PERFLUOROALKYL SUBSTANCES IN PREGNANCY AND THE RISK OF PREECLAMPSIA

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Epidemiology.

Chapel Hill
2013

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ABSTRACT

ANNE P. STARLING: Perfluoroalkyl Substances in Pregnancy and the Risk of Preeclampsia
(Under the direction of Dr. Stephanie M. Engel)

Perfluoroalkyl substances (PFASs) are persistent, ubiquitous environmental contaminants and may be related to preeclampsia, a common pregnancy complication. Previous studies have found serum concentrations of PFASs to be positively associated with serum cholesterol in non-pregnant individuals, and also associated with pregnancy-induced hypertension, including preeclampsia. Using data from the large, population-based Norwegian Mother and Child Cohort (MoBa) Study, we estimated associations between PFAS concentrations measured during pregnancy and an independently validated diagnosis of preeclampsia. Additionally, we estimated associations between mid-pregnancy PFAS concentrations and plasma lipid concentrations in order to evaluate one possible mechanism of association between PFASs and preeclampsia.

A case-cohort study was conducted to estimate associations between mid-pregnancy plasma PFAS concentrations and preeclampsia. In proportional hazards models adjusted for maternal age, pre-pregnancy body mass index, education and smoking, we observed no positive associations between PFASs and preeclampsia. However, we found an inverse association between perfluoroundecanoic acid (PFUnDA) and preeclampsia, with a hazard ratio of 0.8 (95% confidence interval [CI]=0.7, 0.9) per natural-log unit increase in PFUnDA.

In a separate cross-sectional analysis, we found positive associations between mid-pregnancy plasma concentrations of seven PFASs and high-density lipoprotein cholesterol (HDL). The greatest change in HDL per natural-log unit of exposure was associated with PFUnDA. HDL increased 3.7 mg/dL per interquartile shift in PFUnDA (95% CI=2.5, 4.9). A multi-pollutant model for HDL including seven PFAS exposures also showed the strongest association with PFUnDA compared with the other six PFASs. Additionally, perfluorooctane sulfonate (PFOS) was positively associated with plasma total cholesterol. Total cholesterol increased 4.2 mg/dL per interquartile shift in PFOS (95% CI=0.8, 7.7) in adjusted models.

We found positive associations between certain PFASs and plasma lipid parameters, and we observed that different PFASs may have different strengths of association with lipid parameters during pregnancy. We did not observe any positive associations between mid-pregnancy plasma PFAS concentrations and a validated diagnosis of preeclampsia. The plasma concentrations of PFOA in this study, while within the range of previous studies of non-occupationally exposed populations, are substantially lower than exposure levels in the previous study that found associations between PFOA and preeclampsia.

ACKNOWLEDGEMENTS

There are many people to whom I would like to express my gratitude for making this work possible. I would like to thank my advisor, Dr. Stephanie Engel, and my NIEHS mentor, Dr. Matthew Longnecker, for their tireless support, advice, and encouragement over the course of my dissertation research. I would also like to thank Dr. Julie Daniels, Dr. David Richardson, and Dr. Alison Stuebe for serving on my committee, and for their insightful comments and suggestions which greatly improved the quality of the work.

In addition to my dissertation committee, I also received guidance and assistance from scientists in the Epidemiology Branch at NIEHS. I would especially like to thank Dr. Jane Hoppin for her patient instruction as I explored research questions, Dr. Kristina Whitworth for helping to orient me to the MoBa study, and Dr. Donna Baird for her experienced and perceptive critiques of my work. I would like to thank the staff of the Norwegian Institute of Public Health and the Medical Birth Registry of Norway for their generous collaboration.

The support of my family and friends is immeasurably valuable to me in this and all of my endeavors. I am enormously grateful to my parents, Susan Starling and Kenneth Starling, who have always been my biggest advocates, and to Dr. Peter Maniloff, who inspires me to strive toward my best work, and my best self.

This research was supported in part by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences, and by an extramural award

(1-F30-ES022126-01) from the National Institute of Environmental Health Sciences. The content is solely the responsibility of the author and does not necessarily represent the official views of the National Institutes of Health. The validation of preeclampsia diagnosis was funded by the National Institute of Child Health and Human Development (R01HD058008, PI Stephanie Engel). The Norwegian Mother and Child Cohort Study is supported by the Norwegian Ministry of Health and the Ministry of Education and Research, NIH/NIEHS (contract no. N01-ES-75558), NIH/NINDS (grant no.1 UO1 NS 047537-01, grant no.2 UO1 NS 047537-06A1), and the Norwegian Research Council/ FUGE (grant no. 151918/S10).

I would like to acknowledge the support of the UNC Medical Scientist Training Program (grant no. 5T32GM008719-10, PI: Eugene Orringer) and the UNC Clinical Translation Science Award, T32 Program (grant no. 5TL1RR025745-03, PI: Marschall Runge).

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LIST OF ABBREVIATIONS

BMI	Body mass index
CI	Confidence interval
DAG	Directed acyclic graph
FOSA	Perfluoroalkyl sulfonamide
FOSE	Perfluoroalkyl sulfonamidoethanol
FTOH	Fluorotelomer alcohol
GFR	Glomerular filtration rate
HDL	High-density lipoprotein
HR	Hazard ratio
IQR	Interquartile range
LDL	Low-density lipoprotein
LOQ	Limit of quantification
MBRN	Medical Birth Registry of Norway
MoBa	Norwegian Mother and Child Cohort Study
OR	Odds ratio
PAPS	Polyfluoroalkyl phosphate surfactants
PFAS	Perfluoroalkyl substance
PFCA	Perfluorinated carboxylic acid
PFDA	Perfluorodecanoic acid
PFHpS	Perfluoroheptane sulfonate
PFHxS	Perfluorohexane sulfonate
PFNA	Perfluorononanoic acid

PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PFSA	Perfluorinated sulfonic acid
PFUnDA	Perfluoroundecanoic acid
PPAR	Peroxisome proliferator-activated receptor

CHAPTER 1: BACKGROUND AND REVIEW OF LITERATURE

1.1. Overview

Perfluoroalkyl substances (PFASs) are persistent environmental chemicals that are detectable in the blood of nearly all Americans (1) and many other populations worldwide (2). The most commonly measured species of PFAS, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), have been widely used in industrial and consumer products, including surface treatments for fabrics and carpets, food packaging, fire-fighting foam and other wetting agents (3). In both highly exposed populations as well as general population samples, PFASs have been positively associated with altered lipid profiles that are consistent with certain disturbances found in cardiovascular disease, including elevated plasma cholesterol, triglycerides, and uric acid (4-7).

A recent study of non-occupationally exposed women living in an area of high PFOA contamination in drinking water found estimated concentrations of PFOA to be positively associated with preeclampsia in both linear and quintile-based analyses (8). Preeclampsia is a serious complication of pregnancy, consisting of new-onset hypertension combined with proteinuria. Preeclampsia commonly leads to preterm delivery, and carries a high risk of maternal and infant morbidity and mortality. The possibility that plasma concentrations of widespread environmental contaminants during

pregnancy may increase the risk of preeclampsia is of substantial public health concern and merits further examination.

1.2. Critical Review of Literature

1.2.1. Humans are widely exposed to PFASs

PFASs are man-made chemicals with a variety of consumer and industrial uses, including surface treatments for textiles and carpets, coatings for food packaging and non-stick cookware, fire-fighting foam, hydraulic fluids, and various waxes, polishes, adhesives, lubricants and surfactants (3, 9, 10). These compounds are persistent in the natural environment and in the human body (11). Exposure to PFOS and PFOA is near-universal in the general U.S. population; over 99% of 2007-2008 NHANES participants age 12 or older had detectable levels in their blood, with mean serum concentrations of 13.2 ng/mL of PFOS and 4.1 ng/mL of PFOA (12). Sources of exposure in the general population may include food, drinking water, house dust, air, and breast milk (13, 14). The widespread presence of PFASs in human blood and in the environment is of substantial public health concern because toxicologic and epidemiologic studies have suggested a number of potential adverse effects.

The unique chemical structure of a PFAS consists of a fully-fluorinated carbon backbone and one or more hydrophilic heads (15). PFASs are highly resistant to degradation in the environment or metabolism in the body, owing to their extremely strong carbon-fluorine bonds (11, 16). PFASs are not lipophilic, and while the tissue distribution in humans is unknown, rodent studies suggest that PFASs likely reside primarily in the liver, kidney, and blood (17). PFASs bind strongly to the protein component of plasma (18). The geometric mean half-lives of PFOS, perfluorohexane

sulfonate (PFHxS), and PFOA in human serum were estimated in one study as 4.8 years, 7.3 years, and 3.5 years, respectively (19).

1.2.2. Sources and pathways of human exposure to PFASs

PFASs have been produced since the 1950s for a variety of industrial purposes. The unique chemical properties of PFASs render them valuable for surface treatments to repel both oil and water (20). PFASs comprise a large group of chemicals, of which we focus on two major groups of concern: (1) perfluorinated carboxylic acids (PFCAs), such as PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnDA); and (2) perfluorinated sulfonic acids (PFSAs), such as PFOS, perfluorohexane sulfonate (PFHxS), and perfluoroheptane sulfonate (PFHpS). In addition to being intentionally produced, certain PFAS species may also be generated through the degradation or metabolism of precursor molecules. PFCAs may be generated from the breakdown of fluorotelomer alcohols (FTOHs) and polyfluoroalkyl phosphate surfactants (PAPS) (20, 21), while PFSAs may result from the breakdown of perfluoroalkyl sulfonamides and sulfonamidoethanols (FOSAs, FOSEs) (15, 22, 23).

PFASs are widely detected in wildlife and environmental media (15, 24), and the levels of PFASs in human serum likely represent the accumulation of exposures from a variety of sources (25). PFCAs are still being manufactured worldwide (26), while PFSAs are no longer produced in the U.S. or Europe following the listing of PFOS as a persistent organic pollutant (POP) under the Stockholm Convention and a voluntary phase-out by the U.S. manufacturer 3M starting in 2000 (27). While PFCAs and PFSAs have traditionally been produced by separate processes and do not interconvert, the high

correlations between PFOS and PFOA concentrations detected in many species suggest a common source of exposure (25).

In non-occupationally exposed populations, ingestion of PFASs in food is believed to be the principal source of exposure (13, 24, 28). A study in 2004 estimated that average Canadians ingest 250 ng/day of PFCAs and PFOS via contaminated food products, while the total intake from all sources was estimated to be 410 ng/day (29). In addition to food sources, other pathways of PFAS exposure in the general population may include contaminated drinking water (25), breast milk (30), and ingestion or absorption from house dust. House dust is believed to contribute a greater proportion of total exposure for small children, who may spend time crawling on carpets and floors and engage in significant hand-to-mouth activity (24).

Other sources of PFAS exposure in non-occupationally exposed populations may include migration of PFASs from materials used for food packaging and food preparation (9). Fifteen species of PFAS are approved by the FDA for treating paper and other materials that come into contact with food, including microwave popcorn bags, french fry packages, pizza liners, burger boxes, and sandwich wrappers (26). These compounds may migrate from the packaging into the food at a much higher rate than was anticipated, particularly in the presence of emulsified oils (26). Furthermore, PFOA-precursor PAPS are approved by the FDA for food contact materials, and as defoaming agents in pesticides (20). It is unknown whether the toxicological consequences of direct exposure to PFCAs may differ from the consequences of exposure to precursor molecules, as the pathway from FTOH to PFCA includes several reactive intermediates (20). A recent study of indoor air, house dust, and clothes-dryer lint in the homes of pregnant women in

Vancouver, Canada, found detectable levels of FTOH as well as PFASs and PFAS-precursors FOSAs and FOSEs (23). This finding suggests that humans are routinely exposed to combinations of these chemicals in the household environment via inhalation and ingestion (23).

1.2.3. Levels of PFASs among different populations, dietary and other predictors, and trends in human exposure over time

PFASs are detectable in the serum of nearly all inhabitants of developed countries, whether or not PFAS production actually occurs in that country (31). PFASs have been measured in serum and plasma collected from populations in North America (1, 32), South America (2), Asia (33-35), Europe (36) and Australia (37). A few highly exposed populations have been studied, including retired fluorochemical workers (19, 38) and populations with PFOA-contaminated water supplies (39, 40). A summary of the detected concentrations of PFASs in various biomonitoring studies is shown in Table 1.1 and Table 1.2.

Table 1.1. Concentrations (ng/mL) of commonly measured perfluoroalkyl substances among selected populations with background levels of exposure, by matrix and year of collection.

Study Authors	Country/Region	Year	Matrix	Sex	PFOS	PFOA	PFHxS	PFNA
Current Study	Norway	2003-2008	plasma	Female	13*	2.8*	0.7	0.5
Whitworth et al. (41)	Norway	2003-2004	plasma	Female	13*	2.2*		
Inoue et al. (42)	Japan	2003	serum	Female	8.1*			
Harada et al. (43)	Japan/Kyoto	2003	serum	Female	13.8	7.1		
Harada et al. (43)	Japan/Kyoto	2003	serum	Male	28.1	12.4		
Harada et al. (43)	Japan/Akita	2003	serum	Female	6.9	2.5		
Harada et al. (43)	Japan/Akita	2003	serum	Male	12.9	3.4		
Harada et al. (43)	Japan/Miyagi	2003	serum	Female	3.5	2.8		
Harada et al. (43)	Japan/Miyagi	2003	serum	Male	5.7	3.3		
Haug et al. (44)	Norway	2003	serum	Both sexes	32	4.1	2.2	1.1
Calafat et al. (1)	US/NHANES	2003-2004	serum	Female	18.4	3.5	1.7	0.9
Calafat et al.(1)	US/NHANES	2003-2004	serum	Male	23.3	4.5	2.2	1.1
Kato et al. (12)	US/NHANES	2003-2004	serum	Both sexes	20.7	3.95	1.93	0.966
Midasch et al. (45)	Germany	2003	plasma	Female	13*	2.6*		
Karrman et al. (46)	Sweden	2004	serum	Female	20.7	3.8	4.7	0.8
Haug et al. (36)	Norway	2004	serum	Male	18	3.4		0.78
Monroy et al. (47)	Canada	2004-2005	serum	Female	16.2	2.2	4.1	0.8
von Ehrenstein et al. (48)	US/NC	2004-2005	serum	Female	21.9	3.9	1.9	1.2
Grandjean et al. (49)	Faroe Islands	2004-2006	serum	Both sexes	16.7	4.1	0.63	1
Haug et al. (36)	Norway	2005	serum	Male	21	3.5	1.6	0.85
Kato et al. (12)	US/NHANES	2005-2006	serum	Both sexes	17.1	3.92	1.67	1.09
Chan et al. (50)	Canada	2005-2006	serum	Female	7.4	1.4	1.1	
Haug et al. (36)	Norway	2006	serum	Male	12	2.7		0.55

Table 1.1, cont.

Study Authors	Country/Region	Year	Matrix	Sex	PFOS	PFOA	PFHxS	PFNA
Holzer et al. (40)	Germany	2006	plasma	Both sexes	4.6	4.8	0.8	
Holzer et al. (40)	Germany	2006	plasma	Female	5.2	2.8	0.6	
Holzer et al. (40)	Germany	2006	plasma	Male	9.7	5.8	2.2	
Lin et al. (51)	Taiwan	2006-2008	plasma	Female	8.1*	2.45*		1.81
Lin et al. (51)	Taiwan	2006-2008	plasma	Male	11.8*	0.75*		1.52
Kato et al. (12)	US/NHANES	2007-2008	serum	Both sexes	13.2	4.13	1.96	1.49
Kato et al. (12)	US/NHANES	2007-2008	serum	Female	10.7	3.56	1.46	1.33
Kato et al. (12)	US/NHANES	2007-2008	serum	Male	16.3	4.8	2.63	1.66
Fromme et al. (14)	Germany	2007-2009	plasma	Female	3.5	2.3	0.6	0.8
Haines & Murray (52)	Canada	2007-2009	plasma	Female	7.07	2.17		
Haines & Murray (52)	Canada	2007-2009	plasma	Male	11.13	2.94		
Kim et al. (53)	Korea	2007	serum	Female	5.6	1.6	0.89	0.79
Haug et al. (36)	Norway	2007	serum	Female	10	1.9	0.94	0.94
Haug et al. (36)	Norway	2007	serum	Male	10	2.1	0.75	0.62
Schecter et al. (54)	US/Texas	2009	serum	Both sexes	4.1*	2.85*	1.2*	1.2*
Wang et al. (55)	US/California	2009	serum	Female	9.44	2.21	0.88	1.01

Note: All values are means, with the exception of medians which are marked with a (*).

Table 1.2. Concentrations (ng/mL) of commonly measured perfluoroalkyl substances among selected populations with high levels of exposure, by matrix and year of collection.

Study Authors	Country/Region	Year	Matrix	Sex	PFOS	PFOA	PFHxS	PFNA
Olsen et al. (4)	Belgium	2000	serum	Female	130	70		
Olsen et al. (4)	Belgium	2000	serum	Male	960	1030		
Olsen et al. (4)	US/Alabama	2000	serum	Female	1400	1900		
Olsen et al. (4)	US/Alabama	2000	serum	Male	930	1230		
Sakr et al. (56)	US	2004	serum	Both sexes		428		
Frisbee et al. (57)	US/WV&OH	2005-2006	serum	Both sexes	23.3	82.9	5.1	1.6
Holzer et al. (40)	Germany	2006	plasma	Both sexes	4.9	22.1	1.2	
Holzer et al. (40)	Germany	2006	plasma	Female	5.8	23.4	1.1	
Holzer et al. (40)	Germany	2006	plasma	Male	10.5	25.3	2.5	
Zhang et al. (35)	China	2008-2009	serum	Both sexes	14.18	6.93	0.45	
Zhang et al. (35)	China	2008-2010	serum	Female	6.42*	3.51*		
Zhang et al. (35)	China	2008-2011	serum	Male	16.38*	3.49*		
Freberg et al. (58)	Norway	2009	serum	Male	26*	57*	1.5*	12*

Note: All values are means, with the exception of medians which are marked with a (*).

The predictors of PFAS levels in background-exposed populations have varied across studies. Some studies have noted positive associations between serum PFASs and intake of red meat or packaged snack foods (59), while others have emphasized intake of seafood and especially shellfish (28, 44). A study from Denmark found intake of eggs, dairy, and cereals to be weakly predictive of PFOS (31). This variation in dietary predictors is not surprising among studies conducted in different countries or regions, as the PFAS content of food sources likely varies substantially between geographic areas (29). One study found that preparing food by frying was positively associated with PFAS levels, while body mass index, active smoking, and alcohol consumption were negatively associated (31). Another study, however, reported positive associations between body mass index and serum PFAS concentration (60).

Unlike many persistent pollutants, PFASs have not been shown to be higher in adults than children (25). However, among 300 children in Texas, increasing age (up to age 12) was associated with increasing levels of PFOS, PFOA, PFNA, and PFHxS (54). Due to changes in PFAS production that occurred at the same time as this study, it is unclear whether the observed association between PFAS levels and age in children is actually due to accumulating exposures during childhood, or to lower cumulative intake among younger children following the cessation of PFOS production in the U.S., discussed below. Among adults, age may be a predictor of PFASs in some populations but not in others. No relationship with age was found in studies from Denmark (31), China (33), or the United States (61); however, studies from Australia (37) and Norway (36) observed a trend of increasing PFOS with age.

In some populations, levels of certain PFASs appear to be higher in men than women (12, 43, 61, 62). Two studies from China reported higher levels of PFOS in men than women, but no statistically significant sex difference in levels of PFCAs (33, 35). Studies of children have generally reported no significant sex difference in PFAS levels (54, 62).

PFAS levels in the United States declined between the 1999-2000 and 2003-2004 NHANES, by approximately 32% for PFOS and 25% for PFOA (1). This drop may be partially attributed to the 2000-2002 phase-out of PFOS manufacturing in the United States, and to the simultaneous reduction in global PFCA manufacturing emissions (10). Declines of similar magnitude in both PFOS and PFOA were observed in pooled blood samples from Norway between 2000 and 2004 (36). However, there is some evidence in the United States that PFOA levels have remained relatively constant between 2004 and 2008, while serum concentrations of PFNA have increased (12). Table 1.3 shows serum PFAS concentrations in the U.S. since 1999, as well as comparable levels in Norway.

Table 1.3. Mean serum concentrations (ng/mL) of selected PFASs among adults with background levels of exposure in the United States and Norway, by year and sex.

Country	Year	Sex	PFOS	PFOA	PFHxS	PFNA
Norway	1998	Female	15	3.1	1.2	0.46
Norway	1998	Male	31	4.3	1.9	0.67
Norway	2002	Male	27	3.9	--	0.75
Norway	2004	Male	18	3.4	--	0.78
Norway	2005	Male	21	3.5	1.6	0.85
Norway	2006	Male	12	2.7	--	0.55
Norway	2007	Male	10	2.1	0.75	0.62
US/NHANES	1999-2000	Both sexes	30.4	5.21	2.13	0.55
US/NHANES	2003-2004	Both sexes	20.7	3.95	1.93	0.97
US/NHANES	2005-2006	Both sexes	17.1	3.92	1.67	1.09
US/NHANES	2007-2008	Both sexes	13.2	4.13	1.96	1.49
US/NHANES	2007-2008	Female	10.7	3.56	1.46	1.33
US/NHANES	2007-2008	Male	16.3	4.80	2.63	1.66

Note: Sources of data (12, 36). Values shown as missing were not reported.

1.2.4. Potential health effects of PFAS exposure

In animal studies, PFAS exposure has been associated with a variety of adverse effects, including hepatotoxicity, immunotoxicity, tumorigenesis, and developmental and reproductive toxicity (11). The hepatotoxic effects of PFAS exposure in animals may manifest as liver tumors, elevated liver enzymes, and hepatomegaly, as well as altered hepatic immune function (63). In rats and non-human primates, liver hypertrophy associated with PFOS has also been accompanied by decreases in total cholesterol and HDL cholesterol (64, 65), possibly due to impaired lipoprotein production (66).

Immunotoxic effects of PFASs demonstrated in animal studies include reductions in lymphoid organ weights, reduced lymphoid cell numbers, and reduced antibody synthesis (67). Studies in mice suggest that PFAS exposure impairs the adaptive immune response (including lymphocyte proliferation and NK cell activity) while activating

certain components of the innate immune response (68-71). T-cell dependent antibody responses, which require the integrated functioning of the humoral and cell-mediated immune systems, may be impaired in mice at doses of PFOS that are within the equivalent range of human background exposures (72). Tumors associated with PFAS exposure in rats include liver, Leydig cell, and pancreatic acinar cell tumors (17). Non-genotoxic carcinogenic effects in cells *in vitro* have also been observed (73).

Developmental and reproductive effects associated with prenatal PFAS exposure in rats and mice include low birth weight (74), structural abnormalities, and increased neonatal mortality (75). Additionally, pregnant rats exposed to PFOS developed significantly depressed plasma thyroxine (T4) and triiodothyronine (T3) without the normally expected increase in thyroid-stimulating hormone (74). There may also be delayed effects on offspring due to PFAS exposure in utero. In mice, low-dose PFOA exposure during prenatal development was associated with excess weight gain in midlife, as well as elevated insulin and leptin levels, among gonadally intact females only (76).

The mechanism of the observed toxic effects of PFAS exposure is not fully known. In rodents, PFASs appear to act as agonists of peroxisome proliferator-activated receptor alpha (PPARα), a nuclear receptor which regulates a number of genes involved in fatty acid oxidation, lipid transport, glucose metabolism, and other metabolic functions (77). PFASs may also activate other PPARs, including PPARγ (78). The impact of PPAR agonism by PFASs on lipid metabolism *in vivo* is unclear; the result of high-dose PFAS exposure in laboratory animals is generally a reduction in total cholesterol and triglycerides (17, 64, 65), whereas the opposite pattern is detected in highly exposed humans, as described below. Agonism of PPARα is also believed to be responsible for

the liver toxicity and hepatocarcinogenesis caused by PFAS exposure in rodents (11), although this explanation remains controversial (79). PFAS exposure may also alter the expression of genes involved in steroid and glucose metabolism (80, 81) via interaction with other nuclear receptors, including the constitutive androstane receptor and the pregnane X receptor (82-85).

The developmental and immunotoxic effects in animals may be only partially mediated by PPARα agonism. PPARα knockout mice did not show reduced neonatal survival after PFOA exposure, in contrast to PFOA-exposed wild type mice (83); however, PFOS exposure produced similar effects on neonatal mortality in wild type and knockout mice, suggesting a mechanism of developmental toxicity by PFOS that operates independently of PPARα (82). Finally, *in vitro* human peripheral leukocytes exposed to PFASs show reduced cytokine production that appeared to be dependent on PPARα for PFOA but independent for PFOS exposure (86).

Of interest for the etiology of preeclampsia, PFOS exposure has been linked in animal studies to a shift in the balance of Th-1 and Th-2 mediated immunity (69, 70). The predominance of Th-2 (humoral) immunity over Th-1 (cell-mediated) immunity is normal during healthy pregnancy, and has been hypothesized to play a role in preventing the maternal T-cells from attacking the fetus (87). Alterations of the balance between Th-1 and Th-2 immunity during pregnancy could potentially lead to increased risk of fetal rejection and other adverse pregnancy outcomes. However, the shift demonstrated by the response of *ex vivo* splenocytes from male mice exposed to PFOS was a reduction in Th-1 associated cytokines (IL-2 and IFN-γ) and an increase in a Th-2 associated cytokine (IL-4), which would not promote over-activation of cell-mediated immunity (69,

70). The effects of PFAS exposure on the adaptive immune system during pregnancy are unknown.

Despite the ubiquitous presence of PFASs in human plasma, the potential for adverse health effects resulting from chronic, low-level exposure is not well-established. In both highly exposed populations as well as general population samples, PFASs have been associated with altered lipid profiles that are consistent with risk factors for cardiovascular disease, including elevated plasma cholesterol, triglycerides, and uric acid (4-7). These findings have not been consistent across populations, however, possibly owing to the age and sex distribution of the population studied, or to differences in the magnitude of the exposure.

One highly exposed population that has been the subject of extensive study is located in a region of West Virginia and Ohio where drinking water was contaminated with PFOA from a nearby factory. Among 46,294 adults in this population, both PFOA and PFOS (which was not elevated above background levels) were positively associated with total cholesterol and LDL cholesterol, and positively though less strongly associated with triglycerides (5). The ratio of total cholesterol to HDL cholesterol, an important indicator of cardiovascular risk, was also positively associated with both PFOS and PFOA, although there was no linear association between PFOS or PFOA and HDL cholesterol (5). Among 12,476 children and adolescents in the same PFOA-exposed population, serum PFOA was positively associated with total cholesterol, LDL cholesterol, and triglycerides, while serum PFOS was positively associated with total cholesterol, LDL cholesterol, and HDL cholesterol (57). Both of these studies included roughly equal numbers of males and females.

Occupational studies of predominantly male, adult workers have generally reported positive cross-sectional associations between PFOA and total cholesterol, and inconsistent results for associations with other lipid parameters. One study observed positive associations between PFOA and total cholesterol and LDL cholesterol, but no association with HDL cholesterol or triglycerides (56). Another occupational study observed significant positive associations between PFOA and both total cholesterol and uric acid (38). Yet another cross-sectional study of predominantly male workers found positive associations between both PFOS and PFOA and serum total cholesterol and triglycerides, and no associations with HDL cholesterol (4). A recent study of fluorochemical plant workers in China found a negative association between PFOA and HDL cholesterol (88).

Associations between serum PFASs and lipid concentrations have also been studied cross-sectionally in non-pregnant general population samples. In a sample of the U.S. population aged 12 and older, using data from the 2003-2004 NHANES survey, three of the four PFASs examined (PFOS, PFOA, and PFNA) were positively associated with total cholesterol and non-HDL cholesterol, while PFHxS was negatively associated (7). However, the study excluded women who were pregnant or breastfeeding. By contrast, a cross-sectional study using the Canadian Health Measures Survey found significant positive associations between PFHxS and total cholesterol, LDL cholesterol, and non-HDL cholesterol among non-pregnant adults (89). The same study observed positive unweighted associations between PFOA and PFOS and total cholesterol, but the findings did not remain significant after appropriately weighting for the survey design (89). In a study of 723 non-pregnant Inuit adults with background levels of PFASs, a

positive association was observed between PFOS and HDL cholesterol in both men and women, and no association was observed between PFOS and LDL cholesterol or non-HDL cholesterol (90). To our knowledge, no previous studies of the associations between PFAS levels and plasma lipid concentrations have been conducted among pregnant women.

Outcomes related to pregnancy and reproductive health have been investigated in highly exposed as well as in background-exposed populations. Increased time to pregnancy was associated with PFOA and PFOS in one general population study (91), but two other studies failed to replicate this finding (41, 92). In the highly PFOA-exposed C8 cohort, researchers concluded that there was a "probable link" between estimated PFOA concentration and pregnancy-induced hypertension (including preeclampsia) (93). In two studies of the C8 cohort, self-reported preeclampsia was positively associated with maternal PFOA levels (8, 94). Neither study measured maternal PFOA concentration during the pregnancies of interest; one study employed a predictive model to assign PFOA exposure at the time of pregnancy (8), while the other study used measured PFOA and PFOS serum concentrations up to 5 years after the pregnancy (94). Some of the potential pathways by which PFASs may be related to preeclampsia are discussed in Section 1.2.6, below.

While high-dose PFAS exposure in laboratory animals has toxic effects on the fetus, there is currently no scientific consensus as to whether the relatively low levels of exposure found in the general population may result in adverse developmental effects in humans (95). However, at least certain PFASs appear to cross the human placenta (47, 96), and some recent evidence suggests that prenatal PFAS exposure may have long-term

effects on the health of offspring. For example, in a prospective cohort of Danish infants, maternal serum PFOA concentration during pregnancy was associated with an elevated risk of obesity at 20 years in female offspring only (97); which parallels findings in mice described above (76).

1.2.5. Epidemiology of preeclampsia

Preeclampsia is a disease of pregnancy, defined by the new onset of hypertension and proteinuria after 20 weeks of pregnancy (98, 99). This study used a definition based on the American College of Obstetricians and Gynecologists (ACOG) diagnostic criteria for preeclampsia (100), and required both of the following, documented at the same clinic visit: (1) systolic blood pressure of at least 140 mm Hg *or* diastolic blood pressure of at least 90 mm Hg, occurring after 20 completed weeks of gestation in a woman with previously normal blood pressure; and (2) proteinuria, defined as at least +1 on urine dipstick measurement.

The proximate cause of preeclampsia is believed to be reduced placental perfusion, likely resulting from the failure of the spiral arteries of the placental bed to undergo normal physiological changes of pregnancy (101). The consequent mismatch of fetal requirements and maternal supply leads to a suite of maternal inflammatory responses, including vasospasm, activation of the coagulation cascade, and reduced plasma volume through release of fluid into the intravascular space (102). The resultant maternal disease may include signs of hypoperfusion in organs throughout the body (103). The reason for the initial reduction in placental perfusion initiating the process is the subject of intensive study (104).

Preeclampsia affects approximately 3% of pregnancies in the United States (105, 106), and remains a leading cause of maternal mortality in both developed and developing countries. Approximately 16% of maternal deaths in industrialized countries are caused by preeclampsia (107). The incidence of preeclampsia has increased in the United States (105) and in Norway (108, 109) during the past three decades. Approximately 42% of indicated (non-spontaneous) preterm births in the United States, and 15% of all preterm births, may be attributed to preeclampsia (102, 110).

The short-term consequences of preeclampsia may include adverse health outcomes for both the pregnant woman and the fetus or infant. Preeclamptic women are at elevated risk for developing eclampsia (seizures), coma, death, stroke, acute renal failure, pulmonary edema, acute respiratory distress, elevated transaminases, liver hematoma, and hepatic failure (111, 112). Possible adverse outcomes for infants of preeclamptic pregnancies include stillbirth or preterm birth, neonatal mortality, low Apgar scores, febrile seizures, encephalopathy, and growth restriction (112).

Furthermore, evidence suggests that adverse maternal and infant outcomes may extend far beyond the preeclamptic pregnancy. Women who experience pregnancies complicated by preeclampsia are at elevated risk of cardiovascular disease, including hypertension, stroke and venous thromboembolism later in life (113). This association may be due to the physiologic stress of pregnancy unmasking pre-existing tendencies toward cardiovascular disease (114). Long-term health consequences may exist for the infants of preeclamptic pregnancies as well, especially if the management of the disease requires preterm delivery. Preterm infants are at increased risk of motor and cognitive deficits, relative to infants born at term (115). Finally, infants who experience a

preeclamptic uterine environment are at elevated risk for hypertension in adolescence and coronary heart disease later in life (116, 117); however, it is unclear whether the risk in the offspring is due to the environment *in utero*, or to a genetic predisposition shared with the mother (118).

The etiology of preeclampsia remains obscure, but is likely multifactorial with both genetic and environmental contributions. Known risk factors include nulliparity, personal or family history of preeclampsia, chronic hypertension, diabetes mellitus, autoimmune disorders, kidney disease, obesity, multiple gestation, history of subfertility, African-American race, and maternal age (112, 119). For unknown reasons, cigarette smoking during pregnancy is associated with a 30% reduction in the risk of preeclampsia (120). Limited exposure to the sperm of the partner who fathered the current pregnancy has been cited as a risk factor, but it is unclear whether conceiving with a new partner is truly a risk factor, or if the increased risk actually results from the longer interpregnancy interval that often accompanies a change in partners (121). First pregnancies have more than twice the risk of preeclampsia compared with later pregnancies (122). There is a strong risk of recurrence: among women who have had a previous preeclamptic pregnancy, the risk of preeclampsia in the second pregnancy is 14.7% (122).

Some researchers have proposed that preeclampsia may be classified into two subtypes, defined by early (prior to 34 weeks of gestation) versus late (after the completion of 34 weeks) onset (123, 124). Early onset preeclampsia is associated with high vascular resistance and low cardiac output, while later onset is associated with low vascular resistance and occurs more frequently in women with obesity, chronic hypertension, diabetes mellitus, or other preexisting disease (124). Furthermore, these

two subtypes may have distinct etiologies, as indicated by different lipid profiles at 18 weeks of gestation in women who subsequently developed early versus late preeclampsia (125).

In the clinical setting, preeclampsia is categorized as mild or severe, based on blood pressure measurements and the degree of perturbation of lab values. The ACOG diagnostic criteria for mild preeclampsia include: 1) new onset elevated blood pressure (at least 140 mm Hg systolic or at least 90 mm Hg diastolic) occurring after 20 weeks of gestation, and 2) urinary protein excretion of at least 0.3g/day in a 24-hour urine specimen. Severe preeclampsia includes both of these features, and also one or more of the following criteria: 1) blood pressure of at least 160 mm Hg systolic or at least 110 mm Hg diastolic on at least two occasions, measured 6 hours apart and while patient is at rest, 2) urinary protein excretion of at least 5g/day in a 24-hour urine specimen or at least 3+ urine dipstick protein measurement on two samples collected 4 hours apart, 3) oliguria of less than 500mL of urine in 24 hours, 4) cerebral or visual disturbances, 5) pulmonary edema or cyanosis, 6) epigastric or right upper quadrant pain, 7) impaired liver function, 8) thrombocytopenia, or 9) fetal growth restriction (100).

Several genes linked to maternal endothelial function, vasoactive proteins, coagulation and immune function have been associated with preeclampsia (126), suggesting that pre-existing, asymptomatic conditions of the vascular system might predispose women to the disorder. Women with low pre-pregnancy plasma volume, and intolerance to the normal plasma volume expansion of pregnancy, have elevated risk of developing preeclampsia (127, 128). Similarly, women who have habitually elevated sympathetic tone may be at elevated risk for preeclampsia (129).

The maternal immune system is carefully regulated during normal placentation, and disruption of this system is one possible mechanism for the development of preeclampsia. While normal pregnancy is characterized by a predominance of Th-2 (humoral) immunity and associated cytokines (87), the cytokine profile detected in preeclamptic patients appears to reflect an altered balance of Th-1 and Th-2 type CD4 positive helper T-cells (130). Specifically, preeclamptic patients demonstrate an excess of Th-1 cells, which secrete the pro-inflammatory cytokines IL-2, IFN-γ and TGFβ, and a relative decrease in Th-2 type cells, which secrete cytokines IL-4, IL-5, IL-6, and IL-13 (130). An excess of Th-1-type cytokines could increase the risk of cell-mediated immune rejection of the fetus.

Exposure to certain environmental contaminants may increase a woman's risk of developing preeclampsia. Engaging in activities related to pesticide exposure in the first trimester of pregnancy was associated with an elevated risk of preeclampsia and pregnancy-induced hypertension among the wives of farmers in Iowa and North Carolina (131). Additionally, a study of air pollution in California showed significantly elevated risks of preeclampsia associated with nitrogen oxides and with $PM_{2.5}$ (132). Most relevant to this study, among women in a region with PFOA-contaminated drinking water (the C8 cohort), higher estimated PFOA levels during pregnancy were associated with increased risk of preeclampsia (8).

1.2.6. Potential links between PFAS exposure and preeclampsia

As described above, preeclampsia shares many features with cardiovascular disease in non-pregnant individuals, including endothelial dysfunction, elevated

cholesterol and triglycerides, and hyperuricemia (99). Serum PFAS concentrations have been associated with altered lipid profiles as well as elevated uric acid in non-pregnant individuals (5, 6). If PFASs were shown to be causally associated with elevations in cholesterol, uric acid, and triglycerides in non-pregnant individuals, it is plausible that the same biological mechanisms could also increase the risk of preeclampsia in pregnant women. For example, the partial agonism of peroxisome proliferator-activated receptors (PPARs) by PFASs may alter the expression of genes involved in lipid and glucose metabolism, as well as vascular inflammation (77). Notably, administration of a PPARγ antagonist to pregnant rats produced a preeclampsia-like syndrome, including hypertension, proteinuria, and endothelial dysfunction (133).

It is plausible that the lipid pattern associated with PFAS exposure may differ in pregnant women as compared with non-pregnant women. Normal pregnancy is characterized by dramatic changes in plasma volume, glomerular filtration rate, and the concentration of various clinical chemistry analytes relative to the non-pregnant state. The pattern of changes in plasma lipid concentrations during normal pregnancy has been well-documented (134). Total cholesterol rises 25-50% over non-pregnant levels (135). High-density lipoproteins (HDL) begin to increase early in pregnancy and reach their peak at approximately the 28th week of pregnancy (136). Low-density lipoproteins (LDL) are characterized by a slight initial decrease followed by a gradual increase. Very low-density lipoproteins, the principal carriers of triglycerides, increase continuously from between the 14th and 36th week (134). By late pregnancy, triglycerides are typically elevated 200-400% times over pre-pregnant levels (134, 135). In addition to these changes in plasma concentration of lipoproteins, there are qualitative changes in the HDL

and LDL particles during normal pregnancy. In some women, particularly those with steep increases in triglyceride levels during pregnancy, there is a subclass shift toward smaller, denser LDL particles (137). Among HDL particles, there is a subclass shift to larger HDL species (136).

In addition, the kidneys in normal pregnancy adopt a state of hyperfiltration, in which the renal plasma flow and glomerular filtration rate (GFR) increase. The GFR begins to increase in the first trimester and peaks in the 2nd half of pregnancy at approximately 40-60% above the non-pregnant level (138). There is also an expansion of plasma volume in normal pregnancy and a reduction in plasma oncotic pressure along the glomerular capillaries (138). The increased GFR leads to increased excretion of numerous substances normally found in plasma, including uric acid, a metabolic product linked to cardiovascular disease risk and to preeclampsia (139, 140).

Studies of the association between lipid profile and preeclampsia risk have produced inconclusive results, possibly related to the etiologic heterogeneity of the preeclampsia syndrome. One study found that maternal dyslipidemia at 15-20 weeks gestation was associated with mild, but not severe, preeclampsia (141). Another study found that hypertriglyceridemic dyslipidemia before 20 weeks of gestation was associated with early-onset but not late-onset preeclampsia (125). Each of these studies measured non-fasting serum lipids. The severe and mild variants of the preeclampsia syndrome may have distinct lipid profiles as early as mid-pregnancy (141), suggesting a latent phase of the disorder that may be present before the clinical signs of preeclampsia appear. There is currently no definitive test prior to 20 weeks to predict the subsequent

development of preeclampsia, although certain combinations of maternal characteristics and biochemical markers may prove clinically useful (142).

Biomarkers measured early in pregnancy that are associated with subsequent preeclampsia risk may provide insight into the mechanisms leading to certain types of preeclampsia. For example, cystatin C is a cysteine protease inhibitor produced by the maternal decidua, which is believed to play a role in limiting the trophoblast invasion process of normal placentation (143). In non-pregnant individuals, cystatin C is a sensitive indicator of changes in glomerular filtration rate (142). In a recent nested case-control study, maternal serum cystatin C at 11 to 16 weeks of pregnancy was significantly higher in women who subsequently developed preeclampsia than in women who completed normal pregnancies (144).

While the clinical symptoms of preeclampsia resolve with delivery of the placenta, women who develop preeclampsia have an elevated post-pregnancy risk of developing cardiovascular disease, including three to four times the risk of hypertension, and two times the risk of death from cerebrovascular or cardiovascular disease, compared with women with no history of preeclampsia (113). These findings have led some researchers to view the state of pregnancy as a "stress test" that uncovers cardiovascular disease at an early, otherwise asymptomatic, stage (114). One of the features of subclinical cardiovascular disease may be chronic inflammation. Inflammation has long been described as a characteristic of the preeclamptic placenta (145). C-reactive protein (CRP), an inflammatory acute phase reactant, was found to be elevated in post-menopausal women who had developed eclampsia in a pregnancy that occurred 30 years

prior to examination (146). Elevated CRP in non-pregnant women is a marker of increased cardiovascular disease risk (147).

The immunotoxic effects of PFAS exposure may be relevant to preeclampsia as well. In vitro, PFOS is associated with a decrease in human NK cell activity (148). This finding is significant for the pathogenesis of preeclampsia because NK cells are believed to mediate the invasion of trophoblasts into decidua and spiral arteries in normal placentation (149). Insufficient invasion of the spiral arteries due to impaired NK cell function could potentially contribute to preeclampsia (150). Table 1.4 summarizes some of the observed associations with PFASs that may also relate to the pathogenesis of preeclampsia.

Table 1.4. Summary of conditions associated with perfluoroalkyl substances that may also be involved in the pathogenesis of preeclampsia.

Observed Association	Proposed Mechanism	Potential Link to Preeclampsia
Elevated lipids, triglycerides(5, 57)	Partial agonism of peroxisome proliferator activated receptors (PPAR)	Early-onset preeclampsia is characterized by elevated cholesterol and triglycerides (125); administration of PPAR-γ antagonist in rats leads to preeclampsia-like syndrome (133).
Immunotoxicity (11), decreased adaptive immune response (151), including impaired natural killer (NK) cell activity (148)	Indirect inhibition of lymphocyte proliferation (151)	NK cells are involved in the normal invasion of trophoblasts into the uterine spiral arteries to supply the placenta with adequate blood (149), and placental hypoperfusion is a possible etiologic factor in preeclampsia (150).

CHAPTER 2: STATEMENT OF SPECIFIC AIMS

2.1. Specific Aims

2.1.1. To quantify the association between mid-pregnancy plasma PFAS levels and a validated diagnosis of preeclampsia among nulliparous women.

PFAS concentrations were measured in plasma collected at mid-pregnancy from 500 validated nulliparous preeclampsia cases, and 567 pregnancies randomly selected from all eligible nulliparous women without regard to case status. Of the 567 members of the subcohort, 17 were also cases. Preeclampsia diagnosis was reported to the MBRN at delivery and subsequently validated by independent review of antenatal medical records. An association between PFAS levels and preeclampsia has been suggested by a previous study of women highly exposed to PFOA, and our study design employs a well-characterized exposure and outcome in order to estimate this association among nulliparous women.

2.1.2. To determine the cross-sectional associations between mid-pregnancy PFAS levels and several clinical chemistries pertinent to lipid metabolism and cardiovascular health.

PFAS concentrations were measured in plasma from 950 women in MoBa, along with the following substances related to cardiovascular risk and inflammation: total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), C-reactive protein, triglycerides and uric acid. PFASs have been associated with disturbances in lipid metabolism in non-pregnant individuals, and may be linked to chronic

inflammation. The cross-sectional associations between plasma PFASs and lipids among pregnant women have not previously been studied.

2.2. Hypotheses and Interpretation

2.2.1. Specific Aim 1

If the hazard ratio for the association between mid-pregnancy PFAS concentrations and preeclampsia were elevated, this would indicate that nulliparous women with higher PFAS levels at mid-pregnancy are at greater risk of developing preeclampsia, conditional on gestational age, than women with lower PFAS levels. A possible interpretation of this result is a causal relationship between PFAS levels in plasma and preeclampsia. However, other possible explanations for this finding include confounding by an unmeasured variable that is related to both PFAS concentration at mid-pregnancy and to preeclampsia, or chance.

Similarly, if an elevated odds ratio were produced by a logistic regression model of preeclampsia as a function of mid-pregnancy PFAS concentration, this would indicate that the risk of developing preeclampsia over the entire duration of pregnancy increases with increased PFAS concentration. This result could also be the result of a causal relationship, or due to chance or bias from unmeasured confounders. If the logistic regression analysis produced a negative result, but the Cox proportional hazards model produced a positive result, this would suggest that the overall risk of preeclampsia does not vary over the entire duration of pregnancy, but that women with higher PFAS levels tend to develop the disease earlier in gestation than women with lower PFAS levels at mid-pregnancy. A potential alternative interpretation of a positive result could be reverse causality: if a pre-symptomatic stage of the preeclampsia disease process had already

influenced PFAS levels by mid-pregnancy, this could lead to an apparent association between PFASs and preeclampsia.

If an inverse association between mid-pregnancy PFAS concentration and preeclampsia were found in Cox proportional hazards models, it would indicate that nulliparous women with higher levels of PFASs are at lower risk of developing preeclampsia, conditional on gestational age, than women with lower PFAS concentrations. This finding could also be interpreted as a causal relationship (although there is no biological evidence to support this hypothesis), or as the result of confounding by unmeasured variables. Finally, if no association between mid-pregnancy PFAS concentrations and preeclampsia were detected, this could suggest either that there is no association between PFAS concentrations and preeclampsia in this population with background exposure levels, or that the study did not have sufficient power to detect the small magnitude of association that is present.

2.2.2. Specific Aim 2

Based on the results of previous epidemiologic studies, it was expected that the concentrations of seven PFASs at mid-pregnancy would be positively associated with total cholesterol, LDL cholesterol, triglycerides, and uric acid. This finding would lend support to the hypothesis that PFASs during pregnancy are associated with elevations in certain risk factors for cardiovascular disease, and would provide a potential mechanistic explanation for a positive association between PFAS concentration and preeclampsia. A null or negative association with these substances would not support that hypothesis.

A positive association with C-reactive protein would suggest an inflammatory process co-occurring with higher concentrations of PFASs. This could either indicate that PFASs cause inflammation, or that a common cause of PFAS concentration and inflammation is present. An inverse association between PFASs and C-reactive protein could indicate that PFASs protect against certain inflammatory processes, or that a common cause of increased PFASs and decreased C-reactive protein is present. Finally, a null association between PFASs and C-reactive protein would suggest that inflammation is not related to mid-pregnancy PFAS concentration.

2.3. Rationale and Innovation

This dissertation is the first study to examine PFAS levels measured at mid-pregnancy, in relation to the subsequent development of preeclampsia in nulliparous women (Aim 1). Although PFASs are persistent in the body, a decline in maternal serum levels has been observed during pregnancy and lactation (30, 47, 60) and so it may be important to measure PFASs early in pregnancy to accurately estimate any association with pregnancy outcomes. We restricted our analysis to women with no previous live or stillbirths because the risk factors for preeclampsia in later pregnancies may differ from the risk factors in first pregnancies. This study will make an important contribution to the epidemiology of PFASs in nulliparous pregnant women with background levels of exposure.

Furthermore, this will be the first study to examine PFASs in relation to clinical chemistries at mid-pregnancy (Aim 2), including total cholesterol, HDL and LDL cholesterol, triglycerides, uric acid, and C-reactive protein. Blood lipids and uric acid levels have been previously associated with PFASs in cross-sectional studies of non-

pregnant individuals (5-7, 56, 89), but the presence of such associations in pregnant women has not been explored. Additionally, this dissertation will inform our understanding of the possible mechanisms by which PFAS levels in blood may be linked to preeclampsia, by examining mid-pregnancy alterations in clinically important biomarkers.

Given that human exposure to PFASs is ubiquitous, it is a critical public health priority to evaluate whether any adverse health effects may result from chronic, low-level exposure to these compounds, particularly in pregnant women. If a causal association is established, then public health measures may be warranted to reduce exposure to PFASs in the environment, by identifying and minimizing sources of exposure. Moreover, quantifying the association between PFAS concentration and preeclampsia risk may inform the biological mechanisms underlying this common pregnancy complication. In summary, this dissertation will provide a precise estimate of the association between PFASs in pregnancy and the development of preeclampsia, and will also provide information on a possible mechanism of any association.

CHAPTER 3: METHODS

3.1. Overview of Methods

The first aim was addressed using a case-cohort study design within the large Norwegian Mother and Child Cohort (MoBa) Study. The majority of eligible validated preeclampsia cases among nulliparous women enrolled in MoBa in 2003-2007 were included in the study, as well as a random subcohort of women at risk for preeclampsia who enrolled during the same time frame. Cox proportional hazards and logistic regression models were used to determine whether plasma PFAS concentrations at mid-pregnancy were associated with onset of preeclampsia or delivery due to preeclampsia.

The second aim was addressed using a cross-sectional study of a subsample within MoBa. Weighted linear regression models were used to estimate the associations between mid-pregnancy plasma PFAS concentrations and several clinical chemistries of interest, specifically: total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, uric acid, and C-reactive protein. Multi-pollutant models were fitted for HDL in order to determine whether correlated PFASs could produce confounding in single-pollutant models. A hierarchical empirical Bayes model was also fitted in order to shrink the estimates produced by the multi-pollutant model to a common mean.

3.2. Approach

3.2.1. Subject Identification

3.2.1.1. Study population

The source population from which samples were drawn for both aims was the Norwegian Mother and Child Cohort Study (MoBa), a large prospective cohort of pregnant women and their offspring, recruited for the purpose of identifying causes of disease in both mother and child (152). The study was designed and conducted by the Norwegian Institute of Public Health (NIPH), and additional resources for the collection of biological materials were provided by the National Institute of Environmental Health Sciences (NIEHS). The study enrolled approximately 108,000 pregnancies to 90,700 women in Norway between 1999 and 2008. The majority of pregnant women in Norway who scheduled a prenatal care visit between 17 and 20 weeks of gestation were invited by mail to participate in the study. Of all women invited to participate, 39% enrolled, and 87% of enrolled women donated blood samples for analysis. The study was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate. Informed consent was obtained from each participant at enrollment. Identifying information is maintained securely by the Norwegian Institute of Public Health, which provides de-identified data sets to researchers. Further details about the study are available at http://www.fhi.no/morogbarn.

All enrolled women completed a questionnaire with details about their health history, lifestyle, and pregnancy history. Maternal plasma samples were collected at mid-pregnancy (median gestational week = 18), information on preeclampsia was obtained by record linkage with the Medical Birth Registry of Norway (MBRN), and outcome validation was performed on all recognized cases of preeclampsia as well as a random

subset of non-cases (Klungsøyr et al., submitted). Validation methods are described below.

3.2.1.2. Aim 1

For the case-cohort study, 500 cases were selected from among eligible MoBa participants who were reported to have preeclampsia on the MBRN, and whose case status was confirmed by an independent validation study. The validation study requested the antenatal records for all 4,081 women who were recorded on the MBRN as having preeclampsia, as well as 2,000 additional women without reported preeclampsia. Records were received for 90% of eligible pregnancies. These records were reviewed by study personnel for evidence of preeclampsia according to the criteria described in detail in Section 3.2.2.2., below. Additionally, women eligible to be cases in our study also met the following criteria:

1. Enrolled in MoBa during 2003-2007
2. No previous live births or stillbirths
3. Singleton pregnancy
4. Maternal mid-pregnancy plasma sample collected in EDTA tube

For the subcohort sample, 567 individuals who met the criteria described above were randomly selected from the MoBa cohort without regard to case status. Seventeen validated cases randomly selected into the subcohort sample were also included in the study as cases. Participants were excluded from this study if they had evidence of hypertension prior to pregnancy, defined as any of the following: 1) chronic hypertension

reported to the MBRN or on the MoBa questionnaire, or 2) an ICD-9 code corresponding to chronic hypertension on the antenatal medical chart, or 3) a documented blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic prior to 20 weeks of gestation on the antenatal medical chart.

3.2.1.3. Aim 2

For the cross-sectional study, we used an existing data set pertaining to 950 MoBa women selected for a previous case-control study of subfecundity. Women eligible for selection into the subfecundity study met the following criteria:

1. Enrolled in MoBa during 2003-2004
2. Planned pregnancy that resulted in a live-born child
3. Maternal mid-pregnancy plasma sample collected in EDTA tube
4. Non-missing responses to the MoBa questionnaire items regarding time-to-pregnancy

For the previous study, 400 cases were randomly selected from all eligible subfecund women (defined as a self-reported time-to-pregnancy greater than 12 months (41)), and 550 were randomly selected from all women who met the four eligibility criteria described above (with time-to-pregnancy of any length). For our cross-sectional study of mid-pregnancy PFASs and clinical chemistries, only pregnancies with complete outcome and covariate information were included in the analysis.

3.2.2. Study Design

3.2.2.1. Classification of Exposure

Maternal plasma samples were collected in EDTA tubes at mid-pregnancy antenatal visits, and samples were shipped at ambient temperature to the Norwegian Institute of Public Health in Oslo for storage and analysis. The majority of samples were received the day after collection (153). Samples were maintained at -80 degrees Celsius at the MoBa biobank in Oslo (153). The Laboratory Information Management System (LIMS) at the biobank maintains the identifying information associated with each sample and stores the locations of each aliquot within the freezer system. An evaluation of the freeze-thaw cycles of this system and the associated changes in various biomarkers has recently been published, and found minimal changes in most analytes studied after 10 freeze-thaw cycles (154).

Concentrations (ng/mL) of 19 PFAS species were measured in thawed aliquots of plasma using high-performance liquid chromatography/tandem mass spectrometry at the Norwegian Institute of Public Health. The lower limits of detection using this method are in the range of 0.0020-0.050 ng PFAS/mL serum (155). For PFOS, the total area of linear and branched isomers was integrated. Further details about the laboratory methods have been published previously (155). PFASs are chemically stable and any changes in plasma concentration during transport are believed to be negligible (156).

3.2.2.2. Classification of Outcome for Aim 1

Preeclampsia was reported to the MBRN via a standard form completed by midwives at delivery. The form has 5 check boxes pertaining to the outcome: 1)

hemolysis, elevated liver enzymes, and low platelet count (HELLP syndrome); 2) eclampsia; 3) early preeclampsia (diagnosed prior to 34 weeks of gestation); 4) mild preeclampsia; and 5) severe preeclampsia. All MoBa participants who had any form of preeclampsia reported to the MBRN had their antenatal charts requested from hospitals for the purpose of independent review and validation of the preeclampsia diagnosis. Validated cases had evidence in their medical records of meeting the following definition for preeclampsia, requiring both of the following criteria at the same clinic visit:

1. Systolic blood pressure of at least 140 mm Hg *or* diastolic blood pressure of at least 90 mm Hg, occurring after 20 completed weeks of gestation in a woman with previously normal blood pressure; *and*
2. Proteinuria, defined as at least +1 on urine dipstick measurement.

The validated preeclampsia outcome was coded as a binary variable. For cases, the gestational week at diagnosis of preeclampsia and the gestational day at delivery due to preeclampsia were recorded as continuous variables, using ultrasound-based estimates of gestational age.

3.2.2.3. Classification of Outcomes for Aim 2

Among the 950 women in the existing sample, several clinical chemistry analytes of interest were measured in the same mid-pregnancy (non-fasting) plasma sample used to evaluate PFAS concentrations. The analytes that were measured and recorded as continuous variables were: uric acid, C-reactive protein, LDL cholesterol, HDL cholesterol, triglycerides, and total cholesterol. All analytes were measured with an

Olympus AU400e Clinical Chemistry Analyzer at the National Institute of Environmental Health Sciences, using reagents from Beckman Coulter (for triglycerides and cholesterol) and Genzyme Diagnostics (for all others). The method used to measure LDL was direct enzymatic (N-geneous® LDL-ST cholesterol reagent). The within- and between-batch CV for all clinical chemistries were <5%, except for the between-batch CV for triglycerides, which was 6.3%. Binary outcome variables were created for the highest 25% of each clinical chemistry analyte (or lowest 25%, in the case of HDL cholesterol).

3.2.2.4. Covariate Ascertainment

Several covariates of interest were obtained from the MoBa enrollment questionnaire, which was filled out by participants at approximately 18 weeks of gestation, and the MBRN, which was filled out by midwives at delivery. These covariates include:

- *Maternal age at delivery.* This variable was recorded on the MBRN.
- *Pre-pregnancy BMI.* This variable was calculated based on two self-reported questionnaire items: "What did you weigh at the time you became pregnant and what do you weigh now (in kilograms)?" and "How tall are you?"
- *Smoking*. This variable was based on the question, "Do you smoke now (after you became pregnant)?" and classified as a binary variable.
- *History of chronic hypertension*. Women reporting a previous diagnosis of "high blood pressure" were excluded from the case-cohort study.

- *Education completed*. This variable was based on a questionnaire item that asked women to indicate "the highest level of education you [and the baby's father] have completed and current studies if you are still studying." Seven options were provided: 1) 9-year secondary school, 2) 1-2 year high school, 3) technical high school, 4) 3-year high school general studies or junior college, 5) regional technical college or 4-year university degree, 6) university, technical college, more than 4 years, and 7) other education. The seven options were grouped into fewer categories for the purpose of analysis.
- *Gestational age at blood draw*. This information was derived from the MoBa biospecimen records.
- *Parity*. The sample for Aim 1 was restricted to nulliparous women. Nulliparous was defined as having no previous live births or stillbirths after 16 weeks of gestation reported to the MBRN.

Additionally for Aim 1, in order to assess whether any alterations in glomerular filtration rate occurred by mid-pregnancy among women who later developed preeclampsia, the following markers of kidney function were measured in the mid-pregnancy plasma sample:

- *Creatinine*
- *Cystatin C*

Both of these analytes were measured at the National Institute of Environmental Health Sciences using an Olympus AU400e chemistry immuno-analyzer. The creatinine

measurement procedure is a modification of the Jaffe procedure in which creatinine reacts with picric acid at an alkaline pH. The cystatin C assay utilizes the sol particle immunoassay principle, using colloidal gold particles coated with anti-cystatin C specific polyclonal antibodies (157).

For Aim 2, certain variables related to pregnancy history were obtained from the enrollment questionnaire and considered as potential confounders:

- *Parity, length of interpregnancy interval,* and *history of breastfeeding.* The question stem that pertains to these variables is: "Have you ever been pregnant before?" If the respondent answered "yes," further information was requested for each prior pregnancy about the date, outcome, and number of months of breastfeeding for each previous pregnancy.

3.2.2.5. Quality Assurance/Quality Control

For measurement of PFASs in mid-pregnancy plasma, standard procedures for quality control and quality assurance were followed, including cases and non-cases randomly interspersed in each analytic batch. Pooled QC samples were randomly included in batches, and laboratory personnel were masked as to which tubes contained QC samples. Within- and between-batch coefficients of variation were calculated and reported.

For the determination of the outcome in Aim 1, only cases of preeclampsia which were validated by independent review of the antenatal medical records were included. Previous studies may have relied on registries or self-report in order to classify women as having preeclampsia. However, the positive predictive value of self-reported

preeclampsia in one validation study was observed to be less than 60% (158). In MoBa, the outcome of preeclampsia was initially recorded by midwives at birth and reported to the Medical Birth Registry of Norway (MBRN). In the validation study, antenatal medical records were requested for all MoBa participants who were identified as having preeclampsia on the MBRN, and the records were examined to identify women who met the ACOG-derived definition of preeclampsia. The use of a validated outcome should minimize outcome misclassification and improve our ability to detect an association if one exists.

3.2.3. Data Analysis

3.2.3.1. Analyses common to both Aims

The univariate distributions of each of the seven continuous PFASs detectable in greater than 50% of samples were examined, and measures of central tendency and spread were reported. The correlations between each of the seven PFASs were calculated. Quartiles of exposure were created for the assessment of dose-response relationships. The observed concentrations of PFASs were compared to the levels observed in previous studies of the general population in the U.S. and Norway, as well as to highly exposed populations (see Table 1.1 and Table 1.2).

The univariate distributions of the continuous outcome variables for Aim 2 were also examined, and measures of central tendency and spread for each clinical chemistry analyte were reported. Descriptive statistics were calculated for covariates considered potential confounders, and the number of missing values for covariates was assessed.

Bivariate associations between exposures and outcomes, covariates and outcomes, and covariates and exposures, were estimated.

3.2.3.2. Analyses specific to Aim 1

Potential confounders were identified *a priori* based on the existing literature, and a minimally sufficient adjustment set was selected based on a directed acyclic graph (DAG) representing the state of knowledge in this area. The DAG was used to identify potential confounders that are not causal intermediates or colliders. A proposed DAG for Aim 1 is presented in Figure 4.1. For this DAG, the following variables constitute a minimally sufficient adjustment set:

- *Maternal age.* Young and old maternal age have been previously associated with preeclampsia (99), and age has also been associated with PFAS levels, although inconsistently.
- *Pre-pregnancy BMI.* BMI has been previously related to PFAS levels, although both positive and inverse associations have been reported. Pre-pregnancy BMI is positively associated with preeclampsia risk (159).
- *Smoking.* Smoking is inversely associated with preeclampsia risk (120), and may also be related to PFAS levels (31).
- *Education completed*. This variable serves as a proxy for socioeconomic status, which may be associated with PFAS levels (59) and also potentially associated with preeclampsia risk.
- *Cystatin C*. Impaired kidney function, as indicated by cystatin C, may lead to elevated PFASs if urinary excretion is reduced. Additionally,

cystatin C measured at mid-pregnancy may be associated with preeclampsia risk (144). Whether changes in cystatin C may be caused by the preeclampsia disease process remains unknown, and so the status of cystatin C as a confounder is questionable.

The two principal statistical results for Aim 1 are: 1) the hazard ratio for preeclampsia, conditional on gestational age, as a function of plasma PFAS concentration at mid-pregnancy, and 2) the risk ratio of preeclampsia during pregnancy as a function of plasma PFAS concentration at mid-pregnancy. The hazard ratio was estimated using a Cox proportional hazards regression model. The risk ratio was estimated by the odds ratio produced by a multivariate logistic regression model.

There are two event times of interest for the time-to-event analyses: gestational age at diagnosis and gestational age at delivery due to preeclampsia. Each of these times was analyzed in relation to plasma PFAS levels using a multivariate Cox proportional hazards model, weighted for selection into the case-cohort study (160). Non-cases were treated as censored at their gestational age at delivery. Because prenatal care is freely available in Norway, we do not expect that the diagnosis of preeclampsia would be delayed due to limited access to health care. In severe cases of preeclampsia, diagnosis is quickly followed by delivery, often induced by health care providers. However, in more mild cases of preeclampsia, health care providers may not intervene immediately, and women may deliver weeks after the diagnosis. These mild cases may represent a subtype of preeclampsia with a unique etiology. In order to evaluate the influence of these early, mild cases on the effect estimates produced by the time-to-event analysis, both

gestational days at diagnosis and gestational days at delivery were separately evaluated as event times.

The primary advantages of using Cox proportional hazards models are (1) they utilize all available information about the timing of disease onset, and (2) they easily handle the problem of right-censoring of controls due to delivery before 40 weeks of gestation. One assumption of this model is that random censoring (for example, due to delivery of the infant for reasons other than preeclampsia) must be non-informative with regard to the outcome. It is possible that this assumption may be violated if the risk of early delivery from causes other than preeclampsia is associated with the risk of preeclampsia if the pregnancy had continued. However, preterm birth for causes other than preeclampsia is relatively uncommon in this population (<5% of non-cases).

An alternative approach used to address Aim 1 was logistic regression of the cumulative incidence of preeclampsia over the entire risk period. The use of logistic regression to estimate the risk ratio is justified because the outcome is rare in this population (fewer than 4% of pregnancies). The logistic analysis addresses the hypothesis that the overall risk of preeclampsia varies as a function of plasma PFAS levels at mid-pregnancy. However, this approach does not consider women who had pregnancies shorter than 40 weeks, and therefore had a shorter period of time at risk for preeclampsia.

3.2.3.3. Analyses specific to Aim 2

Potential confounders were identified *a priori* based on the existing literature, and a minimally sufficient adjustment set was selected based on a directed acyclic graph

(DAG) representing the state of knowledge in this area. A proposed DAG for the association between PFASs and HDL cholesterol in Aim 2 is presented in Figure 4.2. The DAG was used to identify potential confounders that are not causal intermediates or colliders. For this DAG, the following variables constitute a minimally sufficient adjustment set:

- *Maternal age.* Age has been previously associated with PFAS levels, although inconsistently across studies, and plasma lipid levels typically increase with age (161).
- *Pre-pregnancy BMI.* BMI has been previously associated with PFAS levels, although both positive and inverse associations have been reported. BMI is expected to be positively associated with blood lipid levels (162).
- *Smoking.* Smoking is positively associated with plasma lipid levels (163), and may also be related to PFAS levels (31).
- *Education completed*. This variable serves as a proxy for socioeconomic status, which may be associated with both PFAS levels (59) and with blood lipid levels (164).
- *Gestational age at blood draw.* Adjustment for this variable is important because of the substantial changes in blood volume and hemodynamics that occur throughout pregnancy.
- *Length of interpregnancy interval*. Long interpregnancy intervals are associated with increasing risk of preeclampsia (121), and may also be associated with maternal age.

- *Parity*. Previous pregnancy is associated with reductions in PFASs, possibly through transfer to the fetus or through increased excretion (14, 60).
- *Breastfeeding history*. PFASs decline during pregnancy and breastfeeding (30, 60), and breastfeeding history may be associated with later cardiovascular health and lipid levels (165).

The association between each PFAS concentration and each lipid or clinical chemistry analyte was estimated using weighted multiple linear regression models. Residuals were checked for normality. The regression coefficient for each PFAS exposure variable may be interpreted as the change in the clinical chemistry of interest (or transformed dependent variable) associated with a natural-log unit change in the concentration of PFAS, or an IQR-unit shift in ln-PFAS. Additionally, weighted logistic regression was used to estimate the risk of having high (above the 75th percentile) total cholesterol, LDL cholesterol, triglycerides, uric acid, or C-reactive protein, or having low (below the 25th percentile) HDL cholesterol.

Inverse probability-of-selection weighting was employed in order to account for the selection into the previous case-control study of subfecundity (166). While weighting has a cost in precision, this strategy is preferred in this scenario over adjusting for the selection factor, due to the possibility that subfecundity may share some common causes with the outcomes of interest (167). However, a sensitivity analysis explored the robustness of findings to an alternate strategy of adjusting for subfecundity rather than weighting. To ensure that the over-sampling of subfecund women for the prior study did

not bias the effect estimates in the present cross-sectional study, an additional analysis was restricted to the 550 women who were selected without regard to time-to-pregnancy. Additionally, an analysis was restricted to nulliparous women to examine whether this produced any change in point estimates.

In order to assess whether or not the models met the assumptions of a log-linear relationship between exposure and outcome (or linear in the logit, in the case of logistic models), residual plots were examined. Dose-response relationships were evaluated using quartile-based categories of PFAS exposure, where possible.

Various statistical methods were employed to evaluate the influence of multiple correlated PFAS exposures. The problem posed by multiple correlated exposures in this study is the difficulty in distinguishing which of the PFASs may be primarily responsible for any association detected. A multi-pollutant model for HDL was used to identify PFASs that may be acting as confounders of the PFAS-HDL association and to include them as covariates in a common model. To address concerns about possible multicollinearity in the multiple-pollutant model, the variance inflation factors were examined.

Additionally, empirical Bayes regression was employed to examine how the estimates for the mutually-adjusted PFAS-HDL associations changed when "shrunk" to a common mean (168). Empirical Bayes regression may lend stability to coefficient estimates for multiple, correlated exposures because it assumes that the coefficients are drawn from a common distribution. Imposing groupings of exposures based on prior information may also improve estimates; however, the classification of PFASs into perfluorinated carboxylic acids and perfluorinated sulfonic acids did not aid in estimation

and so coefficients were shrunk to a single common mean. The coefficients produced by single-pollutant, multiple-pollutant, and empirical Bayes models for HDL were compared with regard to precision and bias.

3.2.4. Statistical Power and Sample Size

The sample size of Aim 1 was chosen to provide sufficient statistical power to detect the magnitude of association between PFASs and preeclampsia that was anticipated in this population. Assuming a two-sided hypothesis test with type 1 error rate of 5%, and given the measured distribution of PFOS and PFOA concentration in this population, 500 cases should provide greater than 80% power to detect an odds ratio of greater than or equal to 1.43 associated with PFAS concentration above the median level of exposure. This was smaller than the effect size observed in preliminary studies in this population.

Anticipating the statistical power for Aim 2 was more challenging, as it was an exploratory analysis with several different outcomes, and no comparable data exist in populations with background levels of exposure. On the basis of the distributions of the exposures and the outcome of total cholesterol, the intended sample size of 950 provided statistical power to detect the beta-coefficients shown in Table 3.1, given a type 1 error rate of 5%. These estimates are within the range of estimates found in previous studies; however, no previous studies have been conducted in pregnant women.

Table 3.1. Power to detect associations between PFASs (ng/mL) and total cholesterol (mg/dL).

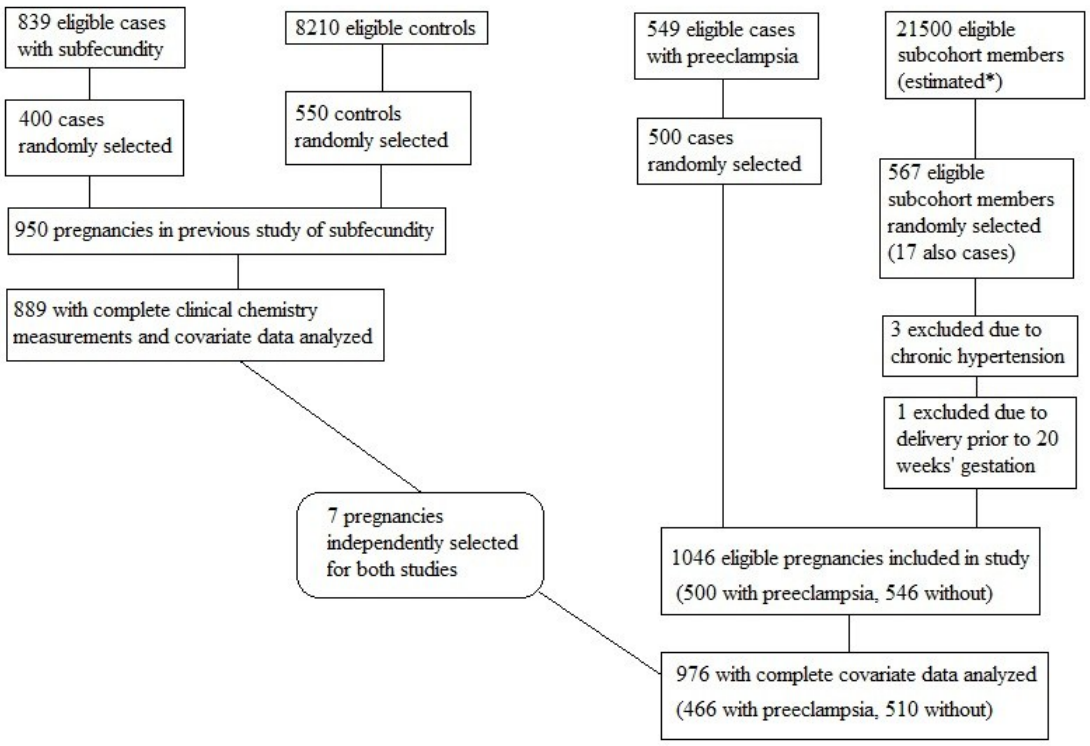
Power	PFOA β*	PFOS β*
0.70	2.35	0.52
0.80	2.65	0.60
0.90	3.05	0.70

*β represents a change in total cholesterol (mg/dL) per 1-unit increase in PFAS (ng/mL).

3.2.5. Participant Eligibility and Selection

The process of selecting eligible participants for each of the two aims is summarized in Figure 3.1.

Figure 3.1. Summary of participant eligibility and participation in both aims.



* The number of eligible subcohort members for the preeclampsia study was estimated because the availability of EDTA plasma samples was not confirmed for all potential subcohort members.

CHAPTER 4: RESULTS

4.1. Perfluoroalkyl substances during pregnancy and validated preeclampsia among nulliparous women in the Norwegian Mother and Child Cohort Study[1]

4.1.1. Introduction

Preeclampsia is a serious complication of pregnancy, consisting of new-onset hypertension combined with kidney dysfunction, which frequently leads to preterm delivery. Preeclampsia affects approximately 3% of pregnancies in the United States (105) and in Norway (109), and it is a leading cause of maternal morbidity and mortality. The etiology of preeclampsia remains obscure, but it is likely multifactorial, with both genetic and environmental contributions. Known risk factors for preeclampsia include nulliparity, personal or family history of preeclampsia, chronic hypertension, diabetes mellitus, autoimmune disorders, kidney disease, obesity, multiple gestation, history of subfertility, and maternal age (112, 119). For unknown reasons, cigarette smoking during pregnancy is associated with a 30% reduction in the risk of preeclampsia (120). Recent studies have raised the concern that blood concentrations of perfluoroalkyl substances during pregnancy may be associated with an increased risk of preeclampsia (8, 94).

Perfluoroalkyl substances (PFASs) are persistent environmental contaminants detectable in the blood of human populations worldwide (1, 2, 16, 36). The most commonly measured species of PFAS, perfluorooctane sulfonate (PFOS) and

[1]A revised version of Section 4.1 was submitted to the *American Journal of Epidemiology* in 2013 with the following co-authors: Stephanie M. Engel, David B. Richardson, Donna D. Baird, Line Småstuen Haug, Rolv Skjærven, Alison M. Stuebe, Kari Klungsøyr, Quaker Harmon, Georg Becher, Cathrine Thomsen, Azemira Sabaredzovic, Merete Eggesbø, Jane A. Hoppin, Gregory S. Travlos, Ralph E. Wilson, Lill Iren Trogstad, Per Magnus, Matthew P. Longnecker.

perfluorooctanoic acid (PFOA), have been widely used in industrial and consumer products, including surface treatments for fabrics and carpets, food packaging, fire-fighting foam and other wetting agents (3). PFASs are highly resistant to degradation in the environment or metabolism in the body (11, 16). The half-lives of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in human serum are estimated to be 4.8 years and 2.3 years, respectively (19, 169).

Despite the ubiquitous presence of PFASs in humans and in the environment, the potential adverse health effects that may result from chronic, low-level exposure have not been adequately studied, particularly among pregnant women. A recent study of non-occupationally exposed women living in an area of high PFOA contamination in drinking water found model-based estimates of pregnancy PFOA levels to be positively associated with preeclampsia (8). Two possible mechanisms by which PFASs may lead to preeclampsia are described here.

One potential mechanism of association may be through increased plasma lipids. While the result of high-dose PFAS exposure in laboratory animals is generally a reduction in cholesterol (64, 65), the opposite trend has been found in highly exposed humans. In studies of humans with occupational or other above-background levels of PFAS exposure, PFOA and PFOS have been associated with elevated total cholesterol and uric acid (5, 6, 38). Similarly, in general population samples, PFOS and PFOA concentrations have been positively associated with total cholesterol and non-HDL cholesterol (7). This observed association between PFASs and elevated lipids is consistent with a potential mechanism by which PFASs could be linked with preeclampsia, because preeclampsia is often preceded by elevated non-HDL cholesterol

and triglycerides (125, 141). However, our recent study of pregnant women in the Norwegian Mother and Child Cohort Study (MoBa) found positive associations between several PFASs and HDL cholesterol, as well as an association between PFOS and total cholesterol (Section 4.2).

A second potential mechanism by which PFAS concentrations during pregnancy may lead to preeclampsia is via immunotoxic effects. In vitro, PFOS is associated with a decrease in human natural killer (NK) cell activity (148). This finding is significant for the pathogenesis of preeclampsia because NK cells are believed to mediate the invasion of trophoblasts into the decidua and spiral arteries in normal placentation (149). Insufficient invasion of the spiral arteries due to impaired NK cell function could potentially contribute to preeclampsia (150).

The possibility that plasma concentrations of these widespread environmental contaminants during pregnancy may increase the risk of preeclampsia is of substantial public health concern and merits further examination. We explored this question in a case-cohort study of women with background levels of exposure enrolled in the large MoBa cohort.

4.1.2. Methods

4.1.2.1. Participants

This is a sub-study within MoBa, a prospective pregnancy cohort study conducted by the Norwegian Institute of Public Health (152, 153, 170). The Study was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate. Participants were recruited from throughout Norway in 1999-2008. The

majority of pregnant women who scheduled a routine ultrasound examination between 17 and 20 weeks of gestation were invited by mail to participate in the study, and 39% of invited women participated. Informed consent was obtained from each MoBa participant upon recruitment. Data were linked to the Medical Birth Registry of Norway (MBRN) (171). Further details may be found at www.fhi.no/morogbarn. The present study is based on version 4.301 of the quality-assured data files released for research.

Eligibility requirements for this analysis were pregnancy with a singleton infant, no previous live births or stillbirths, no history of chronic hypertension, available mid-pregnancy EDTA-preserved plasma sample, and enrollment in MoBa 2003-2007. This study was restricted to women with no previous live births or stillbirths because PFASs have been shown to decline in recent pregnancy and lactation (14, 60), and because nulliparous women may have different risk factors for preeclampsia than women with previous pregnancies (121, 172). We further restricted eligibility to women who enrolled in 2003 and later because the laboratory analysis of PFASs required EDTA anticoagulation, a process that was initiated in the MoBa study in 2003. From 549 eligible cases of preeclampsia in this time frame, 500 were randomly selected into the study. From approximately 21,500 eligible pregnancies, 550 were selected at random as the subcohort sample.

4.1.2.2. Exposure

Maternal non-fasting blood samples were collected at hospitals and maternity units across Norway at the time of study enrollment (median = 18 weeks of gestation) and shipped at ambient temperature to the MoBa biorepository in Oslo. Most samples

were received and processed the day after collection (153). At the biorepository, plasma was separated, aliquoted, and stored at -80 degrees Celsius. Changes in PFAS concentrations in transit are believed to be negligible, as PFASs are chemically stable (173), and a recent study showed no evidence of change over time in concentrations of four PFASs in serum maintained at room temperature for 10 days (156).

Concentrations (ng/mL) of nine PFASs were measured in maternal plasma using high-performance liquid chromatography/tandem mass spectrometry at the Norwegian Institute of Public Health. Details of the analytic process have been published previously (155). Analyses were restricted to the seven PFASs present in at least 50% of samples: perfluorooctane sulfonate (PFOS), perfluoroheptane sulfonate (PFHpS), perfluorohexane sulfonate (PFHxS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnDA). The limit of quantification (LOQ) was 0.05 ng/mL for these seven PFASs. For quantification of PFOS, the total area of linear and branched isomers was integrated. A total of 25 blinded specimens from a single pool were analyzed in the same batches as the sample specimens for quality assurance/quality control (QA/QC) purposes. Inter-assay coefficients of variation were calculated for each PFAS, and ranged from 8.6 for PFOA to 29.8 for PFHpS.

4.1.2.3. Outcome

At the time of delivery, birth attendants reported the presence or absence of preeclampsia to the MBRN using a standard form. A validation study was conducted to quantify the performance of the MBRN in identifying preeclampsia (Klungsøyr et al.,

submitted). Antenatal medical records were reviewed for evidence of meeting diagnostic criteria for preeclampsia based on a definition published by the American College of Obstetricians and Gynecologists (ACOG) (100). The validation study found the sensitivity of MBRN report of preeclampsia was 97% and the specificity was 76% (Klungsøyr et al., submitted).

Validated preeclampsia in the present study required evidence of both of the following criteria, at the same antenatal clinic visit:

1) Systolic blood pressure of at least 140 mm Hg, or diastolic blood pressure of at least 90 mm Hg, occurring after 20 completed weeks of gestation; and
2) Proteinuria, defined as at least 1+ on urine dipstick measurement.

Our definition differs from the ACOG definition in that we relied on a single urine dipstick measurement, while ACOG recommends the use of 24-hour urine collection to diagnose proteinuria. The use of 24-hour urine collection was not standard practice in Norway at the time of the study and therefore this information was generally not available to us in antenatal records. Additionally, our definition required that hypertension and proteinuria be documented at the same clinic visit. The gestational week of the clinic visit during which these diagnostic criteria were met was recorded.

We excluded both cases and cohort members with evidence of chronic hypertension, given that diagnosis of preeclampsia in the presence of chronic hypertension is clinically complicated and often times ambiguous (174). Chronic hypertension was defined as any of the following: 1) chronic hypertension reported to the MBRN or on the MoBa questionnaire, or 2) an ICD-9 code corresponding to chronic hypertension on the antenatal medical chart, or 3) a documented blood pressure of at least

140 mm Hg systolic or 90 mm Hg diastolic prior to 20 weeks of gestation on the antenatal medical chart.

4.1.2.4. Other variables

At the time of enrollment, women provided information via questionnaire regarding a number of demographic and lifestyle characteristics, as well as events in their reproductive and medical history. Maternal characteristics reported to the MBRN were also available. Covariate information was obtained from the following sources: maternal age at delivery was reported to the MBRN; pre-pregnancy body mass index (BMI) was calculated based on the participant's self-reported height and weight before she became pregnant; maternal education completed was self-reported in response to a questionnaire item asking women to report the highest level of education completed; maternal smoking during pregnancy was self-reported in response to the question, "Do you smoke now (after you became pregnant)?" at a median of 18 weeks gestation.

Creatinine (mg/dL) and cystatin C (mg/L) were measured in mid-pregnancy plasma samples to detect changes in kidney function and glomerular filtration rate. Both substances were measured at the National Institute of Environmental Sciences using an Olympus AU400e chemistry immuno-analyzer. The procedure used to measure creatinine was a kinetic modification of the Jaffe procedure in which creatinine reacted with picric acid at an alkaline pH. The cystatin C assay was based on the sol particle immunoassay (157), using colloidal gold particles coated with anti-cystatin C specific polyclonal antibodies.

4.1.2.5. Statistical analysis

We used weighted Cox proportional hazards models to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between each PFAS and preeclampsia. Weights were based on the inverse probability of selection into the case-cohort study (160). The 17 individuals who were randomly selected into both case and subcohort samples were assigned two separate intervals of person-time; one interval for their time at risk in the subcohort during which they were included in the risk sets for other cases, and one interval for their time immediately prior to becoming a case (160). Cases not in the subcohort were considered to enter the study immediately prior to becoming a case. The proportional hazards assumption was verified through graphical inspection of weighted Schoenfeld residuals; there was no evidence to reject the null hypothesis of proportional hazards.

We considered two separate event times: the gestational week of the clinic visit during which both diagnostic criteria were met, and the gestational day of delivery due to preeclampsia. The separate methods were expected to provide different results only in the instances where diagnosis and delivery were separated by several weeks. Additionally, we used logistic regression to examine a binary outcome of preeclampsia diagnosis without regard to the timing of diagnosis or delivery.

Modeled covariates were selected based on a directed acyclic graph (DAG) representing associations reported in the existing literature, and the identification of a minimally sufficient set of covariates to control confounding (Figure 4.1). The minimally sufficient adjustment set was identified using DAGitty v1.0 (www.dagitty.net). The modeled covariates and their categorizations were as follows: maternal age at delivery

(continuous); pre-pregnancy BMI (continuous); maternal education (<=12 years, 13-16 years, >16 years); and smoking at mid-pregnancy (yes/no).

Concentrations of PFASs were treated in two ways for the purposes of analysis: (1) as quartiles, with the lowest quartile serving as the referent category (with the exception of PFDA, which was categorized only at or above versus below the median due to >25% of values below the LOQ) and (2) as natural-log transformed continuous variables to assess linear trends. For all PFASs, values below the LOQ were replaced by the expected value of the log-normal distribution, given that the value was below the limit of quantification; this was calculated as the mean of values below the limit of quantification randomly drawn from the estimated log-normal distribution (175). We examined the shape of the dose-response function using logistic regression models of the association between each continuous PFAS exposure, modeled as a restricted cubic spline function with 5 knots at the 5^{th} , 25^{th}, 50^{th}, 75^{th}, and 95^{th} percentiles, and validated preeclampsia as a binary outcome. All statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC). The logistic spline models were fitted using a SAS macro created by Desquilbet and Mariotti (176).

4.1.3. Results

The principal analysis included 976 women (466 cases and 510 non-cases) with complete information on modeled covariates. Three of the original 550 subcohort members reported chronic hypertension to the MBRN and therefore were excluded. Additionally, one member of the subcohort had a gestational age at delivery reported to the MBRN of less than 20 weeks, and so was excluded because she was never at risk to

develop preeclampsia. Fewer than 6% of participants were missing data for any modeled covariate.

The participants ranged in age from 16 to 44 years old (Table 4.1). There was no notable difference in age distribution between cases and controls. BMI ranged from 15.4 to 48.8 kg/m^2, and there were more cases than controls with a BMI greater than or equal to 30 kg/m^2 (16% of cases versus 7% of controls). The majority of participants had between 13 and 16 years of education (75% of cases and 70% of controls). Overall, 7% of participants reported current smoking at mid-pregnancy, with a slightly lower percentage of cases reporting smoking (6%). The median duration of pregnancy was shorter among cases (274 days) than among controls (282 days).

As noted above, this analysis is restricted to the seven PFASs that were detectable in plasma samples from greater than 50% of participants (Table 4.2). PFOS had the highest median concentration (12.87 ng/mL) and was detectable in 100% of samples. PFOA had the second highest median concentration (2.78 ng/mL) and was also detectable in 100% of samples. The other measured PFASs in descending order of median concentration were PFHxS, PFNA, PFUnDA, PFHpS, and PFDA. PFDA was detectable in 71% of samples. The seven PFASs were moderately to highly correlated with one another (Table 4.3). The lowest correlation observed was between PFDA and PFHpS (ρ=0.18) and the highest was between PFNA and PFDA (ρ=0.75). PFOS and PFOA were correlated at ρ=0.64.

None of the seven PFASs had positive associations with preeclampsia, either in quartile analyses or when PFASs were entered as continuous natural-log transformed exposure variables (Table 4.4). Interestingly, there was an inverse association between

PFUnDA and preeclampsia: the highest quartile of PFUnDA had an adjusted HR of 0.55 (95% CI=0.38, 0.81) relative to the lowest quartile, and each increase of 1 ln-ng/mL of PFUnDA was associated with an adjusted HR of 0.78 (95% CI=0.66, 0.92). Additional adjustment for plasma creatinine (Table 4.5) or plasma cystatin C (Table 4.6) did not produce meaningful changes in any effect estimates.

Similarly, none of the seven PFASs were positively associated with delivery due to preeclampsia (Table 4.7). Each upper quartile of PFUnDA was associated with decreased risk of delivery due to preeclampsia relative to the lowest quartile, and each increase of 1 ln-ng/mL of PFUnDA was associated with an HR for delivery due to preeclampsia of 0.71 (95% CI=0.59, 0.84). There was no indication in the quartile analysis of a linear dose-response relationship for PFUnDA; all three of the upper quartiles of PFUnDA were associated with reduced preeclampsia risk relative to the lowest quartile of PFUnDA.

In logistic regression analyses of binary preeclampsia diagnosis, without regard to timing, the second quartile of PFUnDA was associated with reduced risk of preeclampsia (HR=0.60, 95% CI=0.42, 0.87) relative to the lowest quartile, as was the highest quartile of PFUnDA (HR=0.65, 95% CI=0.44, 0.96) (Table 4.8). Each increase of 1 ln-ng/mL of PFUnDA was associated with an HR for preeclampsia of 0.86 (95% CI=0.73, 1.01).

The shape of the dose-response function was characterized using restricted cubic spline functions for the association between each continuous PFAS exposure variable and preeclampsia. The spline graphs suggested weakly inverse associations between PFNA, PFDA, PFUnDA and preeclampsia. There was some indication in the spline graphs of an increase in preeclampsia risk at the higher concentrations of PFHpS. Overall the results

of the logistic regression analysis using restricted cubic spline functions appeared to parallel the results of the Cox proportional hazards models. There were no strong linear trends observed within the range of exposure concentrations measured in this population.

4.1.4. Discussion

We observed no positive associations between plasma PFAS concentrations and preeclampsia in this sample of 976 nulliparous Norwegian women, at background levels of PFAS exposure. A series of related studies on pregnancy-induced hypertension and preeclampsia conducted in a population highly exposed to PFOA through contaminated drinking water reported positive associations with both PFOA and PFOS (8, 94). The difference in results between our study and those previous studies may be partly attributed to differences in exposure assessment, to differences in preeclampsia case definition, or to the restriction of our study to nulliparous women.

Our study directly measured perfluoroalkyl substances at mid-pregnancy, prior to the onset of preeclampsia, while previous studies used indirect methods to assess perfluoroalkyl substances during pregnancy. Specifically, one study employed predictive modeling to estimate exposure at the time of pregnancy (8); another measured exposure concentration in serum up to 5 years after the pregnancy of interest (94). Exposures assessed in different ways could be biased differently by unmeasured factors. For example, recall bias could be present if ascertainment of preeclampsia history took place after the participants became aware of PFOA contamination of their drinking water.

The PFOA serum concentrations measured or predicted in previous studies were substantially higher than the plasma concentration measured in the present study. It is

possible that shape of the PFOA-preeclampsia dose-response function is non-linear, and that effects are only observable at higher exposure concentrations than those present in our study. However, differences in exposure magnitude do not explain the lack of association between PFOS and preeclampsia found in our study, because the median PFOS concentration in the previous study was approximately equal to ours (94).

We employed a definition of preeclampsia that was validated through review of antenatal medical records, while previous studies used self-reported preeclampsia (8, 94). The positive predictive value of self-reported preeclampsia in a previous study was approximately 50-60% (177). The use of medical records in our study may have served to reduce outcome misclassification, possibly through the exclusion of other hypertensive disorders of pregnancy that did not meet the diagnostic criteria for preeclampsia.

Our study was restricted to nulliparous women. This restriction ensures that observed PFAS levels do not reflect recent declines in body burden due to previous pregnancy and lactation (14, 60, 178). Nulliparous women also have a higher risk of preeclampsia as compared to parous women (122). Women who develop preeclampsia in their second or later pregnancies may have a different underlying set of risk factors and clinical characteristics as compared to nulliparous women. Approximately half of the women in the previous studies (8, 94) were nulliparous. It is possible that the association between mid-pregnancy PFASs and preeclampsia may differ between nulliparous and parous women.

We observed an inverse (protective) association between PFUnDA and preeclampsia. This finding must be considered preliminary as we are not aware of any previous studies that have evaluated this association. There are several possible

explanations for this finding, which may result from chance, causality, confounding, or pharmacokinetics.

One possible explanation for the inverse association between PFUnDA and preeclampsia is the mediation of high-density lipoprotein (HDL) cholesterol. In a separate study, also within the MoBa cohort, all seven PFASs studied were positively associated with HDL cholesterol at mid-pregnancy, and PFUnDA showed the strongest association with HDL (Section 4.2). As higher levels of HDL are considered to be protective against cardiovascular disease (179), and low HDL cholesterol during pregnancy has been associated with increased risk of preeclampsia (180), it is possible than an environmental factor leading to increased HDL levels could in fact lower the risk of preeclampsia. However, if the association between PFUnDA and preeclampsia is mediated by HDL, then adjustment for HDL may produce estimates that are biased or difficult to interpret (181). Furthermore, it is unknown whether the previously observed association between PFASs and HDL in pregnant women is causal, or the result of unmeasured confounding or pharmacokinetics. For example, there may be a special affinity between certain PFASs and HDL cholesterol in circulating blood, leading to a positive cross-sectional association (182).

Another possible explanation for observed associations between PFASs and preeclampsia may be immune-mediated. PFASs have a number of immunotoxic effects (67), and altered immunologic responses may also be involved in the pathogenesis of preeclampsia (183). However, it is unclear how the immunotoxic effects of PFASs could produce the observed inverse association between PFUnDA and preeclampsia.

Strengths of this study include the use of a validated diagnosis of preeclampsia, which serves to reduce outcome misclassification, and the use of a highly sensitive assay for PFASs, allowing the quantification of several PFASs that have not been studied extensively. Additionally, our restriction to nulliparous women eliminates the possibility of bias resulting from recent changes in PFAS concentration due to pregnancy and lactation. By contrast with previous studies (8, 94), we were able to directly measure PFASs in mid-pregnancy plasma, prior to the onset of preeclampsia. However, the possibility remains that an asymptomatic, unidentified stage of the disease may already be present at mid-pregnancy. If this hypothetical pre-symptomatic stage were to influence kidney function or glomerular filtration, it could also influence excretion and therefore plasma concentrations of PFASs. However, adjustment for plasma creatinine and cystatin C did not affect our results, suggesting that the estimates were not confounded by measurably impaired kidney function. We cannot rule out the possibility of bias due to confounding by an unmeasured variable.

As previously noted, the participation rate into the MoBa study was 39% of eligible women. It is possible that selection bias may have influenced the results of this study in unpredictable ways. If unmeasured variables were related to both exposure and to selection into the study, or related to both selection and the outcome, bias could result. However, we are unaware of any strong confounders that were unmeasured in this study. Additionally, the process used to validate cases of preeclampsia excluded pregnancies lacking antenatal medical records, or for whom the absence of chronic hypertension prior to 20 weeks of gestation could not be established. Another potential limitation of this study may be the lower median BMI in this population as compared to US populations.

Only 11% of participants had a BMI of 30 kg/m^2 or greater, whereas 32% of US women aged 20-39 in the US had a BMI in this range (184). This difference may limit generalizability of the findings to US populations.

4.1.5. Conclusions

Our study does not support a positive association between plasma PFAS concentration at mid-pregnancy and preeclampsia among nulliparous women. Our results do suggest that different PFASs may have different associations with preeclampsia, and that it is important to examine associations with each PFAS separately. Further research may wish to examine whether the observed inverse association between PFUnDA and preeclampsia is present in other populations, and to investigate mechanisms that may underlie this association.

Table 4.1. Characteristics of 976 eligible pregnant women enrolled in the Norwegian Mother and Child Cohort Study in 2003-2007 and selected into a case-cohort study of preeclampsia.

	Cases (N=466)		Controls (N=510)		Total	
	N	%	N	%	N	%
Maternal age at delivery (years)						
16-24	78	17	80	16	158	16
25-29	202	43	210	41	412	42
30-34	139	30	169	33	308	32
35-44	47	10	51	10	98	10
Body mass index (kg/m^2)*						
15.4-24.9	263	56	367	72	630	65
25.0-29.9	130	28	105	20	235	24
30.0-48.8	73	16	38	7	111	11
Maternal education (years)						
12 or less	13	3	11	2	24	2
13-16	351	75	359	70	710	73
More than 16	97	21	130	25	227	23
Other education	5	1	10	2	15	2
Smoking during pregnancy						
Yes	28	6	38	7	66	7
No	438	94	472	93	910	93
Duration of pregnancy (weeks)						
24-<28	4	1	0	0	4	0.4
28-<32	17	4	2	0.4	19	2
32-<37	73	16	21	4	95	10
37-<42	356	77	440	87	798	82
42-<44	13	3	44	9	57	6
Missing	3		3		6	

*Pre-pregnancy body mass index

Table 4.2. Plasma concentrations (ng/mL) of seven perfluoroalkyl substances detectable in at least 50% of samples, among 976 eligible pregnant women enrolled in the Norwegian Mother and Child Cohort Study in 2003-2007.

		%	Chain	Percentile					
Name	Abbrev	>LOQ	Length	5th	25th	50th	75th	95th	CV*
Perfluorooctanoic acid	PFOA	100.0	8	1.43	2.14	2.78	3.57	5.15	8.6
Perfluorononanoic acid	PFNA	99.7	9	0.24	0.39	0.54	0.74	1.12	13.3
Perfluorodecanoic acid	PFDA	71.1	10	<LOQ	<LOQ	0.10	0.18	0.34	27.0
Perfluoroundecanoic acid	PFUnDA	83.7	11	<LOQ	0.08	0.17	0.27	0.47	22.2
Perfluorohexane sulfonate	PFHxS	99.5	6	0.27	0.49	0.69	0.95	1.78	14.6
Perfluoroheptane sulfonate	PFHpS	83.6	7	<LOQ	0.09	0.15	0.23	0.37	29.8
Perfluorooctane sulfonate	PFOS	100.0	8	6.07	9.69	12.87	17.03	25.53	11.4

* Inter-assay coefficient of variation was calculated as (standard deviation / mean)*100 for 25 pooled QA/QC samples.

Table 4.3. Spearman correlation coefficients between plasma concentrations of perfluoroalkyl substances detectable in >50% of samples, among 976 pregnant women in the Norwegian Mother and Child Cohort Study selected for a case-cohort study of preeclampsia.

	PFOA	PFNA	PFDA	PFUnDA	PFHxS	PFHpS	PFOS
PFOA	-	0.61	0.45	0.31	0.54	0.53	0.64
PFNA		-	0.75	0.51	0.45	0.31	0.42
PFDA			-	0.54	0.27	0.18	0.25
PFUnDA				-	0.40	0.24	0.33
PFHxS					-	0.50	0.58
PFHpS						-	0.74
PFOS							-

Table 4.4. Hazard ratios for preeclampsia diagnosis associated with plasma PFAS concentration (ng/mL) among 976 eligible pregnant women in the Norwegian Mother and Child Cohort Study, of whom 466 developed preeclampsia.

		Crude HR	95% CI	Adjusted* HR	95% CI
PFOA	(Q1) 0.32-2.11	1.		1.	
	(Q2) 2.12-2.77	0.99	0.70, 1.42	1.03	0.70, 1.50
	(Q3) 2.77-3.56	0.93	0.65, 1.32	0.92	0.63, 1.35
	(Q4) 3.56-11.28	0.88	0.61, 1.25	1.01	0.69, 1.48
	Per ln-unit PFOA	0.84	0.63, 1.13	0.89	0.65, 1.22
PFNA	(Q1) <LOQ-0.39	1.		1.	
	(Q2) 0.39-0.54	0.85	0.60, 1.22	0.88	0.60, 1.30
	(Q3) 0.54-0.74	0.92	0.64, 1.31	1.04	0.71, 1.53
	(Q4) 0.74-3.54	0.80	0.56, 1.15	0.88	0.60, 1.29
	Per ln-unit PFNA	0.84	0.66, 1.07	0.90	0.70, 1.16
PFDA	<LOQ-0.10 (median)	1.		1.	
	0.10-1.74	0.81	0.63, 1.05	0.88	0.67, 1.16
	Per ln-unit PFDA	0.86	0.74, 1.00	0.88	0.75, 1.04
PFUnDA	(Q1) <LOQ-0.08	1.		1.	
	(Q2) 0.08-0.17	0.55	0.38, 0.79	0.51	0.35, 0.76
	(Q3) 0.17-0.27	0.57	0.40, 0.82	0.60	0.41, 0.88
	(Q4) 0.27-1.01	0.49	0.34, 0.71	0.55	0.38, 0.81
	Per ln-unit PFUnDA	0.76	0.65, 0.89	0.78	0.66, 0.92
PFHxS	(Q1) <LOQ-0.49	1.		1.	
	(Q2) 0.49-0.69	0.94	0.66, 1.34	0.86	0.59, 1.26
	(Q3) 0.69-0.95	1.00	0.70, 1.42	1.01	0.69, 1.49
	(Q4) 0.95-11.47	0.84	0.59, 1.19	0.93	0.64, 1.36
	Per ln-unit PFHxS	0.86	0.70, 1.06	0.91	0.72, 1.14
PFHpS	(Q1) <LOQ-0.09	1.		1.	
	(Q2) 0.09-0.15	1.22	0.85, 1.74	1.30	0.88, 1.92
	(Q3) 0.15-0.22	1.01	0.71, 1.45	1.01	0.69, 1.48
	(Q4) 0.22-1.19	1.04	0.73, 1.48	1.12	0.77, 1.63
	Per ln-unit PFHpS	1.01	0.85, 1.20	1.03	0.86, 1.24
PFOS	(Q1) 1.44-9.66	1.		1.	
	(Q2) 9.67-12.79	1.18	0.82, 1.68	1.12	0.76, 1.65
	(Q3) 12.80-16.91	0.85	0.59, 1.21	0.88	0.60, 1.29
	(Q4) 16.91-56.61	1.08	0.75, 1.53	1.09	0.75, 1.58
	Per ln-unit PFOS	1.08	0.82, 1.42	1.13	0.84, 1.52

*Adjusted for maternal age, pre-pregnancy BMI, education completed, and smoking during pregnancy.

Table 4.5. Hazard ratios for preeclampsia diagnosis associated with plasma PFAS concentration (ng/mL) among 975 eligible pregnant women with complete covariate and outcome information in the Norwegian Mother and Child Cohort Study, of whom 466 developed preeclampsia, additionally adjusted for plasma creatinine (mg/dL).

		Unadjusted HR	95% CI	Adjusted* HR	95% CI
PFOA	(Q1) 0.32-2.11	1.		1.	
	(Q2) 2.12-2.77	0.99	0.69, 1.41	1.02	0.70, 1.49
	(Q3) 2.77-3.56	0.92	0.64, 1.32	0.91	0.62, 1.34
	(Q4) 3.56-11.28	0.87	0.61, 1.24	1.02	0.70, 1.50
	Per ln-unit PFOA	0.84	0.63, 1.12	0.90	0.66, 1.24
PFNA	(Q1) <LOQ-0.39	1.		1.	
	(Q2) 0.39-0.54	0.86	0.60, 1.23	0.89	0.61, 1.32
	(Q3) 0.54-0.74	0.92	0.64, 1.31	1.06	0.72, 1.56
	(Q4) 0.74-3.54	0.80	0.56, 1.15	0.91	0.62, 1.33
	Per ln-unit PFNA	0.84	0.66, 1.07	0.92	0.71, 1.19
PFDA	<LOQ-0.10 (median)	1.		1.	
	0.10-1.74	0.81	0.63, 1.04	0.89	0.67, 1.18
	Per ln-unit PFDA	0.86	0.73, 1.00	0.89	0.76, 1.05
PFUnDA	(Q1) <LOQ-0.08	1.		1.	
	(Q2) 0.08-0.17	0.55	0.38, 0.79	0.50	0.34, 0.74
	(Q3) 0.17-0.27	0.57	0.40, 0.82	0.59	0.40, 0.87
	(Q4) 0.27-1.01	0.49	0.34, 0.71	0.56	0.38, 0.82
	Per ln-unit PFUnDA	0.76	0.65, 0.89	0.78	0.66, 0.92
PFHxS	(Q1) <LOQ-0.49	1.		1.	
	(Q2) 0.49-0.69	0.93	0.65, 1.33	0.87	0.59, 1.28
	(Q3) 0.69-0.95	0.99	0.70, 1.41	1.04	0.70, 1.53
	(Q4) 0.95-11.47	0.83	0.58, 1.18	0.93	0.64, 1.36
	Per ln-unit PFHxS	0.86	0.70, 1.06	0.91	0.73, 1.14
PFHpS	(Q1) <LOQ-0.09	1.		1.	
	(Q2) 0.09-0.15	1.21	0.85, 1.73	1.29	0.87, 1.92
	(Q3) 0.15-0.22	1.01	0.70, 1.44	0.99	0.68, 1.45
	(Q4) 0.22-1.19	1.03	0.72, 1.47	1.12	0.77, 1.64
	Per ln-unit PFHpS	1.01	0.85, 1.19	1.03	0.86, 1.24
PFOS	(Q1) 1.44-9.66	1.		1.	
	(Q2) 9.67-12.79	1.18	0.83, 1.69	1.13	0.76, 1.68
	(Q3) 12.80-16.91	0.85	0.59, 1.21	0.89	0.61, 1.30
	(Q4) 16.91-56.61	1.08	0.75, 1.53	1.10	0.75, 1.60
	Per ln-unit PFOS	1.08	0.82, 1.42	1.15	0.85, 1.55

* Adjusted for maternal age, pre-pregnancy BMI, education completed, smoking, and creatinine (mg/dL).

Table 4.6. Hazard ratios for preeclampsia diagnosis associated with plasma PFAS concentration (ng/mL) among 972 eligible pregnant women with complete covariate and outcome information in the Norwegian Mother and Child Cohort Study, of whom 464 developed preeclampsia, additionally adjusted for plasma cystatin C (mg/L).

		Unadjusted HR	95% CI	Adjusted* HR	95% CI
PFOA	(Q1) 0.32-2.11	1.		1.	
	(Q2) 2.12-2.77	0.99	0.69, 1.41	1.02	0.70, 1.49
	(Q3) 2.77-3.56	0.94	0.66, 1.34	0.94	0.64, 1.37
	(Q4) 3.56-11.28	0.87	0.61, 1.24	1.00	0.68, 1.46
	Per ln-unit PFOA	0.84	0.63, 1.13	0.89	0.65, 1.22
PFNA	(Q1) <LOQ-0.39	1.		1.	
	(Q2) 0.39-0.54	0.86	0.60, 1.23	0.88	0.60, 1.30
	(Q3) 0.54-0.74	0.91	0.64, 1.30	1.03	0.70, 1.52
	(Q4) 0.74-3.54	0.80	0.56, 1.15	0.88	0.60, 1.29
	Per ln-unit PFNA	0.84	0.66, 1.07	0.90	0.70, 1.16
PFDA	<LOQ-0.10 (median)	1.		1.	
	0.10-1.74	0.81	0.63, 1.05	0.88	0.67, 1.17
	Per ln-unit PFDA	0.86	0.74, 1.00	0.88	0.75, 1.04
PFUnDA	(Q1) <LOQ-0.08	1.		1.	
	(Q2) 0.08-0.17	0.54	0.38, 0.78	0.51	0.34, 0.75
	(Q3) 0.17-0.27	0.57	0.40, 0.82	0.60	0.40, 0.87
	(Q4) 0.27-1.01	0.50	0.34, 0.71	0.56	0.38, 0.82
	Per ln-unit PFUnDA	0.76	0.66, 0.89	0.78	0.66, 0.92
PFHxS	(Q1) <LOQ-0.49	1.		1.	
	(Q2) 0.49-0.69	0.92	0.65, 1.32	0.84	0.57, 1.24
	(Q3) 0.69-0.95	1.00	0.70, 1.42	1.02	0.69, 1.50
	(Q4) 0.95-11.47	0.85	0.59, 1.20	0.94	0.64, 1.38
	Per ln-unit PFHxS	0.87	0.70, 1.07	0.91	0.73, 1.14
PFHpS	(Q1) <LOQ-0.09	1.		1.	
	(Q2) 0.09-0.15	1.22	0.85, 1.74	1.29	0.87, 1.92
	(Q3) 0.15-0.22	1.00	0.70, 1.44	1.00	0.68, 1.46
	(Q4) 0.22-1.19	1.04	0.73, 1.49	1.12	0.77, 1.64
	Per ln-unit PFHpS	1.01	0.85, 1.20	1.03	0.86, 1.24
PFOS	(Q1) 1.44-9.66	1.		1.	
	(Q2) 9.67-12.79	1.18	0.82, 1.68	1.12	0.75, 1.65
	(Q3) 12.80-16.91	0.84	0.58, 1.20	0.87	0.59, 1.27
	(Q4) 16.91-56.61	1.08	0.76, 1.55	1.10	0.75, 1.60
	Per ln-unit PFOS	1.09	0.83, 1.43	1.14	0.84, 1.53

*Adjusted for maternal age, pre-pregnancy BMI, education completed, smoking, and cystatin C (mg/L).

Table 4.7. Hazard ratios for delivery due to preeclampsia associated with plasma PFAS concentration (ng/mL) among 970 eligible pregnant women in the Norwegian Mother and Child Cohort Study, of whom 463 developed preeclampsia.

		Unadjusted HR	95% CI	Adjusted* HR	95% CI
PFOA	(Q1) 0.32-2.11	1.		1.	
	(Q2) 2.12-2.77	0.94	0.65, 1.38	0.96	0.64, 1.45
	(Q3) 2.77-3.56	0.90	0.62, 1.32	0.92	0.62, 1.38
	(Q4) 3.56-11.28	0.85	0.58, 1.25	0.99	0.66, 1.48
	Per ln-unit PFOA	0.84	0.61, 1.15	0.92	0.65, 1.29
PFNA	(Q1) <LOQ-0.39	1.		1.	
	(Q2) 0.39-0.54	0.80	0.55, 1.16	0.84	0.56, 1.27
	(Q3) 0.54-0.74	0.86	0.59, 1.25	0.98	0.66, 1.48
	(Q4) 0.74-3.54	0.72	0.50, 1.06	0.79	0.53, 1.19
	Per ln-unit PFNA	0.78	0.60, 1.02	0.85	0.64, 1.12
PFDA	<LOQ-0.10 (median)	1.		1.	
	0.10-1.74	0.79	0.60, 1.02	0.84	0.63, 1.12
	Per ln-unit PFDA	0.83	0.71, 0.98	0.84	0.71, 1.00
PFUnDA	(Q1) <LOQ-0.08	1.		1.	
	(Q2) 0.08-0.17	0.48	0.32, 0.70	0.45	0.30, 0.68
	(Q3) 0.17-0.27	0.48	0.33, 0.71	0.48	0.31, 0.72
	(Q4) 0.27-1.01	0.44	0.30, 0.65	0.48	0.32, 0.72
	Per ln-unit PFUnDA	0.71	0.60, 0.83	0.71	0.59, 0.84
PFHxS	(Q1) <LOQ-0.49	1.		1.	
	(Q2) 0.49-0.69	0.92	0.63, 1.34	0.88	0.58, 1.32
	(Q3) 0.69-0.95	0.96	0.66, 1.40	0.99	0.66, 1.49
	(Q4) 0.95-11.47	0.81	0.56, 1.18	0.92	0.62, 1.38
	Per ln-unit PFHxS	0.84	0.67, 1.05	0.90	0.70, 1.16
PFHpS	(Q1) <LOQ-0.09	1.		1.	
	(Q2) 0.09-0.15	1.23	0.84, 1.79	1.40	0.92, 2.11
	(Q3) 0.15-0.22	1.06	0.73, 1.55	1.06	0.70, 1.59
	(Q4) 0.22-1.19	1.07	0.74, 1.55	1.22	0.82, 1.82
	Per ln-unit PFHpS	1.03	0.86, 1.24	1.07	0.88, 1.30
PFOS	(Q1) 1.44-9.66	1.		1.	
	(Q2) 9.67-12.79	1.08	0.74, 1.57	1.02	0.67, 1.54
	(Q3) 12.80-16.91	0.83	0.57, 1.21	0.86	0.57, 1.29
	(Q4) 16.91-56.61	1.04	0.72, 1.52	1.09	0.74, 1.62
	Per ln-unit PFOS	1.07	0.80, 1.43	1.15	0.84, 1.57

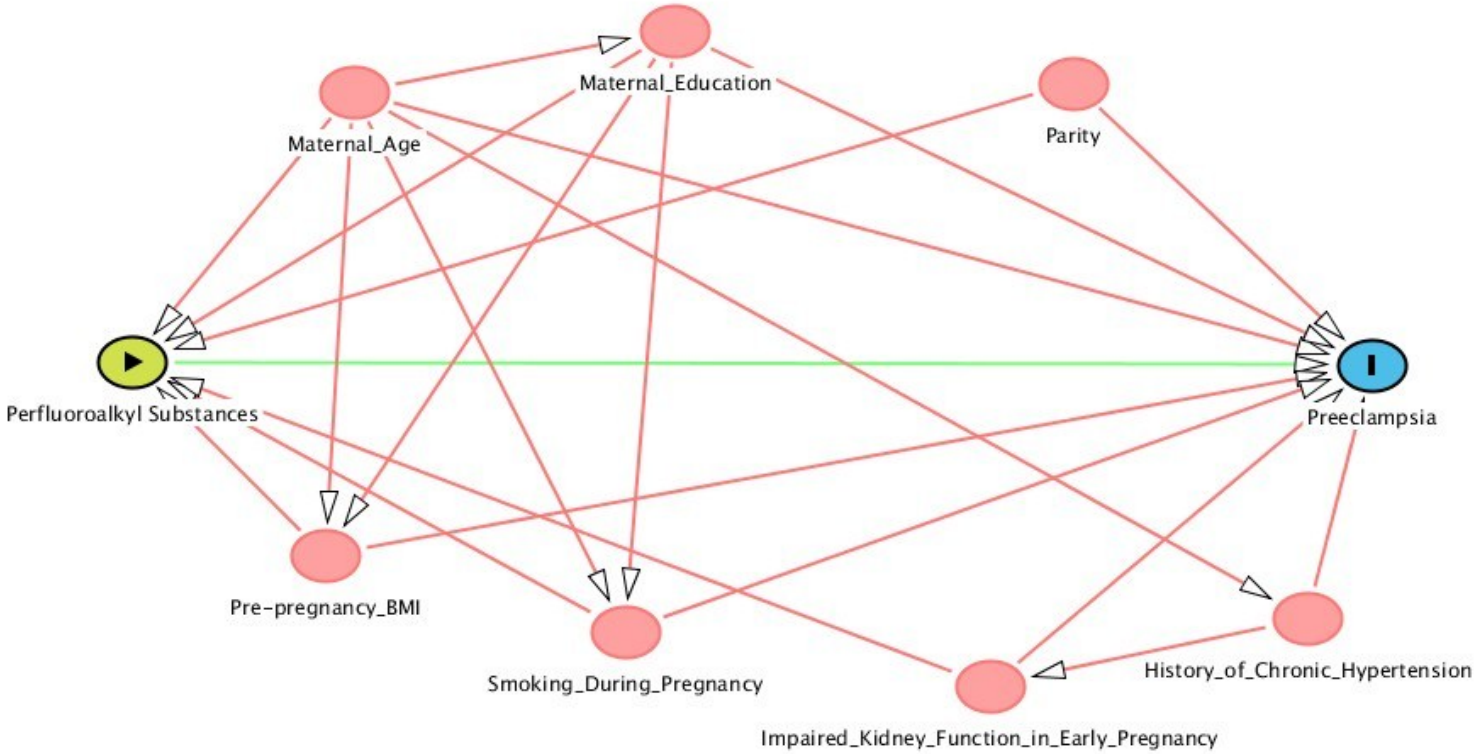
*Adjusted for maternal age, pre-pregnancy BMI, education completed, and smoking during pregnancy.

Table 4.8. Odds ratios for preeclampsia diagnosis associated with plasma PFAS concentration (ng/mL) among 976 eligible pregnant women in the Norwegian Mother and Child Cohort Study.

		Unadjusted OR	95% CI	Adjusted* OR	95% CI
PFOA	(Q1) 0.32-2.11	1.		1.	
	(Q2) 2.12-2.77	1.03	0.72, 1.47	1.04	0.72, 1.50
	(Q3) 2.77-3.56	0.97	0.68, 1.38	1.03	0.72, 1.49
	(Q4) 3.56-11.28	0.89	0.62, 1.27	1.02	0.70, 1.47
	Per ln-unit PFOA	0.85	0.63, 1.17	0.94	0.68, 1.29
PFNA	(Q1) <LOQ-0.39	1.		1.	
	(Q2) 0.39-0.54	0.91	0.64, 1.30	1.01	0.70, 1.46
	(Q3) 0.54-0.74	0.98	0.68, 1.39	1.17	0.81, 1.70
	(Q4) 0.74-3.54	0.86	0.60, 1.23	1.01	0.69, 1.47
	Per ln-unit PFNA	0.89	0.69, 1.14	0.99	0.76, 1.30
PFDA	(0) <LOQ-0.10	1.		1.	
	(1) 0.10-1.74	0.84	0.66, 1.08	0.97	0.74, 1.28
	Per ln-unit PFDA	0.89	0.76, 1.03	0.96	0.82, 1.13
PFUnDA	(Q1) <LOQ-0.08	1.		1.	
	(Q2) 0.08-0.17	0.57	0.40, 0.81	0.60	0.42, 0.87
	(Q3) 0.17-0.27	0.64	0.45, 0.92	0.72	0.50, 1.06
	(Q4) 0.27-1.01	0.53	0.37, 0.77	0.65	0.44, 0.96
	Per ln-unit PFUnDA	0.79	0.68, 0.92	0.86	0.73, 1.01
PFHxS	(Q1) <LOQ-0.49	1.		1.	
	(Q2) 0.49-0.69	0.88	0.62, 1.26	0.84	0.58, 1.21
	(Q3) 0.69-0.95	0.96	0.67, 1.37	0.94	0.66, 1.36
	(Q4) 0.95-11.47	0.85	0.59, 1.21	0.95	0.65, 1.37
	Per ln-unit PFHxS	0.88	0.72, 1.07	0.92	0.75, 1.13
PFHpS	(Q1) <LOQ-0.09	1.		1.	
	(Q2) 0.09-0.15	1.17	0.82, 1.67	1.14	0.79, 1.64
	(Q3) 0.15-0.22	0.99	0.69, 1.42	0.98	0.68, 1.41
	(Q4) 0.22-1.19	1.02	0.72, 1.46	1.01	0.70, 1.46
	Per ln-unit PFHpS	1.01	0.85, 1.20	1.01	0.85, 1.20
PFOS	(Q1) 1.44-9.66	1.		1.	
	(Q2) 9.67-12.79	1.19	0.83, 1.70	1.20	0.83, 1.73
	(Q3) 12.80-16.91	0.85	0.60, 1.22	0.90	0.62, 1.29
	(Q4) 16.91-56.61	1.10	0.77, 1.56	1.13	0.78, 1.62
	Per ln-unit PFOS	1.11	0.84, 1.47	1.14	0.85, 1.53

* Adjusted for maternal age, pre-pregnancy BMI, education completed, and smoking during pregnancy.

Figure 4.1. Directed acyclic graph describing the hypothesized associations between perfluoroalkyl substances, preeclampsia, and covariates.



4.2. Perfluoroalkyl substances and cardiometabolic clinical chemistries in plasma during pregnancy among women in the Norwegian Mother and Child Cohort Study[2]

4.2.1. Introduction

Perfluoroalkyl substances (PFASs) are persistent environmental contaminants detectable in the blood of human populations worldwide (2, 16), including nearly all Norwegians (36) and Americans (1). PFASs are used in the manufacturing process of numerous industrial and consumer products, including surface treatments for fabrics and food packaging, fire-fighting foam, surfactants and pesticide additives (15). They may enter the environment through release from industrial sources or through consumer products (10).

Sources of exposure to PFASs in the general population may include food, drinking water, house dust, air, and breast milk (13, 14). PFASs are highly resistant to degradation in the environment or metabolism in the body (11, 16). The half-lives of perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), and perfluorooctanoate (PFOA) are estimated to be 4.8 years, 7.3 years, and 2.3 years, respectively (19, 169). PFASs are not lipophilic, and while the tissue distribution in humans is unknown, animal studies suggest that PFASs likely reside primarily in the liver, kidneys, and blood (17).

Animal studies have demonstrated a number of adverse health effects associated with high-dose oral exposure to PFOS and PFOA, including hepatotoxicity, tumorigenesis, immunotoxicity, and developmental toxicity (11, 185). Some adverse

[2]A revised version of Section 4.2 was submitted to the journal *Environment International* in 2013 with the following co-authors: Stephanie M. Engel, Kristina W. Whitworth, David B. Richardson, Alison M. Stuebe, Julie L. Daniels, Line Småstuen Haug, Merete Eggesbø, Georg Becher, Azemira Sabaredzovic, Cathrine Thomsen, Ralph E. Wilson, Gregory S. Travlos, Jane A. Hoppin, Donna D. Baird, Matthew P. Longnecker.

effects in animals are believed to be mediated through the binding of PFASs to the peroxisome proliferator-activated receptor alpha (PPARα), which plays a role in the regulation of lipid and glucose metabolism in humans and rodents (83).

In epidemiologic studies of highly exposed populations as well as populations with background levels of exposure, PFAS concentrations have been associated with altered lipid profiles that are consistent with increased risk of cardiovascular disease. These lipid alterations have included elevated plasma cholesterol, triglycerides, and uric acid (4-7). The two PFASs typically present in human blood at the highest concentrations, PFOS and PFOA, have been studied most extensively, but recently researchers have begun to examine the lipid correlates of PFASs present at lower concentrations, including PFHxS and perfluorononanoic acid (PFNA) (7, 89). The observed associations between PFASs and lipid concentrations have not been consistent across studies or across populations, possibly owing to the different age and sex distributions of the groups studied, the different sizes of the study populations, or the different settings (occupational versus general populations).

To our knowledge, no previous studies of the associations between PFAS levels and plasma lipid concentrations have been conducted among pregnant women. The relation between PFASs and lipids during pregnancy is particularly important to quantify for the following reasons: 1) pregnant women differ in their relative and absolute lipid concentrations (135), and may show different associations between PFASs and lipids as compared with non-pregnant women; and 2) altered plasma lipids during pregnancy, particularly elevated plasma triglycerides, are associated with a number of adverse outcomes, including preeclampsia (186) and pregnancy-induced hypertension (187).

A retrospective study of pregnancy outcomes among women in Ohio and West Virginia exposed to PFOA-contaminated drinking water found elevated odds of preeclampsia associated with higher levels of PFOA (8). If pregnant women with higher PFAS levels also demonstrate the altered lipid patterns previously described in non-pregnant populations, then this may suggest a mechanism by which plasma PFAS concentrations could be linked to an increased risk of preeclampsia. In particular, elevations in triglycerides and certain types of low-density lipoprotein (LDL) particles are considered "pro-atherogenic" during pregnancy and may promote oxidative stress and endothelial damage leading to preeclampsia (188). Elevated uric acid (hyperuricemia) during pregnancy is also a feature of preeclampsia (99). Elevated C-reactive protein (CRP) in non-pregnant women is a marker of increased cardiovascular disease risk (147).

We therefore measured cross-sectional associations between PFAS concentrations at mid-pregnancy and the levels of the following cardiometabolic clinical chemistries: total cholesterol, LDL cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, uric acid, and CRP. The purpose of this study was to increase understanding of the physiologic correlates of plasma PFAS concentration during pregnancy and to evaluate a possible mechanism of any associations that may exist between plasma PFAS concentrations and adverse pregnancy outcomes, including preeclampsia.

4.2.2. Material and Methods

4.2.2.1. Cohort description and eligibility criteria

The Norwegian Mother and Child Cohort Study (MoBa) is a prospective population-based pregnancy cohort conducted by the Norwegian Institute of Public Health (152, 153, 170). The study was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate. Participants were recruited from all over Norway in 1999-2008. In total, 39% of invited women participated. Informed consent was obtained from each MoBa participant upon recruitment. At the time of enrollment, women provided information via questionnaire regarding a number of demographic and lifestyle characteristics, as well as events in their reproductive and medical history. Blood samples were obtained from the mother during pregnancy (median = 18 weeks of gestation). Data obtained in MoBa were linked to the Medical Birth Registry of Norway (171). Further details may be found at www.fhi.no/morogbarn. The current study is based on version 4.301 of the quality-assured data files released for research.

The current analysis uses data that were previously collected for a case-control study of PFASs and subfecundity (41). For the previous study, 400 pregnancies were randomly selected from all eligible MoBa participants who met criteria for subfecundity (defined as self-reported time to pregnancy of greater than 12 months), and 550 pregnancies were randomly selected from all eligible MoBa participants who reported a time to pregnancy of any duration. To be eligible for selection into the previous study, women must have enrolled in MoBa in 2003-2004, delivered a live-born child, provided a mid-pregnancy plasma sample, and provided complete information about time to

pregnancy on the enrollment questionnaire. From the 950 participants in the previous study, 889 women with complete information on covariates and outcomes of interest were included in the present analysis.

4.2.2.2. Collection and analysis of plasma samples

At the time of study enrollment, maternal non-fasting blood samples were collected in EDTA tubes at hospitals and maternity units across Norway and shipped at ambient temperature to the MoBa biorepository in Oslo. The majority of samples were received and processed the day after collection (153). At the biorepository, plasma was separated, aliquoted, and stored at -80 degrees Celsius. Changes in PFAS concentrations in transit are believed to be negligible, as PFASs are chemically stable (173), and a recent study showed no evidence of change over time in concentrations of four PFASs in serum maintained at room temperature for 10 days (156). Lipid concentrations are also expected to be relatively unchanged during shipping; a previous study demonstrated that lipid measurements changed by only a small percentage (<7% for LDL, <4% for HDL, total cholesterol, and triglycerides) when whole blood was kept at room temperature (21 degrees Celsius) in EDTA tubes for up to 7 days (189).

4.2.2.3. Exposure measurement

Concentrations (ng/mL) of nineteen PFASs were measured in maternal plasma using high-performance liquid chromatography/tandem mass spectrometry at the Norwegian Institute of Public Health. Details of the analytic process have been published previously (155). Analyses were restricted to the seven PFASs present in at least 50% of

samples: perfluorooctane sulfonate (PFOS), perfluoroheptane sulfonate (PFHpS), perfluorohexane sulfonate (PFHxS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnDA). The limit of quantification (LOQ) was 0.05 ng/mL for these seven PFASs. For quantification of PFOS, the total area of linear and branched isomers was integrated. A total of 50 blinded, pooled specimens were analyzed in the same batches as the sample specimens for quality assurance/quality control (QA/QC) purposes. Inter-assay coefficients of variation were calculated for each PFAS.

4.2.2.4. Outcome measurement

Plasma lipid parameters (total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides) as well as uric acid and CRP were measured in the same mid-pregnancy, non-fasting plasma sample that was used to evaluate PFAS concentrations. All analytes were measured with an Olympus AU400e Clinical Chemistry Analyzer at the National Institute of Environmental Health Sciences, using reagents from Beckman Coulter and Genzyme Diagnostics. The method used to measure LDL was direct enzymatic (N-geneous® LDL-ST cholesterol reagent). Inter-assay coefficients of variation were calculated for all lipid parameters based on the 50 QA/QC samples.

4.2.2.5. Covariates

Information on maternal characteristics was derived from the MoBa baseline questionnaire and from the Medical Birth Registry of Norway (MBRN). Variables considered to be potential confounders based on the prior literature were: maternal age (5,

7, 89, 90), pre-pregnancy body mass index (BMI) (60), nulliparous or most recent interpregnancy interval (41, 60), duration of breastfeeding most recent child (41), maternal years of education (5, 89), current smoking at mid-pregnancy (5, 7, 89, 90), gestational weeks at blood draw (60), and amount of oily fish consumed daily at the time of the mid-pregnancy questionnaire (90, 178).

Additionally, for models with HDL cholesterol as the outcome, adjustment for plasma albumin concentration (g/dL; quartiles) was considered due to the positive correlation observed between HDL and plasma albumin. Albumin was measured in the mid-pregnancy maternal plasma sample with an Olympus AU400e Clinical Chemistry Analyzer at the National Institute of Environmental Health Sciences, using reagents from Beckman Coulter. The method of measurement was direct and utilized a neutral buffered solution of bromocresol green as a dye binding indicator.

4.2.2.6. Statistical analysis

Weighted multiple linear regression was used to estimate the association between each PFAS concentration and each continuous clinical chemistry outcome. Weighted logistic regression was used to estimate the association between each PFAS concentration and each binary clinical chemistry outcome. Weights were based on the inverse probability of selection into the original case-control study (41), as described in Richardson et al. (166). A sensitivity analysis was performed to examine whether restricting to the women who were selected without regard to subfecundity (the 'base sample') would produce results that closely resembled the results of the weighted analysis. Additional sensitivity analyses examined whether (1) adjustment for

subfecundity rather than weighting, or (2) restriction of the study population to nulliparous women only, would influence results. The analysis of nulliparous women only was performed only with adjustment for subfecundity rather than weighting because the additional restrictions on eligibility rendered the previous selection weights invalid.

Concentrations of PFASs were treated in three ways for the purposes of analysis: (1) as quartiles, with the lowest quartile serving as the referent category (with the exception of PFDA, which was categorized only at or above versus below the median due to >25% of values below the limit of quantification), (2) as natural-log transformed continuous variables to assess linear trends, and (3) combined into a single "multiple high exposure" variable which was equal to 1 if all PFAS concentrations measured for a given participant were in the highest category of the observed distribution. In models using quartiles of exposure, beta-coefficients represent the change in clinical chemistry outcome associated with each of the upper quartiles, relative to the lowest quartile of PFAS concentration. In models using natural log-transformed continuous exposures, the beta-coefficients represent the change in clinical chemistry outcome associated with each natural log-unit increase in each PFAS. Additionally, the change in clinical chemistry outcome associated with an interquartile-range (IQR) shift in each continuous ln-PFAS (from the 25^{th} percentile to the 75^{th} percentile of the observed exposure distribution) is reported. For PFDA, because more than 25% of values were below the LOQ, the IQR was estimated from the observed portion of the log-normal distribution. For all PFASs, values below the LOQ were replaced by the expected value of the log-normal distribution, given that the value was below the limit of quantification; this was

calculated as the mean of values below the limit of quantification randomly drawn from the estimated log-normal distribution (175).

Each lipid parameter or clinical chemistry outcome was treated as a continuous outcome variable in a separate model with a single PFAS exposure variable. Plasma triglycerides and CRP were natural-log transformed in order to meet the modeling assumption of normally distributed residuals. For simplicity and comparability, models for all six lipid parameters and clinical chemistry outcomes were adjusted for the same covariate set. Covariates were selected through the construction of a directed acyclic graph (DAG) representing the existing literature (Figure 4.2), and the identification of a minimally sufficient set of variables to control confounding. The DAG was primarily based on expected confounders of the PFAS-HDL association but was also generalizable to the other outcomes. The minimally sufficient adjustment set was identified using DAGitty v1.0 (www.dagitty.net).

All covariates were modeled as categorical variables to allow for non-linear associations. The categorization of covariates was as follows: maternal age (<24, 25-29, 30-34, >=35 years); pre-pregnancy BMI (<25, 25-29.99, >=30 kg/m^2); maternal education (less than high school, completed high school, some college, 4 or more years of college); smoking at mid-pregnancy (yes/no), oily fish consumed (0-3.0, 3.1-7.5, 7.6-14.9, 15-100 g/day); gestational age at blood draw (12-16, 17-18, 19-20, 21-37 weeks); nulliparous or most recent interpregnancy interval (nulliparous, 4-23, 24-47, 48-245 months); and breastfeeding duration in previous pregnancy (nulliparous or <1, 1-5, 6-11, 12-17, 18-36 months). Subjects were excluded from the analysis if they had missing values for any modeled covariates.

Some PFASs were moderately or highly correlated with other measured PFASs. Spearman rank-order correlations were calculated between each pair of PFASs. In order to explore the possible influence of confounding by correlated PFASs in single-pollutant models, a multiple-pollutant model was estimated for HDL cholesterol. In the multiple-pollutant model, all seven PFASs detectable in >50% of samples were included in the same model as natural-log transformed continuous variables. The variance inflation factors for each of the exposures in this model were examined to assess collinearity. Finally, an empirical Bayes model was fitted for HDL in which all coefficients for each ln-PFAS were shrunk to a common mean. The empirical Bayes model lends stability to estimates of highly correlated exposures by assuming that all coefficients for each exposure-outcome association are drawn from a common distribution. Each individual coefficient is shrunk toward the mean of this common distribution. All statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC).

4.2.3. Results

The sample size for this analysis was 889 women with complete data on all covariates. Each of the individual covariates had missing data for <3% of participants. Participants ranged in age from 19 to 44 (Table 4.9). Half of the women had no previous live births or stillbirths (50%), while 3% had three or more previous pregnancies. A total of 8% reported smoking during pregnancy. The range of gestational age at the time of blood draw was from 12 weeks to 37 weeks; however, the majority of women (99%) provided plasma samples during their second trimester of pregnancy (14-26 weeks'

gestation), and 73% of participants provided plasma samples between 17 and 20 weeks of gestation.

Of the nineteen PFASs measured, only seven were detectable in greater than 50% of samples: PFOA, PFNA, PFDA, PFUnDA, PFHxS, PFHpS, and PFOS. Further analyses were restricted to these seven compounds. PFDA was detectable in 70% of samples, while the six other PFASs were detectable in at least 88% of samples (Table 4.10). PFOS and PFOA were detectable in 100% of samples. The highest median concentration was observed for PFOS (13.03 ng/mL), followed by PFOA, then PFHxS, PFNA, PFUnDA, PFHpS, and finally PFDA (0.09 ng/mL).

A number of the PFASs demonstrated moderate to high pairwise correlations (Table 4.11). The most strongly correlated PFASs were PFNA and PFDA (ρ=0.71). The least correlated PFASs among the seven were PFUnDA and PFHpS (ρ=0.17). In general, the sulfonates (PFHxS, PFHpS, PFOS) were relatively highly correlated with one another (ρ=0.53-0.59) while the pairwise correlations among the carboxylates (PFOA, PFNA, PFDA, PFUnDA) were more variable (ρ=0.26-0.71).

The median plasma concentration for total cholesterol was 211 mg/dL, for HDL was 67 mg/dL, for LDL was 125 mg/dL, for triglycerides was 124 mg/dL, for uric acid was 3.2 (mg/dL), and for CRP was 4.4 mg/L (Table 4.12). One participant was missing data for all clinical chemistry analytes; another participant had a missing value for C-reactive protein only. The distribution of plasma total cholesterol, HDL cholesterol, and LDL cholesterol was approximately normal while the distributions of plasma triglycerides and C-reactive protein demonstrated positive skew. Triglycerides and C-reactive protein were therefore natural-log transformed in subsequent analyses. Two

implausibly low values for HDL cholesterol (2.5 mg/dL) and triglycerides (2.5 mg/dL), and one implausibly high value for C-reactive protein (250 mg/L) were excluded and the values were treated as missing.

Lipid concentrations were moderately to highly correlated with one another; the highest correlation was observed between total cholesterol and LDL (ρ=0.88), while total cholesterol was only moderately correlated with HDL (ρ=0.36) and ln-triglycerides (ρ=0.34). HDL and LDL were very weakly correlated (ρ=0.06, $p>0.05$), while HDL and ln-triglycerides were negatively correlated (ρ= -0.29, $p<0.05$). Uric acid and C-reactive protein were moderately correlated with one another (ρ=0.23), and C-reactive protein was moderately correlated with triglycerides (ρ=0.26)

Total cholesterol was positively associated with ln-PFOS as a continuous variable (Table 4.13). Each ln-unit increase in PFOS was associated with an increase of 8.89 mg/dL (95% CI=1.64, 16.14) in total cholesterol. For each IQR-unit increase in ln-PFOS, total cholesterol increased by 4.22 mg/dL (95% CI=0.78, 7.66). The latter change represents an increase of 2.0% over the median concentration of total cholesterol in this population. The third and fourth quartiles of PFOS had elevated total cholesterol as compared to the first quartile, but the confidence intervals were imprecise.

The single pollutant model for the association between ln-PFOS and total cholesterol was additionally adjusted for ln-PFUnDA to evaluate whether this PFAS, which appeared to confound the PFAS-HDL associations, also might have produced bias in the PFOS-total cholesterol association observed. There was no evidence of confounding bias by ln-PFUnDA on the PFOS-total cholesterol association in the adjusted model. The covariate-adjusted change in total cholesterol associated with a 1 ln-

unit change in PFOS without adjustment for ln-PFUnDA was 8.89 (95% CI=1.64, 16.14); in the model further adjusted for ln-PFUnDA the change in total cholesterol for a 1-ln-unit change in PFOS was 10.26 (95% CI=2.35, 18.16).

None of the other PFASs had notable linear associations with total cholesterol; in addition, the quartile estimates generally did not provide support for monotonic dose-response relationships between PFASs and total cholesterol.

In adjusted quartile analyses, all seven PFASs had higher HDL cholesterol associated with the highest quartile of exposure, relative to the lowest quartile of exposure (or, in the case of PFDA, associated with concentration at or above the median versus below the median) (Table 4.14). Additionally, PFOS, PFNA, PFDA, PFUnDA, and PFHxS showed positive linear associations with HDL cholesterol in adjusted models. The strongest evidence supporting a monotonic dose-response relationship with HDL was observed for PFUnDA, which had the highest quartile-specific associations as well as the largest associated change in HDL. For each natural log-unit increase in PFUnDA, HDL increased by 4.05 mg/dL (95% CI=2.76, 5.35). For each IQR-unit increase in ln-PFUnDA, HDL increased by 3.71 mg/dL (95% CI=2.52, 4.90). This change represents an increase of 5.5% over the median concentration of HDL cholesterol in this sample.

The other six PFASs also demonstrated some evidence of an exposure-response relationship with HDL in adjusted quartile analyses, although in general the association was driven by the highest quartile of exposure. Each IQR-unit increase in ln-PFNA was associated with an increase of 1.67 mg/dL HDL (95% CI=0.57, 2.76); each IQR-unit increase in ln-PFDA was associated with an increase of 2.57 mg/dL HDL (95% CI=1.23,

3.90); and each IQR-unit increase in ln-PFOS was associated with an increase of 2.08 mg/dL HDL (95% CI=1.12, 3.04).

Adjustment for plasma albumin concentration (quartiles) tended to reduce the magnitude of the associations between each PFAS quartile and HDL cholesterol (Table 4.15). On average, the difference in HDL cholesterol between the lowest and highest quartile of PFAS was reduced by 15% with the inclusion of plasma albumin in the model. The coefficients for the linear associations between continuous natural-log transformed PFAS variables and continuous HDL were attenuated by 16% on average, but remained elevated.

All seven PFASs were included as continuous natural-log transformed exposure variables in the same model to determine their mutually-adjusted associations with HDL. The coefficients for all PFASs were attenuated, with the exception of PFUnDA, which remained strongly associated with HDL (Table 4.16). The change in HDL for each ln-unit of PFNA was reduced dramatically from 2.84 mg/dL (95% CI=0.97, 4.71) to -2.04 (95% CI=-5.09, 1.00) in the adjusted multiple pollutant model. By contrast, the change in HDL for each ln-unit of PFUnDA decreased only slightly from 4.05 mg/dL (95% CI=2.76, 5.35) to 3.96 mg/dL (95% CI=2.05, 5.88). The multiple pollutant model therefore confirmed the strong association between PFUnDA and HDL. The influence of including the other six PFASs in the multiple pollutant model was generally much stronger than the influence of adjustment for covariates in the single pollutant models. The multiple pollutant model did not show evidence of multicollinearity; all variance inflation factors for the PFAS variables were <4.

A multiple pollutant empirical Bayes model was also fitted for HDL, including only the 524 participants selected without regard to subfecundity (base sample). In the empirical Bayes model, beta-coefficients were shrunk to a common mean (Table 4.17). The changes in beta-coefficients between the multiple pollutant model and the empirical Bayes model were minimal, as compared to the changes between the single pollutant and multiple pollutant model estimates (Figure 4.3).

In adjusted models for LDL cholesterol, the beta-coefficient for a 1 ln-unit change in PFOS was elevated, but the confidence interval was wide and included the null (Table 4.18); each ln-unit shift in PFOS was associated with a change of 6.43 mg/dL LDL (95% CI= -0.12, 12.97). The second quartile of PFOS concentration was associated with a decrease in LDL concentration relative to the first quartile, while the third and fourth quartiles of PFOS concentration were associated with elevated LDL. None of the other six PFASs were associated with LDL.

Linear associations between PFASs and triglycerides were not observed (Table 4.19). The fourth quartile of PFUnDA concentration was associated with small decrease in ln-triglycerides (-0.08 ln-mg/dL, 95% CI= -0.16, -0.01) relative to the first quartile. None of the other PFASs were associated with triglycerides in quartile-based or continuous models. Similarly, no associations between ln-PFAS and uric acid were observed, either in linear or quartile-based analyses (Table 4.20).

There was some indication of an inverse association between PFNA and natural log-transformed CRP (Table 4.21). The change in CRP for each ln-ng/mL change in PFNA was -0.15 ln-mg/L (95% CI = -0.28, -0.03); each IQR-unit shift in PFNA was associated with a change of -0.09 ln-mg/L of CRP (95% CI = -0.16, -0.02). The fourth

quartile of PFNA was also associated with lower ln-C-reactive protein relative to the first quartile (-0.23 ln-mg/L, 95% CI = -0.41, -0.05). None of the other PFASs were associated with CRP in quartile-based or continuous models.

When the lipid parameters and cardiometabolic clinical chemistries were examined as binary outcome variables corresponding to above or below the upper quartile of the observed distribution, the results were similar to the results of the continuous outcome models (Tables 4.22-4.27). PFUnDA and PFDA were both associated with reduced odds of low HDL (below the 25th percentile; Table 4.23). For PFDA, the adjusted odds ratio for low HDL associated with PFDA concentration at or above the median relative to below the median was OR=0.55 (95% CI=0.36, 0.84). For PFUnDA, the adjusted odds ratio for low HDL associated with the highest quartile of PFUnDA concentration relative to the lowest quartile was OR=0.29 (95% CI=0.16, 0.55). The second quartile of PFUnDA was associated with reduced odds of high triglycerides (above the 75th percentile; Table 4.25), OR=0.52 (95% CI=0.30, 0.89), as was the highest quartile of PFHpS (OR=0.56, 95% CI=0.31, 0.99). The highest categories of PFDA, PFUnDA, and PFHxS were associated with reduced odds of high CRP (Table 4.27).

Additional sensitivity analyses were performed to examine the robustness of results to different modeling strategies. Restricting the analysis to the 524 women with complete covariate and outcome data who were selected without regard to subfecundity produced quartile effect estimates that were similar to those produced by the weighted models, although the confidence limits were wider due to the smaller sample size (Tables 4.28-4.33).

The influence of weighting for prior selection by subfecundity was evaluated by adjusting for subfecundity (prior case status) and performing unweighted analyses. Estimates did not change systematically from the weighted estimates (Tables 4.34-4.39).

The unweighted analysis was additionally restricted to nulliparous women only, in order to examine whether observed associations differed in this subpopulation as compared to the larger sample. When adjusted analyses were restricted to nulliparous women only, confidence intervals widened further but the overall interpretation of results did not change, with the possible exception of stronger inverse associations with natural log-transformed triglycerides (Tables 4.40-4.45).

Finally, exposure was defined as a binary variable, "multiple high exposure," which was equal to 1 when the measured concentrations of all seven PFASs were in the highest quartile of the observed distribution. The results of linear regression of each clinical chemistry outcome on this binary exposure variable were similar to the results of the single pollutant analyses, showing a positive linear association with HDL cholesterol, and a weak inverse association with natural-log transformed triglycerides (Table 4.46).

4.2.4. Discussion

Among pregnant women from a population with background levels of PFAS exposure, plasma concentrations of all seven PFASs examined were positively associated with HDL cholesterol. The observed associations are consistent with the findings of some previous cross-sectional studies, but inconsistent with others. A recent study of 723 non-pregnant Inuit adults with background levels of PFOS exposure showed a positive association between PFOS and HDL cholesterol in both men and women (90). Among

12,476 children and adolescents from a highly PFOA-exposed community in Ohio and West Virginia, serum PFOS was positively associated with HDL cholesterol (57). However, other studies have reported no association between PFASs and HDL cholesterol (5, 7), and one recent study of highly PFOA-exposed male workers in China reported an inverse association between PFOA and HDL (88).

Evidence of a linear association between PFOS and total cholesterol was also observed in the present study, but no evidence of association between other PFASs and total cholesterol. A number of previous studies have described positive associations between multiple PFASs and total cholesterol. Among adults and children in the highly PFOA-exposed Ohio and West Virginia population, both PFOA and PFOS (the latter was not elevated above background levels) were positively associated with serum total cholesterol (5, 57). Studies of highly exposed, predominantly male, adult workers have reported positive cross-sectional associations between PFOA and total cholesterol (4, 38, 56). However, some studies have reported null or inverse associations. A study of non-pregnant adults in the general U.S. population described positive associations between PFOS, PFOA, PFNA and total cholesterol, but an inverse association between PFHxS and total cholesterol (7). A recent study of non-pregnant adult Canadians reported a positive association between PFHxS and total cholesterol, but no significant associations between PFOA or PFOS and total cholesterol in weighted analyses (89).

We observed no associations between any PFAS and LDL cholesterol, either in quartile analyses or when each PFAS was treated as a continuous variable. Similarly, there were no linear associations between PFASs and triglycerides, although the highest quartile of PFUnDA exposure and the second quartile of PFHpS were associated with

slight decreases in triglycerides. The existing literature on this topic is inconclusive, with previous studies reporting either positive or no associations between PFASs and LDL cholesterol or triglycerides. Among adults and children in the highly PFOA-exposed Ohio and West Virginia population, PFOA concentrations were positively associated with LDL cholesterol and triglycerides; PFOS was positively associated with LDL cholesterol in both adults and children, and with triglycerides in adults only (5, 57). However, among non-pregnant Inuit adults, no association was observed between PFOS and LDL cholesterol (90). In one occupational study, PFOA concentration was positively associated with serum LDL cholesterol but not with triglycerides (56), while in another occupational study both PFOS and PFOA were positively associated with triglycerides (LDL not analyzed) (4).

Our study provided no strong evidence for associations between PFASs and uric acid or CRP. A previous study of highly PFOA-exposed adults found positive associations with uric acid for both PFOS and PFOA (6). In occupationally exposed individuals, PFOA was positively associated with uric acid, but not with CRP (38).

The median plasma concentrations of the seven PFASs in this study were comparable to reported serum levels from 2006 in Norway (36) and somewhat lower than reported serum levels from the United States in the same year (12). Plasma and serum measurements of PFASs have been shown to be approximately equal for a particular subject at a given time (190). Therefore the PFAS concentrations measured in the current study are comparable to the magnitude of exposure observed in previous studies of background-exposed populations.

This study is the first to specifically examine associations between PFASs and cardiometabolic clinical chemistries in pregnant women. Some previous studies (7, 90) excluded pregnant women, who are known to have altered lipid metabolism relative to non-pregnant women. Normal pregnancy is characterized by substantial changes in the concentration of various constituents of plasma relative to the non-pregnant state. Notably, total cholesterol rises 25-50% over non-pregnant levels (135) and triglycerides are typically elevated 200-400% times over pre-pregnant levels by late pregnancy (134, 137). HDL cholesterol also increases by approximately 40% over non-pregnant levels (134). The composition of HDL particles also changes during pregnancy, such that each particle contains a greater proportion of cholesterol and acylglycerols, but without an increase in phospholipid content (191).

PFOA and PFOS concentrations in pregnant women have been reported to be somewhat lower than in non-pregnant women (192); in one study this difference persisted after adjustment for plasma volume changes (via hemoglobin/hematocrit ratio) as well as demographic and lifestyle factors (193). The observed differences may be due to the residual confounding effects of normal plasma volume expansion during pregnancy, but may also suggest transfer of PFASs to the fetus, or increased excretion of PFASs during pregnancy (194). Given these differences in both lipids and PFASs during pregnancy, it is plausible that whatever causal or non-causal mechanism leads to observed associations between PFASs and lipids, the associations may differ in pregnant women relative to non-pregnant women.

The biological mechanism that may lead to positive associations between plasma PFAS concentrations and lipids in humans is largely unknown. PFAS are known to

activate PPARα, a receptor involved in regulating gene expression related to lipid and glucose metabolism, but the activation of this receptor in humans tends to produce lower lipid concentrations (195). While some of the toxic effects of PFOA in animals are believed to operate through activation of PPARα, evidence suggests that the toxicity of PFOS may occur through alternate mechanisms, independent of PPARα (82, 83). Recently, toxicologists have begun to study PFASs other than PFOA and PFOS, and have demonstrated varying strength of PPARα activation associated with PFASs of different chain lengths (196, 197). The relative proportion of biological effects due to PPARα-dependent mechanisms and PPARα-independent mechanisms may vary across PFASs.

The investigation of potential health effects due to PFASs other than PFOA and PFOS is particularly important given that human exposure to these less-studied PFASs may be increasing (12). Certain PFASs that previously appeared in human serum at low concentrations, such as PFHxS, have been used to replace PFOS in household stain-proofing and other applications following the phase-out of PFOS in 2000-2002, and therefore we may expect higher human exposures in the future (198, 199). As previously noted, a study of non-pregnant individuals in the US general population found opposite lipid associations with PFHxS as compared to the other PFASs studied (PFOA, PFOS, PFNA) (7), highlighting the importance of examining the effects of different PFASs separately.

As multiple PFASs tend to be correlated with one another, it is possible that the results observed in single-exposure models are partially due to the confounding influence of one or more correlated PFAS species. In the present study, the results of the model that included all seven PFASs suggested that PFUnDA had the strongest positive

association with HDL, and that the associations between HDL and other PFASs observed in single-exposure models may have been biased due to confounding by exposure to PFUnDA. PFUnDA was the longest-chain PFAS examined here, but we are not aware of any research suggesting that it has higher potency or biological activity in humans relative to the shorter-chain PFASs. Toxicology studies will be required to establish the relative biological activity of PFASs of different chain lengths.

A limitation of this study is the cross-sectional design, which does not allow causal interpretation of the findings. Non-causal explanations for the findings may include unmeasured confounding or pharmacokinetics. A recent laboratory study explored whether previously observed cross-sectional associations between PFASs and plasma lipid parameters could be explained by the distribution of PFASs into lipoprotein fractions, and concluded that this partitioning was not a sufficient explanation for the dose-response associations observed (182). However, the same study found that of the small proportion of each PFAS (for example, <25% of PFOS) that resided in the non-lipoprotein-depleted portion of the plasma, a somewhat larger percentage was recovered from the HDL subfraction relative to the LDL and VLDL subfractions (182), raising the possibility of a special affinity between HDL and some PFASs. Researchers may wish to further examine whether this affinity could account for the association observed here between HDL and PFASs in plasma.

Another potential limitation of this study is the use of non-fasting plasma lipid parameters. Fasting for 8-12 hours is typically recommended prior to lipid screening. However, researchers have observed minimal changes in lipid profiles following typical food intake (200). Additionally, non-fasting triglycerides may be more strongly

associated with cardiovascular disease risk in women than fasting triglycerides (201). Our study also employed a single measurement of plasma lipid concentrations. As these measurements may vary over time, multiple measures are preferred to establish lipid profiles for cardiovascular risk (202). Finally, our study did not consider dietary components other than oily fish that may influence lipid concentrations.

In general, the findings lend only modest support to a possible mechanism by which PFASs concentrations during pregnancy may be related to adverse pregnancy outcomes. Lipid disturbances in early and late pregnancy associated with preeclampsia include elevated triglycerides (141, 186), as well as higher total cholesterol and LDL, and typically lower HDL cholesterol (203, 204), although one study noted slightly higher HDL at mid-pregnancy among women who subsequently developed severe preeclampsia as compared with controls (141). We found no evidence of elevated triglycerides associated with PFAS concentrations in pregnant women. The positive associations we observed between multiple PFASs and HDL cholesterol are not consistent with elevated risk of preeclampsia. However, we did observe a positive linear association between PFOS and total cholesterol, and higher total cholesterol is associated with elevated risk of preeclampsia.

4.2.5. Conclusions

This study provides evidence of PFAS-specific associations with lipids in pregnant women. Specifically, plasma PFOS concentration during pregnancy was positively associated with total cholesterol, and multiple PFASs were positively associated with HDL cholesterol. While the latter finding is not consistent with lipid

profiles that have been associated with adverse pregnancy outcomes, the association between PFOS concentrations and cholesterol during pregnancy merits further investigation in a longitudinal study to determine whether or not it is likely to be causal. Elevated total cholesterol during pregnancy is associated with adverse pregnancy outcomes, including preeclampsia, which can result in harm to the mother and developing fetus.

Table 4.9. Characteristics of 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study (2003-2004) and previously selected for a case-control study of subfecundity.

	N	%		N	%
Age (years)			Time to pregnancy (months)		
19-24	54	6	<3	292	33
25-29	262	29	3-6	145	16
30-34	397	45	7-12	54	6
35-44	176	20	>12	396	45
Pre-pregnant body mass index (kg/m^2)			Previous live births or stillbirths		
14.9-<25.0	528	59	0	446	50
25.0-<30.0	240	27	1	307	35
30.0-45.4	121	14	2	111	12
			3 +	25	3
Education completed					
Less than high school	73	8	Interpregnancy interval (months)		
Completed high school	287	32	No previous births/stillbirths	446	50
Some college	372	42	4-23	106	12
4 or more years college	157	18	24-47	177	20
			48-245	160	18
Smoking at mid-pregnancy					
Yes	72	8			
No	817	92	Breastfeeding duration in previous pregnancy (months)		
			No previous births/stillbirths	446	50
Oily fish consumed (g/day)			<1	560	13
0-3.0	230	26	1-5	39	4
3.1-7.5	224	25	6-11	146	16
7.6-14.9	215	24	12-17	119	13
15-100	220	25	18-36	25	3
Gestational week at blood draw			Trimester of pregnancy at blood draw		
12-16	151	17	First (12-13 weeks)	6	0.7
17-20	651	73	Second (14-26 weeks)	879	99
21-37	87	10	Third (27-37 weeks)	4	0.4

Table 4.10. Plasma concentrations (ng/mL) of seven perfluoroalkyl substances detectable in at least 50% of samples from 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004.

	Abbreviation	Percent Detectable	Carbon Chain Length[a]	Percentile 5th	25th	50th	75th	95th	CV[b]
Perfluorocarboxylic Acids									
Perfluorooctanoic acid	PFOA	100.0	8	1.05	1.67	2.25	3.03	4.43	6.7
Perfluorononanoic acid	PFNA	99.9	9	0.17	0.29	0.39	0.51	0.81	15.6
Perfluorodecanoic acid	PFDA	70.3	10	<LOQ[c]	<LOQ	0.09	0.15	0.27	18.1
Perfluoroundecanoic acid	PFUnDA	94.0	11	<LOQ	0.13	0.22	0.33	0.57	32.8
Perfluorosulfonic Acids									
Perfluorohexane sulfonate	PFHxS	99.8	6	0.27	0.44	0.60	0.87	1.87	13.2
Perfluoroheptane sulfonate	PFHpS	88.0	7	<LOQ	0.09	0.13	0.19	0.32	44.5
Perfluorooctane sulfonate	PFOS	100.0	8	6.90	10.32	13.03	16.60	24.34	11.3

[a] Chain length for perfluorocarboxylic acids includes the carbon in the carboxyl group.
[b] Coefficient of variation, inter-assay, was calculated as (weighted standard deviation of batch means / weighted mean of batch means)*100 for the 50 pooled control samples, weighted by the number of pooled control samples in each analytic batch.
[c] Limit of quantification.

Table 4.11. Spearman correlation coefficients among plasma concentrations (ng/mL) of seven perfluoroalkyl substances detectable in at least 50% of samples from 889[a] pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004.

	PFOA	PFNA	PFDA	PFUnDA	PFHxS	PFHpS	PFOS
PFOA	-						
PFNA	0.62	-					
PFDA	0.42	0.71	-				
PFUnDA	0.26	0.56	0.60	-			
PFHxS	0.57	0.51	0.32	0.32	-		
PFHpS	0.48	0.43	0.24	0.17	0.53	-	
PFOS	0.64	0.66	0.44	0.44	0.59	0.59	-

[a] Values below the limit of quantification are excluded; sample size differs for each pairwise correlation.

Table 4.12. Distribution of plasma lipid concentrations among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004.

	Percentile					
	5th	25th	50th	75th	95th	CV[a]
Total cholesterol (mg/dL)	158	189	211	233	276	2.5
High-density lipoprotein cholesterol (mg/dL)	47	58	67	75	88	4.0
Low-density lipoprotein cholesterol (mg/dL)	81	107	125	147	178	4.3
Triglycerides (mg/dL)	74	98	124	158	217	8.7
Uric Acid (mg/dL)	2.2	2.7	3.2	3.6	4.3	2.4
C-reactive protein (mg/L)	1.1	2.8	4.4	7.9	17.1	2.3

[a] Coefficient of variation, inter-assay, was calculated as (weighted standard deviation of batch means / weighted mean of batch means)*100 for the 50 pooled control samples, weighted by the number of pooled control samples in each analytic batch.

Table 4.13. Weighted[a] linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and total cholesterol (mg/dL) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004.

		Adjusted[b] LSM[c] or β	95% CI[d]	
PFOA	Quartile 1 (referent)	206.93		
	Quartile 2	1.27	-6.73,	9.26
	Quartile 3	3.39	-4.67,	11.46
	Quartile 4	3.74	-5.17,	12.64
	Per ln-unit (PFOA)[e]	2.48	-4.42,	9.38
	Per IQR(ln-PFOA)[f]	1.49	-2.65,	5.64
PFNA	Quartile 1 (referent)	210.01		
	Quartile 2	-5.05	-12.54,	2.43
	Quartile 3	-3.84	-11.55,	3.87
	Quartile 4	2.25	-6.43,	10.94
	Per ln-unit (PFNA)	0.00	-6.00,	5.99
	Per IQR(ln-PFNA)	0.00	-3.52,	3.51
PFDA	Below median (referent)	207.91		
	At or above median	1.93	-3.47,	7.33
	Per ln-unit (PFDA)	1.70	-2.26,	5.67
	Per IQR(ln-PFDA)[g]	1.71	-2.27,	5.69
PFUnDA	Quartile 1 (referent)	207.62		
	Quartile 2	0.73	-6.34,	7.79
	Quartile 3	0.16	-7.30,	7.61
	Quartile 4	4.35	-3.52,	12.21
	Per ln-unit (PFUnDA)	0.84	-3.32,	5.01
	Per IQR(ln-PFUnDA)	0.77	-3.04,	4.59
PFHxS	Quartile 1 (referent)	207.26		
	Quartile 2	0.41	-7.13,	7.94
	Quartile 3	1.42	-6.29,	9.13
	Quartile 4	4.04	-4.10,	12.18
	Per ln-unit (PFHxS)	2.87	-1.89,	7.62
	Per IQR(ln-PFHxS)	1.92	-1.26,	5.10
PFHpS	Quartile 1 (referent)	209.26		
	Quartile 2	-1.01	-8.52,	6.50
	Quartile 3	-2.37	-10.03,	5.29
	Quartile 4	0.94	-7.20,	9.08
	Per ln-unit (PFHpS)	-0.61	-5.12	3.91
	Per IQR(ln-PFHpS)	-0.48	-4.09	3.12
PFOS	Quartile 1 (referent)	207.78		
	Quartile 2	-3.62	-10.63,	3.38
	Quartile 3	2.79	-5.21,	10.79
	Quartile 4	7.34	-0.68,	15.36
	Per ln-unit (PFOS)	8.89	1.64,	16.14
	Per IQR(ln-PFOS)	4.22	0.78,	7.66

[a] Weighted for prior selection by subfecundity.
[b] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[c] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[d] 95% confidence interval.
[e] Coefficient represents the change in total cholesterol for each 1 ln-(ng/mL) increase in PFAS concentration.
[f] Coefficient represents the change in total cholesterol for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[g] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.14. Weighted[a] linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and HDL cholesterol (mg/dL) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004.

		Adjusted[b] LSM[c] or β	95% CI[d]	
PFOA	Quartile 1 (referent)	61.15		
	Quartile 2	0.21	-2.40,	2.83
	Quartile 3	2.31	-0.59,	5.21
	Quartile 4	3.42	0.56,	6.28
	Per ln-unit (PFOA)[e]	2.14	-0.25,	4.52
	Per IQR(ln-PFOA)[f]	1.28	-0.15,	2.72
PFNA	Quartile 1 (referent)	61.60		
	Quartile 2	-0.06	-2.60,	2.49
	Quartile 3	0.49	-2.09,	3.07
	Quartile 4	3.27	0.48,	6.06
	Per ln-unit (PFNA)	2.84	0.97,	4.71
	Per IQR(ln-PFNA)	1.67	0.57,	2.76
PFDA	Below median (referent)	61.28		
	At or above median	2.73	0.89,	4.57
	Per ln-unit (PFDA)	2.56	1.22,	3.89
	Per IQR(ln-PFDA)[g]	2.57	1.23,	3.90
PFUnDA	Quartile 1 (referent)	59.31		
	Quartile 2	3.05	0.57,	5.54
	Quartile 3	4.40	1.86,	6.95
	Quartile 4	7.62	4.98,	10.25
	Per ln-unit (PFUnDA)	4.05	2.76,	5.35
	Per IQR(ln-PFUnDA)	3.71	2.52,	4.90
PFHxS	Quartile 1 (referent)	60.39		
	Quartile 2	1.57	-0.95,	4.10
	Quartile 3	2.69	0.07,	5.32
	Quartile 4	3.22	0.77,	5.66
	Per ln-unit (PFHxS)	1.46	0.19,	2.74
	Per IQR(ln-PFHxS)	0.98	0.13,	1.83
PFHpS	Quartile 1 (referent)	61.60		
	Quartile 2	-0.27	-2.59,	2.05
	Quartile 3	0.32	-2.22,	2.86
	Quartile 4	2.99	0.52,	5.46
	Per ln-unit (PFHpS)	1.30	-0.07,	2.66
	Per IQR(ln-PFHpS)	1.04	-0.05,	2.12
PFOS	Quartile 1 (referent)	60.33		
	Quartile 2	1.98	-0.38,	4.33
	Quartile 3	2.49	0.00,	4.99
	Quartile 4	4.46	2.04,	6.88
	Per ln-unit (PFOS)	4.39	2.36,	6.42
	Per IQR(ln-PFOS)	2.08	1.12,	3.04

[a] Weighted for prior selection by subfecundity.
[b] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[c] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[d] 95% confidence interval.
[e] Coefficient represents the change in lipid outcome for each 1 ln-(ng/mL) increase in PFAS concentration.
[f] Coefficient represents the change in lipid outcome for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[g] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.15. Weighted[a] linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and HDL cholesterol (mg/dL) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, additionally adjusted for plasma albumin concentration (quartiles).

		Adjusted[b] LSM[c] or β	95% CI[d]	
PFOA	Quartile 1 (referent)	60.95		
	Quartile 2	-0.07	-2.64,	2.50
	Quartile 3	2.13	-0.73,	4.98
	Quartile 4	3.01	0.12,	5.89
	Per ln-unit (PFOA)[e]	1.76	-0.63,	4.16
	Per IQR(ln-PFOA)[f]	1.06	-0.38,	2.50
PFNA	Quartile 1 (referent)	61.56		
	Quartile 2	-0.56	-3.09,	1.97
	Quartile 3	0.17	-2.39,	2.73
	Quartile 4	2.35	-0.46,	5.17
	Per ln-unit (PFNA)	2.30	0.41,	4.19
	Per IQR(ln-PFNA)	1.35	0.24,	2.46
PFDA	Below median (referent)	61.05		
	At or above median	2.37	0.54,	4.20
	Per ln-unit (PFDA)	2.22	0.87,	3.56
	Per IQR(ln-PFDA)[g]	2.22	0.87,	3.58
PFUnDA	Quartile 1 (referent)	59.11		
	Quartile 2	3.00	0.54,	5.46
	Quartile 3	4.29	1.77,	6.82
	Quartile 4	7.16	4.56,	9.75
	Per ln-unit (PFUnDA)	3.79	2.50,	5.07
	Per IQR(ln-PFUnDA)	3.47	2.29,	4.64
PFHxS	Quartile 1 (referent)	60.21		
	Quartile 2	1.37	-1.14,	3.88
	Quartile 3	2.59	0.00,	5.17
	Quartile 4	2.77	0.32,	5.23
	Per ln-unit (PFHxS)	1.14	-0.14,	2.42
	Per IQR(ln-PFHxS)	0.98	0.13,	1.83
PFHpS	Quartile 1 (referent)	61.47		
	Quartile 2	-0.56	-2.90,	1.79
	Quartile 3	0.06	-2.42,	2.53
	Quartile 4	2.46	-0.04,	4.96
	Per ln-unit (PFHpS)	1.01	-0.36,	2.37
	Per IQR(ln-PFHpS)	0.81	-0.28,	1.90
PFOS	Quartile 1 (referent)	60.08		
	Quartile 2	1.89	-0.48,	4.26
	Quartile 3	2.58	0.09,	5.07
	Quartile 4	4.00	1.60,	6.40
	Per ln-unit (PFOS)	3.89	1.84,	5.93
	Per IQR(ln-PFOS)	1.84	0.87,	2.82

[a] Weighted for prior selection by subfecundity.
[b] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily, plasma albumin concentration.
[c] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[d] 95% confidence interval.
[e] Coefficient represents the change in HDL cholesterol (mg/dL) for each 1 ln-(ng/mL) increase in PFAS concentration.
[f] Coefficient represents the change in HDL cholesterol (mg/dL) for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[g] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.16. Weighted[a] linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and HDL cholesterol (mg/dL) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004. Single pollutant model and multiple pollutant model.

	Single pollutant model, adjusted[b]		Multiple pollutant model, adjusted	
	β per ln-unit[c]	95% CI[d]	β per ln-unit	95% CI
PFOA	2.14	-0.25, 4.52	0.51	-3.00, 4.03
PFNA	2.84	0.97, 4.71	-2.04	-5.09, 1.00
PFDA	2.56	1.22, 3.89	0.30	-1.86, 2.45
PFUnDA	4.05	2.76, 5.35	3.96	2.05, 5.88
PFHxS	1.46	0.19, 2.74	0.06	-1.60, 1.72
PFHpS	1.30	-0.07 2.66	0.14	-1.63, 1.92
PFOS	4.39	2.36 6.42	2.24	-1.37, 5.84

[a] Weighted for prior selection by subfecundity.
[b] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[c] Coefficient represents the change in HDL cholesterol (mg/dL) for each 1 ln-(ng/mL) increase in PFAS concentration.
[d] 95% confidence interval.

Table 4.17. Linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and HDL cholesterol (mg/dL) among 524 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, selected without regard to subfecundity (base sample). Multiple pollutant model compared to hierarchical empirical Bayes shrinkage model.

	Multiple pollutant model, adjusted[a]		Empirical Bayes model, adjusted	
	β per ln-unit[b]	95% CI[c]	β per ln-unit	95% CI
PFOA	0.68	-3.36, 4.71	0.41	-1.97, 2.67
PFNA	-2.30	-6.12, 1.52	-0.18	-3.10, 1.70
PFDA	0.22	-2.19, 2.62	0.51	-1.28, 2.01
PFUnDA	4.02	1.74, 6.30	2.38	0.58, 4.81
PFHxS	0.14	-2.06, 2.34	0.36	-1.34, 1.85
PFHpS	0.07	-2.10, 2.23	0.37	-1.26, 1.83
PFOS	2.42	-1.98, 6.81	1.11	-1.00, 3.85

[a] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[b] Coefficient represents the change in HDL cholesterol (mg/dL) for each 1 ln-(ng/mL) increase in PFAS concentration.
[c] 95% confidence interval.

Table 4.18. Weighted[a] linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and LDL cholesterol (mg/dL) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004.

		Adjusted[b] LSM[c] or β	95% CI[d]	
PFOA	Quartile 1 (referent)	125.96		
	Quartile 2	0.77	-6.27,	7.81
	Quartile 3	4.05	-3.30,	11.41
	Quartile 4	3.24	-4.48,	10.95
	Per ln-unit (PFOA)[e]	2.19	-4.03,	8.41
	Per IQR(ln-PFOA)[f]	1.32	-2.42,	5.06
PFNA	Quartile 1 (referent)	129.39		
	Quartile 2	-4.87	-11.62,	1.89
	Quartile 3	-3.81	-10.70,	3.09
	Quartile 4	-0.77	-8.27,	6.73
	Per ln-unit (PFNA)	-2.15	-7.32,	3.01
	Per IQR(ln-PFNA)	-1.26	-4.29,	1.77
PFDA	Below median (referent)	127.29		
	At or above median	0.79	-3.97,	5.54
	Per ln-unit (PFDA)	0.10	-3.40,	3.59
	Per IQR(ln-PFDA)[g]	0.10	-3.41,	3.61
PFUnDA	Quartile 1 (referent)	129.04		
	Quartile 2	-1.77	-8.02,	4.49
	Quartile 3	-3.55	-10.35,	3.25
	Quartile 4	-2.45	-9.49,	4.60
	Per ln-unit (PFUnDA)	-2.39	-6.00,	1.22
	Per IQR(ln-PFUnDA)	-2.19	-5.49,	1.11
PFHxS	Quartile 1 (referent)	127.12		
	Quartile 2	0.25	-6.40,	6.91
	Quartile 3	0.36	-6.31,	7.02
	Quartile 4	1.33	-6.05,	8.70
	Per ln-unit (PFHxS)	1.82	-2.60,	6.24
	Per IQR(ln-PFHxS)	1.22	-1.74,	4.17
PFHpS	Quartile 1 (referent)	127.03		
	Quartile 2	2.73	-3.81,	9.28
	Quartile 3	-1.07	-7.83,	5.70
	Quartile 4	2.07	-4.98,	9.12
	Per ln-unit (PFHpS)	-0.04	-4.00,	3.93
	Per IQR(ln-PFHpS)	-0.03	-3.20,	3.14
PFOS	Quartile 1 (referent)	127.18		
	Quartile 2	-3.43	-9.50,	2.65
	Quartile 3	2.40	-4.71,	9.51
	Quartile 4	5.32	-1.82,	12.46
	Per ln-unit (PFOS)	6.43	-0.12,	12.97
	Per IQR(ln-PFOS)	3.05	-0.06,	6.15

[a] Weighted for prior selection by subfecundity.
[b] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[c] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[d] 95% confidence interval.
[e] Coefficient represents the change in lipid outcome for each 1 ln-(ng/mL) increase in PFAS concentration.
[f] Coefficient represents the change in lipid outcome for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[g] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.19. Weighted[a] linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and natural-log transformed triglycerides (ln-mg/dL) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004.

		Adjusted[b] LSM[c] or β	95% CI[d]	
PFOA	Quartile 1 (referent)	4.92		
	Quartile 2	0.03	-0.04,	0.10
	Quartile 3	0.00	-0.08,	0.08
	Quartile 4	-0.04	-0.12,	0.04
	Per ln-unit (PFOA)[e]	0.00	-0.07,	0.06
	Per IQR(ln-PFOA)[f]	0.00	-0.04,	0.04
PFNA	Quartile 1 (referent)	4.94		
	Quartile 2	-0.03	-0.10,	0.04
	Quartile 3	-0.02	-0.09,	0.05
	Quartile 4	-0.02	-0.09,	0.06
	Per ln-unit (PFNA)	-0.02	-0.07,	0.03
	Per IQR(ln-PFNA)	-0.01	-0.04,	0.02
PFDA	Below median (referent)	4.95		
	At or above median	-0.06	-0.11,	0.00
	Per ln-unit (PFDA)	-0.03	-0.07,	0.00
	Per IQR(ln-PFDA)[g]	-0.03	-0.07,	0.00
PFUnDA	Quartile 1 (referent)	4.97		
	Quartile 2	-0.06	-0.13,	0.00
	Quartile 3	-0.07	-0.14,	0.00
	Quartile 4	-0.08	-0.16,	-0.01
	Per ln-unit (PFUnDA)	-0.04	-0.08,	0.00
	Per IQR(ln-PFUnDA)	-0.04	-0.07,	0.00
PFHxS	Quartile 1 (referent)	4.95		
	Quartile 2	-0.05	-0.11,	0.02
	Quartile 3	-0.02	-0.10,	0.05
	Quartile 4	-0.02	-0.10,	0.05
	Per ln-unit (PFHxS)	-0.01	-0.05,	0.03
	Per IQR(ln-PFHxS)	-0.01	-0.03,	0.02
PFHpS	Quartile 1 (referent)	4.96		
	Quartile 2	-0.07	-0.14,	0.00
	Quartile 3	-0.01	-0.08,	0.06
	Quartile 4	-0.06	-0.14,	0.01
	Per ln-unit (PFHpS)	-0.03	-0.07,	0.01
	Per IQR(ln-PFHpS)	-0.02	-0.06,	0.01
PFOS	Quartile 1 (referent)	4.93		
	Quartile 2	0.00	-0.07,	0.07
	Quartile 3	-0.03	-0.10,	0.04
	Quartile 4	-0.01	-0.08,	0.07
	Per ln-unit (PFOS)	-0.02	-0.09,	0.04
	Per IQR(ln-PFOS)	-0.01	-0.04,	0.02

[a] Weighted for prior selection by subfecundity.
[b] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[c] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[d] 95% confidence interval.
[e] Coefficient represents the change in lipid outcome for each 1 ln-(ng/mL) increase in PFAS concentration.
[f] Coefficient represents the change in lipid outcome for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[g] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.20. Weighted[a] linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and uric acid (mg/dL) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004.

		Adjusted[b] LSM[c] or β	95% CI[d]	
PFOA	Quartile 1 (referent)	3.29		
	Quartile 2	0.02	-0.13,	0.18
	Quartile 3	0.02	-0.14,	0.18
	Quartile 4	-0.01	-0.19,	0.16
	Per ln-unit (PFOA)[e]	0.03	-0.13,	0.20
	Per IQR(ln-PFOA)[f]	0.02	-0.08,	0.12
PFNA	Quartile 1 (referent)	3.29		
	Quartile 2	-0.03	-0.19,	0.13
	Quartile 3	0.02	-0.14,	0.19
	Quartile 4	0.04	-0.12,	0.21
	Per ln-unit (PFNA)	0.03	-0.10,	0.16
	Per IQR(ln-PFNA)	0.02	-0.06,	0.10
PFDA	Below median (referent)	3.32		
	At or above median	-0.07	-0.17,	0.04
	Per ln-unit (PFDA)	-0.03	-0.10,	0.04
	Per IQR(ln-PFDA)[g]	-0.03	-0.10,	0.04
PFUnDA	Quartile 1 (referent)	3.35		
	Quartile 2	-0.10	-0.26,	0.05
	Quartile 3	-0.07	-0.24,	0.10
	Quartile 4	-0.09	-0.26,	0.08
	Per ln-unit (PFUnDA)	-0.04	-0.13,	0.04
	Per IQR(ln-PFUnDA)	-0.04	-0.12,	0.04
PFHxS	Quartile 1 (referent)	3.27		
	Quartile 2	0.08	-0.07,	0.23
	Quartile 3	0.05	-0.09,	0.19
	Quartile 4	-0.03	-0.19,	0.12
	Per ln-unit (PFHxS)	-0.02	-0.13,	0.09
	Per IQR(ln-PFHxS)	-0.01	-0.09,	0.06
PFHpS	Quartile 1 (referent)	3.29		
	Quartile 2	-0.05	-0.19,	0.10
	Quartile 3	0.00	-0.15,	0.14
	Quartile 4	0.07	-0.08,	0.22
	Per ln-unit (PFHpS)	0.04	-0.05,	0.13
	Per IQR(ln-PFHpS)	0.03	-0.04,	0.10
PFOS	Quartile 1 (referent)	3.32		
	Quartile 2	-0.09	-0.25,	0.06
	Quartile 3	0.02	-0.14,	0.17
	Quartile 4	0.01	-0.15,	0.17
	Per ln-unit (PFOS)	0.00	-0.19,	0.19
	Per IQR(ln-PFOS)	0.00	-0.09,	0.09

[a] Weighted for prior selection by subfecundity.
[b] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[c] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[d] 95% confidence interval.
[e] Coefficient represents the change in lipid outcome for each 1 ln-(ng/mL) increase in PFAS concentration.
[f] Coefficient represents the change in lipid outcome for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[g] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.21. Weighted[a] linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and natural log-transformed C-reactive protein (ln-mg/L) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004.

		Adjusted[b] LSM[c] or β	95% CI[d]	
PFOA	Quartile 1 (referent)	1.77		
	Quartile 2	-0.01	-0.18.	0.15
	Quartile 3	-0.02	-0.20,	0.16
	Quartile 4	-0.11	-0.31,	0.08
	Per ln-unit (PFOA)[e]	-0.11	-0.27,	0.04
	Per IQR(ln-PFOA)[f]	-0.07	-0.16,	0.03
PFNA	Quartile 1 (referent)	1.84		
	Quartile 2	-0.16	-0.34,	0.02
	Quartile 3	-0.15	-0.32,	0.03
	Quartile 4	-0.23	-0.41,	-0.05
	Per ln-unit (PFNA)	-0.15	-0.28,	-0.03
	Per IQR(ln-PFNA)	-0.09	-0.16,	-0.02
PFDA	Below median (referent)	1.78		
	At or above median	-0.10	-0.22,	0.03
	Per ln-unit (PFDA)	-0.07	-0.16,	0.02
	Per IQR(ln-PFDA)[g]	-0.07	-0.16,	0.02
PFUnDA	Quartile 1 (referent)	1.79		
	Quartile 2	-0.05	-0.22,	0.12
	Quartile 3	-0.03	-0.19,	0.13
	Quartile 4	-0.12	-0.30,	0.07
	Per ln-unit (PFUnDA)	-0.05	-0.14,	0.04
	Per IQR(ln-PFUnDA)	-0.05	-0.13,	0.04
PFHxS	Quartile 1 (referent)	1.76		
	Quartile 2	-0.01	-0.20,	0.17
	Quartile 3	0.03	-0.15,	0.20
	Quartile 4	-0.10	-0.28,	0.07
	Per ln-unit (PFHxS)	-0.11	-0.21,	0.00
	Per IQR(ln-PFHxS)	-0.07	-0.14,	0.00
PFHpS	Quartile 1 (referent)	1.70		
	Quartile 2	0.15	-0.02,	0.32
	Quartile 3	0.00	-0.16,	0.16
	Quartile 4	0.13	-0.04,	0.30
	Per ln-unit (PFHpS)	0.06	-0.03,	0.15
	Per IQR(ln-PFHpS)	0.05	-0.03,	0.12
PFOS	Quartile 1 (referent)	1.73		
	Quartile 2	0.06	-0.12,	0.24
	Quartile 3	0.05	-0.12,	0.22
	Quartile 4	-0.02	-0.20,	0.15
	Per ln-unit (PFOS)	-0.02	-0.17,	0.13
	Per IQR(ln-PFOS)	-0.01	-0.08,	0.06

[a] Weighted for prior selection by subfecundity.
[b] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[c] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[d] 95% confidence interval.
[e] Coefficient represents the change in lipid outcome for each 1 ln-(ng/mL) increase in PFAS concentration.
[f] Coefficient represents the change in lipid outcome for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[g] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.22. Weighted[a] logistic regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and high total cholesterol (above the 75th percentile) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004.

		Adjusted[b] OR	95% CI[c]	
PFOA	Quartile 1 (referent)	1.		
	Quartile 2	1.13	0.66,	1.91
	Quartile 3	1.33	0.75,	2.34
	Quartile 4	1.05	0.57,	1.93
PFNA	Quartile 1 (referent)	1.		
	Quartile 2	0.71	0.42,	1.20
	Quartile 3	0.74	0.43,	1.25
	Quartile 4	1.24	0.71,	2.16
PFDA	Below median (referent)	1.		
	At or above median	1.35	0.92,	1.98
PFUnDA	Quartile 1 (referent)	1.		
	Quartile 2	0.98	0.57,	1.69
	Quartile 3	1.03	0.60,	1.77
	Quartile 4	1.45	0.83,	2.53
PFHxS	Quartile 1 (referent)	1.		
	Quartile 2	0.83	0.49,	1.42
	Quartile 3	1.15	0.68,	1.94
	Quartile 4	1.10	0.64,	1.90
PFHpS	Quartile 1 (referent)	1.		
	Quartile 2	0.94	0.56,	1.57
	Quartile 3	0.91	0.54,	1.51
	Quartile 4	0.98	0.57,	1.67
PFOS	Quartile 1 (referent)	1.		
	Quartile 2	0.64	0.38,	1.10
	Quartile 3	1.05	0.60,	1.83
	Quartile 4	1.56	0.90,	2.68

[a] Weighted for prior selection by subfecundity.
[b] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[c] 95% confidence interval.

Table 4.23. Weighted[a] logistic regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and low HDL cholesterol (below the 25th percentile) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004.

		Adjusted[b] OR	95% CI[c]	
PFOA	Quartile 1 (referent)	1.		
	Quartile 2	0.86	0.49,	1.53
	Quartile 3	0.74	0.38,	1.42
	Quartile 4	0.50	0.24,	1.00
PFNA	Quartile 1 (referent)	1.		
	Quartile 2	1.09	0.60,	1.98
	Quartile 3	1.26	0.70,	2.24
	Quartile 4	0.92	0.46,	1.84
PFDA	Below median (referent)	1.		
	At or above median	0.55	0.36,	0.84
PFUnDA	Quartile 1 (referent)	1.		
	Quartile 2	0.52	0.31,	0.88
	Quartile 3	0.36	0.20,	0.66
	Quartile 4	0.29	0.16,	0.55
PFHxS	Quartile 1 (referent)	1.		
	Quartile 2	0.86	0.48,	1.52
	Quartile 3	0.91	0.50,	1.66
	Quartile 4	0.81	0.44,	1.50
PFHpS	Quartile 1 (referent)	1.		
	Quartile 2	0.96	0.55,	1.69
	Quartile 3	1.33	0.76,	2.33
	Quartile 4	0.76	0.41,	1.42
PFOS	Quartile 1 (referent)	1.		
	Quartile 2	1.06	0.62,	1.82
	Quartile 3	0.96	0.55,	1.69
	Quartile 4	0.53	0.27,	1.04

[a] Weighted for prior selection by subfecundity.
[b] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[c] 95% confidence interval.

Table 4.24. Weighted[a] logistic regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and high LDL cholesterol (above the 75th percentile) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004.

		Adjusted[b] OR	95% CI[c]	
PFOA	Quartile 1 (referent)	1.		
	Quartile 2	1.12	0.66,	1.89
	Quartile 3	1.11	0.63,	1.98
	Quartile 4	1.03	0.55,	1.94
PFNA	Quartile 1 (referent)	1.		
	Quartile 2	0.63	0.37,	1.07
	Quartile 3	0.55	0.32,	0.93
	Quartile 4	0.85	0.49,	1.49
PFDA	Below median (referent)	1.		
	At or above median	1.12	0.77,	1.65
PFUnDA	Quartile 1 (referent)	1.		
	Quartile 2	0.79	0.46,	1.36
	Quartile 3	0.76	0.44,	1.33
	Quartile 4	0.95	0.55,	1.66
PFHxS	Quartile 1 (referent)	1.		
	Quartile 2	0.82	0.48,	1.42
	Quartile 3	0.99	0.58,	1.68
	Quartile 4	1.04	0.59,	1.82
PFHpS	Quartile 1 (referent)	1.		
	Quartile 2	1.17	0.68,	1.99
	Quartile 3	0.90	0.52,	1.55
	Quartile 4	1.24	0.71,	2.17
PFOS	Quartile 1 (referent)	1.		
	Quartile 2	0.63	0.37,	1.08
	Quartile 3	0.81	0.46,	1.44
	Quartile 4	1.65	0.96,	2.85

[a] Weighted for prior selection by subfecundity.
[b] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[c] 95% confidence interval.

Table 4.25. Weighted[a] logistic regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and high triglycerides (above the 75th percentile) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004.

		Adjusted[b] OR	95% CI[c]	
PFOA	Quartile 1 (referent)	1.		
	Quartile 2	1.08	0.62,	1.89
	Quartile 3	0.65	0.35,	1.20
	Quartile 4	0.52	0.26,	1.00
PFNA	Quartile 1 (referent)	1.		
	Quartile 2	0.88	0.49,	1.58
	Quartile 3	0.90	0.50,	1.63
	Quartile 4	1.03	0.56,	1.89
PFDA	Below median (referent)	1.		
	At or above median	0.78	0.52,	1.16
PFUnDA	Quartile 1 (referent)	1.		
	Quartile 2	0.52	0.30,	0.89
	Quartile 3	0.64	0.36,	1.13
	Quartile 4	0.68	0.38,	1.21
PFHxS	Quartile 1 (referent)	1.		
	Quartile 2	0.68	0.39,	1.20
	Quartile 3	0.89	0.51,	1.56
	Quartile 4	0.75	0.40,	1.41
PFHpS	Quartile 1 (referent)	1.		
	Quartile 2	0.43	0.24,	0.76
	Quartile 3	0.73	0.44,	1.23
	Quartile 4	0.56	0.31,	0.99
PFOS	Quartile 1 (referent)	1.		
	Quartile 2	0.95	0.55,	1.64
	Quartile 3	0.67	0.38,	1.18
	Quartile 4	0.86	0.47,	1.58

[a] Weighted for prior selection by subfecundity.
[b] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[c] 95% confidence interval.

Table 4.26. Weighted[a] logistic regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and high uric acid (above the 75th percentile) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004.

		Adjusted[b] OR	95% CI[c]	
PFOA	Quartile 1 (referent)	1.		
	Quartile 2	0.80	0.45,	1.42
	Quartile 3	0.97	0.52,	1.80
	Quartile 4	1.27	0.67,	2.43
PFNA	Quartile 1 (referent)	1.		
	Quartile 2	0.84	0.48,	1.46
	Quartile 3	1.00	0.58,	1.75
	Quartile 4	1.10	0.62,	1.95
PFDA	Below median (referent)	1.		
	At or above median	0.82	0.55,	1.21
PFUnDA	Quartile 1 (referent)	1.		
	Quartile 2	0.77	0.45,	1.32
	Quartile 3	0.75	0.42,	1.31
	Quartile 4	0.77	0.44,	1.37
PFHxS	Quartile 1 (referent)	1.		
	Quartile 2	1.50	0.85,	2.65
	Quartile 3	1.44	0.82,	2.50
	Quartile 4	1.22	0.69,	2.15
PFHpS	Quartile 1 (referent)	1.		
	Quartile 2	1.01	0.57,	1.79
	Quartile 3	1.51	0.89,	2.56
	Quartile 4	1.60	0.91,	2.81
PFOS	Quartile 1 (referent)	1.		
	Quartile 2	1.11	0.62,	1.97
	Quartile 3	1.46	0.83,	2.55
	Quartile 4	1.32	0.73,	2.38

[a] Weighted for prior selection by subfecundity.
[b] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[c] 95% confidence interval.

Table 4.27. Weighted[a] logistic regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and high C-reactive protein (above the 75th percentile) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004.

		Adjusted[b] OR	95% CI[c]	
PFOA	Quartile 1 (referent)	1.		
	Quartile 2	1.03	0.57,	1.86
	Quartile 3	1.16	0.62,	2.16
	Quartile 4	0.78	0.40,	1.52
PFNA	Quartile 1 (referent)	1.		
	Quartile 2	0.76	0.42,	1.38
	Quartile 3	0.48	0.27,	0.85
	Quartile 4	0.46	0.24,	0.87
PFDA	Below median (referent)	1.		
	At or above median	0.60	0.40,	0.92
PFUnDA	Quartile 1 (referent)	1.		
	Quartile 2	0.83	0.45,	1.50
	Quartile 3	0.73	0.40,	1.35
	Quartile 4	0.52	0.27,	0.99
PFHxS	Quartile 1 (referent)	1.		
	Quartile 2	0.83	0.47,	1.46
	Quartile 3	0.89	0.51,	1.54
	Quartile 4	0.36	0.19,	0.66
PFHpS	Quartile 1 (referent)	1.		
	Quartile 2	1.78	0.97,	3.26
	Quartile 3	1.11	0.61,	2.02
	Quartile 4	1.50	0.80,	2.81
PFOS	Quartile 1 (referent)	1.		
	Quartile 2	2.10	1.18,	3.73
	Quartile 3	1.07	0.58,	1.95
	Quartile 4	0.66	0.35,	1.23

[a] Weighted for prior selection by subfecundity.
[b] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[c] 95% confidence interval.

Table 4.28. Linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and total cholesterol (mg/dL) among 524 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, selected without regard to subfecundity status (base sample).

		Adjusted[a] LSM[b] or β	95% CI[c]	
PFOA	Quartile 1 (referent)	208.02		
	Quartile 2	1.38	-7.35,	10.11
	Quartile 3	3.43	-6.36,	13.21
	Quartile 4	3.90	-6.48,	14.29
	Per ln-unit (PFOA)[d]	2.53	-5.93,	10.98
	Per IQR(ln-PFOA)[e]	1.52	-3.56,	6.60
PFNA	Quartile 1 (referent)	211.56		
	Quartile 2	-6.47	-15.37,	2.43
	Quartile 3	-4.68	-13.53,	4.18
	Quartile 4	1.95	-7.61,	11.51
	Per ln-unit (PFNA)	-0.25	-7.03,	6.53
	Per IQR(ln-PFNA)	-0.15	-4.12,	3.83
PFDA	Below median (referent)	208.99		
	At or above median	2.00	-4.36,	8.36
	Per ln-unit (PFDA)	1.90	-2.71,	6.51
	Per IQR(ln-PFDA)[f]	1.91	-2.72,	6.53
PFUnDA	Quartile 1 (referent)	209.14		
	Quartile 2	0.20	-8.61,	9.02
	Quartile 3	-0.95	-10.04,	8.13
	Quartile 4	4.16	-5.47,	13.78
	Per ln-unit (PFUnDA)	0.62	-4.21,	5.45
	Per IQR(ln-PFUnDA)	0.57	-3.86,	4.99
PFHxS	Quartile 1 (referent)	208.32		
	Quartile 2	1.03	-7.64,	9.70
	Quartile 3	0.84	-7.76,	9.43
	Quartile 4	4.19	-4.94,	13.31
	Per ln-unit (PFHxS)	2.87	-2.48,	8.22
	Per IQR(ln-PFHxS)	1.92	-1.66,	5.49
PFHpS	Quartile 1 (referent)	210.43		
	Quartile 2	-0.44	-8.85,	7.98
	Quartile 3	-2.84	-11.32,	5.64
	Quartile 4	1.09	-8.13,	10.31
	Per ln-unit (PFHpS)	-0.55	-5.60,	4.50
	Per IQR(ln-PFHpS)	-0.44	-4.48,	3.60
PFOS	Quartile 1 (referent)	208.50		
	Quartile 2	-3.53	-11.98,	4.92
	Quartile 3	3.53	-5.52,	12.57
	Quartile 4	8.29	-0.90,	17.48
	Per ln-unit (PFOS)	9.13	0.97,	17.29
	Per IQR(ln-PFOS)	4.33	0.46,	8.20

[a] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[b] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[c] 95% confidence interval.
[d] Coefficient represents the change in total cholesterol for each 1 ln-(ng/mL) increase in PFAS concentration.
[e] Coefficient represents the change in total cholesterol for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[f] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.29. Linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and HDL cholesterol (mg/dL) among 524 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, selected without regard to subfecundity status (base sample).

		Adjusted[a] LSM[b] or β	95% CI[c]	
PFOA	Quartile 1 (referent)	61.23		
	Quartile 2	-0.03	-2.95,	2.89
	Quartile 3	2.28	-0.99,	5.55
	Quartile 4	3.49	0.02,	6.96
	Per ln-unit (PFOA)[d]	2.11	-0.73,	4.94
	Per IQR(ln-PFOA)[e]	1.27	-0.44,	2.97
PFNA	Quartile 1 (referent)	61.76		
	Quartile 2	-0.37	-3.36,	2.61
	Quartile 3	0.08	-2.89,	3.05
	Quartile 4	3.03	-0.18,	6.23
	Per ln-unit (PFNA)	2.67	0.40,	4.93
	Per IQR(ln-PFNA)	1.56	0.24,	2.89
PFDA	Below median (referent)	61.28		
	At or above median	2.62	0.50,	4.75
	Per ln-unit (PFDA)	2.47	0.94,	4.00
	Per IQR(ln-PFDA)[f]	2.48	0.94,	4.02
PFUnDA	Quartile 1 (referent)	59.40		
	Quartile 2	2.87	-0.03,	5.77
	Quartile 3	4.07	1.08,	7.06
	Quartile 4	7.62	4.45,	10.78
	Per ln-unit (PFUnDA)	4.04	2.45,	5.62
	Per IQR(ln-PFUnDA)	3.70	2.25,	5.15
PFHxS	Quartile 1 (referent)	60.33		
	Quartile 2	1.56	-1.34,	4.46
	Quartile 3	2.71	-0.17,	5.58
	Quartile 4	3.27	0.22,	6.32
	Per ln-unit (PFHxS)	1.51	-0.28,	3.31
	Per IQR(ln-PFHxS)	1.01	-0.19,	2.21
PFHpS	Quartile 1 (referent)	61.68		
	Quartile 2	-0.28	-3.09,	2.53
	Quartile 3	-0.01	-2.85,	2.82
	Quartile 4	3.19	0.11,	6.27
	Per ln-unit (PFHpS)	1.25	-0.44,	2.95
	Per IQR(ln-PFHpS)	1.00	-0.35,	2.35
PFOS	Quartile 1 (referent)	60.32		
	Quartile 2	1.89	-0.95,	4.72
	Quartile 3	2.44	-0.60,	5.47
	Quartile 4	4.44	1.35,	7.52
	Per ln-unit (PFOS)	4.38	1.65,	7.11
	Per IQR(ln-PFOS)	2.08	0.78,	3.37

[a] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[b] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[c] 95% confidence interval.
[d] Coefficient represents the change in HDL cholesterol for each 1 ln-(ng/mL) increase in PFAS concentration.
[e] Coefficient represents the change in HDL cholesterol for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[f] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.30. Linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and LDL cholesterol (mg/dL) among 524 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, selected without regard to subfecundity status (base sample).

		Adjusted[a] LSM[b] or β	95% CI[c]	
PFOA	Quartile 1 (referent)	126.77		
	Quartile 2	0.91	-6.75,	8.56
	Quartile 3	4.03	-4.55,	12.62
	Quartile 4	3.12	-5.99,	12.23
	Per ln-unit (PFOA)[d]	2.08	-5.34,	9.50
	Per IQR(ln-PFOA)[e]	1.25	-3.21,	5.71
PFNA	Quartile 1 (referent)	130.46		
	Quartile 2	-5.85	-13.68,	1.98
	Quartile 3	-4.34	-12.12,	3.44
	Quartile 4	-1.03	-9.44,	7.38
	Per ln-unit (PFNA)	-2.34	-8.28,	3.61
	Per IQR(ln-PFNA)	-1.37	-4.86,	2.12
PFDA	Below median (referent)	128.02		
	At or above median	0.95	-4.63,	6.53
	Per ln-unit (PFDA)	0.35	-3.69,	4.40
	Per IQR(ln-PFDA)[f]	0.36	-3.70,	4.42
PFUnDA	Quartile 1 (referent)	130.05		
	Quartile 2	-1.92	-9.66,	5.83
	Quartile 3	-4.26	-12.24,	3.72
	Quartile 4	-2.64	-11.09,	5.81
	Per ln-unit (PFUnDA)	-2.52	-6.76,	1.71
	Per IQR(ln-PFUnDA)	-2.31	-6.19,	1.57
PFHxS	Quartile 1 (referent)	127.84		
	Quartile 2	0.80	-6.82,	8.41
	Quartile 3	0.06	-7.49,	7.61
	Quartile 4	1.41	-6.60,	9.43
	Per ln-unit (PFHxS)	1.89	-2.81,	6.59
	Per IQR(ln-PFHxS)	1.26	-1.88,	4.40
PFHpS	Quartile 1 (referent)	127.80		
	Quartile 2	3.32	-4.06,	10.70
	Quartile 3	-1.14	-8.57,	6.30
	Quartile 4	2.03	-6.06,	10.11
	Per ln-unit (PFHpS)	0.05	-4.38,	4.49
	Per IQR(ln-PFHpS)	0.04	-3.50,	3.58
PFOS	Quartile 1 (referent)	127.52		
	Quartile 2	-3.20	-10.62,	4.22
	Quartile 3	3.16	-4.79,	11.10
	Quartile 4	6.41	-1.66,	14.48
	Per ln-unit (PFOS)	6.66	-0.51,	13.83
	Per IQR(ln-PFOS)	3.16	-0.24,	6.56

[a] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[b] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[c] 95% confidence interval.
[d] Coefficient represents the change in LDL cholesterol for each 1 ln-(ng/mL) increase in PFAS concentration.
[e] Coefficient represents the change in LDL cholesterol for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[f] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.31. Linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and natural-log transformed triglycerides (ln-mg/dL) among 524 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, selected without regard to subfecundity status (base sample).

		Adjusted[b] LSM[c] or β	95% CI[d]	
PFOA	Quartile 1 (referent)	4.93		
	Quartile 2	0.04	-0.04,	0.12
	Quartile 3	0.01	-0.08,	0.10
	Quartile 4	-0.03	-0.12,	0.07
	Per ln-unit (PFOA)[d]	0.01	-0.07,	0.09
	Per IQR(ln-PFOA)[e]	0.00	-0.04,	0.05
PFNA	Quartile 1 (referent)	4.95		
	Quartile 2	-0.03	-0.11,	0.05
	Quartile 3	-0.02	-0.10,	0.06
	Quartile 4	-0.01	-0.10,	0.08
	Per ln-unit (PFNA)	-0.01	-0.07,	0.05
	Per IQR(ln-PFNA)	-0.01	-0.04,	0.03
PFDA	Below median (referent)	4.95		
	At or above median	-0.05	-0.11,	0.01
	Per ln-unit (PFDA)	-0.03	-0.07,	0.01
	Per IQR(ln-PFDA)[f]	-0.03	-0.07,	0.01
PFUnDA	Quartile 1 (referent)	4.98		
	Quartile 2	-0.06	-0.14,	0.02
	Quartile 3	-0.07	-0.15,	0.01
	Quartile 4	-0.08	-0.17,	0.01
	Per ln-unit (PFUnDA)	-0.04	-0.09,	0.00
	Per IQR(ln-PFUnDA)	-0.04	-0.08,	0.00
PFHxS	Quartile 1 (referent)	4.96		
	Quartile 2	-0.05	-0.13,	0.03
	Quartile 3	-0.03	-0.11,	0.05
	Quartile 4	-0.02	-0.10,	0.06
	Per ln-unit (PFHxS)	-0.01	-0.06,	0.04
	Per IQR(ln-PFHxS)	-0.01	-0.04,	0.03
PFHpS	Quartile 1 (referent)	4.96		
	Quartile 2	-0.07	-0.15,	0.00
	Quartile 3	-0.01	-0.08,	0.07
	Quartile 4	-0.06	-0.14,	0.02
	Per ln-unit (PFHpS)	-0.03	-0.07,	0.02
	Per IQR(ln-PFHpS)	-0.02	-0.06,	0.02
PFOS	Quartile 1 (referent)	4.94		
	Quartile 2	0.00	-0.07,	0.08
	Quartile 3	-0.02	-0.11,	0.06
	Quartile 4	0.00	-0.08,	0.09
	Per ln-unit (PFOS)	-0.02	-0.09,	0.06
	Per IQR(ln-PFOS)	-0.01	-0.05,	0.03

[a] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[b] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[c] 95% confidence interval.
[d] Coefficient represents the change in ln-triglycerides for each 1 ln-(ng/mL) increase in PFAS concentration.
[e] Coefficient represents the change in ln-triglycerides for a shift in PFAS concentration from the 25^{th} percentile to the 75^{th} percentile of the observed exposure distribution.
[f] IQR of ln(PFDA) estimated as 2*(75^{th} percentile-median) because the 25^{th} percentile of PFDA was below the limit of quantification.

Table 4.32. Linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and uric acid (mg/dL) among 524 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, selected without regard to subfecundity status (base sample).

		Adjusted[a] LSM[b] or β	95% CI[c]	
PFOA	Quartile 1 (referent)	3.29		
	Quartile 2	0.02	-0.15,	0.19
	Quartile 3	0.00	-0.19,	0.19
	Quartile 4	-0.04	-0.24,	0.17
	Per ln-unit (PFOA)[d]	0.02	-0.14,	0.18
	Per IQR(ln-PFOA)[e]	0.01	-0.09,	0.11
PFNA	Quartile 1 (referent)	3.29		
	Quartile 2	-0.04	-0.21,	0.14
	Quartile 3	0.02	-0.15,	0.19
	Quartile 4	0.04	-0.15,	0.22
	Per ln-unit (PFNA)	0.03	-0.11,	0.16
	Per IQR(ln-PFNA)	0.01	-0.06,	0.09
PFDA	Below median (referent)	3.31		
	At or above median	-0.08	-0.20,	0.05
	Per ln-unit (PFDA)	-0.03	-0.12,	0.05
	Per IQR(ln-PFDA)[f]	-0.03	-0.12,	0.05
PFUnDA	Quartile 1 (referent)	3.35		
	Quartile 2	-0.11	-0.28,	0.06
	Quartile 3	-0.07	-0.25,	0.10
	Quartile 4	-0.10	-0.29,	0.08
	Per ln-unit (PFUnDA)	-0.05	-0.14,	0.04
	Per IQR(ln-PFUnDA)	-0.05	-0.13,	0.04
PFHxS	Quartile 1 (referent)	3.27		
	Quartile 2	0.08	-0.08,	0.25
	Quartile 3	0.05	-0.11,	0.22
	Quartile 4	-0.05	-0.23,	0.12
	Per ln-unit (PFHxS)	-0.03	-0.13,	0.08
	Per IQR(ln-PFHxS)	-0.02	-0.09,	0.05
PFHpS	Quartile 1 (referent)	3.29		
	Quartile 2	-0.05	-0.21,	0.11
	Quartile 3	0.01	-0.16,	0.17
	Quartile 4	0.06	-0.12,	0.24
	Per ln-unit (PFHpS)	0.04	-0.06,	0.14
	Per IQR(ln-PFHpS)	0.03	-0.05,	0.11
PFOS	Quartile 1 (referent)	3.31		
	Quartile 2	-0.10	-0.26,	0.07
	Quartile 3	0.02	-0.16,	0.19
	Quartile 4	-0.01	-0.19,	0.17
	Per ln-unit (PFOS)	-0.02	-0.17,	0.14
	Per IQR(ln-PFOS)	-0.01	-0.08,	0.07

[a] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[b] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[c] 95% confidence interval.
[d] Coefficient represents the change in uric acid for each 1 ln-(ng/mL) increase in PFAS concentration.
[e] Coefficient represents the change in uric acid for a shift in PFAS concentration from the 25^{th} percentile to the 75^{th} percentile of the observed exposure distribution.
[f] IQR of ln(PFDA) estimated as 2*(75^{th} percentile-median) because the 25^{th} percentile of PFDA was below the limit of quantification.

Table 4.33. Linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and natural-log transformed C-reactive protein (ln-mg/L) among 524 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, selected without regard to subfecundity status (base sample).

		Adjusted[a] LSM[b] or β	95% CI[c]	
PFOA	Quartile 1 (referent)	1.74		
	Quartile 2	-0.01	-0.21,	0.19
	Quartile 3	-0.01	-0.23,	0.22
	Quartile 4	-0.12	-0.35,	0.12
	Per ln-unit (PFOA)[d]	-0.12	-0.31,	0.08
	Per IQR(ln-PFOA)[e]	-0.07	-0.19,	0.05
PFNA	Quartile 1 (referent)	1.84		
	Quartile 2	-0.20	-0.40,	0.00
	Quartile 3	-0.16	-0.37,	0.04
	Quartile 4	-0.27	-0.48,	-0.05
	Per ln-unit (PFNA)	-0.18	-0.33,	-0.03
	Per IQR(ln-PFNA)	-0.11	-0.20,	-0.02
PFDA	Below median (referent)	1.76		
	At or above median	-0.11	-0.25,	0.04
	Per ln-unit (PFDA)	-0.07	-0.18,	0.03
	Per IQR(ln-PFDA)[f]	-0.07	-0.18,	0.03
PFUnDA	Quartile 1 (referent)	1.77		
	Quartile 2	-0.06	-0.26,	0.14
	Quartile 3	-0.03	-0.24,	0.18
	Quartile 4	-0.14	-0.36,	0.08
	Per ln-unit (PFUnDA)	-0.06	-0.17,	0.05
	Per IQR(ln-PFUnDA)	-0.06	-0.16,	0.05
PFHxS	Quartile 1 (referent)	1.75		
	Quartile 2	-0.03	-0.23,	0.17
	Quartile 3	0.03	-0.17,	0.23
	Quartile 4	-0.11	-0.31,	0.10
	Per ln-unit (PFHxS)	-0.12	-0.24,	0.00
	Per IQR(ln-PFHxS)	-0.08	-0.16,	0.00
PFHpS	Quartile 1 (referent)	1.67		
	Quartile 2	0.17	-0.02,	0.37
	Quartile 3	0.00	-0.19,	0.20
	Quartile 4	0.16	-0.05,	0.37
	Per ln-unit (PFHpS)	0.07	-0.05,	0.18
	Per IQR(ln-PFHpS)	0.05	-0.04,	0.15
PFOS	Quartile 1 (referent)	1.71		
	Quartile 2	0.05	-0.14,	0.25
	Quartile 3	0.05	-0.16,	0.26
	Quartile 4	-0.02	-0.23,	0.19
	Per ln-unit (PFOS)	-0.02	-0.21,	0.17
	Per IQR(ln-PFOS)	-0.01	-0.10,	0.08

[a] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[b] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[c] 95% confidence interval.
[d] Coefficient represents the change in ln-C-reactive protein for each 1 ln-(ng/mL) increase in PFAS concentration.
[e] Coefficient represents the change in ln-C-reactive protein for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[f] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.34. Linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and total cholesterol (mg/dL) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, additionally adjusted for subfecundity rather than weighted.

		Adjusted[a] LSM[b] or β	95% CI[c]	
PFOA	Quartile 1 (referent)	203.97		
	Quartile 2	1.30	-5.74,	8.34
	Quartile 3	3.91	-3.61,	11.43
	Quartile 4	3.19	-4.82,	11.19
	Per ln-unit (PFOA)[d]	2.28	-4.21,	8.78
	Per IQR(ln-PFOA)[e]	1.37	-2.53,	5.28
PFNA	Quartile 1 (referent)	205.87		
	Quartile 2	-0.58	-7.37,	6.20
	Quartile 3	-1.94	-8.85,	4.97
	Quartile 4	2.71	-4.48,	9.89
	Per ln-unit (PFNA)	0.24	-4.91,	5.38
	Per IQR(ln-PFNA)	0.14	-2.88,	3.15
PFDA	Below median (referent)	205.35		
	At or above median	1.35	-3.58,	6.28
	Per ln-unit (PFDA)	0.88	-2.57,	4.32
	Per IQR(ln-PFDA)[f]	0.88	-2.58,	4.33
PFUnDA	Quartile 1 (referent)	204.13		
	Quartile 2	1.36	-5.45,	8.16
	Quartile 3	3.08	-3.84,	9.99
	Quartile 4	4.56	-2.57,	11.70
	Per ln-unit (PFUnDA)	1.33	-2.25,	4.92
	Per IQR(ln-PFUnDA)	1.22	-2.06,	4.50
PFHxS	Quartile 1 (referent)	204.68		
	Quartile 2	-0.94	-7.71,	5.84
	Quartile 3	3.29	-3.58,	10.15
	Quartile 4	3.53	-3.56,	10.62
	Per ln-unit (PFHxS)	2.68	-1.44,	6.81
	Per IQR(ln-PFHxS)	1.79	-0.97,	4.55
PFHpS	Quartile 1 (referent)	206.38		
	Quartile 2	-2.95	-9.66,	3.76
	Quartile 3	-0.54	-7.24,	6.16
	Quartile 4	0.89	-6.05,	7.84
	Per ln-unit (PFHpS)	-0.55	-4.43,	3.33
	Per IQR(ln-PFHpS)	-0.44	-3.54,	2.66
PFOS	Quartile 1 (referent)	206.15		
	Quartile 2	-3.88	-10.56,	2.79
	Quartile 3	0.36	-6.59,	7.30
	Quartile 4	4.21	-2.83,	11.25
	Per ln-unit (PFOS)	8.19	1.88,	14.51
	Per IQR(ln-PFOS)	3.89	0.89,	6.88

[a] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily, and subfecundity (self-reported time to pregnancy >12 months).
[b] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[c] 95% confidence interval.
[d] Coefficient represents the change in total cholesterol for each 1 ln-(ng/mL) increase in PFAS concentration.
[e] Coefficient represents the change in total cholesterol for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[f] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.35. Linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and HDL cholesterol (mg/dL) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, additionally adjusted for subfecundity rather than weighted.

		Adjusted[a] LSM[b] or β	95% CI[c]	
PFOA	Quartile 1 (referent)	60.92		
	Quartile 2	1.24	-1.08,	3.56
	Quartile 3	2.65	0.17,	5.13
	Quartile 4	3.32	0.68,	5.96
	Per ln-unit (PFOA)[d]	2.24	0.09,	4.38
	Per IQR(ln-PFOA)[e]	1.34	0.05,	2.63
PFNA	Quartile 1 (referent)	61.20		
	Quartile 2	1.00	-1.24,	3.23
	Quartile 3	1.83	-0.45,	4.10
	Quartile 4	3.95	1.59,	6.31
	Per ln-unit (PFNA)	3.32	1.63,	5.00
	Per IQR(ln-PFNA)	1.95	0.96,	2.94
PFDA	Below median (referent)	61.41		
	At or above median	3.01	1.39,	4.63
	Per ln-unit (PFDA)	2.78	1.66,	3.90
	Per IQR(ln-PFDA)[f]	2.79	1.66,	3.92
PFUnDA	Quartile 1 (referent)	59.25		
	Quartile 2	3.48	1.28,	5.68
	Quartile 3	5.41	3.18,	7.64
	Quartile 4	7.63	5.33,	9.94
	Per ln-unit (PFUnDA)	4.10	2.95,	5.26
	Per IQR(ln-PFUnDA)	3.75	2.70,	4.81
PFHxS	Quartile 1 (referent)	60.69		
	Quartile 2	1.68	-0.55,	3.92
	Quartile 3	2.68	0.41,	4.94
	Quartile 4	3.03	0.70,	5.37
	Per ln-unit (PFHxS)	1.33	-0.03,	2.69
	Per IQR(ln-PFHxS)	0.89	-0.02,	1.80
PFHpS	Quartile 1 (referent)	61.51		
	Quartile 2	-0.28	-2.49,	1.93
	Quartile 3	1.54	-0.67,	3.75
	Quartile 4	2.56	0.27,	4.85
	Per ln-unit (PFHpS)	1.47	0.19,	2.75
	Per IQR(ln-PFHpS)	1.18	0.15,	2.20
PFOS	Quartile 1 (referent)	60.43		
	Quartile 2	2.35	0.16,	4.55
	Quartile 3	2.74	0.45,	5.02
	Quartile 4	4.57	2.25,	6.88
	Per ln-unit (PFOS)	4.44	2.36,	6.51
	Per IQR(ln-PFOS)	2.11	1.12,	3.09

[a] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily, and subfecundity (self-reported time to pregnancy >12 months).
[b] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[c] 95% confidence interval.
[d] Coefficient represents the change in total cholesterol for each 1 ln-(ng/mL) increase in PFAS concentration.
[e] Coefficient represents the change in total cholesterol for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[f] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.36. Linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and LDL cholesterol (mg/dL) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, additionally adjusted for subfecundity rather than weighted.

		Adjusted[a] LSM[b] or β	95% CI[c]	
PFOA	Quartile 1 (referent)	123.78		
	Quartile 2	0.41	-5.67,	6.50
	Quartile 3	4.52	-1.99,	11.02
	Quartile 4	3.56	-3.36,	10.48
	Per ln-unit (PFOA)[d]	2.53	-3.09,	8.15
	Per IQR(ln-PFOA)[e]	1.52	-1.86,	4.90
PFNA	Quartile 1 (referent)	126.60		
	Quartile 2	-1.97	-7.85,	3.90
	Quartile 3	-2.76	-8.75,	3.22
	Quartile 4	-0.35	-6.58,	5.87
	Per ln-unit (PFNA)	-1.98	-6.43,	2.47
	Per IQR(ln-PFNA)	-1.16	-3.77,	1.45
PFDA	Below median (referent)	125.55		
	At or above median	0.10	-4.17,	4.36
	Per ln-unit (PFDA)	-0.80	-3.78,	2.18
	Per IQR(ln-PFDA)[f]	-0.81	-3.80,	2.18
PFUnDA	Quartile 1 (referent)	126.66		
	Quartile 2	-1.83	-7.72,	4.07
	Quartile 3	-1.72	-7.71,	4.27
	Quartile 4	-2.03	-8.20,	4.15
	Per ln-unit (PFUnDA)	-2.13	-5.23,	0.97
	Per IQR(ln-PFUnDA)	-1.95	-4.79,	0.89
PFHxS	Quartile 1 (referent)	125.37		
	Quartile 2	-1.04	-6.91,	4.83
	Quartile 3	1.38	-4.56,	7.33
	Quartile 4	1.13	-5.01,	7.28
	Per ln-unit (PFHxS)	1.50	-2.07,	5.07
	Per IQR(ln-PFHxS)	1.00	-1.38,	3.39
PFHpS	Quartile 1 (referent)	125.17		
	Quartile 2	0.71	-5.10,	6.52
	Quartile 3	-0.57	-6.37,	5.23
	Quartile 4	2.35	-3.66,	8.36
	Per ln-unit (PFHpS)	-0.16	-3.52,	3.20
	Per IQR(ln-PFHpS)	-0.13	-2.81,	2.56
PFOS	Quartile 1 (referent)	126.59		
	Quartile 2	-4.36	-10.14,	1.43
	Quartile 3	-0.14	-6.15,	5.88
	Quartile 4	1.80	-4.30,	7.90
	Per ln-unit (PFOS)	5.76	0.29,	11.24
	Per IQR(ln-PFOS)	2.73	0.14,	5.33

[a] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily, and subfecundity (self-reported time to pregnancy >12 months).
[b] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[c] 95% confidence interval.
[d] Coefficient represents the change in total cholesterol for each 1 ln-(ng/mL) increase in PFAS concentration.
[e] Coefficient represents the change in total cholesterol for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[f] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.37. Linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and natural-log transformed triglycerides (ln-mg/dL) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, additionally adjusted for subfecundity rather than weighted.

		Adjusted[a] LSM[b] or β	95% CI[c]	
PFOA	Quartile 1 (referent)	4.91		
	Quartile 2	0.01	-0.05,	0.07
	Quartile 3	-0.02	-0.08,	0.05
	Quartile 4	-0.07	-0.14,	0.00
	Per ln-unit (PFOA)[d]	-0.04	-0.10,	0.02
	Per IQR(ln-PFOA)[e]	-0.03	-0.06,	0.01
PFNA	Quartile 1 (referent)	4.92		
	Quartile 2	-0.01	-0.07,	0.05
	Quartile 3	-0.03	-0.09,	0.03
	Quartile 4	-0.05	-0.11,	0.02
	Per ln-unit (PFNA)	-0.04	-0.09,	0.00
	Per IQR(ln-PFNA)	-0.03	-0.05,	0.00
PFDA	Below median (referent)	4.92		
	At or above median	-0.07	-0.11,	-0.03
	Per ln-unit (PFDA)	-0.05	-0.08,	-0.02
	Per IQR(ln-PFDA)[f]	-0.05	-0.08,	-0.02
PFUnDA	Quartile 1 (referent)	4.95		
	Quartile 2	-0.07	-0.13,	-0.01
	Quartile 3	-0.07	-0.13,	-0.01
	Quartile 4	-0.10	-0.16,	0.04
	Per ln-unit (PFUnDA)	-0.05	-0.08,	-0.02
	Per IQR(ln-PFUnDA)	-0.04	-0.07,	-0.01
PFHxS	Quartile 1 (referent)	4.92		
	Quartile 2	-0.03	-0.09,	0.03
	Quartile 3	-0.01	-0.07,	0.06
	Quartile 4	-0.03	-0.10,	0.03
	Per ln-unit (PFHxS)	-0.01	-0.05,	0.03
	Per IQR(ln-PFHxS)	-0.01	-0.03,	0.02
PFHpS	Quartile 1 (referent)	4.94		
	Quartile 2	-0.07	-0.13,	-0.01
	Quartile 3	-0.02	-0.08,	0.04
	Quartile 4	-0.07	-0.13,	-0.01
	Per ln-unit (PFHpS)	-0.04	-0.07,	0.00
	Per IQR(ln-PFHpS)	-0.03	-0.06,	0.00
PFOS	Quartile 1 (referent)	4.92		
	Quartile 2	-0.01	-0.07,	0.05
	Quartile 3	-0.05	-0.11,	0.01
	Quartile 4	-0.04	-0.10,	0.02
	Per ln-unit (PFOS)	-0.04	-0.10,	0.02
	Per IQR(ln-PFOS)	-0.02	-0.05,	0.01

[a] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily, and subfecundity (self-reported time to pregnancy >12 months).
[b] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[c] 95% confidence interval.
[d] Coefficient represents the change in total cholesterol for each 1 ln-(ng/mL) increase in PFAS concentration.
[e] Coefficient represents the change in total cholesterol for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[f] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.38. Linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and uric acid (mg/dL) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, additionally adjusted for subfecundity rather than weighted.

		Adjusted[a] LSM[b] or β	95% CI[c]	
PFOA	Quartile 1 (referent)	3.27		
	Quartile 2	0.04	-0.09,	0.17
	Quartile 3	0.10	-0.05,	0.24
	Quartile 4	0.06	-0.09,	0.21
	Per ln-unit (PFOA)[d]	0.08	-0.04,	0.21
	Per IQR(ln-PFOA)[e]	0.05	-0.02,	0.12
PFNA	Quartile 1 (referent)	3.29		
	Quartile 2	0.01	-0.12,	0.14
	Quartile 3	0.03	-0.10,	0.16
	Quartile 4	0.07	-0.06,	0.21
	Per ln-unit (PFNA)	0.05	-0.05,	0.14
	Per IQR(ln-PFNA)	0.03	-0.03,	0.08
PFDA	Below median (referent)	3.32		
	At or above median	-0.04	-0.13,	0.06
	Per ln-unit (PFDA)	-0.01	-0.07,	0.06
	Per IQR(ln-PFDA)[f]	-0.01	-0.07,	0.06
PFUnDA	Quartile 1 (referent)	3.35		
	Quartile 2	-0.09	-0.22,	0.04
	Quartile 3	-0.08	-0.21,	0.05
	Quartile 4	-0.03	-0.17,	0.10
	Per ln-unit (PFUnDA)	-0.02	-0.09,	0.05
	Per IQR(ln-PFUnDA)	-0.02	-0.08,	0.05
PFHxS	Quartile 1 (referent)	3.27		
	Quartile 2	0.08	-0.05,	0.20
	Quartile 3	0.05	-0.08,	0.18
	Quartile 4	0.05	-0.09,	0.18
	Per ln-unit (PFHxS)	0.02	-0.05,	0.10
	Per IQR(ln-PFHxS)	0.02	-0.04,	0.07
PFHpS	Quartile 1 (referent)	3.31		
	Quartile 2	-0.03	-0.16,	0.10
	Quartile 3	-0.04	-0.16,	0.09
	Quartile 4	0.11	-0.02,	0.24
	Per ln-unit (PFHpS)	0.04	-0.03,	0.12
	Per IQR(ln-PFHpS)	0.03	-0.02,	0.09
PFOS	Quartile 1 (referent)	3.32		
	Quartile 2	-0.08	-0.21,	0.05
	Quartile 3	0.03	-0.10,	0.16
	Quartile 4	0.07	-0.06,	0.20
	Per ln-unit (PFOS)	0.06	-0.06,	0.18
	Per IQR(ln-PFOS)	0.03	-0.03,	0.08

[a] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily, and subfecundity (self-reported time to pregnancy >12 months).
[b] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[c] 95% confidence interval.
[d] Coefficient represents the change in total cholesterol for each 1 ln-(ng/mL) increase in PFAS concentration.
[e] Coefficient represents the change in total cholesterol for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[f] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.39. Linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and natural-log transformed C-reactive protein (ln-mg/dL) among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, additionally adjusted for subfecundity rather than weighted.

		Adjusted[a] LSM[b] or β	95% CI[c]	
PFOA	Quartile 1 (referent)	1.85		
	Quartile 2	-0.04	-0.20,	0.11
	Quartile 3	-0.09	-0.26,	0.08
	Quartile 4	-0.11	-0.29,	0.07
	Per ln-unit (PFOA)[d]	-0.11	-0.26,	0.03
	Per IQR(ln-PFOA)[e]	-0.07	-0.15,	0.02
PFNA	Quartile 1 (referent)	1.85		
	Quartile 2	-0.05	-0.20,	0.10
	Quartile 3	-0.10	-0.25,	0.06
	Quartile 4	-0.13	-0.28,	0.03
	Per ln-unit (PFNA)	-0.06	-0.18,	0.05
	Per IQR(ln-PFNA)	-0.04	-0.10,	0.03
PFDA	Below median (referent)	1.82		
	At or above median	-0.06	-0.16,	0.05
	Per ln-unit (PFDA)	-0.04	-0.11,	0.04
	Per IQR(ln-PFDA)[f]	-0.04	-0.11,	0.04
PFUnDA	Quartile 1 (referent)	1.81		
	Quartile 2	-0.01	-0.16,	0.14
	Quartile 3	-0.02	-0.17,	0.14
	Quartile 4	-0.05	-0.20,	0.11
	Per ln-unit (PFUnDA)	-0.02	-0.10,	0.06
	Per IQR(ln-PFUnDA)	-0.02	-0.09,	0.06
PFHxS	Quartile 1 (referent)	1.80		
	Quartile 2	0.03	-0.12,	0.18
	Quartile 3	0.01	-0.14,	0.16
	Quartile 4	-0.08	-0.24,	0.08
	Per ln-unit (PFHxS)	-0.06	-0.16,	0.03
	Per IQR(ln-PFHxS)	-0.04	-0.10,	0.02
PFHpS	Quartile 1 (referent)	1.79		
	Quartile 2	0.05	-0.09,	0.20
	Quartile 3	-0.01	-0.16,	0.14
	Quartile 4	0.04	-0.12,	0.19
	Per ln-unit (PFHpS)	0.03	-0.06,	0.11
	Per IQR(ln-PFHpS)	0.02	-0.05,	0.09
PFOS	Quartile 1 (referent)	1.77		
	Quartile 2	0.09	-0.05,	0.24
	Quartile 3	0.05	-0.10,	0.20
	Quartile 4	-0.03	-0.18,	0.13
	Per ln-unit (PFOS)	-0.01	-0.15,	0.13
	Per IQR(ln-PFOS)	0.00	-0.07,	0.06

[a] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily, and subfecundity (self-reported time to pregnancy >12 months).
[b] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[c] 95% confidence interval.
[d] Coefficient represents the change in total cholesterol for each 1 ln-(ng/mL) increase in PFAS concentration.
[e] Coefficient represents the change in total cholesterol for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[f] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.40. Linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and total cholesterol (mg/dL) among 446 nulliparous pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, additionally adjusted for subfecundity rather than weighted.

		Adjusted[a] LSM[b] or β	95% CI[c]	
PFOA	Quartile 1 (referent)	200.24		
	Quartile 2	4.58	-9.09,	18.25
	Quartile 3	9.10	-3.83,	22.02
	Quartile 4	5.47	-7.04,	17.98
	Per ln-unit (PFOA)[d]	3.52	-4.90,	11.93
	Per IQR(ln-PFOA)[e]	2.11	-2.94,	7.17
PFNA	Quartile 1 (referent)	208.36		
	Quartile 2	-1.30	-11.57,	8.97
	Quartile 3	-3.83	-14.00,	6.35
	Quartile 4	-1.42	-11.40,	8.55
	Per ln-unit (PFNA)	-1.61	-8.55,	5.32
	Per IQR(ln-PFNA)	-0.95	-5.01,	3.12
PFDA	Below median (referent)	207.17		
	At or above median	-1.16	-7.89,	5.56
	Per ln-unit (PFDA)	-1.63	-6.20,	2.94
	Per IQR(ln-PFDA)[f]	-1.64	-6.22,	2.95
PFUnDA	Quartile 1 (referent)	205.11		
	Quartile 2	3.08	-6.47,	12.63
	Quartile 3	1.93	-7.46,	11.31
	Quartile 4	2.51	-7.20,	12.22
	Per ln-unit (PFUnDA)	0.29	-4.44,	5.03
	Per IQR(ln-PFUnDA)	0.27	-4.07,	4.60
PFHxS	Quartile 1 (referent)	203.22		
	Quartile 2	0.57	-10.34,	11.47
	Quartile 3	6.92	-3.50,	17.34
	Quartile 4	4.31	-5.91,	14.53
	Per ln-unit (PFHxS)	3.15	-2.09,	8.39
	Per IQR(ln-PFHxS)	2.11	-1.40,	5.61
PFHpS	Quartile 1 (referent)	207.75		
	Quartile 2	-6.13	-16.20,	3.93
	Quartile 3	1.07	-8.73,	10.88
	Quartile 4	-0.08	-9.34,	9.18
	Per ln-unit (PFHpS)	1.13	-3.91,	6.17
	Per IQR(ln-PFHpS)	0.90	-3.12,	4.93
PFOS	Quartile 1 (referent)	207.64		
	Quartile 2	-3.12	-13.51,	7.26
	Quartile 3	-1.57	-11.44,	8.31
	Quartile 4	2.18	-7.49,	11.86
	Per ln-unit (PFOS)	7.44	-0.41,	15.29
	Per IQR(ln-PFOS)	3.53	-0.20,	7.25

[a] Adjusted for age, pre-pregnant body mass index, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily, and subfecundity (self-reported time to pregnancy >12 months).
[b] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[c] 95% confidence interval.
[d] Coefficient represents the change in total cholesterol for each 1 ln-(ng/mL) increase in PFAS concentration.
[e] Coefficient represents the change in total cholesterol for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[f] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.41. Linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and HDL cholesterol (mg/dL) among 446 nulliparous women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, additionally adjusted for subfecundity rather than weighted.

		Adjusted[a] LSM[b] or β	95% CI[c]	
PFOA	Quartile 1 (referent)	57.52		
	Quartile 2	3.54	-1.24,	8.32
	Quartile 3	5.13	0.60,	9.65
	Quartile 4	4.88	0.51,	9.26
	Per ln-unit (PFOA)[d]	2.60	-0.35,	5.55
	Per IQR(ln-PFOA)[e]	1.56	-0.21,	3.34
PFNA	Quartile 1 (referent)	58.42		
	Quartile 2	2.90	-0.64,	6.45
	Quartile 3	4.38	0.87,	7.89
	Quartile 4	6.78	3.34,	10.23
	Per ln-unit (PFNA)	4.88	2.48,	7.27
	Per IQR(ln-PFNA)	2.86	1.46,	4.26
PFDA	Below median (referent)	60.38		
	At or above median	4.42	2.09,	6.75
	Per ln-unit (PFDA)	3.38	1.81,	4.96
	Per IQR(ln-PFDA)[f]	3.39	1.81,	4.97
PFUnDA	Quartile 1 (referent)	58.40		
	Quartile 2	3.70	0.46,	6.94
	Quartile 3	6.32	3.14,	9.50
	Quartile 4	9.17	5.87,	12.46
	Per ln-unit (PFUnDA)	4.70	3.09,	6.30
	Per IQR(ln-PFUnDA)	4.30	2.83,	5.77
PFHxS	Quartile 1 (referent)	58.61		
	Quartile 2	2.92	-0.88,	6.73
	Quartile 3	3.84	0.20,	7.48
	Quartile 4	5.16	1.59,	8.73
	Per ln-unit (PFHxS)	1.69	-0.15,	3.52
	Per IQR(ln-PFHxS)	1.13	-0.10,	2.36
PFHpS	Quartile 1 (referent)	60.78		
	Quartile 2	-1.71	-5.21,	1.80
	Quartile 3	2.21	-1.20,	5.62
	Quartile 4	2.98	-0.24,	6.20
	Per ln-unit (PFHpS)	2.66	0.91,	4.41
	Per IQR(ln-PFHpS)	2.13	0.72,	3.53
PFOS	Quartile 1 (referent)	57.66		
	Quartile 2	4.21	0.64,	7.78
	Quartile 3	6.14	2.74,	9.53
	Quartile 4	7.54	4.21,	10.87
	Per ln-unit (PFOS)	6.09	3.38,	8.80
	Per IQR(ln-PFOS)	2.89	1.60,	4.17

[a] Adjusted for age, pre-pregnant body mass index, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily, and subfecundity (self-reported time to pregnancy >12 months).
[b] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[c] 95% confidence interval.
[d] Coefficient represents the change in total cholesterol for each 1 ln-(ng/mL) increase in PFAS concentration.
[e] Coefficient represents the change in total cholesterol for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[f] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.42. Linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and LDL cholesterol (mg/dL) among 446 nulliparous women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, additionally adjusted for subfecundity rather than weighted.

		Adjusted[a] LSM[b] or β	95% CI[c]	
PFOA	Quartile 1 (referent)	119.40		
	Quartile 2	3.94	-7.71,	15.58
	Quartile 3	9.15	-1.87,	20.16
	Quartile 4	6.17	-4.49,	16.82
	Per ln-unit (PFOA)[d]	3.68	-3.49,	10.86
	Per IQR(ln-PFOA)[e]	2.21	-2.10,	6.53
PFNA	Quartile 1 (referent)	130.50		
	Quartile 2	-5.30	-14.04,	3.44
	Quartile 3	-6.60	-15.26,	2.06
	Quartile 4	-5.73	-14.23,	2.76
	Per ln-unit (PFNA)	-4.05	-9.96,	1.86
	Per IQR(ln-PFNA)	-2.38	-5.84,	1.09
PFDA	Below median (referent)	126.99		
	At or above median	-2.81	-8.54,	2.92
	Per ln-unit (PFDA)	-2.86	-6.75,	1.04
	Per IQR(ln-PFDA)[f]	-2.87	-6.77,	1.04
PFUnDA	Quartile 1 (referent)	127.40		
	Quartile 2	-0.40	-8.54,	7.73
	Quartile 3	-3.94	-11.93,	4.06
	Quartile 4	-4.18	-12.45,	4.09
	Per ln-unit (PFUnDA)	-3.10	-7.13,	0.93
	Per IQR(ln-PFUnDA)	-2.84	-6.53,	0.85
PFHxS	Quartile 1 (referent)	123.89		
	Quartile 2	1.15	-8.15,	10.45
	Quartile 3	5.24	-3.65,	14.13
	Quartile 4	0.68	-8.04,	9.40
	Per ln-unit (PFHxS)	0.79	-3.69,	5.27
	Per IQR(ln-PFHxS)	0.53	-2.46,	3.52
PFHpS	Quartile 1 (referent)	125.34		
	Quartile 2	0.67	-7.95,	9.28
	Quartile 3	0.67	-7.72,	9.06
	Quartile 4	1.21	-6.72,	9.13
	Per ln-unit (PFHpS)	-0.11	-4.41,	4.19
	Per IQR(ln-PFHpS)	-0.09	-3.53,	3.35
PFOS	Quartile 1 (referent)	129.19		
	Quartile 2	-5.21	-14.06,	3.65
	Quartile 3	-4.66	-13.08,	3.76
	Quartile 4	-2.46	-10.71,	5.80
	Per ln-unit (PFOS)	3.66	-3.05,	10.38
	Per IQR(ln-PFOS)	1.74	-1.45,	4.92

[a] Adjusted for age, pre-pregnant body mass index, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily, and subfecundity (self-reported time to pregnancy >12 months).
[b] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[c] 95% confidence interval.
[d] Coefficient represents the change in total cholesterol for each 1 ln-(ng/mL) increase in PFAS concentration.
[e] Coefficient represents the change in total cholesterol for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[f] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.43. Linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and natural-log transformed triglycerides (ln-mg/dL) among 446 nulliparous women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, additionally adjusted for subfecundity rather than weighted.

		Adjusted[a] LSM[b] or β	95% CI[c]	
PFOA	Quartile 1 (referent)	4.97		
	Quartile 2	-0.04	-0.17,	0.09
	Quartile 3	-0.08	-0.21,	0.05
	Quartile 4	-0.12	-0.25,	0.00
	Per ln-unit (PFOA)[d]	-0.09	-0.17,	-0.01
	Per IQR(ln-PFOA)[e]	-0.05	-0.10,	0.00
PFNA	Quartile 1 (referent)	4.97		
	Quartile 2	-0.05	-0.15,	0.04
	Quartile 3	-0.11	-0.21,	-0.02
	Quartile 4	-0.15	-0.25,	-0.06
	Per ln-unit (PFNA)	-0.11	-0.18,	-0.04
	Per IQR(ln-PFNA)	-0.06	-0.10,	-0.02
PFDA	Below median (referent)	4.94		
	At or above median	-0.14	-0.20,	-0.07
	Per ln-unit (PFDA)	-0.10	-0.15,	-0.06
	Per IQR(ln-PFDA)[f]	-0.10	-0.15,	-0.06
PFUnDA	Quartile 1 (referent)	4.95		
	Quartile 2	-0.05	-0.14,	0.04
	Quartile 3	-0.10	-0.19,	-0.01
	Quartile 4	-0.18	-0.28,	-0.09
	Per ln-unit (PFUnDA)	-0.08	-0.13,	-0.03
	Per IQR(ln-PFUnDA)	-0.07	-0.12,	-0.03
PFHxS	Quartile 1 (referent)	4.94		
	Quartile 2	-0.05	-0.15,	0.06
	Quartile 3	-0.06	-0.16,	0.04
	Quartile 4	-0.08	-0.18,	0.02
	Per ln-unit (PFHxS)	-0.02	-0.08,	0.03
	Per IQR(ln-PFHxS)	-0.02	-0.05,	0.02
PFHpS	Quartile 1 (referent)	4.94		
	Quartile 2	-0.08	-0.18,	0.01
	Quartile 3	-0.02	-0.11,	0.08
	Quartile 4	-0.10	-0.19,	-0.01
	Per ln-unit (PFHpS)	-0.05	-0.10,	0.00
	Per IQR(ln-PFHpS)	-0.04	-0.08,	0.00
PFOS	Quartile 1 (referent)	4.96		
	Quartile 2	-0.04	-0.14,	0.06
	Quartile 3	-0.12	-0.22,	-0.03
	Quartile 4	-0.12	-0.21,	-0.03
	Per ln-unit (PFOS)	-0.09	-0.17,	-0.01
	Per IQR(ln-PFOS)	-0.04	-0.08,	-0.01

[a] Adjusted for age, pre-pregnant body mass index, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily, and subfecundity (self-reported time to pregnancy >12 months).
[b] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[c] 95% confidence interval.
[d] Coefficient represents the change in total cholesterol for each 1 ln-(ng/mL) increase in PFAS concentration.
[e] Coefficient represents the change in total cholesterol for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[f] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.44. Linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and uric acid (mg/dL) among 446 nulliparous women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, additionally adjusted for subfecundity rather than weighted.

		Adjusted[a] LSM[b] or β	95% CI[c]	
PFOA	Quartile 1 (referent)	3.36		
	Quartile 2	-0.07	-0.34,	0.20
	Quartile 3	0.02	-0.23,	0.28
	Quartile 4	-0.03	-0.28,	0.21
	Per ln-unit (PFOA)[d]	0.08	-0.08,	0.25
	Per IQR(ln-PFOA)[e]	0.05	-0.05,	0.15
PFNA	Quartile 1 (referent)	3.30		
	Quartile 2	0.07	-0.13,	0.27
	Quartile 3	0.05	-0.15,	0.25
	Quartile 4	0.09	-0.11,	0.28
	Per ln-unit (PFNA)	0.06	-0.08,	0.19
	Per IQR(ln-PFNA)	0.03	-0.05,	0.11
PFDA	Below median (referent)	3.39		
	At or above median	-0.11	-0.24,	0.02
	Per ln-unit (PFDA)	-0.02	-0.11,	0.07
	Per IQR(ln-PFDA)[f]	-0.02	-0.11,	0.07
PFUnDA	Quartile 1 (referent)	3.39		
	Quartile 2	-0.07	-0.26,	0.11
	Quartile 3	-0.13	-0.31,	0.05
	Quartile 4	0.03	-0.15,	0.22
	Per ln-unit (PFUnDA)	0.00	-0.10,	0.09
	Per IQR(ln-PFUnDA)	0.00	-0.09,	0.08
PFHxS	Quartile 1 (referent)	3.28		
	Quartile 2	0.10	-0.12,	0.31
	Quartile 3	0.06	-0.14,	0.27
	Quartile 4	0.10	-0.10,	0.30
	Per ln-unit (PFHxS)	0.06	-0.04,	0.16
	Per IQR(ln-PFHxS)	0.04	-0.03,	0.11
PFHpS	Quartile 1 (referent)	3.32		
	Quartile 2	-0.04	-0.24,	0.15
	Quartile 3	0.03	-0.16,	0.22
	Quartile 4	0.11	-0.08,	0.29
	Per ln-unit (PFHpS)	0.08	-0.02,	0.18
	Per IQR(ln-PFHpS)	0.06	-0.01,	0.14
PFOS	Quartile 1 (referent)	3.26		
	Quartile 2	0.05	-0.16,	0.25
	Quartile 3	0.18	-0.01,	0.37
	Quartile 4	0.14	-0.05,	0.33
	Per ln-unit (PFOS)	0.08	-0.08,	0.23
	Per IQR(ln-PFOS)	0.04	-0.04,	0.11

[a] Adjusted for age, pre-pregnant body mass index, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily, and subfecundity (self-reported time to pregnancy >12 months).
[b] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[c] 95% confidence interval.
[d] Coefficient represents the change in total cholesterol for each 1 ln-(ng/mL) increase in PFAS concentration.
[e] Coefficient represents the change in total cholesterol for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[f] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.45. Linear regression coefficients between plasma concentrations of perfluoroalkyl substances (ng/mL) and natural-log transformed C-reactive protein (ln-mg/dL) among 446 nulliparous women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004, additionally adjusted for subfecundity rather than weighted.

		Adjusted[a] LSM[b] or β	95% CI[c]	
PFOA	Quartile 1 (referent)	1.88		
	Quartile 2	-0.17	-0.50,	0.16
	Quartile 3	-0.25	-0.56,	0.07
	Quartile 4	-0.20	-0.51,	0.10
	Per ln-unit (PFOA)[d]	-0.14	-0.34,	0.06
	Per IQR(ln-PFOA)[e]	-0.08	-0.21,	0.04
PFNA	Quartile 1 (referent)	1.70		
	Quartile 2	0.04	-0.21,	0.29
	Quartile 3	-0.06	-0.31,	0.19
	Quartile 4	-0.07	-0.31,	0.17
	Per ln-unit (PFNA)	-0.08	-0.25,	0.09
	Per IQR(ln-PFNA)	-0.05	-0.15,	0.05
PFDA	Below median (referent)	1.70		
	At or above median	-0.06	-0.22,	0.10
	Per ln-unit (PFDA)	-0.05	-0.16,	0.06
	Per IQR(ln-PFDA)[f]	-0.05	-0.16,	0.06
PFUnDA	Quartile 1 (referent)	1.65		
	Quartile 2	0.11	-0.12,	0.34
	Quartile 3	-0.03	-0.26,	0.20
	Quartile 4	0.00	-0.24,	0.23
	Per ln-unit (PFUnDA)	-0.05	-0.17,	0.06
	Per IQR(ln-PFUnDA)	-0.05	-0.15,	0.06
PFHxS	Quartile 1 (referent)	1.69		
	Quartile 2	0.02	-0.24,	0.29
	Quartile 3	0.00	-0.25,	0.25
	Quartile 4	-0.05	-0.30,	0.20
	Per ln-unit (PFHxS)	-0.03	-0.16,	0.10
	Per IQR(ln-PFHxS)	-0.02	-0.11,	0.06
PFHpS	Quartile 1 (referent)	1.74		
	Quartile 2	-0.09	-0.33,	0.15
	Quartile 3	-0.12	-0.35,	0.12
	Quartile 4	-0.03	-0.26,	0.19
	Per ln-unit (PFHpS)	0.02	-0.11,	0.14
	Per IQR(ln-PFHpS)	0.01	-0.09,	0.11
PFOS	Quartile 1 (referent)	1.59		
	Quartile 2	0.18	-0.08,	0.43
	Quartile 3	0.14	-0.10,	0.38
	Quartile 4	0.03	-0.20,	0.27
	Per ln-unit (PFOS)	0.08	-0.11,	0.27
	Per IQR(ln-PFOS)	0.04	-0.05,	0.13

[a] Adjusted for age, pre-pregnant body mass index, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily, and subfecundity (self-reported time to pregnancy >12 months).
[b] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.
[c] 95% confidence interval.
[d] Coefficient represents the change in total cholesterol for each 1 ln-(ng/mL) increase in PFAS concentration.
[e] Coefficient represents the change in total cholesterol for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.
[f] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.

Table 4.46. Weighted[a] linear regression of each clinical chemistry outcome on "multiple high exposure" group, consisting of individuals with exposure in highest category for all seven perfluoroalkyl substances measured in mid-pregnancy plasma among 889 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003-2004.

	Unadjusted			Adjusted[b]		
	β	95% CI[c]		β	95% CI	
Total Cholesterol	4.73	-8.99,	18.44	4.06	-10.26,	18.38
HDL Cholesterol	8.88	4.94,	12.82	7.41	3.52,	11.30
LDL Cholesterol	-2.31	-14.38,	9.76	-0.27	-12.53	11.99
Ln-Triglycerides	-0.13	-0.25,	-0.01	-0.12	-0.22,	-0.02
Uric Acid	0.19	-0.08,	0.46	0.13	-0.15,	0.40
Ln-C-reactive protein	-0.15	-0.43,	0.12	-0.09	-0.37,	0.20

[a] Weighted for prior selection by subfecundity.
[b] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.
[c] 95% confidence interval.

Figure 4.2. Directed acyclic graph describing the hypothesized associations between perfluoroalkyl substances, HDL cholesterol, and modeled covariates.

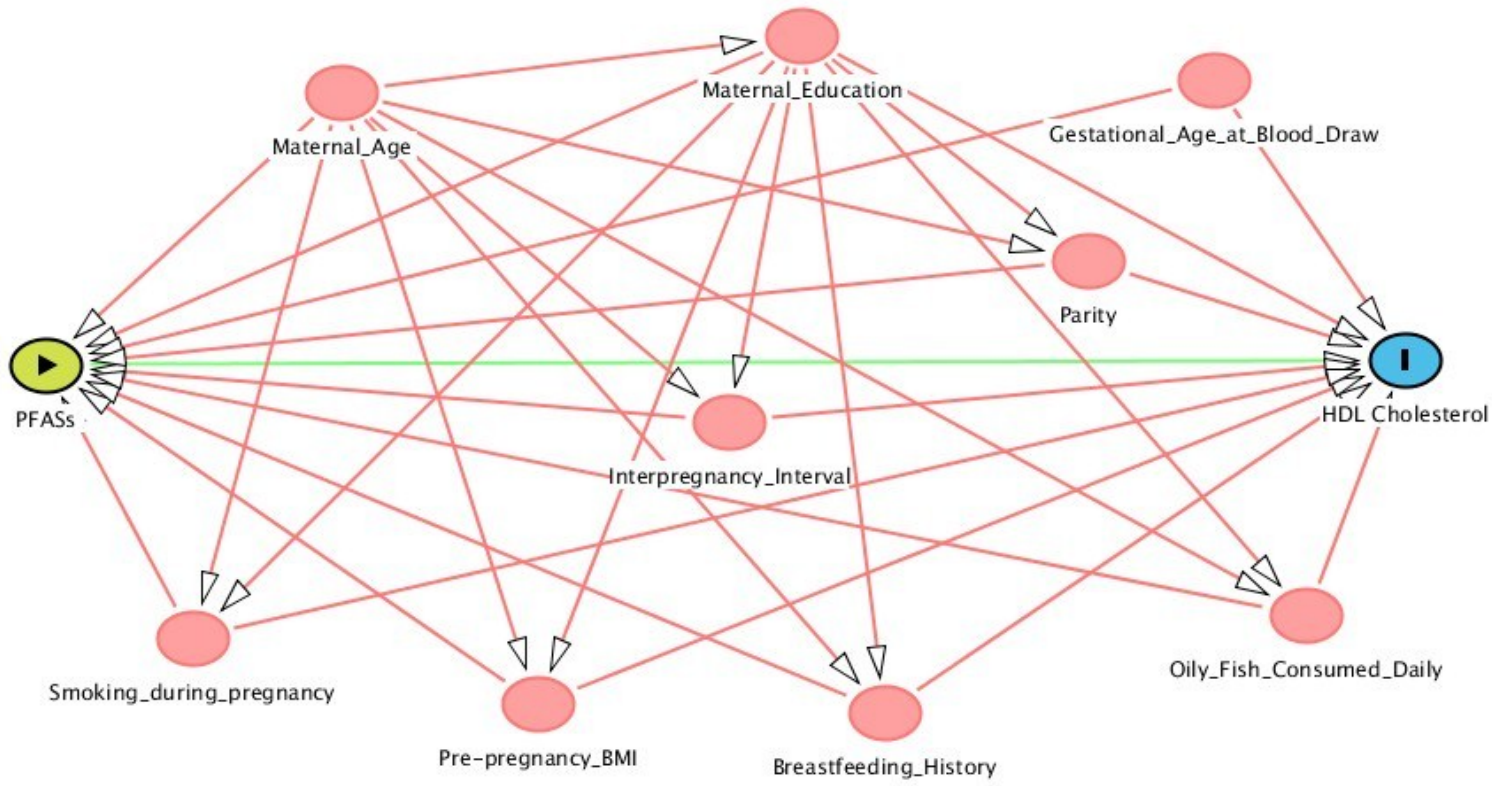
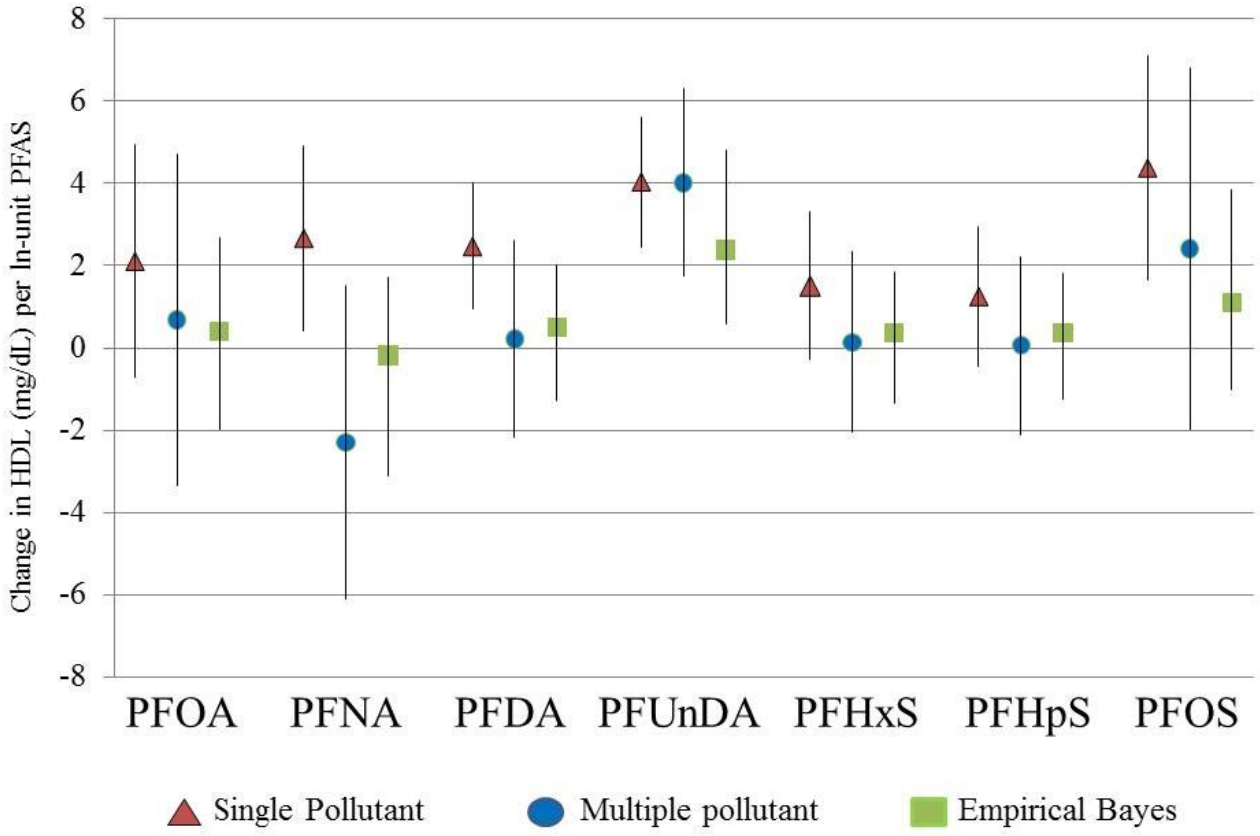


Figure 4.3. Single and multiple pollutant models for the association between perfluoroalkyl substances and HDL cholesterol (mg/dL), among 524 women selected without regard to subfecundity (base sample) enrolled in the Norwegian Mother and Child Cohort Study 2003-2004.



CHAPTER 5: CONCLUSIONS

5.1. Overall Study Aims, Findings, and Interpretation

The aims of this study were twofold: 1) to estimate the association between mid-pregnancy perfluoroalkyl substances (PFASs) and a validated diagnosis of preeclampsia among nulliparous women, and 2) to determine the cross-sectional associations between mid-pregnancy PFASs and several clinical chemistries of interest for cardiovascular health. These aims were accomplished using separate samples of eligible pregnancies from the large Norwegian Mother and Child Cohort (MoBa) study. The aims are interconnected, in that the cross-sectional associations with lipid parameters and other clinical chemistries were intended to evaluate one possible biological mechanism linking PFASs with preeclampsia.

The first aim was accomplished through the design and analysis of a case-cohort study within MoBa. Cases were randomly selected from eligible cases of preeclampsia whose diagnosis was validated by independent medical record review, and the subcohort was randomly selected from all eligible women enrolled in the same time frame. PFASs were measured in mid-pregnancy plasma samples and weighted Cox proportional hazards regression models were used to estimate the association between each of seven PFASs and preeclampsia, using as the event time either the gestational week at which diagnostic criteria were met, or the gestational day of delivery due to preeclampsia. In both circumstances, none of the seven PFASs examined were positively associated with preeclampsia risk, and PFUnDA was inversely associated with preeclampsia.

Based on a series of previous studies conducted among women highly exposed to PFOA through contaminated drinking water (8, 94), we expected positive associations between PFASs and preeclampsia risk. The differences in results between our study and the previous studies may be partially attributed to differences in outcome ascertainment method and exposure assessment, to lower PFOA concentrations in our study as compared with the previous studies, or to underlying differences between the populations studied.

Self-reported preeclampsia has been previously found to have a relatively low positive predictive value of 50-60% (177). The use of self-report in previous studies of preeclampsia may have identified "case" groups that inadvertently included women with other conditions, such as underlying chronic hypertension or pregnancy-induced hypertension, as well as those with true preeclampsia. Moreover, recall bias could be present if ascertainment of preeclampsia history took place after the participants became aware of PFOA contamination of their drinking water.

Our study also differed from previous studies in the method of exposure assessment. We directly measured PFAS concentrations in plasma at mid-pregnancy, while previous studies either estimated PFAS concentrations during pregnancy based on a predictive model (8) or measured PFASs in serum up to 5 years after the pregnancy of interest (94). Estimated PFOA concentrations in the previous studies were higher than concentrations measured in our study; for example, the upper boundary of the lowest category of PFOA exposure in one of the previous studies was higher than the 95th percentile of the PFOA distribution in our study (8) (Table 4.2). However, our restricted cubic spline models did not show any indication of increasing risk of preeclampsia

associated with PFOA exposure, even at the highest observed concentrations. Moreover, the median estimated PFOS concentration in the previous study did not differ notably from the concentration measured in our study. The previous studies did not examine associations with PFASs other than PFOS and PFOA, nor did they find linear dose-response relationships between PFOS or PFOA and preeclampsia (8, 94).

Like many epidemiologic studies, this investigation was prompted by a reported association in a highly exposed population. The fact that our study, conducted in a background-exposed population, failed to replicate the findings of the previous study may reflect unmeasured differences in underlying population characteristics. For example, the women who were most highly exposed to contaminated drinking water in Ohio and West Virginia may have had other risk factors that predisposed them to preeclampsia, or other environmental or genetic factors that interacted with high PFOA exposure to produce an elevated risk of preeclampsia. Additional studies conducted in different populations may help to resolve these questions of possible bias due to unmeasured confounders or effect modifiers.

The inverse association we found between PFUnDA and preeclampsia (Section 4.1) has not been previously reported. We had no specific hypothesis regarding the association between PFUnDA and preeclampsia, and therefore this finding must be interpreted with some caution. However, if an inverse association between PFUnDA and preeclampsia is observed consistently in other studies, it could potentially be explained by the causal mediation of HDL cholesterol. We observed a strong positive association between PFUnDA and HDL in our cross-sectional analyses (Section 4.2). This is notable because preeclampsia is typically characterized by reduced HDL (205), and it is

conceivable that an environmental exposure leading to increased HDL in pregnant women could actually be protective against preeclampsia. However, we cannot determine from a cross-sectional study whether or not the observed associations between PFUnDA and HDL are causal, nor do we know whether elevations in HDL actually prevent preeclampsia, so this mechanism remains speculative. Moreover, an attempt to adjust for HDL as a causal intermediate in order to "decompose" the observed association into direct and indirect effects would be unlikely to produce interpretable results, as the strict assumptions required for this procedure are unlikely to be met in this case (181).

Another consideration in the interpretation of these findings is the fact that we restricted our study population to women with no previous live births or stillbirths. The goal of this restriction was to simplify interpretation, as PFASs decline through pregnancy and lactation (14, 60) then may increase again during the interval between pregnancies (41), and this may produce biased associations, particularly if the outcome of interest is related to fertility. Restricting analyses to women with no previous live births or stillbirths may eliminate bias due to previous pregnancy history. Moreover, the pathogenesis of preeclampsia in nulliparous women may differ from the pathogenesis in parous women. Associations between PFASs and preeclampsia may differ between nulliparous and parous women; therefore these findings may not be generalizable to parous women.

Finally, the interpretation of observed associations between PFASs and preeclampsia is somewhat complicated by the heterogeneity of the disease. Several researchers have suggested that early-onset preeclampsia (prior to 34 weeks' gestation) may have a distinct etiology from late-onset preeclampsia, as evidenced by the fact that

early and late manifestations of the disease are characterized by different hemodynamic states and different lipid profiles (123, 124). While we attempted to restrict our analysis to a clearly defined phenotype of preeclampsia, it is possible that the cases of preeclampsia with onset prior to 34 weeks (14% of cases in our study) may show different associations with PFAS concentrations than the cases with later onset. Unfortunately our study was not powered to detect these differences.

The principal outcome in Aim 1 was the gestational age at onset of preeclampsia. This study design was intended to account for the varying lengths of pregnancy, due to the competing risk of early delivery for reasons other than preeclampsia. However, the results of the time-to-event model using gestational age at preeclampsia onset did not differ substantially from the results of the logistic analysis, which considered preeclampsia as a binary outcome. This suggests that any detected differences in preeclampsia risk associated with PFAS concentrations were differences in the diagnosis of preeclampsia at any point during pregnancy, rather than differences in the timing of diagnosis. By contrast, if women with higher concentrations of PFASs had earlier onset of preeclampsia than women with lower concentrations of PFASs, but no differences in overall incidence of preeclampsia, then we would have seen null results from the logistic regression models but positive results from the Cox proportional hazards models.

Similarly, the Cox proportional hazards models using the gestational age at identified onset of preeclampsia did not produce substantially different results from the models using gestational age at delivery due to preeclampsia, with the exception of a small inverse association with PFDA in the model using gestational age at delivery. This finding could be due to chance; however it could also suggest a subset of women with

higher PFDA concentrations who tended to have mild preeclampsia and deliver slightly later than women with lower PFDA concentrations who also have preeclampsia. Women with mild disease may be able to remain pregnant for longer and therefore deliver later than women with more severe disease with the same gestational age at onset. The small difference in the results of these two analyses is intriguing, but will need to be replicated in other studies.

The second aim was accomplished through the analysis of data from a subset of women in the MoBa study with PFASs and certain clinical chemistries measured during pregnancy. Based on previous studies of non-pregnant individuals, we expected to observe positive associations between PFASs and total cholesterol, as well as LDL cholesterol and triglycerides (5, 89). We did observe a positive linear association between PFOS and total cholesterol, but no associations with other PFASs. Moreover, the regression models based on PFAS quartile estimates did not indicate linear dose-response associations between PFASs and total cholesterol. We observed no associations between PFASs and LDL cholesterol or triglycerides.

We did observe positive associations between each of the seven PFASs and HDL cholesterol, and the strongest association was observed between PFUnDA and HDL. We cannot deduce from this single study whether this finding is due to confounding, pharmacokinetics, or to a causal association. Previous studies of PFASs and lipid parameters in non-pregnant populations have generally shown positive associations between PFASs and total cholesterol and non-HDL cholesterol (89), although one study noted an inverse association between PFHxS and total cholesterol (7). Lipid parameters change dramatically during the course of pregnancy (134) and it is possible that

associations between PFASs and lipid parameters in blood may differ in pregnant women as compared with non-pregnant individuals.

PFASs are often correlated in biological samples, but a simply summary measure is not recommended due to the possibility that each PFAS may have independent biological activity through multiple pathways (206). We explored a number of different approaches to this problem, including single pollutant, multiple pollutant, and empirical Bayes (EB) models. In the cross-sectional study of PFASs and HDL cholesterol, the differences between the results of the single pollutant and multiple pollutant models were substantial, and the differences between the multiple pollutant model and the EB model were more modest. Both types of multiple pollutant models (conventional and empirical Bayes) adjusted for possible confounding of each PFAS-HDL association by other, correlated PFAS exposures. We were only able to adjust for the seven PFASs detectable in greater than 50% of participants; other PFASs were present at very low concentrations but their relative potency remains unknown. While the use of multiple modeling strategies was highly informative about the potential influence of mutual confounding by correlated exposures, we cannot conclude based on this study alone which model provided the most unbiased estimates of the associations of interest.

Most previous studies have focused on PFOS and PFOA, which are found at the highest concentrations in most populations. However, it has become evident in recent studies that individual PFASs may differ in their associations with outcomes of interest. Some laboratory studies have suggested that certain properties of PFASs may depend on the carbon chain length of the molecule, and PFUnDA was the longest-chain PFAS examined here. However, chain length alone does not appear to explain the relative

potency of PFAS types, at least with regard to activation of peroxisome-proliferator activated receptor alpha (196, 197).

We cannot rule out the possibility that these results may be biased by unobserved confounding. The predictors of plasma PFAS concentrations are not well-established, and may vary between populations. There may be shared causes of elevated PFASs and elevated HDL in pregnancy that we did not adjust for. Additionally, there may be different confounders of each PFAS-lipid association. The relative toxicologic and pharmacokinetic properties of each of the PFASs in humans have not yet been adequately documented. There may also be affinities between HDL and PFASs in circulating blood (182) that could explain the observed association.

5.2. Strengths

This is the first study to utilize PFAS concentrations measured during pregnancy to estimate associations with lipid parameters and other clinical chemistries, as well as with a validated diagnosis of preeclampsia. Some previous studies have excluded pregnant women from cross-sectional analyses of PFASs and lipids (7, 90), while some have retrospectively estimated pregnancy PFAS concentrations rather than measuring during pregnancy (8, 94). Our study addresses the important question of whether there may be adverse effects of elevated plasma PFAS concentrations during pregnancy, and specifically whether concentrations of PFASs may be associated with preeclampsia risk.

This study used data from the large MoBa pregnancy cohort, which collected detailed questionnaire data as well as biological samples at mid-pregnancy (152), and high-quality PFAS assays performed by the Norwegian Institute of Public Health (155). The sensitivity of these assays allowed for the analysis of several PFASs, rather than only

the most prevalent chemicals. Additionally, we used an independently validated diagnosis of preeclampsia, based on a review of antenatal medical records (Klungsoyr et al., submitted). The use of a validated diagnosis of preeclampsia may have reduced outcome misclassification and improved our ability to detect an association. Finally, this study employed a novel treatment of correlated exposures, including a comparison of single pollutant, multiple pollutant, and empirical Bayes shrinkage models.

5.3. Limitations

We measured PFASs in plasma samples collected at mid-pregnancy, when participants were recruited into the MoBa study, by contrast with previous studies which employed indirect methods of exposure assessment (8, 94). However, we cannot rule out the possibility that an early manifestation of preeclampsia may have already been present at the time that samples were drawn. If kidney function were altered at mid-pregnancy, this could lead to decreased urinary excretion of PFASs. The likely result of this hypothetical scenario would be a positive association between PFASs at mid-pregnancy and the eventual development of preeclampsia; we did not observe such an association in this study.

Another potential limitation of our study design was the assumption of non-informative censoring in Cox proportional hazards models for preeclampsia. If the risk of censoring (or early delivery due to reasons other than preeclampsia) were related to the risk of preeclampsia, then this assumption would be violated and our results could be biased. However, the number of women delivering prior to 37 weeks due to reasons other than preeclampsia is small in this population (<5% of non-cases), and so the magnitude of this bias is expected to be minor.

It is possible that selection bias may have influenced the results of our study. The participation rate in the MoBa study was 39% of eligible women, and a previous study suggests that MoBa participants differed from the general population of women giving birth in Norway in a number of ways (170). For example, women under 25 years old were under-represented in MoBa, as were single women, women with two or more previous births, women with previous stillbirths, and women with pregestational diabetes (170). If selection into the MoBa study were related to both PFAS exposure and to preeclampsia, this could produce bias in our results. Our study was restricted to nulliparous women, and we adjusted for variables known to be strong confounders, therefore we believe that the magnitude of this potential bias is likely to be low. However, our results may not be generalizable to a population of women with different underlying characteristics.

5.4. Public Health Implications

PFASs have been produced for over 50 years, and are now detectable in the blood of humans in nearly all industrialized countries, including countries with no history of domestic PFAS production (31). PFASs are believed to reside in human blood, liver, and kidneys (17) and to have half-lives in the human body of 2-7 years (19, 169). It is unknown whether adverse health effects may result from chronic, low-level exposure in humans. At high levels, PFASs are known to be hepatotoxic, immunotoxic, tumorigenic, and developmentally toxic to fetuses (75, 82). It is therefore highly important to establish whether these widespread contaminants may adversely affect the health of pregnant women and their infants. Regulatory bodies in the United States and Europe have begun to restrict the production of PFOS and to work towards the replacement of PFOA with

alternative products. Proposed replacement chemicals must also be evaluated for health risks and environmental persistence. Perhaps one lesson to be learned from the ubiquitous presence of PFASs in the environment and in human bodies is that, in the future, we must evaluate not only the high-dose toxicity of a chemical but also the potential health effects of low-dose, chronic exposure before allowing widespread production and use.

5.5. Clinical Relevance

While the study of the population health effects of environmental contaminants is clearly within the scope of public health research, the implications of such research for clinical practice are less clear. If PFASs are eventually shown to have a causal relationship with preeclampsia or lipids during pregnancy, then it may be appropriate for obstetricians and gynecologists to communicate these risks to women in the context of prenatal or pre-conception counseling. This counseling could include education on sources of exposure to PFASs, so that women could potentially make more informed choices about their exposures (207). This may be particularly important if a woman has had occupational or other high-dose exposure to PFASs. For the general population, however, such an evaluation may cause unnecessary anxiety because individuals have limited ability to reduce their exposure to PFASs in their daily environments. More research will be needed to determine the most common sources of PFAS exposure in order to develop effective screening questions for clinicians to evaluate exposure risk (208). Ultimately, policy and regulatory changes leading to reduction or elimination of exposure to environmental pollutants are likely to be the most promising route to preventing pregnancy complications with environmental causes.

While we observed an inverse association between mid-pregnancy PFUnDA concentration and preeclampsia risk, it would be premature to suggest that the observed protective association is due to a causal effect of PFUnDA. However, the further investigation of possible causes of the observed inverse association may lead to a greater understanding of the pathogenesis of preeclampsia. We hope that increased knowledge of risk factors for preeclampsia may eventually aid in the prevention of this serious and common complication of pregnancy.

5.6. Future Directions

This study produced several novel findings, the interpretation of which will spur future research in this area. In order to establish whether the observed cross-sectional association between PFASs and HDL cholesterol in pregnant women may be causal, it would be informative to conduct a prospective study, in which plasma concentrations of PFASs are measured prior to the beginning of pregnancy. Cohorts of women intending to become pregnant are challenging to assemble, but given the stability of PFASs in stored plasma, it is possible that this question could be examined using stored plasma from a study of fertility or early pregnancy that required recruitment prior to pregnancy. If elevated PFASs prior to pregnancy were associated with higher HDL cholesterol during pregnancy, this would be more suggestive of a causal association, particularly if adequately adjusted for diet, exercise, alcohol consumption and other known causes of high HDL. However, the biological mechanism of such an association is unknown, and this effect would likely need to be demonstrated in an animal model in order to make a convincing argument for causality.

Similarly, the interpretation of the observed inverse association between mid-pregnancy PFUnDA and the subsequent diagnosis of preeclampsia is complicated by the timing of plasma collection. It is possible that an early, subclinical manifestation of the disease state may already be present, and may influence the measured concentration of PFASs in plasma, possibly through altered excretion of PFASs by the kidneys. It is unclear why such a process might only influence the association with PFUnDA. However, further toxicology studies are needed to establish the relative biological activity and potentially diverse modes of action of several widespread PFASs.

The fact that such a limited number of comparable studies have been conducted suggests that more epidemiologic studies of environmental exposures should consider including pregnant women. Pregnant women are often excluded from general population studies because the altered hemodynamics and lipid metabolism of pregnancy make it difficult to directly compare measured values from pregnant women to non-pregnant individuals. Given that all individuals in industrialized and developing areas are regularly exposed to environmental contaminants, it is important to study the potential adverse effects of PFASs and other common pollutants during pregnancy, a state in which both the mother and infant may be highly susceptible to harm.

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