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Fish consumption and time trends in blood concentrations of six perfluoroalkyl acids

A longitudinal study in the Tromsø Study

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Disclosure

I used ChatUiT version 3.5 for improving my written English language in this thesis. The front-page image was created using an AI image generator from Microsoft.

Abstract

Background: The European food safety authority (EFSA) has established a new upper limit for weekly intake of four different perfluoroalkyl acids (PFAAs) at 4.4 ng/kg/week. The Norwegian population already exceeds the upper limit through current fish consumption, despite not meeting the Norwegian Directorate of Health's recommendations for fish intake. A person's blood concentrations of PFAAs depends on a wide range of factors such as birth year, sex, parity, and diet. Additionally, PFAA concentrations in the environment has undergone significant changes in the last 30 years. However, the extent to which fish consumption has contributed to the changes in human blood concentrations remain unstudied.
Aim: The aim of this thesis was to assess the potential associations between fish consumption and time trends in blood concentrations of the selected PFAAs in repeated measures from the same individuals, utilizing data from participants in the Tromsø Study (1986-2016).
Material and method: The control group of an existing nested case-control study within the Tromsø Study, comprised of 76 females and 69 males, were investigated in this thesis. The participants had participated in between three to five surveys in the given period. Intake of total, lean, and fatty fish were extracted from questionnaires answered by the participants at each survey. PFAA concentrations were measured in blood samples collected at each survey. Linear mixed models for repeated measures were used to estimate time trends of PFAA concentrations among high and low consumers of fish. Interaction terms between fish groups and time was used to assess whether there were significantly different time trends.
Results: In the first survey, 53% of the participants reported to consume two or more dinner serving of fish per week. The fish consumption increased throughout the study period. High consumers of fish generally had higher PFAA concentrations compared to low consumers, and high consumers of total and lean fish generally had faster increasing and slower decreasing time trends than low consumers for most of the investigated PFAAs. Fatty fish consumer had minimal differences in PFAA concentrations between high and low consumers and did not experience significantly different time trends between the groups.
Conclusion: High consumers of total and lean fish had higher PFAA concentrations than low consumers and faster increasing and slower declining PFAA time trends. This indicates that high consumers sustained higher body burden of some PFAAs for an extended duration compared to low consumers, even as environmental concentrations declined. However, both high and low fish consumers exhibited high PFAA concentrations, indicating that reduced fish consumption did not guarantee PFAA blood concentrations within recommended limits.

Abstrakt

Bakgrunn: Den europeiske myndighet for næringsmiddeltrygghet (EFSAs) har satt en ny øvre grense for ukentlig inntak av fire utvalgte perfluoroalkylsyrer (PFAAs) på 4,4 ng/kg/uke. Den norske befolkningen overskrider allerede den øvre grensen gjennom nåværende fiskekonsum på tross av at fiskeinntaket ikke når helsemyndighetenes anbefalinger. En persons blodkonsentrasjoner av PFAAs avhenger av en lang rekke faktorer som blant annet fødselsår, kjønn, antall fødte barn og kosthold. I tillegg har konsentrasjonen av PFAAs i miljøet endret seg mye de siste 30 årene. Hvordan fiskekonsum har bidratt til denne endringen i konsentrasjon av PFAAs i blodet gjennom perioden, er derimot ikke studert tidligere.
Formål: Målet med studien var å finne mulige sammenhenger mellom fiskekonsum og tidstrender i konsentrasjoner av PFAAs i blodet gjennom gjentatte målinger fra samme personer som har deltatt i Tromsøundersøkelsen mellom 1986 og 2016.
Metode: Studiedeltagerne besto av 76 kvinner og 69 menn som hadde deltatt som kontroller i en eksisterende nøstet kasus-kontroll studie. Deltagerne måtte ha deltatt i mellom tre og fem undersøkelser i den aktuelle tidsperioden. Data på total, mager og fet fiskekonsum ble samlet inn ved hjelp av selvadministrerte spørreskjemaer. PFAA-konsentrasjonene ble målt i blodprøvene som ble samlet inn ved hver undersøkelse. Lineær blandet effekt-modell for repeterte målinger ble brukt som statistisk metode for å undersøke trendene i PFAA konsentrasjoner hos de som rapporterte høyt og lavt inntak av fisk. Et interaksjonsledd mellom fiskegrupper og tid ble brukt for å vurdere om trenden var ulik i de to gruppene.
Resultat: Ved den første undersøkelsen rapporterte 53% av deltagerne at de spiste fisk til middag to eller flere ganger per uke. Fiskekonsumet økte gjennom studieperioden. De som konsumerte mye total og mager fisk, hadde generelt raskere økende og saktere avtagende PFAA tidstrender sammenlignet med de som konsumerte lite total og mager fisk. De absolutte forskjellene i PFAA konsentrasjoner mellom høy og lavkonsumentene av fet fisk var minimale og det ble ikke observerte forskjeller i tidstrender mellom høy- og lavkonsumenter av fet fisk.
Konklusjon: Personene som konsumerte mye fisk, både total og mager, hadde høyere konsentrasjoner av PFAA enn de som konsumerte lite fisk, og konsentrasjonene økte raskere og avtok saktere. Dette indikerer at høyforbrukere opprettholdt en høyere belastning av PFAAs over lengre tid, selv når miljøkonsentrasjonene gikk ned. Allikevel hadde både de som konsumerte mye og lite fisk høye konsentrasjoner av PFAA, noe som tyder på at selv et redusert fiskekonsum ikke sikret PFAA-blodkonsentrasjoner under anbefalte grenser.

List of abbreviations

EFSA	The European Food Safety Authority
BMI	Body mass index
CHD	Coronary heart disease
CVD	Cardiovascular disease
DAG	Directed Acyclic Graph
LC n-3 FA	Long-chained omega 3 fatty acids
ng/mL	Nanograms per milliliter
PFAS	Per- and polyfluoroalkyl substances
PFDA	Perfluorodecanoic acid
PFHxS	Perfluorohexane sulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonic acid
PFUdA	Perfluoroundecanoic acid
PFAAs	Perfluoroalkyl acids
POPs	Persistent organic pollutions
SD	Standard deviation
T_n	Time point n
TWI	Tolerable weekly intake
VKM	The Norwegian Scientific Committee for Food and Environment

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1 Introduction

1.1 Fish consumption

Throughout history, fish and seafood has been an important food source and part of a healthy diet for populations across the globe (1). Although fish is considered healthy food, fish is also a source of contaminants such as lead, dioxins, mercury, polychlorinated biphenyls, and brominated flame retardants, raising health-related concerns (2).

The global fish consumption is increasing and was growing with about 1.5 percent each year between 1961 and 2018 (3). The observed growth is mostly due to improved access through international trade (3), making fish available for more people. While global fish consumption is on the rise, certain high-income countries have experienced a decrease in fish consumption (3). In Norway, fish consumption has dropped with approximately 12% since 2015 (4). Although fish consumption has declined on a national level, there are disparities in fish consumption within Norway. Individuals with higher education and those living in northern Norway tend to consume more fish, as do older people (4, 5). However, younger adults who prioritize their health, reporting more physical activity and less smoking, also consume more fish compared to their peers (5). It is not only the amount of fish we are eating that is changing, but also the way we are consuming fish. Historically, fish was consumed as whole fish or filet, but today, more and more fish are processed into minced blends used in products such as fish cakes, fish sticks and other highly processed foods (6). Fish is also one of the food items that has experienced the greatest price increase in Norway, along with the price of vegetables. This contributes to increase the socioeconomic disparity in the consumption of healthy food, such as fish, in Norway (7).

Before Norway found oil in the 1960s, fish was an important source of income for the country (8). All over Norway's long coastline, people have been living of fish both for income and as an important food source (9). After the 1970s, fish farming and especially farming of salmon, has increased substantially in Norway, and today Norway provides more than half of the world's farmed salmon (10). Even though salmon production provides many jobs and contributes to a large part of Norway's Gross domestic product today, salmon production also has negative consequences by affecting wildlife in several ways by pollution on the sea floor, infect wild stocks with sea lice, and diluting the genes of natural salmon by escapements (11).

1.1.1 Health benefits of fish consumption

Fish consumption offers several health benefits by being an important source of essential nutrients like long chained omega 3 fatty acids (LC n-3 FA), vitamin D, iodine, selenium, and vitamin B12 (12).

Fatty fish like salmon or trout are a rich source of LC n-3 FA, which improve heart health by reducing blood pressure and boosting brain development and health (13-15). LC n-3 FA should contribute to more than 1% of our total daily energy intake (16), which can be provided with under one portion of salmon. Fatty fish is also a good source of vitamin D, a crucial vitamin for calcium absorption and metabolism that benefits bone health (17). Additionally, vitamin D is suggested to decrease the risk of certain types of cancer, type 1 diabetes, and multiple sclerosis (18).

Lean fish is a good source of iodine, an essential nutrient for growth and development through its role in thyroid hormone production (19). Fish also provides selenium, an important antioxidant that plays an important role in thyroid function (20), and vitamin B12 that is an essential nutrient which is crucial in production of red blood cells and function of the nervous system (16, 21).

A review from 2023 evaluated evidence from meta-analyses regarding fish consumption and different health outcomes (22). The review concluded that there was moderate to high evidence for a reduction in risks of 17 different negative health outcomes when associated with fish consumption. Three of the outcomes with the strongest evidence were all-cause mortality, prostate cancer mortality and cardiovascular disease (CVD) mortality. Fish consumption was also associated with better vitamin D status and increase in the HDL-cholesterol, which is positive for health (22).

In 2022 The Norwegian Scientific Committee for Food and Environment (VKM) also evaluated the weight of evidence between exposure and health outcomes for fish intake (16). Fish consumption was reported associated with a reduction in risks of all-cause mortality, mortality from CVD, coronary heart disease (CHD), stroke, myocardial infarction, dementia and Alzheimer's disease in adults, and preterm birth and low birth weight (16). The associations between fish consumption and CVD are mostly ascribed to the content of LC n-3 FA and therefore mostly linked to consumption of fatty fish (23). However, lean fish has also

been associated with beneficial health outcomes such as beneficial changes in lipid profiles (24).

1.1.2 Dietary recommendations

Based on the beneficial effect of a sufficient intake of fish, the Norwegian Directorate of Health suggests that the Norwegian population should eat 300-450 grams of fish per week, with 200 grams being fatty fish like salmon, trout, herring, or mackerel (12). This corresponds to about two to three dinner servings of fish per week (12). According to the national dietary survey "Norkost 3", only a third of the Norwegian population meets the recommendations (25).

In a Norwegian diet, more than 20% of the total intake of LC n-3 FA, vitamin D, iodine, selenium and vitamin B12 comes from consumption of fish (16). Especially LC n-3 FA, iodine and vitamin D are nutrients that are not found in many other food sources in the Norwegian diet. By increasing fish consumption in Norway, VKM states that the amount of people not meeting the recommendations for iodine, selenium and vitamin B12 could be reduced and the intake of vitamin D could be closer to the recommendations, which would benefit the Norwegian population (16). Also, an increase in fish consumption would reduce the populations risk of CVD, CHD, dementia and Alzheimer's which accounts for a large part of the countries' disease burden (16).

1.2 Per- and polyfluoroalkyl substances

Per- and polyfluoroalkyl substances (PFAS) is a collective term for over 10,000 fluorinated substances (16, 26). As PFAS are man-made chemicals, they were first introduced to the world in the 1940s (27). PFAS are stable chemicals with water-, oil- and stain-resistant properties that are commonly used in products like non-sticky cookware, waterproof clothing, firefighting foams and food packaging, to mention some (28). Humans get exposed to PFAS mainly through what we eat and drink, but also the air we breathe and the products we use in everyday life (29). Almost all humans have PFAS in their blood, and because PFAS has been shown to have hazardous effects on human health, they are considered a global concern (30).

1.2.1 The PFAS Family

The primary backbone of PFAS consists of a hydrophobic alkyl chain that can vary in length, and a hydrophilic functional group. The charge of the end group can vary from neutral, positive or negative, which will affect the chemical property of the substance (31).

The PFAS family can be divided into polymers and non-polymers (Figure 1). Non-polymers can be further divided into perfluoroalkyl substances and polyfluoroalkyl substances. In per-fluoroalkyl substances, all hydrogen atoms, except the ones in functional groups, are replaced with fluorine. In poly-fluoroalkyl substances however, at least one carbon needs to have all its hydrogens replaced with fluorine (31). The covalent bond between carbon and fluor is considered very strong and is what makes the chemicals so persistent to degradation (32). This thesis will manly focus on perfluoroalkane sulfonic acids and perfluoroalkyl carboxylic acids found under the subclass perfluoroalkyl substances and the group perfluoroalkyl acids (PFAAs) as seen in the figure below (Figure 1).

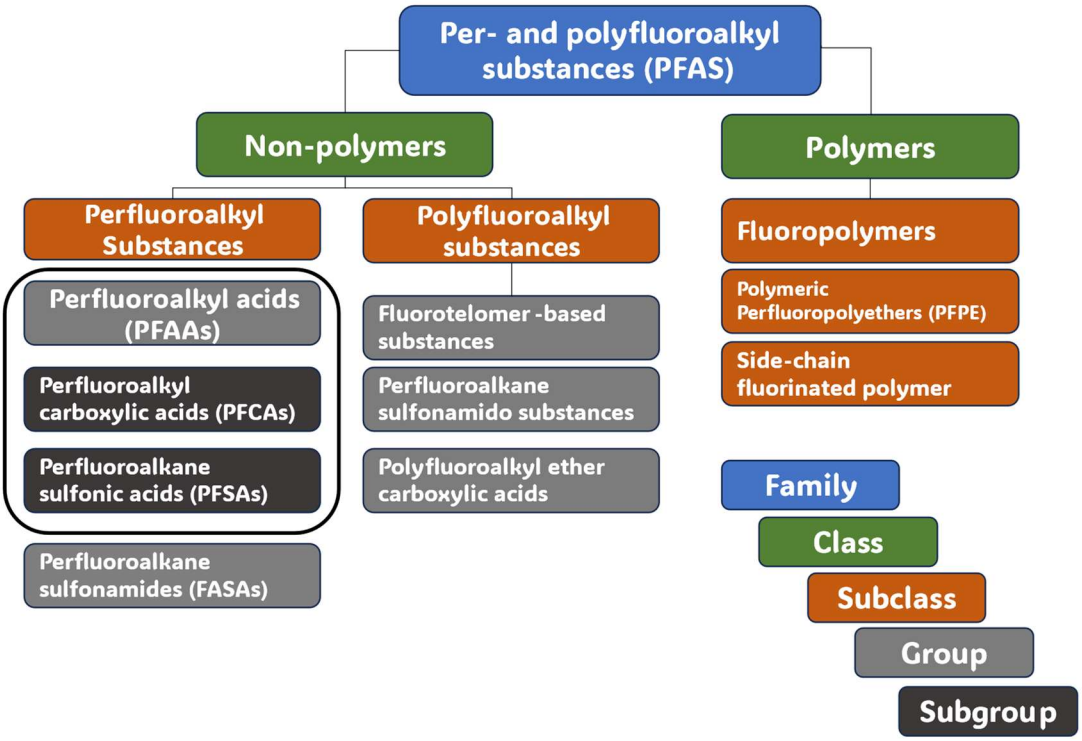

Figure 1: Summary of the per- and polyfluoroalkyl substances family tree. Figure made with inspiration from an existing figure by PFAS naming convention 2023 (33).

PFAAs are often categorised into long-chained and short-chained compounds. Perfluoroalkyl carboxylic acids are considered long-chained if they contain seven or more perfluorinated carbons. Perfluoroalkane sulfonic acids, however, are considered long-chained if they contain six or more perfluorinated carbons (31). Some PFAAs can also be separated into linear and branched isomers. Isomers can be created when the carbon-backbone consist of more than two carbon atoms (31).

1.2.2 Exposure pathways and contributing factors to PFAAs blood concentrations

Every day we are exposed to PFAAs. PFAAs can be introduced in the human body through contaminated food and water, inhalation or contact with contaminated products (29). PFAAs also get transferred from mother to child through the placenta and breast milk (34). As illustrated in Figure 2, PFAAs used in industry are released into the environment and find their way to humans through consumer products, foods, and water (35).

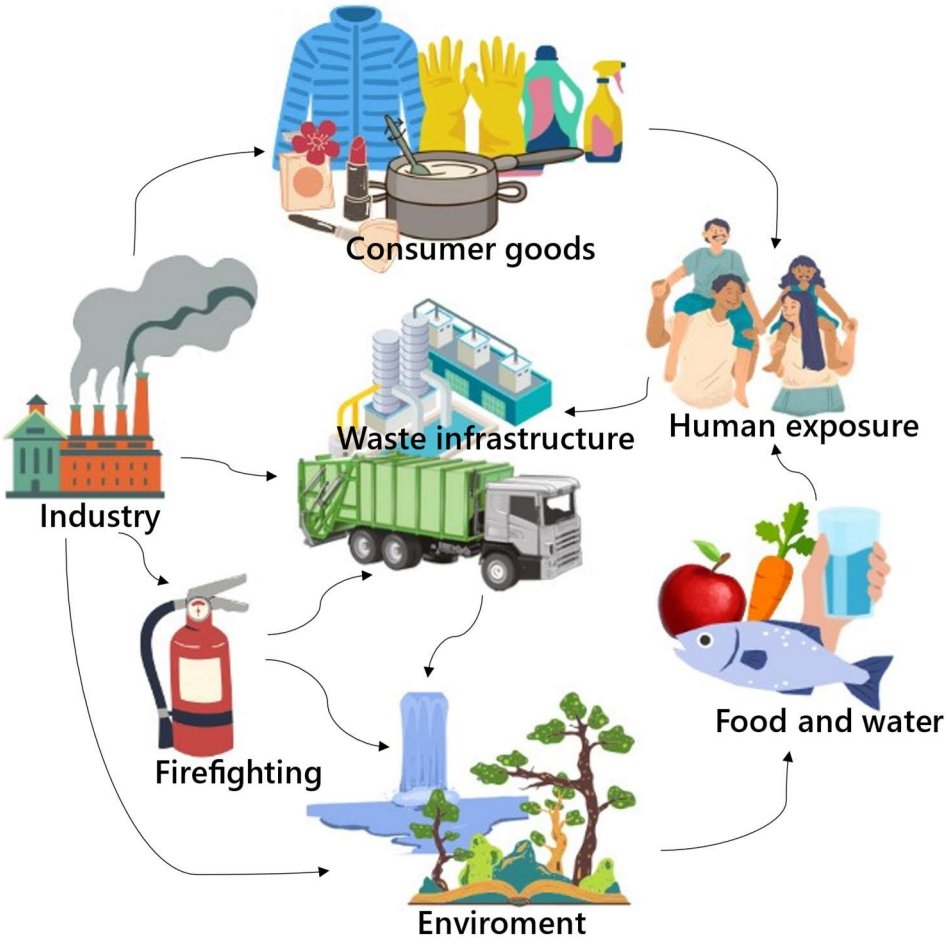

Figure 2: Perfluoroalkyl acids human exposure pathways scheme. Figure made with inspiration from an existing figure by the European Environment Agency (57).

In addition to being exposed to PFAAs trough everyday life, there are other factors that contribute to human blood concentration of PFAAs. Among them are age/birth year, sex, history of reproduction, profession and where you live.

Females generally have lower PFAA concentrations in their blood compared to males (36). This is mainly because females excrete some PFAAs through menstruation, pregnancy, and lactation (37). How many children a female give birth to will therefore affect her PFAA

concentrations. Even though menstruation, pregnancy and lactation count for some of the differences in PFAA concentration between females and males, they do not fully explain the sex differences according to Jein et al. (38). Also, there have been large changes in the types of PFAAs most commonly used in industry over the past 30 years and, for example, the use of perfluorooctane sulfonic acid (PFOS) has been drastically reduced after the early 2000s (39). Due to this shift, the concentration of PFOS in females' bodies is influenced by if they gave birth before or after this period (40). Historical use of PFAAs also affects the PFAA concentrations in individuals born in different years (or birth cohorts) (41), as some will have lived through periods with higher environmental exposure to certain types of PFAAs than others, thus experiencing greater exposure. Therefore, while it can be said that older individuals generally have higher PFAA concentrations than younger people (42), the composition and the concentrations of specific PFAAs will vary.

How often you vacuum clean can also affect your exposure to PFAAs, because house dust can contain PFAAs and therefore expose us through the air we breathe at home (43). Some studies suggest that inhalation only represents a minor contributor to human exposure of PFAAs, and others conclude that this is an underestimated factor (44).

Exposure to PFAAs is highest close to industrial sites where PFAAs are used in production (45). In Norway there are no PFAAs production sites, however people living close to military sites and airports, where firefighters have been training with lots of firefighting foams containing PFAAs, have been considered more at risk of being exposed (45). The use of PFAAs-containing fire foams is considered to be the largest source of PFAA emissions to the environment in Norway (46). This is because they contaminate soil and drinking water and the process of cleaning up after use of firefighting foams is technically very difficult and expensive (46). Because of this, the European Union (EU) wants to ban all use of PFAA containing firefighting foams. The regulations are assumed to take place in 2025/2026 (46).

People working as ski waxers has also been at risk of having significantly higher concentration of PFAAs, especially perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA), than the general population. This was the case because ski wax previously contained these fluorinated compounds (47). In 2020, the Norwegian Environment Agency banned products containing PFOA, including ski wax (47).

1.2.3 Bioaccumulation and contamination of food

Bioaccumulation happens when a chemical is taken up and stored faster than the body can metabolize or excrete the compound (48). This results in increased concentrations in the body compared to the concentrations in the surrounding environment. Biomagnification refers to an increased concentration of a chemical from one individual at one level in the food chain to the next tropical level (48). Already in 2001, researchers could observe that fish-eating animals had higher concentrations of PFOS than the concentration in their diet (49), and we now know for sure that PFAAs both accumulate in the body and magnify at different trophic levels in the food chain. This results in older and bigger fish containing more of some PFAAs than younger and smaller fish, and fish-eating animals have higher PFAA concentrations than the fish itself. Bioaccumulation of different PFAAs generally increases with increasing chain length (50, 51).

Plants and animals become exposed to PFAAs through contaminated air, soil, and waters, subsequently leading to accumulation within our food. The contamination of food is thought to be mainly caused by accumulation in the food chain and transfer from materials like food packaging and cookware that contain PFAAs (32). Transfer from materials in food production, packaging and cooking might reveal PFAAs currently used, while PFAAs from food itself can reflect bioaccumulation over time (32).

1.2.4 Absorption, distribution, and elimination of PFAAs in the human body

PFAAs have been shown to be easily absorbed through the gastrointestinal tract in humans (52). After entering the body, PFAAs attach to proteins in the blood, mostly albumin, and are then distributed throughout the entire body, especially in the areas with high blood circulation such as the liver and the kidneys (52). Humans do not metabolise PFAAs, they are slowly excreted from the body through urine, faces, blood loss and lactation (32, 53). Half-lives of PFAAs in the human body depends on whether they are short-chained or long-chained. Short-chained PFAAs are less likely to be reabsorbed from the urine, intestine or liver and therefore have a shorter half-life between a few days to a month, whereas long-chained PFAAs can have a half-life up to several years (32). The half-life of PFOS and perfluorohexane sulfonic acid (PFHxS) are approximately 5.4 and 8.5 years, respectively, in humans (53).

1.2.5 Health effects of PFAAs

PFAAs have been linked to several adverse health effects in humans (Figure 3) such as oxidative stress, decreased fertility, high cholesterol, hormone disruption, and elevated cancer risk (28, 54-56).

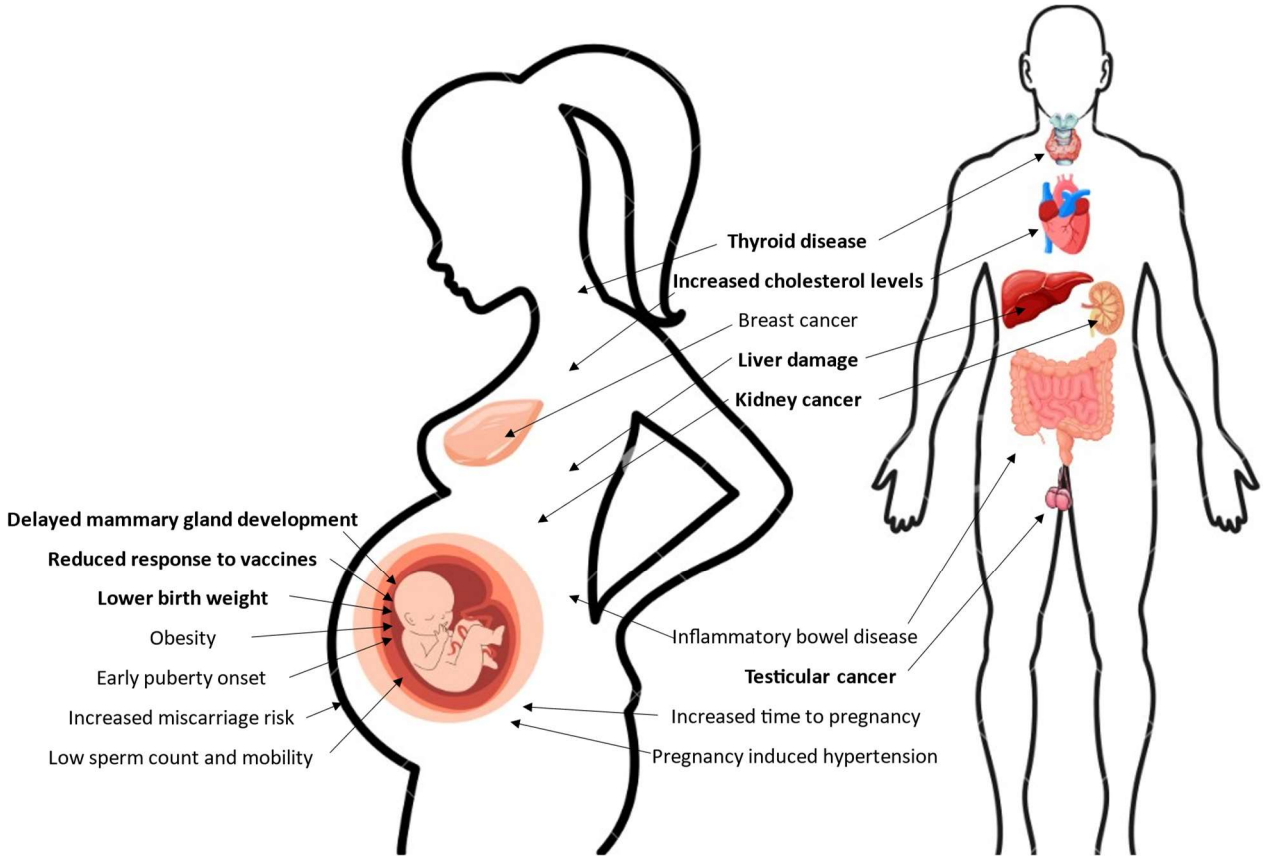

Figure 3: Effects of perfluoroalkyl acids on human health. Bold text represents higher certainty. Figure made with inspiration from an existing figure from The European Environment Agency (57).

The health effect that humans is considered most sensitive for at the lowest PFAA concentrations, is the inhibited development of antibodies after vaccination (34). PFAAs, especially PFOS, PFOA and PFHxS, effect on antibody response have been found in both cross-sectional and longitudinal studies. A systematic review and meta-analysis from 2022, addressing the effect of PFAS exposure on production of antibodies after vaccination of children, concluded that any type of PFAS may cause vaccination to be less effective than expected (57). This is of concern because vaccines reduce risk of diseases and are of great importance for reducing deaths and morbidity worldwide (57). If the immune system is compromised or dysregulated it can cause increased risk of infections, more severe infection symptoms and increased risk of chronic disease.

The International Agency for Research on Cancer (IARC) has classified PFOA and PFOS as "possibly carcinogenic to humans" (Group 2B), based on evidence in humans that it can cause testicular and kidney cancer, and evidence that it can cause cancer in experimental animals (58). The evidence is though limited and that's why it's only classified as "possibly carcinogenic" (58). A systematic review and meta-analysis from 2022 concluded that there was consistent evidence for PFAS hepatoxicity from rodent studies and that the findings were supported by markers of liver cell damage, such as alanine transaminase (ALT), associated with PFAS exposure in human studies (59). Testicular cancer and kidney cancer are the two types of cancer with most evidence for being associated with PFOA and PFOS exposure (60).

Evidence regarding PFAAs association with unnormal lipid concentrations is strong, but the direction of association and the strength of the association differ between different PFAAs and different lipid types (61). A systematic review from 2022 found evidence of a positive association between PFOA and PFNA and low-density lipoprotein-cholesterol, they also found evidence of a positive association between PFOA, PFOS and total cholesterol (61).

Several studies have identified associations of high PFAS exposure and irregularities of females' menstrual cycle, earlier menopause and reduced levels of oestrogen and androgens (62). PFHxS is especially known to affect the endocrine system and can reduce other hormone concentrations such as thyroid hormones T3 and T4 (63). PFHxS is also known to affect the development of the brain.

Often in studies addressing associations of PFAS and health effects, they address single PFAS. Therefore, associations are more often linked to PFOS, PFOA and other single PFAS. PFNA and PFHxS has the same chemical structures and physiochemical properties as PFOA and PFOS, therefore it is assumed that they have many of the same effects even though they are less studied (34). There are also over 10,000 PFAS chemicals whose health effects are not well understood, and the potential mixture effect of the sum of PFAS compounds adds an additional layer of complexity to this problem. According to De Silva et al., our ability to investigate how transition from use of PFOS and PFOA to newer PFAS have influenced human health is limited due to the limited time newer PFAS have been in the environment and due to limited assessment methods to detect newer PFAS (29). So far, the total health cost resulting from PFAS exposure have been estimated at 52-84 billion euros per year in the EU/EEA (46).

1.2.6 Regulations

In the early 2000s, PFOS and PFOA were found in human blood samples around the world. The first one to report PFOS in tissue of wildlife animals was Giesy and Kannan in 2001 (49). They found PFOS not only in samples close to industrial sites, but also in remote areas. As the reports on PFAS in nature and humans increased, the concern about their potential hazardous effect also started to rise. Due to this concern, PFOS and PFOA were voluntarily phased-out by some of the major manufactures in the United States around the year 2000-2002 (31).

In 2001, PFOS was restricted in the United States and in 2006 the European Union followed. Later PFOS and PFOA was banned globally under the Stockholm convention on persistent organic pollutions (POPs) in 2009 and 2019, respectively. PFHxS was added to the POPs regulation in the Stockholm convention in 2022 (64, 65).

Although regulations of some PFAS is an important step in preventing further exposure, PFOS, PFOA and PFHxS represent only a small number of the total number of PFAS (29). As the Director of Food Policy for Consumer Report, Brian Ronholm, said to Consumer Reports in 2022 “Trying to ban individual PFAS is an impossible game of whack-a-mole. As soon as one is addressed, industry comes up with another” (66). Even though many companies use PFAS compounds in their products, other companies manage to make the same products without the use of PFAS. This proves that in many cases there are alternatives, and we can manage without the use of PFAS. But as long as PFAS are allowed in production, most companies will not take the cost to find good alternatives (67). Importantly, on January 7th, 2023, Norway, Netherlands, Germany, Sweeden and Denmark presented a proposal to ban all use of PFAS. A decision on the PFAS proposal is expected within 2025/2026 (26).

1.2.7 Time trends

The production of PFAS is now shifting from “legacy PFAS” towards short-chained “emerging PFAS” with lower bioaccumulation capacity. The term “legacy PFAS” refers to PFAS that have been phased out, while “emerging PFAS” refers to more recently produced and so far, unregulated PFAS (55). Although the concentrations of legacy PFAS in the environment are showing decreasing trends since early 2000, the presence of emerging PFAS are on the rise (34, 56). As a result, young people today will have a different composition of PFAS in their blood compared to young people 20 years ago.

Among previous studies that have assessed time trend of PFAAs in Norway, are a longitudinal study with cross-sectional measurements from 2009 which analysed serum samples from 57 Norwegian adults to assess the changes in time trends of PFAAs between 1976 and 2007. This study identified a pronounced peak in concentrations of PFOS and PFOA around the year 2000, along with an increase in concentration of PFHxS, PFNA and perfluorodecanoic acid (PFDA) from 1976 until the early 90s where they appeared to stabilize (68).

Berg et al. conducted a study in Northern Norway, where they analysed serum samples from 30-year-old males and females collected in 1986, 1994, 2001, and 2007. Their findings indicate rising trends for PFHxS and PFOS from 1986 until 2001, at which point the concentrations peaked. PFOA reached its highest concentrations in 1994 before showing a decline by 2007. PFNA and PFDA, on the other hand, exhibited increasing trends throughout the study period. No specific temporal trends were observed for perfluoro-undecanoic acid (PFUdA) (69).

Nøst et al., in contrast to the two other mentioned studied, analysed the time trends through repeated measures from the same individuals. Their study covered 10 PFAAs in 53 males across five time points between 1979 and 2007. They reported a peak for PFOA and PFOS in the years 1994 and 2001, respectively. PFHxS also reached its highest concentrations in 2001, but the decrease towards 2007 was not as pronounced as that observed for PFOS and PFOA. PFNA, PFDA and PFUdA all showed increasing trends from 1979 to 2007 (41).

1.3 PFAAs and fish consumption

The association between fish consumption and PFAA concentrations has been established in several studies. In a Norwegian study from 2010, Haug et. al, concluded that consumption of fish and shellfish was significantly associated with an increase in serum PFAS concentrations (70). Another study published in the same year by Rylander et al. found that females who ate more fish had higher plasma concentrations of PFOS, PFNA and PFHxS (71). Berg et al. found in 2014 that high consumers of marine foods had significantly higher serum concentrations of PFOS, PFNA, PFDA and PFUdA (40). This was also later confirmed by a study conducted in six European countries by Papadoplulou et al, in 2019 (72). Bjorke-Monsen et al. collected data on never-pregnant Norwegian females between June 2012 and March 2015, while Christensen et al. investigated data from 2007-2014 collected in the cycles of the National Health and Nutrition Examination on a representative sample of the U.S

population. They both concluded that fish consumption was a strong predictor for serum PFAS concentrations (28, 73).

In 2020, the European food safety authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM panel) preformed a risk assessment for the sum of PFOA, PFNA, PFHxS and PFOS after request from the European Commission (32). These four PFAAs were selected based on observations of concentrations in humans and animals, where they made up over 90% of the analysed PFAAs (32). The EFSA report concluded that the food categories that contributed to highest exposure to PFOA, PFNA, PFHxS, and PFOS combined, were fish meat, fruit and fruit products, and eggs and egg products (32).

1.4 Research rationale

In EFSAs risk assessment they reassessed the tolerable weekly intake (TWI) that was in 2018 set to 13 ng/kg/week for PFOS and 6 ng/kg/week for PFOA (74). The new recommendations now recommend a TWI on the four PFAAs (PFOA, PFNA, PFHxS and PFOS) in total to be 4.4 ng/kg/week. The new guidelines shows that the intake in Europe exceeds the new recommendations (16, 75). According to EFSA, TWI is the maximum intake of a substance that can be consumed weekly over a lifetime without risking adverse health effects (76). In the case of PFAAs, the TWI was described as: “This TWI should prevent that mothers reach a body burden that results in levels in milk that would lead to serum levels in the infant, associated with decrease in vaccination response” (77).

The Norwegian Directorate of Health suggests that the Norwegian population should eat 300-450 grams of fish per week, with 200 grams being fatty fish (12). At the same time, the media is overflowing with headlines about the risk of eating fish due to its high levels of environmental pollutions. Is it still advisable to recommend a high fish intake considering the exposure to environmental pollutants? VKM performed a risk assessment of fish in the Norwegian diet in 2022 (16). The report discovered that the average adult population in Norway has a 1.7-fold higher exposure to PFAAs compared to the TWI recommendations with the current fish consumption which is lower than recommended. However, the report concludes that the health benefits of consuming the recommended 300-450 grams of fish per week outweigh the risk for all age groups (16). The conclusion is supported by evidence that increased fish consumption would reduce the incidence of stroke, CHD, dementia, and Alzheimer’s in the Norwegian population. These diseases are contributing to a large part of

the disease burden in Norway, especially with an increasingly older population as these diseases often come with older age. These diseases are therefore considered important to prevent. As PFAAs are found in almost all foods, the VKM concludes “a reduction of fish intake probably will cause some reduction in exposure, it may not suffice to get an exposure below the TWIs” (16). At the same time, an increased fish intake up to the recommended amounts will only increase the intake of PFOA, PFOA, PFHxS and PFNA in total from 1.7 times the TWI to 1.9 times the TWI. This is why the VKM has concluded that the benefits from increasing fish intake up to the recommended amounts outweighs the risks (16).

A person’s blood concentration of PFAAs depends on a wide range of factors such as birth year, sex, numbers of children, and diet. Additionally, PFAA concentration in the environment has undergone significant changes in the last 30 years, with some increasing and others decreasing. However, the extent to which fish consumption has contributed to the changes in human blood concentrations remain unstudied. The study period of this thesis falls within the timeframe when the composition of PFAAs in the environment experienced lots of changes. In this master’s thesis I studied time trends of PFAAs in high and low fish consumers. This research can shed light on how fish consumption may contribute to the changes in blood concentrations of PFAAs over time, which may provide useful information for risk benefit assessments of fish consumption.

2 Aim

The main aim of this thesis was to assess the potential associations between fish consumption and time trends of six selected PFAAs in repeated blood samples from the same individuals participating in the Tromsø Study (1986-2016).

Specific objectives:

- Investigate to what extent the study participants achieved the Norwegian recommendations on weekly fish consumption.
- Assess differences in PFAA concentrations in high and low consumers of fish (total/fatty/lean) cross-sectionally at five different time points (T1-1986/87; T2-1994/95; T3-2001; T4-2007/08; T5-2015/16)
- Investigate how the concentrations of different PFAAs in blood from the study participants changed over time (1986-2016) in high vs low consumers of fish.

3 Material and Methods

3.1 Study design

This master thesis used data from The Tromsø Study sampled between 1986-2016, a study with a longitudinal design.

3.2 The Tromsø Study

The Tromsø Study was initiated in 1974 due to observed higher mortality rates from CHD among males in Tromsø compared to the rest of Norway, sparking the interest among researchers to uncover underlying reasons (78). In the second survey of the Tromsø Study (Tromsø2) conducted in 1979/80, females were also included as participants. The Tromsø Study so far consists of seven surveys (Tromsø1-Tromsø7). With over 45,000 individuals participating one or more times over the course of the seven surveys, the Tromsø Study has become the most widely participated population study in Norway (78).

3.2.1 Questionnaires

When participants were invited to the Tromsø Study, they received an invitation letter and a questionnaire (Q1). If attending the physical examination, they received another often more comprehensive questionnaire (Q2) (79). In Tromsø1 and Tromsø2, these questions mainly concerned information about CVD, diabetes, physical activity, smoking and family history of diseases. For each survey, the questionnaires have expanded, including more information about medicine use, health care, sleeping and socioeconomical status (79). The questionnaires have primarily required participants to select a checkbox option that best represented their response. Additionally, many questions have been structured as either yes/no or numeric responses (79).

3.2.2 Physical examinations

In Tromsø1, the physical examination primarily focused on assessing blood pressure, weight, and height (79). However, similarly to the questionnaires, the scope of the physical examination has evolved across the different surveys. In later surveys they have taken measurements like hip-waist ratio, bone mineral density test, ultrasound scans, and electrocardiogram, among others. The most recent addition to the physical examination in Tromsø6 was pain sensitivity tests (79).

3.2.3 Blood samples

In Tromsø1, only serum haemoglobin, non-fasting serum cholesterol, triglycerides, and glucose level were measured routinely in the collected blood samples. In later surveys, more comprehensive analysis has been performed to establish renal function, inflammation markers and markers of hepatic disorder, to mention some (79). Blood samples from participants have been stored as serum at -70 degrees Celsius from each survey. The stored samples from the different surveys makes it possible to study serum characteristics like environmental contaminants from periods of higher usage today.

3.3 Study sample

This master thesis utilized the controls from an existing nested case-control study conducted within the Tromsø Study. The nested case-control study aimed to investigate longitudinal changes of persistent organic pollutants and their association with type 2 diabetes mellitus by utilizing repeated measures from the same individuals at up to five time points (T): 1986/87 (T1), 1994/95 (T2), 2001 (T3), 2007/08 (T4), and 2015/16 (T5) (80). The controls for the study were randomly selected from the participants that had contributed to at least the same surveys as the cases, had no diagnosis of diabetes, and had available serum samples. A total of 145 people (females=76, males=69) were included as controls, and thus as participants in my study (Figure 4). The controls were not individually matched to the cases (80).

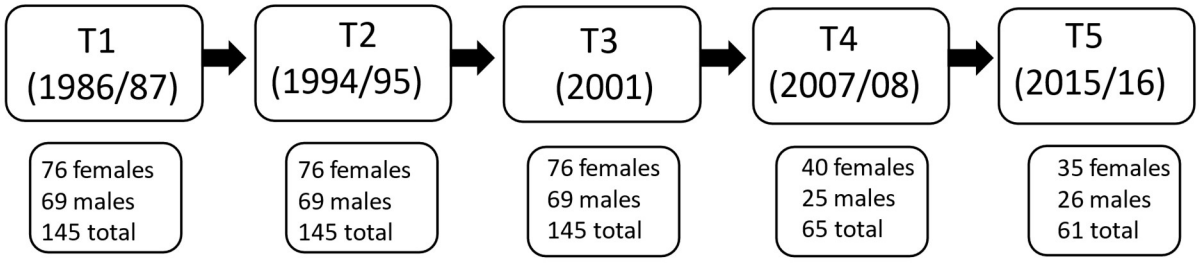

Figure 4: Number of participants (females, males, total) participating at each time point (T1-1986/87; T2-1994/95; T3-2001; T4-2007/08; T5-2015/16).

3.4 Measures of fish consumption

Fish consumption was the main exposure variable in my study. Information regarding fish intake (total/lean/fatty) was generated from the participants responses on the questionnaires (Q1/Q2) administered at each survey. The questions about fish consumption and the corresponding response frequencies varied across the surveys. For example, at T1 the responses to the question about how often you eat lean fish had four response options ranging

less than once per week, once per week, twice per week and three or more times per week. At T2 there were six response options ranging from never to daily and with one option being two to three times per week. This was challenging when classifying the participants according to fish consumption because the threshold/cutoff could fall within the range of one of the answer options in one or several of the surveys.

The participants in this thesis were categorised into "high" or "low" consumers of fish (total/lean/fatty) at each time point. For lean fish, the threshold for being classified as "high consumer" was determined as two or more dinner servings of lean fish per week. For fatty fish, the cutoff was set to one or more dinner servings per week. The reason for the different thresholds for lean and fatty fish is based on traditions in Northern Norway of eating more lean than fatty fish. In cases where participants had missing responses to either the lean or fatty fish intake question, they were categorised as low consumers for the respective fish type they had missing data for. If participants had missing answers on both questions, they were considered as having missing data for that particular time point. At T3, there was no question regarding lean fish consumption and participants could therefore not be categorised based on lean fish consumption at that time point.

Participants were also categorised as high or low consumers of total fish. The variable "total fish consumption" was derived by combining the data on lean and fatty fish consumers at each time point. High consumers of total fish included individuals who were categorised as high consumer of either fatty or lean fish, or both. This means that an individual reporting high consumption of fatty fish and low consumption of lean fish, would be considered a high consumer of total fish. The same applied to an individual reporting high consumption of lean fish and low consumption of fatty fish or an individual reporting high consumption of both lean and fatty fish. Consequently, low consumers of total fish consisted of those who reported low consumption of both lean and fatty fish.

The categorisation of participants into groups based on their consumption of fatty or lean fish was implemented to be able to distinct the contributions of each fish type to the temporal trends and overall exposure to PFAAs. Additionally, incorporating a variable that represented total fish consumption facilitated a more straightforward comparison of my findings with those from major organizations such as EFSA and VKM, as well as with other studies that do not distinguish between fatty and lean fish.

3.5 Measures of PFAAs

The outcome variables in this study were blood concentrations (ng/mL) of six selected PFAAs: PFOS, PFOA, PFHxS, PFNA, PFDA and PFUdA. PFOS, PFOA, PFHxS and PFNA were selected for their relevance in the establishment of the TWI by EFSA, which considers the cumulative exposure to these four PFAAs. Additionally, PFOS, PFOA and PFHxS have been observed to exhibit overall declining concentrations in human populations in the last two decades (34, 41). On the other hand, PDFA, PFUdA and PFNA were included due to indications that their concentrations were increasing in humans in the study period (34, 41). Incorporating three PFAAs that were exhibiting increasing trends in humans, alongside three with decreasing trends, enabled the opportunity to observe how fish exposure may differentially influence concentrations of PFAAs during periods of both increasing and decreasing environmental exposure. The chemical structure of the six PFAAs are shown in the figure below (Figure 5).

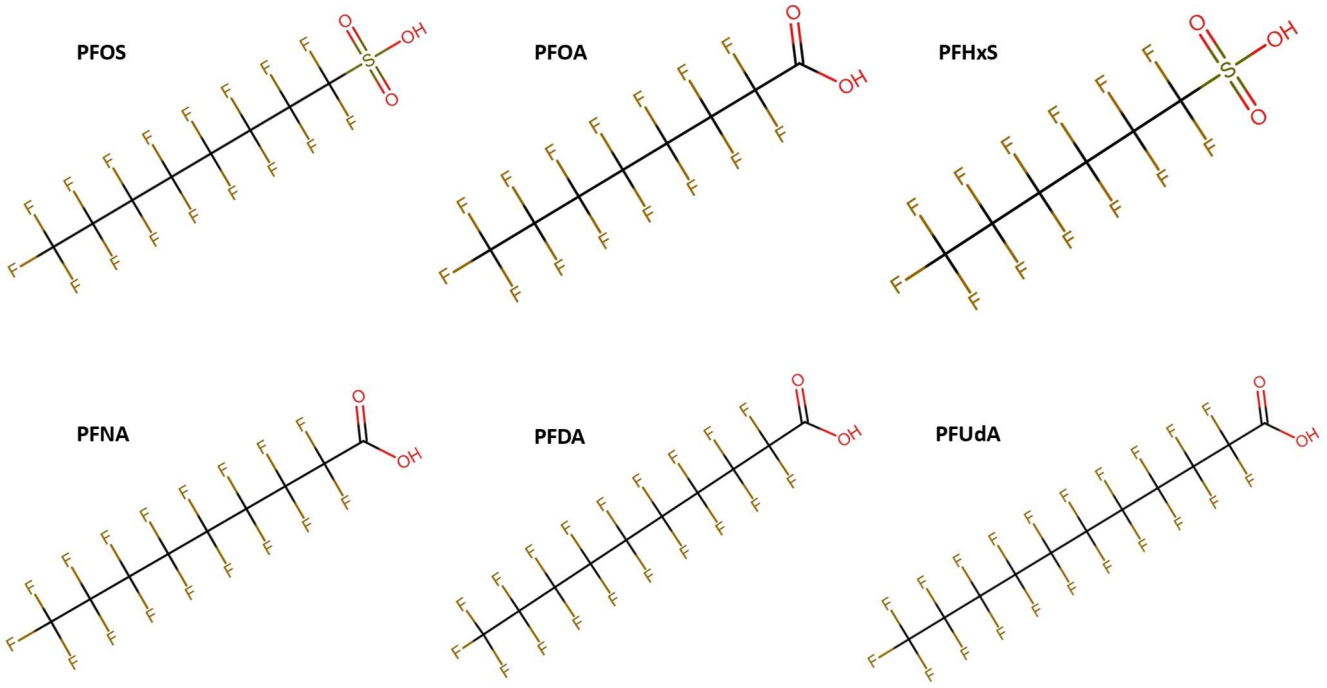

Figure 5: Chemical structure of perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluoro-undecanoic acid (PFUdA). Figures made using a chemical sketch tool (MarvinSketch).

Venous blood samples were collected from participants at the physical examination at each time point. After the samples were taken, they were put on ice for later to be analysed. Data on PFAA concentrations in serum were already obtained in the nested case-control study.

How the PFAAs were analysed in the blood samples have been described in detail elsewhere (81). In short, all sample tests were performed at the University hospital of Northern Norway in the Laboratory Medicine department. As the samples had been stored at freezing temperatures (<−20 °C) they conducted a controlled thawing process including being placed in a fridge at 8 degrees overnight and in room temperature for 30 minutes before further steps. Other preparations were performed by a Freedom evo 200 liquid handler which did automatic sample preparations. Instrumental analyses were performed by using an ultrahigh-pressure liquid chromatography triple quadruple mass spectrometry (UHPL-MS/MS). Masslynx and Targetlynx software were used for quantification. Blank samples and standard reference samples were used to control for background contamination and measure the accuracy of the measurements. The method demonstrated good precision and accuracy overall, with most results showing low variability and only minor deviations from known reference values (<20% for most tests), although sensitivity decreased slightly at lower concentrations (80, 81). All PFAAs mentioned in this thesis were above the limits of detection (LODs) in all individual samples. Linear forms as well as sum of branched and linear forms were quantified for PFOS and PFHxS, but only the sum of branched and linear are used in this thesis.

3.6 Covariates

Data on dietary habits that may be associated with intake of fish and PFAA concentrations, such as meat consumption, dairy consumption, consumption of fruits and vegetables, and egg consumption (32) were extracted from the questionnaires. The participants in the study were categorised into “high” or “low” consumers of each dietary item at each time point. For meat, the threshold for being classified as “high consumer” was determined to three or more dinner servings per week at T1 and T2. At T3, there was no question regarding meat consumption and participants could therefore not be categorised into high and low meat consumers at that time point. At T4 and T5, the range of the response categories in the questionnaire did not make it possible to set the threshold at three dinner-serving per week and it was therefore set to one dinner serving per week (the response alternative in the questionnaire was 1-3 dinner servings per week). The threshold for high consumption of dairy was set to one and a half glass of milk per day. For fruits and vegetables, the threshold for high consumption were set to seven serving per week. There was no data on egg consumption at T1, T3 and T4, but at T2 the threshold for high consumption was set to eating egg one or more times per week and for T5 it was set as eating approximately 20 grams if egg per day.

Body mass index (BMI) was calculated by using data on weight and height collected at the physical examination. Number of births and months of breastfeeding were reported in the self-administered questionnaires.

3.7 Statistical analyses

Statistical analyses were performed in Stata Statistical software version 17, with a predetermined statistical significance threshold set at $p<0.05$. Descriptive statistics, including mean, standard deviation (SD) and the range of minimum and maximum values, were used to describe the study sample characteristics. Confounding factors were identified by creating a Directed Acyclic Graph (DAG) including exposure and outcome variables, and other relevant variables for the study (Supplementary Figure 2).

After classifying all participants into high or low consumers of total, lean, and fatty fish, two sample t-tests were conducted to assess whether there was a significant difference in the mean concentration of the six selected PFAAs in high vs low consumers of fish at each time point. Although PFAA concentrations are typically right skewed with most values being low but some exceptionally high, suggesting the median might better represent central tendency, the differences in PFAA concentrations are normally distributed around the mean. Additionally, given the varying group sizes in this study, the mean may be a more suitable measure for comparing concentrations between groups. Henc, the mean concentrations have been used when comparing concentrations among the groups.

Multivariable linear mixed effect models with random intercept and random slope were used to analyse the time trends of the six selected PFAAs (dependent variables) in low vs high fish consumers (independent variable). In order to meet the model assumption of normality, the PFAA variables were log-transformed. First, a model only including time and fish consumption status, and an interaction term between time and fish consumption, was used to assess if there were significantly different time trends between high and low fish consumers of fish before adjusting for confounding variables. The adjusted models included six PFAAs (dependent variables), fish consumption status, time, and confounders; meat-, dairy-, fruits and vegetables consumption status, age, and sex (independent variables), as well as an interaction term between fish consumption status and time. Fatty fish models were not adjusted for meat status due to missing data on meat consumption at T3 and none of the models were adjusted for eggs due to missing data on egg consumption on T1, T3 and T4. A Wald test was used to assess if the interactions between time and sex were considered

significant, and therefore if the model should also include interactions between time and sex as a confounding variable. A Wald test was used to assess whether the interaction terms between fish groups and time was significant, and thereby if the difference in PFAA time trends were significantly different between the high and low fish consumers.

Because the data used in the mixed-model analysis were log-transformed, it was difficult to assess the actual differences in PFAA concentrations between high and low consumers. Therefore, the predicted values obtained from the linear mixed model analysis were back-transformed by using the exponential function. These values represent the geometric mean concentrations.

3.8 Ethics

In the first, second and third survey of the Tromsø Study, written consent was not required from the participants. It was assumed that participants who met, answered the questionnaires, and agreed to physical examination, also consented to the use of their data. However, starting from the fourth survey written consent has been obtained from all participants (82).

This project used already collected data. Therefore, no additional burden was added on the participants. The research question is relevant, and only a few studies in the world can conduct a similar study since most population surveys do not have repeated measurements from the same individuals. This project was approved by the Regional Committees for Medical and Health Research Ethics (REK, case number: 614867). Consequently, this study represents an approved and research-relevant use of the data.

3.8.1 Privacy and confidentiality

Data was pseudonymized in advance of receiving the dataset and the data file was stored and deleted according to the guidelines on research data at UiT The Arctic University of Norway (83). A Data Protection Impact Assessment (DPIA) for the project was prepared by the Norwegian Agency for Shared Service in Education and Research (SIKT) and UiT (Ref no: 234002).

3.8.2 Conflict of interest

No conflicts of interest.

4 Results

4.1 Sample characteristics

The study sample contained 76 females with a mean (SD) age of 43.9 (8.9) years and 69 males with a mean age of 46.5 (10.5) years at the first time point (T1). The mean (SD) BMI for females at T1 was 23.7 (3.7) kg/m^2, while the mean BMI for males was 24.7 (2.9) kg/m^2. The mean BMI increase through the study period. At the last survey (T5) females had a mean (SD) BMI at 26.8 (5.7) kg/m^2, and males had 28.1 (4.5) kg/m^2.

4.1.1 Dietary intake

At T1, the fish consumption habits for 53% of the participants were according to the Norwegian recommendations about fish consumption, with at least two dinner servings of fish per week. The percentage increased to 60% at T2, 63% at T4 and 72% at T5 (Figure 6).

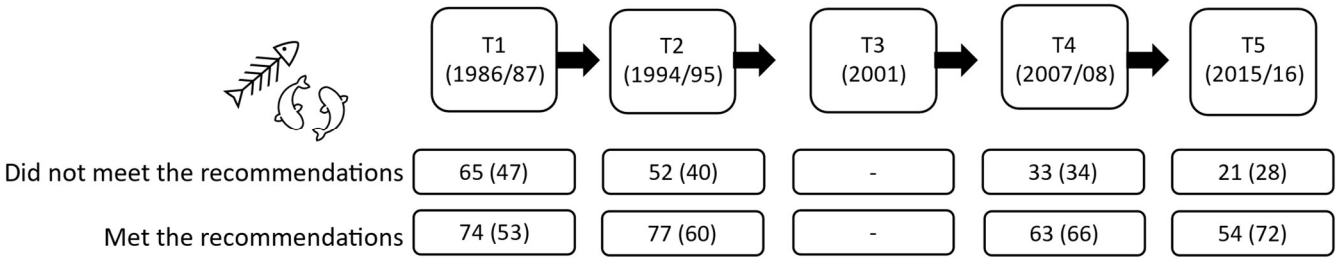

Figure 6: Number of participants (n [%]) that met or did not meet the Norwegian Directorate of Health's recommendations of a minimum of two dinner servings of fish per week. (T1-1986/87; T2-1994/95; T3-2001; T4-2007/08; T5-2015/16). There is no data at T3 due to missing questions regarding lean fish at T3 in the questionnaires.

However, the Norwegian recommendations also specify that out of the recommended weekly intake, 200g should be fatty fish, a target that fewer of the participants achieve. It is challenging to directly convert 200g of fatty fish into number of meals; however, if we assume that a typical fish meal is approximately 150g, then 16% (T1), 41% (T2), 36% (T3), 38% (T4) and 52% (T5) of the participants consumed 150g or more per week. This can be inferred from the number of participants categorised as high fatty fish consumers (Figure 7) since the threshold for high consumers were set to one serving per week.

A high percentage of participants were considered high consumers of lean fish, with at least two servings of lean fish per week (49% [T1], 42% [T2], 77% [T4], 75% [T5]). The numbers of high fatty fish consumers on the other hand were lower with 16% (T1), 41% (T2), 36%

(T3), 38% (T4) and 52% (T5), even though the threshold for high fatty fish consumption were lower than for lean fish. In general, the fish consumption increased from T1 to T5 (Figure 7).

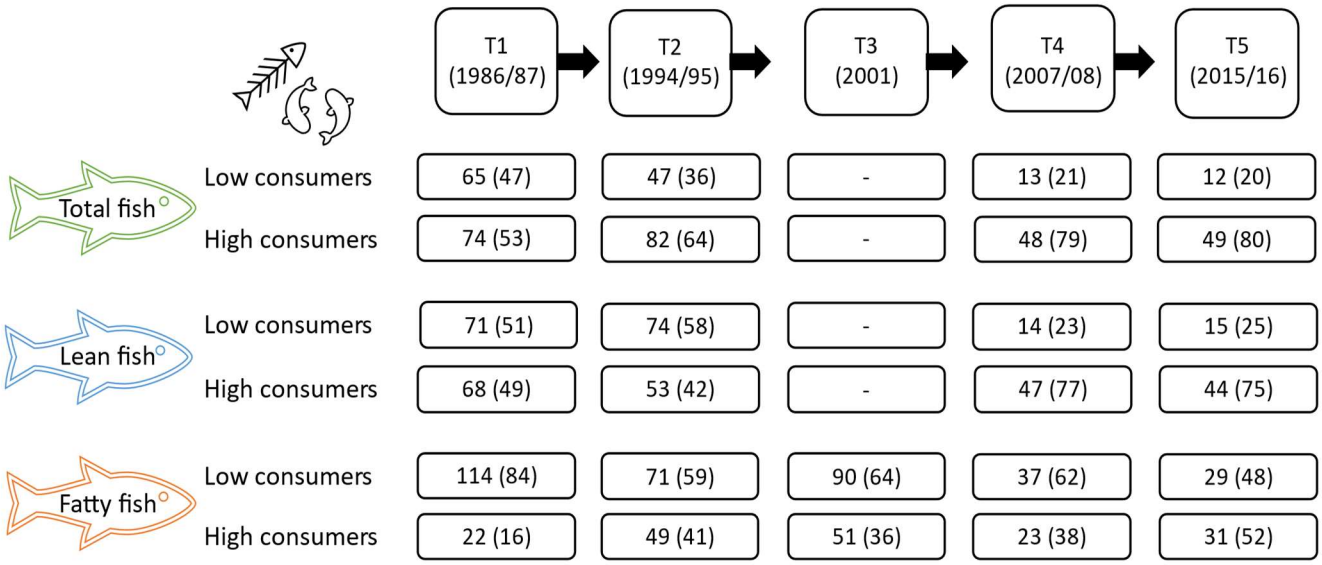

Figure 7*:* Number of participants (n [%]) categorised as high or low consumers of total, lean, and fatty fish at each time point (T1-1986/87; T2-1994/95; T3-2001; T4-2007/08; T5-2015/16). There is no data at T3 on lean and total fish consumption due to missing questions regarding lean fish at T3 in the questionnaires. High intake of lean fish was defined as ≥2 dinner servings per week; high intake of fatty fish≥1 dinner serving per week; high intake of total fish=high lean and/or high fatty fish consumption.

4.1.2 Characteristics of high and low fish consumers

At all timepoints, except at T1 for fatty fish consumers, participants reporting high fish consumption were in general older than those reporting low fish consumption (Table 1, Supplementary table 1-2). BMI was relatively similar between high and low consumers (total/lean/fatty fish) at all time points (Table 1, Supplementary table 1-2). For females, the average number of births fell within the range of 2 to 3 children at each time point for both high and low consumers of total, lean, and fatty fish (Table 1, Supplementary table 1-2).

At the initial time points, T1 and T2, it was a higher percentage of males, compared to females, reporting high total and lean fish consumption, however at T4 and T5 there was a higher percentage of females reporting high consumption compared to males (Table 1, Supplementary table 1, Supplementary figure 1). For fatty fish, low consumption was reported most frequently for both males and females, with the exception of males at T2 and females at T5 where 53% and 57%, respectively, reported high fatty fish consumption (Supplementary table 2, Supplementary figure 1).

Table 1: Participants characteristics at each time point (T1-1986/87; T2-1994/95; T4-2007/08; T5-2015/16) for all participants categorised as high or low consumers of total fish*.

Total fish		T1	T2	T3÷	T4	T5
Females n (%)	Low	37 (50)	28 (42.4)		7 (19.4)	5 (14.3)
	High	37 (50)	38 (57.6)		29 (80.6)	30 (85.7)
Males n (%)	Low	28 (43.1)	19 (30.2)		6 (24.0)	7 (26.9)
	High	37 (56.9)	44 (69.8)		19 (76.0)	19 (73.1)
Total n (%)	Low	65 (46.8)	47 (36.4)		13 (21.3)	12 (19.7)
	High	74 (53.2)	82 (63.6)		48 (76.7)	49 (80.3)
Birth year min/max	Low	1925/1963	1929/1963		1936/1969	1931/1969
	High	1925/1969	1925/1969		1926/1961	1929/1961
Age years mean(min/max)	Low	43.6 (23/61)	51.0 (31/65)		56.6 (38/71)	64.0 (46/84)
	High	47.0 (17/61)	54.6 (25/69)		65.3 (46/81)	71.7 (54/86)
Weight kg mean (SD)	Low	70.9 (13.8)	70.2 (13.1)		75.8 (14.2)	84.3 (12.7)
	High	69.9 (11.4)	75.6 (13.8)		78.8 (17.7)	76.0 (16.2)
BMI kg/m^2 mean (SD)	Low	24.2 (3.60)	24.5 (4.30)		26.0 (3.12)	27.6 (4.72)
	High	24.2 (3.03)	25.9 (3.76)		28.0 (5.38)	27.3 (5.40)
Parity n mean (SD) §	Low	2.14 (1.46)	2.50 (1.32)		2.43 (1.51)	2.40 (1.14)
	High	2.68 (1.51)	2.60 (1.57)		2.72 (1.83)	2.80 (1.42)
Breastfeeding (months) mean (SD) §	Low	-	12.9 (10.4)		16.0 (16.8)	16.0 (9.14)
	High	-	13.3 (11.4)		12.8 (13.5)	18.3 (14.7)

*High lean fish consumption was defined as ≥2 dinner servings per week; high fatty fish consumption ≥1 dinner serving per week; high total fish=high lean and/or high fatty fish consumption.
÷No data on T3 due to missing questions regarding lean fish at T3.
§Only for females that have reported having children.

There were no significant differences in the frequency of reported meat consumption between high and low total fish consumers at T1, T2 and T4 ($p>0.05$). However, at T5 those reporting high fish consumption were more likely to also report high meat consumption, compared to those reporting low fish intake. This was the only time point where the difference in reported meat consumption between high and low fish consumers were significantly different ($p<0.05$). At T1 and T2, the threshold for what was considered high consumption of meat was set to three or more servings per week. At T4 and T5, the threshold was set to one serving of meat per week, due to the questionnaire response option range of one to three servings per week at those time points. This resulted in a higher percentage of participants reporting high

consumption of meat at T4 and T5 compared to T1 and T2 (Figure 8 A). There was no statistical difference in the frequency of reported consumption of dairy, eggs, and fruits and vegetables between high and low total fish consumers at any time point (Figure 8 C, D and E, $p>0.05$), meaning that high and low consumers of total fish in general consumed the same amount of these foods.

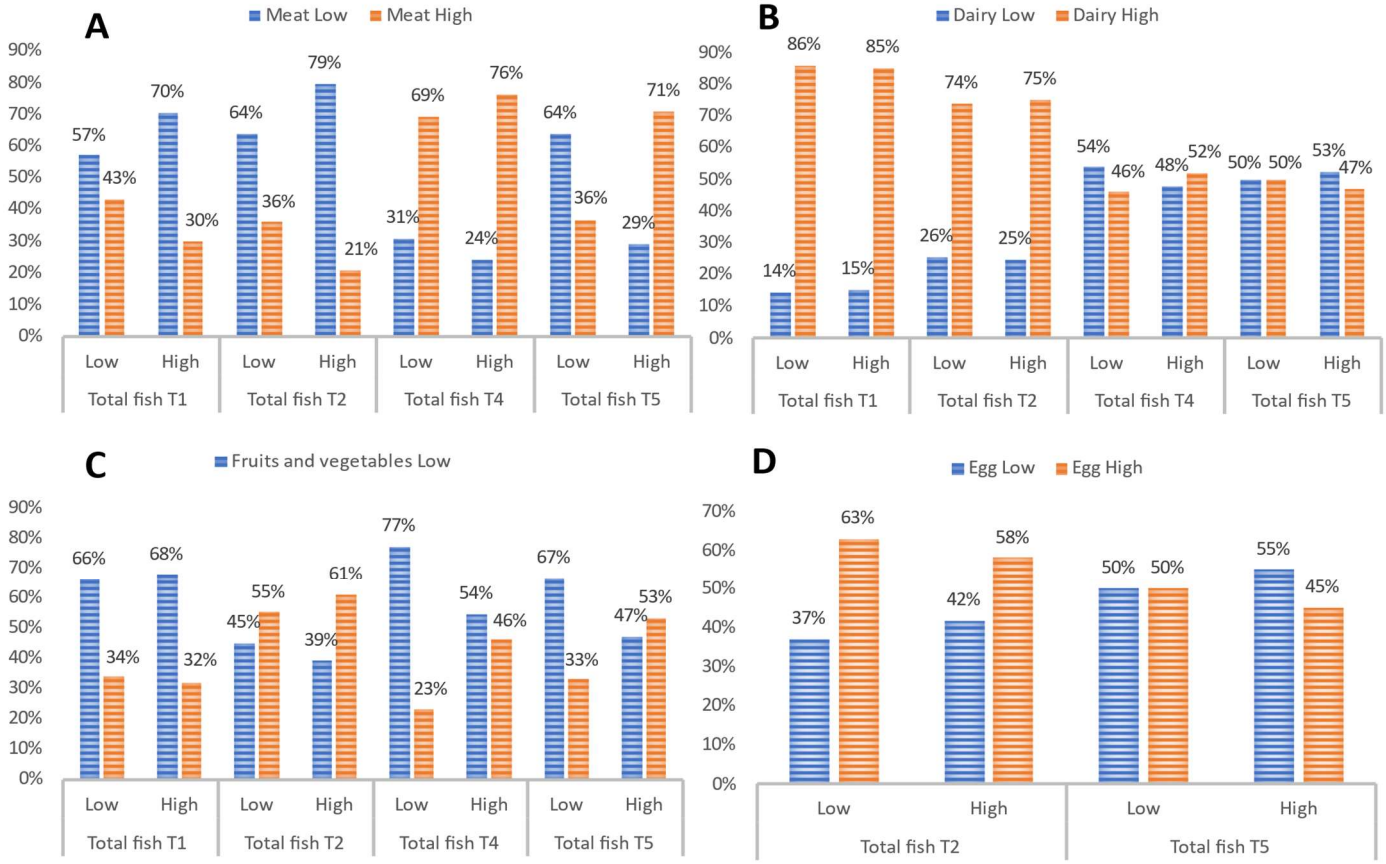

Figure 8: Distribution of high and low meat (A), dairy (B), fruits and vegetables (C), and egg (D) consumers among high and low total fish consumers at four different time points (T1-1986/87; T2-1994/95; T4-2007/08; T5-2015/16). Numbers represent percentages of low or high total fish consumers that is also low or high consumer of the respective dietary component. High intake of meat at T1 and T2 was defined as ≥3 dinner servings per week. At T4 and T5, high intake was defined as ≥1 dinner serving per week. High intake dairy ≥1.5 glasses a day. High intake fruits and vegetables≥7 servings per week. High intake egg≥1 time per week (T2), ≥20g/day (T5)- no information on egg at T1 and T4. High lean fish consumption ≥2 dinner servings per week; high fatty fish consumption≥1 dinner serving per week; high total fish=high lean and/or high fatty fish consumption.

4.2 PFAA concentrations in the study participants

PFOS emerged as the predominant PFAA compound, exhibiting the highest mean serum concentration among the six selected PFAAs among the study participants. The mean (median) concentrations for PFOS ranged from approximately 18 ng/mL (14 ng/mL) to 41 ng/mL (36 ng/mL) at the different time points. Following was PFOA, which demonstrated the next highest mean (median) concentrations, although substantially lower than for PFOS, spanning from around 2.4 ng/mL (2.2 ng/mL) to 4.3 ng/mL (4.1 ng/mL) at the different timepoints. PFHxS displayed mean (median) concentrations within the range of 0.9 ng/mL (0.8 ng/ml) to 2.3 ng/mL (2.0 ng/mL) at the different time points. PFNA, PFDA and PFUdA exhibited mean concentrations below or just above 1 ng/mL throughout the study period.

4.2.1 Sex differences

Across all time points and unadjusted, males exhibited higher mean concentration of PFOS than females. The smallest mean difference among males and females was observed at T4 with 4.34 ng/mL higher concentrations in males ($p>0.05$), and the greatest difference was observed at T3 with 11.5 ng/mL higher mean concentration in males ($p<0.05$) (Figure 9, Supplementary table 6). For PFOA and PFHxS, the mean concentration was higher for males at T1, T2 and T3, however the largest concentration difference among them was at 1.06 ng/mL, observed for PFOA at T3 ($p<0.05$). While men's mean PFHxS concentrations appeared to be decreasing from T3-T4, females' mean concentration continued to increase until T4, resulting in females having higher concentrations at T4 and T5 compared to men, with 0.59 ng/mL higher concentrations in females at T4 ($p>0.05$). This was also observed for PFOA, where males had decreasing concentrations from T2 to T3 while females' concentration continued to increase up until T3. Males had approximately 0.16 ng/mL higher PFNA concentrations at T2 and T3, compared to females ($p<0.05$). At the other time points, the mean concentrations of PFNA were similar between the sexes ($p>0.05$). The mean concentration of PFDA and PFUdA were also relatively similar between males and females; however, males had 0.07 ng/mL ($p<0.05$) and 0.09 ng/mL ($p>0.05$) higher PFDA concentrations, at T2 and T3, respectively, and 0.14 ng/mL and 0.24 ng/mL higher PFUdA concentrations at T2 and T3 ($p<0.05$) (Figure 9).

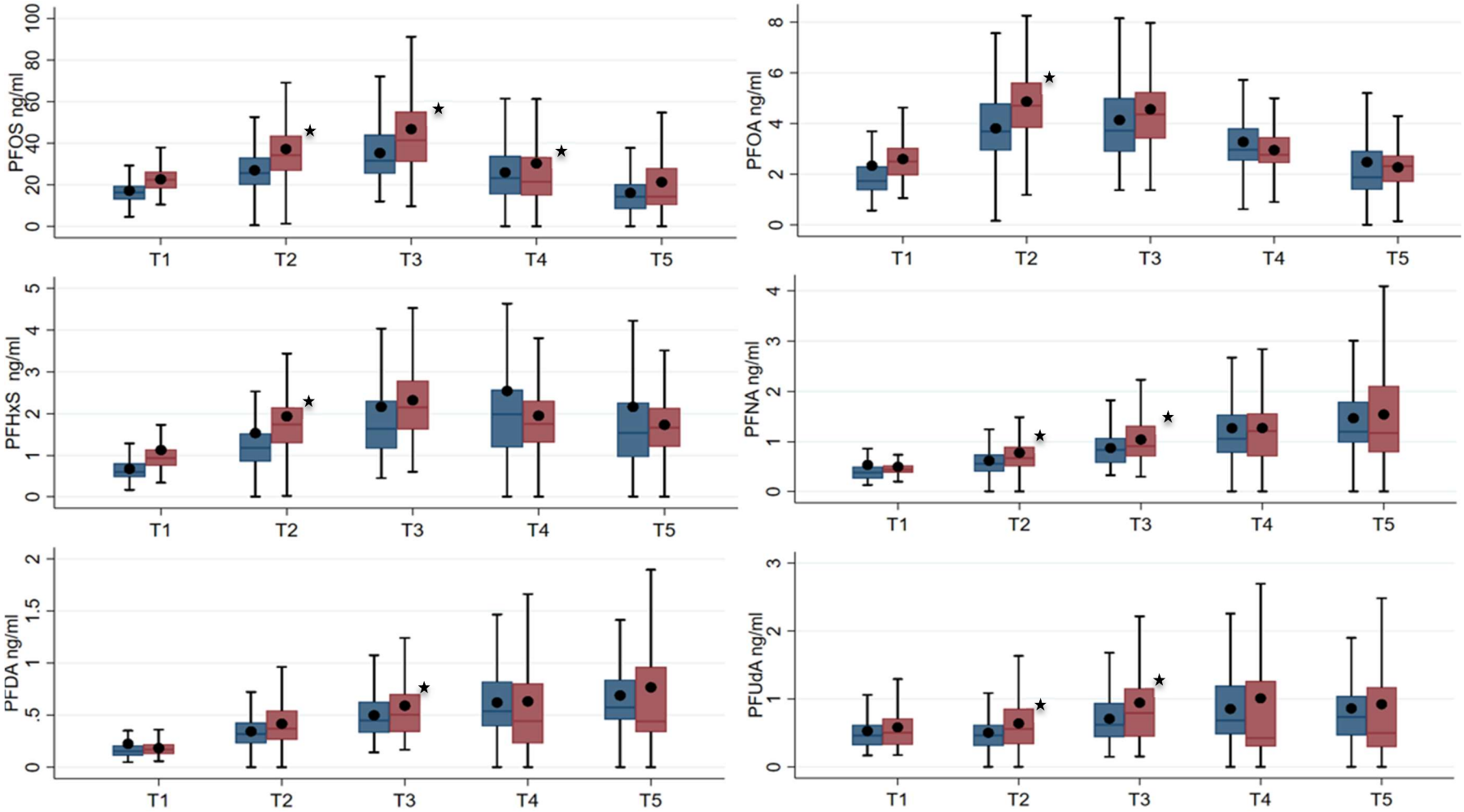

Figure 9: Perfluoroalkyl acid concentrations for participants divided into males (red boxes) and females (blue boxes) at different time points in The Tromsø Study (T1-1986/87; T2-1994/95; T3-2001; T4-2007/08; T5-2015/16). Abbreviations: PFOS: Sum Perfluorooctane sulfonic acid; PFOA: Perfluorooctanoic acid; PFHxS: sum Perfluorohexane sulfonic acid; PFNA: Perfluorononanoic acid; PFDA: Perfluorodecanoic acid; PFUdA: perfluoroundecanoic acid. Boxes represent the 25th–75th percentiles, dot represent the mean, whiskers indicate 1.5 times the length of the interquartile range above and below the 75th and 25th percentiles, star represents significant mean difference between males and females ($p<0.05$).

4.3 Cross-sectional PFAA concentrations in high and low fish consumers

Unadjusted mean PFOS concentrations tended to be higher in high consumers of total and lean fish compared to low consumers across all time points (Figure 10 and 11, Supplementary table 3 and 4). The largest difference between high and low total fish consumers occurred at T4, where high consumers had mean concentrations of PFOS that were 15.6 ng/mL higher than low consumers (16.3 ng/mL vs 31.9 ng/mL, $p<0.05$). Conversely, the smallest difference was observed at T1, with high total fish consumers exhibited PFOS concentrations 2.54 ng/mL higher than low consumers (18.4 ng/mL vs 20.9 ng/mL, $p<0.05$) (Supplementary table 3). For lean fish consumers, the largest and smallest difference in PFOS were observed at T4 and T1, respectively. High consumers of lean fish had a mean PFOS concentration that was 14.7 ng/mL higher than low consumers at T4 (17.2 ng/mL vs 31.9 ng/mL, $p<0.05$) and 2.55 ng/mL higher at T1 (18.5 ng/mL vs 21.1 ng/mL, $p<0.05$) (Supplementary table 4). PFOS concentrations were also found to be higher in high consumers of fatty fish compared to low consumers of fatty fish, although the differences in concentrations were less pronounced than the differences among high and low total and lean fish consumers. The difference in PFOS concentrations among fatty fish consumers was largest at T2 and T3 with high consumers having 10.5 ng/mL (28.2 ng/mL vs 38.7 ng/mL, $p<0.05$) and 9.01 ng/mL (37.4 ng/mL vs 46.5 ng/mL, $p<0.05$) higher concentrations than low consumers, respectively (Figure 12, Supplementary table 5). The time point with the least difference among fatty fish consumers was at T1 with high consumers exhibiting 1.39 ng/mL higher mean concentrations compared to low consumers (19.5 ng/mL vs 20.9 ng/mL, $p>0.05$).

Across all time points, PFOA concentrations were relatively similar between high and low consumers of total, lean, and fatty fish (Figure 10-12, Supplementary table 3-5).
High consumers of total and lean fish had respectively 0.72 ng/mL and 0.81 ng/mL higher concentrations than low consumers at T5 – the timepoints with largest difference in PFOA concentrations (1.81 ng/mL vs 2.53 ng/mL, $p>0.05$, 1.81 ng/mL vs 2.63 ng/mL, $p>0.05$). For fatty fish consumers, the largest difference accrued at T3 where high consumers had 0.66 ng/mL higher concentrations (4.06 ng/mL vs 4.73 ng/mL) compared to low consumers ($p<0.05$).

Overall, the mean PFHxS concentrations were higher in high total and lean fish consumers, but with a non-statistically significant difference at most time points (Figure 10-12, Supplementary table 3-5). The largest difference in PFHxS concentrations between high and low consumers of total, lean, and fatty fish were at T5, T5 and T4, respectively, with high consumers having 0.91 ng/mL, 1.03 ng/mL, and 0.66 ng/mL higher concentrations than low consumers (total: 1.25 ng/mL vs 2.16 ng/mL; lean: 1.22 ng/mL vs 2.24 ng/mL; fatty: 2.16 ng/mL vs 2.82 ng/mL, $p>0.05$). Although the mentioned differences were not considered significant there were significant differences between high and low total and lean fish consumers for PFHxS at T2, but the absolute differences were smaller than those observed at T5 and T4.

High consumers of total, lean, and fatty fish had higher mean concentrations of PFNA compared to low consumers across most time points, except T1 for total and lean fish consumers where the concentrations were similar. The greatest differences in PFNA between high and low consumers of total, lean, and fatty fish were all observed at T5. High consumers of total fish had 0.75 ng/mL higher concentrations (0.90 ng/mL vs 1.65 ng/mL, $p<0.05$), high lean fish consumers had 0.80 ng/mL higher concentrations (0.92 ng/mL vs 1.72 ng/mL, $p<0.05$) and high fatty fish consumer had 0.27 ng/mL higher concentrations (1.38 ng/mL vs 1.65 ng/mL, $p>0.05$), than the low consumers (Figure 10-12, Supplementary table 3-5).

High consumers of total, lean, and fatty fish had higher mean concentrations of PFDA compared to low consumers across most time points. The expectation was at T1 for total, lean, and fatty fish, where the concentrations were similar in high and low consumers. The greatest difference for PFDA among total fish consumers was at T4, with high consumers having 0.39 ng/mL higher concentrations than low consumers (0.34 ng/mL vs 0.73 ng/mL, $p<0.05$). For lean fish consumers the greatest difference was at T5 with high consumers having 0.40 ng/mL higher concentrations than low consumers (0.42 ng/mL vs 0.83 ng/mL, $p<0.05$). For fatty fish consumers the largest difference was at T3 with high consumers exhibiting concentrations 0.13 ng/mL higher than low consumers (0.50 ng/mL vs 0.63 ng/mL, $p<0.05$) (Figure 10-12, Supplementary table 3-5).

Mean PFUdA concentrations tended to be higher in high consumers of total, lean, and fatty fish across all time points. The greatest difference in PFUdA concentrations for total fish consumers was at T4, with high consumers exhibited concentrations that was 0.74 ng/mL

higher than low consumers (0.37 ng/mL vs 1.12 ng/mL, $p<0.05$). The smallest observed difference was at T1, with high consumers exhibiting concentrations 0.19 ng/mL higher than low consumers (0.46 ng/mL vs 0.65 ng/mL, $p<0.05$) (Supplementary table 3). For lean and fatty fish, the largest difference was also observed at T4, with high lean fish consumers having 0.67 ng/mL higher concentrations (0.43 ng/mL vs 1.10 ng/mL, $p<0.05$) and high fatty fish consumers having 0.35 ng/mL higher concentrations (0.83 ng/mL vs 1.18 ng/mL, $p>0.05$), than the low consumers (Figure 10-12, Supplementary table 3-5).

Overall, there were greater differences between high and low consumers of total and lean fish, compared to the difference between high and low consumers of fatty fish. (Figure 10-12, Supplementary table 3-5).

After summarizing the concentrations of the six PFAAs (hereafter referred to as $PFAAs_6$) for each individual at each time point and calculating the average across all participants, I observed that both high and low consumers of total and lean fish exhibited $PFAAs_6$ concentrations exceeding 20 ng/mL at most time points, with the exception of T5. At T5, high consumers of total and lean fish had mean $PFAAs_6$ concentration of 28.1 ng/mL and 29.4 ng/mL, respectively, whereas low consumers of total and lean fish demonstrated an average $PFAAs_6$ concentration of 16.4 ng/mL and 16.3 ng/mL, respectively. For fatty fish, both high and low consumers maintained $PFAAs_6$ concentrations above 24 ng/mL at all time points. The peak concentrations of $PFAAs_6$ occurred at T3 where this study only can separate the participants based on fatty fish consumption due to missing questions regarding lean fish in the questionnaires at T3. At T3, high consumers of fatty fish reached mean $PFAAs_6$ concentrations of 56.3 ng/mL and low consumers of 45.8 ng/mL. PFOS constituted the predominant share of the total $PFAAs_6$ concentrations, contributing with approximately 70-80% to the total at each time point.

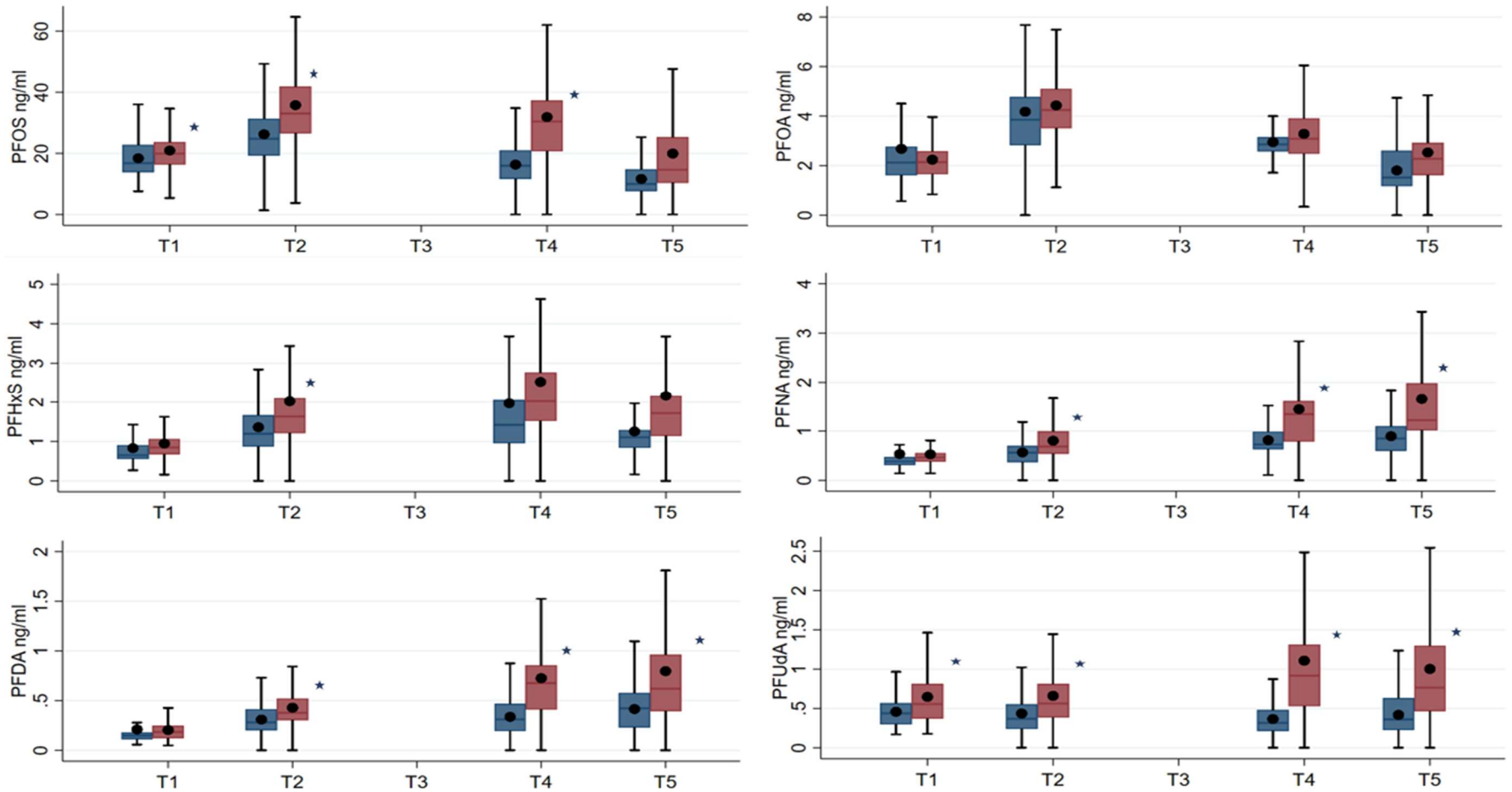

Figure 10: Perfluoroalkyl acid concentrations for all participants categorised as high consumers of total fish (red boxes) and low consumers of total fish (blue boxes) at four different time points (T1-1986/87; T2-1994/95; T4-2007/08; T5-2015/16) in The Tromsø Study. Abbreviation: High lean fish consumption was defined as ≥2 dinner servings per week; high fatty fish consumtion≥1 dinner serving per week; high total fish consumption=high lean and/or high fatty fish consumption. PFOS: Sum Perfluorooctane sulfonic acid; PFOA: Perfluorooctanoic acid; PFHxS: sum Perfluorohexane sulfonic acid; PFNA: Perfluorononanoic acid; PFDA: Perfluorodecanoic acid; PFUdA: perfluoroundecanoic acid. Boxes represent the 25th–75th percentiles, dot represent the mean, whiskers indicate 1.5 times the length of the interquartile range above and below the 75th and 25th percentiles, star represents significant mean difference between high and low consumers ($p<0.05$). There is no data at T3 due to missing questions regarding lean fish at T3 in the questionnaires.

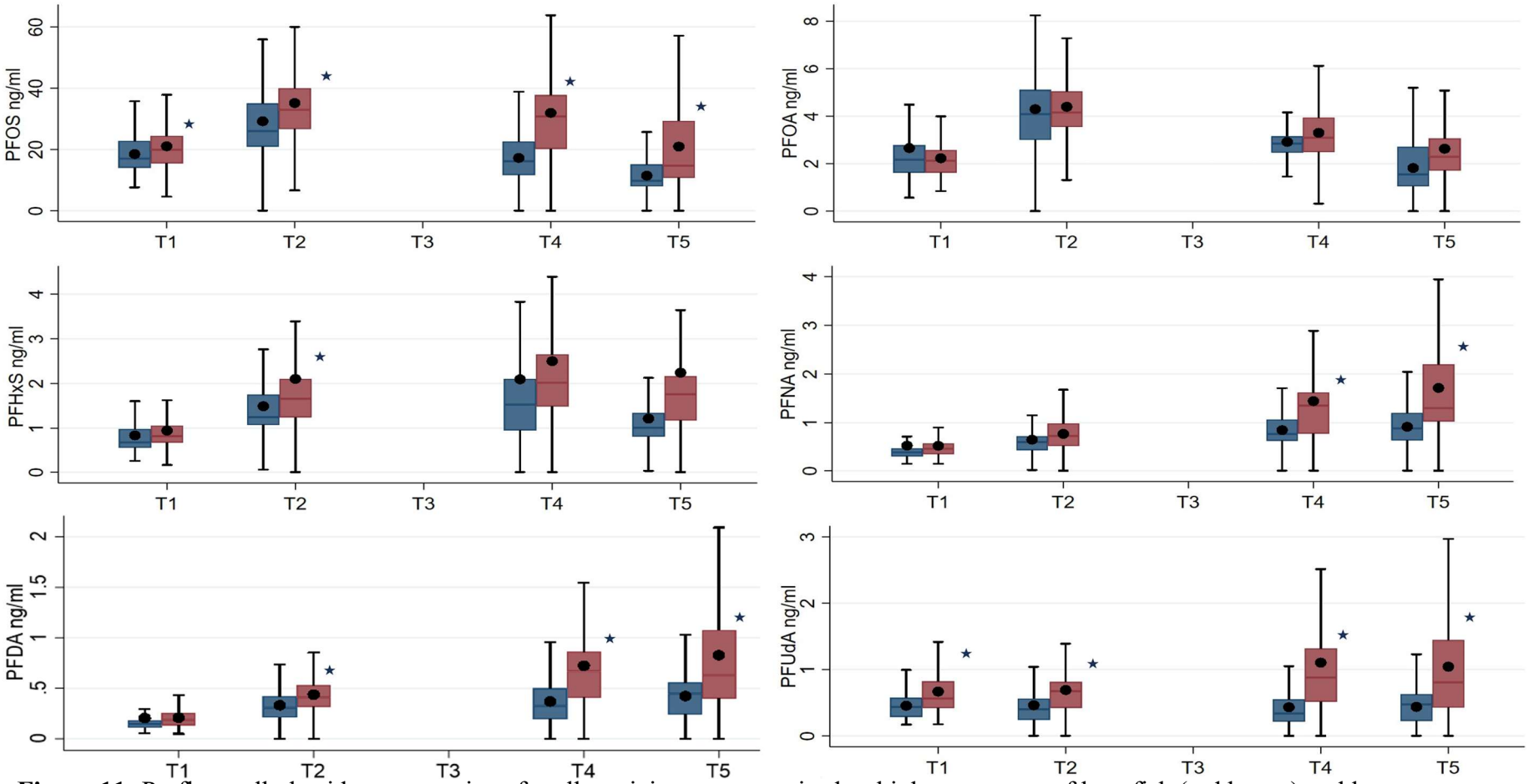

Figure 11: Perfluoroalkyl acid concentrations for all participants categorised as high consumers of lean fish (red boxes) and low consumers of lean fish (blue boxes) at four different time points (T1-1986/87; T2-1994/95; T4-2007/08; T5-2015/16) in The Tromsø Study. Abbreviations: High lean fish consumption was defined as ≥2 dinner servings per week; PFOS: Sum Perfluorooctane sulfonic acid; PFOA: Perfluorooctanoic acid; PFHxS: sum Perfluorohexane sulfonic acid; PFNA: Perfluorononanoic acid; PFDA: Perfluorodecanoic acid; PFUdA: perfluoroundecanoic acid. Boxes represent the 25th–75th percentiles, dot represent the mean, whiskers indicate 1.5 times the length of the interquartile range above and below the 75th and 25th percentiles, star represents significant mean difference between high and low consumers ($p<0.05$). There is no data at T3 due to missing questions regarding lean fish at T3 in the questionnaires.

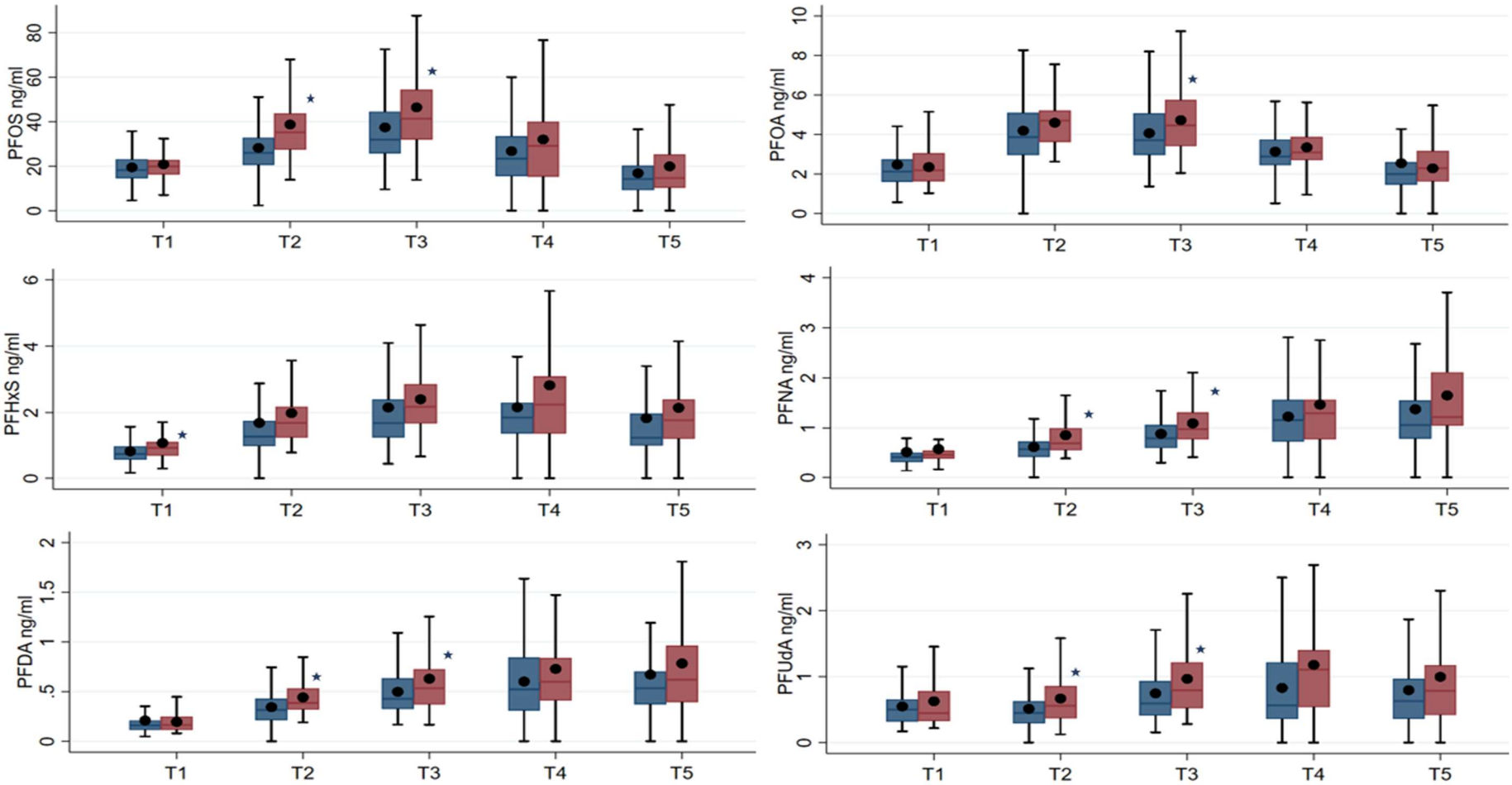

Figure 12: Perfluoroalkyl acid concentrations for all participants categorised as high consumers of fatty fish (red boxes) and low consumers of fatty fish (blue boxes) at five different time points (T1-1986/87; T2-1994/95; T3-2001; T4-2007/08; T5-2015/16) in The Tromsø Study. Abbreviations: High fatty fish consumption was defined as≥1 dinner serving per week; PFOS: Sum Perfluorooctane sulfonic acid; PFOA: Perfluorooctanoic acid; PFHxS: sum Perfluorohexane sulfonic acid; PFNA: Perfluorononanoic acid; PFDA: Perfluorodecanoic acid; PFUdA: perfluoroundecanoic acid. Boxes represent the 25th–75th percentiles, dot represent the mean, whiskers indicate 1.5 times the length of the interquartile range above and below the 75th and 25th percentiles, star represents significant mean difference between high and low consumers ($p<0.05$).

4.4 Longitudinal changes in PFAAs in high vs low fish consumers

For total and lean fish consumers, the time trends of the six included PFAAs appeared similar. High consumers of total and/or lean fish had slightly faster increasing and slower decreasing time trends than those reporting low consumption of total and/or lean fish.

In the unadjusted models, there were significantly different time trends for PFOS and PFUdA between high and low consumers of total fish ($p<0.05$) (Supplementary table 7). For lean fish, the time trends between high and low consumers were significantly different for PFOS, PFNA and PFUdA ($p<0.05$) (Supplementary table 8). After adjusting for confounding variables there were significantly different time trends of PFOS, PFHxS, PFNA and PFUdA between high and low consumers of total fish (Figure 13, Supplementary table 7); high consumers of lean fish had also different time-trends of PFOS, PFNA and PFUdA compared to low consumers ($p<0.05$) (Figure 14, Supplementary table 8). For PFOA and PFDA, the time trends for high and low consumers were not significantly different for any of the fish groups ($p>0.05$).

In both high and low consumers of total and lean fish, the PFOS and PFOA concentrations increased up until T2 (1994), and PFHxS increased up until T4 (2007); thereafter it decreased (Figure 13 and 14). PFNA and PFDA had increasing time trends from T1(1986) to T5(2015), although low consumers of total and lean fish had decreasing time trends of PFNA from T4 to T5. Both high and low consumers of total and lean fish had decreasing/stable time trends of PFUdA from T1 to T2. However, from T2 to T4, low consumers continued to decrease, while high consumers started to demonstrate an increase. Both high and low consumers of total and lean fish exhibited increasing PFUdA concentrations from T4 to T5 (Figure 13 and 14).

None of the studied PFAAs showed significantly different time trends in high vs low consumers of fatty fish, also after adjusting for confounders ($p>0.05$) (Figure 15, Supplementary table). PFOS, PFOA and PFHxS had increasing time trends for both high and low fatty fish consumers from T1 to T2/T3, thereafter it decreased. For PFNA and PFDA the time trends increased from T1 to T5 in both groups. PFUdA had increasing time trends from T2 to T5, also in both groups (Figure 15).

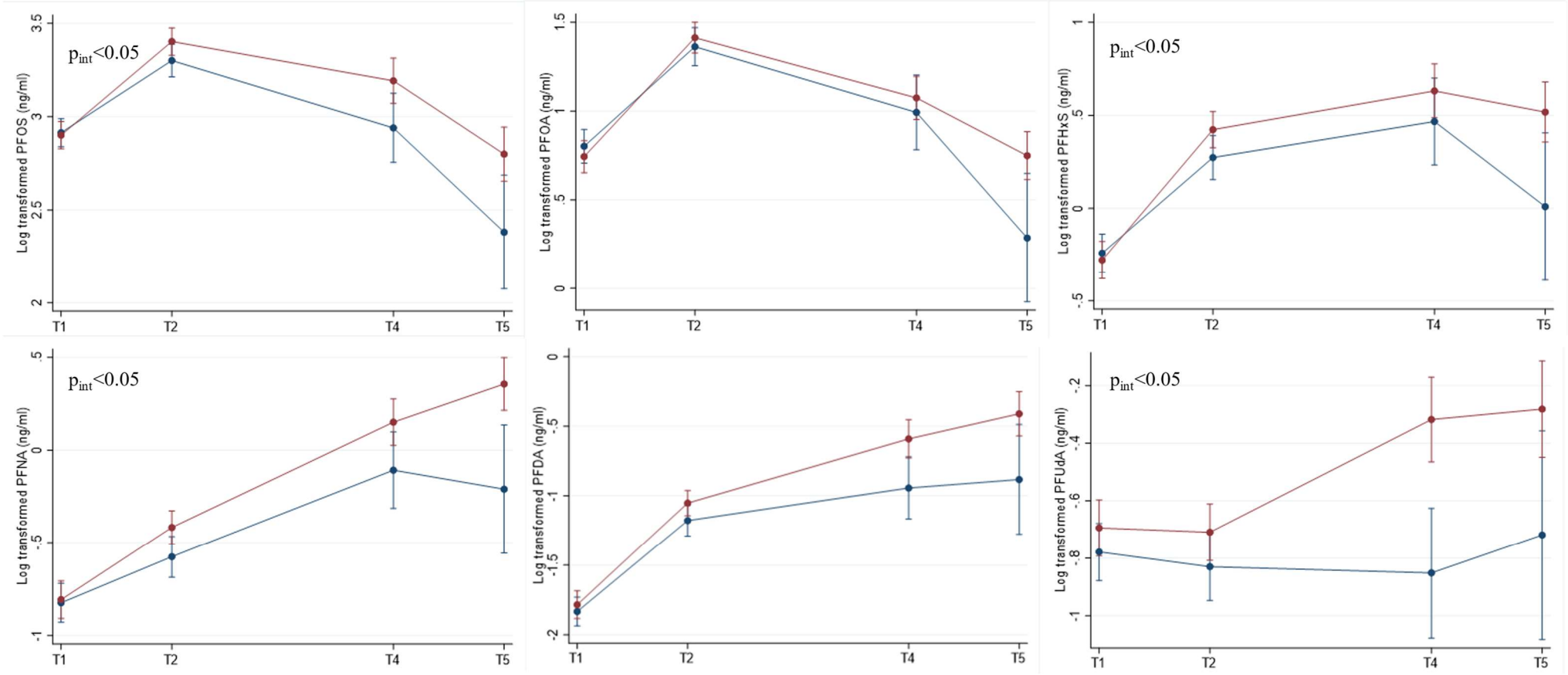

Figure 13: Predicted perfluoroalkyl acid concentrations (log-scale) in high total fish consumers (red line) and low total fish consumers (blue line) after adjusting for confounders at four different time points (T1-1986/87; T2-1994/95; T4-2007/08; T5-2015/16). Abbreviations: High lean fish consumption was defined as ≥2 dinner servings per week; high fatty fish consumtion≥1 dinner serving per week; high total fish consumption=high lean and/or high fatty fish consumption. PFOS: Sum Perfluorooctane sulfonic acid; PFOA: Perfluorooctanoic acid; PFHxS: sum Perfluorohexane sulfonic acid; PFNA: Perfluorononanoic acid; PFDA: Perfluorodecanoic acid; PFUdA: perfluoroundecanoic acid. The models are adjusted for: time(surveys), sex, age, meat-, dairy-, fruits-, and vegetables consumption status, and interactions between time and fish consumption status. PFOA, PFHxS, and PFDA were also adjusted for interactions between time and sex. All time trends were predicted with random intercept and random slope. There is no data at T3 due to missing questions regarding lean fish at T3 in the questionnaires. P_{int} represents significantly different time trends between high and low consumers.

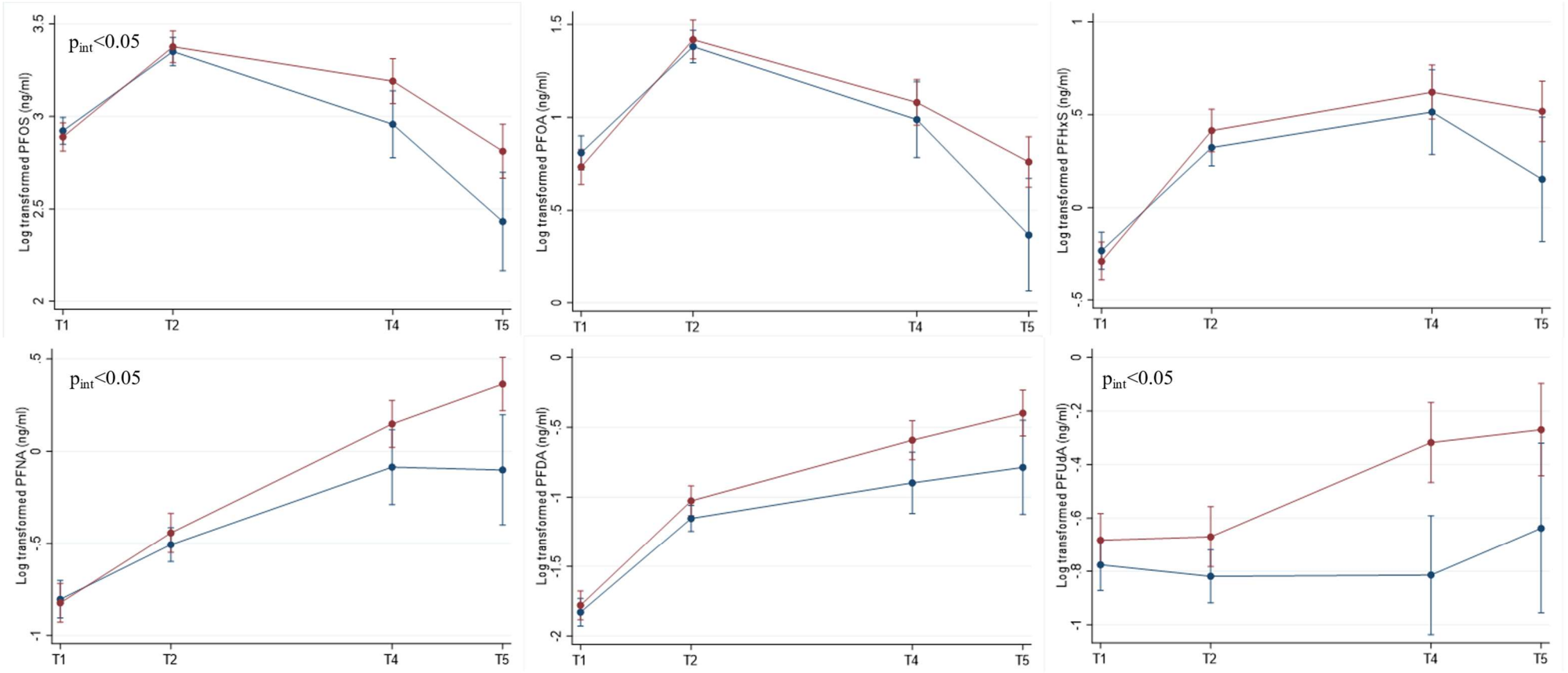

Figure 14: Predicted perfluoroalkyl acid concentrations (log-scale) in high lean fish consumers (red line) and low lean fish consumers (blue line) after adjusting for confounders at four different time points (T1-1986/87; T2-1994/95; T4-2007/08; T5-2015/16). Abbreviations: High lean fish consumption was defined as ≥2 dinner servings per week; PFOS: Sum Perfluorooctane sulfonic acid; PFOA: Perfluorooctanoic acid; PFHxS: sum Perfluorohexane sulfonic acid; PFNA: Perfluorononanoic acid; PFDA: Perfluorodecanoic acid; PFUdA: perfluoroundecanoic acid. The models are adjusted for: time(surveys), sex, age, meat-, dairy-, fruits-, and vegetables consumption status, and interactions between time and fish consumption status. PFOA and PFHxS were also adjusted for interactions between time and sex. All time trends were predicted with random intercept and random slope. There is no data at T3 due to missing questions regarding lean fish at T3 in the questionnaires. P_{int} represents significantly different time trends between high and low consumers.

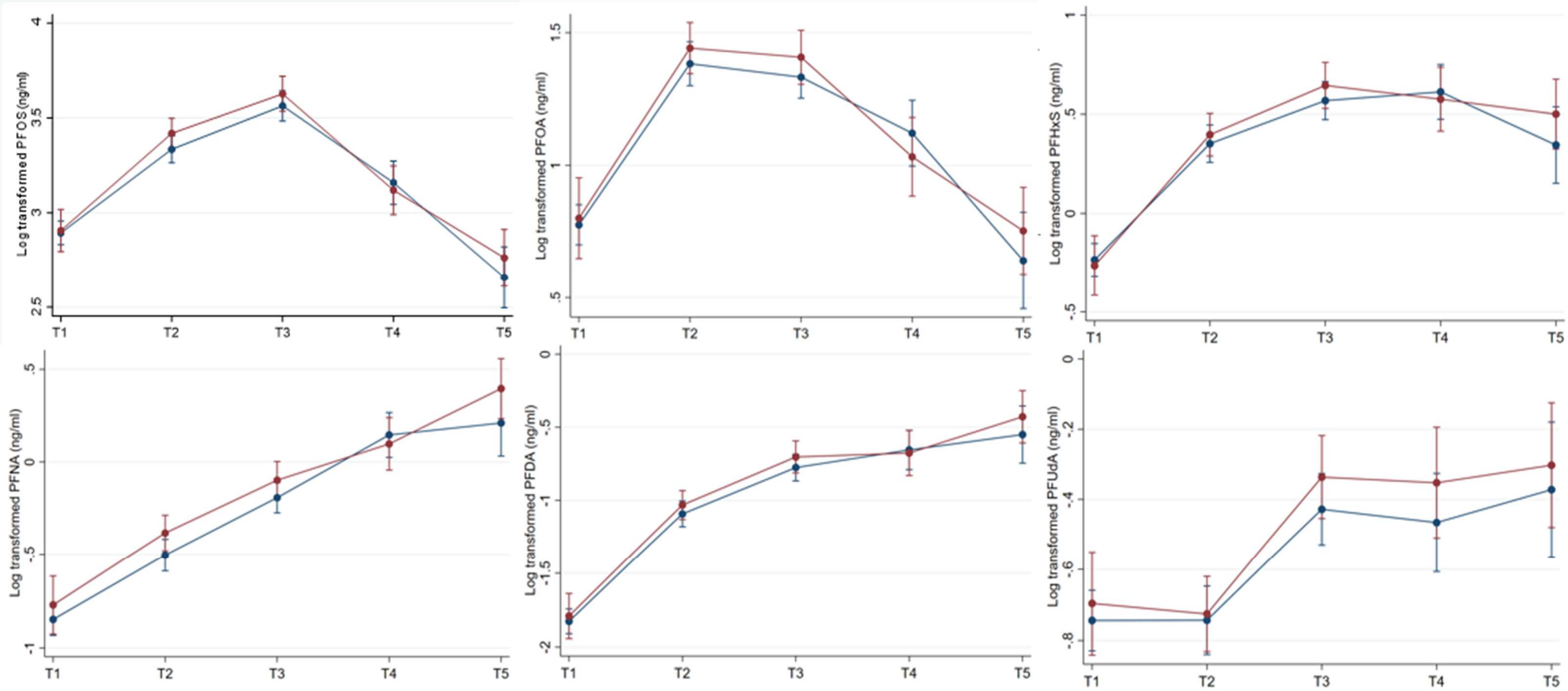

Figure 15: Predicted perfluoroalkyl acid concentrations (log-scale) in high fat fish consumers (red line) and low fatty fish consumers (blue line) after adjusting for confounders at five different time points (T1-1986/87; T2-1994/95; T3-2001; T4-2007/08; T5-2015/16). Abbreviations: High fatty fish consumption was defined as≥1 dinner serving per week; PFOS: Sum Perfluorooctane sulfonic acid; PFOA: Perfluorooctanoic acid; PFHxS: sum Perfluorohexane sulfonic acid; PFNA: Perfluorononanoic acid; PFDA: Perfluorodecanoic acid; PFUdA: perfluoroundecanoic acid. The models are adjusted for: time(surveys), sex, age, dairy-, fruits- and vegetables consumption status, and interactions between time and fish consumption status. PFHxS were also adjusted for interactions between time and sex. All time trends were predicted with random intercept and random slope.

4.5 Back-transformed predicted values and absolute differences

Figures 16-18 illustrate the absolute differences in PFAA concentrations between high and low consumers using the back-transformed predicted values obtained from the adjusted linear mixed model analysis. These values represent the geometric mean concentrations. For total- and lean fish, the differences in PFAA concentrations between high and low consumers increased towards T5. At T1, high consumers of total fish had a predicted PFOS concentration of 18.0 ng/mL, while low consumers had a predicted concentration of 18.3 ng/mL, illustrating similar concentrations in high and low consumers. With increasing time, high consumers experienced faster increasing and slower decreasing concentrations than low consumers, resulting in high consumers having mean predicted concentration of 16.6 ng/mL at T5 and low consumers of 10.9 ng/mL (Figure16). For the remaining five PFAAs, the concentrations were lower, resulting in smaller absolute differences between high and low consumers of total and lean fish, although the percentage difference can be more pronounced. For PFNA, for example, the predicted concentrations at T5 were 1.45 ng/mL in high consumers and 0.82 ng/mL in low consumers, a difference at 0.63 ng/mL. This indicates a 77% higher concentration in high consumers compared to low consumers.

For fatty fish consumers the absolute concentrations were similar in high and low consumers. The biggest difference for PFOS concentrations between high and low fatty fish consumers were at T2 with high consumers exhibiting concentrations of 30.1 ng/mL and low consumers at 28.4 ng/mL, a difference of 1.7 ng/mL.

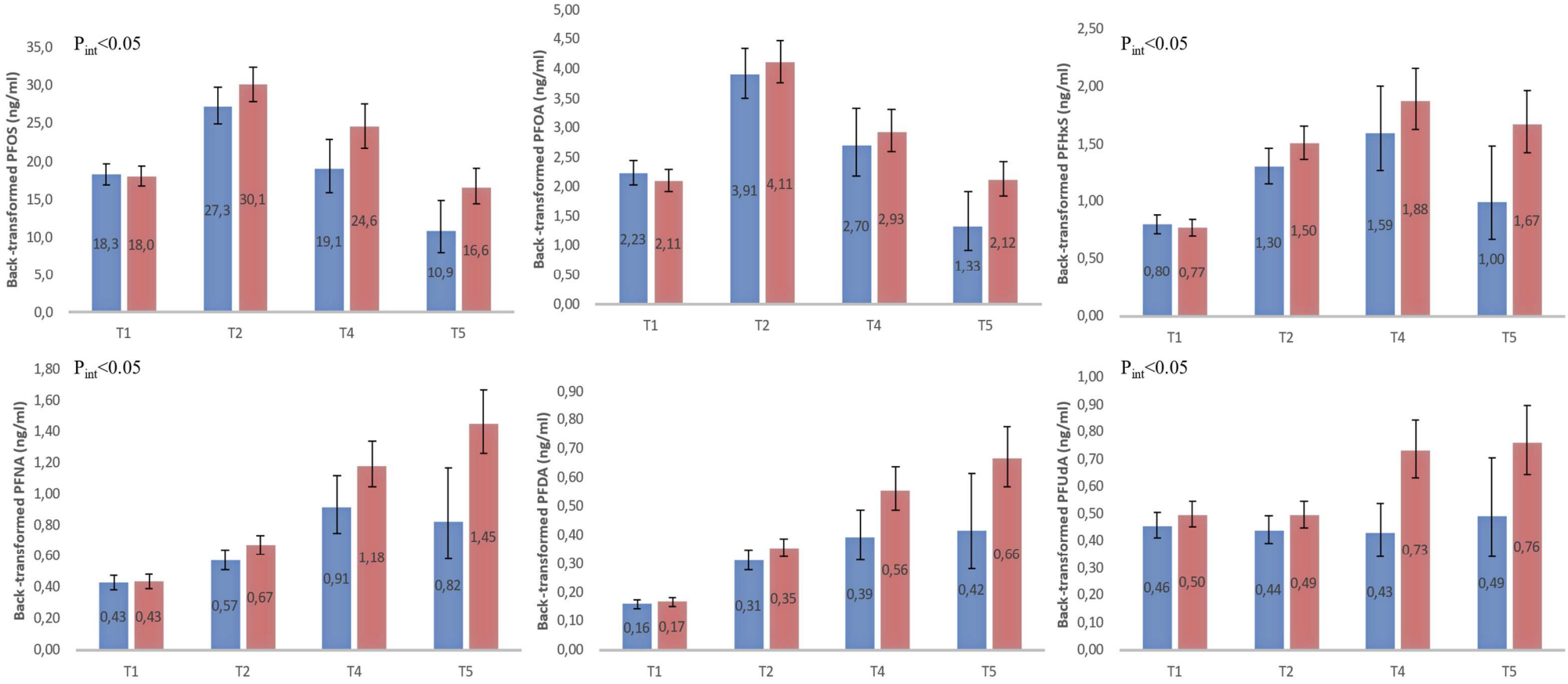

Figure 16: Back-transformed predicted perfluoroalkyl acid concentrations in high total fish consumers (red) and low total fish consumers (blue) adjusting for confounders at four different time points (T1-1986/87; T2-1994/95; T4-2007/08; T5-2015/16). Abbreviations: PFOS: Sum Perfluorooctane sulfonic acid; PFOA: Perfluorooctanoic acid; PFHxS: sum Perfluorohexane sulfonic acid; PFNA: Perfluorononanoic acid; PFDA: Perfluorodecanoic acid; PFUdA: perfluoroundecanoic acid. The models are adjusted for: time(surveys), sex, age, meat-, dairy-, fruits-, and vegetables consumption status, and interactions between time and fish consumption status. PFOA, PFHxS, and PFDA were also adjusted for interactions between time and sex. All time trends were predicted with random intercept and random slope. There is no data on T3 due to missing questions regarding total fish at T3 in the questionnaire. P_{int} represents significantly different time trends between high and low consumers. Whiskers indicates the 95% confidence interval.

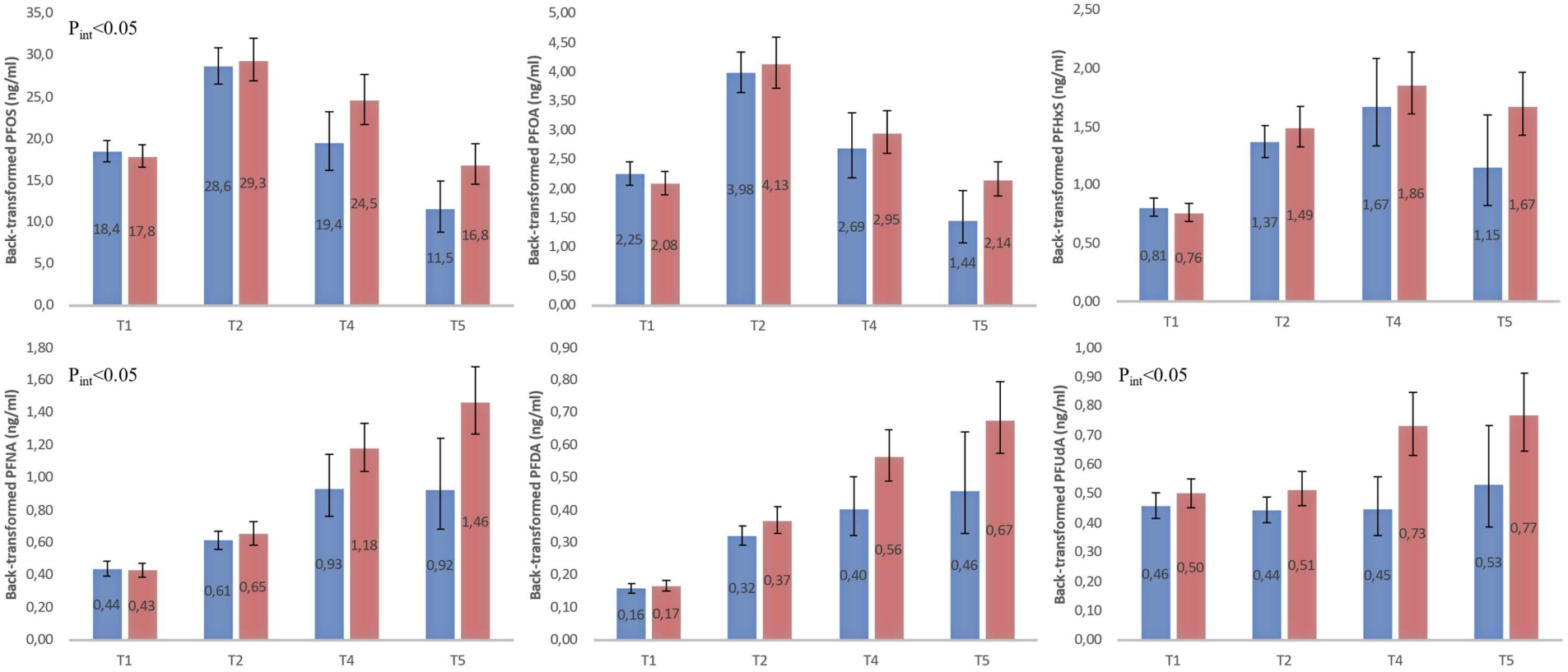

Figure 17: Back-transformed predicted perfluoroalkyl acid concentrations in high lean fish consumers (red) and low lean fish consumers (blue) after adjusting for confounders at four different time points (T1-1986/87; T2-1994/95; T4-2007/08; T5-2015/16). Abbreviations: PFOS: Sum Perfluorooctane sulfonic acid; PFOA: Perfluorooctanoic acid; PFHxS: sum Perfluorohexane sulfonic acid; PFNA: Perfluorononanoic acid; PFDA: Perfluorodecanoic acid; PFUdA: perfluoroundecanoic acid. The models are adjusted for: time(surveys), sex, age, meat-, dairy-, fruits-, and vegetables consumption status, and interactions between time and fish consumption status. PFOA and PFHxS were also adjusted for interactions between time and sex. All time trends were predicted with random intercept and random slope. There is no data at T3 due to missing questions on lean fish at T3 in the questionnaires. P_{int} represents significantly different time trends between high and low consumers. Whiskers indicates the 95% confidence interval.

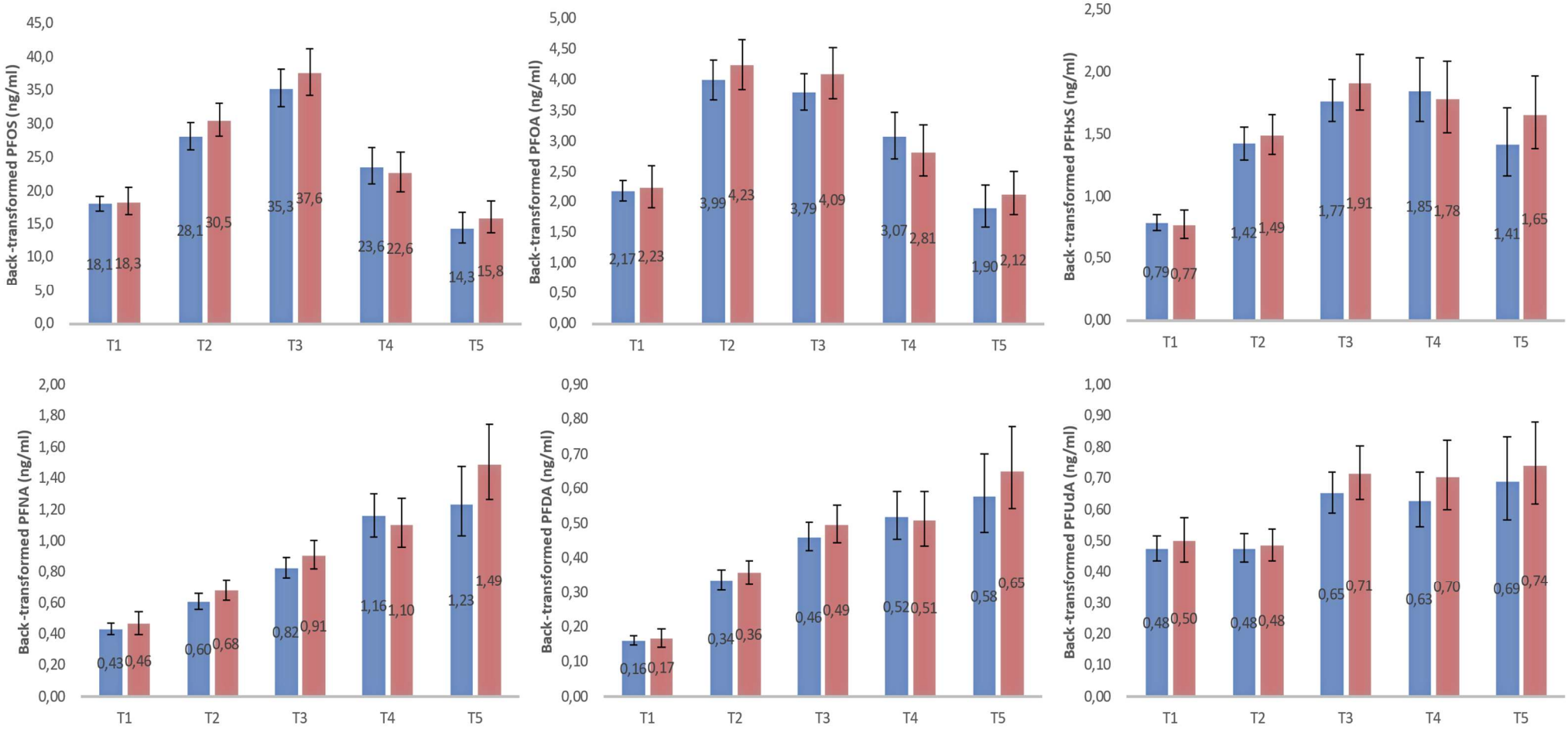

Figure 18: Back-transformed predicted perfluoroalkyl acid concentrations in high fat fish consumers (red) and low fatty fish consumers (blue) after adjusting for confounders at five different time points (T1-1986/87; T2-1994/95; T3-2001; T4-2007/08; T5-2015/16). Abbreviations: PFOS: Sum Perfluorooctane sulfonic acid; PFOA: Perfluorooctanoic acid; PFHxS: sum Perfluorohexane sulfonic acid; PFNA: Perfluorononanoic acid; PFDA: Perfluorodecanoic acid; PFUdA: perfluoroundecanoic acid. The models are adjusted for: time(surveys), sex, age, dairy-, fruits- and vegetables consumption status, and interactions between time and fish consumption status. PFHxS were also adjusted for interactions between time and sex. All time trends were predicted with random intercept and random slope. Whiskers indicates the 95% confidence interval.

5 Discussion

In this thesis, I investigated the potential associations between fish consumption and the time trends of six PFAAs in repeated blood samples from the same individuals participating in the Tromsø Study (1986-2016). I also addressed cross-sectional differences in PFAA concentrations between high and low consumers of total, lean, and fatty fish and assessed if the study participants were following the official dietary guidelines regarding weekly fish consumption. In this section, I will discuss the presented results and the strengths and limitations in the method and material of this thesis.

5.1 Main findings

5.1.1 Fish consumption in the study group

I found that more than 53% of the participants reported to consume two or more dinner serving of fish per week at T1 (1985). The percentage increased though the study period to 66% at T4 (2007/08) and 72% at T5 (2015/16). These findings indicate that the participants consumed a great quantity of fish compared to the average Norwegian, of whom only 33% met the recommended weekly fish intake in 2010/11 (25). Fewer of the participants (T1; 16%, T2; 41%, T3; 36%, T4; 38%, T5; 52%) consumed the recommended 200g of fatty fish per week. The participants who had reported high fish consumption, tended to be older. These observations are consistent with reports that highlight that older individuals and residents of Northern Norway as groups, typically have higher fish consumption than the general Norwegian population (4). The observed increase in fish consumption over the study period may also be explained by the tendency to consume more fish with older age, as the study sample also aged throughout the study period.

My study observed that high and low consumers of fish had similar patterns in consumption of dairy, meat, fruits and vegetables, and eggs. Other research has shown that people who eat more fish, often also eat more fruits and vegetables. This pattern was thus expected as individuals consuming more fish are in general likely more conscious of including healthy foods and maintaining a balanced diet, and therefore include more fruits and vegetables in their diet (7). However, our results don't show higher intake of fruits and vegetables among those who eat a lot of fish. This could be due to where the cutoff for high and low consumption were set, or it could be because fish consumption in Northern Norway is a result of tradition and accessibility, rather than by fish being a healthy food alternative.

5.1.2 Time trends in high vs low fish consumers

I found that participants in the Tromsø Study who reported a high intake of total and lean fish experienced a significantly faster increase and slower decline in the concentration of PFOS, PFHxS, PFNA, and PFUdA for total fish, and PFOS, PFNA, and PFUdA for lean fish compared to low consumers.

As environmental concentrations increased through the study period, all consumers experienced an increase in PFAA concentrations. The increase was greater in high consumers of total and lean fish, who were more exposed to these chemicals due to higher fish consumption. When the environmental concentrations of PFOS and PFHxS were decreasing, and with a time lag also in humans (40), all participants experienced decreasing concentrations. However, the decrease in PFAA concentrations were slower among individuals with high consumption of total and lean fish because they replenished their body's accumulated PFAAs by consuming more fish containing PFAAs. Consequently, it appears that high total/lean fish consumers sustain higher body burden of some PFAAs for an extended duration compared to low consumers, even as environmental concentrations declined.

High consumers of fatty fish did not experience a faster increase or slower decline in PFAA concentration compared to low consumers. Also, the adjusted absolute differences between high and low fatty fish consumers were smaller compared to the differences between high and low total/lean fish consumers. In VKMs report they state that lean and fatty fish contributes approximately equally to PFAA exposure across all age groups, with a little higher contribution from lean fish in adults (16). Given that the participants in this study predominantly consumed lean fish, this might have influenced the results and lean fish could be the primary contributor to PFAA exposure, potentially obscuring the effect that fatty fish consumption may have on PFAA concentrations. At the same time, a study conducted in the Netherlands in 2011 found higher concentrations of PFAAs in fish with lower lipid levels in muscle tissue (lean fish), and lower concentrations in fish with high lipid levels (fatty fish) (84). This could also possibly explain why high fatty fish consumption were not associated with higher adjusted PFAA concentrations and significantly different time trends. In addition to this, a 2019 study investigating PFAA concentrations in both farmed and wild-caught fish, revealed significantly lower PFAA concentrations in farmed fish compared to those caught in the wild (85). The weak association between fatty fish consumption and PFAA concentrations

could therefore potentially be linked to the fact that fatty fish are more likely to be farmed and therefore contain less PFAAs than wild-caught lean fish. Fish farming is predominantly practiced for fatty fish species such as salmon, whereas it is less common for lean fish species like cod (86). However, without specific information from the surveys regarding whether participants consumed farmed or wild-caught fish, we cannot definitively attribute the smaller differences observed among fatty fish consumers to potentially lower concentrations in farmed fatty fish.

5.1.3 Considerations of absolute differences in PFAA concentrations according to fish intake

The faster increase and slower decline in PFAA concentrations among high total/lean fish consumers, compared to low consumers, resulted in increasing absolute differences towards T5. However, was the impact of consuming high amounts of fish greater than the impact of being male or female during this period? At T2 (1994/95) the mean predicted PFOS concentration were 27.3 ng/mL for low total fish consumers and 30.1 ng/mL in high total fish consumers, a difference at 2.8 ng/mL. If we compare this to the unadjusted difference between females and males at the same time point, males had a mean PFOS concentration at 37.2 ng/mL, while females had a mean of 27.0 ng/mL, which is a difference of just above 10 ng/mL. Even though the concentrations for males and females are unadjusted we could observe that the impact of being female or male, is considerably greater than the impact of being high or low total fish consumer, at that time point. Note that females and males exhibited almost the same mean age at that time point. The differences among males and females are less pronounced towards T5, at the same time as the differences between high and low total/lean fish consumers increase. Therefore, males exhibited mean concentrations that were 5.2 ng/mL higher than those of females at T5, which is approximately the same as the difference observed between high and low total/lean fish consumers at that same time point with 5.7 ng/mL higher concentrations in high consumers of total fish. So, even though the PFOS differences between the sexes are decreasing and the differences between high and low fish consumers are increasing over time in the study participants, the impact of being a female or a male is still on par with the impact of being a high or low fish consumer towards the end of this survey.

By T4 (2007/08) the mean predicted PFOS concentration were 19.1 ng/mL for low consumers of total fish and 24.6 ng/mL for high consumers, a difference at 5.5 ng/mL. Over an eight-

year period to T5 (2015/16), the concentrations in low consumers declined to 10.9 ng/mL and in high consumers to 16.6 ng/mL. Hence, in eight years, PFOS concentrations in high consumers declined to concentrations below what low consumers experienced eight years earlier. This indicates that even with slower declining trends, high consumers still experienced substantial declines in PFOS concentrations, even though the absolute differences between high and low consumers increased further from T4 to T5. For PFHxS, the predicted concentrations at T4 (2007/08) for high total fish consumers were 1.88 ng/mL, and for low consumers 1.59 ng/mL, a difference at 0.29 ng/mL. Over eight years, concentrations decreased to 1.67 ng/mL for high consumers and to 1.00 ng/mL for low consumers, a difference at 0.67 ng/mL. For PFHxS, the concentration in high consumers did not decrease below what low consumers showed eight years earlier. However, the differences were at less than 1 ng/mL, and whether such small differences are clinically relevant can be difficult to determine. The same can be said for PFNA, PFDA and PFUdA, where the concentration difference between high and low total/lean fish consumers were large in relative terms, although not in absolute numbers. For instance, at T5, the concentrations of PFNA are 77% higher in high consumers compared to low consumers of total fish. However, the absolute difference in the predicted concentration were only 0.63 ng/mL.

Although PFOS concentrations in high consumers of total/lean fish eventually fell below those of low consumers from eight years earlier, high consumers maintained substantially higher absolute concentrations of PFOS, PFHxS, PFNA and PFUdA than low consumers for over 20 years, spanning from T2 to T5 (1994/95-2015/16). However, it's worth noting that low consumers also experience substantially high concentrations over the same period.

The absolute PFAA differences between high and low fatty fish consumers were minimal at T1, similarly as for total and lean fish consumers. However, since the time trends between high and low consumers of fatty fish did not differ from each other, the absolute differences between high and low fatty fish consumers remained small throughout the study period.

5.1.4 Overall body burden of six PFAAs

As high total/lean fish consumers sustain higher body burden of some PFAAs for an extended duration compared to low consumers, it could possibly have an impact on their health over time. In 2022, the National Academies of Science, Engineering, and Medicine issued a guidance on PFAS exposure, testing, and clinical monitoring (87). They asserted that adverse

health effects linked to PFAS exposure (specifically referencing the sum of N-Methyl-perfluorooctane sulfonamido acetic acid, PFHxS, PFOA, PFDA, PFUdA, PFOS, and PFNA in serum or plasma) are not anticipated at concentrations below 2 ng/mL. Concentrations ranging from 2 to 20 ng/mL are estimated to pose a potential risk of adverse effects, particularly in sensitive populations. Beyond 20 ng/mL, there is an estimated increased risk of adverse health effects. The guidance underscores the importance of clinicians consistently using the sum of PFAS when considering health risk, instead of looking at single PFAS concentrations (87). Therefore, if we examine the sum of the six PFAAs (PFOS, PFOA, PFHxS, PFNA, PFDA, and PFUdA) to determine if the participants were at risk of negative health effects, both high and low consumers of total, lean, and fatty fish had unadjusted mean $PFAAs_6$ concentration above 20 ng/mL at all time points. With the exceptions of low consumers of total and lean fish at T5 with $PFAAs_6$ concentrations of 16.4 ng/mL and 16.3 ng/mL, respectably. This means that, according to National Academies of Science, Engineering, and Medicine, regardless of if you were a high or a low consumer, between T1 and T4 (1986-2008), you were still in the category of increased risk of adverse health effect if you experienced $PFAAs_6$ concentration above 20 ng/mL. At T5 (2015/16) on the other hand, low consumers of total and lean fish were in the group of potential risk, although in the upper range of the category. Even though both high and low consumers fell into the same risk category (>20 ng/mL) between T1 and T4, the elevated concentrations observed in high consumers compared to low consumers could still potentially increase disease risk. Given the absence of clear thresholds at higher concentrations then 20 ng/mL (87), it remains challenging to ascertain whether the observed differences between high and low consumers over time hold clinical significance.

The concentration differences in PFOS between high and low consumers were greater and represented a more substantial portion of the difference in $PFAAs_6$ between high and low consumers than for the other five PFAAs. At T5, high total fish consumers had a mean $PFAAs_6$ concentration of 28.1 ng/mL and low consumers had 16.4 ng/mL, a difference at 11.7 ng/mL. When considering this difference, the contribution of the 0.63 ng/mL difference that was observed between high and low total fish consumers for PFNA, is quite minimal. The same can be said about the concentration difference among high and low consumers for the other PFAAs with concentrations just above or below one. Given the expected continued decline in PFOS concentrations over the years since the last survey (T5), it is anticipated that both high and low fish consumers will experience further reductions in PFOS concentrations,

and consequently, in $PFAA_6$ concentrations. Simultaneously, concentrations of other emerging PFAAs are likely to increase further. This trend suggests that these emerging PFAAs may represent an increasing proportion of the $PFAA_6$ concentrations/body burden in future assessments. Also, if the current trend continues, with high total and lean fish consumers experiencing a faster increase in concentration of emerging PFAAs compared to low consumers, we can anticipate that the differences between high and low consumers will become even more pronounced with time. Without regulations, newer PFAAs could follow a similar shape in time, as we observed for PFOS in this thesis, reaching high concentrations in humans and potentially even higher concentrations in high fish consumers. This underscores the importance of regulating emerging PFAAs to prevent them from reaching high concentrations in humans and wildlife over time. To be able to continue advocation for the health benefits of fish consumption, we must limit the use of PFAAs in industry to ensure that fish PFAA concentrations do not escalate to a point where we can no longer recommend fish consumption.

5.1.5 Sex differences

Males generally had higher mean concentrations of PFOS than females at all time points, although the difference decreased towards T4 and T5. For males the mean concentration of PFOA and PFHxS were higher at T1, T2 and T3, compared to females. Thereafter, the time trends appeared to be different between males and females for PFOA and PFHxS, when only assessing mean concentration at the different time points. Females experienced increasing mean concentrations from T3 to T4 for PFHxS and from T2 to T3 for PFOA, while males had decreasing mean concentrations, resulting in females having higher mean concentrations in the last surveys compared to males. For PFNA, PFDA and PFUdA there was relatively similar mean concentrations between males and females, only with a few time points where males had higher concentrations. At T3, the mean age of the females in the study was 58.9 years old. As most females reach menopause by the age of 52 (73), we can assume that they no longer excrete PFAAs trough childbearing/pregnancy, lactation, or menstruation. This might be why the concentration differences among males and females are reduced towards T5.

5.1.6 Overlap in time trends and concentrations with other studies

Among the study participants, I generally observed a peak in PFOA and PFOS concentrations around T2 (1994) and T3 (2001), respectively. These findings are in line with other research

from Norway and other European studies (41, 68, 69, 88-91). My results also demonstrated a rise from T1 (1986) through T5 (2015) in PFNA concentrations, while PFDA and PFUdA stabilized more after T3 (2001) in low consumers of fish. Haug et al., also reported a clear increase in PFNA, PFDA and PFUdA from 1976 to the early 1990s but followed by a stabilization in the concentrations of these compounds, including PFNA, until the study ended in 2007 (68). Norèn et al. investigated PFAA concentrations in Swedish adolescents between 2000 and 2017 and observed that PFDA and PFNA increased to 2009 and decreased thereafter (88), which was not observed in my study in high consumers of total/lean fish. However, for low consumers of total/lean fish, I observe a small decrease for PFNA after T4 (2007/8). PFHxS had increasing time trends towards T3 (2001) when investigating fatty fish consumers which had data on T3, and towards T4 (2007/08) for total fish and lean fish consumers which had no data at T3. The increase up until around the year 2000 and the decrease after 2000 has also been observed in other European countries (68, 69, 88, 89, 91). The decrease in PFHxS was more pronounced in low consumers of total and lean fish compared to high consumers. However, the decrease in general was not as great as for PFOS and PFOA, which was also illustrated by Nøst et al. (41).

In my study sample, PFOS, PFOA and PFHxS were the PFAAs substances with the highest mean concentrations, and with PFOS being the absolute major contributor. These findings are in agreement with findings from other European studies (68, 69, 88-91). When examining the data cross-sectionally, it was observed that individuals with high fish consumption generally had elevated concentrations of PFOS, PFNA, PFDA and PFUdA compared to those with low fish consumption. This is in line with results from previous studies that have investigated the link between fish consumption and PFAA concentrations (70, 72, 92).

PFOA, was not associated with fish consumption in the same way as the other PFAAs when investigating the cross-sectional differences and did also not show significantly different time trends between high and low fish consumers. This finding is in line with a separate study conducted in Norway, which explored the relationship between dietary patterns and perfluorinated compounds, which found no significant association between dietary habits, including fish consumption, and increased PFOA concentrations (71). Later, Berg et al. identified associations between higher intake of salty snacks and beef with elevated PFOA concentrations, yet they did not observe a similar association with fish consumption (40). This might explain why the time trends of PFOA was not significantly different between high and low fish consumers, as fish does not seem to contribute to increased PFOA concentrations.

5.1.7 Should we reduce fish consumption based on EFSA's TWI?

Because both high and low fish consumers experienced high PFAA concentrations, it is challenging to conclude if the differences in PFAA concentrations between high and low fish consumers over time were large enough to make a difference in terms of risk of developing diseases such as cancer. However, EFSA based their TWI on trying to prevent mothers to reach a body burden that would lead to serum concentrations in the infant associated with decrease in vaccination response (77). As vaccine response are the outcome that is observed at lowest PFAA concentrations, and the vaccine response is not something that needs time to develop, like cancer, the differences in high and low fish consumers concentrations and time trends might be more relevant. Given that the results in my study are representative for younger women who eat fish, we should consider the implications for pre-pregnancy planning. To achieve a body burden below what is considered safe for a child, a woman might need to reduce her total and lean fish consumption many years before planning to conceive. This reduction could help ensure a faster decline in her PFAA concentrations and/or maintain low concentrations towards pregnancy. However, this might lead to other health concerns as females of childbearing age represent the segment of the population with the highest need for iodine. Lean fish is the main source of iodine in the Norwegian diet (93), together with milk products, therefore recommending decreasing lean fish consumption in this period would demand more comprehensive recommendations and information on other sources for iodine. With that being said, the intake of iodine is already low in fertile woman, because of low lean fish consumption, and more information on how to increase iodine consumption should be provided (94). However, EFSA projected that in order for the child to be breastfed for 12 months without reaching unacceptable concentrations, the mother's concentration for the sum of the four PFAAs (PFOS, PFOA, PFNA, and PFHxS) should not exceed 6.9 ng/mL. Notably, both high and low fish consumers surpassed these recommendations. Given that these results are representative for fertile women, it indicates that even with a reduction in fish consumption, PFAA concentrations would still surpass the recommended thresholds in the period examined.

As EFSA's TWI guidelines are primarily designed to prevent females from reaching a body burden that could potentially lead to reduced vaccine response in infants, it raises questions about the applicability of these limitations for females not planning to have children, females above childbearing age, and males. Should individuals in these categories also adhere to the TWI restrictions? Considering that males and older individuals are more prone to developing

CVD and CHD (95), and fish consumption has been reported associated with a reduction in risks of CHD and mortality from CVD (16), it's plausible that males and older populations might derive greater benefits from higher fish intake. Moreover, there's stronger evidence supporting the health benefits of fatty fish consumption compared to lean fish consumption (23). Consuming higher amounts of fatty fish could potentially yield positive outcomes in reducing disease risk without presenting the same drawbacks associated with elevated PFAA concentrations, given that fatty fish didn't exhibit the same differences between high and low consumers in terms of adjusted PFAA concentrations and time trends. It's worth noting that the Norwegian health recommendations for fish consumption already advocate making 200g to be fatty fish out of the recommended 300-450g, recognizing the potential health advantages associated with this specific type of fish.

5.1.7.1 What if we stop eating fish?

Would the intake of PFAAs be reduced to zero if we stopped consuming fish? The answer is no. As PFAAs are ubiquitous in the environment, regardless of the alternative dietary choices we might make instead of fish, it is likely that we would still ingest PFAAs from those food sources (77). People that are omnivores, often choose between meat, chicken, or fish. Since meat also contains PFAAs and red meat consumption is associated with certain negative health outcomes (96), the choice to eat fish, even though it can contain some more PFAAs than meat, might still be considered a better choice considering negative health effects from red meat compared to the positive health effect of fish. Meaning, if you stop consuming fish, in order to reduce your exposure to PFAAs, you will still have considerable amounts of PFAAs in your blood, and at the same time you will reduce the beneficial health effects of risk reduction for stroke, Alzheimer’s, dementia and heart disease that higher fish consumption provides. Consequently, the solution isn't to exclude specific food items to reduce intake of PFAAs, but to maintain a diverse diet encompassing a range of food groups, including meat, chicken, fish, vegetables, fruits, corn, and legumes to guarantees the consumption of essential nutrients required by the body. And most importantly, rather than focusing on restricting foods out of fear of PFAAs, the focus should instead be on limiting PFAAs emission.

5.2 Strengths, limitations, and methodological considerations

In my study I categorised the participants into total, lean, and fatty fish consumers, and reported findings from all categories. This is quite unusual but has been made possible by the information available to me. This approach ensures that my results are relevant and comparable across different scopes of research within the field, and it makes it possible to distinct the contributions of lean and fatty fish to the temporal trends and overall exposure to PFAAs.

5.2.1 Sample size

The sample size in this thesis is limited, driven by the expense of laboratory analyses of PFAAs in blood and the availability of blood samples. However, Therese Nøst conducted a study on time trends of three different PFAAs during the same period covered in this thesis. She found that analysing samples from just 5 individuals for PFOS, 7 for PFOA and 3 PFUdA would be enough to detect any changes in the three PFAAs over 10%, with 80% power and significance level of 5% (97). Therefore, the sample size of 145 individuals included and with no categories with under 12 participants, the numbers are considered sufficient to identify potential changes in time trends.

Due to the limited sample size, the participants are solely categorised into two consumer groups: high and low consumers. This is done to ensure sufficient number of individuals within each category at each time point. Therefore, a sensitivity analysis within subgroups was not performed. Dividing the sample into three or four categories would possibly have resulted in insufficient number of participants in each group, thereby compromising the statistical power and reliability of the findings.

5.2.2 Dietary assessments

This thesis assessed fish consumption through self-reported questionnaires, posing a potential challenge as participants tend to report their dietary habits in a way that they believe is socially accepted (98). This bias may result in overreporting of healthy food choices, such as fish, and underreporting of less healthy food options, potentially leading to misclassification. However, B.E. Birgisdottir et al. suggested that the use of food frequency questionnaire (FFQ) can effectively identify individuals with both high and low fish consumption, with minimal risk of gross misclassifications (99). In the mentioned study however, the participants were between 20 and 40 years old, living in Spain, Irland and Island, and the mean reported frequency of fish consumption was 1.6 times per week according to the FFQ.

Therefore, the observed trends may not be directly applicable to my study sample, as my study sample was characterised by a higher average age and a generally higher baseline fish consumption.

Another challenge comes with the fact that there are discrepancies among the questionnaires used in the different surveys in terms of number of questions related to fish, as well as the frequencies in the answer options. This might have resulted in misclassification if participants over- or underreported their fish consumption differently in the different surveys due to varying questionnaire structure. This also made the process of dividing participants into high and low consumers challenging. At T1, the frequency options ranged from less than once per week to four or more times per week. By T2, the options expanded to include 'never' and specific frequencies up to 'every day.' At T4 and T5, the options included a range of 1-3 dinner servings per week. Consequently, to maintain a consistent definition of high consumption across all time points while ensuring sufficient sample sizes, the cutoff did in some cases fall within the range of one of the answer options in one or several of the surveys. For instance, the cutoff at two servings per week for lean fish consumers might include those who only consumed one serving per week at T4 and T5, potentially misclassifying them as high consumers. This also means that the cutoff for fatty fish at one serving per week, and lean fish at two servings per week were within the same response option at T4 and T5. So even though there were different cutoffs for lean and fatty fish, they were the same at T4 and T5. At T3 the questionnaire did not ask about lean fish, which made the possibility to examine data from T3 and association with lean and total fish, impossible. So even if the questionnaires have expanded over the years, when assessing time trends using data from several surveys, the discrepancies among the questionnaire might introduce measurement errors and can limits the way to process the data.

The distribution of participants in the high and low consumer categories varied considerably across the time points. For example, at T5, 80% were classified as high consumers of total fish, which make only 20% classified as low consumers. When one group is much smaller than the other, the wider confidence intervals associated with the smaller group may make it more challenging to detect a statistically significant difference between the groups. This is because the overlap between the confidence intervals of the two groups is more likely when one interval is wide, which could lead to a failure to reject the null hypothesis of no difference.

5.2.3 Confounding variables

As discussed earlier, the categorisation of participants into high and low consumers were challenging due to inconsistencies in the structure of survey questions and the response options provided. This difficulty extended to the classification of participants' consumption levels of foods that could act as confounders. If participants were inaccurately classified as high or low consumers of confounding foods due to these measurement errors, the adjustment for these confounding variables in the analysis may have been incomplete or incorrect. Additionally, in this thesis there was used an internal exposure approach looking at concentrations in serum samples in combination with data on fish consumption to evaluate the association between them (29). The serum samples reflect total PFAAs exposure, not only from fish, and we cannot truly say what the difference in PFAA serum concentrations that was observed between high and low consumers comes from the fish consumption itself. Because of possible information bias, the analysis might not fully account for confounding factors, leading to residual confounding. This means that the observed association between the exposure and the outcome may still be influenced by these confounding factors, despite attempts to adjust for them.

By the use of DAG, factors such as number of children and months of breastfeeding were not identified as confounding factors. This is because I could not identify that the number of children a woman has had, or the duration of breastfeeding would influence her long-term fish consumption. But this might be a wrong assumption due to recommendations regarding consumption of specific fish species during pregnancy. These recommendations, however, is limited to the duration of the pregnancy, and is not assumed to make females change her fish consumption also after giving birth. Also, fish consumption is not assumed to be affected by whether you have given birth to one or more children. Moreover, the species of fish that pregnant females are advised to avoid are generally not those that are consumed regularly by the general population. Since most females in this study also had completed childbirth before the first time point (T1), it is unlikely that the number of children they have had would serve as a confounding variable in the analysis of fish consumption patterns. Additionally, analyses were conducted adjusting for the number of children, but the results remained relatively unchanged. This further suggests that the number of children does not have a substantial impact on the findings in my study.

5.2.4 External validity and selection bias

This work examined the temporal trends of PFAAs from 1986 to 2016, a period that encompasses the increase, peak, and subsequent decline in concentrations of PFOS, PFOA, and PFHxS. It also covers the timeframe in which concentrations of newer emerging PFAAs began to increase. My findings are influenced by the fact that I analysed PFAA trends during this specific period. This is due to the clear association between historical PFAA use and emissions and concentrations found in human serum (41). Therefore, discussions of PFAA trends should always take into account the era under consideration.

The Tromsø Study is a health-focused research initiative, and individuals who are more concerned about their own health or more aware of health issues may be more motivated to participate in such studies. My study required participants to engage in at least three surveys, which increased the need for motivation to continue answering questionnaires and attending physical examinations. This requirement might have introduced selection bias, as these individuals might have different health characteristics and lifestyles than individuals with less interest in health matters. Consequently, my results might be more representative for people with a heightened interest in health.

My study participants had a mean age 45 years at T1 and 70 years at T7, they also reported higher fish consumption than the general Norwegian population and high lean fish consumption. This indicates that my results are most representative for older populations and populations that consume a lot of fish, especially lean fish. However, I might suggest that although the concentrations observed in this thesis might not be generalizable for younger populations, the differences in time trends between high and low total/lean fish consumers that are observed in this thesis, are likely to be observed also in younger populations with similar fish consumption.

5.3 Future research

To gain deeper insights into how fish consumption affects the trends of PFAAs over time, more extensive studies with a larger number of participants and with more comprehensive FFQs, combined with other methods for measuring dietary intake like food diary, should be conducted. This would allow for more detailed grouping of individuals based on their level of fish consumption, including non-consumers and extreme consumers. Such categorisation would give more accurate analysis of the contribution of fish consumption to the temporal trends of PFAA concentrations in humans.

In future studies, it would also be interesting to include if the fish the participants consumed were mostly wild-caught or farmed, and where it was farmed or caught. This is because studies suggest that some fish species contain higher concentrations of PFAAs than others and that some geographical regions and waters have elevated PFAA concentrations, making the fish from such areas more contaminated (56). Also, farmed and wild-caught fish has been found to have significant different PFAA concentrations, with lower concentrations in farmed fish compared to wild caught (85). How the fish have been cooked and types of cooking equipment is also interesting as it might make a difference in terms of PFAA concentrations in the consumed fish (100), and this should therefore also be included in future studies.

Longitudinal studies investigating PFAA time trends with repeated measurements from the same individuals should also be carried out in populations that consume high amounts of fatty fish, as well as populations with a diet rich in meat or chicken, and vegetarians and vegans. In my study sample, the participants consumed mostly lean fish, and this might have affected whether I observed significant finding in lean or fatty fish consumers. Enhanced understanding of the contribution of various foods such as meat, chicken, vegetables, and fruits to the temporal trend of PFAA concentrations could influence our evaluation of fish consumption. If it is determined that other dietary items contribute substantially to PFAA exposure and time trends, this could serve as an argument in favour of fish consumption, as it would suggest that fish may not be a worse alternative in terms of PFAA time trends compared to other foods. This approach would provide knowledge about to which degree fish consumption poses a risk compared to other foods, or if the concern truly varies based on the predominant foods in the populations diet over time.

Research that examines all PFAAs collectively, as well as the total exposure of contaminants from fish consumption, could offer deeper insight. Such studies would help us better understand the cumulative impact of these pollutants on our health and how fish contributes to health outcomes overall. Further research, also including younger women, could assist in determining whether more detailed guidance on fish consumption should be provided for woman of childbearing age.

6 Conclusion

In the first survey, 53% of the participants consumed the recommended two or more dinner servings of fish per week. The fish consumption increased throughout the study period. Participants who reported higher intake of total and lean fish, experienced a significantly faster increase and slower decline in the concentration of PFOS, PFHxS, PFNA, and PFUdA for total fish and PFOS, PFNA, and PFUdA for lean fish compared to low consumers. This resulted in high consumers of total/lean fish generally having higher PFAA concentrations compared to low consumers over time and indicates that high consumers sustained higher body burden of some PFAAs for an extended duration compared to low consumers. Fatty fish consumer had minimal differences in absolute PFAA concentrations between high and low consumers and did not experience significantly different time trends between the groups. As environmental concentrations increased, the gap in emerging PFAA concentrations between high and low total/lean fish consumers widened, potentially increasing further without regulation. However, both high and low fish consumers experienced high PFAA concentrations at all time points and eating less fish did not guarantee PFAA blood concentrations below recommended levels.

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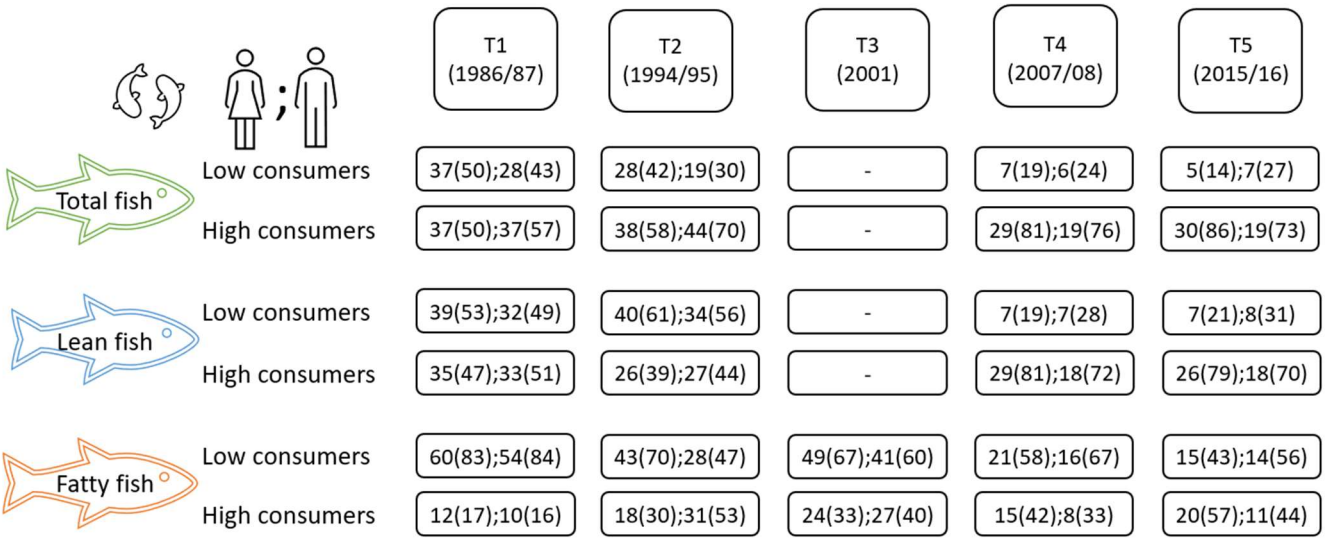
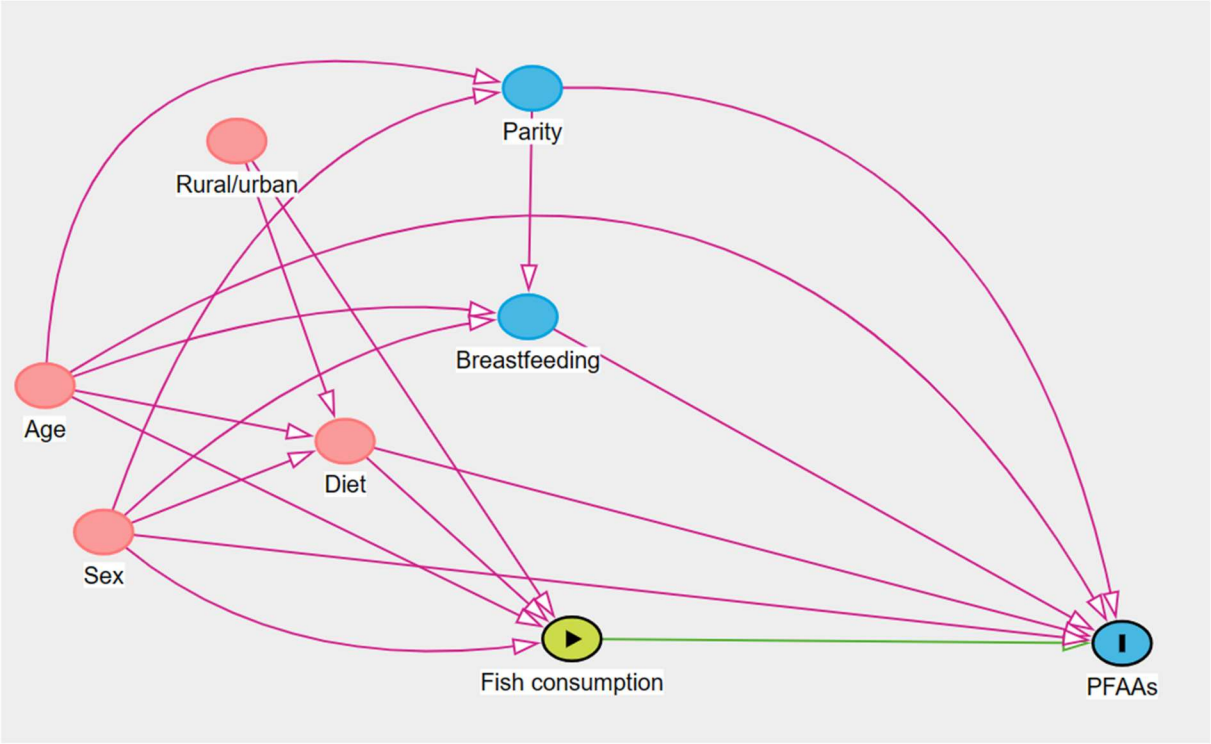
Appendix 1



Supplementary Figure 1: Number of males and females (n[%] females; n[%] males) categorised as high or low consumers of total, lean, and fatty fish at each time point (T1-1986/87; T2-1994/95; T3-2001; T4-2007/08; T5-2015/16). High lean fish consumption was defined as≥2 dinner servings per week; high fatty fish consumption≥1 dinner serving per week; high total fish consumption=high lean and/or high fatty fish consumption.

Appendix 2



Supplementary Figure 2: Directed Acyclic Graph (DAG) for identifying confounding variables between fish consumption and perfluoroalkyl acids (PFAAs).

Argumentations for my assumptions in DAG. Arrows below represents directions of arrows in the DAG:

Age → Fish consumption/diet

- People of older age tend to eat more fish compared to younger adults (4). People may change their diet as they age due to health reasons, interests, and practical considerations.

Age → PFAAs

- Historical use of PFAAs affects the PFAA concentrations in individuals born in different years (or birth cohorts) (41).

Age → Parity/Breastfeeding

- The older you are, the more likely you are to have had more children and therefore breastfed for a longer period.

Sex → Diet/Fish consumption

- Females are more likely to take healthy food choices than males, therefore more likely to consume for example more fish. In Tromsø, males who have spent most time at sea, probably have consumed more fish compared to females.

Sex → Breastfeeding/parity

- Only females can give birth and breastfeed.

Sex → PFAAs

- Males tend to have higher PFAA concentrations compared to females.

Rural/urban → Diet/fish consumption

- Rural areas, especially near freshwater or coastal areas, often have easier access to fish. In these areas where fishing is part of culture and tradition, fish consumption is more common.

Diet → Fish consumption

- Individuals who maintain a healthy diet are more likely to incorporate fish into their meals compared to those who do not prioritize healthy eating habits (7).

Diet → PFAAs

- PFAAs are present to varying degrees in nearly all types of food (77). Therefore, the type of food you consume frequently will affect the concentration of PFAAs in your blood.

Breastfeeding/parity → PFAAs

- Females excrete some PFAAs through menstruation, pregnancy, and lactation (37).

Appendix 3

Supplementary Table 1: Participants characteristics at each time point (T1-1986/87; T2-1994/95; T4-2007/08; T5-2015/16) for all participants categorised as low consumers of lean fish or high consumers of lean fish*.

Lean fish		T1	T2	T3÷	T4	T5
Females n (%)	Low	39 (52.7)	40 (60.6)		7 (19.4)	7 (21.2)
	High	35 (47.3)	26 (39.4)		29 (80.6)	26 (78.8)
Males n (%)	Low	32 (49.2)	34 (55.7)		7 (28.0)	8 (30.8)
	High	33 (50.8)	27 (44.3)		18 (72.0)	18 (69.2)
Total n (%)	Low	71 (51.1)	74 (58.3)		14 (23.0)	15 (25.4)
	High	68 (48.9)	53 (41.7)		47 (77.1)	44 (74.6)
Birth year min/max	Low	1925/1969	1928/1969		1936/1969	1931/1969
	High	1925/1961	1925/1960		1926/1961	1929/1961
Age years mean(min/max)	Low	43 (17/61)	52.4 (25/66)		56.2(38/71)	62.9 (46/84)
	High	47 (25/61)	54.4 (34/69)		65.6(46/81)	72.4 (54/86)
Weight kg mean (SD)	Low	71.3 (14.1)	71.6 (13.2)		79.3 (18.7)	81.5 (14.0)
	High	69.4 (10.7)	76.2 (14.2)		77.9 (16.6)	77.4 (15.7)
BMI kg/m^2 mean (SD)	Low	24.3 (3.72)	24.8 (3.92)		27.0 (4.74)	27.4 (4.95)
	High	24.1 (2.81)	26.2 (4.07)		27.7 (5.15)	27.5 (4.49)
Parity n mean (SD) §	Low	2.15 (1.42)	2.58 (1.22)		2.43 (1.51)	2.43 (0.98)
	High	2.69 (1.55)	2.54 (1.79)		2.72 (1.83)	2.81 (1.50)
Breastfeeding months Mean (SD) §	Low	-	13.5 (10.2)		16.0 (16.8)	18.7 (9.79)
	High	-	12.6 (12.2)		12.8 (13.5)	18.4 (15.3)

*High lean fish consumption was defined as ≥2 dinner servings per week
÷No data on T3 due to missing questions regarding lean fish at T3.
§Only for females that have reported having children

Supplementary Table 2: Participants characteristics at each time point (T1-1986/87; T2-1994/95; T3-2001; T4-2007/08; T5-2015/16) for all participants categorised as low consumers of fatty fish or high consumers of fatty fish*.

Fatty fish		T1	T2	T3	T4	T5
Females n (%)	Low	60 (83.3)	43 (70.5)	49 (67.1)	21 (58.3)	15 (42.9)
	High	12 (16.7)	18 (29.5)	24 (32.9)	15 (41.7)	20 (57.1)
Males n (%)	Low	54 (84.4)	28 (47.5)	41 (60.3)	16 (66.7)	14 (56.0)
	High	10 (15.6)	31 (52.5)	27 (39.7)	8 (33.0)	11 (44.0)
Total n (%)	Low	114 (83.8)	71 (59.2)	90 (63.8)	37 (61.7)	29 (48.3)
	High	22 (16.2)	49 (40.8)	51 (36.2)	23 (38.3)	31 (51.7)
Birth year min/max	Low	1925/1963	1925/1963	1925/1969	1926/1969	1931/1969
	High	1927/1969	1925/1969	1925/1961	1931/1958	1929/1961
Age years mean(min/max)	Low	45.6 (23/61)	51.6 (31/69)	59.5(32/76)	62.7(38/81)	69.6 (46/84)
	High	43.8 (17/59)	55.4 (25/69)	61.2(40/76)	64.6(49/76)	70.9 (54/86)
Weight kg mean (SD)	Low	70.6 (12.2)	71.6 (13.8)	75.8(14.1)	76.3 (15.7)	77.0 (15.3)
	High	69.9 (13.6)	76.3 (13.8)	77.1 (15)	79.5 (17.5)	77.6 (16.6)
BMI kg/m^2 mean (SD)	Low	24.3 (3.28)	25.1 (4.54)	26.5(4.43)	26.9 (4.92)	24.3 (3.28)
	High	23.9 (3.35)	25.5 (3.34)	26.5(3.92)	28.0 (4.60)	23.9 (3.35)
Parity n mean (SD) §	Low	2.40 (1.60)	2.53 (1.61)	2.65 (1.49)	2.90 (1.79)	2.93 (1.22)
	High	2.42 (1.08)	2.72 (1.23)	2.50 (1.44)	2.33 (1.72)	2.60 (1.50)
Breastfeeding months Mean (SD) §	Low	-	12.7 (11.7)	13.9 (11.0)	16.6 (14.8)	21.8 (12.9)
	High	-	13.8 (9.84)	14.6 (12.2)	8.50 (11.0)	14.9 (14.3)

*High fatty fish consumption was defined as ≥1 dinner serving per week
§Only for females that have reported having children

Appendix 4

Supplementary Table 3: Perfluoroalkyl acid concentrations (ng/mL) at each time point (T1-1986/87; T2-1994/95; T3-2001; T4-2007/08; T5-2015/16) for all participants categorised as low consumers of total fish and high consumers of total fish.

		T1 mean;median(p25-p75)	T2 mean;median(p25-p75)	T3÷	T4 mean;median(p25-p75)	T5 mean;median(p25-p75)
PFOS	Low	18.4;16.8(13.9-22.8)	26.3;24.8(19.3-31.3)		16.3;16.0(11.7-21.0)	11.7;10.0(7.71-14.8)
	High	20.9;19.9(16.4-23.7)	35.8;33.1(26.6-41.8)		31.9;30.4(20.7-37.2)	19.9;14.7(10.4-25.3)
PFOA	Low	2.68;2.13(1.61-2.77)	4.18;3.86(2.83-4.77)		2.95;2.86(2.57-3.15)	1.81;1.52(1.17-2.60)
	High	2.24;2.14(1.66-2.58)	4.43;4.25(3.51-5.10)		3.28;3.08(2.48-3.91)	2.53;2.28(1.62-2.91)
PFHxS	Low	0.83;0.65(0.56-0.91)	1.36;1.19(0.87-1.66)		1.97;1.42(0.96-2.05)	1.25;1.10(0.84-1.29)
	High	0.95;0.85(0.68-1.06)	2.02;1.63(1.21-2.10)		2.53;2.02(1.52-2.76)	2.16;1.72(1.14-2.16)
PFNA	Low	0.53;0.38(0.31-0.47)	0.57;0.56(0.37-0.70)		0.82;0.73(0.64-0.99)	0.90;0.85(0.60-1.10)
	High	0.53;0.46(0.39-0.55)	0.80;0.69(0.54-0.99)		1.44;1.34(0.79-1.61)	1.65;1.22(1.01-1.98)
PFDA	Low	0.21;0.15(0.11-0.18)	0.31;0.28(0.20-0.41)		0.34;0.31(0.19-0.47)	0.41;0.42(0.23-0.58)
	High	0.20;0.18(0.12-0.25)	0.43;0.38(0.31-0.52)		0.73;0.68(0.41-0.86)	0.80;0.62(0.40-0.96)
PFUdA	Low	0.46;0.44(0.30-0.57)	0.44;0.37(0.24-0.55)		0.37;0.32(0.22-0.48)	0.42;0.36(0.23-0.63)
	High	0.65;0.56(0.37-0.81)	0.66;0.56(0.39-0.81)		1.11;0.92(0.53-1.31)	1.00;0.76(0.47-1.30)

÷No data on T3 due to missing questions regarding lean fish at T3 in the questionnaires. Abbreviations: PFOS: Sum Perfluorooctane sulfonic acid; PFOA: Perfluorooctanoic acid; PFHxS: sum Perfluorohexane sulfonic acid; PFNA: Perfluorononanoic acid; PFDA: Perfluorodecanoic acid; PFUdA: perfluoroundecanoic acid; High lean fish consumption≥2 dinner servings per week; high fatty fish consumption≥1 dinner serving per week; high total fish consumption=high lean and/or high fatty fish consumption.

Supplementary Table 4: Perfluoroalkyl acid concentrations at each time point (T1-1986/87; T2-1994/95; T3-2001; T4-2007/08; T5-2015/16) for all participants categorised as low consumers of lean fish and high consumers of lean fish.

		T1 mean;median(p25-p75)	T2 mean;median(p25-p75)	T3÷	T4 mean;median(p25-p75)	T5 mean;median(p25-p75)
PFOS	Low	18.5;17.0(14.1-22.8)	29.2;26.0(21.0-35.0)		17.2;16.2(11.7-22.6)	11.5;9.77(7.98-15.1)
	High	21.1;19.9(15.5-24.4)	35.2;32.9(26.7-40.0)		31.9;30.8(20.3-37.7)	21.0;14.7(10.8-29.3)
PFOA	Low	2.66;2.17(1.62-2.77)	4.30;4.08(3.00-5.10)		2.92;2.84(2.47-3.15)	1.82;1.55(1.05-2.71)
	High	2.23;2.12(1.63-2.57)	4.40;4.15(3.55-5.04)		3.30;3.09(2.49-3.94)	2.63;2.29(1.71-3.06)
PFHxS	Low	0.84;0.68(0.57-0.98)	1.49;1.25(1.07-2.11)		2.09;1.53(0.96-2.11)	1.22;1.01(0.82-1.34)
	High	0.95;0.82(0.68-1.06)	2.10;1.66(1.25-2.10)		2.50;2.02(1.49-2.65)	2.24;1.76(1.18-2.16)
PFNA	Low	0.53;0.39(0.32-0.48)	0.65;0.61(0.44-0.73)		0.85;0.77(0.64-1.07)	0.92;0.88(0.64-1.20)
	High	0.53;0.47(0.36-0.58)	0.77;0.73(0.53-0.99)		1.45;1.35(0.78-1.62)	1.72;1.30(1.03-2.20)
PFDA	Low	0.21;0.15(0.11-0.18)	0.33;0.31(0.21-0.42)		0.37;0.33(0.19-0.50)	0.42;0.45(0.24-0.56)
	High	0.21;0.19(0.13-0.25)	0.44;0.41(0.32-0.53)		0.73;0.67(0.41-0.86)	0.83;0.63(0.40-1.08)
PFUdA	Low	0.46;0.44(0.29-0.57)	0.46;0.40(0.24-0.56)		0.43;0.34(0.22-0.55)	0.44;0.47(0.23-0.63)
	High	0.67;0.56(0.42-0.82)	0.69;0.67(0.43-0.81)		1.10;0.88(0.52-1.32)	1.04;0.81(0.43-1.44)

÷No data on T3 due to missing questions regarding lean fish at T3 in the questionnaires. Abbreviations: PFOS: Sum Perfluorooctane sulfonic acid; PFOA: Perfluorooctanoic acid; PFHxS: sum Perfluorohexane sulfonic acid; PFNA: Perfluorononanoic acid; PFDA: Perfluorodecanoic acid; PFUdA: perfluoroundecanoic acid; High lean fish consumption≥2 dinner servings per week.

Supplementary Table 5: Perfluoroalkyl acid concentrations at each time point (T1-1986/87; T2-1994/95; T3-2001; T4-2007/08; T5-2015/16) for all participants categorised as low consumers of fatty fish and high consumers of fatty fish.

		T1 mean;median(p25-p75)	T2 mean;median(p25-p75)	T3 mean;median(p25-p75)	T4 mean;median(p25-p75)	T5 mean;median(p25-p75)
PFOS	Low	19.5;18.3(14.7-23.1)	28.2;26.0(20.7-32.8)	37.4;31.9(25.9-44.5)	26.8;23.4(15.6-33.4)	16.9;14.3(9.24-20.2)
	High	20.9;19.9(16.4-22.8)	38.8;35.2(27.5-43.7)	46.5;41.4(32.1-54.3)	32.0;29.1(15.4-39.9)	19.9;14.7(10.4-25.3)
PFOA	Low	2.47;2.12(1.61-2.73)	4.19;3.87(2.97-5.09)	4.06;3.71(2.97-5.07)	3.13;2.88(2.45-3.74)	2.55;2.00(1.47-2.60)
	High	2.35;2.18(1.64-3.05)	4.60;4.70(3.63-5.20)	4.73;4.46(3.41-5.74)	3.35;3.09(2.70-3.87)	2.29;2.29(1.62-3.16)
PFHxS	Low	0.83;0.75(0.59-0.98)	1.69;1.28(1.00-1.75)	2.15;1.68(1.26-2.39)	2.16;1.85(1.38-2.30)	1.83;1.24(1.01-1.96)
	High	1.09;0.94(0.71-1.11)	1.98;1.69(1.25-2.18)	2.40;2.18(1.67-2.86)	2.82;2.24(1.37-3.09)	2.14;1.77(1.22-2.40)
PFNA	Low	0.52;0.42(0.32-0.51)	0.62;0.58(0.43-0.73)	0.89;0.79(0.60-1.06)	1.22;1.16(0.73-1.56)	1.38;1.06(0.79-1.55)
	High	0.58;0.47(0.40-0.55)	0.86;0.70(0.56-1.00)	1.09;0.98(0.77-1.31)	1.47;1.29(0.77-1.57)	1.65;1.22(1.05-2.11)
PFDA	Low	0.21;0.16(0.12-0.21)	0.34;0.32(0.21-0.43)	0.50;0.43(0.33-0.63)	0.6;0.52(0.31-0.84)	0.67;0.53(0.37-0.70)
	High	0.19;0.16(0.12-0.25)	0.44;0.39(0.32-0.53)	0.63;0.53(0.37-0.72)	0.73;0.6(0.41-0.84)	0.78;0.62(0.40-0.96)
PFUdA	Low	0.55;0.50(0.32-0.65)	0.51;0.45(0.29-0.63)	0.75;0.59(0.41-0.93)	0.83;0.56(0.36-1.22)	0.79;0.63(0.36-0.96)
	High	0.63;0.45(0.33-0.78)	0.67;0.56(0.37-0.85)	0.97;0.79(0.52-1.22)	1.18;1.11(0.54-1.40)	1.00;0.79(0.42-1.17)

Abbreviations: PFOS: Sum Perfluorooctane sulfonic acid; PFOA: Perfluorooctanoic acid; PFHxS: sum Perfluorohexane sulfonic acid; PFNA: Perfluorononanoic acid; PFDA: Perfluorodecanoic acid; PFUdA: perfluoroundecanoic acid; high fatty fish consumption≥1 dinner serving per week.

Supplementary Table 6: Perfluoroalkyl acid concentrations at each time point (T1-1986/87; T2-1994/95; T3-2001; T4-2007/08; T5-2015/16) for all participants categorised as males or females.

		T1 mean;median(p25-p75)	T2 mean;median(p25-p75)	T3 mean;median(p25-p75)	T4 mean;median(p25-p75)	T5 mean;median(p25-p75)
PFOS	Females	17.1;16.3(13.0-19.5)	27.0;25.6(20.5-33.1)	35.3;31.6(25.3-44.0)	26.0;23.2(15.3-33.8)	16.1;14.3(8.47-20.2)
	Males	22.8;22.4(18.4-26.2)	37.2;34.2(26.7-43.7)	46.8;41.4(31.0-55.1)	30.3;21.4(14.9-33.4)	21.3;14.3(10.2-28.0)
PFOA	Females	2.34;1.73(1.37-2.30)	3.81;3.69(2.94-4.79)	4.14;3.72(2.89-4.99)	3.27;2.96(2.53-3.81)	2.48;1.88(1.39-2.91)
	Males	2.60;2.50(1.97-3.03)	4.87;4.70(3.84-5.60)	4.56;4.36(3.41-5.23)	2.95;2.76(2.44-3.46)	2.27;2.32(1.70-2.74)
PFHxS	Females	0.68;0.61(0.49-0.81)	1.54;1.19(0.86-1.53)	2.17;1.64(1.17-2.31)	2.54;1.99(1.20-2.57)	2.17;1.54(0.97-2.27)
	Males	1.13;0.94(0.76-1.15)	1.94;1.74(1.30-2.16)	2.32;2.15(1.63-2.79)	1.95;1.76(1.31-2.31)	1.74;1.67(1.22-2.14)
PFNA	Females	0.54;0.39(0.28-0.51)	0.63;0.56(0.41-0.75)	0.88;0.84(0.58-1.08)	1.27;1.06(0.79-1.54)	1.47;1.20(0.99-1.80)
	Males	0.51;0.45(0.39-0.53)	0.78;0.68(0.51-0.90)	1.05;0.91(0.71-1.32)	1.27;1.21(0.72-1.57)	1.54;1.18(0.79-2.11)
PFDA	Females	0.22;0.15(0.11-0.21)	0.34;0.32(0.23-0.43)	0.50;0.45(0.33-0.63)	0.62;0.54(0.39-0.82)	0.69;0.57(0.46-0.84)
	Males	0.18;0.17(0.13-0.22)	0.42;0.37(0.27-0.54)	0.59;0.50(0.34-0.70)	0.63;0.44(0.23-0.80)	0.77;0.44(0.34-0.96)
PFUdA	Females	0.53;0.46(0.32-0.62)	0.50;0.46(0.31-0.62)	0.71;0.62(0.44-0.94)	0.85;0.68(0.48-1.19)	0.86;0.74(0.47-1.04)
	Males	0.58;0.50(0.33-0.71)	0.64;0.56(0.36-0.85)	0.95;0.79(0.45-1.16)	1.01;0.43(0.31-1.26)	0.92;0.50(0.30-1.17)

Abbreviations: PFOS: Sum Perfluorooctane sulfonic acid; PFOA: Perfluorooctanoic acid; PFHxS: sum Perfluorohexane sulfonic acid; PFNA: Perfluorononanoic acid; PFDA: Perfluorodecanoic acid; PFUdA: perfluoroundecanoic acid.

Appendix 5

Supplementary Table 7: Multivariable adjusted regression coefficients, standard errors (SE), and 95% confidence level from linear mixed effect models to assess the longitudinal changes in perfluoroalkyl acid concentrations (log- scale, ng/mL) from 1986/87-2015/16 according to fish consumption in the Tromsø Study (1986-2016).

	Model 1		**Model 2**	
	β – coefficient (SE)	**95% CI / p-value for Wald test**	**β – coefficient (SE)**	**95% CI / p-value for Wald test**
PFOS				
- Total fish	-0.03 (0.05)	-0.12, 0.06	-0.01 (0.05)	-0.10, 0.08
- Survey				
T2	0.39 (0.05)	0.30, 0.48	0.40 (0.05)	0.31, 0.49
T4	-0.06 (0.09)	-0.12, 0.24	0.04 (0.10)	-1.45, 0.23
T5	-0.45 (0.11)	-0.66, -0.23	-0.52 (0.16)	-0.82, -0.21
- Interactions				
Total fishxT2	0.11 (0.06)	-0.01, 0.24	0.11 (0.06)	-0.01, 0.24
Total fishxT4	0.25 (0.10)	0.04, 0.45	0.27 (0.11)	0.56, 0.48
Total fishxT5	0.34 (0.12)	0.10, 0.58	0.44 (0.17)	0.11, 0.76
- *Wald test for Total fish x Time interaction term*		**0.02**		**0.02**
Sex x T2				
Sex x T4				
Sex x T5				
- *Wald test for Sex x Time interaction term*				
- Sex			0.30 (0.05)	0.19, 0.41
- Age			0.01 (0.003)	0.002, 0.01
- Meat			0.004 (0.04)	-0.06, 0.07
- Fruits and vegetables			-0.01 (0.03)	-0.08, 0.05
- Dairy			-0.004 (0.04)	-0.09, 0.08
Constant	2.93 (0.04)	2.86, 3.01	2.78 (0.06)	2.66, 2.90
PFOA				
- Total fish	-0.06 (0.07)	-0.19, 0.07	-0.06 (0.06)	-0.18, 0.07

- Survey				
T2	0.56 (0.07)	0.43, 0.68	0.56 (0.07)	0.41, 0.70
T4	0.26 (0.11)	0.04, 0.48	0.34 (0.13)	0.09, 0.58
T5	-0.29 (0.13)	-0.55, -0.04	-0.40 (0.21)	-0.82, 0.01
- Interactions				
Total fishxT2	0.14 (0.09)	-0.04, 0.31	0.11 (0.09)	-0.07, 0.28
Total fishxT4	0.10 (0.13)	-0.16, 0.37	0.14 (0.14)	-0.13, 0.41
Total fishxT5	0.29 (0.15)	-0.004, 0.58	0.52 (0.21)	0.12, 0.92
- *Wald test for Total fishxtime interaction term*		0.20		0.07
Sex x T2			0.004 (0.08)	-0.15, 0.16
Sex x T4			-0.31 (0.12)	-0.54, -0.08
Sex x T5			-0.24 (0.14)	-0.51, 0.03
- *Wald test for Sex x time interaction term*				**0.02**
- Sex			0.25 (0.07)	0.12, 0.38
- Age			-0.001 (0.003)	-0.01, 0.005
- Meat			-0.01 (0.05)	-0.10, 0.08
- Fruits and vegetables			0.01 (0.04)	-0.08, 0.09
- Dairy			-0.13(0.05)	-0.23, -0.03
Constant	0.79 (0.05)	0.70, 0.89	0.78 (0.07)	0.64, 0.93
PFHxS				
- Total fish	-0.05 (0.07)	-0.19, 0.08	-0.04 (0.06)	-0.16, 0.09
- Survey				
T2	0.51 (0.07)	0.38, 0.64	0.54 (0.07)	0.34, 0.68
T4	0.74 (0.12)	0.50, 0.98	0.94 (0.13)	0.68, 1.20
T5	0.46 (0.15)	0.18, 0.75	0.34 (0.23)	-0.05, 0.84
- Interactions				
Total fishxT2	0.19 (0.09)	0.01, 0.37	0.18 (0.09)	0.01, 0.36
Total fishxT4	0.16 (0.14)	-0.17, 0.45	0.20 (0.14)	-0.08, 0.48
Total fishxT5	0.32 (0.17)	0.00, 0.65	0.55 (0.22)	0.12, 0.98
- *Wald test for Total fishxTime*		0.10		**0.03**

interaction term				
Sex x T2			-0.10 (0.08)	-0.26, 0.05
Sex x T4			-0.54 (0.12)	-0.79, -0.30
Sex x T5			-0.37 (0.15)	-0.67, -0.08
- *Wald test for Sex x Time interaction term*				**<0.001**
- Sex			0.49 (0.08)	0.34, 0.64
- Age			0.01 (0.004)	0.0005, 0.01
- Meat			-0.03 (0.05)	-0.13, 0.06
- Fruits and vegetables			0.09 (0.05)	-0.01, 0.18
- Dairy			-0.07 (0.05)	-0.18, 0.05
Constant	-0.22 (0.06)	-0.33, -0.10	-0.42 (0.08)	-0.58, -0.26
PFNA				
- Total fish	0.02 (0.06)	-0.11, 0.15	0.01 (0.06)	-0.11, 0.14
Survey				
T2	0.27 (0.06)	0.16, 0.39	0.23 (0.06)	0.17, 0.41
T4	0.78 (0.11)	0.57, 1.00	0.76 (0.11)	0.54, 0.98
T5	0.79 (0.13)	0.55, 1.04	0.65 (0.18)	0.30, 1.01
- Interactions				
Total fishxT2	0.15 (0.08)	-0.02, 0.31	0.14 (0.08)	-0.02, 0.31
Total fishxT4	0.21 (0.13)	-0.04, 0.46	0.24 (0.13)	-0.01, 0.50
Total fishxT5	0.36 (0.14)	0.07, 0.64	0.55 (0.20)	0.17, 0.93
- *Wald test for Total fishxTime interaction term*		0.06		**0.02**
Sex x T2				
Sex x T4				
Sex x T5				
- *Wald test for Sex x Time interaction term*				
- Sex			0.11 (0.07)	-0.02, 0.24

- Age			0.01 (0.003)	0.004, 0,02
- Meat			-0.03 (0.05)	-0.12, 0.06
- Fruits and vegetables			-0.09 (0.04)	-0.17, 0.0004
- Dairy			-0.10 (0.05)	-0.21, 0.003
Constant	-0.84 (0.05)	-0.94, -0.73	-0.76 (0.08)	-0.92, -0.61
PFDA				
- Total fish	0.05 (0.06)	-0.08, 0.17	0.05 (0.06)	-0.08, 0.17
- Survey				
T2	0.66 (0.06)	0.54, 0.79	0.60 (0.07)	0.46, 0.74
T4	0.86 (0.12)	0.64, 1.09	0.95 (0.13)	0.69, 1.20
T5	1.09 (0.13)	0.83, 1.35	0.96 (0.23)	0.51, 1.41
- Interactions				
Total fishxT2	0.10 (0.09)	-0.08, 0.27	0.08 (0.09)	-0.09, 0.25
Total fishxT4	0.33 (0.14)	0.06, 0.60	0.31 (0.14)	0.03, 0.58
Total fishxT5	0.21 (0.15)	-0.09, 0.51	0.42 (0.22)	-0.01, 0.86
- *Wald test for Total fishxTime interaction term*		0.10		0.09
Sex x T2			0.17 (0.08)	0.03, 0.32
Sex x T4			-0.09 (0.12)	-0.34, 0.15
Sex x T5			0.02 (0.16)	-0.29, 0.32
- *Wald test for Sex x Time interaction term*				**0.04**
- Sex			-0.05 (0.08)	-0.20, 0.10
- Age			0.02 (0.003)	0.01, 0.02
- Meat			-0.09 (0.05)	-0.18, 0.01
- Fruits and vegetables			-0.07 (0.05)	-0.16, 0.02
- Dairy			-0.03 (0.06)	-0.14, 0.07
Constant	-1.81 (0.05)	-1.92, -1.71	-1.71 (0.08)	-1.87, -1.55
PFUdA				
- Total fish	0.09 (0.06)	-0.03, 0.21	0.08 (0.06)	-0.03, 0.20
- Survey				
T2	-0.02 (0.06)	-0.14, 0.10	-0.04 (0.06)	-0.16, 0.08
T4	-0.05 (0.11)	-0.27, 0.17	-0.06 (0.11)	-0.28, 0.16
T5	0.19 (0.13)	-0.07, 0.44	0.07 (0.18)	-0.29, 0,43

- Interactions				
Total fishxT2	0.01 (0.08)	-0.15, 0.18	0.04 (0.08)	-0.12, 0,20
Total fishxT4	0.42 (0.13)	0.17, 0.69	0.45 (0.13)	0.19, 0.71
Total fishxT5	0.19 (0.15)	-0.10, 0.48	0.35 (0.20)	-0.03, 0.74
- *Wald test for Total fishxTime interaction term*		**0.01**		**0.006**
Sex x T2				
Sex x T4				
Sex x T5				
- *Wald test for Sex x Time interaction term*				
- Sex			0.06 (0.07)	-0.09, 0.20
- Age			0.02 (0.004)	0.01, 0.03
- Meat			-0.08 (0.04)	-0.17, 0.007
- Fruits and vegetables			-0.02 (0.04)	-0.10, 0.07
- Dairy			-0.02 (0.05)	-0.13, 0.09
Constant	-0.76 (0.05)	-0.87, -0.66	-0.74 (0.08)	-0.89, -0.58

Model 1: Adjusted for time and interaction between total fish consumption status and time. All time trends were predicted with random intercept and random slope. There is no data at T3 due to missing questions regarding lean fish in the questionnaires at T3.

Model 2: Adjusted for time, sex, age, meat-, fruits and vegetables-, and dairy consumption status, interaction between total fish consumption status and time. PFOA, PFHxS, and PFDA were also adjusted for interactions between time and sex. All time trends were predicted with random intercept and random slope. There is no data at T3 due to missing questions regarding lean fish in the questionnaires at T3.

Abbreviations: T2: Time-point 2 (1994/95); T4: Time-point 4 (2007/08); T5: Time-point 5 (2015/16); PFOS: Sum Perfluorooctane sulfonic acid; PFOA: Perfluorooctanoic acid; PFHxS: sum Perfluorohexane sulfonic acid; PFNA: Perfluorononanoic acid; PFDA: Perfluorodecanoic acid; PFUdA: perfluoroundecanoic acid.

Supplementary Table 8: Multivariable adjusted regression coefficients, standard errors (SE), and 95% confidence level from linear mixed effect models to assess the longitudinal changes in perfluoroalkyl acid concentrations (log- scale, ng/mL) from 1986/87-2015/16 according to lean fish consumption in the Tromsø Study (1986-2016).

	Model 1		**Model 2**	
	β – coefficient (SE)	**95% CI / p-value for Wald test**	**β – coefficient (SE)**	**95% CI / p-value for Wald test**
PFOS				
- High lean fish	-0.05 (0.05)	-0.14, 0.05	-0.03 (0.05)	-0.12, 0.06
- Survey				
T2	0.43 (0.04)	0.35, 0.51	0.44 (0.04)	0.36, 0.52
T4	0.07 (0.09)	-0.10, 0.25	0.05 (0.09)	-0.13, 0.23
T5	-0.44 (0.10)	-0.64, -0.25	-0.47 (0.14)	-0.74, -0.21
- Interactions				
Lean fishxT2	0.06 (0.06)	-0.06, 0.19	0.06 (0.06)	-0.07, 0.18
Lean fishxT4	0.25 (0.10)	0.05, 0.45	0.27 (0.11)	0.06, 0.48
Lean fishxT5	0.35 (0.11)	0.13, 0.58	0.42 (0.15)	0.13, 0.70
- *Wald test for Lean fish x Time interaction term*		**0.01**		**0.01**
Sex x T2				
Sex x T4				
Sex x T5				
- *Wald test for Sex x Time interaction term*				
- Sex			0.30 (0.5)	0.20, 0.41
- Age			0.01 (0.003)	0.003, 0.01
- Meat			0.007 (0.04)	-0.06, 0.08
- Fruits and vegetables			-0.01 (0.03)	-0.08, 0.06
- Dairy			-0.01 (0.04)	-0.09, 0.07
Constant	2.90 (0.04)	2.86, 3.02	2.79 (0.06)	2.67, 2.91
PFOA				
- Lean fish	-0.08 (0.06)	-0.21, 0.04	-0.08 (0.06)	-0.20, 0.05
- Survey				
T2	0.58 (0.06)	0.47, 0.69	0.57 (0.07)	0.43, 0.70
T4	0.25 (0.11)	0.03, 0.46	0.32 (0.13)	0.07, 0.56
T5	-0.27 (0.11)	-0.49, -0.04	-0.33 (0.18)	-0.69, 0.03
- Interactions				

Lean fishxT2	0.13 (0.09)	-0.04, 0.30	0.11 (0.09)	-0,06, 0.29
Lean fishxT4	0.14 (0.13)	-0.11, 0.40	0.17 (0.13)	-0.09, 0.43
Lean fishxT5	0.30 (0.14)	0.04, 0.57	0.47 (0.18)	0.12, 0.82
- *Wald test for Lean fishxtime interaction term*		0.13		0.055
Sex x T2			0.01 (0.08)	-0.15, 0.17
Sex x T4			-0.30 (0.12)	-0.53, -0.07
Sex x T5			-0.25 (0.14)	-0.51, 0.02
- *Wald test for Sex x time interaction term*				**0.02**
- Sex			0.25 (0.07)	0.12, 0.38
- Age			-0.0005 (0.003)	-0.01, 0.005
- Meat			-0.01 (0.05)	-0.10, 0.08
- Fruits and vegetables			0.01 (0.04)	-0.08, 0.09
- Dairy			-0.14 (0.05)	-0.24, -0,04
Constant	0.80 (0.05)	0.71, 0.89	0.80 (0.07)	0.65, 0.94
PFHxS				
- Lean fish	-0.07 (0.07)	-0.21, 0.06	-0.06 (0.07)	-0.19, 0.07
- Survey				
T2	0.56 (0.06)	0.44, 0.67	0.58 (0.07)	0.44, 0.71
T4	0.77 (0.12)	0.53, 1.00	0.97 (0.13)	0.71, 1.23
T5	0.50 (0.13)	0.24, 0.75	0.54 (0.19)	0.15, 0.92
- Interactions				
Lean fishxT2	0.14 (0.09)	-0.04, 0.32	0.15 (0.09)	-0.03, 0.32
Lean fishxT4	0.14 (0.14)	-0.13, 0.42	0.17 (0.14)	-0.11, 0.44
Lean fishxT5	0.29 (0.15)	-0.01, 0.60	0.43 (0.19)	0.06, 0.81
- *Wald test for Lean fishxTime interaction term*		0.21		0.09
Sex x T2			-0.10 (0.08)	-0.26, 0.05
Sex x T4			-0.53 (0.12)	-0.77, -0.28
Sex x T5			-0.39 (0.15)	-0.68, -0.10
- *Wald test for Sex x Time*				**0.003**

interaction term				
- Sex			0.49 (0.08)	0.34, 0.64
- Age			0.01 (0.004)	0.001, 0.02
- Meat			-0.02, 0.05	-0.12, 0.07
- Fruits and vegetables			0.08 (0.05)	-0.01, 0.18
- Dairy			-0.08 (0.06)	-0.19, 0.04
Constant	-0.21 (0.05)	-0.32, -0.10	-0.41 (0.08)	-0.57, -0.25
PFNA				
- Lean fish	-0.01 (0.06)	-0.14, 0.11	-0.02 (0.06)	-0.15, 0.11
Survey				
T2	0.33 (0.05)	0.23, 0.43	0.34 (0.06)	0.23, 0.45
T4	0.79 (0.10)	0.58, 0.99	0.76 (0.11)	0.54, 0.97
T5	0.82 (0.11)	0.06, 1.04	0.75 (0.16)	0.44, 1.05
- Interactions				
Lean fishxT2	0.07 (0.08)	-0.09, 0.24	0.08 (0.09)	-0.08, 0.25
Lean fishxT4	0.23 (0.13)	-0.01, 0.48	0.25 (0.13)	-0.003, 0.51
Lean fishxT5	0.37 (0.13)	0.11, 0.63	0.48 (0.17)	0.14, 0.82
- *Wald test for Lean fishxTime interaction term*		**0.03**		**0.03**
Sex x T2				
Sex x T4				
Sex x T5				
- *Wald test for Sex x Time interaction term*				
- Sex			0.12 (0.07)	-0.02, 0.25
- Age			0.01 (0.004)	0.004, 0.02
- Meat			-0.03 (0.05)	-0.12, 0.06
- Fruits and vegetables			-0.09 (0.04)	-0.17, 0.002
- Dairy			-0.11 (0.05)	-0.22, -0.01
Constant	-0.82 (0.05)	-0.92, -0.72	-0.74 (0.08)	-0.90, -0.58
PFDA				
- Lean fish	0.06 (0.06)	-0.07, 0.19	0.05 (0.07)	-0.08, 0.17
- Survey				
T2	0.70 (0.06)	0.59, 0.81	0.70 (0.06)	0.59, 0.82
T4	0.89 (0.11)	0.67, 1.11	0.93 (0.12)	0.70, 1.17
T5	1.09 (0.12)	0.86, 1.33	1.06 (0.17)	0.72, 1.40
- Interactions				

Lean fishxT2	0.06 (0.09)	-0.11, 0.23	0.09 (0.09)	-0.08, 0.26
Lean fishxT4	0.30 (0.13)	0.04, 0.56	0.29 (0.14)	0.01, 0.56
Lean fishxT5	0.22 (0.14)	-0.06, 0.50	0.34 (0.19)	-0.03, 0.71
- *Wald test for Lean fishxTime interaction term*		0.11		0.13
Sex x T2				
Sex x T4				
Sex x T5				
- *Wald test for Sex x Time interaction term*				
- Sex			0.01 (0.07)	-0.12, 0.14
- Age			0.02 (0.003)	0.01, 0.02
- Meat			-0.07 (0.05)	-0.17, 0.02
- Fruits and vegetables			-0.09 (0.05)	-0.18, -0.0002
- Dairy			-0.05 (0.06)	-0.16, 0.06
Constant	-1.82 (0.05)	-1.92, -1.72	-1.73 (0.08)	-1.88, -1.57
PFUdA				
- Lean fish	0.11 (0.06)	-0.01, 0.23	0.09 (0.06)	-0.02, 0.21
- Survey				
T2	-0.004 (0.05)	-0.12, 0.10	-0.03 (0.05)	-0.13, 0.07
T4	-0.02 (0.11)	-0.23, 0.20	-0.02, 0.11	-0.24, 0.20
T5	0.21 (0.12)	-0.02, 0.44	0.15 (0.16)	-0.16, 0.47
- Interactions				
Lean fishxT2	0.01 (0.08)	-0.15, 0.18	0.06 (0.08)	-0.10, 0.22
Lean fishxT4	0.39 (0.13)	0.14, 0.64	0.40 (0.13)	0.14, 0.66
Lean fishxT5	0.15 (0.14)	-0,12, 0.42	0.27 (0.18)	-0.07, 0.62
- *Wald test for Lean fishxTime interaction term*		**0.02**		**0.02**
Sex x T2				
Sex x T4				
Sex x T5				
- *Wald test for Sex x Time*				

interaction term				
- Sex			0.06 (0.07)	-0.08, 0.21
- Age			0.02 (0.004)	0.01, 0.03
- Meat			-0.07 (0.05)	-0.16, 0.01
- Fruits and vegetables			-0.03 (0.05)	-0.012, 0.06
- Dairy			-0.03 (0.05)	-0.14, 0.08
Constant	-0.77 (0.05)	-0.87, -0.66	-0.73 (0.08)	-0.88, -0.58

Model 1: Adjusted for time and interaction between lean fish consumption status and time. All time trends were predicted with random intercept and random slope. There is no data at T3 due to missing questions regarding lean fish in the questionnaires at T3.

Model 2: Adjusted for time, sex, meat-, fruits and vegetables-, and dairy consumption status, interaction between lean fish consumption status and time. PFOA and PFHxS were also adjusted for interactions between time and sex. All time trends were predicted with random intercept and random slope. There is no data at T3 due to missing questions regarding lean fish in the questionnaires at T3.

Abbreviations: T2: Time-point 2 (1994/95); T4: Time-point 4 (2007/08); T5: Time-point 5 (2015/16); PFOS: Sum Perfluorooctane sulfonic acid; PFOA: Perfluorooctanoic acid; PFHxS: sum Perfluorohexane sulfonic acid; PFNA: Perfluorononanoic acid; PFDA: Perfluorodecanoic acid; PFUdA: perfluoroundecanoic acid.

Supplementary Table 9: Multivariable adjusted regression coefficients, standard errors (SE), and 95% confidence level from linear mixed effect models to assess the longitudinal changes in perfluoroalkyl acid concentrations (log- scale, ng/mL) from 1986/87-2015/16 according to fatty fish consumption in the Tromsø Study (1986-2016).

	Model 1		**Model 2**	
	β – coefficient (SE)	**95% CI / p-value for Wald test**	**β – coefficient (SE)**	**95% CI / p-value for Wald test**
PFOS				
- Fatty fish	-0.01 (0.06)	-0.13, 0.11	0.01 (0.06)	-0.10, 0.13
- Survey				
T2	0.43 (0.03)	0.36, 0.49	0.44 (0.03)	0.37, 0.51
T3	0.66 (0.04)	0.59, 0.73	0.67 (0.04)	0.59, 0.74
T4	0.27 (0.05)	0.16, 0.38	0.26 (0.06)	0.15, 0.38
T5	-0.26 (0.07)	-0.40, -0.12	-0.24 (0.08)	-0.40, -0.08
- Interactions				
Fatty fishxT2	0.09 (0.07)	-0.05, 0.23	0.07 (0.07)	-0.06, 0.21
Fatty fishxT3	0.07 (0.07)	-0.08, 0.21	0.05 (0.07)	-0.09, 0.19
Fatty fishxT4	-0.05 (0.09)	-0.22, 0.12	-0.05 (0.09)	-0.22, 0.12
Fatty fishxT5	0.11 (0.10)	-0.08, 0.30	0.09 (0.12)	-0.12, 0.30
- *Wald test for Fatty fish x Time interaction term*		0.25		0.46
Sex x T2				
Sex x T3				
Sex x T4				
Sex x T5				
- *Wald test for Sex x Time interaction term*				
- Sex			0.30 (0.05)	0.20, 0.41
- Age			0.01 (0.003)	0.003, 0.01
- Fruits and vegetables			0.002 (0.03)	-0.05, 0.05
- Dairy			0.003 (0.03)	-0.06, 0.06
Constant	2.92 (0.03)	2.85, 2.98	2.76 (0.05)	2.66, 2.85
PFOA				
- Fatty fish	0.02 (0.08)	-0.14, 0.18	0.02 (0.08)	-0.14, 0.19
- Survey				
T2	0.61 (0.04)	0.52, 0.70	0.61 (0.05)	0.51, 0.70
T3	0.60 (0.05)	0.51, 0.69	0.56 (0.05)	0.45, 0.66
T4	0.37 (0.07)	0.24, 0.50	0.35 (0.07)	0.21, 0.49
T5	-0.11 (0.08)	-0.28, 0.05	-0.14 (0.10)	-0.33, 0.06

- Interactions				
Fatty fishxT2	0.05 (0.10)	-0.15, 0.24	0.03 (0.10)	-0.16, 0.23
Fatty fishxT3	0.06 (0.10)	-0.14, 0.26	0.05 (0.10)	-0.15, 0.25
Fatty fishxT4	-0.13 (0.12)	-0.37, 0.11	-0.11 (0.12)	-0.36, 0.13
Fatty fishxT5	0.04 (0.13)	-0.21, 0.30	0.09 (0.15)	-0.20, 0.37
- *Wald test for Fatty fishxtime interaction term*		0.46		0.57
Sex x T2				
Sex x T3				
Sex x T4				
Sex x T5				
- *Wald test for Sex x time interaction term*				
- Sex			0.18 (0.05)	0.08, 0.28
- Age			0.002 (0.003)	-0.003, 0.01
- Fruits and vegetables			0.023 (0.04)	-0.05, 0.09
- Dairy			-0.10 (0.04)	-0.18, -0.03
Constant	0.76 (0.04)	0.69, 0.83	0.75 (0.06)	0.63, 0.86
PFHxS				
- Fatty fish	-0.10 (0.09)	-0.26, 0.07	-0.03 (0.08)	-0.18, 0.13
- Survey				
T2	0.59 (0.05)	0.50, 0.68	0.62 (0.06)	0.51, 0.73
T3	0.84 (0.05)	0.75, 0.93	0.92 (0.06)	0.80, 1.05
T4	0.85 (0.07)	0.71, 0.99	1.05 (0.09)	0.88, 1.22
T5	0.64 (0.09)	0.46, 0.82	0.75 (0.12)	0.51, 0.98
- Interactions				
Fatty fishxT2	0.16 (0.10)	-0.03, 0.36	0.07 (0.10)	-0.12, 0.27
Fatty fishxT3	0.17 (0.10)	-0.04, 0.37	0.11 (0.10)	-0.09, 0.30
Fatty fishxT4	0.04 (0.12)	-0.20, 0.29	-0.01 (0.12)	-0.25, 0.23
Fatty fishxT5	0.14 (0.14)	-0.13, 0.40	0.18 (0.14)	-0.09, 0.46
- *Wald test for Fatty fishxTime interaction term*		0.40		0.58
Sex x T2			-0.07 (0.08)	-0.22, 0.08
Sex x T3			-0.26 (0.08)	-0.41, -0,11
Sex x T4			-0.44 (0.11)	-0.66, -0.21
Sex x T5			-0.36 (0.14)	-0.64, -0.08

- *Wald test for Sex x Time interaction term*				**<0.001**
- Sex			0.48 (0.08)	0.34, 0.63
- Age			0.01 (0.004)	0.001, 0.02
- Fruits and vegetables			0.11 (0.04)	0.03, 0.18
- Dairy			-0.03 (0.04)	-0.11, 0.06
Constant	-0.23 (0.05)	-0.32, -0.14	-0.49 (0.07)	-0.62, -0.36
PFNA				
- Fatty fish	0.07 (0.08)	-0.08, 0.23	0.08 (0.08)	-0.08, 0.24
Survey				
T2	0.33 (0.04)	0.25, 0.42	0.34 (0.04)	0.26, 0.43
T3	0.66 (0.04)	0.58, 0.75	0.66 (0.05)	0.56, 0.75
T4	1.01 (0.06)	0.88, 1.13	0.99 (0.07)	0.86, 1.13
T5	1.03 (0.08)	0.87, 1.19	1.06 (0.10)	0.87, 1.25
- Interactions				
Fatty fishxT2	0.06 (0.09)	-0.13, 0.24	0.04 (0.09)	-0.14, 0.23
Fatty fishxT3	0.02 (0.10)	-0.17, 0.21	0.02 (0.10)	-0.17, 0.21
Fatty fishxT4	-0.15 (0.11)	-0.37, 0.07	-0.13 (0.12)	-0.35, 0.10
Fatty fishxT5	0.10 (0.12)	-0.14, 0.35	0.11 (0.14)	-0.16, 0.38
- *Wald test for Fatty fishxTime interaction term*		0.22		0.41
Sex x T2				
Sex x T3				
Sex x T4				
Sex x T5				
- *Wald test for Sex x Time interaction term*				
- Sex			0.12 (0.06)	-0.002, 0.24
- Age			0.01 (0.003)	0.01, 0.02
- Fruits and vegetables			-0.03 (0.03)	-0.09, 0.04
- Dairy			-0.05 (0.04)	-0.13, 0.03
Constant	-0.84 (0.04)	-0.92, -0.75	-0.85 (0.06)	-0.97, -0.73
PFDA				
- Fatty fish	0.02 (0.08)	-0.14, 0.18	0.04 (0.08)	-0.12, 0.19
- Survey				
T2	0.71 (0.04)	0.63, 0.80	0.73 (0.04)	0.64, 0.82

T3	1.03 (0.05)	0.94, 1.12	1.05 (0.05)	0.95, 1.15
T4	1.17 (0.07)	1.04, 1.30	1.17 (0.73)	1.03, 1.31
T5	1.25 (0.08)	1.08, 1.42	1.28 (0.10)	1.07, 1.48
- Interactions				
Fatty fishxT2	0.05 (0.10)	-0.14, 0.24	0.03 (0.09)	-0.16, 0.21
Fatty fishxT3	0.05 (0.10)	-0.15, 0.24	0.04 (0.10)	-0.16, 0.23
Fatty fishxT4	-0.07 (0.12)	-0.30, 0.16	-0.05 (0.12)	-0.29, 0.18
Fatty fishxT5	0.08 (0.13)	-0.17, 0.33	0.09 (0.14)	-0.19, 0.36
- *Wald test for Fatty fishxTime interaction term*		0.75		0.85
Sex x T2				
Sex x T3				
Sex x T4				
Sex x T5				
- *Wald test for Sex x Time interaction term*				
- Sex			0.03 (0.07)	-0.10, 0.16
- Age			0.02 (0.003)	0.01, 0.03
- Fruits and vegetables			-0.03 (0.03)	-0.10, 0.04
- Dairy			0.005 (0.04)	-0.08, 0.08
Constant	-1.80 (0.04)	-1.88, -1.71	-1.81 (0.06)	-1.94, -1.69
PFUdA				
- Fatty fish	0.03 (0.08)	-0.12, 0.18	0.05 (0.07)	-0.10, 0.19
- Survey				
T2	-0.01 (0.04)	-0.09, 0.08	0.001 (0.04)	-0.08, 0.08
T3	0.34 (0.04)	0.23, 0.40	0.32 (0.05)	0.22, 0.41
T4	0.31 (0.06)	0.18, 0.43	0.28 (0.07)	0.15, 0.41
T5	0.33 (0.08)	0.17, 0.49	0.37 (0.09)	0.19, 0.56
- Interactions				
Fatty fishxT2	-0.01 (0.09)	-0.19, 0.17	-0.03 (0.09)	-0.20, 0.14
Fatty fishxT3	0.03 (0.09)	-0.15, 0.22	0.04 (0.09)	-0.14, 0.23
Fatty fishxT4	0.03 (0.11)	-0.19, 0.25	0.07 (0.11)	-0.15, 0.28
Fatty fishxT5	0.04 (0.12)	-0.20, 0.27	0.02 (0.13)	-0.24, 0.28
- *Wald test for Fatty fishxTime interaction term*		0.98		0.84
Sex x T2				
Sex x T3				

Sex x T4 Sex x T5 - *Wald test for Sex x Time interaction term*				
- Sex - Age - Fruits and vegetables - Dairy			0.07 (0.08) 0.02 (0.004) 0.001 (0.03) 0.01 (0.04)	-0.08, 0.22 0.02, 0.03 -0.06, 0.07 -0.07, 0.09
Constant	-0.72 (0.05)	-0.81, -0.63	-0.76 (0.07)	-0.89, -0.64

Model 1: Adjusted for time and interaction between fatty fish consumption status and time. All time trends were predicted with random intercept and random slope.

Model 2: Adjusted for time, sex, age, fruits, and vegetables-, and dairy consumption status interaction between fatty fish consumption status and time. PFHxS was also adjusted for interactions between time and sex. All time trends were predicted with random intercept and random slope.

Abbreviations: T2: Time-point 2 (1994/95); T3: Time-point 3 (2001); T4: Time-point 4 (2007/08); T5: Time-point 5 (2015/16); PFOS: Sum Perfluorooctane sulfonic acid; PFOA: Perfluorooctanoic acid; PFHxS: sum Perfluorohexane sulfonic acid; PFNA: Perfluorononanoic acid; PFDA: Perfluorodecanoic acid; PFUdA: perfluoroundecanoic acid.