sulfonate	∑ PFOS	100	0.001, 0.925	100	0.001, 0.392
Sum perfluorononane sulfonate	∑ PFNS	3.2	0.001, 0.026	12.4	0.001, 0.025
Sum perfluorodecane sulfonate	∑ PFDS	21.6	0.001, 0.03	21.6	0.001, 0.053
Perfluorododecane sulfonate	PFDoDS	2.6	0.001, 0.011	11.8	0.001, 0.009
Sum perfluorooctane sulfonamide	∑ PFOSA	6.5	0.001, 0.017	11.8	0.001, 0.011

Abbreviations: T1, time 1 (2001/2); T2, time 2 (2005/6); MDL, method detection limit.

Table A.2. Spearman correlations to assess the relationship between different PFAA compounds at T1 and T2

	PFOA	PFNA	PFDA	PFUnDA	∑PFHxS	∑PFHpS	∑PFOS
T1 (2001/2)							
PFOA	-						
PFNA	0.70	-					
PFDA	0.60	0.91	-				
PFUnDA	0.52	0.82	0.91	-			
∑PFHxS	0.61	0.64	0.57	0.52	-		
∑PFHpS	0.76	0.79	0.68	0.60	0.76	-	
∑ PFOS	0.76	0.82	0.76	0.67	0.66	0.92	-
T1 (2005/6)							
PFOA	-						
PFNA	0.77	-					
PFDA	0.65	0.89	-				
PFUnDA	0.57	0.78	0.90	-			
∑PFHxS	0.58	0.58	0.54	0.49	-		
∑PFHpS	0.78	0.81	0.72	0.64	0.74	-	
∑ PFOS	0.74	0.85	0.82	0.74	0.62	0.88	-

Abbreviations: T1, time 1 (2001/2); T2, time 2 (2005/6); PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUnDA, perfluoroundecanoic acid; ∑PFHxS, sum perfluorohexane sulfonate; ∑PFHpS, sum perfluoroheptane sulfonate; ∑PFOS, sum perfluorooctane sulfonate.

Table A.3. Demographic characteristics and longitudinal changes in PFAAs concentrations from T1 to T2 within cases and controls.

	Cases (n=46)		**Control group 1 (n=44)**		**Control group 2 (n=41)**	
Variables	**ΔMean $_{T2-T1}$ (SD)**	**(95%CI) p-value**	**ΔMean $_{T2-T1}$ (SD)**	**(95%CI) p-value**	**ΔMean $_{T2-T1}$ (SD)**	**(95%CI) p-value**
Weight (kg)	0.07 (10.1)	(-3.05, 3.19) 0.96	2.38 (4.90)	(0.85, 3.91) **<0.01**	2.28 (5.36)	(0.54, 4.02) **<0.05**
BMI (kg/m$^{2)}$	0.63 (3.81)	(-0.54, 1.80) 0.28	1.11 (1.74)	(0.57, 1.65) **<0.001**	1.57 (2.50)	(0.75, 2.40) **<0.001**
Parity	0.09 (0.47)	(-0.05, 0.23) 0.21	0.02 (0.15)	(-0.02, 0.07) 0.32	0.03 (0.16)	(-0.03, 0.08) 0.32
Breastfeeding (months)	-	-	-	-	-	-
Fish intake (g/day)	0.37 (42.0)	(-12.1, 12.8) 0.95	0.74 (52.0)	(-15.1, 16.6) 0.93	0.30 (53.9)	(-16.7, 17.3) 0.97
Meat intake (g/day)	-1.57 (43.1)	(-14.4, 11.2) 0.81	-10.9 (54.1)	(-27.4, 5.51) 0.19	20.2 (60.2)	(1.17, 39.1) **<0.05**
Dairy intake (g/day)	-36.2 (163)	(-84.6, 12.1) 0.14	-64.4 (184)	(-120, -8.38) **<0.05**	-49.9 (175)	(-105, 5.37) 0.08
Fruits and vegetables intake (g/day)	70.4 (249)	(-3.48, 144) 0.06	68.5 (156)	(21.3, 116) **<0.01**	133 (125)	(93.6, 173) **<0.001**
PFAA compounds (ng/mL)						
PFOA	-0.16 (0.48)	(-0.31, -0.02) <0.05	-0.13 (0.64)	(-0.33, 0.06) 0.19	-0.14 (0.56)	(-0.31, 0.04) 0.12
PFNA	0.20 (0.17)	(0.15, 0.25) **<0.001**	0.19 (0.16)	(0.14, 0.24) **<0.001**	0.19 (0.14)	(0.15, 0.23) **<0.001**
PFDA	0.07 (0.07)	(0.05, 0.09) **<0.001**	0.08 (0.09)	(0.06, 0.11) **<0.001**	0.07 (0.05)	(0.05, 0.09) **<0.001**
PFUnDA	0.09 (0.13)	(0.05, 0.13) **<0.001**	0.09 (0.14)	(0.04, 013) **<0.001**	0.05 (0.08)	(0.03, 0.08) **<0.001**
∑ PFHxS	0.13 (0.30)	(0.04, 0.22) **<0.01**	0.0005 (0.42)	(-0.13, 0.13) 0.99	0.10 (0.43)	(-0.04, 0.23) 0.16
∑ PFHpS	-0.02 (0.07)	(-0.04, 0.003) 0.09	-0.02 (-0.05)	(-0.05, 0.01) 0.19	-0.01 (0.06)	(-0.03, 0.006) 0.18
∑ PFOS	-3.67 (4.54)	(-5.02, -2.32) **<0.001**	-3.46 (6.05)	(-5.30, -1.62) **<0.001**	-3.19 (3.65)	(-4.35, -2.04) **<0.001**

Abbreviations: T1, time 1 (2001/2); T2, time 2 (2005/6); SD, standard deviation; BMI, body mass index; PFAA, perfluoroalkyl acid; PFOA, perfluorooctanoic acid; PFA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUnDA, perfluoroundecanoic acid; ∑PFHxS, sum perfluorohexane sulfonate; ∑PFHpS, sum perfluoroheptane sulfonate; ∑PFOS, sum perfluorooctane sulfonate.

Table A.4. Longitudinal changes in perfluoroalkyl acid concentrations between cases and control groups 1 and 2.

Variables		**Difference in cases (T2-T1)-Difference in Controls (T2-T1)**			
		Case-Control group 1 (n=44)	**p-value**	**Case-Control group 2 (n=41)**	**p-value**
Weight (kg)	Mean (95% CI)	1.36 (-2.50, 5.22)	0.48	4.38 (2.09, 6.67)	**<0.01**
Body mass index (kg/m$^{2)}$	Mean (95% CI)	-0.39 (-1.80, 1.02)	0.58	-1.40 (-2.24, -0.55)	**<0.01**
Fish intake (g/day)	Mean (95% CI)	-0.07 (-20.9, 20.8)	1.00	5.29 (-13.6, 24.0)	0.58
Meat intake (g/day)	Mean (95% CI)	9.62 (-10.8, 30.0)	0.35	-20.1 (-45.1, 4.95)	0.11
Dairy products intake (g/day)	Mean (95% CI)	24.9 (-43.9, 93.7)	0.50	20.1 (-64.2, 104)	0.63
Fruits and vegetables intake (g/day)	Mean (95% CI)	-12.7 (-92.7, 67.3)	0.75	-61.5 (-148, 25.0)	0.16
PFAA compounds (ng/mL)					
PFOA	Mean (95% CI)	-0.03 (-0.27, 0.20)	0.77	-0.04 (-0.29, 0.20)	0.74
PFNA	Mean (95% CI)	0.01 (-0.06, 0.08)	0.72	0.0003 (-0.05, 0.05)	0.99
PFDA	Mean (95% CI)	-0.01 (-0.04, 0.02)	0.51	0.004 (-0.02, 0.03)	0.77
PFUDA	Mean (95% CI)	0.007 (-0.04, 0.06)	0.78	0.05 (-0.003, 0.10)	0.07
∑ PFHxS	Mean (95% CI)	0.14 (-0.0006, 0.27)	0.05	0.02 (-0.13, 0.18)	0.76

∑ PFHpS	Mean (95% CI)	-0.40 (-0.51, -0.29)	**<0.01**	-0.38 (-0.44, -0.32)	**<0.01**
∑ PFOS	Mean (95% CI)	-0.21 (-2.29, 1.88)	0.84	-0.26 (-1.73, 1.21)	0.72

Abbreviations: PFAA, perfluoroalkyl acid ; T1, time 1 (2001/2); T2, time 2 (2005/6); PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUnDA, perfluoroundecanoic acid; ∑PFHxS, sum perfluorohexane sulfonate; ∑PFHpS, sum perfluoroheptane sulfonate; ∑PFOS, sum perfluorooctane sulfonate. *p<0.05, **p<0.01

Table A.5. Multivariable adjusted estimates for associations between changes in PFAAs (T2-T1), T2DM status and covariates.

	T2-T1 (Case-control group 1)	**T2-T1 (Case-control group 2)**
	β -coefficient (95% CI)	β -coefficient (95% CI)
PFOA		
T2DM	-0.07 (-0.34, 0.20)	0.001 (-0.25, 0.25)
Age	0.007 (-0.02, 0.03)	0.01 (-0.01, 0.04)
Δ Fish intake	**0.08 (0.02, 0.13)**	**0.06 (0.005, 0.11)**
Δ Meat intake	0.02 (-0.04, 0.07)	0.03 (-0.02, 0.08)
Δ Dairy intake	-0.02 (-0.05, 0.02)	0.03 (-0.01, 0.06)
Δ Fruits & vegetables intake	-0.01 (-0.05, 0.02)	-0.01 (-0.04, 0.02)
BMI	0.01 (-0.02, 0.04)	-0.03 (-0.05, 0.002)
Δ Weight	0.004 (-0.01, 0.02)	-0.004 (-0.02, 0.01)
constant	-0.15 (-0.34, 0.05)	-0.09 (-0.29, 0.12)
PFNA		
T2DM	0.01 (-0.06, 0.09)	0.02 (-0.06, 0.09)
Age	0.006 (-0.0009, 0.01)	0.006 (-0.002, 0.01)
Δ Fish intake (20 g/day)	0.01 (-0.005, 0.03)	-0.002 (-0.02, 0.01)
Δ Meat intake (20 g/day)	0.01 (-0.005, 0.02)	0.002 (-0.01, 0.02)
Δ Dairy intake (50 g/day)	-0.01 (-0.02, 0.0005)	0.006 (-0.006, 0.01)
Δ Fruits & vegetables intake (50 g/day)	0.001 (-0.009, 0.01)	0.003 (-0.006, 0.01)
BMI	-0.0008 (-0.008, 0.007)	-0.006 (-0.01, 0.002)
Δ Weight	-0.0007 (-0,005, 0.004)	0.0008 (-0.006, 0.004)
Constant	**0.18 (0.12, 0.23)**	**0.19 (0.13, 0.25)**
PFDA		
T2DM	-0.01 (-0.05, 0.02)	0.008 (-0.03, 0.04)
Age	**0.004 (0.00006, 0.007)**	0.002 (-0.001, 0.006)
Δ Fish intake (20 g/day)	0.005 (-0.003, 0.01)	-0.0002 (-0.007, 0.007)
Δ Meat intake (20 g/day)	0.003 (-0.005, 0.01)	0.0006 (-0.006, 0.007)
Δ Dairy intake (50 g/day)	-0.004 (-0.01, 0.0007)	-0.0008 (-0.006, 0.004)
Δ Fruits & vegetables intake (50 g/day)	-0.00005 (-0.005, 0.005)	0.0009 (-0.003, 0.005)
BMI	-0.0007 (-0.004, 0.003)	-0.002 (-0.006, 0.001)
Δ Weight	-0.0008 (-0.003, 0.002)	-0.0009 (-0.003, 0.001)
constant	**0.08 (0.06, 0.11)**	**0.07 (0.04, 0.10)**
PFUnDA		
T2DM	0.007 (-0.06, 0.07)	0.05 (-0.003, 0.11)
Age	0.005 (-0.001, 0.01)	0.003 (-0.003, 0.009)
Δ Fish intake (20 g/day)	-0.003 (-0.02, 0.01)	-0.01 (-0.02, 0.002)
Δ Meat intake (20 g/day)	0.008 (-0.005, 0.02)	-0.002 (-0.01, 0.009)
Δ Dairy intake (50 g/day)	-0.0005 (-0.009, 0.008)	0.004 (-0.004, 0.01)
Δ Fruits & vegetables intake (50 g/day)	-0.003 (-0.005, 0.01)	0.006 (-0.0006, 0.01)
BMI	-0.004 (-0.01, 0.002)	-0.004 (-0.01, 0.002)
Δ Weight	-0.003 (-0.007, 0.001)	-0.002 (-0.005, 0.002)
constant	**0.09 (0.05, 0.14)**	**0.05 (0.01, 0.10)**
∑PFHxS		
T2DM	0.14 (-0.03, 0.31)	0.05 (-0.13, 0.23)
Age	0.006 (-0.01, 0.02)	0.01 (-0.006, 0.03)
Δ Fish intake (20 g/day)	0.02 (-0.01, 0.06)	0.008 (-0.03, 0.05)
Δ Meat intake (20 g/day)	-0.008 (-0.04, 0.03)	0.003 (-0.03, 0.04)
Δ Dairy intake (50 g/day)	**-0.03 (-0.05, -0.004)**	0.02 (-0.005, 0.05)
Δ Fruits & vegetables intake (50 g/day)	0.005 (-0.02, 0.03)	0.005 (-0.02, 0.03)
BMI	0.007 (-0.02, 0.03)	-0.01 (-0.03, 0.009)
Δ Weight	0.0005 (-0.01, 0.01)	0.001 (-0.01, 0.01)

constant	-0.06 (-0.18, 0.06)	0.12 (-0.03, 0.26)
∑PFHpS		
T2DM	-0.002 (-0.04, 0.04)	-0.009 (-0.04, 0.02)
Age	0.003 (-0.0004, 0.007)	0.001 (-0.002, 0.004)
Δ Fish intake (20 g/day)	0.008 (-0.0002, 0.02)	0.002 (-0.004, 0.009)
Δ Meat intake (20 g/day)	-0.001 (-0.008, 0.006)	-0.001 (-0.007, 0.005)
Δ Dairy intake (50 g/day)	-0.005 (-0.01, 0.0003)	**0.005 (0.0009, 0.01)**
Δ Fruits & vegetables intake (50 g/day)	0.0002 (-0.005, 0.005)	0.0002 (-0.003, 0.004)
BMI	0.003 (-0.001, 0.006)	-0.0005 (-0.004, 0.003)
Δ Weight	0.0009 (-0.001, 0.003)	0.0004 (-0.001, 0.002)
constant	**-0.03 (-0.06, -0.003)**	-0.007 (-0.03, 0.02)
∑PFOS		
T2DM	-0.94 (-3.42, 1.54)	-0.37 (-2.16, 1.43)
Age	0.17 (-0.07, 0.41)	0.13 (-0.06, 0.32)
Δ Fish intake (20 g/day)	0.48 (-0.03, 0.99)	0.10 (-0.29, 0.49)
Δ Meat intake (20 g/day)	-0.13 (-0.61, 0.35)	-0.09 (-0.44, 0.26)
Δ Dairy intake (50 g/day)	-0.14 (-0.49, 0.20)	0.26 (-0.009, 0.53)
Δ Fruits & vegetables intake (50 g/day)	0.0002 (-0.31, 0.31)	0.02 (-0.20, 0.24)
BMI	0.20 (-0.04, 0.44)	-0.08 (-0.28, 0.12)
Δ Weight	-0.03 (-0.18, 0.13)	-0.0001 (-0.11, 0.11)
constant	**-3.82 (-5.62, -2.01)**	**-2.77 (-4.23, -1.31)**

Abbreviations: T1, time 1 (2001/2); T2, time 2 (2005/6); PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUnDA, perfluoroundecanoic acid; ∑PFHxS, sum perfluorohexane sulfonate; ∑PFHpS, sum perfluoroheptane sulfonate; ∑PFOS, sum perfluorooctane sulfonate. The models are adjusted for centered age at T1, changes in fish, meat, dairy, fruits and vegetables intake (T2-T1), centered BMI at T1 and change in weight (T2-T1).

Paper II

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Longitudinal changes in concentrations of persistent organic pollutants (1986-2016) and their associations with type 2 diabetes mellitus.

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Longitudinal changes in concentrations of persistent organic pollutants (1986–2016) and their associations with type 2 diabetes mellitus

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ABSTRACT

Background: Positive associations have been reported between persistent organic pollutants (POPs) and type 2 diabetes mellitus (T2DM); however, causality has not been established. Over the last decades, environmental exposure to legacy POPs has decreased, complicating epidemiological studies. In addition, physiological risk factors for T2DM may also influence POP concentrations, contributing to a complex network of factors that could impact associations with T2DM. Longitudinal studies on this topic are lacking, and few have assessed prospective and cross-sectional associations between repeated POP measurements and T2DM in the same individuals, which may shed light on causality.

Objectives: To compare longitudinal trends in concentrations of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) in T2DM cases and controls, and to examine prospective and cross-sectional associations between PCBs, OCPs and T2DM at different time-points before and after T2DM diagnosis in cases.

Methods: We conducted a longitudinal, nested case-control study (1986–2016) of 116 T2DM cases and 139 controls from the Tromsø Study. All participants had three blood samples collected before T2DM diagnosis in cases, and up to two samples thereafter. We used linear mixed-effect models to assess temporal changes of POPs within and between T2DM cases and controls, and logistic regression models to investigate the associations between different POPs and T2DM at different time-points.

Results: PCBs, *trans*-nonachlor, *cis*-nonachlor, oxychlordane, *cis*-heptachlor epoxide, *p,p'*-DDE, and *p,p'*-DDT declined more slowly in cases than controls, whereas *β*-HCH and HCB declined similarly in both groups. Most POPs showed positive associations between both pre- and post-diagnostic concentrations and T2DM, though effect estimates were imprecise. These associations were most consistent for cis-heptachlor epoxide.

Discussion: The observed positive associations between certain POPs and T2DM may be because of higher POP concentrations within prospective T2DM cases, due to slower temporal declines as compared to controls.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a global health challenge, affecting nearly 463 million people worldwide (prevalence: 9.3%) in 2019 (Saeedi et al., 2019). Conventional risk factors for T2DM include older age, obesity, genetic predisposition, and sedentary lifestyle. Recent research has also focused on other risk factors, like persistent organic pollutants (POPs), and has established positive associations between T2DM and several polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) (Magliano et al., 2014; Taylor et al.,

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2013), although causality has not been established. PCBs and OCPs are classified as endocrine disrupting chemicals; they circulate in the human body and are stored in adipose tissue. Researchers have proposed several endocrine mechanisms that may link POPs and T2DM, including endocrine disruption of estrogen, androgen, thyroid hormone, and glucocorticoid homeostasis. Other proposed mechanisms include disruption of mitochondrial function, which results in the accumulation of diacylglycerol and other metabolites of fatty acid metabolism. This eventually suppresses the insulin signaling pathways, leading to insulin-resistance (Howell and Mangum, 2011; Yang et al., 2017).

Although positive associations between several POPs and T2DM have been reported in many published studies, including some meta-analyses (Song et al., 2016; Tang et al., 2014), discrepancies between them reflect the fact that there are many challenges in the study of POPs and T2DM. One of these is the overall declining time-trend in human concentrations of legacy POPs (Abass et al., 2018; Nost et al., 2013). Also, the vast majority of previous studies investigated the association between POPs and T2DM in a single blood sample collected either before or after T2DM diagnosis, which does not necessarily reflect life-long exposure to POPs, or past peak exposure, which could be relevant for disease etiology. Physiological factors related to T2DM, like age (birth year), weight change, and adiposity have also been shown to influence POP concentrations (Nost et al., 2013; Schade and Heinzow, 1998; Stubleski et al., 2018; Tornevi et al., 2019), thus affecting POPs-T2DM associations. Additionally, individuals with T2DM usually change their lifestyle after diagnosis, often resulting in stabilized or decreased body weight and improved lipid profiles (Ford et al., 2013). These factors could also influence POP concentrations, thereby affecting cross-sectional associations between POPs and prevalent T2DM. Until now, only two studies have assessed intra-individual changes in POPs within T2DM cases and controls using repeated samples from the same individuals (one pre- and one post-diagnostic sample) (Berg et al., 2021; Tornevi et al., 2019). Both studies suggested that lifestyle changes related to T2DM at least partly affect POP concentrations and their associations with T2DM. Thus, having repeated POP measurements from the same individuals taken several years apart may extend our knowledge of how POPs are associated with T2DM before and after T2DM diagnosis.

To explore the above-mentioned knowledge gaps, we designed a longitudinal, nested case-control study with three to five repeated POP measurements per participant over a period of 15–30 years. The present study aimed to compare longitudinal trends in POP concentrations between T2DM cases and controls, and to examine prospective and cross-sectional associations between POP concentrations and T2DM at different time-points before and after T2DM diagnosis in cases.

2. Materials and methods

2.1. The Tromsø Study

The Tromsø Study, initiated in 1974, is an ongoing population-based health survey conducted within the Tromsø municipality in Northern Norway. At present, the study consists of seven surveys conducted from 1974 to 2015/16, with a survey conducted approximately every 7 years (Jacobsen et al., 2012). Over 15,000 participants have participated in three or more surveys. At each survey, the participants answered a questionnaire, gave a blood sample, and submitted to a thorough physical examination.

2.2. Study design and participants

We used a longitudinal, nested case-control study design, with repeated blood samples collected from the same individuals at up to five surveys: 1986/87 (T1), 1994/95 (T2), 2001 (T3), 2007/08 (T4) and 2015/16 (T5). To be included, cases had to have a T2DM diagnosis recorded in a local diabetes registry between T3 and T4, and available pre-diagnostic serum samples (T1, T2, and T3). These criteria were fulfilled by 76 women and 69 men. If cases also had post-diagnostic samples (T4 and/or T5) available, they were also included. We randomly selected 76 women and 69 men as controls who had participated in at least the same surveys as the cases, had no T2DM diagnosis recorded in a local diabetes registry, and had available serum samples. The Tromsø Study has HbA1c% results for all included participants for T2-T5. Twenty-nine cases had HbA1c $\geq$ 6.5% in pre-diagnostic samples, and five controls had HbA1c $\geq$ 6.5% at one of the time-points; therefore, they were excluded from the study. Participation in the different surveys is represented by four sample sets (Fig. 1). Two controls and one case had insufficient serum at T2. Thus, the number of samples at each time-point was 255 at T1 and T3, 252 at T2, 120 at T4, and 108 at T5, adding up to 990 samples in total, of which the maximum number of cases and controls at any one time-point was 116 and 139, respectively (at T1 and T3) (Fig. 1).

2.3. Questionnaire, clinical examinations, and laboratory data

Tromsø Study participants completed and underwent clinical examinations at each survey. Questionnaires collected information on participant characteristics, use of medications, parity, and breastfeeding (in women, only available for T2-T5), and physical activity. Health professionals took measures of height and weight and collected blood samples by venous puncture at the clinical examinations. Samples were kept at room temperature for 30 min, after which the coagulated samples were centrifuged at 2000 *g* for 10 min. Aliquots of serum were transferred to secondary plastic sample containers within 1 h and stored at −70 °C (Eggen et al., 2013; Jacobsen et al., 2012).

2.4. Chemical analyses, and data handling

For the POPs and lipids analyses, serum samples of included participants were thawed on ice and aliquoted into two separate vials (Sarstedt, cat.nr 72.694.600). Lipid analyses were performed immediately, whereas the other aliquot was stored at −30 °C for another 3–6 months, until POP analyses were performed. Serum concentrations of triglycerides, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol were analyzed with coulometric methods on a Cobas® 8000 platform (Roche diagnostics) at the Department of Laboratory Medicine, University Hospital of North Norway, which is certified according to ISO 151189 (Accredition, 2020). The analyses are routinely used in the clinic for diagnostic purposes. Quality control samples of three different concentrations were ran each day and their CVs were <3%. The laboratory also participates in the Lab Quality external quality assessment program, and results have been within the acceptance limits (Labquality, 2020). HbA1c% was measured after each survey by high-performance liquid chromatography on a Tosoh G8 analyzer (Tosoh Bioscience); CV was <3%.

All POP analyses were also performed at the Department of Laboratory Medicine, University Hospital of North Norway. The samples from the same individuals were measured in the same batch and processed under identical conditions. Each batch had same number of cases and controls, men and women, from the same time-point with randomized positions. Any information that could identify the samples were blinded to the lab staff.

The method for POP analysis has been described in detail elsewhere (Huber et al., 2020). The procedure includes a Freedom Evo 200 (Tecan, Männedorf, Switzerland) liquid handling workstation, which is used for sample preparation. Laboratory personnel extracted 150 μL of the diluted serum samples and cleaned them using automated solid phase extraction. Gas chromatography atmospheric pressure ionization coupled to tandem mass spectrometers (Waters, Milford, MA, USA) were used for the instrumental analyses of PCB congeners and OCPs. Atmospheric pressure ionization was conducted in positive mode under charge transfer conditions. The multiple reaction monitoring mode with

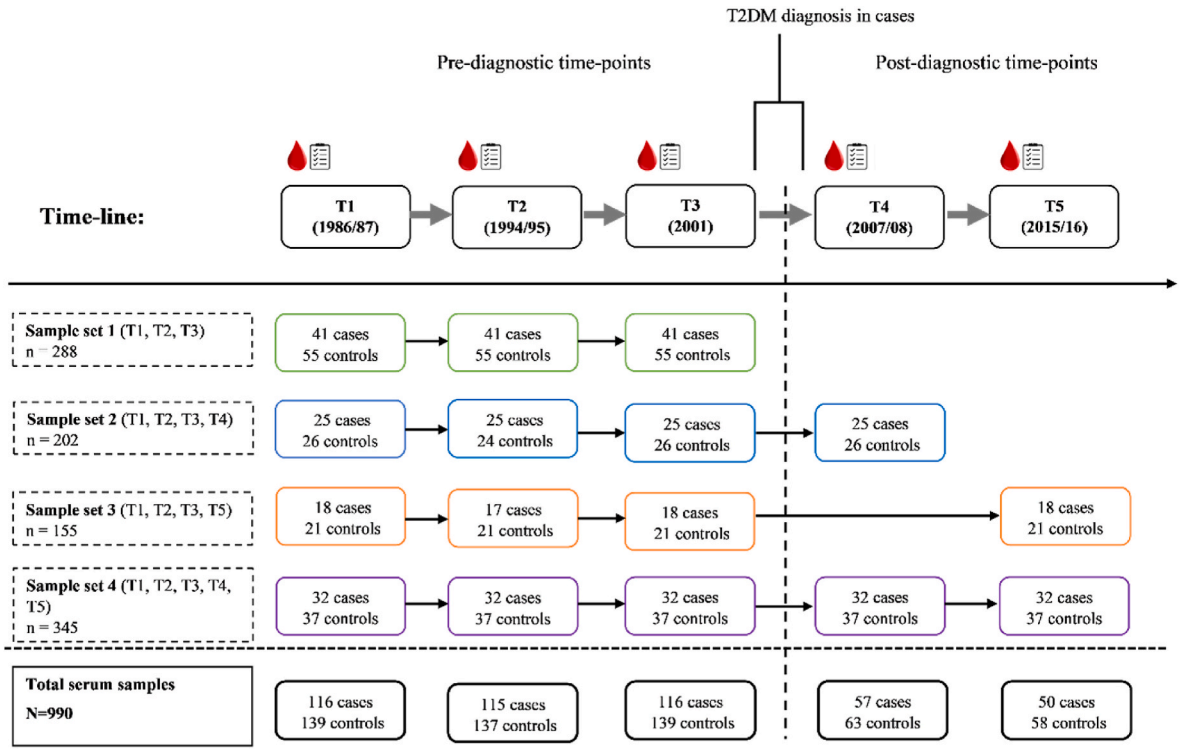

Fig. 1. Overview of the study design, sample size, and sample sets (mutually exclusive groups) based on participation in different surveys (time-points, T) of the Tromsø Study. Abbreviations: T2DM: type 2 diabetes mellitus.

two specific transitions for the individual analytes was applied for detection on the mass spectrometers. Quantification was performed using Masslynx and Targetlynx software (Version 4.1, Waters) and achieved by the internal-standard method with isotope-labeled compounds. For quality assurance, four blank samples, four SRM 1957/1958 (NIST, Gaithersburg, MD, USA) samples, and three bovine serum samples (Sigma Aldrich, Steinheim, Germany) were analyzed within each batch of 96 samples to control for background and carry-over effects. The coefficients of variation (CVs) for the measured POPs ranged from 6 to 24% in the present study, which was within previously established acceptable limits (Huber et al., 2020). All concentrations of the measured POPs were within ±20% of the certified reference materials from the National Institute of Standards and Technology. The laboratory successfully participates in the Arctic Monitoring and Assessment Ring Test for Persistent Organic Pollutants in Human Serum, organized by the Laboratoire de Toxicologie, Institut National de Santé Publique du Québec, Canada, which ensures that the measured POP concentrations are comparable across laboratories.

A total of 13 PCB congeners (PCB-28, 52, 74, 99, 118, 138, 153, 156, 170, 180, 183, 187, and 194) and 13 OCPs (alpha-hexachlorocyclohexane [α-HCH], beta-hexachlorocyclohexane [β-HCH], gamma-hexachlorocyclohexane [γ-HCH], *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor, oxychlordane, heptachlor, *cis*-heptachlor epoxide, hexachlorobenzene [HCB], 1,1,1-trichloro-2,2-bis-(4-chlorophenyl) ethane [*p,p'*-DDT], and 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene [*p,p'*-DDE]) were detected in the analyses (Supplementary Table S1). Concentrations below the sample-specific method of detection limit (MDL) were replaced by MDL divided by the square root of 2. Only those PCBs and OCPs with a detection frequency over 70% at each time-point were included in the present study (Supplementary Table S1). The sum of PCB 118 and 156 is referred to as 'dioxin-like PCBs' ($\sum$DL-PCBs). The sum of all PCB congeners (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187, and 194) included in the analyses is presented as $\sum$PCBs. POP concentrations were lipid-normalized (ng/g lipid) by dividing the wet weight concentrations (pg/mL) by the total lipid concentrations (g/L) according to the formula by Phillips et al.: Total lipids = 2.27*total cholesterol + triglycerides + 0.623 (g/L) (Phillips et al., 1989).

2.5. Statistical analyses

Descriptive statistics of lipid-normalized and wet-weight POP concentrations at each time-point (T1-T5) are presented as means and standard deviations (SD). The mean and median of the different POPs at the different time-points are also presented as box plots. We calculated the mean differences and 95% confidence intervals (CIs) for participant characteristics and POP concentrations between cases and controls, and between men and women, at each time-point. Spearman's rank order correlations were used to assess monotonic relationships between the different POPs at each time-point and also between POPs and BMI at the different time-points.

To assess the time trends in POPs from T1 (1986/87) to T5 (2015/16) in cases and controls, we used multivariable linear mixed-effect models with a random intercept for individuals, while accounting for the dependencies between repeated measures. As the number of samples was considerably larger than the number of measurement occasions, no assumptions were made on the covariance pattern of the random effect; therefore, we fitted an unstructured variance covariance matrix (Fitzmaurice, 2008). Log-transformed POP concentrations were considered dependent variables. Among the independent variables, T2DM status and sex were considered to be constant over time, whereas time-indicator variables of each survey, age, weight change, parity, breastfeeding, total lipids, physical activity, and BMI (normal: $\leq$24.9 kg/m^2, overweight: $\geq$25.0 to $\leq$29.9 kg/m^2, obese: $\geq$30 kg/m^2) were considered to be time-dependent. Interaction terms between T2DM status and time were included to assess whether the time-trends of POPs were different in cases compared to controls. We also included interaction terms between sex and time to assess whether the time-trends of POPs were different between men and women. Predicted POP concentrations after adjusting for the above-mentioned covariates were plotted

for T2DM cases and controls at each time-point.

We used logistic regression models to assess linear associations between POP concentrations (independent variable) and T2DM status (dependent variable) for the different time-points. As our outcome variable (T2DM) is non-time-varying, methods like generalized estimating equations (GEE) that accounts for repeated measurements of POPs is not possible to use (Chen et al., 2015). Instead, we calculated the area under the curve (AUC) for the three pre-diagnostic measurements to quantify the cumulative exposure to POPs. AUCs were then used as independent variables in the logistic regression models. To further take advantage of our repeated measurement design, we modelled the pre-diagnostic POP concentrations as a function of time, using linear mixed effects models with random intercepts and random slopes and unstructured covariance patterns. From the models, we extracted the best linear unbiased prediction (BLUP) of POP concentrations of each individual and used the subject-specific predicted slope as independent variables in logistic regression models. The predicted subject-specific slope then represents a measure of each individual's pre-diagnostic time-trend in POP concentrations. We have presented all results from the logistic regression models as odds ratios (ORs) per 1-SD increase in POP measure in controls along with 95% CIs. To determine which covariates to include in the regression models, we drew a directed acyclic graph (DAG) depicting the hypothesized relationship (based on previous literature) between POPs and T2DM, and the covariates considered (Aune et al., 2014; Bellou et al., 2018; Li et al., 2016). Based on the DAG, covariates included in the regression models were sex, age (in years), weight change (kg), parity, breastfeeding (months), total lipids (g/L), physical activity (categorized into active/inactive), and body mass index (BMI, kg/m^2) (Supplementary Figure S1). Weight change was calculated for time-points T2-T5 using weight information from two adjacent time-points (for example: weight change at T2 = [weight at T2] - [weight at T1]). Weight change at T1 was set to zero, as we had no information on weight from the previous Tromsø survey. Cumulative breastfeeding at each time-point was calculated by summing the reported number of months of breastfeeding per child. As some previous studies have demonstrated sex-specific associations, we also assessed the relationship between POPs and T2DM stratified by sex. Since many analyses were conducted in this work, we controlled for multiple comparisons, and present 99.5% CIs as well, which corresponds to a Bonferroni correction for 10 tests. All statistical analyses were performed using STATA software, version 16 (StataCorp, 4905 Lakeway Drive, College Station, TX, USA).

3. Results

3.1. Sample characteristics

Our study sample consisted of 54% and 52% of females among cases and controls, respectively. The mean age of cases and controls at T1 was 47.5 ± 7.63 and 45.0 ± 9.85 years, respectively. At T1, the cases were ~7.9 (CI: 4.63, 11.2) kg heavier and had a BMI that was 3.15 (CI: 2.25, 4.04) kg/m^2 higher than controls, and this trend persisted through all time-points. There were no differences in parity or breastfeeding between female cases and controls. Total lipids were higher in cases compared to controls in pre-diagnostic time-points, but not in post-diagnostic time-points (Table 1). Men and women showed no differences in BMI or total lipids, except at T1, where men had higher total lipids than women (Supplementary Table S2).

At T1, concentrations of ∑PCBs, *β*-HCH, *trans*-nonachlor, *cis*-nonachlor, oxychlordane, HCB, and *p,p'*-DDE were similar between cases and controls, whereas concentrations of ∑DL-PCBs, *cis*-heptachlor epoxide, and *p,p'*-DDT were higher in cases. However, from T2 to T5, cases had higher mean concentrations of several lipid-normalized POPs compared to controls (Fig. 2, Supplementary Table S3). Similarly, cases experienced higher mean wet-weight concentrations of all POPs at pre-diagnostic time-points, but concentrations were more comparable at post-diagnostic time-points for several POPs (Supplementary Table S4). Cases had in general higher cumulative pre-diagnostic exposure of most

Table 1
Participant characteristics presented as means and standard deviations (SD) for cases and controls, and mean differences (Δ) between type 2 diabetes mellitus cases and controls at each time-point (T). The Tromsø Study (1986–2016).

Characteristics		Pre-diagnostic time-points						Post-diagnostic time-points			
		T1 (1986/87)		T2 (1994/95)		T3 (2001)		T4 (2007/08)		T5 (2015/16)	
		Mean ± SD	ΔMean (95% CI)	Mean ± SD	ΔMean (95% CI)	Mean ± SD	ΔMean (95% CI)	Mean ± SD	ΔMean (95% CI)	Mean ± SD	ΔMean (95% CI)
Age (years)	Cases	47.5 ± 7.63	2.49 (0.28, 4.69)	55.5 ± 7.65	2.48 (0.25, 4.71)	62.5 ± 7.63	2.49 (0.28, 4.69)	65.9 ± 7.38	2.58 (0.50, 5.67)	73.6 ± 7.02	3.72 (0.27, 7.16)
	Controls	45.0 ± 9.85		53.0 ± 9.90		60.0 ± 9.85		63.4 ± 9.44		69.9 ± 10.4	
Weight (kg)	Cases	78.0 ± 14.2	7.91 (4.63, 11.2)	82.1 ± 14.6	9.02 (5.54, 12.5)	86.0 ± 15.2	10.0 (6.38, 13.6)	84.5 ± 14.2	7.50 (1.88, 13.1)	84.5 ± 16.5	7.63 (1.61, 13.7)
	Controls	70.0 ± 12.3		73.1 ± 13.4		76.0 ± 14.1		77.0 ± 16.7		76.8 ± 15.0	
Parity[a]	Cases	2.88 ± 1.56	0.46 (−0.08, 0.99)	2.97 ± 1.50	0.42 (−0.09, 0.93)	2.97 ± 1.50	0.37 (−0.14, 0.87)	2.89 ± 1.28	0.24 (−0.47, 0.95)	2.66 ± 1.32	−0.11 (−0.80, 0.58)
	Controls	2.43 ± 1.54		2.55 ± 1.46		2.60 ± 1.45		2.65 ± 1.75		2.76 ± 1.39	
Breastfeeding[b] **(months)**	Cases	–	–	12.7 ± 11.0	0.64 (−3.40, 4.67)	13.5 ± 12.1	−0.49 (−4.77, 3.79)	13.3 ± 13.3	−0.86 (−7.88, 6.16)	11.3 ± 8.87	−6.75 (−13.2, −0.27)
	Controls			12.0 ± 11.1		14.0 ± 11.7		14.1 ± 14.7		18.0 ± 14.8	
Total Lipids (g/L)	Cases	8.05 ± 1.84	0.90 (0.51, 1.30)	8.30 ± 1.82	0.72 (0.23, 1.20)	7.53 ± 1.33	0.43 (0.11, 0.76)	7.33 ± 1.49	0.08 (−0.42, 0.59)	6.35 ± 1.47	−0.24 (−0.75, 0.27)
	Controls	7.15 ± 1.39		7.58 ± 2.05		7.10 ± 1.29		7.25 ± 1.31		6.59 ± 1.21	
Body Mass Index (kg/m^2)	Cases	27.3 ± 3.91	3.15 (2.25, 4.04)	29.0 ± 4.27	3.61 (2.59, 4.63)	30.6 ± 4.79	4.05 (2.94, 5.15)	30.8 ± 4.78	3.46 (1.70, 5.21)	30.5 ± 5.82	3.33 (1.36, 5.30)
	Controls	24.2 ± 3.34		25.4 ± 3.97		26.5 ± 4.15		27.3 ± 4.91		27.1 ± 4.48	

T1: n = 255, 116 cases; T2: n = 252, 115 cases; T3: n = 255, 116 cases; T4: n = 120, 57cases; T5: n = 108, 50 cases.
[a] Only in women: T1-T3 = 135, 60 cases.
[b] T4 = 76, 36 cases; T5 = 63, 29 cases.

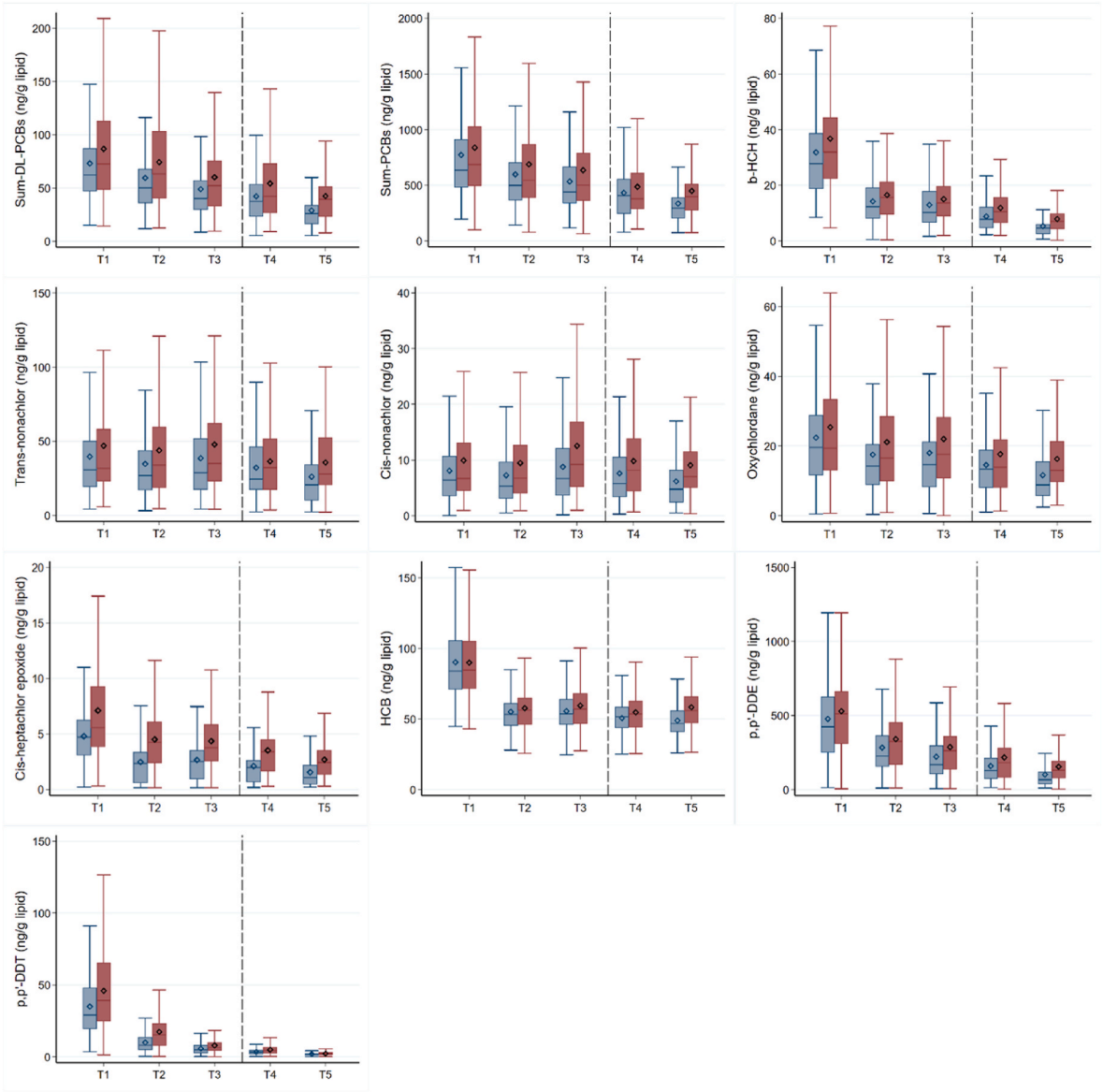

Fig. 2. Lipid-normalized persistent organic pollutant concentrations for type 2 diabetes mellitus cases (red boxes) and controls (blue boxes) at different time-points (T) in the Tromsø Study (1986–2016). Abbreviations: ∑DL-PCBs: dioxin-like polychlorinated biphenyls (PCB 118,156); ∑PCBs: sum polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); *β*-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane. T1-1986/87 (n = 255, 116 cases); T2-1994/95 (n = 252, 115 cases); T3-2001 (n = 255, 116 cases); T4-2007/08 (n = 120, 57 cases); T5-2015/16 (n = 108, 50 cases). Boxes represent the 25th–75th percentiles, horizontal lines within the boxes represent the median, whiskers indicate 1.5 times the length of the interquartile range above and below the 75th and 25th percentiles, respectively, ◊ denotes the mean. The vertical stifled line on the x-axis separates the pre-diagnostic samples from the post-diagnostic samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

POPs as estimated by the AUC (year * ng/g lipid). The predicted subject-specific pre-diagnostic slopes were negative for most individuals, however less for cases than controls.

Positive correlations were observed between the different POPs at each time-point, with correlation coefficients (r_s) ranging between 0.09 and 0.98. The highest correlations were seen between *trans*-nonachlor, *cis*-nonachlor and oxychlordane (>0.85) at all time-points, and the lowest correlation coefficient were seen between HCB and *p,p'*-DDT (0.09) at T5 (Supplementary Tables S5-S9). There were also positive correlations between BMI and POPs at the different time points ranging between 0.01 for ∑PCBs at T3 and 0.42 for *cis*-heptachlor epoxide at T2 (Supplementary Table S10). We observed sex differences in mean lipid-normalized concentrations of most POPs at both pre- and post-diagnostic time-points, except for HCB and *p,p'*-DDE, which were similar in men and women at all time-points (Supplementary Table S2).

3.2. Longitudinal changes in POPs from T1 to T5 in cases versus controls

In both cases and controls, concentrations of all POPs declined from T1 (1986/87) to T5 (2015/16), also after adjusting for sex, age, previous weight change, parity, breastfeeding, total lipids, physical activity, BMI, interaction between T2DM status and time (survey) and interaction between sex and time (Fig. 3). However, the overall decline in POP concentrations was smaller in T2DM cases than in controls, except for *β*-HCH and HCB, which declined similarly in cases and controls (Fig. 3, Supplementary Table S11). Adjusting for weight or BMI as a continuous predictor (instead of applying BMI categories) did not change overall findings. Similar results for all POPs were observed for the wet-weight

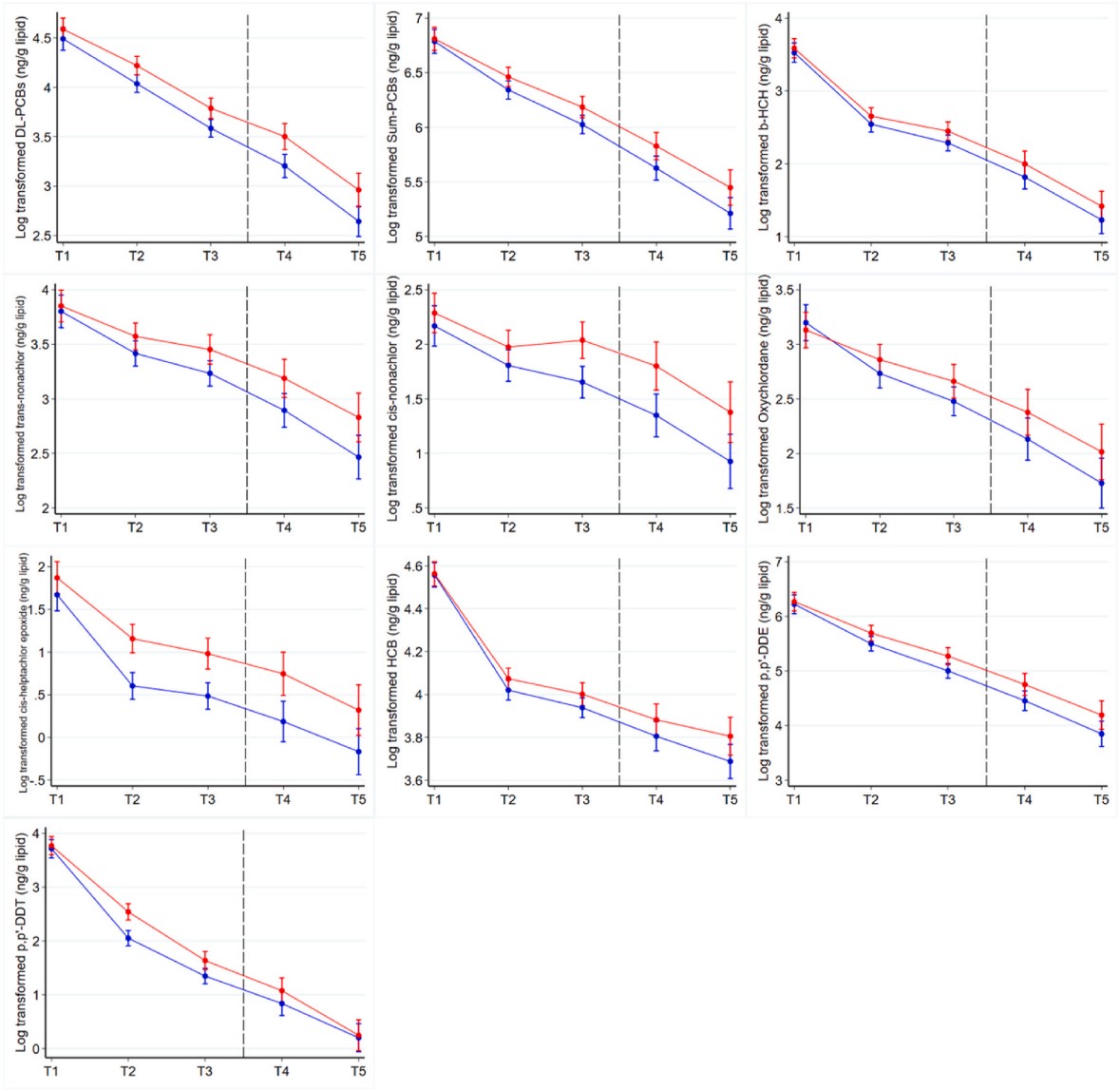

Fig. 3. Predicted lipid-normalized persistent organic pollutant concentrations in type 2 diabetes mellitus cases (in red) and controls (in blue) after adjusting for covariates at different time-points (T) in the Tromsø Study (1986–2016) (n = 990). T1-1986/87; T2-1994/95; T3-2001; T4-2007/08; T5-2015/16. Abbreviations: ∑DL-PCBs: dioxin-like polychlorinated biphenyls (PCB 118,156); ∑PCBs: sum polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); *β*-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane. The vertical stifled line on the x-axis separates the pre-diagnostic time-points from the post-diagnostic time-points. The models are adjusted for time (survey), sex, age, weight change, parity, breastfeeding, total lipids, BMI, interaction between T2DM status and time (survey) and interaction between sex and time (survey). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

concentrations as well (Supplementary Figure S2; Supplementary Table S12). However, when adjusting the confidence intervals and p-values for multiple testing, the stricter criteria for the precision of the estimates still indicated significant differences in longitudinal trends between cases and controls for several of the POPs, except for *β*-HCH, HCB, oxychlordane, *cis*-heptachlor epoxide and *p,p'*-DDT. There were indications of sex differences in temporal trends of all POPs except for *cis*- and *trans*-nonachlor, oxychlordane, and *p,p'*-DDT, with women experiencing slower declines than men. In addition to T2DM status and time, sex, age, weight change, parity, total lipids, physical activity, and BMI were important predictors of POP concentrations, although their relative importance varied according to the compound (Supplementary Tables S11 & S12).

Cases experienced a smaller decrease in lipid-normalized concentrations of ∑DL-PCBs, ∑PCBs, *trans*-nonachlor, oxychlordane, *cis*-heptachlor epoxide, *p,p'*-DDE, and *p,p'*-DDT from T1 to both subsequent pre-diagnostic time-points (T2 and T3) in comparison to controls. A smaller decline in cases was also observed for *cis*-nonachlor, specifically, from T1 to T3. The decline in *β*-HCH and HCB at all pre-diagnostic time-points and for *cis*-nonachlor from T1 to T2 were similar between cases and controls (Fig. 3, Supplementary Table S11). Similar results for the time-trends were observed when only the pre-diagnostic time-points were used in the linear mixed models (data not shown). After controlling for multiple testing, significant differences in declines were still evident for *cis*-heptachlor epoxide, and *p,p'*-DDT from T1 to T2; for ∑DL-PCBs, *trans*-nonachlor, *cis*-nonachlor and *p,p'*-DDE from T1 to T3; and ∑PCBs to both T2 and T3 (Supplementary Table S11 & S12).

When considering the longitudinal changes in POP concentrations from T1 to the post-diagnostic time-points (T4 and T5), cases experienced slower declines in lipid-normalized concentrations of ∑DL-PCBs, ∑PCBs, *trans*-nonachlor, *cis*-nonachlor, oxychlordane, and *p,p'*-DDE compared to controls. The decline for *cis*-heptachlor epoxide (from T1 to

T4) and HCB (from T1 to T5) was smaller in cases than controls, but similar for the other post-diagnostic time-point. Both cases and controls showed similar decreases in concentrations of *p,p'*-DDT and *β*-HCH from T1 to both post-diagnostic time-points (Fig. 3, Supplementary Table S11). After adjusting for multiple comparisons, there were no longer evidence of significant differences in declines between cases and controls for *β*-HCH, oxychlordane, *cis*-heptachlor epoxide, HCB and *p,p'*-DDT to both post-diagnostic time-points. However, there were still significantly slower declines in cases for ∑DL-PCBs, ∑PCBs, *trans*-nonachlor, *cis*-nonachlor and *p,p'*-DDE from T1 to both post-diagnostic time-points compared to controls (Supplementary Tables S11 & S12).

3.3. Associations between POPs and T2DM

After adjusting for confounding factors, several pre-diagnostic POP concentrations were positively associated with subsequent development of T2DM: *cis*-heptachlor epoxide (at T1, T2, and T3), *p,p'*-DDT (at T2), and *cis*-nonachlor (at T3) had 95% CIs around the effect estimates that did not include 1.0 (Fig. 4, Supplementary Table S13). The strengths of the positive associations varied from OR = 1.00 to OR = 1.98 and were stronger closer to the time of diagnosis (T1<T2<T3). The strongest association was observed for *cis*-nonachlor at T3 (OR = 1.98, 95% CI: 1.27–3.08). The wet-weight concentrations demonstrated similar results (Supplementary Figure S3; Supplementary Table S14). The conclusions were similar after adjusting the CIs (at level 99.5%) for multiple testing, except for *cis*-heptachlor epoxide at T1 which now had a wide CI including 1.0 (Supplementary Tables S13 & S14). In the models stratified by sex, we observed comparable results, but the associations were stronger for men for *cis*-nonachlor at T3, and *cis*-heptachlor epoxide and *p,p'*-DDT at T2 (data not shown). Results from the models estimating the associations between cumulative exposure to POPs, measured as AUC, and T2DM, showed similar results as the logistic regression models done separately at T1, T2 and T3. In the multivariable AUC models, only cis-heptachlor epoxide (OR = 1.75, CI: 1.29, 2.37) and *p,p'*-DDT (OR = 1.46, CI:1.12, 1.91) were relatively strongly associated with T2DM with CI intervals not including 1.0, also after controlling for multiple testing (Supplementary Table S15). The predicted pre-diagnostic time-trends of POPs were positively associated with T2DM, indicating that a slower decline was associated with T2DM. The 95% and 99.5% CIs indicated though poor precision of the estimates, which limited further interpretations (Supplementary Table S15).

Positive associations between POPs and T2DM were observed in the post-diagnostic time-points (except for ∑PCBs, *trans*-nonachlor, and oxychlordane), but corresponding 95% CIs were wide and included 1.0, except for *cis*-heptachlor epoxide at T4 (OR = 1.74, 95% CI: 1.07–2.83) (Fig. 4, Supplementary Table S13). Wet-weight concentrations of the same POPs were also positively associated with T2DM; however, the CIs indicated low precision of the point estimates (Supplementary Figure S3; Supplementary Table S14). After controlling for multiple comparisons, all POPs showed very wide CIs, indicating imprecise effect estimates (Supplementary Tables S13 & S14). Associations at post-diagnostic time-points stratified by sex demonstrated positive point estimates similar to those in the overall study sample, but the 95% CIs suggested poor precision, which hampered further interpretations (data not shown).

4. Discussion

This is the first study to fully embrace the chronologic aspects of the complexity of the relationship between POPs and T2DM by including repeated POP measurements from the same individuals collected before and after T2DM diagnosis. We observed a slightly smaller decline in the concentration of several POPs during the observation period (1986–2016) in T2DM cases compared to controls. The difference in decline between cases and controls was consistent in both pre- and post-diagnostic time-points. Our study sample also demonstrated evidence that *cis*-nonachlor, *cis*-heptachlor epoxide, and *p,p'*-DDT are positively associated with T2DM up to 7 years before diagnosis (*cis*-heptachlor epoxide and *p,p'*-DDT), and the strength of these associations increased closer to time of diagnosis. However, after T2DM diagnosis, none of the POPs were associated with T2DM after Bonferroni correction for

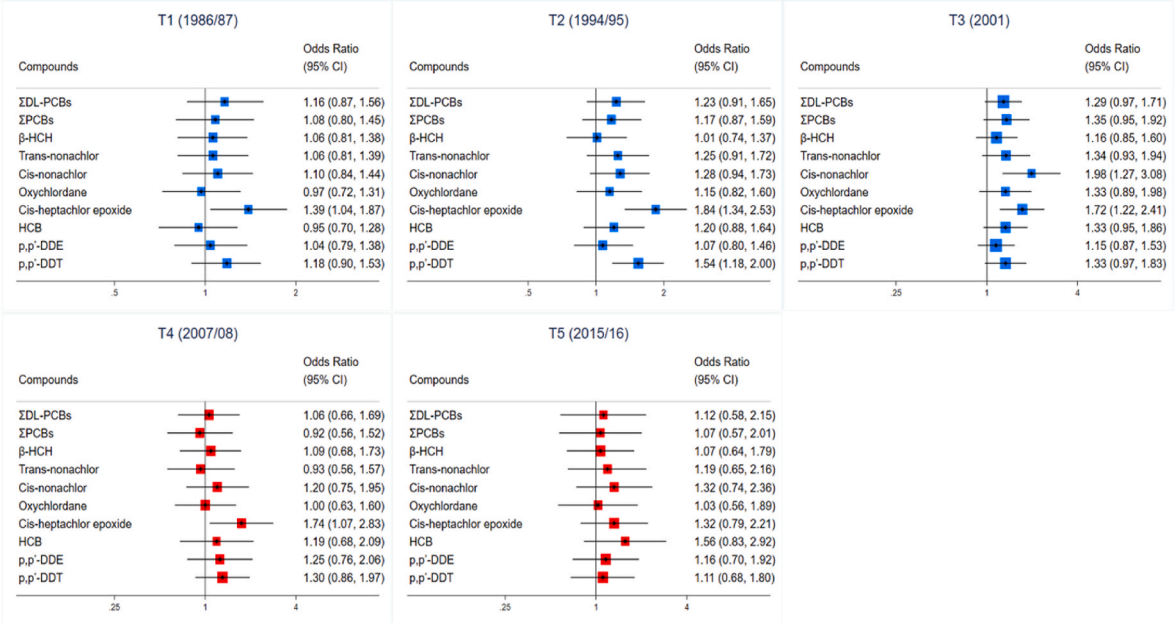

Fig. 4. Odds ratios and 95% confidence intervals (CIs) for the associations between a one-standard deviation (SD) increase in lipid-normalized concentrations of persistent organic pollutants (among controls) and type 2 diabetes mellitus at different time points (T) in the Tromsø Study (1986–2016). T1: (n = 254); T2: (n = 235); T3: (n = 225); T4: (n = 100); T5: (n = 93). Abbreviations: ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); *β*-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane. Odds ratios are adjusted for sex, age, weight change, parity, breastfeeding, total lipids, physical activity, and BMI (except for weight change and breastfeeding at T1).

multiple comparisons. Overall, we observed higher POP concentrations in T2DM cases before diagnosis, and slower temporal declines of several POPs in the same individuals. Our observations suggest that the biological mechanisms related to T2DM or risk factors for the disease also lower the elimination rate of POPs. Thus, increased retention of POPs in individuals at high risk for T2DM could explain the positive associations between POPs and T2DM we observed as early as 15 years before clinical diagnosis of T2DM. Indeed, several factors associated with T2DM can influence POP concentrations, their metabolism, and/or excretion. For instance, toxicokinetic modelling indicates that the greater the BMI, the slower the decline of POP concentrations in the body (Wolff et al., 2007; Wood et al., 2016). Other factors that determine temporal trends of POPs include age (birth year), weight change, and blood lipid levels (Nost et al., 2013; Schade and Heinzow, 1998; Stubleski et al., 2018; Tornevi et al., 2019). In our study, T2DM cases were heavier and had higher BMIs than controls throughout the study period. This may be attributed to dietary habits and other lifestyle factors that influenced body weight before T1. The fact that cases had smaller decreases in the concentrations of several POPs after controlling for differences in sex, age, weight change, parity, breastfeeding, total lipids, physical activity, and BMI, and adjusting for multiple testing suggests that additional mechanisms may be entangled in the underlying mechanisms behind the time-trends of POPs. For instance, physiological factors associated with obesity and T2DM may alter several molecular mechanisms within the body, which further affect elimination rates of POPs. Accordingly, obesity is associated with cytochrome P450 (CYP) enzyme activities (Tomankova et al., 2017; Zanger and Schwab, 2013), processes that are critical for the detoxification of xenobiotics and the metabolism of numerous drugs. Particularly, CYP 3A (a subfamily of CYP), the most plentiful phase I drug-metabolizing enzyme which is found abundantly in the liver and intestines of humans, has been shown to have reduced metabolizing capacity in obese individuals (Krogstad et al., 2021; Zanger and Schwab, 2013). Reduced metabolizing capacity of these enzymes in prospective T2DM cases years before clinical manifestation of the disease could partly explain smaller decreases in POP concentrations, as several cases were already overweight or obese at the start of the study. It is also possible that the adjustment for BMI in our models do not fully account for all aspects of overweight and obesity.

Only two prior studies have compared POP measurements in one pre- and one post-diagnostic sample from the same T2DM cases and controls (Berg et al., 2021; Tornevi et al., 2019). In line with our observations for ∑PCBs, Tornevi and colleagues (Tornevi et al., 2019) reported that T2DM cases experienced smaller declines compared to the controls. Conversely, in our recently published pilot study (Berg et al., 2021), we observed that lipid-normalized concentrations of several OCPs and PCBs increased from the pre-to the post-diagnostic time-point in cases (~4 years), whereas a declining trend based on environmental exposure or a slight increase was observed in controls. The wet-weight concentrations in the same study did not demonstrate an increasing trend. We hypothesized that lifestyle changes provoked by T2DM diagnosis could lead to rapid improvements in lipid profiles and at least partly explain these observations. However, the present study does not support that hypothesis, as we observed weaker associations in post-diagnostic samples. Instead, our study suggests that differences in POP concentrations between cases and controls are present years before T2DM diagnosis.

Our results further suggest that men and women had different temporal trends for several POPs, with a slower decline observed in women, even though most women were above childbearing age at T1. However, this interaction did not affect the overall observations of slower declines in cases compared to controls. This aspect still deserves further in-depth study, as this is beyond the scope of the present publication. In addition to sex, time, and T2DM status, other factors such as age, weight change, total lipids, physical activity, and BMI influenced the time-trends of POPs, clearly emphasizing the complex network of factors that both influence POP concentrations and are independent risk factors of T2DM. To move the research forward in the field of POPs and T2DM, longitudinal studies with repeated measurements like this one are important.

We observed overall positive associations between pre-diagnostic concentrations of many POPs and T2DM with variations in the size and precision of effect estimates at the different time-points. In fact, *cis*-heptachlor epoxide, *p,p'*-DDT, and *cis*-nonachlor were the only compounds that displayed relatively strong and precise associations (with 95% and 99.5% CIs that did not include 1.0) after adjusting for confounding factors and multiple testing. Of these, *cis*-heptachlor epoxide and *p,p'*-DDT were consistently associated with T2DM at T2 and T3 and had distinctly higher concentrations among cases compared to controls even at T1. In addition, our results indicate differential elimination rates of *cis*-heptachlor epoxide and *p,p'*-DDT between cases and controls in the pre-diagnostic period, but not overall after controlling for multiple testing. Also, for unknown reasons, the concentrations of *cis*-nonachlor increased from T2 to T3 among cases, which could explain the strong positive association between *cis*-nonachlor and T2DM at T3. Our measures of cumulative exposure to POPs prior to T2DM diagnosis (AUCs) and the subject-specific pre-diagnostic time-trends confirmed the results observed at the separate pre-diagnostic time-points, as well as our analysis of differences in time-trends between cases and controls. Former prospective studies based on background exposure in general populations have reported an increased risk of T2DM with increasing concentrations of *p,p'*-DDE (Rignell-Hydbom et al., 2009; Turyk et al., 2009); *trans*-nonachlor, oxychlordane, and highly chlorinated PCBs (Lee et al., 2010); heptachlor and *cis*-heptachlor epoxide (Everett and Matheson, 2010; Montgomery et al., 2008); and HCB (Tornevi et al., 2019; Wu et al., 2013); or that gender modifies the association between POPs and T2DM (Vasiliu et al., 2006; Zong et al., 2018). However, the most recently published case-cohort study reported no associations between POPs and incident T2DM (Magliano et al., 2021). Additionally, when comparing results across studies, there is no uniform consistency about which POPs are positively associated with T2DM, which is an important, but not exclusive criteria for causality. Our observations of positive associations between *cis*-heptachlor epoxide and T2DM at two out of three pre-diagnostic time-points, as well as *p,p'*-DDT at T2 add to the range of publications that have reported positive associations between one or several POPs and T2DM. This could reflect a causal association; however, based on the lack of consistency in previous research and our indications of differential elimination rates of POPs according to T2DM status, there are also other explanations that need to be considered. Additionally, very few previous publications control for multiple comparisons, and our work highlight the necessity of this.

Differences in the timing of blood collection with respect to T2DM diagnosis in different studies could affect the final results. Our study is the first to investigate repeated pre-diagnostic measurements of POPs within the same individuals, and our results suggest that the associations between POPs and T2DM vary over time. Therefore, studies with only one pre-diagnostic measurement could reach different conclusions depending on how long before T2DM diagnosis blood samples were collected. Other factors that could influence study results include sample size, selection of confounders, and statistical modeling. Stratification by sex did not change our overall findings. However, the associations were stronger in men compared to women, which could also be a result of smaller sample size and less precision of effect estimates in women.

Our study also assessed the relationship between POPs and T2DM at two post-diagnostic time-points. All post-diagnostic POP measurements showed mainly positive associations, but 95% CIs were wide, exhibiting no evidence for increased odds of prevalent T2DM after Bonferroni correction. Previous reviews of epidemiological studies have consistently indicated that POPs are associated with increased odds of prevalent T2DM (Evangelou et al., 2016; Lee et al., 2014; Taylor et al., 2013). In cross-sectional studies of prevalent T2DM, it is not certain whether elevated concentrations of POPs preceded T2DM diagnosis. Thus, it is important to remember that, in these studies, POPs were measured after clinical manifestations of T2DM. In the present study, cases showed no

major weight loss. However, they showed stable post-diagnostic weight and BMI, and decreased total lipids, and weak positive post-diagnostic associations were demonstrated. Thus, as previously mentioned, our results do not support that lifestyle changes after T2DM diagnosis affect POP concentrations, thereby creating strong positive associations, as has been proposed in previous studies (Berg et al., 2021; Tornevi et al., 2019). However, slower declines in POP concentrations in cases compared to controls was observed even at post-diagnostic time-points. Therefore, our findings must be interpreted with caution, as the weakened associations in our post-diagnostic samples may also be attributed to the smaller sample sizes at T4 and T5.

The most unique feature of this study is the study design itself, with three to five repeated POP measurements for every individual, which has enabled us to examine temporal trends of POPs over a period of 30 years and to assess repeated associations between the different POPs and T2DM. These aspects have not been explored in previously published epidemiological studies. All T2DM cases in our study were identified using a local diabetes registry. Additionally, HbA1c% measurements from the individuals were available at the different time-points, which further enabled us to confirm T2DM status. Another important strength is that height and weight of all participants were objectively measured at each survey by health care professionals and were not self-reported. In addition, complete data were available on most of covariates for which we adjusted. Therefore, to the best of our knowledge, we adjusted for all potential confounders in both the logistic regression and mixed-model analyses. Meticulous laboratory quality control measures for the chemical analyses are an added strength of the present study. However, there are limitations that also need to be considered. For instance, there were fewer participants with post-diagnostic measurements, which may have influenced the strength and precision of effect estimates of the post-diagnostic POP concentrations and T2DM. Another limitation to be considered is that we could not investigate the interactions between POP concentrations and BMI in relation to T2DM as several of the cases were already either overweight/obese at T1 leaving very few participants in the stratified BMI categories. It is also important to remember that BMI does not necessarily reflect fat mass, but also muscle mass. Including a better measure of body fat mass than BMI and a larger sample size so that stratification by BMI status could be possible is highly relevant for future research in this field. This would be particularly interesting as the different POPs clearly displayed different correlations with BMI. It should also be noted that the generalization of these findings may be limited to populations similar to the adult Norwegian population.

Taken together, our results indicate that POPs have an extremely complex relationship with T2DM, as factors related to T2DM also affect POP concentrations. We suggest that slower elimination rates of POPs in people who develop T2DM can explain the observed positive associations between POPs and T2DM. The higher retention is not necessarily caused only by obesity as previously suggested (Wolff et al., 2007) but could also be a result of reduced activity in detoxifying enzymes or other molecular events related to risk factors for T2DM. We hope this study will trigger further longitudinal assessments on the relationship between T2DM and POPs, along with studies of factors such as obesity, lipids, and enzyme activities, which may play key roles in the temporal changes of POPs in individuals with T2DM, thereby influencing POPs-T2DM associations.

5. Conclusion

Our results suggest that higher retention of POPs in people that later develop T2DM can explain the observed positive associations between POP concentrations and T2DM.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

AUC	area under the curve
BLUP	best linear unbiased prediction
BMI	body mass index
CI	confidence interval
CVs	Coefficients of variations
CYP	cytochrome P450
DAG	directed acyclic graph
DL-PCBs	dioxin-like polychlorinated biphenyls
γ-HCH	gamma-hexachlorocyclohexane
HCB	hexachlorobenzene
MDL	method of detection limit
OCPs	Organochlorine pesticides
OR	odds ratio
p,p'-DDE	1,1-bis-(4-chlorophenyl)-2,2-dichloroethene
p,p'-DDT	1,1,1-trichloro-2,2-bis-(4-chlorophenyl) ethane
PCBs	Polychlorinated biphenyls
POPs	Persistent Organic Pollutants
r_s	correlation coefficients
SD	standard deviation
T	time-point
T2DM	Type 2 Diabetes Mellitus
α-HCH	alpha-hexachlorocyclohexane
β-HCH	beta-hexachlorocyclohexane

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.112129.

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Supplemental Material

Longitudinal changes in concentrations of persistent organic pollutants (1986-2016) and their associations with type 2 diabetes mellitus.

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Table S12. Multivariable adjusted regression coefficients, standard errors (SE), 95% and 99.5% confidence intervals (CIs) from linear mixed effect models to assess the longitudinal changes in wet-weight concentrations (pg/mL) of persistent organic pollutants from 1986/87-2015/16 according to type 2 diabetes mellitus status (T2DM) (n=990) in the Tromsø Study (1986-2016).

Table S13. Odds ratios (ORs), 95% and 99.5% confidence intervals (CIs) for the associations between one-standard deviation (SD) increase in lipid-normalized concentrations of persistent organic pollutants (among controls) and type 2 diabetes mellitus (T2DM) at different time-points (T) in the Tromsø Study (1986-2016).

Table S14. Odds ratios (ORs), 95% and 99.5% confidence intervals (CIs) for the associations between one-standard deviation (SD) increase in wet-weight concentrations of persistent organic pollutants (among controls) and type 2 diabetes mellitus (T2DM) at different time points (T) in the Tromsø Study (1986-2016).

Table S15. Odds ratios (ORs), 95% and 99.5% confidence intervals (CIs) for the associations between lipid-normalized concentrations of persistent organic pollutants and type 2 diabetes mellitus (T2DM) for the pre-diagnostic time-points in the Tromsø Study (1986-2001).

Figure S1. Directed acyclic graph (DAG) illustrating assumptions about the causal relationship between persistent organic pollutants (POPs) and type 2 diabetes mellitus (T2DM) and potential confounders.

Figure S2. Predicted wet-weight concentrations (pg/mL) of persistent organic pollutants in type 2 diabetes mellitus cases (in red) and controls (in green) after adjusting for covariates from 1986/87 to 2015/16 (n=990) at different time-points (T).

Figure S3. Odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between a one-standard deviation (SD) increase in wet-weight concentrations of persistent organic pollutants (among controls) and type 2 diabetes mellitus at different time points (T) in the Tromsø Study (1986-2016).

Table S1. Detection frequencies of the different persistent organic pollutants at different time-points (T) in the Tromsø Study (1986-2016).

	Pre-diagnostic time-points			Post-diagnostic time-points	
Compounds	**T1 (1986/87) n=290 %**	**T2 (1994/95) n=290 %**	**T3 (2001) n=290 %**	**T4 (2007/08) n=130 %**	**T5 (2015/16) n=122 %**
PCBs (pg/mL)					
PCB 28	87.6	51.7	50	20	13.9
PCB 52	86.2	54.5	45.9	10.8	8.2
PCB 74	100	100	100	97.7	95.1
PCB 99	100	100	99.2	99.2	99.2
PCB 118	100	100	100	100	100
PCB 138	100	100	100	100	100
PCB 153	100	100	100	100	100
PCB 156	100	100	100	100	100
PCB170	100	100	100	100	100
PCB 180	100	100	100	100	100
PCB 183	100	100	100	99.2	99.2
PCB 187	100	100	100	100	100
PCB 194	100	100	100	100	100
Pesticides (pg/mL)					
α-HCH	29.3	7.50	1.20	16.7	11.9
γ-HCH	24.2	29.6	16.4	0	0.1
β-HCH	100	98.4	100	100	98.2
Heptachlor	11.3	17.8	10.9	17.5	0.1
***Trans*-chlordane**	25.4	20.6	15.2	36.7	8.3
***Cis*-chlordane**	54.7	39.9	30.5	30	30.3
***Trans*-nonachlor**	100	100	100	100	100
***Cis*-nonachlor**	98.8	99.6	99.6	100	100
Oxychlordane	91.4	98.8	98.4	98.3	100
***Cis*-heptachlor epoxide**	89.1	75.9	84.0	80.0	74.3
HCB	100	100	100	100	100
***p,p´*-DDE**	100	100	99.6	99.2	100
***p,p´*-DDT**	99.6	89.3	91.0	91.7	83.5

Abbreviations: PCB: Polychlorinated biphenyl; α-HCH: alpha-hexachlorocyclohexane; β-HCH: beta-hexachlorocyclohexane; ɣ-HCH: gamma-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene.

Table S2. Participant characteristics, and lipid-normalized concentrations of persistent organic pollutants (ng/g lipid) presented as means, standard deviations (SD), and mean differences with 95% confidence intervals (CIs) between men and women at different time-points (T) in the Tromsø Study (1986-2016).

		Pre-diagnostic time-points						Post-diagnostic time-points			
Characteristics		**T1 (1986/87)**		**T2 (1994/95)**		**T3 (2001)**		**T4 (2007/08)**		**T5 (2015/16)**	
		Mean ±SD	**ΔMean (95% CI)**	**Mean ±SD**	**ΔMean (95% CI)**	**Mean ±SD**	**ΔMean (95% CI)**	**Mean ±SD**	**ΔMean (95% CI)**	**Mean ±SD**	**ΔMean (95% CI)**
Age (years)	Men	47.5 ±9.88	2.62	55.5 ±9.88	2.62	62.5 ±9.88	2.62	64.3 ±10.1	-0.40	71.2 ±10.2	-0.66
	Women	44.9 ±7.93	(0.42, 4.82)	52.9 ±7.93	(0.42, 4.82)	59.9 ±7.93	(0.42, 4.82)	64.7 ±7.63	(-3.63, 2.84)	71.9 ±8.38	(-4.21, 2.90)
Weight (kgs)	Men	81.7 ±11.6	15.3	84.9 ±12.3	14.4	87.6 ±13.4	13.4	91.4 ±13.0	17.2	87.6 ±12.6	12.5
	Women	66.4 ±11.3	(12.5, 18.2)	70.5 ±13.3	(11.2, 17.5)	74.2 ±14.3	(9.95, 16.8)	74.2 ±14.0	(12.1, 22.3)	75.2 ±16.4	(6.70, 18.3)
Body Mass Index (kg/m^2)	Men	26.1 ±3.41	0.86	27.1 ±3.54	0.2	28.2 ±3.87	-0.33	29.3 ±3.73	0.53	28.3 ±3.84	-0.67
	Women	25.2 ±4.31	(-0.11, 1.83)	26.9 ±5.17	(-0.91, 1.31)	28.5 ±5.64	(-1.54, 0.88)	28.8 ±5.80	(-1.40, 2.46)	29.0 ±6.28	(-2.76, 1.42)
Total Lipids (g/L)	Men	7.94 ±1.78	0.71	8.11 ±2.16	0.38	7.30 ±1.36	0.02	7.26 ±1.25	-0.03	6.36 ±1.66	-0.21
	Women	7.23 ±1.50	(0.30, 1.11)	7.73 ±1.79	(-0.11, 0.87)	7.28 ±1.29	(-0.31, 0.35)	7.30 ±1.48	(-0.56, 0.49)	6.57 ±1.05	(-0.73, 0.31)
PCBs (ng/g lipid)											
ΣDL-PCBs	Men	95.2 ±54.4	30.2	78.2 ±46.8	22.5	64.2 ±40.1	19.2	55.8 ±39.5	12.6	35.7 ±25.6	1.11
	Women	65.0 ±34.5	(19.1, 41.3)	55.6 ±34.3	(12.4, 32.6)	44.9 ±26.1	(11.0, 27.5)	43.2 ±27.9	(0.36, 24.9)	34.6 ±24.5	(-8.55, 10.8)
ΣPCBs	Men	1018 ±563.2	407.2	793.7 ±467.4	290.9	724.2 ±427.7	271.6	573.7 ±346.1	183.4	437.2 ±267.5	85.42
	Women	610.7 ±321	(295.6, 518.7)	502.8 ±286.3	(196.0, 385.8)	452.6 ±252.4	(186.1, 357.1)	390.4 ±200.2	(84.73, 282.0)	351.8 ±207.5	(-5.238, 176.1)
Pesticides (ng/g lipid)											
β-HCH	Men	37.7 ±29.2	6.91	14.1 ±9.13	-2.03	12.7 ±7.95	-2.09	10.1 ±7.75	-0.15	5.63 ±3.54	-1.43
	Women	30.8 ±14.2	(1.34, 12.5)	16.2 ±8.66	(-4.23, 0.18)	14.8 ±8.28	(-4.10, -0.08)	10.3 ±5.54	(-2.57, 2.26)	7.06 ±5.31	(-3.23, 0.37)
***Trans*-nonachlor**	Men	56.8 ±46.3	26.2	50.0 ±38.4	21.0	54.2 ±44.4	21.6	43.2 ±29.1	14.2	35.3 ±25.2	8.20
	Women	30.7 ±20.3	(17.5, 34.8)	29.0 ±22.4	(13.3, 28.7)	32.6 ±24.6	(12.8, 30.3)	29.0 ±19.8	(5.30, 23.0)	27.1 ±20.2	(-0.48, 16.9)
***Cis*-nonachlor**	Men	11.9 ±9.93	5.58	10.8 ±8.54	4.83	13.6 ±12.2	5.81	11.5 ±8.41	4.53	9.03 ±6.88	2.62
	Women	6.29 ±4.34	(3.73, 7.44)	6.01 ±4.93	(3.13, 6.54)	7.74 ±6.04	(3.48, 8.15)	6.99 ±4.73	(2.15, 6.90)	6.41 ±4.79	(0.39, 4.84)
Oxychlordane	Men	31.2 ±23.4)	14.1	23.6 ±18.1	8.49	24.8 ±19.1	9.44	20.2 ±14.4	6.62	14.3 ±10.4	0.94
	Women	17.1 ±11.1	(9.69, 18.6)	15.2 ±11.7	(4.74, 12.2)	15.4 ±11.2	(5.62, 13.3)	13.6 ±9.23	(2.36, 10.9)	13.4 ±9.95	(-2.99, 4.87)
***Cis*-heptachlor epoxide**	Men	7.67 ±5.19	3.43	4.22 ±2.73	1.54	4.27 ±3.03	1.59	3.50 ±2.87	1.13	2.38 ±1.97	0.53
	Women	4.24 ±2.58	(2.44, 4.42)	2.68 ±2.38	(0.90, 2.17)	2.68 ±1.94	(0.97, 2.22)	2.37 ±1.64	(0.32, 1.94)	1.86 ±1.56	(-0.15, 1.20)
HCB	Men	93.6 ±28.8	6.62	55.6 ±15.4	-1.25	58.6 ±17.8	2.40	50.9 ±13.1	-2.58	54.2 ±15.6	54.2
	Women	86.9 ±26.8	(-0.23, 13.5)	56.8 ±14.7	(-4.98, 2.48)	56.2 ±14.6	(-1.59, 6.39)	53.5 ±12.2	(-7.28, 2.11)	52.6 ±13.7	(-4.00, 7.25)
***p,p'*-DDE**	Men	525 ±335	45.3	320 ±211	20.8	255 ±173	6.67	169 ±100	-28.4	108 ±73.6	-30.6
	Women	479 ±296	(-32.5, 123)	300 ±212	(-31.8, 73.5)	248 ±195	(-39.1, 52.4)	197 ±185	(-88.2, 31.4)	139 ±154	(-79.7, 18.6)
***p,p'*-DDT**	Men	44.7 ±26.7	9.07	15.5 ±12.8	4.13	7.27 ±5.09	1.03	4.80 ±3.18	1.07	2.19 ±1.48	0.21
	Women	35.7 ±23.5	(2.88, 15.2)	11.4 ±8.89	(1.42, 6.83)	6.24 ±4.69	(-0.18, 2.23)	3.73 ±2.39	(0.05, 2.08)	1.97 ±1.05	(-0.27, 0.69)

T1: n=255, 120 men; T2: n=252, 118 men, T3: 255, 120 men; T4: 120, 44 men, T5: 108, 45 men. Abbreviations: SD: standard deviation; Δ: denotes mean differences between men and women with 95% confidence intervals at each time-point; ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); β-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane.

Table S3. Lipid-normalized concentrations (ng/g lipid) of persistent organic pollutants presented as means, standard deviations (SD), and mean differences with 95% confidence intervals (CIs) between type 2 diabetes mellitus cases and controls at different time-points (T) in the Tromsø Study (1986-2016).

		Pre-diagnostic time-points						Post-diagnostic time-points			
Compounds (ng/g lipid)		**T1 (1986/87)**		**T2 (1994/95)**		**T3 (2001)**		**T4 (2007/08)**		**T5 (2015/16)**	
		Mean ±SD	**ΔMean (95% CI)**	**Mean ±SD**	**ΔMean (95% CI)**	**Mean ±SD**	**ΔMean (95% CI)**	**Mean ±SD**	**ΔMean (95% CI)**	**Mean ±SD**	**ΔMean (95% CI)**
ΣDL-PCBs	Cases Controls	86.7 ±50.5 73.1 ±43.8	13.6 (1.96, 25.2)	74.3 ±45.9 59.4 ±37.5	14.8 (4.51, 25.2)	60.1 ±35.5 48.9 ±33.3	11.2 (2.65, 19.7)	54.2 ±38.1 42.1 ±26.8	12.0 (0.20, 23.9)	42.3 ±28.4 28.8 ±19.5	13.5 (4.32, 22.7)
ΣPCBs	Cases Controls	838 ±522 773 ±470	65.0 (-57.4, 187)	689 ±439 597 ±376	91.1 (-10.1, 192)	636 ±402 534 ±338	101 (10.2, 193)	486 ±306 432 ±246	54.0 (-46.1, 154)	449 ±260 334 ±203	115 (26.1, 203)
β-HCH	Cases Controls	36.7 ±23.1 31.8 ±22.4	4.92 (-0.70, 10.5)	16.5 ±8.85 14.2 ±8.88	2.34 (0.13, 4.55)	15.0 ±7.86 12.9 ±8.34	2.09 (0.07, 4.10)	11.8 ±7.35 8.80 ±5.06	3.03 (0.77, 5.30)	7.85 ±5.31 5.27 ±3.73	2.58 (0.85, 4.32)
***Trans*-nonachlor**	Cases Controls	46.9 ±42.5 39.7 ±32.1	7.20 (-2.02, 16.4)	43.8 ±35.3 34.7 ±29.7	9.16 (1.10, 17.2)	47.8 ±41.5 38.5 ±32.0	9.25 (0.18, 18.3)	36.5 ±25.6 32.1 ±23.5	4.38 (-4.49, 13.2)	35.7 ±23.4 26.1 ±21.2	9.61 (1.09, 18.1)
***Cis*-nonachlor**	Cases Controls	9.92 ±9.22 8.08 ±6.72	1.83 (-0.14, 3.81)	9.48 ±8.20 7.25 ±6.22	2.23 (0.43, 4.02)	12.5 ±11.9 8.77 ±7.37	3.76 (1.35, 6.17)	9.82 ±7.27 7.60 ±5.93	2.22 (-0.17, 4.61)	9.05 ±6.33 6.16 ±5.13	2.89 (0.70, 5.07)
Oxychlordane	Cases Controls	25.4 ±20.1 23.4 ±18.5	3.00 (-1.77, 7.76)	21.1 ±15.6 17.5 ±15.5	3.59 (-0.28, 7.46)	22.0 ±16.8 18.0 ±15.3	3.99 (0.01, 7.96)	17.7 ±13.7 14.6 ±9.52	3.10 (-1.14, 7.34)	16.3 ±11.0 11.6 ±8.80	4.71 (0.93, 8.49)
***Cis*-heptachlor epoxide**	Cases Controls	7.12 ±5.37 4.80 ±2.93	2.32 (1.28, 3.37)	4.51 ±2.96 2.48 ±1.95	2.03 (1.42, 2.64)	4.35 ±2.87 2.66 ±2.15	1.69 (1.07, 2.31)	3.53 ±2.55 2.12 ±1.64	1.41 (0.64, 2.18)	2.69 ±1.90 1.55 ±1.43	1.15 (0.51, 1.78)
HCB	Cases Controls	89.9 ±26.9 90.2 ±28.7	-0.31 (-7.23, 6.60)	57.7 ±15.7 55.1 ±14.4	2.59 (-1.14, 6.31)	59.5 ±18.0 55.6 ±14.3	3.92 (-0.06, 7.91)	54.7 ±13.9 50.6 ±10.9	4.05 (-0.44, 8.54)	58.3 ±15.5 48.8 ±12.1	9.47 (4.20, 14.7)
***p,p'*-DDE**	Cases Controls	529 ±307 477 ±321	52.1 (-25.8, 130)	340 ±213 283 ±208	56.8 (4.45, 109)	286 ±199 222 ±167	64.2 (19.0, 109)	217 ±175 159 ±139	57.5 (0.61, 114)	155 ±140 101 ±110	54.1 (6.30, 102)
***p,p'*-DDT**	Cases Controls	45.9 ±27.9 35.0 ±22.0	10.9 (4.77, 17.1)	17.4 ±12.9 9.87 ±7.71	7.51 (4.91, 10.1)	7.90 ±5.57 5.74 ±4.03	2.15 (0.97, 3.34)	4.89 ±3.24 3.43 ±1.98	1.46 (0.50, 2.42)	2.23 ±1.27 1.92 ±1.21	0.32 (-0.16, 0.79)

T1: n=255, 116 cases; T2: n=252, 115 cases; T3: n=255, 116 cases; T4: n=120, 57cases; T5: n=108, 50 cases. Abbreviations: ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); β-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane.

Table S4. Wet-weight concentrations (pg/mL) of persistent organic pollutants presented as means, standard deviations (SD), and mean differences with 95% confidence intervals (CIs) between cases and type 2 diabetes mellitus controls at different time-points (T) in the Tromsø Study (1986-2016).

		Pre-diagnostic time-points						Post-diagnostic time-points			
Compounds (ng/g lipid)		**T1 (1986/87)**		**T2 (1994/95)**		**T3 (2001/02)**		**T4 (2007/08)**		**T5 (2015/16)**	
		Mean ±SD	**ΔMean (95% CI)**	**Mean ±SD**	**ΔMean (95% CI)**	**Mean ±SD**	**ΔMean (95% CI)**	**Mean ±SD**	**ΔMean (95% CI)**	**Mean ±SD**	**ΔMean (95% CI)**
ΣDL-PCBs	Cases Controls	709.8 ±468.2 540.0 ±374.4	169.8 (65.85, 273.7)	641.0 ±531.0 467.3 ±402.8	173.7 (57.72, 605.2)	454.3 ±288.1 351.5 ±257.8	102.7 (35.38, 170.1)	391.5 ±292.5 311.4 ±214.5	80.09 (-12.05, 172.2)	263.6 ±172.8 190.5 ±157.6	73.15 (14.24, 132.1)
ΣPCBs	Cases Controls	6860 ±4722 5709 ±3956	1151 (81.19, 2221)	5920 ±4842 4684 ±3867	1236 (155.2, 2316)	4792 (3169) 3840 (2616)	952.3 (238.9, 1666)	3523 ±2331 3190 ±1990	332.8 (-448.7, 1114)	2786 ±1602 2214 ±1400	572.7 (-0.074, 1145)
β-HCH	Cases Controls	305.2 ±244.8 234.5 ±192.5	70.70 (16.75, 124.7)	140.4 ±86.24 112.3 ±96.98	28.13 (5.156, 51.11)	114.0 (67.42) 91.99 (65.17)	22.01 (5.611, 38.40)	86.12 ±56.64 65.88 ±46.18	20.25 (1.636, 38.86)	40.23 ±34.50 34.95 ±28.54	14.28 (2.253, 26.31)
***Trans*-nonachlor**	Cases Controls	397.8 ±409.8 296.6 ±271.9	101.3 (16.69, 185.9)	386.1 ±391.1 276.9 ±308.2	109.2 (22.38, 196.0)	361.6 (323.1) 273.8 (237.0)	87.76 (18.57, 157.0)	264.0 ±188.4 239.0 ±184.1	24.95 (-42.44, 92.35)	223.7 ±152.1 173.6 ±150.7	50.14 (-7.780, 108.1)
***Cis*-nonachlor**	Cases Controls	84.39 ±87.99 60.21 ±55.35	24.18 (6.337, 42.02)	83.22 ±89.02 58.38 ±68.19	24.84 (5.317, 44.36)	94.27 (91.00) 62.14 (52.29)	32.12 (14.17, 50.08)	70.89 ±53.21 56.49 ±46.33	14.40 (-3.600, 32.39)	56.69 ±39.81 40.76 ±35.53	15.94 (1.561, 30.31)
Oxychlordane	Cases Controls	213.6 ±194.1 168.1 ±157.3	45.56 (2.218, 88.90)	186.1 ±188.4 140.8 ±172.0	45.30 (0.562, 90.05)	167.0 (133.1) 128.0 (115.2)	39.02 (8.391, 69.64)	128.9 ±106.1 108.4 ±77.40	20.52 (-12.84, 53.88)	103.1 ±73.61 76.78 ±61.05	26.34 (0.651, 52.04)
***Cis*-heptachlor epoxide**	Cases Controls	62.71 ±79.96 35.77 ±24.46	26.94 (12.86, 41.02)	39.27 ±35.87 19.70 ±18.13	19.57 (12.68, 26.46)	33.27 (24.06) 18.86 (16.02)	14.41 (9.435, 19.38)	25.94 ±18.81 15.74 ±12.84	10.20 (4.423, 15.97)	17.03 ±12.37 10.08 ±9.296	6.953 (2.811, 11.10)
HCB	Cases Controls	724.4 ±283.8 643.3 ±236.6	81.16 (16.98, 145.3)	476.2 ±164.6 411.6 ±140.3	64.54 (26.72, 102.4)	441.5 (134.4) 388.5 (102.4)	52.92 (23.69, 82.15)	390.2 ±94.01 360.3 ±74.38	29.97 (-0.535, 60.48)	358.0 ±83.47 313.9 ±66.31	44.13 (15.53, 72.72)
***p,p'*-DDE**	Cases Controls	4302 ±2745 3442 ±2381	860.1 (227.9, 1492)	2920 ±2145 2146 ±1602	774.2 (308.7, 1240)	2167 (1556) 1568 (1208)	598.9 (257.8, 940.0)	1565 ±1276 1182 ±1104	382.9 (-47.43, 813.2)	975.1 ±937.5 678.1 ±811.8	297.0 (-36.73, 630.7)
***p,p'*-DDT**	Cases Controls	380.6 ±275.7 254.2 ±173.3	126.4 (70.46, 182.2)	150.6 ±128.7 78.10 ±71.88	72.53 (47.18, 97.83)	60.18 ±47.63 40.54 ±28.69	19.64 (10.11, 29.16)	35.11 ±23.45 25.33 ±16.63	9.779 (2.483, 17.08)	14.35 ±8.74 12.67 ±8.271	1.679 (-1.569, 4.928)

T1: n=255, 116 cases; T2: n=252, 115 cases; T3: n=255, 116 cases; T4: n=120, 57cases; T5: n=108, 50 cases. Abbreviations: ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); β-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane.

Table S5. Spearman's rank correlations between the different persistent organic pollutants at time-point 1 (T1, 1986/87), n=255.

	∑DL-PCBs	**∑PCBs**	**b-HCH**	**Trans-nonachlor**	**Cis-nonachlor**	**Oxychlordane**	**Cis-heptachlor epoxide**	**HCB**	***p,p'*-DDE**	***p,p'*-DDT**
∑DL-PCBs	-									
∑PCBs	0.91	-								
β -HCH	0.27	0.34	-							
***Trans*-nonachlor**	0.83	0.82	0.26	-						
***Cis*-nonachlor**	0.78	0.79	0.19	0.96	-					
Oxychlordane	0.84	0.83	0.32	0.88	0.85	-				
***Cis*-heptachlor epoxide**	0.62	0.65	0.45	0.65	0.63	0.66	-			
HCB	0.51	0.57	0.35	0.56	0.54	0.55	0.52	-		
***p,p'*-DDE**	0.46	0.42	0.24	0.32	0.32	0.34	0.36	0.21	-	
***p,p'*-DDT**	0.41	0.47	0.47	0.46	0.42	0.36	0.46	0.32	0.45	-

Abbreviations: ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); β-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane.

Table S6. Spearman's rank correlations between the different persistent organic pollutants at time-point 2 (T2, 1994/95), n=252.

	∑DL-PCBs	**∑PCBs**	**b-HCH**	**Trans-nonachlor**	**Cis-nonachlor**	**Oxychlordane**	**Cis-heptachlor epoxide**	**HCB**	***p,p'*-DDE**	***p,p'*-DDT**
∑DL-PCBs	-									
∑PCBs	0.93	-								
β -HCH	0.37	0.31	-							
***Trans*-nonachlor**	0.85	0.85	0.25	-						
***Cis*-nonachlor**	0.85	0.84	0.26	0.97	-					
Oxychlordane	0.88	0.87	0.33	0.94	0.90	-				
***Cis*-heptachlor epoxide**	0.64	0.59	0.39	0.65	0.65	0.67	-			
HCB	0.57	0.52	0.37	0.49	0.51	0.58	0.41			
***p,p'*-DDE**	0.59	0.60	0.40	0.48	0.46	0.48	0.48	0.32	-	
***p,p'*-DDT**	0.60	0.55	0.42	0.56	0.56	0.53	0.62	0.29	0.64	-

Abbreviations: ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); β-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane.

Table S7. Spearman's rank correlations between the different persistent organic pollutants at time-point 3 (T3, 2001), n=255.

	∑DL-PCBs	∑PCBs	b-HCH	Trans-nonachlor	Cis-nonachlor	Oxychlordane	Cis-heptachlor epoxide	HCB	*p,p'*-DDE	*p,p'*-DDT
∑DL-PCBs	-									
∑PCBs	0.92	-								
β -HCH	0.32	0.25	-							
***Trans*-nonachlor**	0.76	0.76	0.26	-						
***Cis*-nonachlor**	0.76	0.73	0.22	0.97	-					
Oxychlordane	0.78	0.77	0.32	0.95	0.92	-				
***Cis*-heptachlor epoxide**	0.63	0.58	0.30	0.73	0.74	0.71	-			
HCB	0.49	0.45	0.26	0.59	0.61	0.60	0.61	-		
***p,p'*-DDE**	0.53	0.55	0.29	0.49	0.51	0.47	0.49	0.42	-	
***p,p'*-DDT**	0.62	0.55	0.39	0.68	0.69	0.63	0.60	0.45	0.64	-

Abbreviations: ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); β-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane.

Table S8. Spearman's rank correlations between the different persistent organic pollutants at time-point 4 (T4, 2007/08), n=120.

	∑DL-PCBs	∑PCBs	b-HCH	Trans-nonachlor	Cis-nonachlor	Oxychlordane	Cis-heptachlor epoxide	HCB	*p,p'*-DDE	*p,p'*-DDT
∑DL-PCBs	-									
∑PCBs	0.93	-								
β -HCH	0.59	0.53	-							
***Trans*-nonachlor**	0.86	0.86	0.44	-						
***Cis*-nonachlor**	0.85	0.83	0.41	0.98	-					
Oxychlordane	0.89	0.89	0.50	0.95	0.92	-				
***Cis*-heptachlor epoxide**	0.70	0.67	0.52	0.77	0.77	0.75	-			
HCB	0.48	0.45	0.35	0.46	0.44	0.49	0.35			
***p,p'*-DDE**	0.66	0.67	0.55	0.49	0.48	0.49	0.50	0.27	-	
***p,p'*-DDT**	0.78	0.71	0.46	0.73	0.75	0.70	0.66	0.30	0.67	-

Abbreviations: ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); β-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane.

Table S9. Spearman's rank correlations between the different persistent organic pollutants at time-point 5 (T5, 2015/16), n=108.

	∑DL-PCBs	∑PCBs	b-HCH	Trans-nonachlor	Cis-nonachlor	Oxychlordane	Cis-heptachlor epoxide	HCB	*p,p'*-DDE	*p,p'*-DDT
∑DL-PCBs	-									
∑PCBs	0.92	-								
β -HCH	0.66	0.57	-							
***Trans*-nonachlor**	0.88	0.88	0.51	-						
***Cis*-nonachlor**	0.84	0.82	0.47	0.96	-					
Oxychlordane	0.90	0.87	0.58	0.93	0.88	-				
***Cis*-heptachlor epoxide**	0.62	0.62	0.58	0.64	0.65	0.63	-			
HCB	0.49	0.45	0.40	0.44	0.42	0.48	0.35	-		
***p,p'*-DDE**	0.71	0.71	0.57	0.58	0.56	0.57	0.53	0.33	-	
***p,p'*-DDT**	0.52	0.47	0.41	0.51	0.53	0.43	0.56	0.09	0.59	-

Abbreviations: ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); β-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane.

Table S10. Spearman's rank correlations between the different persistent organic pollutants and body mass index for the different time-points (T), Tromsø Study (1986-2015/16).

	T1 (1986/87)	T2 (1994/95)	T3 (2001)	T4 (2007/08)	T5 (2015/16)
BMI-ΣDL-PCBs	0.19	0.21	0.14	0.23	0.21
BMI-ΣPCBs	0.07	0.07	0.01	0.10	0.08
***BMI-β*-HCH**	0.27	0.28	0.16	0.38	0.32
***BMI-Trans*-nonachlor**	0.13	0.15	0.05	0.19	0.16
***BMI-Cis*-nonachlor**	0.16	0.18	0.08	0.23	0.20
BMI-Oxychlordane	0.16	0.15	0.04	0.18	0.14
BMI-*Cis*-heptachlor epoxide	0.41	0.42	0.32	0.38	0.38
BMI-HCB	0.07	0.02	0.10	0.21	0.15
BMI-*p,p'*-DDE	0.19	0.25	0.20	0.23	0.26
BMI-*p,p'*-DDT	0.31	0.38	0.18	0.32	0.15

T1: n=255; T2: n=252; T3: n=255; T4: n=120; T5: n=108. Abbreviations: BMI: body Mass Index; ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); β-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane.

Table S11. Multivariable adjusted regression coefficients, standard errors (SE), 95% and 99.5% confidence intervals (CIs) from linear mixed effect models to assess the longitudinal changes in lipid-normalized concentrations (ng/g lipid) of persistent organic pollutants from 1986/87-2015/16 according to type 2 diabetes mellitus (T2DM) status (n=990) in the Tromsø Study (1986-2016).

	Model 1		Model 2		
	β – coefficient (SE)	95% CI / p-value for Walds test	β – coefficient (SE)	95% CI / p-value for Walds test	Adjusted CIs at 99.5% for multiple comparisons
∑DL-PCBs					
- T2DM	0.16 (0.07)	0.02, 0.30	0.10 (0.06)	-0.03, 0.22	-0.08, 0.28
- *Sampling year*					
T2	-0.22 (0.02)	-0.27, -0.18	-0.42 (0.05)	-0.51, -0.33	-0.55, -0.29
T3	-0.45 (0.03)	-0.50, -0.40	-0.88 (0.06)	-1.00, -0.75	-1.06, -0.70
T4	-0.60 (0.04)	-0.67, -0.53	-1.19 (0.09)	-1.36, -1.02	-1.44, -0.95
T5	-0.91 (0.04)	-0.99, -0.82	-1.74 (0.11)	-1.95, -1.52	-2.05, -1.43
- *Interactions*					
T2DMxT2	0.05 (0.03)	-0.02, 0.11	0.09 (0.04)	0.02, 0.16	-0.01, 0.19
T2DMxT3	0.08 (0.04)	0.004, 0.15	0.11 (0.04)	0.03, 0.18	0.003, 0.21
T2DMxT4	0.18 (0.05)	0.08, 0.29	0.20 (0.05)	0.10, 0.30	0.06, 0.34
T2DMxT5	0.30 (0.07)	0.17, 0.43	0.22 (0.05)	0.12, 0.32	0.08, 0.36
- *Wald test for T2DMxtime interaction term*		**<0.001**		**<0.001**	
Sex x T2			-0.06 (0.04)	-0.14, 0.02	-0.18, 0.05
Sex x T3			-0.06 (0.04)	-0.14, 0.03	-0.17, 0.06
Sex x T4			-0.19 (0.05)	-0.30, -0.09	-0.34, -0.04
Sex x T5			-0.22 (0.06)	-0.33, -0.11	-0.38, -0.07
- *Wald test for sexxtime interaction term*				**<0.001**	
- Sex			0.005 (0.09)	-0.17, 0.17	-0.24, 0.25
- Age			0.03 (0.003)	0.03, 0.04	0.02, 0.04
- Weight change			-0.01 (0.002)	-0.02, -0.01	-0.02, -0.008
- Parity			-0.10 (0.02)	-0.14, -0.06	-0.16, -0.04
- Breastfeeding			0.0007 (0.002)	-0.002, 0.004	-0.004, 0.005
- Total Lipids			-0.003 (0.006)	-0.01, 0.009	-0.02, 0.01
- Physical activity			-0.03 (0.02)	-0.06, 0.01	-0.08, 0.03
- BMI					
Overweight			-0.01 (0.03)	-0.07, 0.04	-0.09, 0.06
Obese			-0.01 (0.04)	-0.09, 0.07	-0.13, 0.10
- constant	4.14 (0.05)	4.05, 4.24	4.30 (0.08)	4.15, 4.45	4.08, 4.52
∑PCBs					
- T2DM	0.06 (0.07)	-0.08, 0.20	0.02 (0.06)	-0.10, 0.14	-0.15, 0.20
- *Sampling year*					
T2	-0.27 (0.02)	-0.31, -0.22	-0.40 (0.04)	-0.48, -0.32	-0.52, -0.29
T3	-0.40 (0.02)	-0.44, -0.35	-0.71 (0.06)	-0.83, -0.60	-0.88, -0.50
T4	-0.58 (0.03)	-0.65, -0.52	-1.06 (0.08)	-1.22, -0.90	-1.29, -0.84
T5	-0.77 (0.04)	-0.85, -0.69	-1.45 (0.10)	-1.65, -1.25	-1.74, -1.16
- *Interactions*					
T2DMxT2	0.06 (0.03)	-0.003, 0.12	0.10 (0.03)	0.04, 0.15	0.01, 0.18
T2DMxT3	0.11 (0.03)	0.04, 0.18	0.14 (0.03)	0.08, 0.20	0.05, 0.22

T2DMxT4	0.16 (0.05)	0.07, 0.26	0.18 (0.04)	0.10, 0.26	0.06, 0.30
T2DMxT5	0.29 (0.06)	0.17, 0.41	0.21 (0.04)	0.13, 0.30	0.09, 0.34
- *Wald test for T2DMxtime interaction term*		**<0.001**		**<0.001**	
Sex x T2			-0.09 (0.03)	-0.16, -0.02	-0.19, 0.009
Sex x T3			-0.10 (0.04)	-0.17, -0.03	-0.20, 0.005
Sex x T4			-0.20 (0.05)	-0.29, -0.11	-0.33, -0.07
Sex x T5			-0.25 (0.05)	-0.35, -0.16	-0.39, -0.12
- *Wald test for sexxtime interaction term*				**<0.001**	
- Sex			0.18 (0.08)	0.02, 0.38	0.18, 0.41
- Age			0.03 (0.003)	0.02, 0.03	0.02, 0.04
- Weight change			-0.01 (0.001)	-0.02, -0.01	-0.02, -0.01
- Parity			-0.09 (0.01)	-0.13, -0.05	-0.15, -0.04
- Breastfeeding			-0.00005 (0.001)	-0.003, 0.003	-0.004, 0.004
- Total Lipids			-0.001 (0.005)	-0.01, 0.009	-0.02, 0.01
- Physical activity			-0.03 (0.02)	-0.07, 0.0002	-0.08, 0.01
- BMI					
Overweight			-0.05 (0.02)	-0.10, -0.004	-0.12, 0.02
Obese			-0.07 (0.04)	-0.14, -0.0002	-0.17, 0.03
- constant	6.51 (0.05)	6.42, 6.60	6.59 (0.07)	6.45, 6.73	6.39, 6.79
***β*-HCH**					
- T2DM	0.15 (0.07)	0.008, 0.30	0.06 (0.08)	-0.09, 0.21	-0.16, 0.28
- *Sampling year*					
T2	-0.85 (0.05)	-0.95, -0.75	-0.85 (0.09)	-1.02, -0.68	-1.10, -0.61
T3	-0.96 (0.05)	-1.07, -0.85	-1.15 (0.10)	-1.35, -0.95	-1.43, -0.87
T4	-1.30 (0.07)	-1.44, -1.15	-1.57 (0.13)	-1.83, -1.32	-1.94, -1.21
T5	-1.74 (0.08)	-1.90, -1.59	-2.14 (0.15)	-2.43, -1.85	-2.55, -1.73
- *Interactions*					
T2DMxT2	0.02 (0.08)	-0.14, 0.17	0.05 (0.08)	-0.11, 0.21	-0.18, 0.28
T2DMxT3	0.07 (0.08)	-0.08, 0.23	0.10 (0.08)	-0.06, 0.27	-0.13, 0.38
T2DMxT4	0.12 (0.11)	-0.09, 0.33	0.12 (0.11)	-0.10, 0.34	-0.19, 0.44
T2DMxT5	0.09 (0.12)	-0.14, 0.31	0.13 (0.12)	-0.10, 0.36	-0.20, 0.46
- *Wald test for T2DMxtime interaction term*		0.76		0.67	
Sex x T2			-0.26 (0.09)	-0.44, -0.08	-0.51, -0.0007
Sex x T3			-0.18 (0.09)	-0.36, 0.004	-0.44, 0.08
Sex x T4			-0.27 (0.12)	-0.51, -0.03	-0.61, 0.07
Sex x T5			-0.31 (0.12)	-0.55, -0.07	-0.66, 0.04
- *Wald test for sexxtime interaction term*				**0.03**	
- Sex			-0.01 (0.11)	-0.22, 0.20	-0.31, 0.29
- Age			0.02 (0.003)	0.009, 0.02	0.006, 0.03
- Weight change			-0.02 (0.004)	-0.03, -0.01	-0.03, -0.009
- Parity			-0.03 (0.03)	-0.09, 0.03	-0.11, 0.06
- Breastfeeding			0.004 (0.003)	-0.002, 0.01	-0.005, 0.01
- Total Lipids			0.02 (0.01)	-0.01, 0.04	-0.02, 0.05
- Physical Activity			-0.04 (0.04)	-0.12, 0.04	-0.16, 0.08
- BMI					
Overweight			0.07 (0.05)	-0.03, 0.18	-0.08, 0.22
Obese			0.19 (0.07)	0.05, 0.33	-0.02, 0.40

- constant	3.31 (0.05)	3.21, 3.40	3.44 (0.10)	3.14, 3.55	3.05, 3.63
***Trans*-nonachlor**					
- T2DM	0.13 (0.09)	-0.06, 0.31	0.05 (0.08)	-0.12, 0.22	-0.19, 0.29
- *Sampling year*					
T2	-0.15 (0.04)	-0.22, -0.08	-0.35 (0.06)	-0.48, -0.23	-0.53, -0.17
T3	-0.07 (0.04)	-0.15, -0.0003	-0.53 (0.09)	-0.70, -0.36	-0.77, -0.28
T4	-0.20 (0.05)	-0.30, -0.10	-0.85 (0.12)	-1.08, -0.62	-1.17, -0.52
T5	-0.36 (0.06)	-0.47, -0.25	-1.26 (0.15)	-1.55, -0.98	-1.67, -0.85
- *Interactions*					
T2DMxT2	0.07 (0.05)	-0.03, 0.18	0.11 (0.05)	0.005, 0.21	-0.04, 0.25
T2DMxT3	0.10 (0.06)	-0.01, 0.20	0.17 (0.05)	0.07, 0.27	0.02, 0.32
T2DMxT4	0.17 (0.08)	0.02, 0.32	0.24 (0.07)	0.10, 0.38	0.04, 0.45
T2DMxT5	0.30 (0.09)	0.13, 0.47	0.32 (0.08)	0.17, 0.46	0.11, 0.52
- *Wald test for T2DMxtime interaction term*		**0.01**		**<0.001**	
Sex x T2			-0.06 (0.06)	-0.18, 0.05	-0.23, 0.10
Sex x T3			-0.08 (0.06)	-0.20, 0.04	-0.25, 0.09
Sex x T4			-0.12 (0.08)	-0.27, 0.03	-0.34, 0.10
Sex x T5			-0.15 (0.08)	-0.30, 0.01	-0.37, 0.08
- *Wald test for sexxtime interaction term*				0.40	
- Sex			0.34 (0.12)	0.11, 0.57	0.02, 0.66
- Age			0.03 (0.004)	0.03, 0.04	0.02, 0.05
- Weight change			-0.01 (0.002)	-0.02, -0.009	-0.02, -0.007
- Parity			-0.05 (0.03)	-0.11, 0.01	-0.13, 0.04
- Breastfeeding			0.002 (0.002)	-0.0007, 0.03	-0.005, 0.008
- Total Lipids			0.02 (0.009)	-0.0007, 0.04	-0.008, 0.04
- Physical Activity			-0.04 (0.03)	-0.10, 0.01	-0.12, 0.04
- BMI					
Overweight			-0.05 (0.04)	-0.12, 0.03	-0.16, 0.06
Obese			-0.10 (0.06)	-0.21, 0.02	-0.26, 0.06
- constant	3.43 (0.06)	3.31, 3.55	3.39 (0.10)	3.19, 3.60	3.10, 3.68
***Cis*-nonachlor**					
- T2DM	0.23 (0.12)	0.005, 0.46	0.12 (0.11)	-0.09, 0.32	-0.18, 0.41
- *Sampling year*					
T2	-0.08 (0.05)	-0.17, 0.02	-0.35 (0.09)	-0.52, -0.18	-0.60, -0.10
T3	0.07 (0.05)	-0.03, 0.17	-0.47 (0.11)	-0.70, -0.25	-0.79, -0.16
T4	-0.02 (0.07)	-0.15, 0.12	-0.80 (0.15)	-1.10, -0.50	-1.22, -0.38
T5	-0.13 (0.08)	-0.28, 0.02	-1.20 (0.18)	-1.56, -0.84	-1.71, -0.68
- *Interactions*					
T2DMxT2	0.01 (0.07)	-0.12, 0.15	0.05 (0.07)	-0.09, 0.19	-0.16, 0.26
T2DMxT3	0.16 (0.07)	0.01, 0.30	0.27 (0.08)	0.12, 0.41	0.05, 0.48
T2DMxT4	0.21 (0.10)	0.02, 0.41	0.33 (0.10)	0.13, 0.54	0.04, 0.62
T2DMxT5	0.24 (0.11)	0.02, 0.47	0.33 (0.11)	0.12, 0.54	0.03, 0.63
- *Wald test for T2DMxtime interaction term*		**0.05**		**<0.001**	
Sex x T2			-0.03 (0.08)	-0.19, 0.14	-0.26, 0.21
Sex x T3			-0.08 (0.09)	-0.25, 0.09	-0.33, 0.16
Sex x T4			-0.04 (0.11)	-0.26, 0.18	-0.36, 0.27

Sex x T5			-0.09 (0.11)	-0.31, 0.13	-0.41, 0.23
- *Wald test for sexxtime interaction term*				0.88	
- Sex			0.30 (0.15)	0.01, 0.58	-0.11, 0.70
- Age			0.04 (0.005)	0.03, 0.05	0.02, 0.05
- Weight change			-0.01 (0.003)	-0.02, -0.005	-0.02, -0.003
- Parity			-0.06 (0.04)	-0.14, 0.01	-0.17, 0.05
- Breastfeeding			0.002 (0.003)	-0.004, 0.008	-0.007, 0.01
- Total Lipids			0.02 (0.01)	-0.002, 0.05	-0.01, 0.06
- Physical Activity			-0.02 (0.04)	-0.10, 0.06	-0.13,0.09
- BMI					
Overweight			0.02 (0.05)	-0.09, 0.13	-0.13, 0.17
Obese			-0.03 (0.08)	-0.19, 0.12	-0.26, 0.19
- constant	1.73 (0.08)	1.58, 1.88	1.69 (0.13)	1.43, 1.95	1.32, 2.06
Oxychlordane					
- T2DM	0.08 (0.11)	-0.14, 0.29	-0.07 (0.10)	-0.25, 0.12	-0.34, 0.20
- *Sampling year*					
T2	-0.20 (0.06)	-0.31, -0.08	-0.42 (0.10)	-0.62, -0.23	-0.70, -0.14
T3	-0.19 (0.06)	-0.30, -0.07	-0.68 (0.12)	-0.91, -0.46	-1.01, -0.36
T4	-0.33 (0.08)	-0.48, -0.17	-1.00 (0.15)	-1.30, -0.70	-1.43, -0.57
T5	-0.52 (0.08)	-0.68, -0.36	-1.31 (0.18)	-1.65, -0.97	-1.80, -0.82
- *Interactions*					
T2DMxT2	0.14 (0.09)	-0.04, 0.31	0.19 (0.09)	0.01, 0.37	-0.06, 0.45
T2DMxT3	0.15 (0.09)	-0.03, 0.32	0.25 (0.09)	0.07, 0.43	-0.01, 0.51
T2DMxT4	0.18 (0.12)	-0.05, 0.41	0.31 (0.13)	0.07, 0.56	-0.04, 0.67
T2DMxT5	0.33 (0.12)	0.09, 0.56	0.35 (0.13)	0.10, 0.61	-0.01, 0.72
- *Wald test for T2DMxtime interaction term*		0.09		**<0.05**	
Sex x T2			-0.08 (0.10)	-0.28, 0.12	-0.37, 0.20
Sex x T3			-0.07 (0.11)	-0.28, 0.13	-0.37, 0.22
Sex x T4			-0.12 (0.14)	-0.39, 0.14	-0.51, 0.26
Sex x T5			-0.32 (0.14)	-0.59, -0.05	-0.71, 0.07
- *Wald test for sexxtime interaction term*				0.23	
- Sex			0.41 (0.13)	0.15, 0.67	0.03, 0.78
- Age			0.03 (0.004)	0.03, 0.04	0.02, 0.05
- Weight change			-0.02 (0.004)	-0.03, -0.008	-0.03, -0.004
- Parity			-0.02 (0.04)	-0.09, 0.05	-0.12, 0.09
- Breastfeeding			0.003 (0.004)	-0.004, 0.01	-0.007, 0.01
- Total Lipids			0.05 (0.01)	0.01, 0.07	-0.0002, 0.09
- Physical Activity			-0.04 (0.05)	-0.14, 0.05	-0.18, 0.09
- BMI					
Overweight			-0.01 (0.06)	-0.13, 0.11	-0.18, 0.16
Obese			-0.06 (0.09)	-0.23, 0.11	-0.31, 0.18
- constant	2.79 (0.08)	2.64, 2.93	2.67 (0.13)	2.43, 2.92	2.32, 3.02
***Cis*-heptachlor epoxide**					
- T2DM	0.51 (0.12)	0.27, 0.75	0.20 (0.11)	-0.02, 0.42	-0.12, 0.51
- *Sampling year*					
T2	-0.76 (0.08)	-0.92, -0.60	-1.08 (0.13)	-1.34, -0.83	-1.45, -0.72
T3	-0.70 (0.08)	-0.86, -0.54	-1.15 (0.15)	-1.44, -0.86	-1.56, -0.74

T4	-0.89 (0.11)	-1.10, -0.68	-1.24 (0.19)	-1.61, -0.86	-1.77, -0.70
T5	-1.08 (0.11)	-1.30, -0.86	-1.72 (0.21)	-2.13, -1.30	-2.31, -1.12
- *Interactions*					
T2DMxT2	0.20 (0.12)	-0.04, 0.44	0.35 (0.13)	0.11, 0.60	0.002, 0.71
T2DMxT3	0.10 (0.12)	-0.14, 0.34	0.30 (0.13)	0.05, 0.55	-0.06, 0.66
T2DMxT4	0.24 (0.16)	-0.07, 0.55	0.36 (0.17)	0.02, 0.70	-0.12, 0.84
T2DMxT5	0.17 (0.16)	-0.15, 0.49	0.29 (0.18)	-0.06, 0.64	-0.21, 0.79
- *Wald test for T2DMxtime interaction term*		0.45		**<0.05**	
Sex x T2			0.03 (0.14)	-0.24, 0.30	-0.36, 0.42
Sex x T3			-0.08 (0.14)	-0.35, 0.20	-0.48, 0.33
Sex x T4			-0.50 (0.18)	-0.86, -0.14	-1.02, 0.02
Sex x T5			-0.24 (0.19)	-0.61, 0.12	-0.77, 0.28
- *Wald test for sexxtime interaction term*				**<0.05**	
- Sex			0.73 (0.15)	0.42, 1.03	0.29, 1.16
- Age			0.02 (0.005)	0.01, 0.03	0.01, 0.04
- Weight change			-0.01 (0.005)	-0.03, -0.004	-0.03, 0.0005
- Parity			0.05 (0.04)	-0.04, 0.13	-0.07, 0.16
- Breastfeeding			0.0005 (0.005)	-0.009, 0.01	-0.01, 0.01
- Total Lipids			0.05 (0.02)	0.01, 0.09	-0.0009, 0.10
- Physical Activity			-0.07 (0.06)	-0.19, 0.06	-0.25, 0.11
- BMI					
Overweight			0.27 (0.08)	0.12, 0.42	0.05, 0.49
Obese			0.46 (0.11)	0.26, 0.67	0.17, 0.76
- constant	1.24 (0.08)	1.07, 1.40	0.80 (0.15)	0.52, 1.09	0.39, 1.21
HCB					
- T2DM	-0.002 (0.04)	-0.07, 0.07	0.005 (0.04)	-0.06, 0.07	-0.09, 0.10
- *Sampling year*					
T2	-0.48 (0.02)	-0.53, -0.44	-0.49 (0.04)	-0.56, -0.42	-0.59, -0.39
T3	-0.47 (0.02)	-0.52, -0.43	-0.59 (0.04)	-0.67, -0.51	-0.71, -0.47
T4	-0.56 (0.03)	-0.62, -0.49	-0.67 (0.05)	-0.78, -0.57	-0.83, -0.52
T5	-0.60 (0.04)	-0.67, -0.53	-0.84 (0.06)	-0.96, -0.72	-1.01, -0.67
- *Interactions*					
T2DMxT2	0.04 (0.03)	0.02, 0.11	0.05 (0.03)	-0.02, 0.11	-0.05, 0.14
T2DMxT3	0.06 (0.04)	-0.006, 0.13	0.06 (0.03)	-0.008, 0.12	-0.04, 0.15
T2DMxT4	0.10 (0.05)	0.006, 0.19	0.07 (0.05)	-0.02, 0.16	-0.06, 0.20
T2DMxT5	0.19 (0.05)	0.08, 0.29	0.11 (0.05)	0.02, 0.21	-0.02, 0.25
- *Wald test for T2DMxtime interaction term*		**0.01**		0.14	
Sex x T2			-0.09 (0.04)	-0.17, -0.02	-0.20, 0.01
Sex x T3			-0.06 (0.04)	-0.13, 0.02	-0.17, 0.05
Sex x T4			-0.16 (0.05)	-0.25, -0.06	-0.30, -0.02
Sex x T5			-0.06 (0.05)	-0.16, 0.03	-0.21, 0.08
- *Wald test for sexxtime interaction term*				**<0.05**	
- Sex			0.04 (0.05)	-0.05, 0.13	-0.09, 0.17
- Age			0.008 (0.001)	0.005, 0.01	0.004, 0.01
- Weight change			-0.006 (0.001)	-0.009, -0.003	-0.01, -0.001

- Parity			-0.02 (0.01)	-0.04, 0.005	-0.06, 0.02
- Breastfeeding			0.003 (0.001)	0.00003, 0.005	-0.001, 0.006
- Total Lipids			-0.05 (0.005)	-0.06, -0.04	-0.06, -0.04
- Physical Activity			-0.02 (0.02)	-0.05, 0.01	-0.07, 0.03
- BMI					
Overweight			0.008 (0.02)	-0.03, 0.05	-0.05, 0.07
Obese			0.06 (0.03)	-0.005, 0.12	-0.03, 0.14
- constant	4.46 (0.02)	4.41, 4.51	4.48 (0.04)	4.39, 4.56	4.36, 4.60
***p,p'*-DDE**					
- T2DM	0.12 (0.09)	-0.07, 0.30	0.05 (0.10)	-0.15, 0.24	-0.23, 0.32
- *Sampling year*					
T2	-0.55 (0.04)	-0.63, -0.48	-0.68 (0.07)	-0.83, -0.54	-0.89, -0.47
T3	-0.84 (0.04)	-0.93, -0.76	-1.17 (0.10)	-1.36, -0.97	-1.45, -0.88
T4	-1.21 (0.06)	-1.33, -1.08	-1.64 (0.13)	-1.91, -1.38	-2.02, -1.27
T5	-1.59 (0.08)	-1.74, -1.44	-2.24 (0.17)	-2.57, -1.91	-2.71, -1.76
- *Interactions*					
T2DMxT2	0.11 (0.05)	-0.0007, 0.21	0.15 (0.06)	0.03, 0.27	-0.02, 0.32
T2DMxT3	0.16 (0.06)	0.04, 0.29	0.22 (0.06)	0.11, 0.34	0.05, 0.39
T2DMxT4	0.22 (0.09)	0.05, 0.40	0.25 (0.08)	0.09, 0.41	0.02, 0.48
T2DMxT5	0.37 (0.11)	0.15, 0.59	0.30 (0.09)	0.13, 0.47	0.06, 0.54
- *Wald test for T2DMxtime interaction term*		**0.02**		**<0.001**	
Sex x T2			-0.08 (0.07)	-0.22, 0.05	-0.27, 0.11
Sex x T3			-0.11 (0.07)	-0.25, 0.03	-0.31, 0.09
Sex x T4			-0.25 (0.09)	-0.42, -0.07	-0.50, 0.008
Sex x T5			-0.27 (0.09)	-0.46, -0.09	-0.53, -0.01
- *Wald test for sexxtime interaction term*				**<0.05**	
- Sex			-0.24 (0.13)	-0.50, 0.02	-0.61, 0.13
- Age			0.02 (0.005)	0.01, 0.03	0.01, 0.04
- Weight change			-0.02 (0.003)	-0.02, -0.01	-0.02, -0.008
- Parity			-0.13 (0.03)	-0.20, -0.06	-0.23, -0.04
- Breastfeeding			0.0006 (0.003)	-0.004, 0.006	-0.007, 0.008
- Total Lipids			-0.02 (0.01)	-0.04, 0.004	-0.04, 0.01
- Physical Activity			-0.07 (0.03)	-0.14, -0.01	-0.17, 0.02
- BMI					
Overweight			0.07 (0.04)	-0.01, 0.16	-0.05, 0.20
Obese			0.16 (0.07)	0.03, 0.30	-0.03, 0.35
- constant	5.94 (0.06)	5.82, 6.07	6.25 (0.12)	6.02, 6.49	5.91, 6.59
***p,p'*-DDT**					
- T2DM	0.26 (0.10)	0.06, 0.46	0.06 (0.10)	-0.14, 0.25	-0.23, 0.35
- *Sampling year*					
T2	-1.43 (0.07)	-1.57, -1.29	-1.72 (0.12)	-1.95, -1.49	-2.05, -1.39
T3	-1.92 (0.07)	-2.06, -1.78	-2.42 (0.13)	-2.68, -2.16	-2.81, -2.06
T4	-2.34 (0.09)	-2.53, -2.15	-2.90 (0.18)	-3.24, -2.55	-3.39, -2.42
T5	-2.89 (0.10)	-3.08, -2.69	-3.46 (0.20)	-3.85, -3.07	-4.03, -2.95
- *Interactions*					
T2DMxT2	0.33 (0.11)	0.12, 0.54	0.43 (0.11)	0.22, 0.65	0.12, 0.75
T2DMxT3	0.12 (0.11)	-0.09, 0.33	0.23 (0.11)	0.01, 0.46	-0.09, 0.55
T2DMxT4	0.09 (0.14)	-0.18, 0.36	0.18 (0.16)	-0.12, 0.49	-0.26, 0.60
T2DMxT5	-0.02 (0.15)	-0.31, 0.26	-0.01 (0.17)	-0.34, 0.32	-0.47, 0.42

- *Wald test for T2DMxtime interaction term*		**0.02**		**<0.01**	
Sex x T2			0.11 (0.12)	-0.13, 0.35	-0.24, 0.45
Sex x T3			0.11 (0.13)	-0.14, 0.36	-0.25, 0.46
Sex x T4			0.04 (0.17)	-0.29, 0.37	-0.43, 0.50
Sex x T5			-0.10 (0.18)	-0.45, 0.24	-0.58, 0.36
- *Wald test for sexxtime interaction term*				0.64	
- Sex			0.04 (0.14)	-0.23, 0.31	-0.36, 0.43
- Age			0.02 (0.004)	0.01, 0.03	0.009, 0.03
- Weight change			-0.01 (0.005)	-0.02, -0.005	-0.03, -0.0004
- Parity			-0.05 (0.04)	-0.13, 0.02	-0.16, 0.05
- Breastfeeding			0.007 (0.004)	-0.001, 0.02	-0.005, 0.02
- Total Lipids			0.04 (0.02)	0.01, 0.08	-0.005, 0.09
- Physical Activity			-0.03 (0.06)	-0.14, 0.08	-0.19, 0.13
- BMI					
Overweight			0.20 (0.07)	0.06, 0.33	-0.01, 0.38
Obese			0.32 (0.10)	0.13, 0.51	0.03, 0.57
- constant	3.36 (0.07)	3.23, 3.50	3.36 (0.13)	3.10, 3.62	3.00, 3.75

Model 1: Adjusted for time (survey) and interaction between T2DM status and time (survey)
Model 2: Adjusted for time (survey), sex, age, weight change, parity, breastfeeding, total lipids, physical activity, BMI categories, interaction between T2DM status and time (survey) and interaction between sex and time (survey).

Abbreviations: T2: Time-point 2 (1994/95); T3: Time-point 3 (2001); T4: Time-point 4 (2007/08); T5: Time-point 5 (2015/16); T2DM: Type 2 Diabetes Mellitus ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); β-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane; BMI: body mass index.

Table S12. Multivariable adjusted regression coefficients, standard errors (SE), 95% and 99.5% confidence intervals (CIs) from linear mixed effect models to assess the longitudinal changes in wet-weight concentrations (pg/mL) of persistent organic pollutants from 1986/87-2015/16 according to type 2 diabetes mellitus status (T2DM) (n=990) in the Tromsø Study (1986-2016).

	Model 1		Model 2		
	β – coefficient (SE)	95% CI / p-value for Walds test	β – coefficient (SE)	95% CI / p-value for Walds test	Adjusted CIs at 99.5% for multiple comparisons
∑DL-PCBs					
- T2DM	0.27 (0.08)	0.11, 0.43	0.11 (0.06)	-0.02, 0.23	-0.07, 0.29
- *Sampling year*					
T2	-0.17 (0.03)	-0.22, -0.12	-0.43 (0.05)	-0.52, -0.34	-0.56, -0.30
T3	-0.45 (0.03)	-0.45, 0.03	-0.89 (0.06)	-1.01, -0.76	-1.07, -0.71
T4	-0.56 (0.04)	-0.56, 0.04	-1.20 (0.09)	-1.37, -1.03	-1.44, -0.96
T5	-0.96 (0.05)	-0.96, 0.05	-1.75 (0.11)	-1.97, -1.53	-2.06, -1.44
- *Interactions*					
T2DMxT2	0.03 (0.04)	-0.05, 0.11	0.09 (0.03)	0.02, 0.15	-0.01, 0.18
T2DMxT3	0.02 (0.04)	-0.06, 0.10	0.10 (0.04)	0.03, 0.17	0.0008, 0.20
T2DMxT4	0.05 (0.06)	-0.06, 0.17	0.18 (0.05)	0.09, 0.28	0.05, 0.32
T2DMxT5	0.11 (0.07)	-0.03, 0.24	0.18 (0.05)	0.08, 0.28	0.04, 0.32
- *Wald test for T2DMxtime interaction term*		0.63		**<0.001**	
Sex x T2			-0.07 (0.04)	-0.15, 0.01	-0.18, 0.04
Sex x T3			-0.06 (0.04)	-0.14, 0.04	-0.18, 0.05
Sex x T4			-0.20 (0.05)	-0.30, -0.09	-0.35, -0.05
Sex x T5			-0.26 (0.05)	-0.37, -0.15	-0.41, -0.11
- *Wald test for sexxtime interaction term*				**<0.001**	
- Sex			0.03 (0.09)	-0.14, 0.20	-0.21, 0.27
- Age			0.03 (0.003)	0.03, 0.04	0.02, 0.04
- Weight change			-0.01 (0.02)	-0.02, -0.009	-0.02, -0.007
- Parity			-0.09 (0.02)	-0.14, -0.05	-0.15, -0.03
- Breastfeeding			0.0007 (0.002)	-0.002, 0.004	-0.004, 0.005
- Total Lipids			0.11 (0.006)	0.10, 0.12	0.09, 0.13
- Physical Activity			-0.02 (0.02)	-0.06, 0.02	-0.07, 0.04
- BMI					
Overweight			-0.02 (0.03)	-0.07, 0.03	-0.09, 0.06
Obesity			-0.02 (0.04)	-0.09, 0.06	-0.13, 0.10
- constant	6.09 (0.06)	5.98, 6.20	6.22 (0.08)	6.07, 6.37	6.00, 6.44
∑PCBs					
- T2DM	0.17 (0.08)	0.02, 0.33	0.03 (0.06)	-0.09, 0.15	-0.14, 0.21
- *Sampling year*					
T2	-0.21 (0.02)	-0.26, -0.17	-0.40 (0.04)	-0.48, -0.33	-0.52, -0.29
T3	-0.40 (0.03)	-0.45, -0.35	-0.72 (0.06)	-0.84, -0.61	-0.89, -0.56
T4	-0.55 (0.04)	-0.62, -0.47	-1.07 (0.08)	-1.23, -0.91	-1.29, -0.85
T5	-0.83 (0.04)	-0.91, -0.74	-1.46 (0.10)	-1.66, -1.26	-1.75, -1.17
- *Interactions*					
T2DMxT2	0.04 (0.04)	-0.03, 0.11	0.10 (0.03)	0.04, 0.15	0.02, 0.18
T2DMxT3	0.06 (0.04)	-0.02, 0.13	0.13 (0.03)	0.07, 0.19	0.05, 0.21

T2DMxT4	0.03 (0.05)	-0.07, 0.14	0.16 (0.04)	0.08, 0.24	0.05, 0.28
T2DMxT5	0.10 (0.06)	-0.02, 0.23	0.17 (0.04)	0.09, 0.26	0.06, 0.29
- *Wald test for T2DMxtime interaction term*		0.49		**<0.001**	
Sex x T2			-0.10 (0.03)	-0.16, -0.03	-0.19, -0.002
Sex x T3			-0.10 (0.03)	-0.17, -0.03	-0.20, -0.005
Sex x T4			-0.20 (0.04)	-0.29, -0.12	-0.33, -0.08
Sex x T5			-0.29 (0.05)	-0.38, 0.20	-0.42, -0.17
- *Wald test for sexxtime interaction term*				**<0.001**	
- Sex			0.21 (0.08)	0.05, 0.36	-0.02, 0.43
- Age			0.03 (0.003)	0.02, 0.04	0.02, 0.04
- Weight change			-0.01 (0.001)	-0.02, -0.01	-0.02, -0.01
- Parity			-0.09 (0.02)	-0.12, -0.05	-0.14, -0.03
- Breastfeeding			-0.00001 (0.001)	-0.003, 0.002	-0.004, 0.004
- Total Lipids			0.11 (0.005)	0.10, 0.12	0.10, 0.13
- Physical Activity			-0.03 (0.02)	-0.06, 0.006	-0.07, 0.02
- BMI					
Overweight			-0.06 (0.02)	-0.10, -0.01	-0.12, 0.007
Obesity			-0.07 (0.03)	-0.14, -0.006	-0.17, 0.02
- constant	8.46 (0.05)	8.35, 8.56	8.52 (0.07)	8.38, 8.65	8.32, 8.71
***β*-HCH**					
- T2DM	0.27 (0.08)	0.10, 0.43	0.07 (0.08)	-0.09, 0.22	-0.15, 0.29
- *Sampling year*					
T2	-0.80 (0.05)	-0.91, -0.69	-0.86 (0.09)	-1.03, -0.68	-1.10, -0.61
T3	-0.96 (0.06)	-1.07, -0.86	-1.16 (0.10)	-1.35, -0.96	-1.44, -0.88
T4	-1.26 (0.07)	-1.40, -1.11	-1.58 (0.13)	-1.83, -1.32	-1.95, -1.21
T5	-1.78 (0.08)	-1.93, -1.63	-2.15 (0.15)	-2.44, -1.87	-2.56, -1.74
- *Interactions*					
T2DMxT2	-0.002 (0.08)	-0.16, 0.16	0.05 (0.08)	-0.11, 0.21	-0.18, 0.28
T2DMxT3	0.02 (0.08)	-0.14, 0.18	0.10 (0.08)	-0.06, 0.26	-0.13, 0.33
T2DMxT4	-0.01 (0.11)	-0.22, 0.20	0.11 (0.11)	-0.11, 0.33	-0.20, 0.43
T2DMxT5	-0.11 (0.11)	-0.33, 0.11	0.09 (0.12)	-0.14, 0.32	-0.23, 0.42
- *Wald test for T2DMxtime interaction term*		0.86		0.76	
Sex x T2			-0.27 (0.09)	-0.46, -0.09	-0.52, -0.01
Sex x T3			-0.19 (0.09)	-0.37, -0.006	-0.45, 0.07
Sex x T4			-0.28 (0.12)	-0.52, -0.04	-0.62, 0.06
Sex x T5			-0.35 (0.12)	-0.59, -0.11	-0.69, -0.001
- *Wald test for sexxtime interaction term*				**<0.05**	
- Sex			0.01 (0.11)	-0.20, 0.22	-0.29, 0.32
- Age			0.02 (0.003)	0.01, 0.02	0.007, 0.03
- Weight change			-0.02 (0.004)	-0.03, -0.01	-0.03, -0.009
- Parity			-0.02 (0.03)	-0.08, 0.04	-0.11, 0.06
- Breastfeeding			0.004 (0.003)	-0.002, 0.01	-0.005, 0.01
- Total Lipids			0.13 (0.01)	0.10, 0.15	0.09, 0.17
- Physical Activity			-0.04 (0.04)	-0.12, 0.05	-0.15, 0.08
- BMI					
Overweight			0.07 (0.05)	-0.03, 0.18	-0.08, 0.22

Obesity			0.20 (0.07)	0.05, 0.34	-0.01, 0.40
- constant	5.25 (0.06)	5.14, 5.36	5.27 (0.10)	5.07, 5.47	4.98, 5.56
***Trans*-nonachlor**					
- T2DM	0.24 (0.10)	0.04, 0.45	0.06 (0.08)	-0.11, 0.22	-0.18, 0.30
- *Sampling year*					
T2	-0.09 (0.04)	-0.17, -0.02	-0.36 (0.06)	-0.48, -0.23	-0.54, -0.18
T3	-0.08 (0.04)	-0.16, 0.002	-0.54 (0.09)	-0.71, -0.37	-0.78, -0.29
T4	-0.17 (0.06)	-0.28, -0.06	-0.85 (0.12)	-1.08, -0.63	-1.18, -0.53
T5	-0.41 (0.06)	-0.53, -0.29	-1.28 (0.15)	-1.56, -0.99	-1.68, -0.87
- *Interactions*					
T2DMxT2	0.06 (0.06)	-0.06, 0.17	0.11 (0.05)	0.007, 0.21	-0.04, 0.25
T2DMxT3	0.04 (0.06)	-0.08, 0.16	0.16 (0.05)	0.06, 0.27	0.02, 0.31
T2DMxT4	0.04 (0.08)	-0.12, 0.19	0.23 (0.07)	0.09, 0.37	0.03, 0.43
T2DMxT5	0.11 (0.09)	-0.07, 0.28	0.28 (0.07)	0.13, 0.42	0.07, 0.48
- *Wald test for T2DMxtime interaction term*		0.77		**<0.001**	
Sex x T2			-0.07 (0.06)	-0.19, 0.04	-0.24, 0.09
Sex x T3			-0.09 (0.06)	-0.20, 0.03	-0.26, 0.08
Sex x T4			-0.13 (0.08)	-0.28, 0.03	-0.34, 0.09
Sex x T5			-0.18 (0.08)	-0.34, -0.03	-0.41, 0.04
- *Wald test for sexxtime interaction term*				0.21	
- Sex			0.37 (0.11)	0.14, 0.60	0.05, 0.69
- Age			0.03 (0.004)	0.03, 0.04	0.02, 0.05
- Weight change			-0.01 (0.002)	-0.02, -0.008	-0.02, -0.006
- Parity			-0.04 (0.03)	-0.10, 0.02	-0.12, 0.04
- Breastfeeding			0.002 (0.002)	-0.003, 0.006	-0.005, 0.008
- Total Lipids			0.13 (0.009)	0.11, 0.15	0.11, 0.15
- Physical Activity			-0.04 (0.03)	-0.09, 0.02	-0.11, 0.04
- BMI					
Overweight			-0.05 (0.04)	-0.13, 0.02	-0.16, 0.06
Obesity			-0.10 (0.06)	-0.21, 0.01	-0.26, 0.06
- constant	5.38 (0.07)	5.24, 5.52	5.31 (0.10)	5.11, 5.52	5.02, 5.60
***Cis*-nonachlor**					
- T2DM	0.35 (0.13)	0.10, 0.60	0.13 (0.11)	-0.08, 0.33	-0.17, 0.42
- *Sampling year*					
T2	-0.02 (0.05)	-0.12, 0.07	-0.35 (0.09)	-0.52, -0.18	-0.60, -0.10
T3	0.07 (0.05)	-0.04, 0.17	-0.48 (0.11)	-0.71, -0.26	-0.80, -0.16
T4	0.02 (0.07)	-0.12, 0.16	-0.81 (0.15)	-1.10, -0.51	-1.23, -0.38
T5	-0.18 (0.08)	-0.34, -0.03	-1.21 (0.18)	-1.57, -0.85	-1.73, -0.69
- *Interactions*					
T2DMxT2	-0.005 (0.07)	-0.15, 0.14	0.05 (0.07)	-0.09, 0.20	-0.16, 0.26
T2DMxT3	0.10 (0.08)	-0.05, 0.25	0.26 (0.08)	0.11, 0.41	0.05, 0.47
T2DMxT4	0.08 (0.10)	-0.12, 0.29	0.32 (0.10)	0.12, 0.52	-0.03, 0.61
T2DMxT5	0.006 (0.12)	-0.17, 0.29	0.29 (0.11)	0.09, 0.50	-0.003, 0.59
- *Wald test for T2DMxtime interaction term*		0.62		**<0.001**	
Sex x T2			-0.04 (0.08)	-0.20, 0.13	-0.27, 0.20
Sex x T3			-0.09 (0.09)	-0.26, 0.08	-0.33, 0.16

Sex x T4			-0.05 (0.11)	-0.27, 0.17	-0.36, 0.27
Sex x T5			-0.13 (0.11)	-0.35, 0.10	-0.45, 0.19
- *Wald test for sexxtime interaction term*				0.78	
- Sex			0.32 (0.15)	0.04, 0.61	-0.08, 0.73
- Age			0.04 (0.005)	0.03, 0.05	0.02, 0.05
- Weight change			-0.01 (0.003)	-0.02, -0.005	-0.02, -0.002
- Parity			-0.05 (0.04)	-0.13, 0.02	-0.16, 0.06
- Breastfeeding			0.002 (0.003)	-0.004, 0.008	-0.007, 0.01
- Total Lipids			0.14 (0.01)	0.11, 0.16	-0.10, 0.17
- Physical Activity			-0.01 (0.04)	-0.09, 0.06	-0.13, 0.10
- BMI					
Overweight			0.02 (0.05)	-0.09, 0.12	-0.08, 0.17
Obesity			-0.03 (0.08)	-0.19, 0.13	-0.26, 0.19
- constant	3.68 (0.09)	3.51, 3.85	3.61 (0.13)	3.35, 3.87	3.24, 3.98
Oxychlordane					
- T2DM	0.19 (0.12)	-0.05, 0.43	-0.06 (0.10)	-0.25, 0.13	-0.38, 0.25
- *Sampling year*					
T2	-0.15 (0.06)	-0.27, -0.02	-0.43 (0.10)	-0.62, -0.23	-0.75, -0.10
T3	-0.19 (0.06)	-0.31, -0.07	-0.69 (0.12)	-0.92, -0.46	-1.08, -0.31
T4	-0.29 (0.08)	-0.46, -0.13	-1.01 (0.15)	-1.31, -0.71	-1.51, -0.51
T5	-0.57 (0.09)	-0.74, -0.40	-1.32 (0.18)	-1.67, -0.98	-1.90, -0.75
- *Interactions*					
T2DMxT2	0.12 (0.09)	-0.06, 0.30	0.20 (0.09)	0.02, 0.37	-0.10, 0.49
T2DMxT3	0.09 (0.09)	-0.09, 0.28	0.25 (0.09)	0.07, 0.43	-0.06, 0.55
T2DMxT4	0.05 (0.12)	-0.18, 0.29	0.30 (0.13)	0.05, 0.55	-0.11, 0.71
T2DMxT5	0.14 (0.13)	-0.11, 0.38	0.32 (0.13)	0.06, 0.57	-0.11, 0.75
- *Wald test for T2DMxtime interaction term*		0.70		**<0.05**	
Sex x T2			-0.09 (0.10)	-0.29, 0.11	-0.43, 0.24
Sex x T3			-0.08 (0.11)	-0.29, 0.13	-0.43, 0.27
Sex x T4			-0.13 (0.14)	-0.40, 0.13	-0.58, 0.31
Sex x T5			-0.36 (0.14)	-0.63, -0.09	-0.81, 0.10
- *Wald test for sexxtime interaction term*				0.14	
- Sex			0.43 (0.13)	0.17, 0.69	-0.008, 0.87
- Age			0.04 (0.004)	0.03, 0.04	0.02, 0.05
- Weight change			-0.02 (0.004)	-0.02, -0.007	-0.03, -0.002
- Parity			-0.01 (0.04)	-0.08, 0.06	-0.13, 0.11
- Breastfeeding			0.003 (0.004)	-0.004, 0.01	-0.009, 0.01
- Total Lipids			0.16 (0.01)	0.13, 0.18	0.11, 0.20
- Physical Activity			-0.04 (0.05)	-0.13, 0.06	-0.20, 0.12
- BMI					
Overweight			-0.009 (0.06)	-0.13, 0.11	-0.21, 0.19
Obesity			-0.06 (0.09)	-0.23, 0.11	-0.35, 0.23
- constant	4.73 (0.08)	4.57, 4.90	4.60 (0.13)	4.45, 4.84	4.19, 5.01
***Cis*-heptachlor epoxide**					
- T2DM	0.62 (0.13)	0.36, 0.89	0.20 (0.11)	-0.02, 0.42	-0.11, 0.52
- *Sampling year*					
T2	-0.71 (0.08)	-0.88, -0.54	-1.08 (0.13)	-1.34, -0.83	-1.45, -0.72

T3	-0.70 (0.08)	-0.87, -0.53	-1.16 (0.15)	-1.44, -0.87	-1.57, -0.74
T4	-0.85 (0.11)	-1.07, -0.63	-1.24 (0.19)	-1.62, -0.87	-1.78, -0.70
T5	-1.13 (0.11)	-1.35, -0.90	-1.73 (0.21)	-2.14, -1.32	-2.32, -1.14
- *Interactions*					
T2DMxT2	0.17 (0.13)	-0.07, 0.43	0.36 (0.13)	0.11, 0.60	0.004, 0.71
T2DMxT3	0.05 (0.13)	-0.20, 0.29	0.30 (0.13)	0.04, 0.55	-0.06, 0.65
T2DMxT4	0.11 (0.16)	-0.21, 0.43	0.35 (0.17)	0.01, 0.69	-0.13, 0.83
T2DMxT5	-0.02 (0.17)	-0.35, 0.31	0.26 (0.18)	-0.09, 0.60	-0.24, 0.75
- *Wald test for T2DMxtime interaction term*		0.61		**<0.05**	
Sex x T2			0.02 (0.14)	-0.25, 0.29	-0.37, 0.41
Sex x T3			-0.09 (0.14)	-0.37, 0.19	-0.49, 0.31
Sex x T4			-0.51 (0.18)	-0.87, -0.15	-1.03, 0.01
Sex x T5			-0.28 (0.19)	-0.65, 0.09	-0.81, 0.25
- *Wald test for sexxtime interaction term*				**<0.05**	
- Sex			0.75 (0.15)	0.44, 1.05	0.31, 1.18
- Age			0.02 (0.005)	0.01, 0.03	0.01,0.04
- Weight change			-0.01 (0.005)	-0.02, -0.004	-0.03, 0.001
- Parity			0.05 (0.04)	-0.03, 0.13	-0.07, 0.17
- Breastfeeding			0.0003 (0.005)	-0.009, 0.009	-0.01, 0.01
- Total Lipids			0.17 (0.02)	0.13, 0.20	0.11, 0.22
- Physical Activity			-0.06 (0.06)	-0.19, 0.06	-0.24, 0.11
- BMI					
Overweight			0.28 (0.08)	0.12, 0.43	0.06, 0.49
Obesity			0.47 (0.11)	0.27, 0.68	0.18, 0.77
- constant	3.18 (0.09)	3.01, 3.36	2.73 (0.15)	2.44, 3.02	2.32, 3.14
HCB					
- T2DM	0.11 (0.04)	0.03, 0.20	0.01 (0.03)	-0.05, 0.07	-0.08, 0.10
- *Sampling year*					
T2	-0.43 (0.02)	-0.47, -0.39	-0.49 (0.03)	-0.56, -0.43	-0.59, -0.40
T3	-0.48 (0.02)	-0.52, -0.43	-0.60 (0.04)	-0.68, -0.52	-0.71, -0.48
T4	-0.53 (0.03)	-0.59, -0.47	-0.68 (0.05)	-0.78, -0.58	-0.853 -0.53
T5	-0.66 (0.03)	-0.72, -0.59	-0.85 (0.06)	-0.97, -0.73	-1.02, -0.68
- *Interactions*					
T2DMxT2	0.03 (0.03)	-0.04, 0.09	0.05 (0.03)	-0.01, 0.11	-0.04, 0.14
T2DMxT3	0.007 (0.03)	-0.06, 0.07	0.06 (0.03)	-0.009, 0.12	-0.05, 0.15
T2DMxT4	-0.03 (0.04)	-0.11, 0.06	0.06 (0.04)	-0.03, 0.15	-0.06, 0.18
T2DMxT5	0.007 (0.05)	-0.09, 0.10	0.08 (0.05)	-0.01, 0.17	-0.05, 0.21
- *Wald test for T2DMxtime interaction term*		0.79		0.31	
Sex x T2			-0.10 (0.04)	-0.17, 0.03	-0.20, -0.002
Sex x T3			-0.07 (0.04)	-0.14, 0.003	-0.17, 0.03
Sex x T4			-0.17 (0.05)	-0.26, -0.07	-0.30, -0.03
Sex x T5			-0.10 (0.05)	-0.20, -0.005	-0.24, 0.04
- *Wald test for sexxtime interaction term*				**<0.01**	
- Sex			0.06 (0.05)	-0.03, 0.15	-0.07, 0.19
- Age			0.009 (0.001)	0.006, 0.01	0.005, 0.01

- Weight change			-0.005 (0.001)	-0.008, -0.002	-0.009, -0.0009
- Parity			-0.01 (0.01)	-0.04, 0.01	-0.05, 0.02
- Breastfeeding			0.002 (0.001)	-0.00006, 0.005	-0.001, 0.006
- Total Lipids			0.06 (0.005)	0.05, 0.07	0.05, 0.08
- Physical Activity			-0.02 (0.02)	-0.05, 0.02	-0.06, 0.03
- BMI					
Overweight			0.01 (0.02)	-0.03, 0.05	-0.05, 0.07
Obesity			0.06 (0.03)	0.002, 0.12	-0.02, 0.15
- constant	6.41 (0.03)	6.35, 6.46	6.41 (0.04)	6.32, 6.50	6.29, 6.53
***p,p'*-DDE**					
- T2DM	0.23 (0.10)	0.04, 0.43	0.06 (0.10)	-0.14, 0.25	-0.22, 0.33
- *Sampling year*					
T2	-0.50 (0.04)	-0.58, -0.43	-0.69 (0.07)	-0.83, -0.54	-0.89, -0.48
T3	-0.85 (0.04)	-0.93, -0.76	-1.18 (0.10)	-1.37, -0.98	-1.46, -0.89
T4	-1.17 (0.06)	-1.29, -1.05	-1.65 (0.13)	-1.92, -1.39	-2.03, -1.27
T5	-1.65 (0.07)	-1.79, -1.50	-2.25 (0.17)	-2.58, -1.92	-2.72, -1.78
- *Interactions*					
T2DMxT2	0.09 (0.06)	-0.03, 0.20	0.15 (0.06)	0.03, 0.26	-0.01, 0.31
T2DMxT3	0.11 (0.06)	-0.02, 0.23	0.22 (0.06)	0.10, 0.34	0.05, 0.39
T2DMxT4	0.10 (0.09)	-0.08, 0.28	0.24 (0.08)	0.08, 0.40	0.006, 0.47
T2DMxT5	0.19 (0.11)	-0.03, 0.40	0.26 (0.08)	0.09, 0.43	0.02, 0.50
- *Wald test for T2DMxtime interaction term*		0.38		**<0.01**	
Sex x T2			-0.09 (0.07)	-0.22, 0.04	-0.28, 0.10
Sex x T3			-0.11 (0.07)	-0.25, 0.02	-0.31, 0.08
Sex x T4			-0.25 (0.09)	-0.43, -0.08	-0.50, 0.0002
Sex x T5			-0.31 (0.09)	-0.49, -0.13	-0.57, -0.05
- *Wald test for sexxtime interaction term*				**<0.01**	
- Sex			-0.21 (0.13)	-0.47, 0.05	-0.59, 0.16
- Age			0.03 (0.005)	0.02, 0.04	0.01, 0.04
- Weight change			-0.02 (0.003)	-0.02, -0.01	-0.02, -0.008
- Parity			-0.13 (0.03)	-0.19, -0.06	-0.22, -0.03
- Breastfeeding			0.0007 (0.003)	-0.004, 0.006	-0.007, 0.008
- Total Lipids			0.10 (0.01)	0.08, 0.12	0.07, 0.13
- Physical Activity			-0.07 (0.03)	-0.13, -0.004	-0.16, 0.02
- BMI					
Overweight			0.07 (0.04)	-0.02, 0.16	-0.06, 0.19
Obesity			0.16 (0.07)	0.03, 0.29	-0.03, 0.35
- constant	7.89 (0.07)	7.76, 8.03	8.17 (0.12)	7.94, 8.41	7.84, 8.51
***p,p'*-DDT**					
- T2DM	0.37 (0.11)	0.15, 0.59	0.06 (0.10)	-0.14, 0.26	-0.23, 0.35
- *Sampling year*					
T2	-1.38 (0.07)	-1.53, -1.24	-1.72 (0.12)	-1.95, -1.49	-2.05, -1.39
T3	-1.92 (0.07)	-2.07, -1.78	-2.44 (0.13)	-2.70, -2.18	-2.82, -2.07
T4	-2.30 (0.10)	-2.50, -2.11	-2.91 (0.18)	-3.25, -2.57	-3.40, -2.43
T5	-2.93 (0.10)	-3.13, -2.72	-3.51 (0.19)	-3.88, -3.13	-4.05, -2.97
- *Interactions*					
T2DMxT2	0.31 (0.11)	0.10, 0.53	0.43 (0.11)	0.21, 0.65	0.12, 0.75
T2DMxT3	0.06 (0.11)	-0.15, 0.28	0.23 (0.11)	0.004, 0.45	-0.09, 0.55
T2DMxT4	-0.04 (0.14)	-0.32, 0.24	0.16 (0.15)	-0.15, 0.46	-0.28, 0.59
T2DMxT5	-0.22 (0.15)	-0.52, 0.08	-0.06 (0.16)	-0.37, 0.25	-0.50, 0.39

- *Wald test for T2DMxtime interaction term*		**<0.01**		**<0.001**	
Sex x T2			0.09 (0.12)	-0.15, 0.34	-0.26, 0.44
Sex x T3			0.10 (0.13)	-0.16, 0.35	-0,26, 0.45
Sex x T4			0.03 (0.17)	-0.30, 0.35	-0.44, 0.49
Sex x T5			-0.15 (0.17)	-0.48, 0.18	-0.62, 0.33
- *Wald test for sexxtime interaction term*				0.56	
- Sex			0.06 (0.14)	-0.22, 0.33	-0.34, 0.45
- Age			0.02 (0.004)	0.01, 0.03	0.01, 0.03
- Weight change			-0.01 (0.005)	-0.02, -0.004	-0.03, 0.0002
- Parity			-0.05 (0.04)	-0.12, 0.03	-0.16, 0.06
- Breastfeeding			0.007 (0.004)	-0.001, 0.02	-0.005, 0.02
- Total Lipids			0.16 (0.02)	0.12, 0.19	0.11, 0.21
- Physical Activity			-0.03 (0.06)	-0.14, 0.08	-0.19, 0.13
- BMI					
Overweight			0.19 (0.07)	0.05, 0.33	-0.34, 0.45
Obesity			0.31 (0.10)	0.12, 0.50	0.01, 0.03
- constant	5.31 (0.08)	5.16, 5.46	5.30 (0.13)	5.04, 5.56	4.92, 5.68

Model 1: Adjusted for time (survey) and interaction between T2DM status and time (survey)
Model 2: Adjusted for time (survey), sex, age, weight change, parity, breastfeeding, total lipids, physical activity, BMI categories, interaction between T2DM status and time (survey) and interaction between sex and time (survey).

Abbreviations: Time-point 2 (1994/95); T3: Time-point 3 (2001); T4: Time-point 4 (2007/08); T5: Time-point 5 (2015/16); T2DM: Type 2 Diabetes Mellitus ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); β-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane; BMI: body mass index.

Table S13. Odds ratios (ORs), 95% and 99.5 % confidence intervals (CIs) for the associations between one-standard deviation (SD) increase in lipid-normalized concentrations of persistent organic pollutants (among controls) and type 2 diabetes mellitus (T2DM) at different time-points (T) in the Tromsø Study (1986-2016).

		Pre-diagnostic time-points			Post-diagnostic time-points	
		T1 (1986/87)	**T2 (1994/95)**	**T3 (2001)**	**T4 (2007/08)**	**T5 (2015/16)**
Compounds (pg/mL)		**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
ΣDL-PCBs	Model 1	1.24 (0.95, 1.62)	1.33 (1.02, 1.72)	1.28 (1.02, 1.62)	1.29 (0.91, 1.84)	1.54 (1.00, 2.37)
	Model 2	1.16 (0.87, 1.56)	1.23 (0.91, 1.65)	1.29 (0.97, 1.71)	1.06 (0.66, 1.69)	1.12 (0.58, 2.15)
	Model 2[a]	1.16 (0.77, 1.77)	1.23 (0.80, 1.88)	1.29 (0.86, 1.94)	1.06 (0.54, 2.07)	1.12 (0.44, 2.85)
ΣPCBs	Model 1	1.04 (0.79, 1.37)	1.15 (0.88, 1.51)	1.22 (0.93, 1.60)	1.09 (0.74, 1.59)	1.45 (0.94, 2.25)
	Model 2	1.08 (0.80, 1.45)	1.17 (0.87, 1.59)	1.35 (0.95, 1.92)	0.92 (0.56, 1.52)	1.07 (0.57, 2.01)
	Model 2[a]	1.08 (0.71, 1.65)	1.17 (0.76, 1.81)	1.35 (0.81, 2.24)	0.92 (0.45, 1.88)	1.07 (0.43, 2.64)
***β*-HCH**	Model 1	1.22 (0.93, 1.58)	1.24 (0.95, 1.63)	1.26 (0.97, 1.64)	1.44 (1.02, 2.02)	1.54 (1.05, 2.27)
	Model 2	1.06 (0.81, 1.38)	1.01 (0.74, 1.37)	1.16 (0.85, 1.60)	1.09 (0.68, 1.73)	1.07 (0.64, 1.79)
	Model 2[a]	1.06 (0.72, 1.55)	1.01 (0.65, 1.57)	1.16 (0.74, 1.84)	1.09 (0.56, 2.12)	1.07 (0.51, 2.23)
***Trans*-nonachlor**	Model 1	1.11 (0.87, 1.42)	1.24 (0.94, 1.62)	1.20 (0.93, 1.54)	1.08 (0.73, 1.61)	1.36 (0.89, 2.09)
	Model 2	1.06 (0.81, 1.39)	1.25 (0.91, 1.72)	1.34 (0.93, 1.94)	0.93 (0.56, 1.57)	1.19 (0.65, 2.16)
	Model 2[a]	1.06 (0.62, 1.81)	1.25 (0.79, 1.98)	1.34 (0.79, 2.27)	0.93 (0.44, 1.96)	1.19 (0.50, 2.80)
***Cis*-nonachlor**	Model 1	1.16 (0.91, 1.48)	1.26 (0.98, 1.63)	1.52 (1.10, 2.11)	1.31 (0.90, 1.92)	1.49 (0.98, 2.26)
	Model 2	1.10 (0.84, 1.44)	1.28 (0.94, 1.73)	1.98 (1.27, 3.08)	1.20 (0.75, 1.95)	1.32 (0.74, 2.36)
	Model 2[a]	1.10 (0.75, 1.62)	1.28 (0.83, 1.98)	1.98 (1.05, 3.72)	1.20 (0.61, 2.39)	1.32 (0.58, 3.02)
Oxychlordane	Model 1	1.06 (0.81, 1.40)	1.18 (0.88, 1.57)	1.21 (0.92, 1.58)	1.17 (0.83, 1.64)	1.40 (0.93, 2.10)
	Model 2	0.97 (0.72, 1.31)	1.15 (0.82, 1.60)	1.33 (0.89, 1.98)	1.00 (0.63, 1.60)	1.03 (0.56, 1.89)
	Model 2[a]	0.97 (0.63, 1.49)	1.15 (0.71, 1.84)	1.33 (0.75, 2.36)	1.00 (0.51, 1.96)	1.03 (0.44, 2.45)
***Cis*-heptachlor epoxide**	Model 1	1.75 (1.34, 2.29)	2.15 (1.64, 2.84)	1.91 (1.45, 2.50)	1.82 (1.26, 2.65)	1.85 (1.22, 2.82)
	Model 2	1.39 (1.04, 1.87)	1.84 (1.34, 2.53)	1.72 (1.22, 2.41)	1.74 (1.07, 2.83)	1.32 (0.79, 2.21)
	Model 2[a]	1.39 (0.91, 2.12)	1.84 (1.17, 2.90)	1.72 (1.06, 2.80)	1.74 (0.87, 3.49)	1.32 (0.63, 2.75)
HCB	Model 1	0.93 (0.72, 1.22)	1.12 (0.88, 1.44)	1.19 (0.95, 1.50)	1.28 (0.91, 1.79)	1.74 (1.19, 2.56)
	Model 2	0.95 (0.70, 1.28)	1.20 (0.88, 1.64)	1.33 (0.95, 1.86)	1.19 (0.68, 2.09)	1.56 (0.83, 2.92)
	Model 2[a]	0.95 (0.61, 1.46)	1.20 (0.77, 1.88)	1.33 (0.82, 2.16)	1.19 (0.53, 2.67)	1.56 (0.64, 3.83)
***p,p'*-DDE**	Model 1	1.12 (0.87, 1.45)	1.23 (0.95, 1.60)	1.32 (1.03, 1.68)	1.32 (0.93, 1.89)	1.42 (0.91, 2.22)
	Model 2	1.04 (0.79, 1.38)	1.07 (0.80, 1.46)	1.15 (0.87, 1.53)	1.25 (0.76, 2.06)	1.16 (0.70, 1.92)
	Model 2[a]	1.04 (0.70, 1.56)	1.07 (0.70, 1.64)	1.15 (0.77, 1.73)	1.25 (0.62, 2.55)	1.16 (0.56, 2.39)
***p,p'*-DDT**	Model 1	1.46 (1.15, 1.86)	1.79 (1.40, 2.29)	1.43 (1.12, 1.82)	1.50 (1.09, 2.08)	1.20 (0.81, 1.77)
	Model 2	1.18 (0.90, 1.53)	1.54 (1.18, 2.00)	1.33 (0.97, 1.83)	1.30 (0.86, 1.97)	1.11 (0.68, 1.80)
	Model 2[a]	1.18 (0.81, 1.71)	1.54 (1.05, 2.25)	1.33 (0.85, 2.10)	1.30 (0.72, 2.36)	1.11 (0.56, 2.21)

T1- (n=254); T2- (n=235); T3- (n=225); T4- (n=100); T5- (n=93).
Model 1: adjusted for age and sex
Model 2: adjusted for age, sex, weight change, parity, breastfeeding, total lipids, physical activity, and BMI (except for weight change and breastfeeding at T1)
Model 2[a]: ORs with 99.5 % CIs for model 2

Abbreviations: ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); β-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane.

Table S14. Odds ratios (ORs), 95% and 99.5% confidence intervals (CIs) for the associations between one-standard deviation (SD) increase in wet-weight concentrations of persistent organic pollutants (among controls) and type 2 diabetes mellitus (T2DM) at different time points (T) in the Tromsø Study (1986-2016).

		Pre-diagnostic time-points			**Post-diagnostic time-points**	
		T1 (1986/87)	**T2 (1994/95)**	**T3 (2001)**	**T4 (2007/08)**	**T5 (2015/16)**
Compounds (pg/mL)		**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
ΣDL-PCBs	Model 1	1.42 (1.08, 1.86)	1.40 (1.04, 1.90)	1.38 (1.04, 1.81)	1.28 (0.87, 1.74)	1.40 (0.93, 2.11)
	Model 2	1.15 (0.85, 1.57)	1.24 (0.89, 1.75)	1.22 (0.85, 1.74)	1.02 (0.61, 1.70)	1.15 (0.57, 2.34)
	Model 2[a]	1.15 0.74, 1.79)	1.24 (0.76, 2.03)	1.22 (0.73, 2.03)	1.02 (0.48, 2.13)	1.15 (0.42, 3.18)
ΣPCBs	Model 1	1.23 (0.93, 1.62)	1.25 (0.95, 1.65)	1.30 (1.00, 1.70)	1.05 (0.72, 1.53)	1.28 (0.85, 1.93)
	Model 2	1.07 (0.78, 1.46)	1.19 (0.84, 1.68)	1.25 (0.87, 1.78)	0.86 (0.50, 1.50)	1.04 (0.53, 2.04)
	Model 2[a]	1.07 (0.69, 1.67)	1.19 (0.73, 1.94)	1.25 (0.75, 2.08)	0.86 (0.39, 1.90)	1.04 (0.40, 2.73)
***β*-HCH**	Model 1	1.35 (1.02, 1.78)	1.33 (0.98, 1.78)	1.35 (1.04, 1.76)	1.36 (0.94, 1.96)	1.43 (0.95, 2.14)
	Model 2	1.05 (0.78, 1.40)	0.97 (0.66, 1.42)	1.17 (0.85, 1.62)	1.07 (0.63, 1.83)	1.06 (0.62, 1.81)
	Model 2[a]	1.05 (0.70, 1.58)	0.97 (0.56, 1.67)	1.17 (0.74, 1.86)	1.07 (0.50, 2.30)	1.06 (0.49, 2.89)
***Trans*-nonachlor**	Model 1	1.24 (0.97, 1.59)	1.29 (0.98, 1.71)	1.28 (0.99, 1.66)	1.04 (0.70, 1.54)	1.23 (0.81, 1.88)
	Model 2	1.07 (0.82, 1.41)	1.29 (0.91, 1.84)	1.27 (0.89, 1.82)	0.89 (0.51, 1.55)	1.16 (0.62, 2.17)
	Model 2[a]	1.07 (0.73, 1.58)	1.29 (0.78, 2.15)	1.27 (0.76, 2.13)	0.89 (0.40, 1.98)	1.16 (0.48, 2.84)
***Cis*-nonachlor**	Model 1	1.29 (1.01, 1.63)	1.31 (0.98, 1.74)	1.48 (1.14, 1.91)	1.25 (0.86, 1.83)	1.37 (0.91, 2.07)
	Model 2	1.12 (0.86, 1.47)	1.30 (0.92, 1.85)	1.60 (1.14, 2.23)	1.18 (0.71, 1.95)	1.32 (0.72, 2.42)
	Model 2[a]	1.12 (0.77, 1.65)	1.30 (0.79, 2.15)	1.60 (0.99, 2.58)	1.18 (0.57, 2.43)	1.32 (0.56, 3.14)
Oxychlordane	Model 1	1.21 (0.93, 1.58)	1.22 (0.92, 1.64)	1.31 (0.99, 1.73)	1.13 (0.81, 1.58)	1.29 (0.87, 1.90)
	Model 2	0.97 (0.72, 1.31)	1.15 (0.80, 1.67)	1.26 (0.85, 1.86)	0.95 (0.57, 1.59)	1.05 (0.57, 1.93)
	Model 2[a]	0.97 (0.63, 1.50)	1.15 (0.68, 1.96)	1.26 (0.72, 2.21)	0.95 (0.45, 1.99)	1.05 (0.44, 2.51)
***Cis*-heptachlor epoxide**	Model 1	1.81 (1.38, 2.37)	2.21 (1.63, 2.99)	1.98 (1.50, 2.60)	1.73 (1.20, 2.50)	1.72 (1.15, 2.58)
	Model 2	1.40 (1.03, 1.91)	1.96 (1.37, 2.81)	1.69 (1.20, 2.38)	1.78 (1.06, 3.00)	1.35 (0.81, 2.24)
	Model 2[a]	1.40 (0.90, 2.18)	1.96 (1.17, 3.28)	1.69 (1.03, 2.76)	1.78 (0.85, 3.75)	1.35 (0.65, 2.79)
HCB	Model 1	1.27 (0.99, 1.63)	1.46 (1.10, 1.93)	1.44 (1.13, 1.85)	1.31 (0.92, 1.85)	1.67 (1.12, 2.50)
	Model 2	0.95 (0.69, 1.31)	1.30 (0.89, 1.88)	1.28 (0.92, 1.79)	1.21 (0.69, 2.11)	1.41 (0.81, 2.47)

	Model 2[a]	0.95 (0.60, 1.50)	1.30 (0.76, 2.21)	1.28 (0.79, 2.06)	1.21 (0.55, 2.68)	1.41 (0.63, 3.15)
***p,p'*-DDE**	Model 1	1.31 (1.02, 1.68)	1.39 (1.08, 1.78)	1.41 (1.11, 1.79)	1.28 (0.88, 1.86)	1.30 (0.84, 2.01)
	Model 2	1.06 (0.80, 1.39)	1.17 (0.88, 1.55)	1.16 (0.88, 1.52)	1.25 (0.74, 2.12)	1.17 (0.69, 1.98)
	Model 2[a]	1.06 (0.71, 1.57)	1.17 (0.78, 1.75)	1.16 (0.78, 1.72)	1.25 (0.59, 2.66)	1.17 (0.55, 2.49)
***p,p'*-DDT**	Model 1	2.04 (1.42, 2.94)	1.83 (1.41, 2.38)	1.49 (1.17, 1.89)	1.46 (1.04, 2.06)	1.13 (0.77, 1.66)
	Model 2	1.32 (0.87, 2.00)	1.64 (1.22, 2.21)	1.29 (0.96, 1.74)	1.31 (0.83, 2.09)	1.16 (0.69, 1.97)
	Model 2[a]	1.32 (0.72, 2.40)	1.64 (1.07, 2.51)	1.29 (0.84, 1.98)	1.31 (0.68, 2.55)	1.16 (0.55, 2.48)

T1- (n=254); T2- (n=235); T3- (n=225); T4- (n=100); T5- (n=93).
Model 1: adjusted for age and sex
Model 2: adjusted for age, sex, weight change, parity, breastfeeding, total lipids, physical activity, and BMI (except for weight change and breastfeeding at T1).
Model 2[a]: ORs with 99.5% CIs for model 2
Abbreviations: ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); β-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane.

Table S15. Odds ratios (ORs), 95% and 99.5% confidence intervals (CIs) for the associations between lipid-normalized concentrations of persistent organic pollutants and type 2 diabetes mellitus (T2DM) for the pre-diagnostic time-points in the Tromsø Study (1986-2001).

	AUC models[a]				**BLUP models[b]**			
	Crude model	**Age+Sex adjusted Model**	**Multivariable model[c]**		**Crude model**	**Age+Sex adjusted Model**	**Multivariable model[c]**	
Compounds (pg/mL)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**CI adjusted at 99.5% level for multiple comparisons**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**CI adjusted at 99.5% level for multiple comparisons**
ΣDL-PCBs	1.39 (1.09, 1.76)	1.33 (1.01, 1.74)	1.17 (0.87, 1.56)	(0.82, 1.47)	1.50 (1.15, 1.97)	1.41 (1.06, 1.89)	0.96 (0.69, 1.35)	(0.59, 1.56)
ΣPCBs	1.23 (0.97, 1.56)	1.15 (0.87, 1.52)	1.10 (0.82, 1.47)	(0.72, 1.67)	1.45 (1.14, 1.84)	1.40 (0.06, 1.83)	1.02 (0.75, 1.39)	(0.65, 1.59)
***β*-HCH**	1.35 (1.02, 1.78)	1.27 (0.97, 1.68)	0.98 (0.73, 1.30)	(0.64, 1.48)	1.39 (1.10, 1.74)	1.33 (1.05, 1.68)	1.03 (0.79, 1.33)	(0.71, 1.48)
***Trans*-nonachlor**	1.29 (1.02, 1.63)	1.22 (0.94, 1.59)	1.13 (0.85, 1.52)	(0.75, 1.72)	1.33 (0.97, 1.82)	1.31 (0.96, 1.78)	1.17 (0.83, 1.65)	(0.72, 1.91)
***Cis*-nonachlor**	1.33 (1.06, 1.67)	1.29 (0.99, 1.66)	1.20 (0.91, 1.59)	(0.81, 1.80)	1.33 (0.95, 1.87)	1.31 (0.93, 1.86)	1.30 (0.91, 1.86)	(0.78, 2.16)
Oxychlordane	1.16 (0.98, 1.38)	1.11 (0.92, 1.34)	1.03 (0.85, 1.26)	(0.78, 1.37)	1.10 (0.87, 1.40)	1.17 (0.91, 1.51)	1.16 (0.88, 1.52)	(0.78, 1.72)

***Cis*-heptachlor epoxide**	2.00 (1.57, 2.58)	2.22 (1.67, 2.96)	1.75 (1.29, 2.37)	(1.13, 2.71)	1.73 (1.29, 2.33)	1.68 (1.24, 2.27)	1.28 (0.92, 1.77)	(0.80, 2.05)
HCB	1.16 (0.91, 1.47)	1.09 (0.86, 1.40)	1.03 (0.77, 1.36)	(0.68, 1.54)	0.87 (0.70, 1.10)	0.93 (0.73, 1.18)	1.01 (0.77, 1.33)	(0.69, 1.49)
***p,p'*-DDE**	1.30 (1.01, 1.67)	1.22 (0.95, 1.59)	1.02 (0.77, 1.35)	(0.68, 1.52)	1.49 (1.09, 2.03)	1.40 (1.01, 1.93)	1.04 (0.73, 1.47)	(0.63, 1.71)
***p,p'*-DDT**	1.82 (1.43, 2.31)	1.82 (1.41, 2.34)	1.46 (1.12, 1.91)	(1.00, 2.14)	1.86 (1.41, 2.45)	1.82 (1.36, 2.45)	1.34 (0.97, 1.85)	(0.85, 2.13)

[a] Models using the area under the curve (AUC) concentrations for T1, T2 and T3. The ORs and 95% CIs are per 1-SD increase in AUC concentrations in controls

[b] Best Linear Unbiased Prediction (BLUP) models for T1, T2 and T3. The ORs and 95% CIs are per 1 SD increase of log-transformed slope of the pre-diagnostic time trend in POP concentrations.

[c] Adjusted for age, sex, total lipids, physical activity, and BMI at T1.

Abbreviations: ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); β-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane.

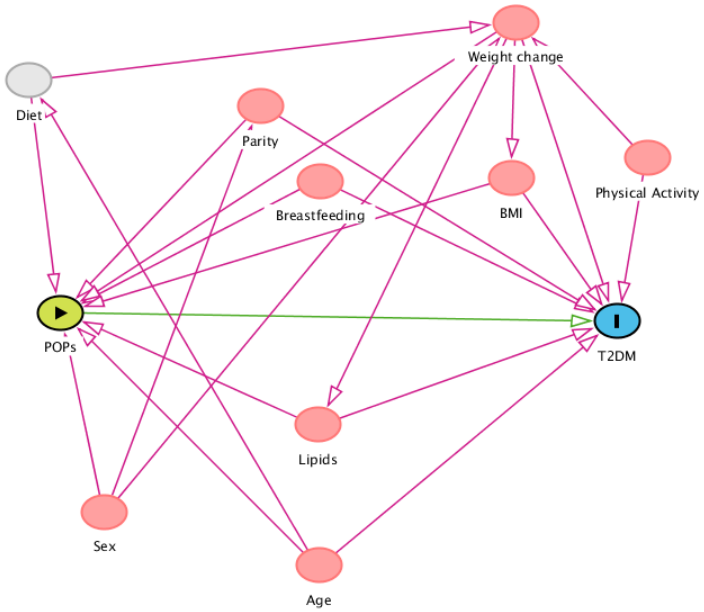

Figure S1. Directed acyclic graph (DAG) illustrating assumptions about the causal relationship between persistent organic pollutants (POPs) and type 2 diabetes mellitus (T2DM) and potential confounders. (The different colors in the DAG represent the following: green-exposure; blue-outcome; pink-confounders; grey-unobserved; arrows-direction of the pathways).

Age, obesity (BMI), increased blood lipids are established risk factors for T2DM and determinants of POP concentrations in the body. Breastfeeding and parity directly influence the POP concentrations in women. Previous studies have shown that low breastfeeding and increased parity also increase the risk of T2DM. Sex directly influences the level of POPs in the body and may increase the risk of T2DM through breastfeeding and/or parity. Decreased physical activity is a known risk factor of T2DM and may influence the POP concentrations through weight change associated with physical activity. Diet (consumption of foods containing POPs) directly influences POP concentrations, although, its effect on T2DM may be mediated through BMI/weight change. Therefore, adjusting for BMI also implies controlling for diet as well as other lifestyle factors.

Thus, the factors (in pink) were considered as confounders that directly/indirectly through mediators (also confounders) affect both POP concentrations and T2DM and thus were adjusted for in our data analyses.

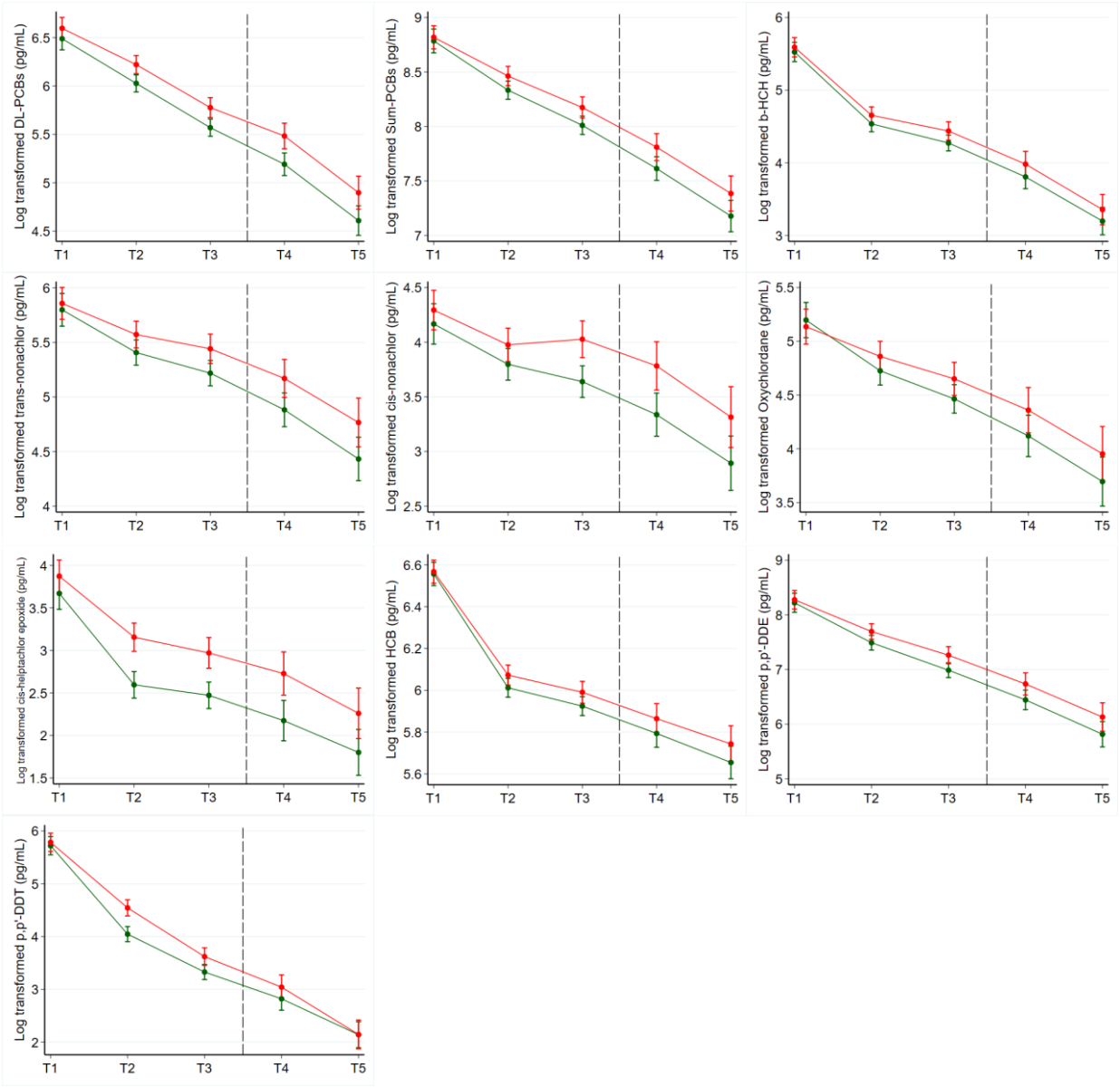

Figure S2. Predicted wet-weight concentrations (pg/mL) of persistent organic pollutants in type 2 diabetes mellitus cases (in red) and controls (in green) after adjusting for covariates from 1986/87 to 2015/16 (n=990) at different time-points (T). T1-1986/87; T2-1994/95; T3-2001; T4-2007/08; T5-2015/16. Abbreviations: ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); *β*-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane. The vertical stifled line on the x-axis separates the pre-diagnostic samples from the post-diagnostic samples. The models are adjusted for time (survey), sex, age, weight change, parity, breastfeeding, total lipids, physical activity, BMI categories, and interaction between T2DM status and time (survey), and sex and time (survey).

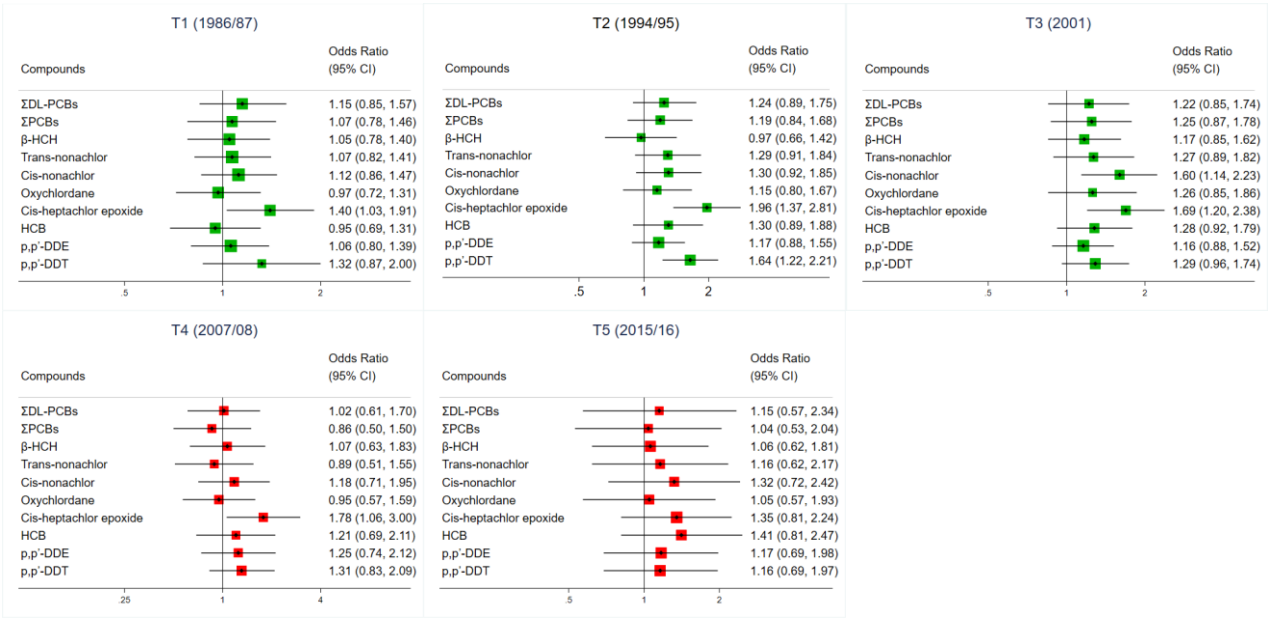

Figure S3. Odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between a one-standard deviation (SD) increase in wet-weight concentrations of persistent organic pollutants (among controls) and type 2 diabetes mellitus at different time points (T) in the Tromsø Study (1986-2016). T1-1986/87 (n=255, 116 cases); T2-1994/95 (n=252, 115 cases); T3-2001 (n=255, 116 cases); T4-2007/08 (n=120, 57cases); T5-2015/16 (n=108, 50 cases). Abbreviations: ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); *β*-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane. The ORs are adjusted for sex, age, weight change, parity, breastfeeding, total lipids, physical activity, and BMI.

Paper III

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Polybrominated diphenyl ethers in type 2 diabetes mellitus cases and controls: repeated measurements prior to and after diagnosis

(submitted to International Journal of Hygiene and Environmental Health)

Polybrominated diphenyl ethers in type 2 diabetes mellitus cases and controls: repeated measurements prior to and after diagnosis

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Abstract

Background

Previous studies have reported associations between certain persistent organic pollutants (POPs) and type 2 diabetes mellitus (T2DM). Polybrominated diphenyl ethers (PBDEs) are a class of POPs that are found in increasing concentrations in humans. Although obesity is a known risk factor for T2DM and PBDEs are fat-soluble, very few studies have investigated associations between PBDEs and T2DM. No longitudinal studies have assessed associations between repeated measurements of PBDE and T2DM in the same individuals and compared time trends of PBDEs in T2DM cases and controls.

Objectives

To investigate associations between pre- and post-diagnostic measurements of PBDEs and T2DM and to compare time trends of PBDEs in T2DM cases and controls.

Methods

Questionnaire data and serum samples from participants in the Tromsø Study were used to conduct a longitudinal nested case-control study among 116 T2DM cases and 139 controls. All included study participants had three pre-diagnostic blood samples (collected before T2DM diagnosis in cases), and up to two post-diagnostic samples after T2DM diagnosis. We used logistic regression models to investigate pre- and post-diagnostic associations between PBDEs and T2DM, and linear mixed-effect models to assess time trends of PBDEs in T2DM cases and controls.

Results

We observed no substantial pre- or post-diagnostic associations between any of the PBDEs and T2DM, except for BDE-154 at one of the post-diagnostic time-points (OR=1.65, 95% CI: 1.00, 2.71). The overall time trends of PBDE concentrations were similar for cases and controls.

Discussion

The study did not support PBDEs increasing the odds of T2DM, prior to or after T2DM diagnosis. T2DM status did not influence the time trends of PBDE concentrations.

Keywords

Polybrominated diphenyl ethers, type 2 diabetes mellitus, time trends, repeated measurements, pre- and post-diagnostic associatins

Funding

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1. Introduction

Type 2 Diabetes Mellitus (T2DM) is considered the world's fastest-growing chronic disease. In 2021, the global prevalence of T2DM was estimated to be 10.5%, i.e., 536.6 million people (Sun et al., 2022). T2DM is a chronic metabolic condition that occurs when the body either cannot produce enough insulin or becomes resistant to the normal effects of insulin. Well-known risk factors for T2DM include obesity, older age, a sedentary lifestyle, and genetic predisposition. In addition, research has addressed the associations between T2DM and factors such as epigenetics, stress, and environmental pollutants (Bellou et al., 2018).

Polybrominated diphenyl ethers (PBDEs) are flame retardants that have been widely used in electronics, plastics, textiles, and furniture to reduce flammability. Primary routes of PBDE exposure for humans are diet and dust inhalation (Costa & Giordano, 2014; Daso et al., 2010). As PBDEs resist degradation, are lipophilic and accumulate in adipose tissues of living organisms (Siddiqi et al., 2003), they have been detected in human samples, such as blood, placental tissue and breast milk (Daso et al., 2010). PBDEs are suspected to be endocrine disrupting chemicals (Birnbaum & Staskal, 2004), are structurally similar to thyroxine 4 (Birnbaum & Staskal, 2004; McDonald, 2002), and have been associated with altered thyroid hormone homeostasis that plays a key role in, for example, adipocyte differentiation and energy storage processes relevant for metabolic disorders such as T2DM (Song et al., 2016). Studies of associations between PBDEs and T2DM have reported inconsistent results: non-linear associations (Lim et al., 2008; Ongono et al., 2019); positive associations (Zhang et al., 2016), and no associations (Airaksinen et al.,

Abbreviations: BDE, bromodiphenyl ether; BMI, Body Mass Index; CI, confidence interval; CVs, coefficients of variation; DAG, Directed Acyclic Graph; GC-MS/MS, gas-chromatography tandem mass-spectrometry; HbA1c, glycated hemoglobin; K_{ow}, n-octanol-water partition coefficient; MDL, method detection limit; OR, odds ratio; PBDEs, polybrominated diphenyl ethers; PCBs, polychlorinated biphenyls; POPs, persistent organic pollutants; r_s, correlation coefficients; SD, standard deviation; T, time point; T2DM, Type 2 Diabetes Mellitus.

2011; Turyk et al., 2009). Most of these studies are cross-sectional in design (Airaksinen et al., 2011; Lim et al., 2008; Turyk et al., 2009; Zhang et al., 2016), and only two studies were prospective; one measured PBDEs in a single blood sample collected prior to disease development and reported inverse, non-significant results (Turyk et al., 2015); and the other estimated the dietary exposure to PBDEs measured in food products and observed increased risk of T2DM only for the second and fourth, versus the first quintile groups (Ongono et al., 2019). Studies with repeated pre-diagnostic PBDE measurements are non-existent in the published literature. Thus, even though intensive research has focused on associations between different groups of persistent organic pollutants and T2DM, knowledge about how PBDEs relate to T2DM is still limited. Additionally, the time trends of individual PBDEs have varied over the years in adult general populations; some concentrations have declined over time, while others have increased, depending on the birth year, study population and sampling time (Thomsen et al., 2002; Toms et al., 2018). PBDEs are stored in adipose and liver tissues, and blood concentrations may therefore be affected by factors related to disease progression, hence time trends of PBDEs may differ in individuals according to T2DM status. In the present study, we thus used a nested case-control design with repeated measurements of PBDEs to i) investigate the associations between PBDEs and T2DM in samples obtained before and after T2DM diagnosis, and ii) compare time trends of PBDEs in serum from T2DM cases and controls.

2. Materials and methods

2.1 The Tromsø Study

The Tromsø Study is an ongoing population-based health survey conducted within the municipality of Tromsø in northern Norway. It was initiated in 1974 and seven surveys have been

conducted approximately every seventh year between 1974 and 2015/16 (Jacobsen et al., 2012). More than 15,000 of the participants took part in three or more Tromsø surveys and answered questionnaires, underwent clinical examination, and donated blood samples for each survey. Participation in the study was voluntary, and an informed consent was provided by all participants. The study was approved by the Regional Committees for Medical Research Ethics.

2.2 *Study design, study participants and data collection*

We used a longitudinal, nested case-control study design, with repeated measurements from the same individuals participating in up to five Tromsø surveys: 1986/87 (T1), 1994/95 (T2), 2001 (T3), 2007/08 (T4) and 2015/16 (T5) (Figure 1). A detailed version of the study design, study participants and data collection has been published (Charles et al., 2022). Briefly, to be included in the study, cases had to have a confirmed T2DM diagnosis recorded in the local diabetes registry between time-points T3 and T4 and also have available pre-diagnostic serum samples (T1, T2, and T3). 76 women and 69 men fulfilled these criteria. If post-diagnostic samples were available for the cases (T4 and/or T5), they were also included in the study sample. We then randomly selected the same number of men and women who had participated in at least the same surveys as the cases, had no T2DM diagnosis recorded in the local diabetes registry, and had available serum samples. They were considered the controls. The participants had also answered questionnaires at each survey with information on participant characteristics, use of medications, parity, and breastfeeding (in women, only available for T2-T5), and physical activity. Health professionals measured height and weight and collected blood samples at the clinical examinations. The Tromsø Study has glycated hemoglobin (HbA1c%) results for all included participants for T2-T5. We excluded twenty-nine cases with HbA1c ≥6.5% in pre-

diagnostic samples, and five controls with HbA1c ≥6.5% at one of the time-points as HbA1c≥6.5% is considered one of the diagnostic criteria for T2DM (International Expert, 2009). In total, 990 blood samples from 116 T2DM cases and 139 healthy controls were included. The number of samples at each time-point were 255 at T1 and T3, 252 at T2, 120 at T4 and 108 at T5 (Figure 1).

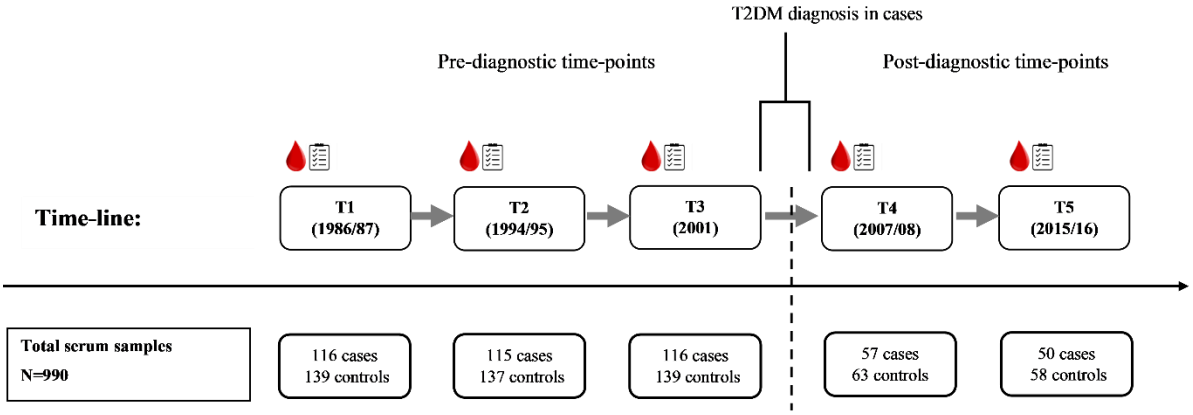

Figure 1. Overview of the study design and sample size in the pre- and post-diagnostic time-points (T) of the Tromsø Study.

2.3 Chemical analyses, and data handling

Frozen serum samples were thawed on ice and split into two aliquots in separate vials (Sarstedt, cat.nr 72.694.600). One aliquot was used for lipid analyses, which were performed immediately after aliquoting, while the other aliquot was stored at -30° C for another 3-6 months, until PBDEs were determined. Both the PBDE analyses and lipid analyses were performed at the Department of Laboratory Medicine, University Hospital of North Norway. A detailed description of the lipid analysis has been described previously (Charles et al., 2022).

A gas-chromatography tandem mass-spectrometry (GC-MS/MS) was used to analyse the PBDEs together with the polychlorinated biphenyls (PCBs). A detailed version of the chemical

analyses procedure has been described elsewhere (Huber et al., 2020). Briefly, the serum samples were prepared in a Freedom Evo 200 (Tecan, Männedorf, Switzerland) liquid handling workstation. Diluted serum samples (150 µL) were extracted and cleaned up by automated solid phase extraction. The instrumental analyses of PBDEs were performed using gas chromatography atmospheric pressure ionization coupled to tandem mass spectrometers (Waters, Milford, MA, USA). Atmospheric pressure ionization was conducted in positive mode under charge transfer conditions. The mass spectrometers were run in multiple reaction monitoring mode using two specific transitions for the individual analytes. Masslynx and Targetlynx software (Version 4.1, Waters) was used for quantification achieved by the internal-standard method with isotope-labeled compounds. Four blank samples, four SRM 1957/1958 (NIST, Gaithersburg, MD, USA) samples, and three bovine serum samples (Sigma Aldrich, Steinheim, Germany) were analyzed within each batch of ninety-six samples, to control for background and carry-over effects as a measure of quality assurance.

The samples from the same participants were analytically determined in the same batch under identical conditions. Each batch had the same number of T2DM cases and controls, and men and women, from identical time-points with randomized positions. The lab staff were blinded to any information that could identify the samples. The measured bromodiphenyl ether (BDE) congeners had coefficients of variation (CVs) ranging between 4% and 26%, which is within previously established acceptable limits (Huber et al., 2020). BDE-47, BDE-99, BDE-100, BDE-153, BDE-154 and BDE-183 were detected above the method detection limit (MDL) in the instrumental analyses (Supplementary Table S1). The PBDE concentrations were lipid-normalized (ng/g lipid) by dividing the wet weight concentrations (pg/mL) by the total lipid concentrations (g/L) where, total lipids = 2.27*total cholesterol + triglycerides + 0.623 (g/L) (Phillips et al., 1989).

2.4 Statistical analyses

In the present study, the PBDE concentrations were right-skewed, with some individuals having high concentrations, and a large proportion of the PBDE concentrations were below the sample-specific MDL. These sample results (<MDL concentrations) were therefore replaced using distribution-based interval regression multiple imputation (Royston, 2007). We imputed values between zero and the individual MDL of each participant at each time-point using the "mi impute chained (intreg)" STATA code. We used twenty imputed datasets for all the statistical analyses presented in this paper.

Descriptive statistics for lipid-normalized PBDE concentrations are presented as means and standard deviations (SD) for each time-point (T). We calculated the mean differences and 95% confidence intervals (CIs) for participant characteristics and PBDE concentrations between T2DM cases and controls. The mean and median concentrations of PBDEs are also presented as box plots for the different time-points. We used Spearman's rank order correlations to assess monotonic relationships at each time-point between the different PBDEs and other legacy POPs measured in the same samples and previously published (Charles et al., 2022).

To investigate linear associations between PBDE concentrations (independent variable) and T2DM status (dependent variable) at each time-point, we used multivariable logistic regression models. We have presented all results from logistic regression models as odds ratios (ORs) per 1-SD increase in PBDE concentrations in controls with 95% CIs. Based on previous literature, we drew a directed acyclic graph (DAG) depicting the hypothesized relationship between PBDEs and T2DM, to determine which covariates to include in the regression models (Aune et al., 2014; Bellou et al., 2018; Li et al., 2016). Age, and increased blood lipids are well-known risk factors for T2DM (Bellou et al., 2018), and determinants of PBDE concentrations in the body (Daniels et

al., 2010; Thomsen et al., 2002; Zhao et al., 2021). Obesity (body mass index: BMI) is an established risk factor of T2DM, however, the role of BMI in the PBDEs-T2DM association is unclear as the research on it is limited. A recent systematic review examining prenatal POPs exposure in relation to obesity development in children found no evidence to support an obesogenic role for PBDEs (Stratakis et al., 2022). Epidemiologic studies in adults were mostly cross-sectional and did not provide consistent evidence for a relation of PBDEs with increased obesity (Daniels et al., 2010; Lee et al., 2012; Lim et al., 2008; Turyk et al., 2010). Therefore, we hypothesized that BMI is a confounder and not in the causal pathway between PBDEs and T2DM. Some previous studies have shown that breastfeeding and parity influence PBDE concentrations in women (Mehta et al., 2020; Zhang et al., 2017). Previous studies have shown that low breastfeeding and increased parity also increase the risk of T2DM (Aune et al., 2014; Li et al., 2016). Sex directly influences concentrations of PBDEs in the body (Zhao et al., 2021), and may increase the risk of T2DM (Huebschmann et al., 2019). Decreased physical activity is a known risk factor of T2DM (Bellou et al., 2018), and may influence PBDE concentrations through changes in weight/BMI associated with physical activity. Diet (consumption of foods containing PBDEs) directly influences PBDE concentrations (Daso et al., 2010), although its effect on T2DM may be mediated through BMI/weight change. Therefore, adjusting for BMI/weight change also implies controlling for diet, as well as other lifestyle factors. Based on the DAG, we included sex, age (in years), parity, breastfeeding (months), physical activity (categorized into active/inactive), total lipids (g/L), weight change (kg) and BMI (kg/m^2) in the analyses (Supplementary Figure S1). We calculated weight change for time-points T2-T5 using weight information from two adjacent time-points (for example: weight change at T2 = [weight at T2] - [weight at T1]). Since we had no information on weight from any previous Tromsø survey, we set weight change at T1 to zero. We summed the reported number of months of breastfeeding

per child to calculate the cumulative breastfeeding at each time-point. We also assessed the associations between PBDEs and T2DM using multivariable logistic regression based on PBDE concentrations divided into tertiles as the independent variable and T2DM as the dependent variable. Furthermore, to compare how different substitution methods of PBDE concentrations <MDL influenced associations between PBDE and T2DM, in addition to the imputation method described above, we also performed multivariable logistic regressions by, i) dividing PBDE concentrations into 2 categories (above/below MDL) and ii) substituting PBDE concentrations below MDL by MDL divided by the square root of 2 and compared these to estimates observed in the multiple imputed data set. The latter methods (MDL/$\sqrt{(2)}$) are commonly used in epidemiological studies of POPs (Airaksinen et al., 2011; Ongono et al., 2019; Turyk et al., 2009) but has been discouraged if the percentages of non-detects are >15% (EPA, 2000).

We assessed the time trends of PBDEs in cases and controls from T1 (1986/87) to T5 (2015/16) using multivariable linear mixed-effect models with a random intercept for individuals, while accounting for the dependencies between repeated measures. Log-transformed PBDE concentrations were considered dependent variables. T2DM status, age at baseline (T1), and sex were time-constant; while indicator variable of each Tromsø survey, weight change, parity, breastfeeding, total lipids, and BMI categories (normal: ≤24.9 kg/m^2, overweight: ≥25.0 to ≤29.9 kg/m^2, obese: ≥30 kg/m^2) were time-varying independent variables. We included interaction terms between T2DM status and time to examine whether the time trends of PBDEs in cases differed from that of controls. We plotted the multivariable-adjusted predicted PBDE concentrations for T2DM cases and controls at each time-point.

All statistical analyses were performed using STATA software, version 16 (StataCorp, 4905 Lakeway Drive, College Station, TX, USA).

3. Results

3.1 Participant characteristics

In our study sample, 54% of the cases and 52% of the controls were females. The mean age at T1 was 47.5±7.63 years in cases and 45.0±9.85 years in controls. At T1, the cases were ~7.9 (95% CI: 4.63, 11.2) kg heavier and had a BMI that was 3.15 (95% CI: 2.25, 4.04) kg/m^2 higher than the controls, and this trend continued through the study period. No differences in parity or breastfeeding between female cases and controls were observed. Cases had higher pre-diagnostic total lipid concentrations compared to controls, but there were no post-diagnostic differences (T4-T5). A detailed description of the participant characteristics has been described previously (Charles et al., 2022).

3.2 Detection frequencies and PBDE concentrations at each time-point

BDE-47 and BDE-153 were the most frequently detected compounds, > 65% and > 42%, respectively, in both cases and controls at all five time-points. The detection frequency for BDE-153 increased with time, while BDE-47 decreased. BDE-154 and BDE-183 were the least detected compounds (< 42% and < 45%, respectively). The detection frequencies for BDE-99 and BDE-100 were higher in the pre-diagnostic time-points compared to the post-diagnostic time-points (Figure 2, Supplementary Table S1).

Cases had higher pre-diagnostic detection frequencies of BDE-153 (T1), BDE-154 (T3), BDE-100 and BDE-183 (T1-T3), compared to controls, and lower detection frequencies of BDE-153 at T2 and T3. In post-diagnostic samples, cases had a higher proportion with concentrations >MDL for BDE-47 and BDE-154 (T4-T5) compared to controls, and a lower proportion for BDE-99 and BDE-183 at T4 (Figure 2).

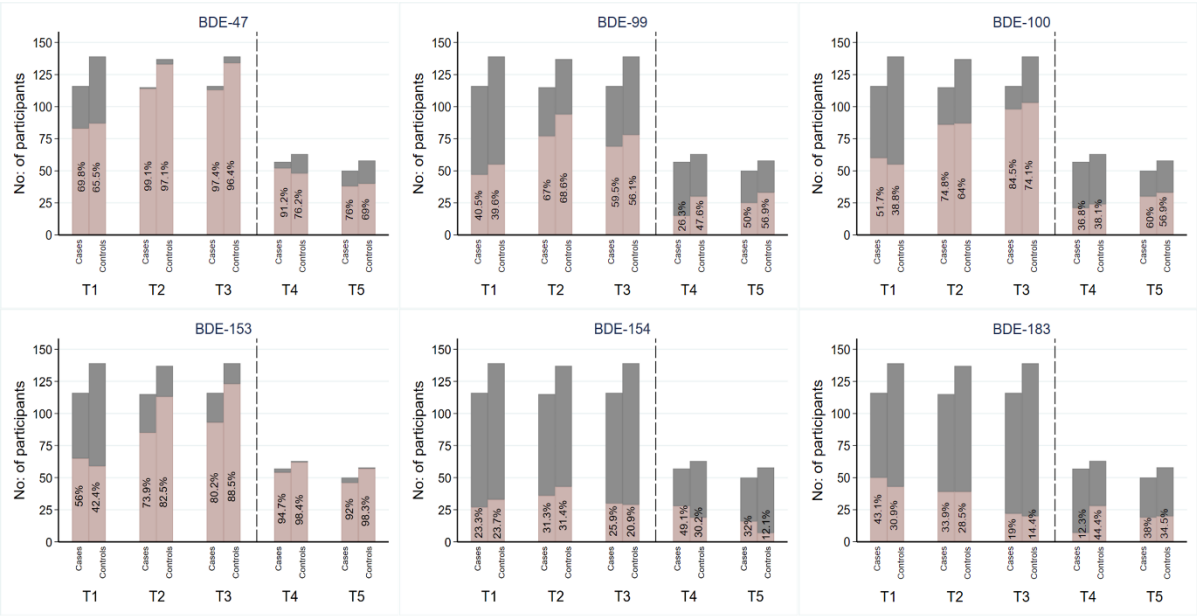

Figure 2. Detection frequencies for polybrominated diphenyl ethers (PBDEs) for type 2 diabetes mellitus cases and controls at different time-points (T) in the Tromsø Study (1986-2016). The pink color represents the proportion of participants with concentrations > the method detection limit (MDL), while the grey color represents the proportion <MDL with the percentages (%) for detected concentrations within each bar for the cases and controls in each survey. The vertical dashed line on the x-axis separates the pre-diagnostic from the post-diagnostic samples. T1, T3: cases: n=116; controls: n=139; T2: cases: n=115, controls: n=137; T4: cases: n=57, controls: n=63; T5: cases: n=50, controls: n=58. Abbreviations: BDE: bromodiphenyl ether.

Spearman correlations between PBDEs and chlorinated POPs (PCBs, organochlorine pesticides) at each time-point had coefficients (r_s) ranging between -0.50 to 0.56. The strongest positive correlations were observed between BDE-47 and ∑PCB (0.56) at T1, BDE-100 and ∑PCBs (0.56) at T2, and BDE-153 and *trans*-nonachlor (0.48) at T4 (Supplementary Tables S2-S6). Cases and controls had similar PBDE concentrations at all time-points except for BDE-99 at

T1, and BDE-100 and BDE-183 at T4, for which controls had higher concentrations compared to cases (Figure 3, Supplementary Table S7).

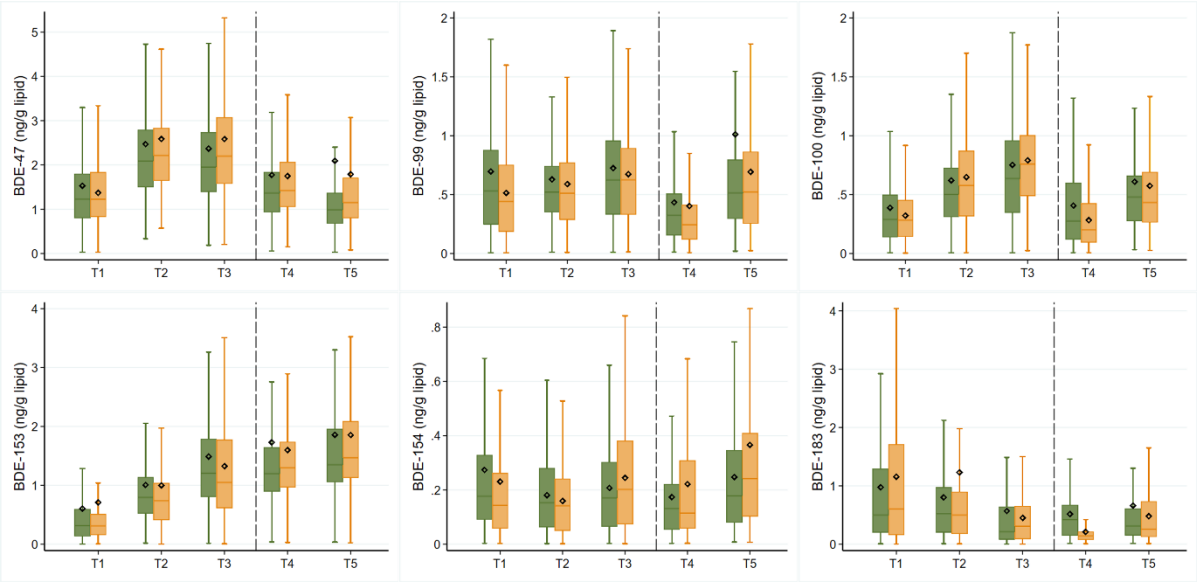

Figure 3. Lipid normalized concentrations (using imputed concentrations) of polybrominated diphenyl ethers (PBDEs) for controls (green boxes) and type 2 diabetes cases (orange boxes) at different time-points (T) in the Tromsø Study (1986-2016). Boxes represent the 25th–75th percentiles, horizontal lines within the boxes denote the median, whiskers indicate 1.5 times the length of the interquartile range above and below the 75th and 25th percentiles, respectively, and ◊ denotes the mean. The vertical dashed line on the x-axis separates the pre-diagnostic from the post-diagnostic samples. T1: n=255; T2: n=252; T3: n=255; T4: n=120; T5: n=108. Abbreviations: BDE: bromodiphenyl ether.

3.3 Associations between PBDEs and T2DM

After adjusting for age, sex, total lipids (g/L), parity, breastfeeding, BMI, weight change and physical activity, we did not observe increased odds for T2DM for any of the PBDEs at any of the time-points, except for BDE-154 at T5 (OR=1.65, 95% CI: 1.00, 2.71). Decreased odds for

T2DM were observed for BDE-183 at T4 (OR=0.32, 95% CI: 0.15, 0.68). Generally, the ORs had wide CIs including 1.0 (Figure 4, Supplementary Table S8). On dividing the PBDE concentrations into tertiles, we observed increased odds for T2DM for BDE-153 at T5, but with poor precision of the point estimates (tertile 2 vs 1: OR= 4.25, 95% CI: 1.13, 15.9; tertile 3 vs 1: OR=4.52, 95% CI:1.02, 20.0) (results not shown).

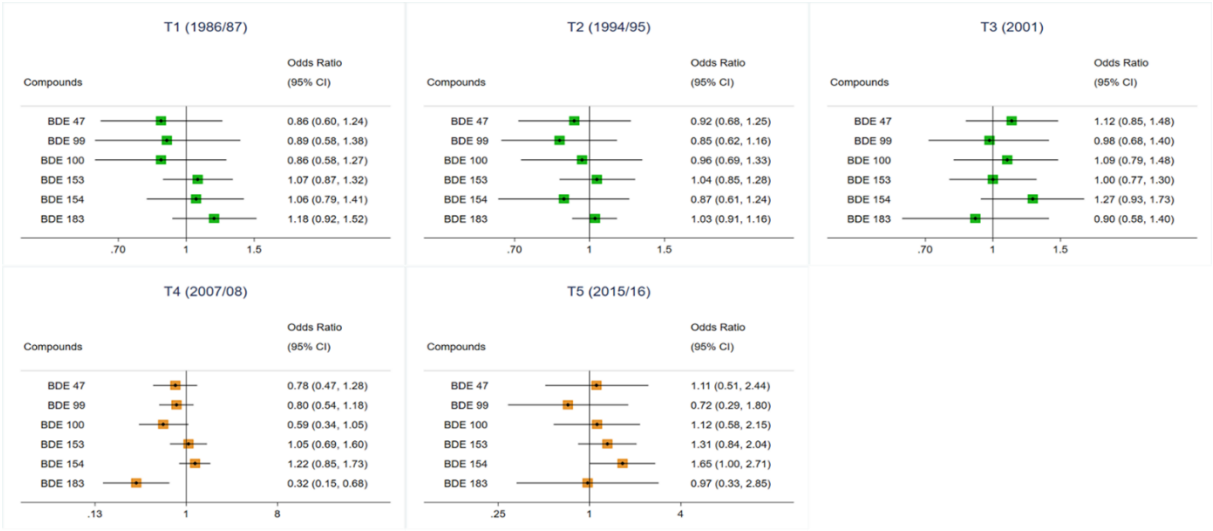

Figure 4. Odds ratios (ORs) with 95% confidence intervals (CIs) for the associations between a one-standard deviation (SD) increase in lipid-normalized concentrations of PBDEs (among controls) and type 2 diabetes mellitus at different time-points (T) in the Tromsø Study (1986-2016). T1: (n=254); T2: (n=235); T3: (n=225); T4: (n=100); T5: (n=93). The ORs are adjusted for age, sex, weight change, parity, breastfeeding, total lipids, physical activity, and BMI (except for weight change and breastfeeding at T1). Abbreviations: BDE: bromodiphenyl ether.

The choice of substitution method for concentrations <MDL had a minor impact on the estimated associations between PBDEs and T2DM. The results from the different regression models were similar in terms of direction and strength. BDE-154 showed an association with

T2DM at T5 in all three substitution methods, and at T4 in the dichotomized substitution method (Figure 5).

Figure 5. Comparison of odds ratios and 95% confidence intervals (CIs) for the associations between lipid-normalized concentrations of PBDEs and type 2 diabetes mellitus at different time-

points (T) for the different methods of substitution for PBDE concentrations below MDL in the Tromsø Study (1986–2016). The ORs are adjusted for age, sex, weight change, parity, breastfeeding, total lipids, physical activity, and BMI (except for weight change and breastfeeding at T1). Abbreviations: BDE: bromodiphenyl ether.

3.4 Time trends of PBDEs

Similar time trends for PBDE concentrations were observed for cases and controls from 1986 to 2016, after adjusting for sex, age at baseline (T1), weight change, parity, breastfeeding, total lipids, BMI categories (normal: ≤24.9 kg/m^2, overweight: ≥25.0 to ≤29.9 kg/m^2, obese: ≥30 kg/m^2) and interaction between T2DM status and time (survey). There was one exception: cases showed a faster decline for BDE-183 from T1 to T4 compared to controls (Figure 6, Supplementary Table S9).

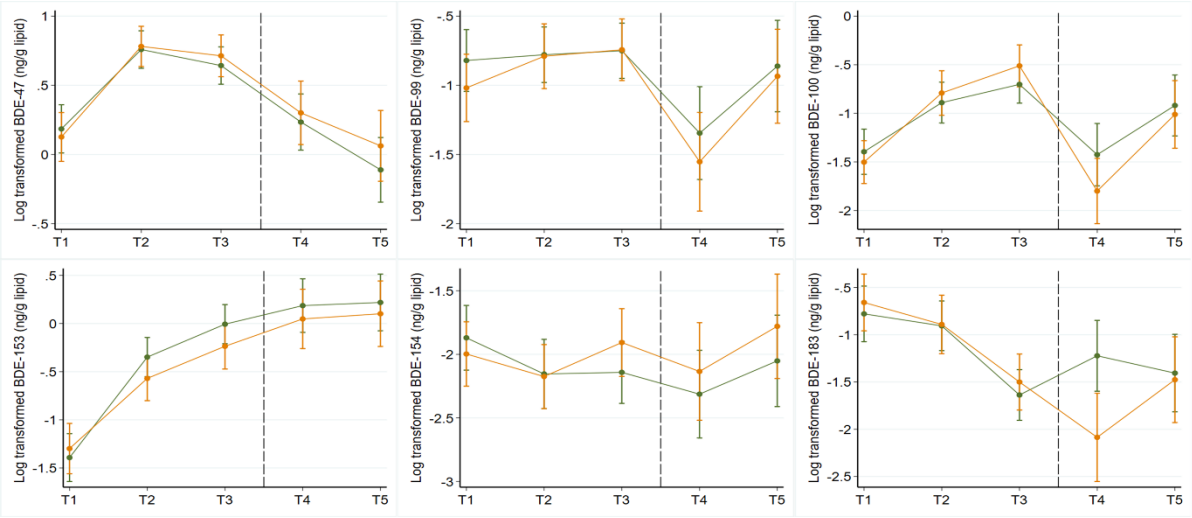

Figure 6. Predicted lipid-normalized polybrominated diphenyl ether concentrations in type 2 diabetes mellitus cases (in orange) and controls (in green) after adjusting for covariates at different time-points (T) in the Tromsø Study (1986–2016) (N = 990). The vertical dashed line on the x-axis separates the pre-diagnostic from the post-diagnostic samples.

Abbreviations: BDE: brominated diphenyl ether.

4. Discussion

This is the first study to assess repeated associations between pre- and post-diagnostic concentrations of PBDEs and T2DM, as well as differences in time trends of PBDEs in T2DM cases and controls. Pre-diagnostic PBDE concentrations did not increase the odds of T2DM substantially. Our findings thus do not support the hypothesis of background exposure to PBDEs as being a risk factor for T2DM. The time trends of PBDEs from 1986 to 2016 were also similar for cases and controls, proposing no differences in metabolism or bioaccumulation of PBDEs according to T2DM status. We did, however, observe increased odds for T2DM for BDE-154 at one of the post-diagnostic time-points. As no similar associations were observed for the pre-diagnostic samples, this finding reflects a physiological change related to the disease itself, an effect of a lifestyle change following the disease, or just a random result.

The lack of association between PBDEs and T2DM in pre-diagnostic samples is in line with one previous prospective study based on concentrations of PBDEs in serum samples (Turyk et al., 2015). The other prospective study by Ongono et al. 2019 found a non-linear association, although this study related exposure to PBDEs (as measured in commonly consumed food products) to T2DM, and several studies have shown poor agreement between dietary intake of POPs and circulating concentrations (Ongono et al., 2019). The null findings in the post-diagnostic (cross-sectional) samples are also in line with previous cross-sectional studies, although the mean concentrations of PBDEs in our post-diagnostic study samples were relatively low or similar compared to both these studies (Airaksinen et al., 2011; Turyk et al., 2009). The other cross-sectional study found positive associations between BDE-47 and prevalent T2DM, although this study measured higher median concentrations of BDE-47, and this could have

contributed to the positive associations reported in the study. Another contributing factor could be the lack of adjustment of potential confounders such as parity, breastfeeding, weight change and physical activity in this study (Zhang et al., 2016).

Our previous study addressing associations between chlorinated POPs and T2DM in the same samples showed slower declines in T2DM cases compared to controls which could explain why prospective T2DM cases experience higher body burdens of chlorinated POPs than healthy controls (Charles et al., 2022). However, both cases and controls had comparable PBDE concentrations in our study. Although PBDEs may be structurally similar to PCBs, have similar log K_{OW} (PCB congeners: 6.04-8.35; PBDE congeners: 6.81-8.27) (ATSDR, 2017, 2000), and be metabolized by the same class of enzymes (Feo et al., 2013; Gross et al., 2015), the time trends for most PBDEs were similar between cases and controls throughout the entire study period. This may be attributed to distinctive characteristics of PBDEs compared to PCBs. For instance, the correlations between PBDEs and the chlorinated POPs ranged from -0.50 to 0.56, depending on the congeners. The fact that these compounds were not correlated to any great extent thus supports the differences in associations and time trends observed in our previous study (Charles et al., 2022), compared to this. Others have also reported weak or moderate correlations between PBDEs and PCBs in human breast milk (She et al., 2007). Furthermore, some studies measuring both PCBs and PBDEs in adipose tissues and liver from the same individuals showed higher PBDE concentrations in the liver, compared to adipose tissues, in some of the samples (Meironyte Guvenius et al., 2001). This may suggest that the bioaccumulation and metabolism of PBDEs is not affected by changes in body fatness and differs from that of PCBs and organochlorine pesticides. This is further supported by the fact that most of the T2DM cases in our study had either overweight or obesity at T1, although their mean PBDE concentrations were similar to that of controls. In fact, it was the controls that had highest concentrations for some

PBDEs at certain time-points (Supplementary Table S8). In line with this, few previous studies have reported no association between BDE-47 and measures of body fatness (Lee et al., 2012; Ronn et al., 2011; Roos et al., 2012).

In the present study, one compound, BDE-183, declined faster among cases compared to controls. From T1 to T3, the detection frequencies for BDE-183 were higher or similar between cases and controls, while, at T4, the detection frequencies for cases and controls were 12.3% and 44.4%, respectively. This suggests that most cases had BDE-183 concentrations that were <MDL at T4, depicting a more rapid decline in the same group, compared to controls (Figure 2 & 6). The smaller sample size at T4 may also have contributed to this difference between the two groups.

A challenge when working with PBDEs is the large proportion of samples with concentrations < MDL. There are different ways of handling non-detects during data analysis, but reassuringly, independent of the method used (multiple imputation, MDL/√2, dichotomization), we observed similar results for the associations between PBDEs and T2DM. Previous research has shown that MDL/√2 substitution of left-censored data may produce less precise estimates (Baccarelli et al., 2005), but we did not observe any considerable differences in associations between using this method and the multiple imputation. Thus, irrespective of the method of substitution used, our overall conclusions remain the same.

The nested longitudinal case-control study design is a major strength of the present study, including three to five repeated PBDE measurements for each study participant. To date, this is the first study to examine both pre- and post-diagnostic associations between PBDEs and T2DM. We could also assess time trends of PBDEs over a period of 30 years within T2DM cases and controls. All T2DM cases were identified and confirmed for diabetes status using a local diabetes registry and HbA1c% measurements of the individuals from the different time-points. Another important strength is that even though we had high non-detects for some of the PBDEs (>20%) at

different time-points, we accounted for the <MDL concentrations using multiple imputation, imputing values between 0 and the individual MDL at the respective time-point for each participant. We had complete data for most of the covariates for which we adjusted. Otherwise, missing values were imputed using multiple imputation. To the best of our knowledge, we identified all potential confounders and adjusted for them in both the logistic regression and mixed-model analyses. Additionally, we used two other substitution methods for the <MDL concentrations, to compare the consistencies in results. Comprehensive quality control measures for the chemical analyses are an added strength of the present study. However, there are limitations that also need to be considered. For instance, not all participants had post-diagnostic measurements, resulting in smaller sample sizes at T4 and T5. This had an impact on the precision of the effect estimates of the post-diagnostic associations. Animal studies suggest that PBDEs may be obesogens (Bondy et al., 2013; Suvorov et al., 2009). If so, then the DAG in our study with BMI as a confounder is wrong. However, previous research has shown no consistent evidence of PBDEs having obesogenic roles in humans thereby rather supporting BMI being a confounder and not a mediating factor (Stratakis et al., 2022). We did not account for multiple testing, i.e., we did not adjust p-values, even though we calculated ORs for six PBDEs at five time-points. With the present overall negative results, adjustments for multiple testing would strengthen the picture of negative findings. The generalization of these findings may be limited to populations similar to the adult Norwegian population, or populations with similar PBDE concentrations. Nevertheless, this study takes advantage of a well-established cohort and is one of the first studies to explore the relationship between PBDEs and T2DM risk.

Our study results support PBDEs not being associated with T2DM before or after T2DM diagnosis. Furthermore, our results show that, despite PBDEs being similar in molecular structure

to PCBs, there may be intra- and inter-individual differences in the bioaccumulation and/or metabolism of PBDEs resulting in similar time trends between cases and controls.

Conclusion

Our results indicate that exposure to PBDEs does not increase the odds of developing T2DM.The time trends were similar for cases and controls, indicating no effect of T2DM-related factors on PBDE concentrations.

Acknowledgements

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Supplemental Material

Polybrominated diphenyl ethers in type 2 diabetes mellitus cases and controls: repeated measurements prior to and after diagnosis

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Table S1. Detection frequencies for the different polybrominated diphenyl ether compounds at different time-points (T) in the Tromsø Study (1986-2016).

	Pre-diagnostic time-points			Post-diagnostic time-points	
Compounds	**T1 (1986/87) n=255**	**T2 (1994/95) n=252**	**T3 (2001) n=255**	**T4 (2007/08) n=120**	**T5 (2015/16) n=108**
	Detected %	**Detected %**	**Detected %**	**Detected %**	**Detected %**
BDE 47	66.7	98.8	96.9	83.3	72.2
BDE 99	40	67.9	57.6	37.5	53.7
BDE 100	45.1	68.7	78.9	37.5	58.3
BDE 153	48.6	79.4	84.7	96.7	95.4
BDE 154	23.5	32.4	23.1	39.2	21.3
BDE 183	36.5	32	16.5	29.2	36.1

Abbreviations: BDE: bromodiphenyl ether

Table S2. Spearman's rank correlations between the different brominated and chlorinated organic pollutants at time-point 1 (T1, 1986/87).

	∑DL-PCBs	∑PCBs	b-HCH	Trans-nonachlor	Cis-nonachlor	Oxychlordane	Cis-heptachlorepoxide	HCB	*p,p'*-DDE	*p,p'*-DDT
BDE 47 (n=176)	0.52	0.56	0.09	0.54	0.55	0.55	0.48	0.33	0.46	0.24
BDE 99 (n=101)	0.04	0.05	0.05	0.06	0.11	0.08	0.005	0.03	0.11	0.03
BDE 100 (n=114)	0.46	0.48	0.06	0.48	0.49	0.48	0.41	0.31	0.29	0.24
BDE 153 (n=120)	0.12	0.20	0.0003	0.13	0.10	0.12	0.07	0.06	-0.03	-0.08
BDE 154 (n=54)	0.07	0.09	0.02	0.15	0.06	0.12	0.07	-0.10	0.10	0.16
BDE 183 (n=93)	0.13	0.14	0.07	0.17	0.12	0.11	0.20	0.24	-0.08	0.33

Abbreviations: ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); β-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane; BDE: bromodiphenyl ether.

Table S3. Spearman's rank correlations between the different brominated and chlorinated organic pollutants at time-point 2 (T2, 1994/95).

	∑DL-PCBs	∑PCBs	b-HCH	Trans-nonachlor	Cis-nonachlor	Oxychlordane	Cis-heptachlorepoxide	HCB	*p,p'*-DDE	*p,p'*-DDT
BDE 47 (n=212)	0.52	0.51	0.18	0.44	0.44	0.45	0.41	0.37	0.47	0.42
BDE 99 (n=153)	0.10	0.14	0.11	0.08	0.07	0.05	0.18	0.06	0.26	0.20
BDE 100 (n=161)	0.54	0.56	0.17	0.45	0.46	0.44	0.42	0.37	0.51	0.40
BDE 153 (n=169)	0.40	0.47	0.08	0.39	0.36	0.45	0.30	0.24	0.20	0.23
BDE 154 (n=76)	0.05	0.10	0.13	0.11	0.09	0.03	0.01	0.05	0.21	0.22
BDE 183 (n=65)	0.15	0.12	0.16	0.03	0.01	0.09	0.02	0.009	0.12	0.06

Abbreviations: ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); β-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane; BDE: bromodiphenyl ether.

Table S4. Spearman's rank correlations between the different brominated and chlorinated organic pollutants at time-point 3 (T3, 2001).

	∑DL-PCBs	∑PCBs	b-HCH	Trans-nonachlor	Cis-nonachlor	Oxychlordane	Cis-heptachlorepoxide	HCB	*p,p'*-DDE	*p,p'*-DDT
BDE 47 (n=237)	0.47	0.48	0.11	0.32	0.33	0.32	0.35	0.27	0.47	0.40
BDE 99 (n=142)	0.07	0.08	-0.04	0.04	0.04	0.04	0.05	0.009	0.14	0.06
BDE 100 (n=195)	0.39	0.41	-0.03	0.28	0.29	0.27	0.21	0.23	0.36	0.29
BDE 153 (n=200)	0.36	0.44	0.002	0.29	0.27	0.31	0.28	0.24	0.13	0.12
BDE 154 (n=63)	0.22	0.25	-0.04	0.21	0.24	0.18	0.15	0.04	0.13	0.17
BDE 183 (n=43)	0.03	0.03	0.16	0.02	0.02	-0.03	-0.04	0.19	0.09	0.03

Abbreviations: ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); β-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane; BDE: bromodiphenyl ether.

Table S5. Spearman's rank correlations between the different brominated and chlorinated organic pollutants at time-point 4 (T4, 2007/08).

	∑DL-PCBs	∑PCBs	b-HCH	Trans-nonachlor	Cis-nonachlor	Oxychlordane	Cis-heptachlorepoxide	HCB	*p,p'*-DDE	*p,p'*-DDT
BDE 47 (n=86)	0.12	0.14	-0.10	0.20	0.18	0.15	0.08	0.02	0.07	0.30
BDE 99 (n=42)	0.16	0.20	0.21	0.19	0.17	0.23	0.26	0.25	0.14	0.28
BDE 100 (n=38)	0.27	0.26	0.22	0.24	0.27	0.22	0.06	-0.10	0.37	0.51
BDE 153 (n=97)	0.36	0.42	-0.09	0.48	0.48	0.45	0.34	0.21	0.06	0.36
BDE 154 (n=43)	0.25	0.25	0.08	0.25	0.27	0.32	0.24	0.33	0.24	0.38
BDE 183 (n=29)	-0.35	-0.39	0.002	-0.41	-0.42	-0.41	-0.21	-0.36	-0.10	-0.50

Abbreviations: ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); β-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane; BDE: bromodiphenyl ether.

Table S6. Spearman's rank correlations between the different brominated and chlorinated organic pollutants at time-point 5 (T5, 2015/16).

	∑DL-PCBs	∑PCBs	b-HCH	Trans-nonachlor	Cis-nonachlor	Oxychlordane	Cis-heptachlorepoxide	HCB	*p,p'*-DDE	*p,p'*-DDT
BDE 47 (n=54)	-0.05	-0.05	-0.05	-0.07	-0.03	-0.09	-0.19	-0.04	0.007	0.10
BDE 99 (n=41)	-0.03	-0.10	-0.05	-0.08	0.03	-0.07	0.13	-0.005	0.02	0.23
BDE 100 (n=39)	-0.30	-0.27	-0.23	-0.32	-0.28	-0.28	-0.08	-0.22	0.06	0.07
BDE 153 (n=74)	0.23	0.29	-0.17	0.26	0.29	0.23	0.20	0.16	0.10	0.26
BDE 154 (n=17)	-0.27	-0.29	-0.10	-0.16	-0.03	-0.16	0.14	-0.13	-0.08	0.28
BDE 183 (n=22)	-0.08	0.09	-0.09	0.06	-0.04	-0.09	0.19	-0.08	0.10	0.17

Abbreviations: ∑DL-PCBs: Dioxin-like Polychlorinated biphenyls (PCB 118,156); ∑PCBs: Sum Polychlorinated biphenyls (PCB 74, 99, 118, 138, 153, 156, 170, 180, 183, 187 and 194); β-HCH: beta-hexachlorocyclohexane; HCB: hexachlorobenzene; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane; BDE: bromodiphenyl ether.

Table S7. Lipid-normalized concentrations (ng/g lipid) of polybrominated diphenyl ether compounds presented as means, standard deviations (SD) and mean differences with 95% confidence intervals (CIs) between type 2 diabetes mellitus cases and controls at different time-points (T) in the Tromsø Study (1986-2016).

	Pre-diagnostic time-points						Post-diagnostic time-points			
Compounds (ng/g lipid)	**T1 (1986/87)**		**T2 (1994/95)**		**T3 (2001)**		**T4 (2007/08)**		**T59 (2015/16**	
	Mean±SD	**ΔMean[a] (95% CI)**	**Mean±SD**	**ΔMean[a] (95% CI)**	**Mean±SD**	**ΔMean[a] (95% CI)**	**Mean±SD**	**ΔMean[a] (95% CI)**	**Mean±SD**	**ΔMean[a] (95% CI)**
BDE 47		-0.16 (-0.44, 0.11)		0.11 (-0.31, 0.54)		0.21 (-0.19, 0.61)		-0.02 (-0.61, 0.56)		-0.31 (-2.16, 1.54)
Cases	1.37±0.82		2.56±1.60		2.58±1.63		1.75±1.52		1.79±2.42	
Controls	1.53±1.26		2.47±1.81		2.37±1.61		1.77±1.71		2.10±6.19	
BDE 99		-0.18 (-0.36, -0.004)		-0.04 (-0.17, 0.09)		-0.05 (-0.20, 0.09)		-0.03 (-0.23, 0.17)		-0.32 (-0.88, 0.24)
Cases	0.51±0.40		0.59±0.45		0.67±0.48		0.40±0.62		0.69±0.74	
Controls	0.70±0.86		0.63±0.53		0.72±0.65		0.43±0.46		1.01±1.86	
BDE 100		-0.07 (-0.15, 0.02)		0.03 (-0.14, 0.20)		0.04 (-0.10, 0.18)		-0.12 (-0.25, -0.002)		-0.03 (-0.26, 0.19)
Cases	0.32±0.23		0.65±0.50		0.79±0.48		0.28±0.25		0.58±0.45	
Controls	0.39±0.37		0.62±0.80		0.75±0.60		0.41±0.38		0.61±0.66	
BDE 153		0.10 (-0.48, 0.68)		-0.006 (-0.38, 0.37)		-0.16 (-0.56, 0.23)		-0.13 (-0.83, 0.56)		-0.002 (-0.67, 0.67)
Cases	0.71±2.79		1.00±1.82		1.33±1.68		1.60±1.86		1.86±1.92	
Controls	0.61±1.86		1.00±1.16		1.49±1.54		1.73±1.99		1.86±1.59	
BDE 154		-0.04 (-0.14, 0.05)		-0.02 (-0.06, 0.02)		0.04 (-0.02, 0.09)		0.05 (-0.03, 0.13)		0.12 (-0.02, 0.26)
Cases	0.23±0.36		0.16±0.13		0.24±0.20		0.22±0.26		0.37±0.42	
Controls	0.27±0.38		0.18±0.14		0.21±0.17		0.17±0.17		0.25±0.24	
BDE 183		0.18 (-0.18, 0.54)		0.43 (-0.76, 1.61)		-0.12 (-0.38, 0.14)		-0.30 (-0.45, -0.16)		-0.18 (-0.73, 0.37)
Cases	1.16±1.66		1.23±6.82		0.45±0.54		0.21±0.27		0.48±0.53	
Controls	0.98±1.22		0.80±1.55		0.57±1.31		0.52±0.47		0.66±1.89	

T1: n=255, 116 cases; T2: n=252, 115 cases; T3: n=255, 116 cases; T4: n=120, 57cases; T5: n=108, 50 cases.

[a] ΔMean=mean in cases-mean in controls

Abbreviations: BDE: bromodiphenyl ether

Table S8. Odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between one-standard deviation (SD) increase in lipid-normalized concentrations (ng/g lipid) of polybrominated diphenyl ethers (among controls) and type 2 diabetes mellitus (T2DM) at different time-points (T) in the Tromsø Study (1986-2016).

	Pre-diagnostic samples			Post-diagnostic samples	
	T1 (1986/87)	T2 (1994/95)	T3 (2001)	T4 (2007/08)	T5 (2015/16)
Compounds (ng/g lipid)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
BDE 47					
Model 1	0.83 (0.60, 1.15)	1.06 (0.80, 1.39)	1.13 (0.85, 1.48)	0.91 (0.61, 1.36)	0.95 (0.54, 1.66)
Model 2	0.86 (0.60, 1.24)	0.92 (0.68, 1.25)	1.12 (0.85, 1.48)	0.78 (0.47, 1.28)	1.11 (0.51, 2.44)
BDE 99					
Model 1	0.66 (0.40, 1.11)	0.91 (0.69, 1.20)	0.94 (0.70, 1.27)	0.91 (0.66, 1.27)	0.70 (0.36, 1.34)
Model 2	0.89 (0.58, 1.38)	0.85 (0.62, 1.16)	0.98 (0.68, 1.40)	0.80 (0.54, 1.18)	0.72 (0.29, 1.80)
BDE 100					
Model 1	0.78 (0.54, 1.12)	1.01 (0.74, 1.38)	1.06 (0.80, 1.40)	0.58 (0.35, 0.97)	0.94 (0.58, 1.51)
Model 2	0.86 (0.58, 1.27)	0.96 (0.69, 1.33)	1.09 (0.79, 1.48)	0.59 (0.34, 1.05)	1.12 (0.58, 2.15)
BDE 153					
Model 1	1.04 (0.86, 1.27)	0.99 (0.82, 1.21)	0.90 (0.69, 1.18)	0.92 (0.62, 1.36)	0.98 (0.69, 1.40)
Model 2	1.07 (0.87, 1.32)	1.04 (0.85, 1.28)	1.00 (0.77, 1.30)	1.05 (0.69, 1.60)	1.31 (0.84, 2.04)
BDE 154					
Model 1	0.90 (0.67, 1.19)	0.84 (0.62, 1.14)	1.22 (0.92, 1.61)	1.19 (0.87, 1.63)	1.34 (0.95, 1.90)
Model 2	1.06 (0.79, 1.41)	0.87 (0.61, 1.24)	1.27 (0.93, 1.73)	1.22 (0.85, 1.73)	1.65 (1.00, 2.71)
BDE 183					
Model 1	1.10 (0.88, 1.38)	1.03 (0.93, 1.14)	0.86 (0.60, 1.23)	0.29 (0.14, 0.61)	0.87 (0.43, 1.78)
Model 2	1.18 (0.92, 1.52)	1.03 (0.91, 1.16)	0.90 (0.58, 1.40)	0.32 (0.15, 0.68)	0.97 (0.33, 2.85)

T1: n=255; T2: n=252; T3: n=255; T4: n=120; T5: n=108.

Model 1: adjusted for age and sex.

Model 2: adjusted for age, sex, weight change, parity, breastfeeding, total lipids, physical activity and BMI (except for weight change and breastfeeding at T1).

Abbreviations: BDE: bromodiphenyl ether.

Table S9. Multivariable adjusted regression coefficients, standard errors (SE) and 95% confidence intervals (CIs) from linear mixed-effect models to assess the longitudinal changes in lipid-normalized concentrations (ng/g lipid) of the different polybrominated diphenyl ether concentrations from 1986/87-2015/16 according to type 2 diabetes mellitus (T2DM) status (N=990) in the Tromsø Study (1986-2016).

	Model 1		**Model 2**	
	β – coefficient (95% CI)	Wald test for T2DMxtime interaction term	β – coefficient (95% CI)	Wald test for T2DMxtime interaction term
BDE-47				
- T2DM	-0.04 (-0.28, 0.19)		-0.06 (-0.30, 0.18)	
- Sampling year				
T2	0.64 (0.44, 0.83)		0.57 (0.36, 0.78)	
T3	0.56 (0.37, 0.76)		0.46 (0.24, 0.68)	
T4	0.12 (-0.12, 0.36)		0.05 (-0.21, 0.31)	
T5	-0.20 (-0.48, 0.09)		-0.30 (-0.60, 0.009)	
- Interactions		0.67		0.77
T2DMxT2	0.10 (-0.18, 0.39)		0.08 (-0.20, 0.36)	
T2DMxT3	0.16 (-0.13, 0.45)		0.13 (-0.16, 0.42)	
T2DMxT4	0.16 (-0.21, 0.52)		0.12 (-0.24, 0.49)	
T2DMxT5	0.25 (-0.13, 0.63)		0.23 (-0.15, 0.61)	
- Sex			0.14 (-0.06, 0.34)	
- Age at T1			0.007 (-0.001, 0.01)	
- Weight change			0.006 (-0.005, 0.02)	
- Parity			-0.04 (-0.11, 0.02)	
- Breastfeeding			0.008 (0.0005, 0.02)	
- Total Lipids			-0.04 (-0.08, -0.007)	
- BMI				
Overweight			0.08 (-0.06, 0.22)	
Obese			0.15 (-0.03, 0.34)	
- constant	0.11 (-0.05, 0.27)		0.08 (-0.16, 0.31)	
BDE-99				
- T2DM	-0.28 (-0.62, 0.05)		-0.20 (-0.54, 0.15)	
- Sampling year				
T2	0.06 (-0.24, 0.37)		0.04 (-0.27, 0.35)	
T3	0.17 (-0.12, 0.46)		0.07 (-0.22, 0.36)	
T4	-0.47 (-0.87, -0.07)		-0.52 (-0.93, -0.12)	
T5	0.11 (-0.29, 0.51)		-0.04 (-0.46, 0.38)	
- Interactions		0.85		0.85
T2DMxT2	0.20 (-0.25, 0.64)		0.19 (-0.25, 0.63)	
T2DMxT3	0.23 (-0.20, 0.65)		0.21 (-0.22, 0.63)	
T2DMxT4	0.04 (-0.60, 0.67)		-0.009 (-0.63, 0.61)	
T2DMxT5	0.17 (-0.46, 0.81)		0.12 (-0.49, 0.74)	
- Sex			0.23 (-0.06, 0.51)	
- Age at T1			-0.002 (-0.01, 0.007)	
- Weight change			0.006 (-0.01, 0.02)	
- Parity			0.01 (-0.08, 0.11)	
- Breastfeeding			0.007 (-0.004, 0.02)	
- Total Lipids			-0.12 (-0.17, -0.08)	
- BMI				
Overweight			0.11 (-0.09, 0.30)	
Obese			-0.04 (-0.28, 0.21)	
- constant	-0.85 (-1.06, -0.63)		-0.99 (-1.30, -0.69)	
BDE-100				

- T2DM	-0.13 (-0.45, 0.20)		-0.11 (-0.43, 0.22)	
- Sampling year				
T2	0.49 (0.19, 0.78)		0.50 (0.20, 0.81)	
T3	0.70 (0.42, 0.99)		0.69 (0.39, 0.99)	
T4	-0.03 (-0.42, 0.36)		-0.03 (-0.43, 0.37)	
T5	0.52 (0.14, 0.89)		0.48 (0.09, 0.86)	
- Interactions		0.26		0.24
T2DMxT2	0.22 (-0.19, 0.62)		0.21 (-0.20, 0.61)	
T2DMxT3	0.33 (-0.08, 0.75)		0.30 (-0.12, 0.72)	
T2DMxT4	-0.21 (-0.77, 0.35)		-0.27 (-0.83, 0.30)	
T2DMxT5	0.08 (-0.43, 0.60)		0.01 (-0.50, 0.53)	
- Sex			0.50 (0.22, 0.77)	
- Age at T1			0.004 (-0.006, 0.01)	
- Weight change			-0.009 (-0.02, 0.008)	
- Parity			0.02 (-0.07, 0.11)	
- Breastfeeding			0.005 (-0.006, 0.02)	
- Total Lipids			-0.04 (-0.09, 0.008)	
- BMI				
Overweight			-0.04 (-0.24, 0.15)	
Obese			0.05 (-0.20, 0.31)	
- constant	-1.40 (-1.62, -1.17)		-1.65 (-1.94, 1.36)	
BDE-153				
- T2DM	0.01 (-0.35, 0.37)		0.09 (-0.28, 0.47)	
- Sampling year				
T2	0.99 (0.71, 1.27)		1.04 (0.76, 1.33)	
T3	1.35 (1.06, 1.63)		1.39 (1.08, 1.69)	
T4	1.54 (1.20, 1.88)		1.58 (1.22, 1.93)	
T5	1.62 (1.27, 1.97)		1.61 (1.24, 1.98)	
- Interactions		0.47		0.48
T2DMxT2	-0.33 (-0.77, 0.12)		-0.31 (-0.76, 0.13)	
T2DMxT3	-0.31 (-0.73, 0.11)		0.32 (-0.74, 0.10)	
T2DMxT4	-0.19 (-0.69, 0.32)		-0.23 (-0.74, 0.27)	
T2DMxT5	-0.12 (-0.64, 0.40)		-0,21 (-0.74, 0.31)	
- Sex			0.41 (0.10, 0.72)	
- Age at T1			-0.003 (-0.01, 0.009)	
- Weight change			-0.01 (-0.03, 0.002)	
- Parity			-0.04 (-0.14, 0.06)	
- Breastfeeding			0.004 (-0.008, 0.02)	
- Total Lipids			-0.04 (-0.09, 0.009)	
- BMI				
Overweight			-0.08 (-0.30, 0.13)	
Obese			-0.13 (-0.40, 0.14)	
- constant	-1.33 (-1.57, -1.10)		-1.44 (-1.79, -1.08)	
BDE-154				
- T2DM	-0.27 (-0.66, 0.12)		-0.13 (-0.51, 0.25)	
- Sampling year				
T2	-0.29 (-0.65, 0.06)		-0.28 (-0.65, 0.08)	
T3	-0.19 (-0.55, 0.17)		-0.27 (-0.65, 0.10)	
T4	-0.43 (-0.85, -0.02)		-0.44 (-0.87, -0.02)	
T5	-0.03 (-0.47, 0.40)		-0.18 (-0.63, 0.26)	
- Interactions		0.38		0.59
T2DMxT2	0.12 (-0.43, 0.66)		0.11 (-0.43, 0.64)	
T2DMxT3	0.40 (-0.13, 0.94)		0.36 (-0.16, 0.88)	
T2DMxT4	0.40 (-0.20, 1.01)		0.31 (-0.29, 0.90)	

T2DMxT5	0.53 (-0.16, 1.22)		0.40 (-0.28, 1.08)	
- Sex			0.27 (-0.04, 0.58)	
- Age at T1			0.0002 (-0.01, 0,01)	
- Weight change			0.003 (-0.02, 0.02)	
- Parity			-0.003 (-0.12, 0.08)	
- Breastfeeding			0.008 (-0.005, 0.02)	
- Total Lipids			-0.17 (-0.23, -0.11)	
- BMI				
Overweight			0.07 (-0.17, 0.31)	
Obese			-0.09 (-0.40, 0.21)	
- constant	-1.86 (-2.11, -1.61)		-1.97 (-2.33, -1.61)	
BDE-183				
- T2DM	0.008 (-0.41, 0.43)		0.12 (-0.30, 0.54)	
- Sampling year				
T2	-0.13 (-0.51, 0.25)		-0.13 (-0.52, 0.26)	
T3	-0.79 (-1.18, -0.40)		-0.86 (-1.27, -0.45)	
T4	-0.41 (-0.90, 0.07)		-0.44 (-0.94, 0.06)	
T5	-0.51 (-1.01, -0.005)		-0.63 (-1.15, -0.10)	
- Interactions		0.08		0.06
T2DMxT2	-0.09 (-0.68, 0.49)		-0.11 (-0.70, 0.48)	
T2DMxT3	0.05 (-0.52, 0.62)		0.02 (-0.56, 0.60)	
T2DMxT4	-0.92 (-1.68, -0.15)		-0.98 (-1.76, -0.21)	
T2DMxT5	-0.10 (-0.81, 0.61)		-0.19 (-0.90, 0.52)	
- Sex			-0.02 (-0.37, 0.34)	
- Age at T1			-0.001 (-0.01, 0.01)	
- Weight change			0.006 (-0.02, 0.03)	
- Parity			-0.03 (-0.15, 0.08)	
- Breastfeeding			0.005 (-0.01, 0.02)	
- Total Lipids			-0.13 (-0.20, -0.06)	
- BMI				
Overweight			0.08 (-0.20, 0.35)	
Obese			-0.06 (-0.36, 0.24)	
- constant	-0.78 (-1.06, -0.49)		-0.74 (-1.14, -0.34)	

Model 1: Adjusted for time (survey) and interaction between T2DM status and time (survey).
Model 2: Adjusted for time (survey), sex, age at baseline (T1), weight change, parity, breastfeeding, total lipids, BMI categories, interaction between T2DM status and time (survey).
Abbreviations: Time-point 2 (1994/95); T3: Time-point 3 (2001); T4: Time-point 4 (2007/08); T5: Time-point 5 (2015/16); T2DM: Type 2 Diabetes Mellitus; BMI: body mass index; BDE: bromodiphenyl ether.

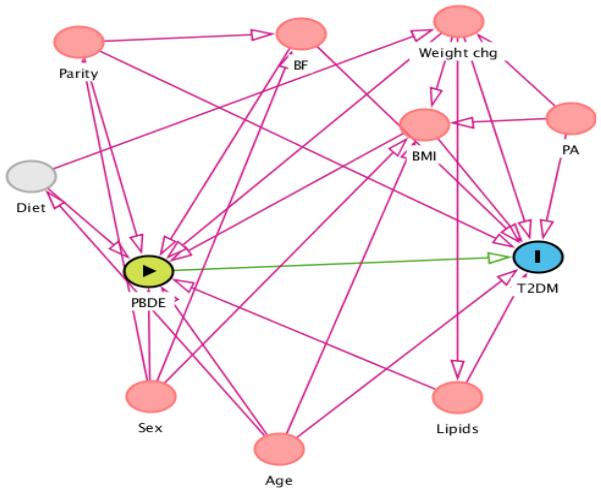

Figure S1. Directed acyclic graph (DAG) illustrating assumptions about the causal relationship between polybrominated diphenyl ethers (PBDEs) and type 2 diabetes mellitus (T2DM) and potential confounders. (The different colors in the DAG represent the following: green-exposure; blue-outcome; pink-confounders; grey-unobserved; arrows-direction of the pathways). Abbreviations: BF: breastfeeding; BMI: body mass index; PA: physical activity