Seafood consumption and hair mercury concentrations in adolescents: FINS-TEENS – a randomized controlled three-armed intervention trial

Anna Kleppe Moe

Master thesis in Clinical Nutrition

Department of Clinical Medicine, Faculty of Medicine

University of Bergen

Institute of Marine Research

2020

Seafood consumption and hair mercury concentrations in adolescents: FINS-TEENS – a randomized controlled three-armed intervention trial

Anna Kleppe Moe

Master thesis in Clinical Nutrition

Department of Clinical Medicine, Faculty of Medicine

University of Bergen

Institute of Marine Research

2020

Supervisors:

Dr. Scient. Lisbeth Dahl[a]

Dr. Scient. Marian Kjellevold[a]

Dr. Scient. Robin Ørnsrud[a,b]

[a]Institute of Marine Research

[b]University of Bergen

Table of Contents

ACKNOWLEDGEMENTS

I am very grateful to have had the opportunity to complete my master's degree at the Institute of Marine Research, Section of Food Security and Nutrition ("Seafood and Nutrition" as of 01.09.19), surrounded by such skilled and knowledgeable people in a pleasant environment. I would like to thank my supervisors Lisbeth Dahl and Marian Kjellevold for providing their knowledge and guidance in the work with this thesis. Thank you for being so encouraging as well as giving constructive feedback for improvements of the thesis, and for being so flexible and willing to adapt the course of the work with the thesis during my pregnancy and maternity leave.

I want to thank co-supervisor Robin Ørnsrud, for invaluable feedback and guidance towards the end, and Berit Solli for taking the time to teach me the method at the lab, and also for spending countless hours trying to repair the analysis machine. A special thanks to Synnøve Næss for always taking the time to help and thoroughly answer all sorts of questions and Ragnhild Marie Mellingen for reading through the thesis and giving feedback.

I would like to thank my fellow students at the University of Bergen from the two classes of clinical nutrition I have been a part of over the course of completing this degree. A special thanks to fellow students writing their thesis at IMR 2018/2019 for great teamwork, a fun and supportive environment, uplifting lunchbreaks and interesting discussions.

Finally, a big thank you to my loving husband Håvard Moe for his endless support and patience, always lifting me up and cheering me on. Thank you to my dear children, Laura and Jona – my biggest pride, joy and motivation in life.

Anna Kleppe Moe

Bergen, May 2020

ABSTRACT

Background: Seafood is recognized as an excellent dietary source to several beneficial nutrients yielding positive health effects and is a recommended part of a healthy diet. Seafood is also a source of contaminants, such as mercury (Hg), which is a neurotoxic heavy metal of food safety concern. Humans are predominately exposed to Hg and more precisely the hazardous organic chemical form methylmercury (MeHg) through seafood consumption. Total hair mercury (THHg) concentration is an accepted proxy for MeHg exposure.

Objective: To investigate seafood consumption, estimate MeHg exposure from seafood consumption, determine THHg concentrations and measure the change in these parameters, among adolescents participating in a three-armed intervention with oily fish, meat or omega-3 supplements in the Fish Intervention Studies Teens (FINS-TEENS) trial.

Methods: Adolescents living in Bergen, Norway, participating in the FINS-TEENS trial were randomized (n=478) to receive school lunch meals with either oily fish or meat, or omega-3 supplements, three days a week for 12 weeks. Seafood consumption and MeHg exposure were estimated using a food frequency questionnaire (FFQ) pre- and post-intervention. Hair samples were collected pre- and post-intervention and THHg concentrations were determined using a direct mercury analyzer method (DMA-80) (n=116).

Results: Pre-intervention median seafood consumption was 277 g/week, median MeHg exposure was 18 μg/week, and median THHg concentration was 127 μg/kg. No significant changes in seafood consumption were found within any groups from pre- to post-intervention, but seafood consumption was significantly higher in the fish group compared to the omega-3 group post-intervention (p=0.13). A significant decrease in estimated MeHg exposure was found in the study population (p=0.007) and omega-3 group (p=0.012), as well as a borderline significant decrease in the fish group (p=0.05). No significant differences were found between the three intervention groups in THHg concentrations (p=0.241) or in the change of THHg concentrations (ΔTHHg) (p=0.914) post-intervention. Medium positive correlations were found pre-intervention between total seafood consumption and THHg concentration (r=0.361, p<0.01), and between

estimated MeHg exposure and THHg concentration (r=0.33, p<0.01). Post-intervention there was also a medium positive correlation between total seafood consumption and THHg concentration (r=0.364, p<0.01). When analyzing by intervention groups, small, medium and medium correlations were found in the fish (r=0.257), meat (r=0.361) and n-3 (r=0.472) group, respectively.

Conclusion: School lunch meals with oily fish did not lead to a significant increase in seafood consumption, estimated MeHg exposure or THHg concentration. Median baseline seafood consumption in this study population is below the dietary recommendations. The median estimated MeHg exposure and median THHg concentration were within the tolerable intake level set by the European Food Safety Authority (EFSA) and reference dose set by the United States Environmental Protection Agency (USEPA), respectively. More research on MeHg exposure and THHg concentrations in adolescents is desired, preferably in a population with seafood consumption in accordance with the Norwegian dietary recommendations.

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BBB	Blood-brain barrier
BMD	Benchmark dose
BMI	Body mass index
bw	Body weight
CI	Confidence intervals
CNS	Central nervous system
CRM	Certified reference material
DEMOCOPHES	Demonstration of a study to coordinate and perform human biomonitoring on a European scale
DHA	Docosahexaenoic acid
dl-PCB	Dioxin-like polychlorinated biphenyl
DMA-80	Direct Mercury Analyzer-80
DPA	Docosapentaenoic acid
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic acid
FAO	Food and Agriculture Organization
Fe	Iron
FFQ	Food frequency questionnaire
FINS	Fish Intervention Studies
GI	Gastrointestinal
HELENA	Healthy Lifestyle in Europe by Nutrition in Adolescence
Hg	Mercury
IQR	Interquartile range
IMR	Institute of Marine Research (Havforskningsinstituttet)
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LCPUFA	Long-chain polyunsaturated fatty acids
LOAEHC	Lowest observable adverse effects hair concentration

LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
LOQ	Limit of quantification
MeHg	Methylmercury
MoBa	The Norwegian Mother and Child Cohort Study
n-3	Omega-3
nm	Nanometer
NOAEL	No observed adverse effect level
NOK	Norwegian krone
PTWI	Provisional tolerable weekly intake
RCT	Randomized controlled trial
RfD	Reference dose
SD	Standard deviation
SDQ	Strengths and difficulties questionnaire
SFA	Saturated fatty acids
SH	Sulfhydryl
SPSS	Statistical package for the social sciences
TEQ	Toxic equivalent
THHg	Total hair mercury
TWI	Tolerable weekly intake
µg	Microgram
USEPA	The United States Environmental Protection Agency
VKM	Vitenskapskomiteen for mat og miljø (Norwegian Scientific Committee for Food and Environment)
WHO	World Health Organization

1. INTRODUCTION

1.1 Nutrition in adolescents

1.1.1 Dietary recommendations

The Norwegian directorate of health has provided national nutritional recommendations to promote public health and prevent chronic diseases. These are summed up in thirteen dietary guidelines addressing diet and nutrition as well as physical activity. Even though they are mainly directed at healthy adults, these advice can also be applied to people in other stages of life, such as children, adolescents, elderly, pregnant and lactating women, as well as individuals with an increased risk of disease (1).

In short, the recommendations are to maintain energy balance by keeping a diet which is mainly plant-based, with plenty of vegetables, fruits and berries, whole grain and fish, whilst limiting the intake of red and processed meat, added sugar, salt, saturated fats and energy dense food products. Fruits, berries, vegetables, whole grain products and low-fat dairy products should all be eaten on a daily basis, and water should be the main choice of beverage (1).

1.1.2 Consumption in adolescents

A nationwide dietary survey published in 2015, "Ungkost 3", mapped the diet of 608 9-year-olds (4^{th} graders) and 657 13-year-olds (8^{th} graders) in primary school and middle school respectively (2). The results implied that the diet among the participants was quite close to the health authorities' recommendations in several areas, though with significant shortcomings in other: Of the total energy intake in the diet of 13-year-olds, saturated fats made up 14% and added sugar amounted to 12%, both being above the recommended <10% (1, 2). Also, the amounts of vegetables, fruits, fish, vitamin D and iron (Fe) in the diet were all below the recommendations, even when including supplement intakes in the calculations. Nutrient supplements were consumed by 43%; 17% took cod liver oil, 11% took omega-3 (n-3) supplements, 22% took multivitamins, 11% took other vitamin/mineral supplements such as vitamin C or Fe (2).

Amongst the 8^{th} graders, 59% reported to eat lunch every day, even though 70% reported to bring a packed lunch to school 5 days a week. 34% reported to buy lunch in the cafeteria once or twice

a week. The survey mentions underreporting from the 8th graders as a weakness with the study, and that the survey seems to present a more favorable average diet than what is actually the case in the general population of this age group (2).

1.2 Seafood

1.2.1 Background

In this thesis, the term *seafood* includes vertebrate and invertebrate aquatic animals, with fish being the largest group within the term. Included are farmed or wild aquatic animals, of freshwater or marine origin, with the exception of jellyfish, aquatic reptiles, echinoderms and aquatic mammals (3). *Fish* and *seafood* are often used interchangeably in literature, and the same will therefore sometimes be seen in this thesis.

Fish and seafood are sources of several beneficial nutrients such as high-quality protein, vitamin D, vitamin B12, the polyunsaturated long chain fatty acids eicosapentaenoic acid (EPA 20:5 n-3) and docosahexaenoic acid (DHA 22:6 n-3), selenium and iodine. On the other hand, fish and seafood can also accumulate high levels of contaminants and toxic elements such as mercury (Hg), dioxins and dioxin-like polychlorinated biphenyls (dl-PCBs) raising human health-related concerns (1, 4-6).

The risk-benefit assessment by the Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) concluded in 2014 that the levels of contaminants in fish present negligible risks and are of no concern for consumers (4). On the other hand, a new assessment by the European Food Safety Authority (EFSA) in 2018 led to a new tolerable weekly intake (TWI) level for dioxins and dl-PCBs being set (7). The new TWI is seven-times lower than the 2001 EU limit set by the European Commission's Scientific Committee on Food. Following this, a new risk-benefit assessment from VKM is in preparation as well, expected to be published in 2021 (8).

1.2.2 Health benefits

Fish consumption has been shown to provide positive health effects, preventing cardiovascular diseases in particular (5, 9). It is important *in utero* as well as during infancy in regard to cognitive development and can contribute to the preventing of cognitive decline and dementia (5). VKM has concluded that these positive health effects of fish consumption can be seen when consuming from 1-2 dinner servings of fish per week, and up to 3-4 servings per week. Because the number of even higher fish consumers was limited in epidemiological studies, no firm conclusions could be drawn on risk and benefit in individuals consuming larger amounts than 3-4 servings of fish per week (4).

1.2.3 Dietary recommendations

When it comes to fish and seafood, it is recommended to eat fish for dinner 2-3 times a week, equivalent to 300-450 grams of pure fish, of which 200 grams ought to be oily fish, such as mackerel, salmon and trout (1). Using fish as bread spread is highly encouraged as well and six portions of bread spread is the equivalent to one dinner portion. Shellfish, though not specifically included in these recommendations, are recommended as part of a healthy diet as well (1). These recommendations on fish intake are similar to, though generally a little higher than, recommendations in other western countries (10-14).

Though these recommendations are applicable for most groups of the population, certain groups, such as pregnant women, are advised to limit consumption of some fish and seafood during pregnancy because of their potential to contain contaminants, such as Hg. Specifically, these are fish liver, freshwater fish such as pike, perch >25cm, trout >1kg, char >1kg, as well as brown meat from crab, seal meat, Greenland halibut >3kg, shark, skate, swordfish, and fresh tuna (15). Choosing to avoid all fish and seafood, or consume less than 1-2 servings per week, may lead to a lack of beneficial nutrients for pregnant women and their fetuses, so these women should follow the general recommendations on fish consumption even though there are some limitations to the specific fish and shellfish they can safely consume (4).

1.2.4 Consumption of fish in adolescents

According to "Ungkost 3", the 13-year-olds had an average fish intake of 24 g/d, equivalent to 168 g/week. Of all participants, 28% reported taking fish-based supplements like cod liver oil or n-3 capsules (2).

A similar nationwide dietary survey published in 2011, "Norkost 3", mapped the diet of the adult population of Norway, aged 18 through 70 years. It concluded that the fish consumption is too low in a large part of the adult Norwegian population, as only one third of the population followed the recommendations (16).

Similar trends have been observed in other European countries: A German survey found the mean fish intake among adolescents age 13-14 years to be 97 g (males) and 71 g (females) per week, but when only including the actual fish consumers in these groups, the mean intake was 243 g (males) and 189 g (females) fish per week (17). In a national diet survey in the UK published in 2017, only 5.3% met the fish recommendations of ≥ 280 g of fish/week among 12-18 year-olds, with a mean total fish intake of 73 g/week (14, 18). In a cohort among Danish adolescents, the median weekly fish intake was 75 g, though higher in boys than girls (19). Adolescents from 10 European cities aged 12.5-17.5 years reported a mean fish intake of 144 g/week (boys) and 139 g/week (girls) in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study (20).

1.3 Mercury

1.3.1 Background

Mercury (Hg) is a heavy metal and a non-essential element, present in the diet mainly via consumption of fish and seafood. It is considered one of the top ten chemicals of major public health concern according to the World Health Organization (WHO) because of its toxic effects on nervous, immune and digestive systems, as well as kidneys, skin, eyes and lungs. It is a serious threat to development of life *in utero* and early life (21).

Hg occurs naturally in the earth's crust and is released into the environment through anthropogenic sources such as coal-burning and industrial processes, and through natural sources such as the weathering of rocks, volcanic activity, soil and water surfaces. It is liquid in room temperature (22).

Ancient and previous use of Hg include medicinal use (in infant teething powders, to treat infections, to treat syphilis), as antifungal agents in seed grain, as antiseptic preservative, in the production of felt hats, in barometers and thermometers. Current use, despite widespread concern of their potential toxic effects, still include in vaccines and dental amalgam, though the use has been phased out in many countries (22, 23).

1.3.2 Chemical forms

Hg exists in two main forms; inorganic Hg (Hg_2^{2+} and Hg^{2+}) including elemental Hg (Hg^0), and organic Hg, which is the main form of exposure through the diet. The toxic effects are different depending on the chemical form (21).

Inorganic Hg includes three oxidation states; Hg^0 (elemental or metallic), Hg_2^{2+} (mercurous) and Hg^{2+} (mercuric) (22, 24). Metallic Hg exists as a silvery liquid and releases a gas called mercury vapor which is chemically stable and can exist in the atmosphere for months or years, leading to a global cycling of Hg. This form of zero oxidation state, Hg^0, can further be oxidized into the two oxidation states of Hg; Hg has either lost one electron and can be found with two Hg atoms linked together, or has lost two electrons and exists as the mercuric ion. When Hg is combined with other elements, such as chlorine or sulphur, these compounds can be called Hg salts. The liquid metallic Hg causes little hazard if ingested, but the Hg vapor is highly toxic if inhaled and can cross the blood-brain barrier (BBB) and damage the central nervous system (CNS) by being oxidized to Hg^{2+} which can be retained in the brain cells for months or years. Other forms of inorganic Hg can also cause kidney damage (22).

Organic Hg are compounds where the mercuric Hg-ion is covalently linked to one or more carbon atoms, such as ethylmercury, phenylmercury and methylmercury (MeHg) which is most common (22). Once emitted to the atmosphere by anthropogenic and natural sources, elemental Hg vapor

is converted to mercuric Hg slowly through oxidation and is then returned to earth in rainwater. The inorganic Hg compounds that end in aquatic sediments are then naturally converted and methylated by the help of bacteria in both marine and freshwater systems into MeHg. It is assumed that these oxidation-reduction and methylation-demethylation reactions between different forms of Hg are widespread in the environment (Figure 1), though methylation is more frequent in aquatic environments than demethylation (24). It is this MeHg which enters the aquatic food chain and further bioaccumulates in fish and shellfish before entering the human diet. MeHg accounts for close to all Hg in fish muscle with a few exceptions, such as pilot whales and other sea mammals where up to 50% can be present as inorganic Hg (22, 25, 26).

As it is the most frequent form of Hg exposure as well as the most hazardous form of Hg for humans, the focus in this thesis will be MeHg (27).

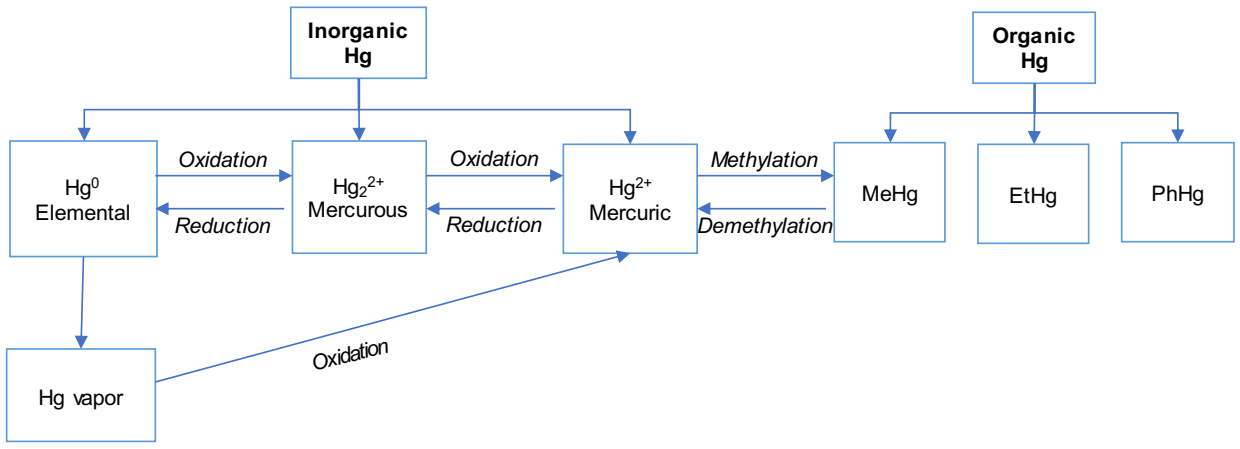

Figure 1: The relationships between different chemical forms of inorganic and organic mercury (Hg)

1.3.3 Toxicokinetics of MeHg

1.3.3.1 Absorption

Inorganic forms of Hg are poorly absorbed in humans (5-10%), but the gastrointestinal (GI) absorption of MeHg into the bloodstream has traditionally been thought to be very efficient, as high as 95-100% (28, 29, p42-46). Recent research challenges this assumption and suggests that the bioavailability of MeHg varies greatly (~2-100%) as it is affected by the Hg source, cooking

methods, interaction with other nutrients, genetics and gut microbiome (30, 31). Some dermal MeHg absorption and absorption through lung inhalation occurs as well, but because of lacking quantitative data, there is uncertainty about to what extent (29, p42, 32, p15).

1.3.3.2 Distribution

In the bloodstream MeHg is distributed by red blood cell transportation to all tissues within about four days, bound to cysteine in hemoglobin, as it has a high affinity for cysteine's thiol groups, also called sulfhydryl groups (SH-groups) (24, 29, p42, 32, p15). Even though distribution is widespread in the body, as much as 10% of the MeHg can accumulate in the CNS. In other tissues, MeHg is converted and stored as inorganic mercury, e.g. in the liver and kidneys. MeHg crosses membrane barriers easily, including the BBB and fetal umbilical cords, both leading to tissues where it accumulates and causes harm; in the brain and the fetus (32, p16).

1.3.3.3 Excretion

Urine, hair and breast milk are all excretion routes for Hg, but fecal excretion is thought to account for 90% of the total elimination of MeHg. This occurs via glutathione carriers as a complex with reduced glutathione. A small fraction is secreted from the liver into bile and is then reabsorbed from the GI-tract where microorganisms can transform and demethylate MeHg into inorganic Hg, which is poorly absorbed and therefore excreted through feces (22, 24, 29, p46-49). Most of it though, is reabsorbed into portal circulation and returned to the liver (33). The whole-body biological halftime of Hg is 70 days on average, and blood-halftime is 50 days, though shorter in lactating women (32, p17).

1.3.4 Toxicodynamics of MeHg

MeHg damages the brain and the CNS causing symptoms such as paresthesia, incoordination, dysarthria, loss of vision and hearing, in worst case coma and eventually death (21, 22). Poisoning has a long latent period which can last for several months (24). It seems the prenatal period is the most sensitive period to MeHg exposure during the life cycle, and that maternal MeHg concentrations during pregnancy highly affect, and can severely damage, the offspring's development of its CNS in a dose-dependent manner (22). It is well established that high exposures to MeHg are harmful, but the threshold of exposure leading to adverse effects is not well defined

(34). Despite attempts to connect risk to relatively low MeHg exposure levels (hair concentrations <1 μg/g total Hg), especially for fetuses and infants through maternal exposure, there is still discrepancy in study findings (34-36). Therefore, there's a lack of consensus in defining a lowest observable adverse effects hair concentration (LOAEHC), but 0.3 μg/g has been suggested (34). There are limited data available on MeHg in children and adolescents, leaving unanswered questions on to what degree adverse effects of exposure can be seen during these years (22). But the Norwegian Fish Intervention Studies Kids (FINS-KIDS) study on preschool children studied the effect of oily fish intake on hair Hg concentrations and cognitive function, and concluded that though lunch meals with oily fish led to an increase in Hg concentrations, values remained lower than risk assessment points that have been set (see section 1.3.6-1.3.7) (37).

The most important way Hg can lead to neurotoxic effects is thought to be by forming complexes with SH-groups. Thiols, such as cysteine, increase the transport of Hg compounds in the body; MeHg-cysteine resembles methionine, an essential amino acid, and MeHg is absorbed into cells bound to cysteine as a methionine mimic by the same mechanism. Such conjugates can then be distributed across cell membranes to all tissues via the large neutral amino acid carrier, as they are treated like neutral amino acids (28, 38). The process of entry into cells as a cysteine complex via the large neutral amino acid carrier is thought to explain MeHg's high affinity and ability to cross the BBB and lead to serious neurotoxic consequences (22, 39).

There have been three large-scale poisoning events caused by MeHg, in Japan and Iraq: For a number of years (1940s-1969) MeHg compounds, byproducts from a chemical factory manufacturing acetaldehyde, were discharged directly into Minamata Bay in Minamata City, Japan. Consuming large amounts of contaminated seafood from the bay, many people in the surrounding village started to show signs of MeHg poisoning in the following years. The first patient suffering from serious neurological symptoms, later known as "Minamata disease", was reported in 1956, and a total of 54 cases including 17 deaths had been reported by 1962. Still, the acetaldehyde production did not stop until 1968 and in 2007 the total number of patients was 2,268 (28, 40). Because of the possibility to study umbilical cords of Minamata babies born during and after the years of poisoning, the Minamata outbreak led to the conclusion that MeHg exposure via the placenta leads to more severe neurological symptoms and consequences for the fetus than for

the mother, who in many cases had mild or no manifestation of the poisoning (28). A similar outbreak after industrial discharge of MeHg took place in Niigata, Japan in 1965, affected more than 1,000 people, and was recognized as the Niigata Minamata disease (40, 41).

In Iraq in 1971-1972 an outbreak of MeHg poisoning resulted in 459 deaths and more than 6000 people being hospitalized. Seed grain had been treated with MeHg fungicide and used to prepare homemade bread which was then eaten. Symptoms were similar to those of Minamata disease (28, 42). There has not been reported any cases of MeHg poisoning from fish consumption where the MeHg present has come only from the natural biomethylation processes (42). In cases such as the Minamata and Niigata outbreaks the high levels of MeHg present in the fish leading to poisoning have come from the industrially discharged MeHg directly (22, 28).

1.3.5 Dietary sources

Organic Hg in the form of MeHg can be found in fish, in mammal meat and poultry following contaminated water and fodder, as well as in foods treated with fungicides, pesticides and insecticides containing MeHg (38, 43). It is, though, mainly the long-term fish consumption which is the dominant predictor almost completely determining the MeHg, and usually total Hg, concentrations in a person. Other sources are seen as insignificant in comparison, in most populations (24, 32, p15, 44). The European project DEMOCOPHES (DEMOnstration of a study to COordinate and Perform Human biomonitoring on a European Scale) provided knowledge on this by demonstrating the association between fish consumption and MeHg concentrations. They did a clustering analysis of the fish consumption of 1799 mother-child pairs from 17 European countries, where Belgium, Denmark, Spain, Portugal and Sweden were eventually placed in the high fish consumption branch, whereas Switzerland, Czech Republic, Hungary, Poland, Romania, Slovenia and Slovakia were placed in the low fish consumption branch (45). There was a strong correlation between consumption of fish products and MeHg exposure shown by Hg concentrations in hair, and between mother and child, supporting findings from previous similar studies (46).

Humans are estimated to have a total daily MeHg exposure of 2.4 μg from all sources, with an uptake of 2.3 μg (32, p15). Even a small fish intake will affect this level greatly as 150 g of fish

(e.g. Atlantic cod fillet) containing 9.4 µg Hg/100 g will yield an intake of as much as 14.1 µg Hg, mainly in the form of MeHg (47).

MeHg rapidly bioaccumulates in the aquatic environment, attaining its highest concentration in the fish on top of the food chain, meaning large predatory species such as trout, tuna, shark, pike, walleye and swordfish contain much higher levels of MeHg than smaller, non-predatory species (Figure 2). Other factors affect MeHg levels in fish as well, such as the age and size of the fish, MeHg in the upper layer of the sediment, microbial activity, salinity, pH and redox potential (24). As the MeHg present in fish is found bound to muscle, the protein level in the fish partly determines the amount of MeHg as well (4).

Fish oil and cod liver oil have been shown to contain very low Hg concentrations at the limit of quantification (LOQ), ranging from "nondetectable" (<6 µg/L) to "negligible" (10-12 µg/L) levels (4, 48).

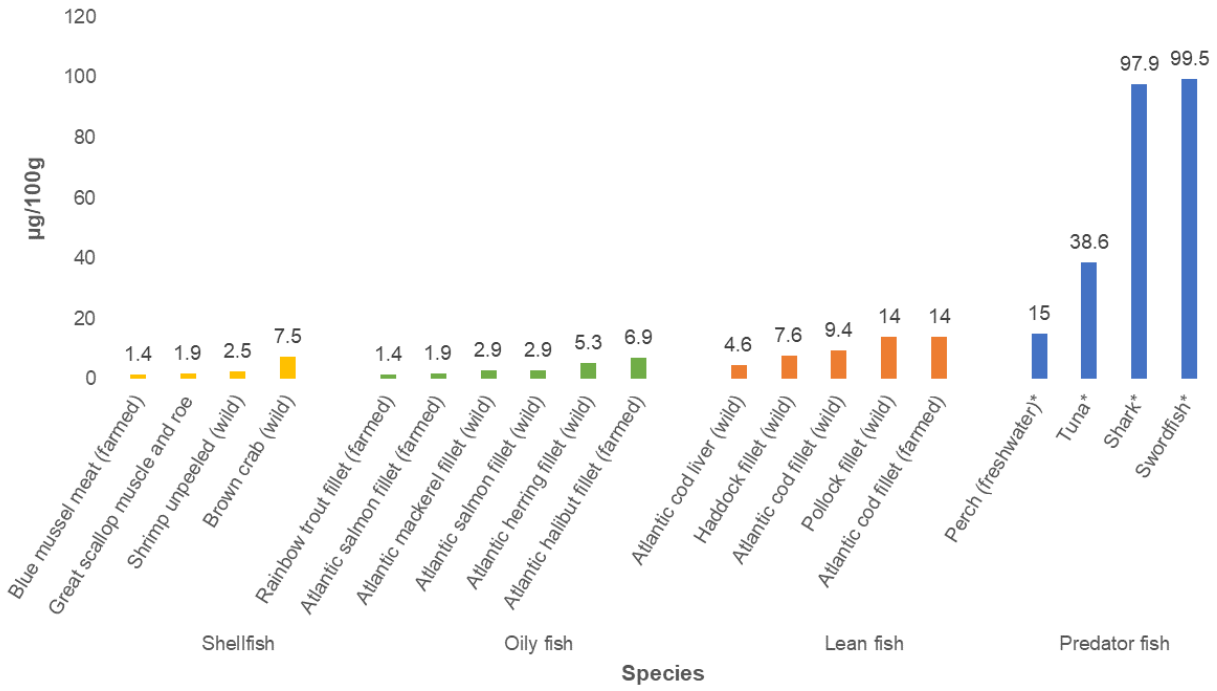

Figure 2: Mercury concentrations in different seafood species
Data retrieved from Seafood data unless otherwise stated (https://sjomatdata.hi.no) (47)
*Data retrieved from United States Food and Drug Administration (FDA) (49).

1.3.6 Tolerable intakes

Tolerable weekly intake (TWI) is defined as the maximum weekly intake of a substance which can be consumed over an entire lifetime without risking adverse health effects (50). The no observed adverse effect level (NOAEL) is the highest concentration of a substance where no adverse effect has occurred in an exposed population whereas the lowest observed adverse effect level (LOAEL) is defined as the lowest concentration of a substance which has caused adverse effects in an exposed population (50). The approach to establishing a TWI is to use NOAEL and/or LOAEL values from the critical studies and apply safety or uncertainty factors to these values (51).

Based on prenatal MeHg neurodevelopmental toxicity, EFSA has established a TWI of 1.3 µg MeHg/kg body weight (bw) (52). The Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) also found neurotoxic effects as a result from *in utero* exposure to be the most sensitive health outcome when evaluating MeHg. Hence, they established a provisional TWI (PTWI) of 1.6 µg/kg bw sufficient to protect developing fetuses, PTWI being a term JECFA uses for contaminants that might accumulate in the body (53). Inorganic Hg has an established TWI of 4.0 µg/kg bw (52). VKM, being EFSA's Norwegian focal point, has not established its own TWI for MeHg but concluded in their benefit-risk assessment report in 2014 that in the amounts consumed by the Norwegian population as of today, the benefits following seafood consumption outweigh the negligible risk the current levels of contaminants like Hg represent (4). In 2019, a new VKM report investigating different scenarios for MeHg exposure from fish stated that people with average, or even high (1000 g/week), fish consumption of the most common fish species (e.g. Atlantic cod and farmed Atlantic salmon) are below the TWI for MeHg as these are regarded as species with low Hg concentrations (54). But in the same report they also said that people who consume more than one portion per week of fish species with high Hg concentrations (e.g. tusk, pike, perch) might be exposed to concentrations exceeding the TWI.

1.3.7 Methods for measuring MeHg exposure

Biomarkers to estimate human exposure of MeHg are hair and blood, with hair levels being up to 250 times more concentrated than blood levels, and therefore a widely used biomarker of Hg exposure to humans (24, 55, 56). MeHg in blood represents recent Hg exposure, and at the time of

formation of a hair strand, the MeHg hair concentrations are proportional to these blood concentrations. But generally, hair concentrations recapitulate previous blood concentrations depending on the growth period of the hair segment measured. As hair grows about one cm per month, the centimeters closest to the root will be representative for the average exposure from the last 1-2 months (24, 57).

A reference dose (RfD) for the total concentration of Hg in hair (THHg), which is an accepted proxy for MeHg exposure (45), has been set to 1000 µg/kg by The United States Environmental Protection Agency (USEPA) (58). This RfD is based upon data from 81 Iraqi children exposed to MeHg *in utero* and the neurological changes which followed, as well as epidemiological studies from Faroe Islands, New Zealand and the Seychelles Islands, three areas where fish consumption and maternal hair Hg concentrations are particularly high (56, 59-61). From this, a benchmark dose (BMD) was set to a hair concentration of 11 000 µg/kg as well as an uncertainty factor of 10 to extrapolate the data to sensitive human subpopulations, in this case; the fetus. As USEPA has taken into account that fetuses are particularly prone to poisoning via maternal exposure, this value is considered safe and applicable to people in all ages and life stages, and people with THHg below the RfD are unlikely to experience adverse effects, expectant mothers and their fetuses included (29, 62).

In Norway, there is a lack of studies on THHg concentrations among adolescents. However, Mommy's Food (63, 64) and the Little in Norway study (65) on mothers and their infants as well as the FINS-KIDS study (37) on Norwegian preschool children, have all shown THHg concentrations below the RfD, with median concentrations ranging from 266-465 µg/kg. Studies in adolescent populations in other countries have found varying THHg concentrations among this age group, though generally well below the established RfD (Table 1).

Table 1: Mean total hair mercury concentrations from different studies in adolescent populations

Country	Population size	Population age	THHg (µg/kg)	Reference
Spain	96	13-16	550	Peña-Fernández 2017 (66)
Spain	96	12-14	560	Ferré-Huguet 2009 (67)
Faroe Islands	860	14	960	Budtz-Jørgensen 2004 (68)
Czech Republic	150	13-14	280, 380, 460 [ab]	Čejchanová 2008 (69)
Czech Republic	3,556	9.9	190[b]	Benes 2003 (70)
Brazil	167	12-18	140	Carneiro 2011 (71)
USA	516	16-19 (only women)	290	McDowell 2004 (55)

[a]Three different regions

[b]Values are medians

1.4 Dietary assessment

Measuring quantity and quality of people's usual dietary intakes is necessary in various research settings but is a very difficult task which no single method can assess perfectly. Therefore, the method should be carefully chosen to fit the research objective, available resources and design (72). Nutritional biomarkers can be used in clinical assessment for objective estimates of a person's diet, and there are also several methods that can be used to provide subjective estimates to assess an individual's or a population's dietary intake: 24-hour dietary recall done once or repeatedly, duplicate diet approach, weighed food records, dietary history and food frequency questionnaire (FFQ) (72, 73). Several of these methods have limitations, making them unsuitable for large-scale epidemiological studies, but the FFQ which was first introduced in the 1960s has multiple benefits making it a widely used tool to collect dietary data in epidemiological studies (74): it is easy to collect and process, it is time- and cost-efficient and imposes less of a burden on respondents than most other methods (73). The FFQ is mainly qualitative and aims to assess which food items or food groups are consumed and with what frequency during a time period. This method can also include some quantification by including portion-size estimates in the questionnaire, a so-called semi-quantitative FFQ. It is done retrospectively and if made with specific food group combinations this method can be used to predict intakes of certain nutrients or non-nutrients and also to estimate energy intakes. Potential limitations are that the FFQ is a subjective measure, and

results are therefore prone to recall bias, and over- or underestimation of intakes are common (72, 75).

Complementary, using a 24-hour dietary recall for example to assess the actual dietary intakes of study participants can provide more detailed intake data, but requires a trained interviewer and is more expensive and time-consuming (72). The long-term diet is not assessed with this method, making it unsuitable for investigating chronic disease or long-term dietary exposure. Repeated 24-hour recalls are needed to measure an average dietary intake of specific food items or food groups, and multiple administrations have been shown to improve the accuracy of the method (75). Minimizing recall bias is a challenge for the interviewer as all information depends on the memory of the respondent (72).

In recent years, a combination of long- and short-term methods, for example FFQ together with 24-hour recall, has been suggested to provide more accurate estimates of dietary intakes (74). Further research on methods and improvements of their accuracy using innovative technologies are still ongoing in attempt to find the best one for nutritional epidemiology (75-77). The main goal is to enhance dietary assessment in terms of decreasing the costs and increasing time-efficiency, while at the same time increasing the accuracy of results (76). To this date, the FFQ is still often the preferred method for assessing dietary intakes in epidemiological studies, despite its limitations.

1.5 Study aims and hypothesis

This thesis is a part of the Fish Intervention Studies Teens (FINS-TEENS) study conducted in 2015 among Norwegian adolescents where the main aim was to study associations between fish consumption and cognition. Adolescents were randomized to receive school lunch meals with either oily fish or meat, or n-3 supplements, three days a week for 12 weeks, and a variety of biological samples as well as questionnaires were collected pre- and post-intervention. In this master thesis, the FFQ and hair samples from the study will be used to investigate the association between dietary seafood consumption and concentration of THHg in adolescents. The means to achieve this, are to

- Estimate habitual seafood consumption and MeHg exposure based on FFQs
- Analyze and evaluate total Hg concentrations of hair samples in adolescents
- Measure the change in seafood consumption, MeHg exposure and THHg concentration from pre- to post-intervention, and differences between intervention groups

The hypothesis for this thesis is that adolescents in the fish intervention group will have an increase in seafood consumption, MeHg exposure and concentration of THHg compared to the meat group and n-3 supplement group after the intervention.

2. METHODS

2.1 Study design – FINS-TEENS

FINS-TEENS is one of seven studies included in FINS where investigating the effects of lean and oily fish on type 2 diabetes and obesity as well as mental health and cognition was the overall aim. FINS-TEENS is designed as a parallel, three-armed, non-blinded, randomized controlled intervention trial (RCT) and was carried out on 9th grade adolescents in Bergen, Norway, aged 14-15 years in February-May 2015 (78).

2.2 Ethics

The protocol was presented for the Regional Committees for Medical and Health Research Ethics and approved by Norwegian Data Protection Official for Research (project number 41030). The trial is registered in ClinicalTrials.gov (NCT02350322) and was performed according to the Declaration of Helsinki. Participation was voluntary, adolescents provided written informed consents together with their caregiver(s) (Appendix I) and had the possibility to withdraw without reason at any time of the study (79, 80).

2.3 Study population

2.3.1 Recruitment

The school recruitment process took place between August and October 2014. All 26 secondary schools in Bergen were invited, by e-mail or telephone, to participate in the FINS-TEENS study,

and eight schools ended up participating. A flow-chart of the recruitment process is presented in Figure 4. Inclusion criteria for the schools were as follows: motivation to participate, three 9th grade classes or more, and rooms to provide for data collection and preparation of meals during the intervention. Exclusion criteria were if the school already had a well-functioning canteen, as this is uncommon in Norwegian schools. The eight participating schools represented five out of eight boroughs in Bergen (Figure 3). The recruitment of participants took place in January 2015, written informed consent was signed by pupil and parent/caregiver, and teachers assisted in collecting these. Exclusion criteria were allergy or intolerance to the study meals or supplements, and a lack of familiarity to the Norwegian language. Of the 785 eligible adolescents invited, 478 (61%) wanted to take part in the study and were randomized to intervention groups, 249 of these were girls, 229 were boys (78) (Figure 4).

Figure 3: Map of the location of the schools enrolled (n=8) in the FINS-TEENS study in Bergen, Norway
Picture: (78)

2.3.2 Randomization

The adolescents were randomized within each school to receive lunch with either oily fish, meat or n-3 long-chain polyunsaturated fatty acid (LCPUFA) supplements after being stratified according to gender. In each school, two boxes, one for girls and one for boys, contained 1/3 pieces of paper marked “Fish”, 1/3 marked “Meat”, and 1/3 marked “Supplements” adding up to the total number of pupils enrolled in that particular school. To determine the intervention group each individual would belong to, a blinded researcher drew notes one by one, from the correct box after being told by a different, not blinded, researcher what each participant’s gender was (79). The researcher not blinded further registered the assigned intervention for each participant in a spreadsheet. Due to the nature of the dietary intervention, it was not possible for either executive researchers nor participants to be blinded.

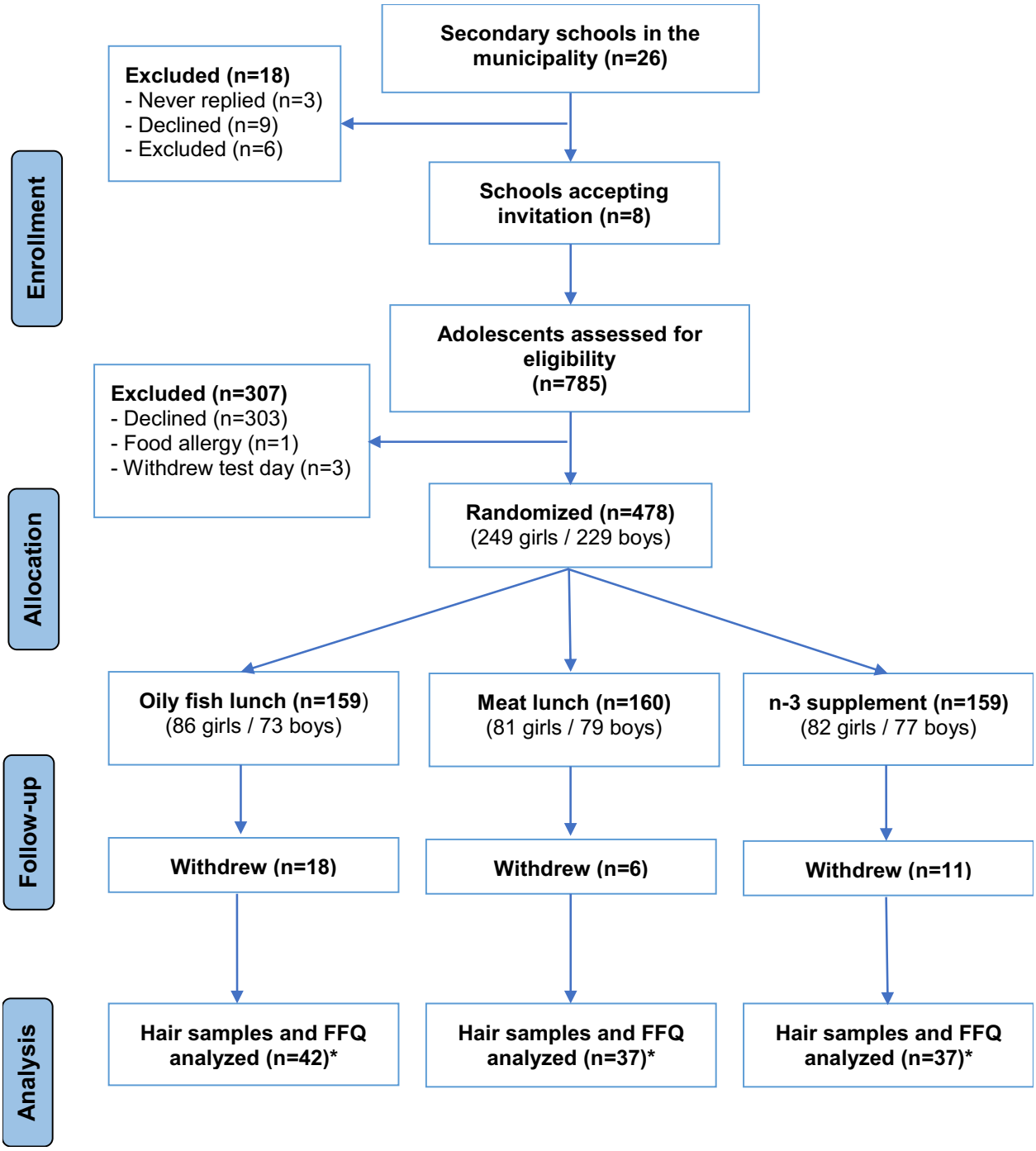

Figure 4: Flow chart of the study population
*Only a selection was analyzed because of technical problems with the instrument (See paragraph 3).
Abbreviations: FFQ, food frequency questionnaire; n-3, omega 3

2.3.3 Sample size and power

The power calculation of sample size assumed a correlation of 0.5 between pre- and post-intervention measurements and was based on the three-armed intervention design. A small to moderate effect size (Cohen's *d*=0.35 (81)) was applied to reveal a meaningful effect of the intervention on the primary outcome ("d2 test of attention"). Based on a significance level of α=0.05 and a power of 80%, a sample size of 119 participants in each group was desired. Enrolling a total of 446 participants was the aim, when considering a 20% drop out rate (78). This sample size estimate was based on the primary outcome, which was measured by the d2 test of attention, not on detecting differences in hair Hg concentrations between the groups, which is the topic of this thesis. The calculations were done using Stata Statistical Software: Release 14. College Station, TX: (STATA-Corp LP®) (82).

2.4 Intervention

For 12 weeks, excluding school holidays, the participating pupils received either school lunch meals with oily fish or meat, or n-3 LCPUFA supplements three days a week, 36 days in total.

2.4.1 Study meals

The research team took part in developing the study meals, and they were made and delivered to the schools by a Bergen-based catering service (Søtt+Salt AS). In the schools, trained study personnel put the participants' name and class on and distributed the meals and supplements into one case for each classroom. Participants took the supplements and ate their lunch in their classrooms under supervision by the study-worker (Figure 5-Figure 6). The meals were similar in content except for the fish and meat (80-100 grams per portion) and had wholegrain pasta/focaccia/baguette/tortilla as well as vegetables/salad and sometimes dressing (Table 2). Levels of some micronutrients differed between the study meals and higher amounts of vitamin D, n-3 fatty acids, dioxins, dl-PCBs and Hg was found in the fish meals whereas levels of saturated fatty acids (SFA) were higher in the meat meals (Table 2) (79). They were designed to follow dietary guidelines for a healthy diet by the Norwegian directorate of health (1). The fish group received different types of oily fish; salmon (average 21 times), mackerel (average 3 times) and herring (average 10 times). The meat group received both white (average 24 times) and red meat

(average 10 times); chicken, turkey, beef and lamb, and cheese was served together with the chicken/turkey ham nine times. Pork meat was not used, and halal meat could be provided upon request. The total number of servings of meals and supplements varied from 30-36 at different schools, with a mean of 34 times (78).

The supplement group received easy to swallow-capsules (Nycoplus® Omega-3, 500 mg, Takeda Nycomed, Asker, Norway) of 500 mg concentrated fish oil, with 105 mg DHA, 158 mg EPA and 13 mg DPA. 90 grams of fish in the fish group corresponded to eight capsules per serving, though this number of capsules was reduced to seven four weeks into the trial because the frequency of mackerel in the fish group was being reduced, after multiple feedbacks that it tasted bad (78). The supplement group will be referred to as “n-3” in the results.

Figure 5: Study meals the way they were handed out to participants in their classrooms
Photo: Emil Breistein

Figure 6: Supplements the way they were handed out to participants in their classrooms
Photo: Emil Breistein

Table 2: Analyzed nutrient and contaminant concentrations of study meals, per portion
Values are medians and retrieved from Skotheim et al. 2017 unless otherwise stated (79).

	Fish meal (n=27)	**Meat meal (n=26)**
Whole portion (g)*	233	227
Fatty fish or meat/cheese (g)*	71	69
Bread/pasta/wraps (g)*	111	110
Vegetables (g)*	42	41
Energy (kcal/100g)	227.4	229.7
Total fat (g/100g)	9.3	8.4
Protein (g/100g)	9.8	11.4
Vitamin D (µg/100g)	1.6	<1[a]
Iodine (µg/100g)	2.2	2.0
n-3 (mg/100g)	774.0	140.5
SFA (g/100g)	1.9	2.84
Dioxin and dl-PCBs (ng TEQ/kg)	0.19	0.12
Mercury (µg/100g)	0.7[b]	0.2[b]

*Values are means

[a]n=4, only 4 meals had vitamin D levels above LOQ

[b]Values calculated for this thesis

Abbreviations: LOQ, limit of quantification; n-3, omega-3; SFA, saturated fatty acids; dl-PCB, dioxin-like polychlorinated biphenyl; TEQ, toxic equivalent

2.4.2 Safety

To evaluate food safety aspects of the intervention, levels of dioxins, dl-PCBs and Hg were assessed and calculated according to the TWI in both meal groups (79). All levels were below set TWI (7).

2.4.3 Dietary compliance

Any leftovers were to be wrapped and put back into the case which was then collected by the study-worker. The number of n-3 LCPUFA capsules left from participants in the supplement group was registered by research assistants into a spreadsheet together with a graded score 0-4 defining how much of the meal had been eaten by participants in the meat and fish groups: '0=nothing eaten', '1=1/4 eaten', '2=2/4 eaten', '3=3/4 eaten' and '4=all eaten'. Both the amount of meat or fish consumed, and amount of the entire meal consumed were registered in such quartiles, though they were not weighed (78). To be able to compare the dietary compliance of contestants across all three intervention groups, the scale for registering n-3 supplement intake was transformed into the scale for registering intake in the meal groups. This was done by dividing each participant's total intake of n-3 capsules by the factor 1.8125. This factor was extracted from the factor 261/144 which represents the maximum number of n-3 capsules a participant could consume during the entire intervention (9 days with 8 capsules, 27 days with 7 capsules = 261), divided by the maximum intake score possible from the meal groups (36 days with score 4 = 144) (79). Hence, it was possible to compare intakes for each participant in all three groups and compliance to the intervention. For this thesis, the dietary compliance data of participants in the fish and meat groups was used together with study meal content (Table 2) to calculate seafood consumption and estimate MeHg exposure from study meals in addition to the background diet from the FFQ data.

2.5 Data collection

Pre- and post-intervention, several data were collected: biological samples (hair, urine, blood), cognitive tests (both reading, spelling and attention performance), a mental health status assessment (the Strengths and Difficulties Questionnaire (SDQ)) and an FFQ for dietary assessment (Appendix II). Collection took place during school hours in classrooms provided for this purpose and was performed by the same study crew. The FFQ and cognitive tests were

administered before lunch, biological samples after lunch. In addition, parents/caregivers received an electronic questionnaire to assess their educational level, household income and marital status, as well as their child's mental health status (SDQ) from their point-of-view (Appendix III) (78). Only the hair samples and FFQ from both pre- and post-intervention as well as small parts of the caregiver questionnaire were used for this thesis.

Throughout this thesis, the term "pre-intervention" will include hair samples taken and the FFQ administered just *before* the intervention with study meals began, which represents the habitual diet three months prior to start of intervention. "Post-intervention" addresses hair samples taken and the FFQ administered just *after* the intervention, which represents the habitual diet during the three-month long intervention period. Post-intervention also includes seafood and Hg content from study meals.

2.5.1 Hair samples

Hair samples for Hg analysis were collected by cutting a 2-5 mm bundle of hair from the occipital area of the scalp with stainless steel scissors (Figure 7). The samples were then tied with a dental floss or similar in the end that had been cut, to make sure hair from this end was later used for analysis (Figure 8). They were put in separate zip-lock bags in room temperature, labelled with ID- and project-numbers as well as "Start" (beginning) or "Slutt" (end) to mark if they were collected pre- or post-intervention, respectively. Pre-intervention samples from one school were stored together in a bigger zip-lock bag, the same for post-intervention. The samples were stored at the Institute of Marine Research (IMR)*.

*Previously NIFES (The National Institute of Nutrition and Seafood Research) until January 1st 2018

Figure 7: Collection of hair samples from participants
Photo: Emil Breistein

Figure 8: Preparation of hair samples from participants
Photo: Emil Breistein

2.5.2 Dietary assessment

An FFQ (Appendix II), assessing the participants' habitual diet the last three months, was completed both pre- and post-intervention. This questionnaire was semi-quantitative retrospective and contained 32 questions concerning the frequencies of consuming different food groups as well

as physical activity and baseline characteristics (83). Post-intervention, participants were told not to include the food served in the trial when answering the FFQ as it was supposed to measure their habitual background diet (80). The FFQ took about 15 minutes and was completed electronically with the survey program Qualtrics.com® (Provo, UT, USA) (80). Each participant was given a personal username and password to login by research staff. Age, weight, height and gender were self-reported and body mass index (BMI) (kg/m^2) was calculated afterwards.

2.6 Data processing

2.6.1 Processing of hair samples

Two cm of hair from the end nearest the scalp was used for the Hg-analysis of the samples pre- and post-intervention. A well-accepted assumption is that the growth rate of human hair in occipital regions of the scalp is approximately 1 cm/month. LeBeau et al.'s research concludes that 1.06 cm/month is more correct, though the actual growth rate will vary between individuals (0.65-2.2 cm/month) (84). They also discovered inconsistencies in the collection of hair samples as the range of hair lengths left when trying to cut the hair as close to the scalp as possible varied from 0.1-3.0 cm between collectors and samples. Based on their research and calculations, the 2 cm used for analysis here will presumably equal Hg exposure from the last 5.2±0.8 to 12.4±1.6 (95% confidence intervals (CI)) weeks prior to collection, when including a two week delay before the new hair forming in the follicle reaches the scalp (84). The hair samples were measured using a ruler and the amount needed for analysis (2 cm) was cut off with stainless steel scissors.

2.6.2 Analysis of hair samples with DMA-80

The hair samples were placed in separate metal boats (nickel) and weighed-in on a calibrated four-decimal scale (Sartorius, CP124S, USA), the weight and ID-number was noted manually, before the boats were placed on a tray with position numbers. Ideally, each 2 cm hair sample should weigh between 10-20 mg for THHg to be within the calibrated area for the method, but the weight of the samples for this thesis varied between 1.9 and 19.6 mg with a mean value of 7 mg.

The boats were placed in the auto sampler of a machine designed to measure total Hg concentrations specifically by direct mercury analysis, DMA-80 (Milestone, Sorisole, Italy), and the weight and ID-number of each sample was manually plotted into the DMA-80 prior to analysis. The DMA-80 has 40 metal boat positions per analysis and was loaded as follows: Positions 1-2 contained empty boats to avoid contamination, positions 3, 4, 21 and 40 contained certified reference material (CRM) to assess analysis accuracy, positions 5-20 and 22-39 contained hair samples. A total of 34 hair samples were analyzed in one full series. Pre- and post-intervention samples with the same ID-number were analyzed in the same series, and there were samples from at least two different schools in each full analysis series. After analysis, the metal boats were burned off with a different program in the DMA-80 for cleaning before they were used again. Results of THHg concentrations were obtained manually from the DMA-80. Some hair samples from FINS-TEENS had already been analyzed prior to this thesis (n=136), and these results were received and included as well as results from the hair analyzed by the undersigned during the work with this thesis (n=96).

2.6.2.1 Quality of analysis

An extern calibration curve was conducted before the analysis of hair samples started in November 2018. CRMs used in the calibrations were: Bovine Muscle BCR184, Bovine Liver 1577, Milkpowder 150, Oyster Tissues, Mussel Tissue, Tort-3, Dorm-3, Dolt-4 and Tuna CE464.

To monitor the quality of the analysis, 10-20 mg of CRM Human hair IAEA-086 (powder, International atomic energy agency, Austria) with a certified reference value of 573 µg Hg/kg was included at four different boat positions in every analysis series, as mentioned. The results from the two first references of each analysis were registered into a control chart at IMR. All results of Hg concentrations in the reference material were within the ± 20% uncertainty limit (458.4 µg Hg/kg – 687.6 µg Hg/kg), and the overall accuracy of these Human hair IAEA-086 results was on average 94%.

2.6.2.2 Principles of DMA-80

The DMA-80 uses atomic absorption spectrophotometry to analyze total Hg concentrations in each hair sample (Figure 9). One by one, the samples are dried, thermally and chemically decomposed,

made to ash and evaporated in an oxygenated furnace at 450ºC to release all Hg. The Hg present is reduced to elemental Hg which is carried by the constant oxygen flow and selectively trapped by a gold amalgamator as Hg has a high affinity to gold. The gold amalgamator is heated to 650ºC to release the Hg vapor, which then travels through two absorbance cuvettes or cells, one of which is long and thin to capture low Hg concentrations, the other short and thick for higher concentrations. Light is sent through the cuvettes and the amount absorbed is proportional to the Hg concentrations present in the cuvette as absorbance is measured at Hg's wavelength, 253.65 nm. By using an external calibration curve together with the specified weight of the sample, Hg concentrations in the hair sample can be determined and results are given in µg/kg (85).

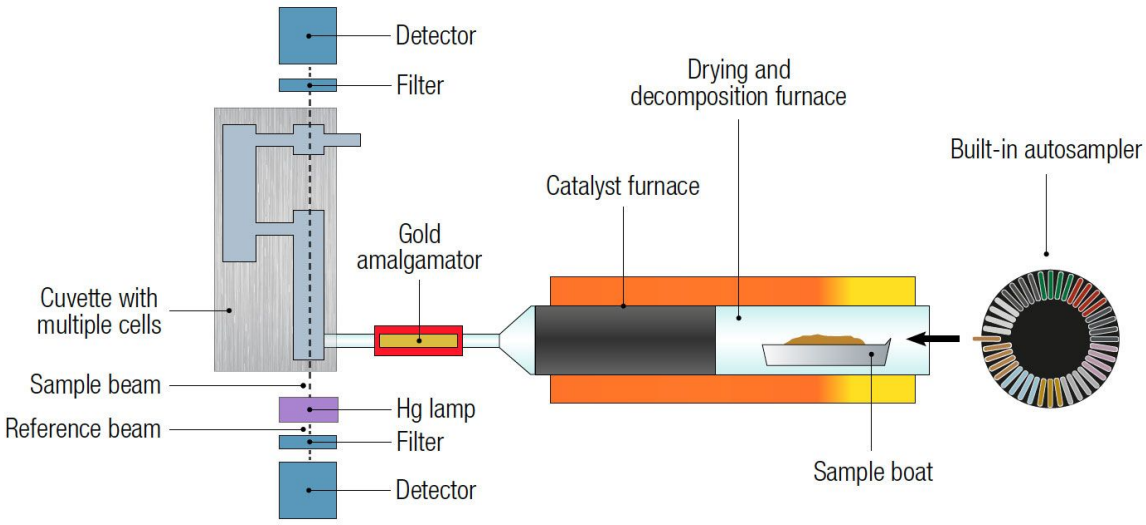

Figure 9: Principles for analysis of total hair mercury concentrations with Direct Mercury Analyzer
Picture: https://www.milestonesrl.com/products/mercury-determination/dma-80-evo (86).

The DMA-80 is calibrated in the linear area of Hg, 1.5 ng-1000 ng. The Limit of Detection (LOD), the minimum Hg amount possible to detect with the method´s given accuracy, is set to 0.02 ng and the Limit of Quantification (LOQ), the minimum Hg amount possible to quantify with the method´s given accuracy, is 0.08 ng. The method´s accuracy for samples within the linear area is ± 20%, whereas samples with Hg amounts below the calibrated area may be less accurately measured. For values below LOQ in this thesis, the LOQ cut-off was divided by two and entered into the dataset (0.04 ng), according to USEPA's guidelines when a small proportion (15%) of samples are below LOQ (87, 88).

2.6.3 Estimated dietary intakes from FFQ

The FFQ completed by participants provided ordinal categorical variables (e.g. <1 time/month, 2-3 times/week etc.) which had to be converted to numerical continuous data to perform further statistical analyses. For this, the validated seafood index from a validated semi-quantitative seafood FFQ established by Markhus et al. (89) was used to calculate indexes. By using this method, it became possible to make an estimation of total seafood consumption in grams per week, summarizing the different types of seafood addressed in the FFQ (e.g. salmon, cod, processed fish products etc.). For summary questions the index chosen was the mean from the range (e.g. 2-3 times/week is equivalent to an index of 2.5 per week). For detail item questions, a more conservative approach was chosen as overestimation of small intakes by respondents is common (90, 91), and therefore the index was based on the lowest frequency from the range (e.g. 2-3 times/week is equivalent to an index of 2 per week). The frequencies of seafood consumption were converted to numerical weekly intervals first and then further to a seafood index representing consumption per week (Table 3, Table 4). As portion sizes were not defined in the completed FFQ, standard portion sizes provided by the Norwegian Food Safety Authority were used to calculate seafood consumption in grams from the seafood index (Table 5) (92). Post-intervention, dietary seafood consumption was calculated using both FFQ responds and calculated intakes from study meals based on each individual´s compliance and content of study meals (see paragraph 2.4.3 and Table 2).

Table 3: Conversion of reported frequencies in FFQ to numerical continuous data and seafood index for questions on seafood intake as dinner
(Questions 1 and 2 in FFQ – Appendix II)

Reported frequency	Numerical interval per week	Seafood index (summary question)	Seafood index (detail item question)
Never	0	0	0
< 1 time/month	> 0-0.25	0.15	0.1*
1-3 times/month	0.25-0.75	0.5	0.25
1 time/week	1	1	1
2-3 times/week	2-3	2.5	2
≥ 4 times/week	≥ 4	4	4

*Seafood index set to 0.1 for distinction of this frequency from the reported frequency "Never".
Abbreviations: FFQ, food frequency questionnaire

Table 4: Conversion of reported frequencies in FFQ to numerical continuous data and seafood index for questions on seafood intake as spread
(Questions 3 and 4 in FFQ – Appendix II)

Reported frequency	Numerical interval per week	Seafood index (summary question)	Seafood index (detail item question)
Never	0	0	0
Rarely	> 0-0.25	0.15	0.1*
1-3 times/month	0.25-0.75	0.5	0.25
1 time/week	1	1	1
2 times/week	2	2	2
3-5 times/week	3-5	4	3
≥ 5 times/week	5	5	5

*Seafood index set to 0.1 for distinction of this frequency from the reported frequency "Never".
Abbreviations: FFQ, food frequency questionnaire

2.6.4 Calculation of dietary MeHg exposure

Average weekly MeHg exposure from the habitual seafood consumption was calculated from the pre-intervention FFQ answers to represent the baseline MeHg exposure. Post-intervention, MeHg exposure was estimated using both FFQ answers and Hg content of study meals together with the participants´ compliance (see paragraph 2.4.3 and Table 2). Total Hg content in different fish and seafood species from the latest analysis year (2008-2018) were retrieved mainly from Seafood Data (47) unless otherwise specified, and used to calculate each participant's exposure based on the frequency information provided by the participant combined with standard portion sizes (92). Data on Hg content and portion sizes used in calculations are presented in

Table 5. The resulting total Hg content is further presented as MeHg exposure, as a conservative approach of expecting 100% Hg to be present in the form of MeHg was chosen, in accordance with VKM´s approach (54). Data on sushi, mackerel in tomato sauce and processed fish products (fish cakes, fish fingers, other fish products) were not available and therefore the seafood species used to calculate MeHg content in these products were the same as the ones chosen in VKM´s benefit-risk assessment of fish and fish products in the Norwegian diet (4). In detail item questions which did not distinguish between several seafood species, a mean value for Hg content from those species was calculated and used, or one species was selected to represent the category. As processed seafood products such as fish cakes and fish fingers only have a pure fish

content of 50-60% results from these calculations were multiplied by 0.55 (4). Sushi has a pure fish content of ~33% and was multiplied by 0.33, caviar and mackerel in tomato sauce has a pure fish content of ~60% and was multiplied by 0.6 (4).

Table 5: Mercury concentrations and estimated portion sizes of seafood addressed in the FFQ

Seafood product	Species used in calculations	µg Hg/100g[a]	Portion size (g)[b]
Oily fish (> 5% fat) for dinner			
Salmon, trout[c]	Atlantic salmon fillet (farmed)	1.9	150
Mackerel	Atlantic mackerel fillet (wild)	2.9	150
Herring	Atlantic herring fillet (wild)	5.3	150
Halibut	Atlantic halibut fillet (wild)	11.0	150
Lean fish (< 5% fat) for dinner			
Cod	Atlantic cod fillet (wild)	9.4	200
Pollock	Pollock fillet (wild)	14	200
Other lean fish[c]	Mean of haddock fillet (wild) and plaice fillet (wild)	6.0	200
Sushi, shellfish and processed seafood for dinner			
Sushi	Atlantic salmon fillet (farmed)	1.9	300
Shellfish[c]	Mean of shrimp peeled (wild), blue mussel meat (wild) and brown crab (wild)	4.4	150
Fish cakes	Atlantic cod fillet (wild)	9.4	150
Fish fingers	Atlantic cod fillet (wild)	9.4	150
Other fish products[c]	Atlantic cod fillet (wild)	9.4	150
Spread/salad/snack			
Mackerel in tomato sauce	Atlantic mackerel fillet (wild)	2.9	40
Canned sardines or herring[c]	Sardines in oil	2.0	25
Other oily fish[c]	Mean of smoked salmon (wild) and smoked trout (wild)	4.6	20
Canned tuna	Canned tuna	7.6[d]	14
Caviar	Atlantic cod roe	1.8	15
Cod roe and liver pate (Svolværpostei/Lofotpostei)[c]	Cod roe/cod liver pate	2.0[e]	15

[a]Data retrieved from Seafood data (https://sjomatdata.hi.no), from latest analysis year (2008-2018), unless otherwise stated (47)

[b]Standard portion sizes retrieved from the Norwegian Food Authority (92)

[c]Detail item question in FFQ does not distinguish between several species/food items

[d]Mercury content retrieved from Nilsen and Måge, 2016 (93)

[e]Mercury content retrieved from Julshamn and Frantzen, 2008 (94)

Abbreviations: FFQ, food frequency questionnaire

2.7 Statistical analysis

The statistical analyses were performed using the Statistical Package for the Social Sciences (IBM® SPSS® version 25, IBM Corporation, Norway) and probability (*p)* values <0.05 were considered significant. Tables and figures were constructed using SPSS®, Microsoft Office Excel 2013 or Microsoft Office Word 2013. The statistical analyses were carried out only on the participants for whom THHg analyses with DMA-80 from pre- and post-intervention had been performed, n=116.

For descriptive results, categorical variables are reported as frequency, n (%), whereas continuous variables are reported as mean (standard deviation (SD)). Normality of results was tested using the Shapiro-Wilk test and visually assessed by inspection of histograms. To test potential between-group differences, the Chi-square test of homogeneity or Fisher's exact test (if >20% of expected values had frequencies <5) was chosen for categorical variables, the one-way analysis of variance (ANOVA) test was chosen for continuous variables.

Some socioeconomic variables were recoded prior to statistical analyses. Educational levels of the participants' parents were recoded from five categories into continuous variables (Table 6). This was to define education as a number of years, and to merge both parents' education years and then divide by two to get a mean as the new variable. The eight categories from the question on the combined income of the parents were merged and re-categorized into three categories defined as "low", "middle" or "high" income (Table 7).

Table 6: Recoding of parents' educational level (highest level completed) into continuous variables

Old variable	New variable (years)
Primary and secondary school ("grunnskole")	10
High school with vocational subjects ("videregående med yrkesfag")	13
High school with general subjects ("videregående med allmennfag")	13
College or university < 4 years	16
College or university > 4 years	17

Table 7: Recoding of parents' combined income (NOK) into categories

Old variable	New variable
< 200 000*	
200 - 349 999	Low income
350 - 549 999	
550 - 749 999	Middle income
750 - 999 999	
1 000 - 1 250 000	
1 250 - 2 000 000	High income
> 2 000 000	

*This alternative was not chosen by any responders (n=94).

Abbreviations: NOK, Norwegian krone

Neither seafood consumption, estimated MeHg exposure nor THHg concentrations were normally distributed, and thus non-parametric statistical tests were applied in analyses; Wilcoxon signed rank-test for within-group comparison of change from pre- to post-intervention and Kruskal-Wallis test for between-group comparison of differences. Also, a one-way analysis of covariance (ANCOVA) was performed using $\log_{10}$ transformed values for THHg concentrations and THHg pre-intervention as a covariate. Continuous variables are expressed as median (5-95th percentile) and mean (SD).

To measure the strength of association between non-parametric variables, the Spearman's rank order correlation coefficient was used. Correlations were assessed between THHg concentrations and total weekly seafood consumption (grams/week), and THHg concentrations and estimated MeHg exposure (µg/week) (95). The correlation strength was considered small if r=0.1-0.29, medium if r=0.3-0.49 and large if r=0.5-1 (81).

3. RESULTS

While working with hair analysis in the laboratory, the DMA-80 instrument stopped working and needed several parts replaced. After having waited for it to be repaired for several months, it was concluded that it could not be fixed, nor replaced in time for this thesis. Having samples being analyzed somewhere else or using a different method presented great challenges both practically, economically and methodically and was ruled out as an option. Due to these unforeseen problems with the DMA-80, only a selection of the available hair samples could be included in the present master thesis; In total, 232 hair samples from 116 participants pre- and post-intervention from five different schools were analyzed. The remaining samples will be analyzed by a lab-technician as soon as the DMA-80 is replaced. Therefore, all results presented in this thesis, from both FFQ and THHg analysis are based only on a selection of the FINS-TEENS study population. This selection (n=116) will from hereon be referred to as the "study population", and if the entire FINS-TEENS study population (n=443) is the population in question, this will be explicitly stated.

3.1 Baseline characteristics

Table 8 displays baseline characteristics of the study population and by intervention groups. No significant differences were found between the three intervention groups with regards to baseline characteristics.

Table 8: Baseline characteristics for the study population and intervention groups
Obtained from pre-intervention FFQ completed by participants (Appendix II) and questionnaire completed by participants' caregiver(s) (Appendix III). Values are given as mean (SD) or n (%).

Variables	n	All (*n*=116)	Fish (*n*=42)	Meat (*n*=37)	n-3 (*n*=37)	*p*-value
Sex, n (%)	116					0.783[d]
Male		55 (47)	20 (48)	19 (51)	16 (43)	
Female		61 (53)	22 (52)	18 (49)	21 (57)	
Weight (kg)	113	57 (11)	59 (14)	55 (9)	56 (9)	0.337[e]
Height (m)	113	1.69 (0.8)	1.7 (0.1)	1.68 (0.1)	1.69 (0.1)	0.781[e]
BMI (kg/m^2)	111	19.9 (3.1)	20.3 (4.0)	19.6 (2.1)	19.7 (2.7)	0.568[e]
Underweight, n (%)[a]		19 (17)	8 (19)	4 (12)	7 (19)	
Normalweight, n (%)[a]		83 (75)	30 (71)	26 (79)	27 (75)	
Overweight, n (%)[a]		9 (8)	4 (10)	3 (9)	2 (6)	
Birth place, Norway, n (%)[b]	94	91 (97)	33 (97)	29 (97)	29 (97)	>0.995[f]
Parents' education (years)[b]	94	15.2 (1.5)	15.3 (1.6)	14.9 (1.6)	15.2 (1.4)	0.488[e]
Parents' combined income before tax, n (%)[b]	94					0.613[f]
Low income[c]		2 (2)	2 (6)	0	0	
Middle income[c]		53 (56)	17 (50)	18 (60)	18 (60)	
High income[c]		39 (42)	15 (44)	12 (40)	12 (40)	

[a]Age and sex specific cut-off points by Juliusson 2017 for age 14.5 years (96)

[b]Answers by participants' caregiver(s)

[c]Low = 200-349 999 NOK, middle = 350-999 999 NOK, high = >1 000 000 NOK

[d]Chi-square test of homogeneity

[e]One way ANOVA test

[f]Fisher's exact test
Abbreviations: ANOVA, analysis of variance; BMI, body mass index; FFQ, food frequency questionnaire; n-3, omega-3; NOK, Norwegian krone; p, probability value; SD, standard deviation.

3.2 Seafood consumption reported in FFQ

Pre-intervention FFQ data were available for 115 of the 116 participants who were analyzed for THHg, whereas the post-intervention FFQ data were available for all 116 participants.

3.2.1 Seafood consumption pre-intervention

Median total seafood consumption was 277 g/week at pre-intervention among the adolescents (n=115), 320 g/week in the fish group, 290 g/week in the meat group and 261 g/week in the n-3 supplement group (Table 9). The adolescents' reported frequency of seafood consumption as dinner and as spread/salad/snack pre-intervention are presented in Figure 10 and Figure 11. Of all respondents, 33% reported a consumption of seafood for dinner in accordance with the recommendations of 2-3 times a week, and 2% reported higher consumption than this (1). Consuming seafood as spread, salad and snack "rarely" or "never" was reported by 57.5%. Mean (SD) and median (5-95%) reported frequency of seafood consumption as dinner was 1.4 (0.9) and 1 (0.2-2.5) times/week, and as spread/salad/snack it was 0.6 (0.9) and 0.2 (0-2.4) times/week. No significant differences in consumption were found between intervention groups pre-intervention (p=0.437).

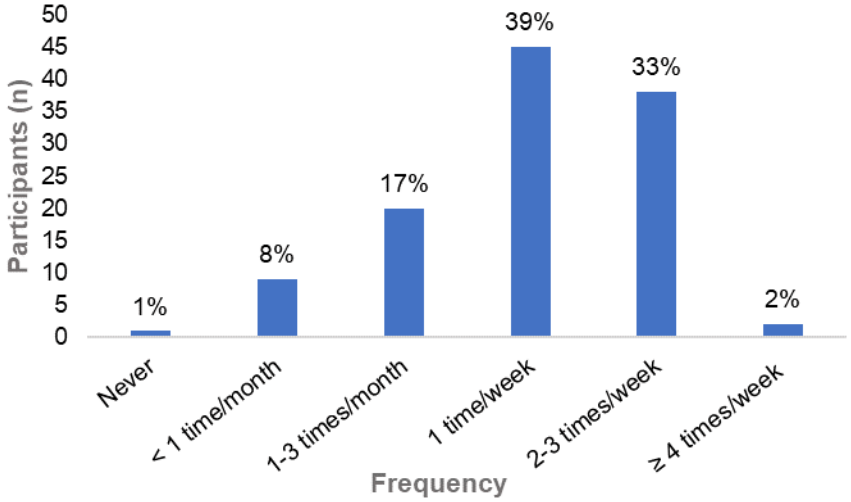

Figure 10: Frequency of seafood consumption as dinner, pre-intervention (n=115), shown as number of participants and percentage

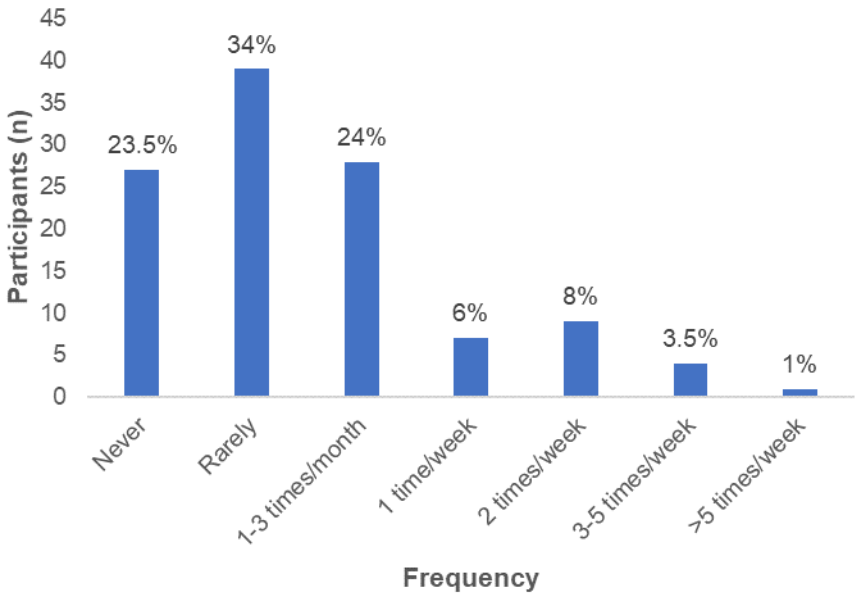

Figure 11: Frequency of seafood consumption as spread, salad and snack, pre-intervention (n=115), shown as number of participants and percentage

3.2.2 Seafood consumption post-intervention

The total seafood consumption post-intervention includes seafood consumption from the intervention study meals. No significant changes were found in any of the groups from pre- to post-intervention. Across all respondents (n=116) median consumption was 261 g/week (p=0.096), 366 g/week in the fish group (p=0.769), 259 g/week in the meat group (p=0.423), and 215 g/week in the n-3 supplement group (p=0.058) (Table 9). In the fish group, a median seafood consumption of 79 g/week came from the weekly three study meals which were served. Post-intervention, a significant difference in seafood consumption was observed between the fish group and n-3 group (p=0.013).

When analyzing the FFQ alone, *not* including fish from study meals, a significant decrease in seafood consumption was found in the study population (p=0.001) and the fish group (p=0.03).

3.2.3 Estimated MeHg exposure from FFQ pre-intervention

The estimated median total MeHg exposure in the study population was 18 µg/week (n=115) pre-intervention (Figure 13). Median estimated MeHg exposure from *seafood as dinner* was 18

µg/week across all respondents, whereas median MeHg exposure from *spread/salad/snack* was only 0.1 µg/week. Estimated median MeHg exposure was 20 µg/week in the fish group, 17 µg/week in the meat group and 13 µg/week in the n-3 supplement group (Figure 13). When considering the mean body weight of 57 kg, the participants were on average exposed to 0.3 µg MeHg/kg bw per week. Compared to EFSA and JECFA's limits of tolerable intakes, this exposure is below the established TWI and PTWI of 1.3 and 1.6 µg MeHg/kg bw, and corresponds to 23% and 19%, respectively. But when analyzing each individual's exposure in relation to their body weight, eleven participants had estimated MeHg exposure levels exceeding EFSA's TWI of 1.3 µg/kg bw/week pre-intervention (Figure 12). No significant differences in MeHg exposure were found between intervention groups pre-intervention (p=0.55).

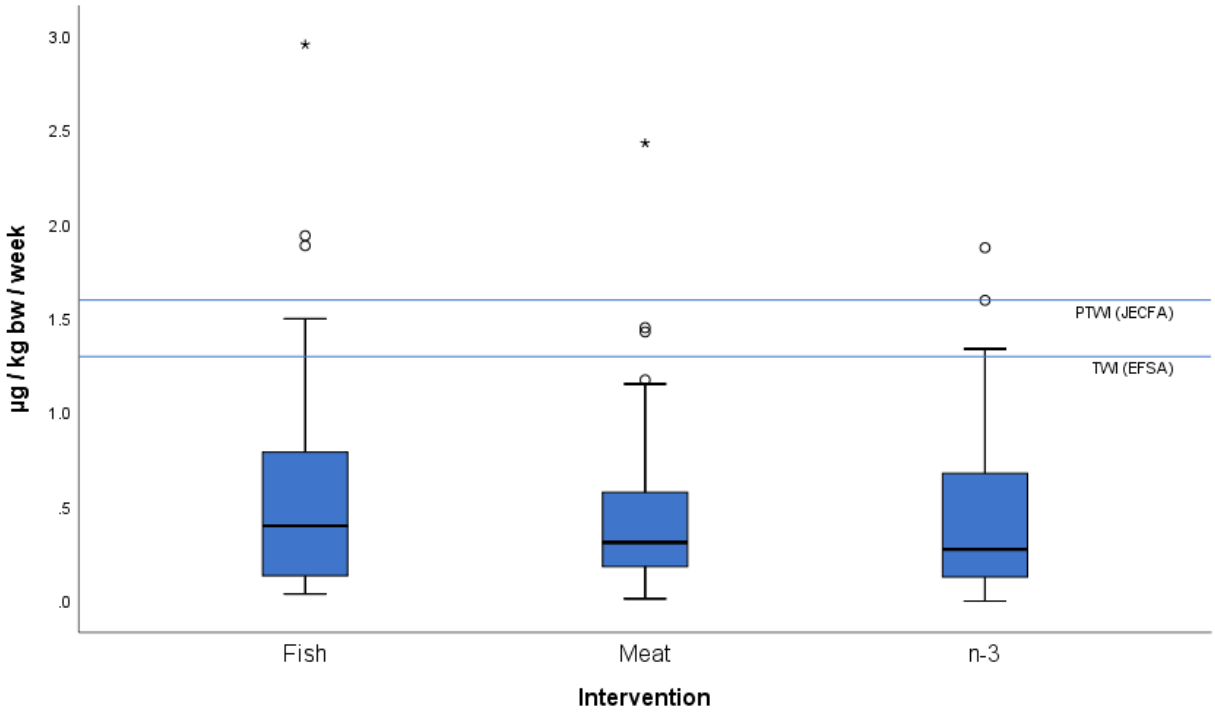

Figure 12: Boxplot displaying estimated methylmercury exposure (µg/kg bw/week) calculated from FFQ seafood consumption, pre-intervention (n=115)

The blue lines represent the limits of tolerable intakes set by JECFA and EFSA (see paragraph 1.3.6).

The horizontal lines through the boxes represent the median value, the lower and upper boundaries of the boxes represent the IQR, the whiskers represent the smallest and largest values in the distribution. According to the software the circles represent outliers (>1.5 box-lengths from edge of box) and the stars represent extreme outliers (>3 box-lengths from edge of box).

Abbreviations: EFSA, European Food Safety Authority; FFQ, food frequency questionnaire; IQR, interquartile range; JECFA, The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives; PTWI, provisional tolerable weekly intake; TWI, tolerable weekly intake

3.2.4 Estimated MeHg exposure from FFQ and study meals post-intervention

Post-intervention, a significant decrease could be seen in estimated MeHg exposure in the study population (p=0.007) and n-3 supplement group (p=0.012). Also, a borderline significant decrease was observed in the fish group (p=0.05). No significant differences in MeHg exposure were found between intervention groups post-intervention (p=0.082). Median MeHg exposure calculated from the post-intervention FFQ and exposure from the fish and meat study meals was 15 µg/week across all respondents, 16 µg/week in the fish group, 19 µg/week in the meat group (p=0.863), and 11 µg/week in the n-3 supplement group (Figure 13, Table 9). Of this, a median of 1.9 µg/week in the fish group and 0.7 µg/week in the meat group came from the fish and meat study meals, respectively. Hypothetically, a 100% compliance to the intervention, consuming the full amounts of all 36 study meals over 12 weeks, would yield an exposure of median 4.9 µg/week to participants in the fish group and 1.4 µg/week to participants in the meat group. Based on the mean body weight, this would equal 6.6% and 1.9% of EFSA´s TWI.

At population level, the median MeHg exposure by mean body weight was 0.3 µg MeHg/kg bw per week post-intervention as well. Whereas on the individual level four participants, two from the fish group, one from the meat group and one from the n-3 group had estimated MeHg exposure levels exceeding EFSA's TWI of 1.3 µg/kg bw/week.

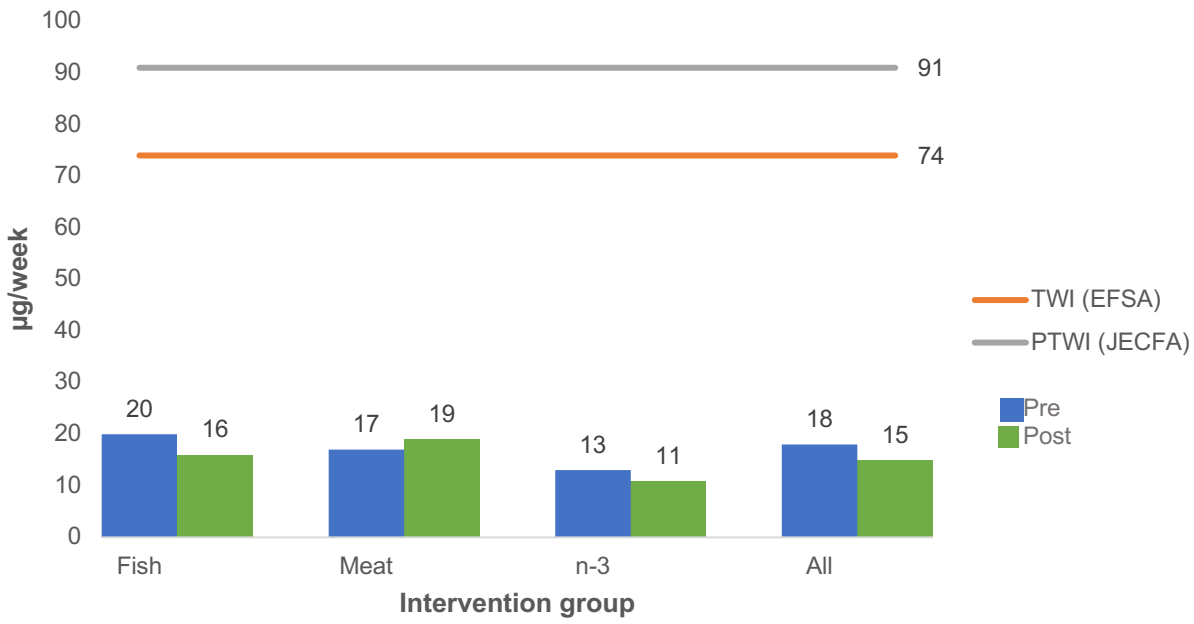

Figure 13: Median methylmercury exposure (µg/week) calculated from FFQ seafood consumption pre-intervention and from FFQ seafood consumption and study meal intake post-intervention, in intervention groups and study population

Grey and orange lines represent the limits of tolerable intake set by JECFA and EFSA for the mean bodyweight reported in the FFQ, 57 kg (see paragraph 1.3.6)

Abbreviations: EFSA, European Food Safety Authority; FFQ, food frequency questionnaire; JECFA, The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives; PTWI, provisional tolerable weekly intake; TWI, tolerable weekly intake

Table 9: Median and mean reported seafood consumption, estimated methylmercury (MeHg) exposure and total hair mercury (THHg) concentrations pre- and post-intervention in all groups, and change (Δ) in THHg concentrations from pre to post

	PRE-INTERVENTION			POST-INTERVENTION			
Variable	**n**	**Median (5-95%)**	**Mean (SD)**	**n**	**Median (5-95%)**	**Mean (SD)**	***p*-value***
Seafood consumption (g/week)							
All	115	277 (41-1352)	422 (407)	116	261 (40-740)	324 (231)	0.096
Fish	42	320 (46-1508)	510 (503)	42	366 (82-851)	396 (253)	0.769
Meat	36	290 (41-1144)	377 (311)	37	259 (21-766)	313 (208)	0.423
n-3	37	261 (18-1134)	367 (358)	37	215 (9-693)	255 (206)	0.058
MeHg exposure (µg/week)							
All	115	18 (2-101)	28 (29)	116	15 (2-53)	20 (17)	0.007↓
Fish	42	20 (3-123)	33 (35)	42	16 (2-62)	23 (22)	0.050
Meat	36	17 (1-98)	26 (25)	37	19 (2-52)	21 (13)	0.863
n-3	37	13 (0-83)	24 (25)	37	11 (0-45)	16 (15)	0.012↓
THHg (µg/kg)							
All	115	127 (6-444)	168 (165)	116	138 (6-502)	192 (215)	0.112
Fish	41	150 (7-589)	188 (175)	42	176 (5-828)	237 (269)	0.193
Meat	37	127 (7-717)	177 (191)	37	139 (6-556)	194 (212)	0.394
n-3	37	102 (5-358)	137 (120)	37	115 (6-471)	140 (125)	0.603
ΔTHHg (µg/kg)							
All				115	2 (-130-169)	13 (86)	0.914[a]
Fish				41	4 (-104-217)	19 (77)	
Meat				37	2 (-124-212)	17 (102)	
n-3				37	2 (-154-129)	3 (78)	

*Wilcoxon signed rank-test for within-group comparison from pre- to post-intervention

[a]Kruskal-Wallis test for between-groups comparison of differences

↓Significantly ($p<0.05$) decreased exposure from pre- to post-intervention

Abbreviations: FFQ, food frequency questionnaire; MeHg, methylmercury; n-3, omega-3; THHg, total hair mercury; SD, standard deviation

3.3 Compliance of intervention

Skotheim et al. (79) reported a significant difference in dietary compliance between the three groups when looking at the full FINS-TEENS study population (n=431). Mean intake of study meals in the fish group was significantly lower than in the meat group and n-3 supplement group. In the fish group, 37% consumed half or more (intake score ≥ 72) of the intervention meal, compared to 66% and 88% in the meat and n-3 supplement groups, respectively. The same was

found to be true in this selection of the FINS-TEENS study population (n=116): only 33% in the fish group consumed half or more of the intervention meals, compared to 57% in the meat group and 95% in the n-3 group. When statistical analysis were carried out with a theoretical compliance of 100% several more significant results were observed: significant differences in seafood consumption between the fish group and meat group (p=0.002) and the fish group and n-3 group (p<0.001), as well as significant differences in estimated MeHg exposure between the fish group and n-3 group (p=0.040).

3.3.1 Hg contents of study meals

Mean (SD) Hg content was 0.7 (0.5) µg/100g in fish meals (n=27) with values ranging from <0.2-1.75 µg/100g. In meat meals (n=26), mean Hg content was 0.2 (0.03) µg/100g with values ranging from <0.2-0.3. A large variation could be seen in Hg contents depending on the fish species used in the study meals; Fish meals with salmon all had Hg contents <0.52 µg/100g, meals with herring had 1.09-1.52 µg Hg/100g, and meals with mackerel had 1.66-1.75 µg Hg/100g. Meals with salmon were served by far the most times and were also the fish meals with the highest compliance.

3.4 Total hair mercury concentration (THHg)

Out of 232 analyzed hair samples, 69% (n=161) had Hg values below the calibrated area (1.5 ng-1000 ng). Out of the samples below the calibrated are, 90% had a lower sample weight than the recommended 10-20 mg for the DMA-80, whereas 71% of the samples within the calibrated area (n=70) weighed <10 mg. 15.9% (n=37) of the analyzed hair samples had values below LOQ (0.08 ng), and one hair sample (0.4%) was reported as “out of range” in the DMA-80 and was hence a missing value.

3.4.1 THHg pre-intervention

Median THHg concentration in the study population pre-intervention was 127 µg/kg, 150 µg/kg in the fish group, 127 µg/kg in the meat group and 102 µg/kg in the n-3 group (Figure 14, Table 9). Values ranged from 4 to 973 µg/kg and all values were below the 1000 µg/kg reference dose set by USEPA (see paragraph 1.3.7). No significant differences were found in THHg concentrations between intervention groups pre-intervention (p=0.452).

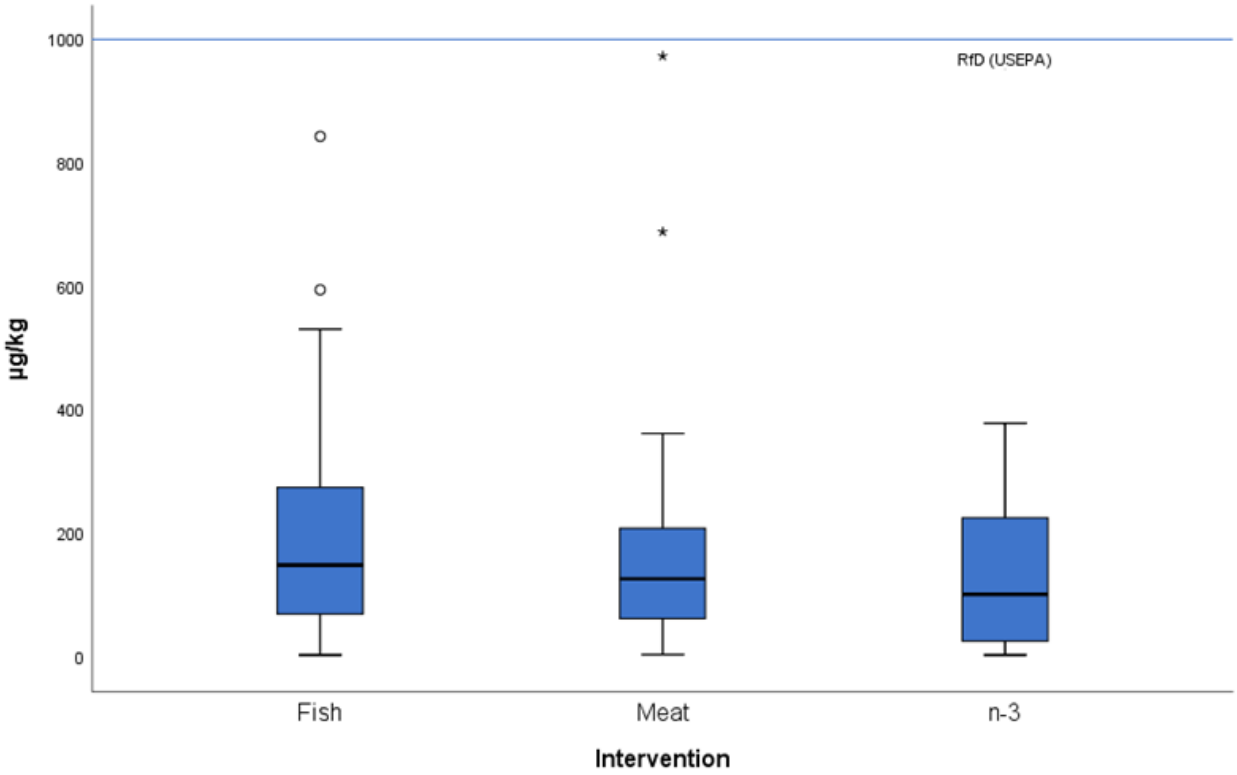

Figure 14: Boxplot of median total hair mercury concentrations (µg/kg) in the three intervention groups (n=115) pre-intervention
The blue line represents the 1000 µg/kg RfD set by USEPA. The horizontal lines through the boxes represent the median value, the lower and upper boundaries of the boxes represent the IQR, the whiskers represent the smallest and largest values in the distribution. According to the software the circles represent outliers (>1.5 box-lengths from edge of box) and the stars represent extreme outliers (>3 box-lengths from edge of box).
Abbreviations: IQR, interquartile range; RfD, reference dose; USEPA, The United States Environmental Protection Agency

3.4.2 THHg post-intervention

No significant change in THHg from pre- to post-intervention was found in the population (p=0.112), nor in the fish, meat or n-3 intervention groups (p=0.193, 0.394, 0.603) (Table 9). Median THHg concentration in the study population post-intervention was 138 µg/kg, 176 µg/kg in the fish group, 139 µg/kg in the meat group and 115 µg/kg in the n-3 group (Table 9). Values ranged from 5 to 1442 µg/kg, with two values being above the 1000 µg/kg reference dose set by USEPA, 1173 µg/kg (meat group) and 1442 µg/kg (fish group) (Figure 15). Pre-intervention THHg concentrations of these two participants were 973 µg/kg (meat group) and missing value (fish group), and they reported a fish consumption of 566 g/week (meat group) and 381 g/week (fish group) post-intervention.

There was no significant difference in THHg concentrations between the three intervention groups post-intervention (p=0.241). A one-way analysis of covariance (ANCOVA) was also performed for between-group comparison of THHg concentrations post-intervention with adjustment for THHg concentrations pre-intervention. This was done both with and without log-transformed data of THHg concentrations pre-intervention, but results, not shown in table, did not differ from unadjusted results (p=0.689 and 0.623), implying no difference between the groups.

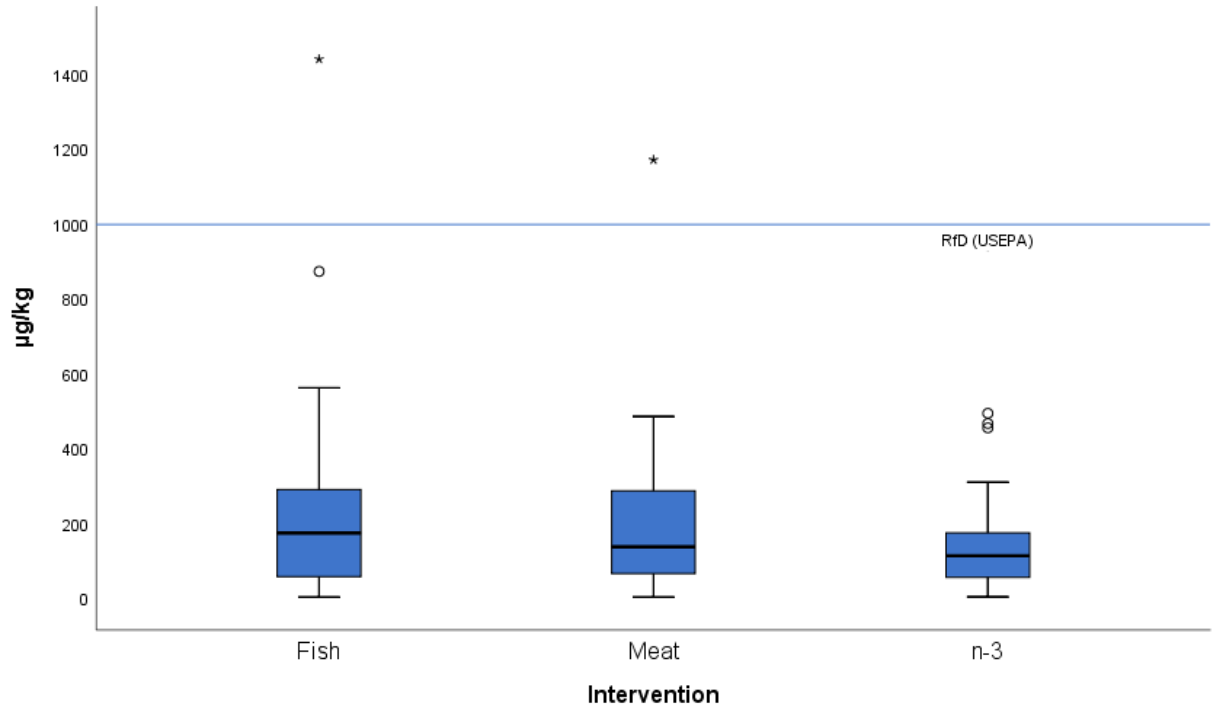

Figure 15: Boxplot of median total hair mercury concentrations (µg/kg) in the three intervention groups (n=116) post-intervention
The blue line represents the 1000 µg/kg RfD set by USEPA. The horizontal lines through the boxes represent the median value, the lower and upper boundaries of the boxes represent the IQR, the whiskers represent the smallest and largest values in the distribution. According to the software the circles represent outliers (>1.5 box-lengths from edge of box) and the stars represent extreme outliers (>3 box-lengths from edge of box).
Abbreviations: IQR, interquartile range; RfD, reference dose; USEPA, The United States Environmental Protection Agency

3.5 Effects of intervention on total hair mercury concentration

No significant differences were found in the median change (Δ) in THHg concentration from pre- to post-intervention between-groups (p=0.914). A small insignificant median variation could be seen in all groups (4, 2 and 2 µg/kg in the fish, meat and n-3 group, respectively) (Table 9).

3.6 Correlation between seafood consumption, estimated MeHg exposure and total hair mercury concentration

Pre-intervention, correlations were assessed between THHg concentration and reported seafood consumption, and the estimated MeHg exposure calculated from FFQ, using Spearman Rank Order Correlation for non-parametric data. There was a medium (81) positive correlation between total seafood consumption and THHg concentration (R^2=0.052) with a rank correlation, *r*, of 0.361 (p<0.01). There was also a medium positive correlation between estimated MeHg exposure and THHg concentration (R^2=0.035) with a rank correlation of 0.33 (p<0.01). Scatterplots demonstrating these relationships are shown in Figure 16 and Figure 17.

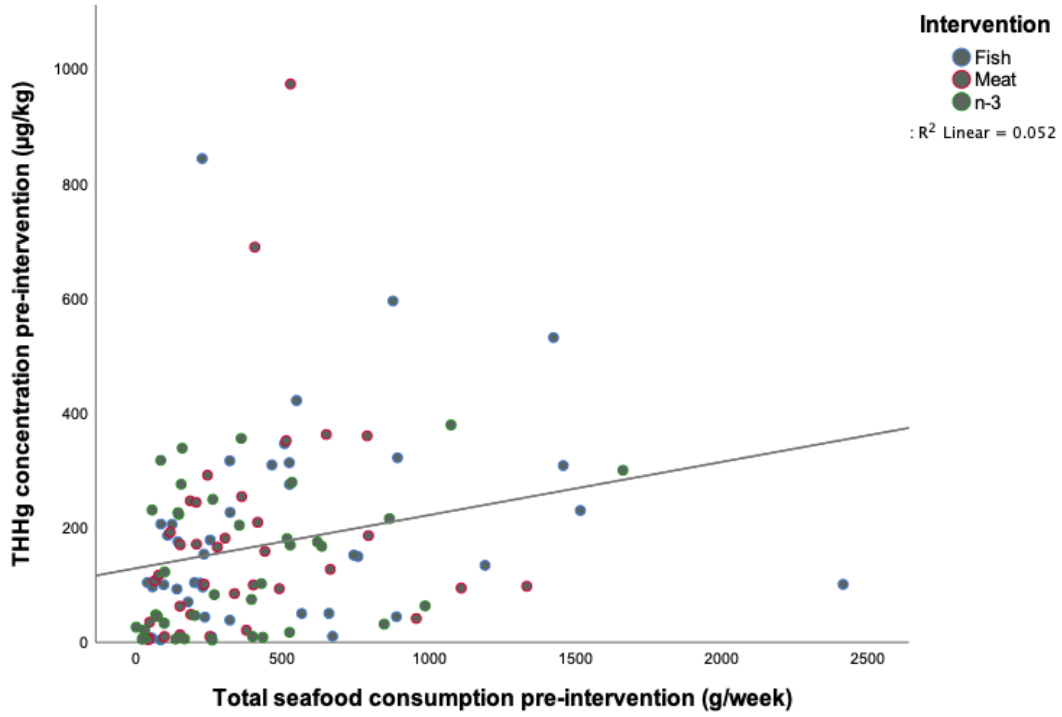

Figure 16: Grouped scatterplot displaying the association between total weekly seafood consumption (g) and total hair mercury concentration (µg/kg) pre-intervention
Abbreviations: THHg, total hair mercury

An outlier from the fish group with an extremely high reported seafood consumption of 2414 g/week can be observed in Figure 16. When examining this participant's FFQ more thoroughly, it seems highly unlikely that the pre-intervention answers are correct, though it *is* theoretically

possible to consume such large amounts of seafood per week. Post-intervention, this participant reports a smaller seafood consumption of 247 g/week, which is much more plausible. When this participant was removed from the correlation analysis however, it did not lead to any big changes; There was still a medium positive correlation between total seafood consumption and THHg concentration (r=0.371, p=0.01), and a low R^2 of 0.076.

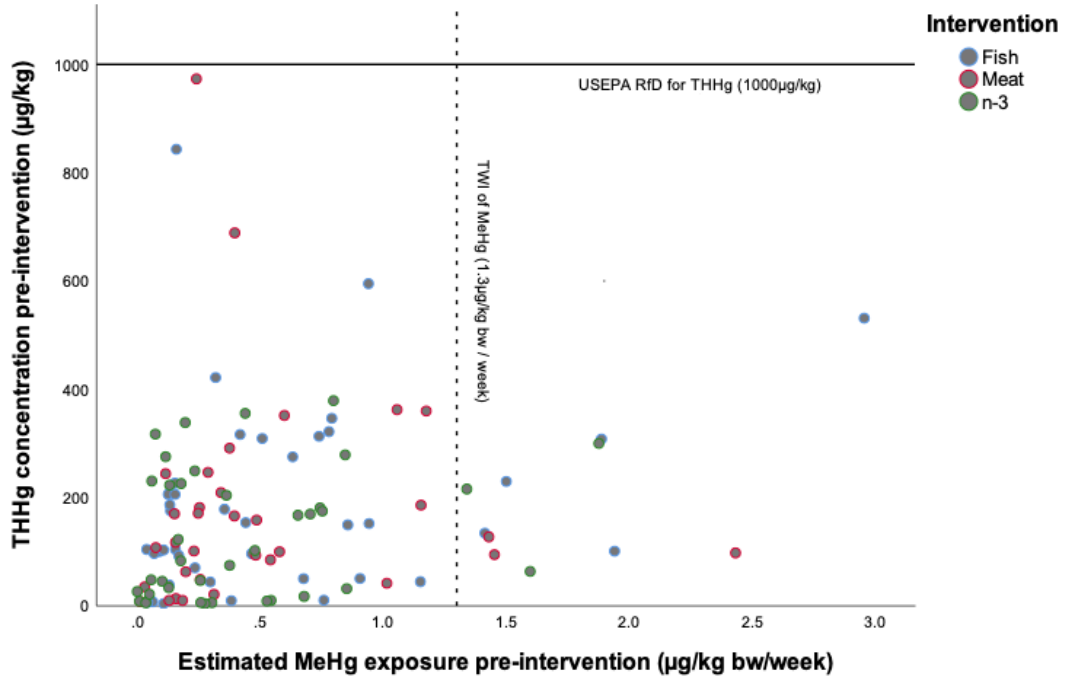

Figure 17: Grouped scatterplot displaying the association between estimated methylmercury exposure (µg/kg bw/week) and total hair mercury concentration (µg/kg) pre-intervention
The dashed line represents the TWI set by EFSA, 1.3 µg/kg bw/week (see paragraph 1.3.6), the firm line represents the 1000 µg/kg RfD for THHg set by USEPA (see paragraph 1.3.7)
Abbreviations: EFSA, European Food Safety Authority; MeHg, methylmercury; RfD, reference dose; THHg, total hair mercury; TWI, tolerable weekly intake; USEPA, The United States Environmental Protection Agency

Post-intervention, correlations were assessed between total seafood consumption including intake from study meals and THHg concentration in the study population and in all three intervention groups. There was a medium positive correlation overall (R^2=0.083) with a rank correlation, r, of 0.364 (p<0.01). There were medium positive correlations in the meat and n-3 groups (r=0.361 p<0.05 and r=0.472 p<0.01), but only a small positive correlation in the fish group (r=0.257) (Figure 18).

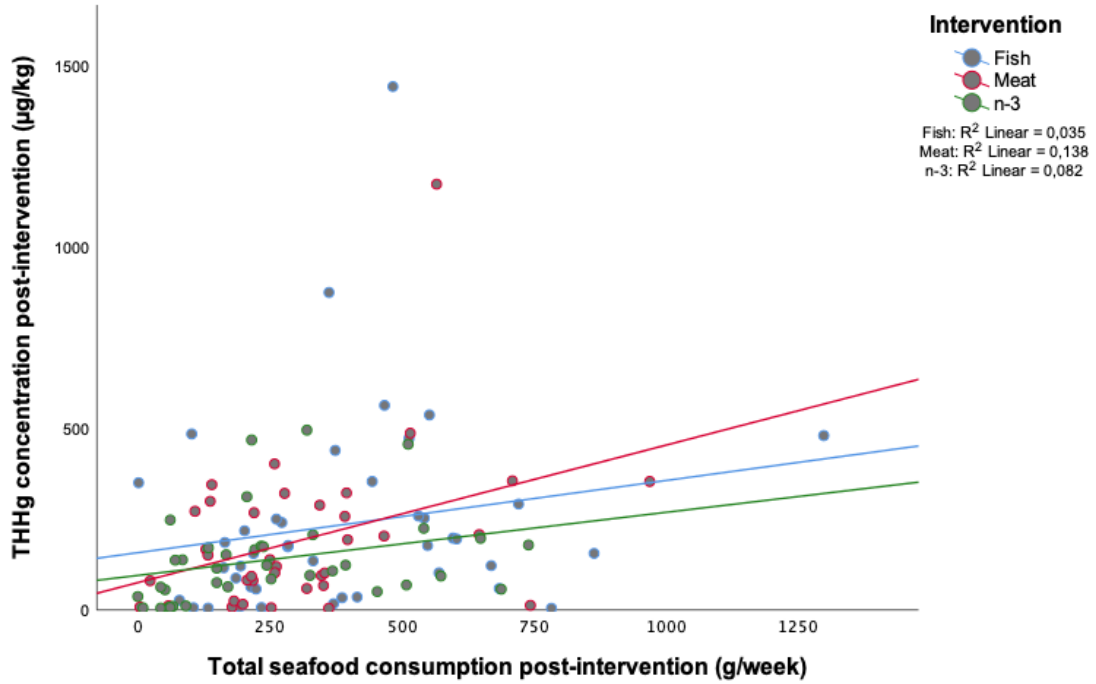

Figure 18: Grouped scatterplot displaying the association between total weekly seafood consumption (g) and total hair mercury concentration (µg/kg) with regression lines for intervention groups, post-intervention

Abbreviations: THHg, total hair mercury

4. DISCUSSION

In this thesis, the aim was to investigate the association between dietary seafood consumption and concentration of THHg in adolescents. The FINS-TEENS RCT was explored for this purpose, in which adolescents received fish or meat meals for lunch, or n-3 supplements, three days a week, for 12 weeks, and FFQ and hair samples for THHg analyses were collected pre- and post-intervention. Habitual seafood consumption and MeHg exposure were estimated based on FFQs, THHg concentrations of hair samples were determined using a DMA-80, and changes in all three parameters from pre- to post-intervention were measured. Mainly, the results showed no significant effects of the intervention on seafood consumption, MeHg exposure or THHg concentrations in the fish group. The results as well as methodological considerations, strengths and limitations with the study design will now be discussed and compared with other studies.

4.1 Reported seafood consumption among adolescents

The median weekly seafood consumption (277 g/week) at baseline found in this study was below the recommended 300-450 g/week. Only 35% of the adolescents reported having a seafood for dinner consumption in accordance with the recommendations of 2-3 times a week or more (1). This consumption could mean that during important years of growth and development, stepping into adulthood, this group may have insufficient intakes of important nutrients provided by seafood (4). The conclusion that consumption of seafood is seemingly too low in the Norwegian population and especially among adolescents is not a new one, but in line with previous national dietary surveys, "Norkost 3" and "Ungkost 3" (2, 16). The reported weekly seafood consumption was greater than what was reported by the 8th graders in "Ungkost 3" (168 g/week) (2).

The reported seafood consumption was still higher compared to other European adolescents (17, 18, 20), Scandinavian adolescents (19, 97) and children (98, 99). This is in line with previous research concluding that seafood consumption is generally greater in Norway than in many other European countries, despite being below the national recommendations (100-102). The reported seafood consumption of the present FINS-TEENS study population (n=115) is similar to the reported consumption in the total FINS-TEENS study population (n=414), both as dinner (1.4 (0.9) (mean(SD)) versus 1.5 (0.9) times/week) and as spread (0.6 (0.9) versus 0.6 (1)) which was reported by Handeland et. al in 2018 (103). In comparison with Norwegian studies in other age groups, the reported FINS-TEENS seafood consumption was lower; 49% of the children (4-6 years old) in the FINS-KIDS study from 2018 followed the recommendations on seafood for dinner 2-3 times a week, according to their caregivers, and so did 43% of the participants in the Mommy's Food study (63, 104).

As reported by Skotheim et. al, the mean amount of fish served per meal in FINS-TEENS was 71 g, equivalent to 213 g/week (79). Due to low compliance, only a median of 79 g/week was consumed by the participants in the fish group. The lack of significantly increased total seafood consumption post-intervention in the fish group, could be due to a decrease in seafood consumption from the background diet as a consequence of the study. According to their FFQ responds, the fish group significantly decreased their habitual seafood consumption during the

intervention (p=0.03). One could expect that a group of adolescents who generally consume not much seafood, do not really like seafood that much, and would downsize the frequency of seafood consumption at home as a result of an increased consumption at school. Still, the changes in reported weekly seafood consumption from pre- to post-intervention did not differ significantly between the three groups, though a significant difference in seafood consumption results between the fish and n-3 group was observed post-intervention (p=0.013). Equally plausible, the observed changes could be due to the uncertainty in the FFQ method associated with multiple administrations of the questionnaire.

4.2 Estimated MeHg exposure among adolescents

The significant decrease in estimated MeHg exposure post-intervention in the study population and n-3 group, and borderline significant decrease in the fish group, is a somewhat peculiar find, as MeHg estimates are calculated based on reported seafood consumption, which did not significantly decrease in those groups. When analyzing only the FFQ post-intervention *not* including study meals, though, a significant decrease in seafood consumption was found in the study population (p=0.001). Though this, too, is somewhat surprising, it could possibly explain the decreased MeHg exposure. The borderline significant decrease in estimated MeHg exposure in the fish group (p=0.05) could also be a result of the significant decrease in reported seafood consumption from the background diet, as well as the fairly low MeHg content of the fish species served in the study meals.

The estimated median MeHg exposure in the study population (0.3 µg/kg bw/week) was similar to the median MeHg exposure estimated in Mommy's Food (0.29 and 0.26 µg/kg bw/week pre- and post-intervention, respectively) and twice the calculated exposure of the mothers in the Norwegian Mother and Child Cohort Study (MoBa) (0.14 µg/kg bw/week) (63, 105). But in both studies stronger correlations between seafood consumption and blood or hair Hg concentration were found, which could mean the accuracy in the FFQ responds on seafood consumption was better than in the FINS-TEENS study. The national dietary survey "Ungkost 3" concluded that among 8th graders the survey seemed to present a more favorable average diet than what is the reality in this age group (2). With this in mind, bias in the form of over-reporting of healthy

foods, such as seafood, is a potential issue in FINS-TEENS as well, as reported seafood consumption was even greater here than in “Ungkost 3”.

The median and mean weekly estimated MeHg exposures were well below the limits of tolerable intakes (1.3 and 1.6 µg/kg bw/week) both pre- and post-intervention (52, 53). These exposure levels are considered safe, and further confirm that in the amounts consumed by the Norwegian adolescents represented in this study, the potential risks of MeHg in seafood are minor and outweighed by the benefits (4). The meat group was the only group where any MeHg from other sources than seafood was represented, as levels from the meat study meals were included in the calculations. Exposure from other food products than seafood is normally considered negligible. Including these amounts in the estimated total exposure, and still observing such low median exposure levels, further strengthen the claim that MeHg levels in the diet present negligible risks and are not of great concern in the levels consumed in the present study.

At the individual level, eleven and four participants had estimated MeHg exposure levels exceeding EFSA’s TWI pre- and post-intervention, respectively. There is reason to believe that at least some of these are due to overreporting of unrealistic weekly seafood consumption, or other report errors, especially pre-intervention, as most of these participants reported much lower seafood consumption post-intervention.

4.3 Total hair mercury concentration in adolescents

The mean and median THHg concentrations reported in this thesis (168 and 127 µg/kg pre-intervention, 192 and 138 µg/kg post-intervention) were far below the USEPA reference dose of 1000 µg/kg. Only two subjects, one from the meat group and one from the fish group, had THHg concentrations exceeding the RfD post-intervention, compared to none pre-intervention. The participant from the meat group increased THHg concentration from 973 µg/kg pre-intervention to 1173 µg/kg post-intervention, equivalent to an increase of 20% which could be only due to the ±20% accuracy of the DMA-80 method. The participant from the fish group had a concentration of 1442 µg/kg post-intervention, but a missing value pre-intervention, which makes it difficult to evaluate the accuracy of the post-intervention result. The FINS-KIDS study with somewhat similar

design reported a mean THHg of 374 µg/kg at baseline (n=210), with values ranging from 15-1017 µg/kg, as well as a significant increase in THHg post-intervention in the fish group (37). This is more than twice the mean of baseline results from this thesis and could be due to the higher habitual seafood intake. The significant increase in THHg post-intervention could be attributed to a higher compliance to the study meals in the FINS-KIDS study. Also, participants in the FINS-KIDS study only received herring and mackerel which contain more Hg than farmed salmon, which was the species served most times in the FINS-TEENS study.

Compared to the studies on European adolescents in Table 1 and the Norwegian studies Mommy's Food and Little in Norway on mothers and their infants, the mean and median values on THHg reported in this thesis are considerably lower (63-70). Also, concentrations were, not surprisingly, much lower than concentrations observed in places where the population is chronically exposed to moderate Hg levels because of high consumption of seafood, such as Amazonia (Brazil), Seychelles, Faroe Island and New Zealand with THHg concentrations ranging from 1850-6090 ug/kg (60, 106-108).

The lack of the hypothesized significant increase in THHg concentration post-intervention can possibly be due to the low compliance in the fish intervention group. Only 33% consumed half or more of the intervention meals, and the compliance was lowest when the meals with the highest Hg contents (mackerel and herring) were served. As we already know, the age group represented in this study population generally consume little fish, and dietary compliance becomes a challenge especially when trying to intervene with food groups that are not already preferred by the test subjects (78). Table 9 shows a trend towards increased THHg concentration and ΔTHHg post-intervention, though not significant. As estimated MeHg exposure decreased significantly, or borderline significantly, in all groups but the meat group, this can further indicate the relatively weak correlation between FFQ responds used for estimating MeHg and THHg concentration.

Considering the hazardous effects of MeHg (21, 22), low THHg concentrations are favorable. But as THHg concentrations are shown to correlate positively with seafood intake both in this study and previous studies (37, 45), low values may indicate, as here, a lower seafood consumption than the recommendation. As VKM has concluded that the benefits of seafood consumption in

accordance with the dietary recommendations (2-3 times/week) outweigh the risks of contaminants, an increased seafood consumption in this population group is desired to reach these recommendations, despite the fact that this will most likely also increase their THHg concentrations (1, 4). Considering the results from this thesis, it is plausible to assume that most adolescents would still have THHg concentrations well below the RfD.

4.4 Methodological discussion

4.4.1 Study design

Choosing a suitable design to match the research question and aim is crucial in any study being conducted. Here, a study originally designed to investigate the effect of an intervention with oily fish, meat or n-3 supplements on cognition in adolescents was used to assess the effect of seafood consumption on THHg concentration (79). If the study were designed to assess seafood consumption and change in THHg concentration primarily, the choice of fish species might have been different, as oily fish, which was used in FINS-TEENS, overall has a lower Hg content than lean fish (Figure 2). Therefore lean fish might have had a greater impact on THHg concentrations in such an intervention (47). Also, VKM has reported that lean fish is the main source of MeHg in Norway (4). The fish study meals containing the highest amounts of Hg, the mackerel meals, were also reduced in serving frequency during the study after reports that it tasted bad, further decreasing the amount of Hg participants were exposed to. The power calculation was also based upon other primary outcomes than THHg. Generally, a study design not chosen with the aims of this thesis as the main outcome, is a limitation to results and conclusions presented here. On the other hand, the RCT design of this study, with individual randomization to reduce the influence of confounding factors and systematic errors is considered a strength.

Conducting an RCT with dietary interventions is difficult for several reasons; it makes it impossible for participants to be blinded as to which food they are receiving, increasing the risk of bias (109). Adding food to someone's diet will also lead to a decrease in other food groups, and this is difficult to control or measure and could influence the results. For example, what the participants with low compliance to the study meals chose to eat for lunch instead is an interesting question which the FFQ did not specifically assess, though it is doubtful THHg concentrations

would have been affected by this. RCTs with dietary interventions are also vulnerable to the food preferences of participants which can increase the risk of attrition bias. Handeland (78) reported that when conducting the study, the meat group was more popular being allocated to than the fish and n-3 supplement groups. Therefore, there is a real chance that dropouts from the fish group did not like fish and had a lower baseline seafood consumption than the median consumption presented here, though no measures were taken to confirm or deny this hypothesis.

As all three groups in the study received some sort of intervention, the FINS-TEENS study design lacks a pure control group which could have unveiled a potential placebo effect of the interventions. The meat group was seen as a control to the fish group as intervention meals were nearly identical except for the fish or meat. Whereas supplements were given to the third group mainly to discover if effects of fish meals were due to the combination of nutrients in the fish or mainly the LCPUFAs, which are also present in n-3 supplements (78). But as neither meat nor n-3 supplements contain significant amounts of Hg, one would not expect a pure control group to contribute to a change in THHg concentrations in the case of this thesis. Therefore, considering the meat and n-3 group as control groups seems sufficient. Also, having a group of adolescents completely avoid fish and/or meat for 12 weeks to provide for a pure control group would be unethical and unfeasible.

Although there originally was a large sample size in the FINS-TEENS study (n=443), which is a strength, the unforeseen problems with the DMA-80 instrument during the work with analyses for this thesis led to a considerably lower sample size (n=116) which was not ideal. Within this new study population, the analyzed participants represented only five of the eight participating schools. For this reason, the results cannot be extrapolated and generalized in the same way they might have if analyses had been done on the entire FINS-TEENS population. Still, a strength was that coincidently the sizes of the three intervention groups ended up being similar. Also, no differences were found in baseline characteristics between the groups and compliance was similar to the compliance in the entire FINS-TEENS population, meaning a potential real effect of the interventions could still have been observed.

The large proportion of 9th graders choosing not to attend in the study might have increased the possibility of selection bias (78). Handeland stated that though data on socioeconomic status among non-attendees were not available, a higher mean educational level was found among FINS-TEENS participants' parents than in the average population in Norway, and the same was true for the smaller study sample in this thesis (110). As healthy habits are shown to be positively associated with socioeconomic status, there is a possibility that the study sample was healthier, e.g. ate more fish at baseline, than non-attendees, and further affected the results from the dietary assessment in this thesis (111, 112). If this was the case, the result would be that the reported seafood consumption would be an overestimation not representative for the general population. The conclusion would still be the same; seafood consumption is lower than recommended among adolescents.

The observed variation in the mean content of fish/meat in intervention meals and especially the lower mean weight of fish (71 g) than first assumed and desired (80-100 g) presents limitations to the study. This could have impacted the amount of Hg the participants were exposed to yielding lower THHg concentrations than if the meals had contained more fish. Though, with the low compliance in mind, it is doubtful that this would have had a significant impact on THHg concentrations post-intervention. On the other hand, having analyzed the study meals for both nutrient and contaminant contents, including Hg, is a strength and an important safety measure when performing such an intervention.

Whether the duration of a study is sufficient to observe a clinically significant change in the parameters being measured, is always an important question to address. For this thesis, measuring THHg, 12 weeks can be expected to be enough to provide a potential change in THHg concentration, considering the growth rate of hair and the time it takes for new hair strands to form in the follicle (84). With this in mind, one can assume that a longer-lasting intervention would not have made an impact, as the length of hair used for analyses would have been the same (2 cm nearest to the scalp) and would still only reflect Hg exposure 5.2-12.4 weeks prior to collection (84).

4.4.2 Dietary assessment

4.4.2.1 Seafood consumption

Other papers from FINS-TEENS have been based upon both validated and not validated questions from the FFQ. The FFQ questions analyzed for this thesis, concerning seafood consumption only, have been validated previously (78, 113) and a validated seafood index was used to calculate seafood consumption (89) which is a strength. It is a well-known fact that respondents are prone to over- and underestimating of food intake in FFQs, especially over-reporting healthy foods like seafood. With this in mind, validating these results by also using a different method for dietary assessment, such as a 24-hour recall would have been optimal (72, 73, 90, 91). Still, compared to a 24-hour recall, the FFQ covers a longer time-period which is relevant when addressing micronutrient intake and contaminant exposure. By using a 24-hour recall assessing dietary intake of specific food groups or micronutrients, one could risk the recall covering a day when respondents have not eaten the food in question (here; seafood), which would lead to an underestimation of MeHg exposure. On the other hand, if the 24-hour recall coincidently covered a day the respondent ate a lot of fish, the opposite could happen, resulting in an overestimation of seafood consumption and MeHg exposure. If combining the FFQ with a 24-hour recall were to be a feasible option, multiple administrations of a recall would have been necessary, imposing a greater burden on participants, and possibly further a decrease in compliance.

The lack of stronger correlations between seafood consumption and MeHg exposure results from the FFQ and THHg concentration could be due to a low reproducibility in the FFQ method and possibly unreliable responses from the adolescents. The uncertainty of the method can have increased if adolescents lacked the motivation to complete the task properly and thoroughly. A stated higher seafood consumption than what was the reality might have led to an overestimation of MeHg exposure as well, which further resulted in smaller correlations with THHg concentration.

A limitation and challenge with the FFQ questions analyzed for this thesis was the lack of pre-defined portion sizes (Appendix II). The national recommendations on seafood consumption are formulated both in terms of frequency and grams, but this FFQ was based on frequencies alone; Respondents were only asked to address the frequency with which they consumed each seafood

product, without providing information on their average portion sizes. The use of standard portion sizes defined by the Norwegian directorate of health raises uncertainty with regard to accuracy. Results could especially be prone to overestimation as these are portion sizes for adults, whereas the FINS-TEENS participants were 14-15 years old and were likely to have smaller portion sizes. It is difficult to determine the importance of combining frequency with amount (portion size) in FFQs, as some researchers have reported that the between-person variations in portion size are smaller than the variation in frequency, and that frequency alone might be sufficient for estimating dietary intakes (114, 115). Still, as the size of the study meals (average 71 g fish) was smaller than the standard portion sizes used for calculations from FFQ (150-200 g) this further confirms the lack of predefined portion sizes being a challenge. Even with small meal sizes, compliance in the fish group was very low, further promoting the suspicion that results might be prone to overestimation.

4.4.2.2 Estimated MeHg exposure from seafood consumption

Levels of Hg in seafood vary greatly both between and within species, which raises uncertainty to the values retrieved and used for calculations to estimate MeHg exposure of the participants in this study. In addition, we lack data on Hg contents of some seafood species and fish products. The FINS-TEENS FFQ also had some questions which did not differentiate between several species. In these cases, the Hg content of another seafood species was used, in accordance with VKM 2015 (4), the mean Hg content of multiple species was used, or one species was chosen to represent the multiple species listed in the FFQ questions, providing only an estimate at best. The observed medium positive correlation between estimated MeHg exposure and THHg concentration could indicate an acceptable estimation despite these uncertainties, though a stronger correlation would have been even better. As previously mentioned, the lack of stronger correlations with THHg could be due to inaccurate FFQ responds in the form of over- and/or under-reporting of seafood consumption, but also due to biological differences between participants, cooking methods or other MeHg exposure sources than seafood (dietary sources, dental amalgam, environmental sources etc.) (31, 33). However, other sources are expected to provide insignificant exposures of MeHg, not affecting THHg concentrations considerably (24, 32, 38, 44). An over-reporting of seafood consumption in the FFQs or overestimation of portion sizes in this thesis is more likely to account for the lack of a stronger correlation, as this is a

known challenge, also observed in this age group (2).

4.4.3 Analysis of total hair mercury concentration

Human errors might have affected the results from the DMA-80 analysis. For this thesis, the initial collecting of hair near the scalp was done by different collectors post-intervention in 2015, and the measuring and cutting of the two cm of hair used for THHg analysis were done by two different people in 2017 and 2018. As LeBeau et al. reported (84), inconsistencies in the cutting of hair next to the scalp between different collectors and in-between collections done by the same collector, are common. This, together with the variability in human head hair growth rate between individuals, affects the interpretation of quantitative segmental analysis of hair (84). But as the collectors in this study cut participants from all three groups, not just one, and results are presented on a group level the impact of these factors are expected to be leveled out.

The assumption that the cut end is from right next to the scalp combined with potential unevenly cut ends which are aligned and assigned a "zero" value for analysis will lead to an underestimation of the time range the hair sample represents. Also, assuming one particular growth rate for all participants in the study could lead to skewed conclusions, as a faster growth rate will lead to less Hg being accumulated and vice versa (37). Still, the timeframe that hair samples were assumed to represent in this thesis (5.2±0.8 to 12.4±1.6 weeks prior to collection), was based upon LeBeau et al.'s calculations which consider the results from their study and take the observed variabilities into account. They also recommend an 8-week delay between the exposure period and the collection of hair to minimize the effects of these variabilities. Because the intervention period of FINS-TEENS was 12 weeks before collecting new hair samples, one can assume this recommendation was complied with. Such inter-individual variation should be similar across all intervention groups following the randomization and the large FINS-TEENS study population size (n=443) which can strengthen conclusions based upon these results, but as the population size was much smaller (n=116) for the THHg analyses for this thesis, it is difficult to make any certain conclusions.

As analyses used for this thesis were performed by two different people, a year apart, different DMA-80 calibrations were used. Also, plotting of hair sample weight, ID-numbers, and THHg results were done manually, further increasing the risk of human error. Despite being analyzed a year apart, and by two different people, it is a strength that pre- and post-intervention hair samples from the same participant always were analyzed in the same analysis series to avoid the effect of day-to-day variations in the method, and so were samples from at least two different schools.

Out of all the analyzed hair samples, 69% had Hg concentrations below the validated area of the DMA-80 which leads to an increased uncertainty of the results of these samples with this method. THHg is computed using the calibration curve and the weight of the sample which is manually plotted into the machine. The uncertainty of the method depends on the amount of weighed-in sample and the homogeneity of the sample. In other words, the amount of hair, the weight, will highly affect whether results are within the calibrated area or not. In this thesis, the mean weight of hair samples (7 mg) was lower than the ideal weight for the DMA-80 validated area (10-20 mg), and some weighed considerably lower, as low as 1.9 mg. Thus, the high percentage of samples with results below the calibrated area is likely to be greatly due to too small hair samples. The collectors of hair were told to cut a 2-5 mm bundle but could inform that often they went for the lowest amount (~2 mm), as many of the study participants were concerned about losing too much hair. Setting a standard sampling size to 5 mm for future studies of THHg may help with the aforementioned challenge and could ensure large enough hair samples to reduce the number of results below the validated area. For results in this thesis though, the initial ±20% uncertainty of the method was further increased by many samples being outside the calibrated area and many samples having a low sample weight. Still, it can be argued, that it is not entirely unlikely that adolescents actually do have very low THHg concentrations considering the known low seafood consumption in this age group (2).

Though hair analyses with DMA-80 is an accepted and validated method for measuring Hg concentrations, the fact that the instrument stopped working during analysis and was deemed impossible to repair, further contributes to increased uncertainty regarding the validity of the THHg results. However, this was impossible to foresee and account for.

4.5 Conclusion

This thesis provides insight on the seafood consumption, estimated MeHg exposure and THHg concentrations of the Norwegian adolescents represented in the present study. An intervention with oily fish did not lead to the expected increase in the aforementioned consumption, exposure and concentration, although a significant higher seafood consumption was observed in the fish group compared to the n-3 group post-intervention. Also, an unexpected significant decrease in estimated MeHg exposure was observed in the study population and n-3 subgroup post-intervention. Therefore, according to our findings, we must reject the hypothesis that adolescents in the fish group have increased seafood consumption, MeHg exposure and THHg concentration compared to the meat and n-3 groups following an intervention with oily fish, at least for this selection of the FINS-TEENS study population (n=116).

Median seafood consumption was below the recommendations, median estimated MeHg exposure was well below EFSA´s TWI and median THHg concentration was well below USEPA´s reference dose. Seafood was shown to contribute moderately to THHg concentration and MeHg exposure as a positive correlation was found between THHg concentration and seafood consumption, and THHg concentration and estimated MeHg exposure.

These findings further support VKM´s 2014 claim that in the amounts consumed in this study, the seafood consumption among Norwegian adolescents is of no concern in regard to mercury exposure (4). The results also show there is a need for increased awareness and education among adolescents in reference to the importance of seafood in the diet. An increased seafood consumption among the adolescents will provide them with several beneficial nutrients important for a better nutrient status. Dietary interventions are difficult to implement, perhaps especially in this particular age group and compliance is a real challenge, affecting the interpretation of the results.

4.6 Future perspectives

Future intervention studies investigating seafood consumption, MeHg exposure and THHg should be designed with this as the primary outcome, and include other fish species than fatty

fish, with higher MeHg contents than the fish used in this study (4). Such studies should be based on methods which make it possible to calculate the weekly seafood consumption of participants more precisely than what was possible here. A larger study population should be included, to be able to generalize findings to a greater extent than in this thesis (n=116). Also, studies investigating THHg concentrations in adolescents should be conducted in populations with higher habitual seafood consumption. Preferably, intakes matching the dietary recommendations from the health authorities, though this is seemingly hard to accomplish with Norwegian adolescent´s low average seafood consumption in mind. More knowledge is needed on the effects of MeHg when exposed to amounts in agreement with the recommended seafood consumption, not only consequences of extreme exposure from poisonings (28, 40-42), or the lack of negative effects following seafood consumption below the dietary recommendations, as in this thesis. Also, the effect different cooking methods has on MeHg levels in seafood is an interesting field of ongoing research, which can also affect future analysis and interpretation of results (31). Finding a more certain threshold for MeHg exposure and thereby also seafood consumption without experiencing negative consequences, is desired.

The rest of the FINS-TEENS hair samples will be analyzed for THHg concentrations in the future and provide results on the entire study population, which will hopefully allow generalization of findings to a greater extent than what was possible in this thesis.

REFERENCES

1. The Norwegian Directorate of Health (Helsedirektoratet). Kostråd for å fremme folkehelsen og forebygge kroniske sykdommer (Nutrition recommendations to promote public health and prevent chronic diseases) Oslo, Norway: Nasjonalt råd for ernæring; 2011 [cited 2018 -09-15]. Available from: https://helsedirektoratet.no/Lists/Publikasjoner/Attachments/400/Kostrad-for-a-fremme-folkehelsen-og-forebygge-kroniske-sykdommer-metodologi-og-vitenskapelig-kunnskapsgrunnlag-IS-1881.pdf.

2. The Norwegian Directorate of Health (Helsedirektoratet). Ungkost 3: Landsomfattende kostholdsundersøkelse blant elever i 4. -og 8. klasse i Norge, 2015 (Ungkost 3: A national dietary survey among students in 4th and 8th grade in Norway, 2015) Oslo, Norway;2015 [cited 2018 -09-15]. Available from: https://www.fhi.no/globalassets/dokumenterfiler/rapporter/2016/ungkost-rapport-24.06.16.pdf.

3. EFSA (European Food Safety Authority). Scientific Opinion on health benefits of seafood (fish and shellfish) consumption in relation to health risks associated with exposure to methylmercury. EFSA Journal. 2014;12(7):3761.

4. VKM (Norwegian Scientific Committee for Food and Environment). Benefit-risk assessment of fish and fish products in the Norwegian diet - an update. Scientific Opinion of the Scientific Steering Committee. Oslo, Norway, 2014 [cited 2018 -09-16]. VKM report 15 [293 pp]:[Available from: https://www.vkm.no/download/18.2994e95b15cc54507161ea1a/1498222018046/0a646edc5e.pdf.

5. Domingo JL. Nutrients and Chemical Pollutants in Fish and Shellfish. Balancing Health Benefits and Risks of Regular Fish Consumption. Crit Rev Food Sci Nutr. 2016;56(6):979-88.

6. Bosch AC, O'Neill B, Sigge GO, Kerwath SE, Hoffman LC. Heavy metals in marine fish meat and consumer health: a review. J Sci Food Agric. 2016;96(1):32-48.

7. EFSA (European Food Safety Authority). Dioxins and related PCBs: tolerable intake level updated 2018 [cited 2019 -01-07]. Available from: https://www.efsa.europa.eu/en/press/news/181120.

8. VKM (Norwegian Scientific Committee for Food and Environment). Risk-benefit assessment of fish in the Norwegian diet Oslo2019 [cited 2020 -04-14]. Available from: https://vkm.no/english/thenorwegianscientificcommitteeforfoodandenvironment/riskbenefitassessmentoffishinthenorwegiandiet.4.7b65040716afa427d7ec5d3a.html.

9. Kris-Etherton PM, Harris WS, Appel LJ, Nutrition C. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Arterioscler Thromb Vasc Biol. 2003;23(2):e20-30.

10. Kromhout D, Spaaij CJ, de Goede J, Weggemans RM. The 2015 Dutch food-based dietary guidelines. Eur J Clin Nutr. 2016;70(8):869-78.

11. National Health and Medical Research Council. Australian Dietary Guidelines Summary Canberra: National Health and Medical Research Council; 2013 [cited 2019 -01-07]. Available from: https://www.eatforhealth.gov.au/sites/default/files/content/The%20Guidelines/n55a_australian_dietary_guidelines_summary_131014_1.pdf.

12. US Department of Health and Human Services and US Department of Agriculture. 2015-2020 Dietary Guidelines for Americans 2015 [cited 2019 -01-07]. 8th:[Available from: https://health.gov/dietaryguidelines/2015/resources/2015-2020_Dietary_Guidelines.pdf.

13. Miljø- og Fødevareministeriet. De officielle Kostråd: Glostrup; 2015 [cited 2019 -01-07]. 3rd:[Available from: https://altomkost.dk/publikationer/publikation/pub/hent-fil/publication/de-officielle-kostraad/.

14. Public Health England. The Eatwell Guide 2016 [cited 2019 -01-07]. Available from: https://www.nhs.uk/live-well/eat-well/the-eatwell-guide/.

15. The Norwegian Directorate of Health (Helsedirektoratet). Råd til spesielle grupper: Gravide (Nutritional Advice for Pregnant Women) 2011 [cited 2018 -10-24]. Available from: http://www.matportalen.no/rad_til_spesielle_grupper/tema/gravide/.

16. The Norwegian Directorate of Health (Helsedirektoratet). Norkost 3: En landsomfattende kostholdsundersøkelse blant menn og kvinner i Norge i alderen 18-70 år, 2010-11 (Norkost 3: A national dietary survey among men and women in Norway between 18-70 years of age, 2010-11). 2012 [cited 2018 -09-21]. Available from: https://helsedirektoratet.no/Lists/Publikasjoner/Attachments/301/Norkost-3-en-landsomfattende-kostholdsundersokelse-blant-menn-og-kvinner-i-norge-i-alderen-18-70-ar-2010-11-IS-2000.pdf.

17. Sichert-Hellert W, Wicher M, Kersting M. Age and time trends in fish consumption pattern of children and adolescents, and consequences for the intake of long-chain n-3 polyunsaturated fatty acids. European Journal of Clinical Nutrition. 2009;63(9):1071-5.

18. Kranz S, Jones NRV, Monsivais P. Intake Levels of Fish in the UK Paediatric Population. Nutrients. 2017;9(4).

19. Lauritzen L, Harslof LB, Hellgren LI, Pedersen MH, Molgaard C, Michaelsen KF. Fish intake, erythrocyte n-3 fatty acid status and metabolic health in Danish adolescent girls and boys. Br J Nutr. 2012;107(5):697-704.

20. Julian-Almarcegui C, Vandevijvere S, Gottrand F, Beghin L, Dallongeville J, Sjostrom M, et al. Association of heart rate and blood pressure among European adolescents with usual food consumption: The HELENA study. Nutr Metab Cardiovasc Dis. 2016;26(6):541-8.

21. World Health Organization. Mercury and health (Fact sheet No. 361) Geneva: WHO; 2017 [cited 2018 -09-20]. Available from: http://www.who.int/news-room/fact-sheets/detail/mercury-and-health.

22. Clarkson TW, Magos L. The toxicology of mercury and its chemical compounds. Crit Rev Toxicol. 2006;36(8):609-62.

23. Gjerdet NR. Nytt om amalgam. Nor Tannlegeforen tid. 2008;118(6):379-80.

24. World Health Organization International Programme on Chemical Safety. Environmental Health Criteria 101: Methylmercury. Geneva; 1990.

25. Julshamn K, Andersen A, Ringdal O, Morkore J. Trace elements intake in the Faroe Islands. I. Element levels in edible parts of pilot whales (Globicephalus meleanus). Sci Total Environ. 1987;65:53-62.

26. World Health Organization International Programme on Chemical Safety. Mercury 2014 [cited 2018 -26-09]. Available from: http://www.who.int/ipcs/assessment/public_health/mercury/en/.

27. Crowe W, Allsopp PJ, Watson GE, Magee PJ, Strain JJ, Armstrong DJ, et al. Mercury as an environmental stimulus in the development of autoimmunity - A systematic review. Autoimmunity Reviews. 2017;16(1):72-80.

28. Sakamoto M, Tatsuta N, Izumo K, Phan PT, Vu LD, Yamamoto M, et al. Health Impacts and Biomarkers of Prenatal Exposure to Methylmercury: Lessons from Minamata, Japan. Toxics. 2018;6(3).

29. The National Academies. Toxicological Effects of Methylmercury. Washington D.C.: National Academy of Sciences; 2000. 368 p.

30. Bradley MA, Barst BD, Basu N. A Review of Mercury Bioavailability in Humans and Fish. Int J Environ Res Public Health. 2017;14(2).

31. Alves RN, Maulvault AL, Barbosa VL, Fernandez-Tejedor M, Tediosi A, Kotterman M, et al. Oral bioaccessibility of toxic and essential elements in raw and cooked commercial seafood species available in European markets. Food Chem. 2018;267:15-27.

32. Nabi S. Toxic Effects of Mercury. 1st ed. New Delhi: Springer; 2014. 268 p.

33. Clarkson TW. The three modern faces of mercury. Environ Health Perspect. 2002;110 Suppl 1:11-23.

34. Schoeman K, Bend JR, Hill J, Nash K, Koren G. Defining a lowest observable adverse effect hair concentrations of mercury for neurodevelopmental effects of prenatal methylmercury exposure through maternal fish consumption: a systematic review. Ther Drug Monit. 2009;31(6):670-82.

35. Karagas MR, Choi AL, Oken E, Horvat M, Schoeny R, Kamai E, et al. Evidence on the human health effects of low-level methylmercury exposure. Environ Health Perspect. 2012;120(6):799-806.

36. Valent F, Mariuz M, Bin M, Little D, Mazej D, Tognin V, et al. Associations of prenatal mercury exposure from maternal fish consumption and polyunsaturated fatty acids with child neurodevelopment: a prospective cohort study in Italy. J Epidemiol. 2013;23(5):360-70.

37. Kvestad I, Vabo S, Kjellevold M, Nostbakken OJ, Midtbo LK, Hysing M, et al. Fatty fish, hair mercury and cognitive function in Norwegian preschool children: Results from the randomized controlled trial FINS-KIDS. Environ Int. 2018.

38. Bjorklund G, Dadar M, Mutter J, Aaseth J. The toxicology of mercury: Current research and emerging trends. Environ Res. 2017;159:545-54.

39. Kerper LE, Ballatori N, Clarkson TW. Methylmercury transport across the blood-brain barrier by an amino acid carrier. Am J Physiol. 1992;262(5 Pt 2):R761-5.

40. Minamata Disease Study Group. Minamata Disease: Its History and Lessons Minamata: Minamata City Planning Division; 2007 [cited 2018 -10-03]. Available from: http://www.minamata195651.jp/pdf/kyoukun_en/kyoukun_eng_all.pdf.

41. Maruyama K, Yorifuji T, Tsuda T, Sekikawa T, Nakadaira H, Saito H. Methyl mercury exposure at Niigata, Japan: results of neurological examinations of 103 adults. J Biomed Biotechnol. 2012;2012:635075.

42. Bakir F, Damluji SF, Amin-Zaki L, Murtadha M, Khalidi A, al-Rawi NY, et al. Methylmercury poisoning in Iraq. Science. 1973;181(4096):230-41.

43. Pietrzkiewicz K, Maliszewski G, Bombik E. Mercury accumulation level in meat and organs of farm and game animals. Folia Pomeranae Universitatis Technologiae Stetinensis Agricultura, Alimentaria, Piscaria et Zootechnica. 2018;343(3):55-62.

44. Seifert B, Becker K, Helm D, Krause C, Schulz C, Seiwert M. The German Environmental Survey 1990/1992 (GerES II): reference concentrations of selected environmental pollutants in blood, urine, hair, house dust, drinking water and indoor air. J Expo Anal Environ Epidemiol. 2000;10(6 Pt 1):552-65.

45. Castano A, Cutanda F, Esteban M, Part P, Navarro C, Gomez S, et al. Fish consumption patterns and hair mercury levels in children and their mothers in 17 EU countries. Environmental Research. 2015;141:58-68.

46. Bjornberg KA, Vahter M, Petersson-Grawe K, Glynn A, Cnattingius S, Darnerud PO, et al. Methyl mercury and inorganic mercury in Swedish pregnant women and in cord blood: influence of fish consumption. Environ Health Perspect. 2003;111(4):637-41.

47. Institute of Marine Research. Seafood data Bergen: Institute of Marine Research; 2018 [cited 2018 -12-12]. Available from: https://sjomatdata.hi.no/#search/.

48. Foran SE, Flood JG, Lewandrowski KB. Measurement of mercury levels in concentrated over-the-counter fish oil preparations: is fish oil healthier than fish? Arch Pathol Lab Med. 2003;127(12):1603-5.

49. US Food and Drug Administration. Mercury Levels in Commercial Fish and Shellfish (1990-2012) Maryland: US Department of Health and Human Services; 2017 [cited 2018 -12-12]. Available from: https://www.fda.gov/food/foodborneillnesscontaminants/metals/ucm115644.

50. EFSA (European Food Safety Authority). Glossary [cited 2018 -10-02]. Available from: https://www.efsa.europa.eu/en/glossary-taxonomy-terms.

51. Herrman JL, Younes M. Background to the ADI/TDI/PTWI. Regulatory Toxicology and Pharmacology. 1999;30(2):S109-S13.

52. EFSA (European Food Safety Authority). Scientific Opinion: Statement on the benefits of fish/seafood consumption compared to the risks of methylmercury in fish/seafood. EFSA Journal. 2015;13(1):3982.

53. FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization). Evaluation of certain food additives and contaminants. Geneva; 2004.

54. VKM (Norwegian Scientific Committee for Food and Environment). Scenario calculations of mercury exposure from fish and overview of species with high mercury concentrations. Oslo, Norway; 2019. Report No.: 2019:3.

55. McDowell MA, Dillon CF, Osterloh J, Bolger PM, Pellizzari E, Fernando R, et al. Hair mercury levels in US children and women of childbearing age: Reference range data from NHANES 1999-2000. Environmental Health Perspectives. 2004;112(11):1165-71.

56. Freire C, Ramos R, Lopez-Espinosa MJ, Diez S, Vioque J, Ballester F, et al. Hair mercury levels, fish consumption, and cognitive development in preschool children from Granada, Spain. Environ Res. 2010;110(1):96-104.

57. Mergler D, Anderson HA, Chan LH, Mahaffey KR, Murray M, Sakamoto M, et al. Methylmercury exposure and health effects in humans: a worldwide concern. Ambio. 2007;36(1):3-11.

58. Rice DC. The US EPA reference dose for methylmercury: sources of uncertainty. Environ Res. 2004;95(3):406-13.

59. Myers GJ, Davidson PW, Cox C, Shamlaye CF, Palumbo D, Cernichiari E, et al. Prenatal methylmercury exposure from ocean fish consumption in the Seychelles child development study. Lancet. 2003;361(9370):1686-92.

60. Crump KS, Kjellstrom T, Shipp AM, Silvers A, Stewart A. Influence of prenatal mercury exposure upon scholastic and psychological test performance: Benchmark analysis of a New Zealand cohort. Risk Analysis. 1998;18(6):701-13.

61. Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, et al. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol Teratol. 1997;19(6):417-28.

62. USEPA (United States Environmental Protection Agency). Mercury Study Report to Congress Volume 1: Executive Summary. 1997.

63. Næss S. Hair mercury levels and seafood consumption in pregnant women - a randomised controlled trial with dietary cod [Master]. Bergen, Norway: University of Bergen; 2018.

64. Rådmannsøy L. Seafood intake and hair mercury levels in infants after a randomized controlled trial with cod consumption during pregnancy [Master]. Bergen, Norway: University of Bergen; 2018.

65. Høgden HAR. Sammenheng mellom sjømatinntak under svangerskap og nivå av kvikksølv i hår fra mor og spedbarn [Master]. Bergen, Norway: University of Bergen; 2014.

66. Pena-Fernandez A, Del Carmen Lobo-Bedmar M, Gonzalez-Munoz MJ. Effects of sex on the levels of metals and metalloids in the hair of a group of healthy Spanish adolescents (13 to 16 years old). Environ Sci Pollut Res Int. 2017;24(30):23666-78.

67. Ferre-Huguet N, Nadal M, Schuhmacher M, Domingo JL. Monitoring metals in blood and hair of the population living near a hazardous waste incinerator: temporal trend. Biol Trace Elem Res. 2009;128(3):191-9.

68. Budtz-Jorgensen E, Grandjean P, Jorgensen PJ, Weihe P, Keiding N. Association between mercury concentrations in blood and hair in methylmercury-exposed subjects at different ages. Environmental Research. 2004;95(3):385-93.

69. Cejchanova M, Spevackova V, Kratzer K, Wranova K, Spevacek V, Benes B. Determination of mercury and methylmercury in hair of the Czech children's population. Biol Trace Elem Res. 2008;121(2):97-105.

70. Benes B, Sladka J, Spevackova V, Smid J. Determination of normal concentration levels of Cd, Cr, Cu, Hg, Pb, Se and Zn in hair of the child population in the Czech Republic. Cent Eur J Public Health. 2003;11(4):184-6.

71. Carneiro MF, Moresco MB, Chagas GR, de Oliveira Souza VC, Rhoden CR, Barbosa F, Jr. Assessment of trace elements in scalp hair of a young urban population in Brazil. Biol Trace Elem Res. 2011;143(2):815-24.

72. Shim JS, Oh K, Kim HC. Dietary assessment methods in epidemiologic studies. Epidemiol Health. 2014;36:e2014009.

73. Gibson RS. Measuring food consumption of individuals. Principles of Nutritional Assessment. 2nd ed. New York: Oxford university press; 2005. p. 41-6.

74. Stumbo PJ. New technology in dietary assessment: a review of digital methods in improving food record accuracy. Proc Nutr Soc. 2013;72(1):70-6.

75. Schatzkin A, Kipnis V, Carroll RJ, Midthune D, Subar AF, Bingham S, et al. A comparison of a food frequency questionnaire with a 24-hour recall for use in an epidemiological cohort study: results from the biomarker-based Observing Protein and Energy Nutrition (OPEN) study. Int J Epidemiol. 2003;32(6):1054-62.

76. Illner AK, Freisling H, Boeing H, Huybrechts I, Crispim SP, Slimani N. Review and evaluation of innovative technologies for measuring diet in nutritional epidemiology. Int J Epidemiol. 2012;41(4):1187-203.

77. Shriver BJ, Roman-Shriver CR, Long JD. Technology-based methods of dietary assessment: recent developments and considerations for clinical practice. Curr Opin Clin Nutr Metab Care. 2010;13(5):548-51.

78. Handeland K. Fatty fish intake, adherence to dietary guidelines and attention performance: a randomized controlled trial in Norwegian adolescents [PhD]. Bergen, Norway: University of Bergen; 2017.

79. Skotheim S, Dahl L, Handeland K, Froyland L, Lie O, Oyen J, et al. Design of the FINS-TEENS study: A randomized controlled trial assessing the impact of fatty fish on cognitive performance in adolescents. Scand J Public Health. 2017;45(6):621-9.

80. Handeland K, Kjellevold M, Wik Markhus M, Eide Graff I, Froyland L, Lie O, et al. A Diet Score Assessing Norwegian Adolescents' Adherence to Dietary Recommendations-Development and Test-Retest Reproducibility of the Score. Nutrients. 2016;8(8).

81. Cohen J. Statistical power analysis for the behavioral sciences. 2nd ed. Hillsdale, NJ: Lawrence Earlbaum Associates1988.

82. Handeland K, Oyen J, Skotheim S, Graff IE, Baste V, Kjellevold M, et al. Fatty fish intake and attention performance in 14-15 year old adolescents: FINS-TEENS - a randomized controlled trial. Nutr J. 2017;16(1):64.

83. Skotheim S, Handeland K, Kjellevold M, Oyen J, Froyland L, Lie O, et al. The effect of school meals with fatty fish on adolescents' self-reported symptoms for mental health: FINS-TEENS - a randomized controlled intervention trial. Food Nutr Res. 2017;61(1):1383818.

84. LeBeau MA, Montgomery MA, Brewer JD. The role of variations in growth rate and sample collection on interpreting results of segmental analyses of hair. Forensic Sci Int. 2011;210(1-3):110-6.

85. USEPA (United States Environmental Protection Agency). Method 7473 (SW-846). Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry. 2007.

86. Milestone s.r.l. [cited 2018 -11-21]. Available from: https://www.milestonesrl.com/products/mercury-determination/dma-80-evo.

87. USEPA (United States Environmental Protection Agency). Guidance for data quality assessment: Practical methods for data analysis. Washington; 2000.

88. Helsel DR. Fabricating data: How substituting values for nondetects can ruin results, and what can be done about it. Chemosphere. 2006;65(11):2434-9.

89. Markhus MW, Graff IE, Dahl L, Seldal CF, Skotheim S, Braarud HC, et al. Establishment of a seafood index to assess the seafood consumption in pregnant women. Food & Nutrition Research. 2013;57.

90. Madden JP, Goodman SJ, Guthrie HA. Validity of the 24-hr. recall. Analysis of data obtained from elderly subjects. J Am Diet Assoc. 1976;68(2):143-7.

91. Gersovitz M, Madden JP, Smiciklas-Wright H. Validity of the 24-hr. dietary recall and seven-day record for group comparisons. J Am Diet Assoc. 1978;73(1):48-55.

92. Mattilsynet, Universitetet i Oslo, Helsedirektoratet. Mål, vekt og porsjonsstørrelser for matvarer (Weights, measures and portion sizes for foods) 2015 [Available from: http://www.matportalen.no/verktoy/kostholdsplanleggeren/maal_vekt_og_porsjonsstorrelser_for_matvarer.

93. Nilsen B, Måge A. Kvikksølv i hermetisert tunfisk. NIFES-rapport (Mercury in canned tuna) Bergen, Norway: National Institute of Nutrition and Seafood Research (NIFES); 2016 [Available from: https://nifes.hi.no/wp-content/uploads/2016/08/rapportforkvikksolvihermetiserttunfisk2016.pdf.

94. Julshamn K, Frantzen S. Miljøgifter i fisk og fiskevarer - En rapport om dioksiner og dioksinlignende PCB, polybromerte flammehemmere og tungmetaller i oljer, makrell, ål og Svolværpostei Bergen, Norway: Mattilsynet; 2008

95. Pallant J. SPSS survival manual - A step by step guide to data analysis using SPSS. 4th ed. Berkshire, England: Open University Press; 2010.

96. Juliusson PB, Hjelmesaeth J, Bjerknes R, Roelants M. New curves for body mass index among children and adolescents. Tidsskr Nor Laegeforen. 2017;137(18).

97. Sjoberg A, Hulthen L. Assessment of habitual meal pattern and intake of foods, energy and nutrients in Swedish adolescent girls: comparison of diet history with 7-day record. Eur J Clin Nutr. 2004;58(8):1181-9.

98. Andersen R, Biltoft-Jensen A, Christensen T, Andersen EW, Ege M, Thorsen AV, et al. What do Danish children eat, and does the diet meet the recommendations? Baseline data from the OPUS School Meal Study. J Nutr Sci. 2015;4.

99. Glynn L, Emmett P, Rogers I, Team AS. Food and nutrient intakes of a population sample of 7-year-old children in the south-west of England in 1999/2000 - what difference does gender make? J Hum Nutr Diet. 2005;18(1):7-19; quiz 21-3.

100. Welch AA, Lund E, Amiano P, Dorronsoro M, Brustad M, Kumle M, et al. Variability of fish consumption within the 10 European countries participating in the European Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr. 2002;5(6b):1273-85.

101. Stratakis N, Roumeliotaki T, Oken E, Barros H, Basterrechea M, Charles MA, et al. Fish Intake in Pregnancy and Child Growth A Pooled Analysis of 15 European and US Birth Cohorts. Jama Pediatr. 2016;170(4):381-90.

102. Leventakou V, Roumeliotaki T, Martinez D, Barros H, Brantsaeter AL, Casas M, et al. Fish intake during pregnancy, fetal growth, and gestational length in 19 European birth cohort studies. American Journal of Clinical Nutrition. 2014;99(3):506-16.

103. Handeland K, Skotheim S, Baste V, Graff IE, Froyland L, Lie O, et al. The effects of fatty fish intake on adolescents' nutritional status and associations with attention performance: results from the FINS-TEENS randomized controlled trial. Nutr J. 2018;17(1):30.

104. Oyen J, Kvestad I, Midtbo LK, Graff IE, Hysing M, Stormark KM, et al. Fatty fish intake and cognitive function: FINS-KIDS, a randomized controlled trial in preschool children. BMC Med. 2018;16(1):41.

105. Vejrup K, Schjolberg S, Knutsen HK, Kvalem HE, Brantsaeter AL, Meltzer HM, et al. Prenatal methylmercury exposure and language delay at three years of age in the Norwegian Mother and Child Cohort Study. Environ Int. 2016;92-93:63-9.

106. Grandjean P, Weihe P, Debes F, Choi AL, Budtz-Jorgensen E. Neurotoxicity from prenatal and postnatal exposure to methylmercury. Neurotoxicol Teratol. 2014;43:39-44.

107. Tavares LM, Camara VM, Malm O, Santos EC. Performance on neurological development tests by riverine children with moderate mercury exposure in Amazonia, Brazil. Cad Saude Publica. 2005;21(4):1160-7.

108. Davidson PW, Leste A, Benstrong E, Burns CM, Valentin J, Sloane-Reeves J, et al. Fish consumption, mercury exposure, and their associations with scholastic achievement in the Seychelles Child Development Study. Neurotoxicology. 2010;31(5):439-47.

109. Schulz KF, Grimes DA. Blinding in randomised trials: hiding who got what. Lancet. 2002;359(9307):696-700.

110. Statistisk sentralbyrå. Befolkningens utdanningsnivå Oslo: SSB; 2018 [cited 2019 -02-26]. Available from: https://www.ssb.no/utdanning/statistikker/utniv/aar.

111. Hanson MD, Chen E. Socioeconomic status and health behaviors in adolescence: a review of the literature. J Behav Med. 2007;30(3):263-85.

112. Skardal M, Western IM, Ask AMS, Overby NC. Socioeconomic differences in selected dietary habits among Norwegian 13-14 year-olds: a cross-sectional study. Food & Nutrition Research. 2014;58.

113. Dahl L, Maeland CA, Bjorkkjaer T. A short food frequency questionnaire to assess intake of seafood and n-3 supplements: validation with biomarkers. Nutr J. 2011;10:127.

114. Samet JM, Humble CG, Skipper BE. Alternatives in the collection and analysis of food frequency interview data. Am J Epidemiol. 1984;120(4):572-81.

115. Hankin JH, Rhoads GG, Glober GA. A dietary method for an epidemiologic study of gastrointestinal cancer. Am J Clin Nutr. 1975;28(9):1055-60.

APPENDICES

Appendix I: Form of request and consent for participation in the FINS-TEENS study

Appendix II: Food frequency questionnaire (FFQ) used pre- and post-intervention

Appendix III: Questionnaire completed by participant's caregiver(s)

Appendix I: Form of request and consent for participation in the FINS-TEENS study

Forespørsel om deltakelse i forskningsprosjektet «Betydning av kosthold for konsentrasjon og læring hos ungdomsskoleelever»

Bakgrunn og hensikt

Dette er et spørsmål til deg som elev på 9.trinn om å delta i studien «Betydningen av kosthold for konsentrasjon og læring hos ungdomsskoleelever». Før du bestemmer deg for om du vil delta er det viktig at du og dine foresatte forstår hvorfor studien gjennomføres, hva det innebærer og hvilke fordeler og eventuelle ulemper som kan være forbundet med å delta.

Hensikten med denne studien er å studere betydningen av kosthold for konsentrasjon og læring hos ungdomsskoleelever. Dette ønsker vi å undersøke ved at elevene blir tilfeldig inndelt i grupper hvor en gruppe elever får servert lunsj med sjømat, en gruppe elever får servert tilsvarende lunsj med ost/kjøtt (ikke sjømat) og en gruppe elever får omega-3 kapsler tre ganger i uken over en periode på 12 uker. Hvis du har kjent matvareallergi/intoleranse kan det hende at du ikke kan delta i studien. Det kommer ikke til å bli servert svinekjøtt og det kan bli tatt hensyn til hvis noen trenger halalkjøtt. Studien er et tverrfaglig forskningsprosjekt mellom Nasjonalt institutt for ernærings- og sjømatforskning (NIFES), Regionalt kunnskapssenter for barn og unge, psykisk helse og barnevern (RKBU Vest, Uni Research Helse) og UiT – Norges arktiske universitet.

Hva innebærer det å være med på studien

- Elevene får servert lunsj på skolen tre ganger i uken i 12 uker, med unntak av elevene som får omega-3 kapsler.
- Det vil bli gjennomført tester knyttet til konsentrasjon og lese- og skriveferdigheter i skoletiden både før og etter forsøksperioden.
- Elevene svarer på spørreskjema i skoletiden knyttet til kosthold, fysisk aktivitet, søvn og psykisk helse både før og etter forsøksperioden.
- Det vil bli tatt blod-, hår- og urinprøver av alle elevene i eller etter skoletid en gang før forsøket starter og en gang etter at forsøket er ferdig.
- En av elevens foresatte vil også få tilsendt ett spørreskjema på e-post hvor de blir bedt om å svare på spørsmål knyttet til sosioøkonomiske forhold, samt spørsmål om elevens søvn, sykdommer og bruk av medisiner både før og etter forsøksperioden.

Blod-, urin- og hårprøvetaking

Prosjektmedarbeider kommer til å gjøre avtale med lærerne på den enkelte skole når blodprøvetakingen skal gjennomføres. Vi vil låne et egnet rom på skolen og elevene kommer i små grupper, slik at det ikke blir mye venting. På noen skoler antar vi at vi kan få til blodprøvetakingen i skoletiden, mens på andre skoler må vi gjøre dette helt på slutten av skoledagen. Vi vil bruke erfarne og godkjente bioingeniører som blodprøvetakere. Når det gjelder urinprøvetaking kommer vi til å be om at eleven tar med en urinprøve hjemmefra den dagen vi har en avtale for blodprøvetaking. Hvis eleven ikke har med urinprøve til blodprøvetakingen ber vi eleven ta den med når vi møtes på skolen. Hårprøven tas på samme tidspunkt som blod og urinprøven.

Fordeler og ulemper

Fordelen ved å bli med i studien er at eleven får servert gratis lunsj eller omega-3 kapsler på skolen tre ganger i uken i hele forsøksperioden. Måltidene vil bli produsert av et cateringfirma, og valg av ingredienser vil være i samsvar med nasjonale kostholdsanbefalinger. Som deltaker kan du få tilbakemelding på din jernstatus. Din deltakelse vil bidra til å gi større forståelse for betydningen av kosthold i forhold til barns konsentrasjon og læringsevne. Alle skolene som deltar i studien vil få tilbud om en felles gjennomgang av resultater fra studien i etterkant. Resultater vil bli presentert på gruppenivå og det vil derfor ikke være mulig å kjenne igjen svarene fra enkeltelever. Mulige ulemper ved å delta på studien kan være ubehag ved blodprøvetaking. Ved å bruke erfarne og godkjente blodprøvetakere sørger vi for at ubehaget blir minst mulig. Blodprøvetaking kan også forårsake ømhet og eventuelt blåmerker på stikkstedet. Svimmelhet og besvimelse kan unntaksvis forekomme.

Hva skjer med prøvene og informasjonen om deg?

Prøvene vil bli analysert for næringsstoffer, biomarkører/gen og uønskede stoffer som er sentrale for å vurdere sjømat som sunn og trygg mat. Alle opplysningene vil bli behandlet uten navn og fødselsdato eller andre direkte gjenkjennende opplysninger (avidentifisert) og lagret på en sikker server på NIFES (biologiske data, kostholdsdata og psykologiske data). Foresatte sine svar vil bli koblet mot barna (elevene) sine svar. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste. Denne listen vil bli lagret på en minnepenn og lagret i et brannsikkert skap ved NIFES. Det er kun prosjektleder og enkelte prosjektmedarbeidere som har adgang til navnelisten og som kan finne tilbake til deg. Prosjektleder og prosjektmedarbeidere har taushetsplikt, og ingen andre har tilgang til personidentifiserbare data.

Prosjektet er vurdert av Regional komité for medisinsk og helsefaglig forskningsetikk (REK sør-øst D) og Norsk samfunnsvitenskapelig datatjeneste (NSD). Biologisk materiale som blod, urin og hår vil bli

oppbevart i godkjent forskningsbiobank ved NIFES til prosjektslutt. Eventuelle rester av disse prøvene vil da bli slettet (destruert). Registrerte data (både biologiske data og andre innsamlede data) vil bli lagret frem til prosjektet avsluttes i 2025 og deretter bli anonymisert og oppbevart hos NSD. Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

Studien er forskerinitiert med finansiering fra Fiskeri og havbruksnæringens forskingsfond (FHF), NIFES, RKBU Vest og UiT etter vitenskapelig vurdering i Norges forskningsråd (NFR222648/FHF900842). Prosjektet er forskerstyrt og alle resultater vil bli publisert. Kommersielle aktører bidrar med produkter og generiske forskningsmidler. Involverte forskere eller institusjoner har ingen økonomiske interesser i studien.

Frivillig deltakelse

Det er frivillig å delta i studien. Om du sier ja til å delta, undertegner du samtykkeerklæringen på siste side. Selv om du sier ja til å delta nå, kan du senere når som helst og uten å oppgi noen grunn, trekke tilbake ditt samtykke. Når elevene fyller 16 år vil vi be om et nytt samtykke fra alle elevene for oppbevaring av innsamlet data og vi ber derfor om at adressen din oppgis i samtykket. Dersom du ønsker å trekke deg fra studien kan du be om at all innsamlet informasjon blir anonymisert og biologisk materiale slettet (destruert). Dersom du/dere har spørsmål knyttet til studien eller ønsker å trekke samtykke kan du kontakte:

Prosjektleder Lisbeth Dahl på telefon: 47291689 eller e-post: lisbeth.dahl@nifes.no eller

Prosjektmedarbeider Siv Skotheim på telefon: 55 58 86 90 eller e-post: siv.skotheim@uni.no.

Samtykke til deltakelse i studien

(Rives av og leveres til kontaktlærer snarest mulig)

Jeg har mottatt informasjon om studien og gir tillatelse til at mitt barn kan delta i studien
" Betydningen av kosthold for konsentrasjon og læring hos ungdomsskoleelever"

Navn på **eleven** (deltaker):

Fornavn: _____ Etternavn: _____

Dato: _____ Underskrift **foresatt**: _____

_____

(E-post adresse til **foresatt** som signerer)

_____

Adresse, postnummer og poststed

☐ Eleven har matvareallergier/intoleranser

Hvis ja, hvilke: _____

☐ Eleven spiser ikke svinekjøtt

☐ Eleven må ha halalkjøtt

Jeg har mottatt informasjon om studien og er villig til å delta i studien
" Betydningen av kosthold for konsentrasjon og læring hos ungdomsskoleelever"

Dato: _____ Underskrift **elev** (fornavn og etternavn): _____

Mobilnummer: _____

Skole: _____ Klasse: _____

Appendix II: Food frequency questionnaire (FFQ) used pre- and post-intervention

NASJONALT INSTITUTT FOR ERNÆRINGS- OG SJØMATFORSKNING

ID: _____

Dato: _____

Spørreskjema om livsstil og hva du spiser

Ha de ***3 siste månedene*** i bakhodet når du fyller ut skjemaet. Vi er klar over at kostholdet varierer fra dag til dag. Prøv likevel så godt du kan å gi et "gjennomsnitt" av ditt inntak. Du skal bare sette ETT kryss på hvert spørsmål med mindre noe annet er spesifisert, og krysset skal være inne i en boks, ikke mellom boksene.

1. Hvor ofte har du spist fisk, fiskeprodukter eller annen sjømat som middagsmat siste 3 måneder?

Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger/ måned	1 gang/uke	2-3 ganger/ uke	4 ganger eller mer/uke
☐	☐	☐	☐	☐	☐

2. Hvor ofte har du spist følgende sjømat <u>som middag</u> siste 3 måneden?

	Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger/ måned	1 gang/uke	2-3 ganger/ uke	4 ganger eller mer/uke
Laks, ørret	☐	☐	☐	☐	☐	☐
Makrell	☐	☐	☐	☐	☐	☐
Sild	☐	☐	☐	☐	☐	☐
Kveite	☐	☐	☐	☐	☐	☐
Torsk	☐	☐	☐	☐	☐	☐
Sei	☐	☐	☐	☐	☐	☐
Annen mager/hvit fisk (lyr, hyse, rød-spette)	☐	☐	☐	☐	☐	☐
Sushi	☐	☐	☐	☐	☐	☐
Skalldyrmiddag (reker, krabbe, blåskjell)	☐	☐	☐	☐	☐	☐
Fiskekaker	☐	☐	☐	☐	☐	☐
Fiskepinner	☐	☐	☐	☐	☐	☐
Andre fiskeprodukter (fiskegrateng, fiskeboller)	☐	☐	☐	☐	☐	☐

NASJONALT INSTITUTT FOR ERNÆRINGS- OG SJØMATFORSKNING

ID: _____

Dato: _____

3. Hvor ofte har du spist sjømat som pålegg, i salat, mellommåltid, snacks eller lignende de siste 3 mnd?

Aldri	Sjelden	1-3 ganger/ måned	1 gang / uke	2 ganger/uke	3-5 ganger / uke	Mer enn 5 ganger / uke
☐	☐	☐	☐	☐	☐	☐

4. Hvor ofte spiser du vanligvis følgende sjømat som pålegg, i salat, mellommåltid, snacks eller lignende de siste tre måneder?

	Aldri	Sjeldent	1-3 ganger/ måned	1 gang/ uke	2 ganger/ uke	3-5 ganger eller mer/uke	Mer enn 5 ganger /uke
Makrell i tomat	☐	☐	☐	☐	☐	☐	☐
Sardiner eller sild på boks	☐	☐	☐	☐	☐	☐	☐
Annen fet fisk som pålegg (peppermakrell, røkt eller gravet laks/ørret)	☐	☐	☐	☐	☐	☐	☐
Tunfisk	☐	☐	☐	☐	☐	☐	☐
Kaviar	☐	☐	☐	☐	☐	☐	☐
Svolværpostei/ Lofotpostei	☐	☐	☐	☐	☐	☐	☐

5. Hvilke brød/knekkebrød type spiser du vanligvis?

Fint (0 -25% sammalt/hele korn) ☐

Halvgrovt (25-50% sammalt/hele korn) ☐

Grovt(50-75% sammalt/hele korn) ☐

Ekstra grovt (75-100% sammalt/hele korn) ☐

Jeg spiser ikke brød eller knekkebrød ☐

NASJONALT INSTITUTT
FOR ERNÆRINGS- OG
SJØMATFORSKNING

6. Bruker du smør eller margarin på brødskiven/knekkebrød/rundstykke?

☐ Ja ☐ Nei

7. Spiser du meieriprodukter (melk, yoghurt, ost)?

Sjelden/aldri	1-3 ganger/uke	4-6 ganger/uke	Hver dag	2 ganger/dag	3-4 ganger eller mer/dag
☐	☐	☐	☐	☐	☐

Hvis ja, hvor mange ganger per uke eller dag spiser/drikker du følgende meieriprodukter?

	Sjelden/aldri	1-3 ganger/uke	4-6 ganger/uke	Hver dag	2 ganger/dag	3-4 ganger eller mer/dag
Helmelk	☐	☐	☐	☐	☐	☐
Lettmelk	☐	☐	☐	☐	☐	☐
Ekstra lett melk	☐	☐	☐	☐	☐	☐
Skummet melk	☐	☐	☐	☐	☐	☐
Sjokolademelk	☐	☐	☐	☐	☐	☐
Drikkeyoghurt	☐	☐	☐	☐	☐	☐
Biola	☐	☐	☐	☐	☐	☐
Cultura	☐	☐	☐	☐	☐	☐
Yoghurt	☐	☐	☐	☐	☐	☐
Hvitost (eks. Norvegia, Jarlsberg, smøreoster)	☐	☐	☐	☐	☐	☐
Brunost	☐	☐	☐	☐	☐	☐

NASJONALT INSTITUTT FOR ERNÆRINGS- OG SJØMATFORSKNING

8. Hvor ofte har du spist retter med rødt kjøtt (pølser, kjøttdeig, biff, koteletter fra svin, storfe, vilt og lam) som middagsmat siste 3 måneder?

Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger/ måned	1 gang/uke	2-3 ganger/ uke	4 ganger eller mer/uke
☐	☐	☐	☐	☐	☐

9. Hvor ofte har du spist retter med kylling/kalkun som middagsmat siste 3 måneder?

Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger/ måned	1 gang/uke	2-3 ganger/ uke	4 ganger eller mer/uke
☐	☐	☐	☐	☐	☐

10. Hvor mange egg spiser du per uke? (stekt, kokt, eggerøre, omelett)

Ingen	1 egg/uke	2-3 egg/uke	4-5 egg /uke	6-7 egg/uke	8 eller flere egg/uke
☐	☐	☐	☐	☐	☐

11. Hvor mange porsjoner grønnsaker eller frukt/bær spiser du i løpet av dagen?
(En porsjon kan for eksempel være 1 middels stor frukt (eple, pære, banan eller annet), eller en håndfull druer, eller ett glass juice. En porsjon grønnsaker kan for eksempel være 1 gulrot eller 3 buketter brokkoli eller en porsjonsbolle med salat. Poteter regnes ikke med.)

	Aldri/sjelden	1-3 porsjoner/uke	4-6 porsjoner/uke	1 porsjon/dag	2 porsjoner /dag	3 porsjoner/dag	4 porsjoner eller mer/dag
Frukt og bær (ikke juice og smoothie)	☐	☐	☐	☐	☐	☐	☐
Grønnsaker	☐	☐	☐	☐	☐	☐	☐
Juice (eks. eple, appelsin)	☐	☐	☐	☐	☐	☐	☐
Smoothie	☐	☐	☐	☐	☐	☐	☐

NASJONALT INSTITUTT FOR ERNÆRINGS- OG SJØMATFORSKNING

12. Har du tatt tran, fiskeolje- eller omega-3 tilskudd (flytende eller som kapsler) de siste 3 mnd?

Ja	Nei
☐	☐

<u>Hvis ja på spm 12</u>, hvilke type tran, fiskeolje- eller omega-3 tilskudd bruker du og hvor ofte?

	Aldri	1-3 ganger/ måned	1-3 ganger/uke	4-6 ganger/uke	Daglig
Tran/Fiskeoljer, flytende	☐	☐	☐	☐	☐
Tran/ Fiskeoljer, kapsler	☐	☐	☐	☐	☐

Spesifiser hvilke(t) merke på tran, fiskeolje- eller omega-3 tilskudd og hvor mye du tar hver gang:

_____

_____

13. Bruker du annet kosttilskudd (vitaminer og mineraler)?

☐ Ja ☐ Nei

<u>14. Hvis ja på spm 13,</u> hvilke type kosttilskudd bruker du og hvor ofte?

	Aldri	1-3 ganger/ måned	1-3 ganger/uke	4-6 ganger/uke	Daglig
Multivitamin (sanasol, vitaminbjørner, biovit, tabletter)	☐	☐	☐	☐	☐
Multimineral	☐	☐	☐	☐	☐
Vitamin D	☐	☐	☐	☐	☐
Jern	☐	☐	☐	☐	☐
Annet	☐	☐	☐	☐	☐

Hvis annet, spesifiser hva:

_____

NASJONALT INSTITUTT FOR ERNÆRINGS- OG SJØMATFORSKNING

Spesifiser hvilke(t) merke på kosttilskudd og hvor mye du tar hver gang:

_____

_____

_____

15. Hvor ofte de siste 3 månedene har du brukt solarium?

☐ Aldri ☐ Sjeldnere enn 1gang/måned ☐ 1 gang/måned ☐ 2-3 ganger/måned ☐ 1-2 ganger/uke

16. Hvor mange uker de siste tre månedene har du vært i Syden?

☐ Har ikke vært i Syden ☐ 1 uke ☐ 2 uker ☐ 3uker ☐ 4 uker eller mer

17. Hvor mange timer er du fysisk aktiv totalt i løpet av en uke? (Moderat til høy intensitet, som rask gåing, løping, ballsport, kampsport og lignende)

☐ En halv time eller mindre/uke ☐ 1 time/uke ☐ 2 timer /uke ☐3 timer/uke eller mer

☐4 timer/uke eller mer

18. Hvor stor vekt legger du på å ha et sunt kosthold?

Veldig liten	Liten	Middels	Stor	Veldig stor
☐	☐	☐	☐	☐

19. Spiser du frokost på morgenen? (Enten hjemme eller når du kommer på skolen)?

☐ Aldri ☐ 1-2 ganger/uke ☐ 3-4 gang/uke ☐ 5-6 ganger/uke ☐ hver dag

NASJONALT INSTITUTT
FOR ERNÆRINGS- OG
SJØMATFORSKNING

20. Hvor ofte spiser du medbrakt matpakke på skolen?

☐ Aldri ☐ 1-2 ganger/uke ☐ 3-4 gang/uke ☐ hver dag

21. Hvor ofte handler du lunsj i kantinen på skolen?

☐ Aldri ☐ 1-2 ganger/uke ☐ 3-4 gang/uke ☐ hver dag

22. Hvor ofte spiser du sjokolade, kaker, kjeks, snop eller lignende <u>på skolen</u>?

☐ Aldri ☐ 1-2 ganger/uke ☐ 3-4 gang/uke ☐ hver dag

23. Hvor ofte spiser du sjokolade, kaker, kjeks, snop eller lignende <u>på fritiden</u>?

☐ Aldri ☐ 1-2 ganger/uke ☐ 3-4 gang/uke ☐ 5-6 ganger/uke ☐ hver dag

24. Hvor ofte drikker du følgende drikker?

	Aldri/sjelden	1-3 ganger/uke	4-6 ganger/uke	Hver dag	2 ganger/dag	3-4 ganger/dag	5 ganger eller mer/dag
Brus/iste/energidrikk (med sukker)	☐	☐	☐	☐	☐	☐	☐
Sukkerfri/lettbrus	☐	☐	☐	☐	☐	☐	☐
Vann	☐	☐	☐	☐	☐	☐	☐

25. Røyker, eller snuser du?

Aldri/sjelden	1 gang / måned	2-3 ganger / måned	1-3 ganger/uke	4-6 ganger/uke	Hver dag	Flere ganger om dagen
☐	☐	☐	☐	☐	☐	☐

NASJONALT INSTITUTT
FOR ERNÆRINGS- OG
SJØMATFORSKNING

Søvn

Her er noen spørsmål om søvn. Svar hvordan det har vært den siste måneden.

26. Når legger du deg vanligvis?

Hverdager (skriv inn klokkeslett i timer og hele kvarter-For eksempel 21.30 eller 22.15)

Helger (skriv inn klokkeslett i timer og hele kvarter-For eksempel 21.30 eller 22.15)

27. Når står du vanligvis opp?

Hverdager (skriv inn klokkeslett i timer og hele kvarter-For eksempel 06.30 eller 07.15)

Helger (skriv inn klokkeslett i timer og hele kvarter-For eksempel 08.00 eller 10.15)

28. Synes du at du får nok søvn?

Ikke nok	Litt lite	Passe	Litt mye	For mye
☐	☐	☐	☐	☐

NASJONALT INSTITUTT
FOR ERNÆRINGS- OG
SJØMATFORSKNING

29. Er du jente eller gutt?

☐ Jente ☐ Gutt

30. Fødselsår:

31. Fødselsmåned (eks januar, februar):

32. Høyde og vekt

Høyde:

Cm

Vekt:

kg

NASJONALT INSTITUTT
FOR ERNÆRINGS- OG
SJØMATFORSKNING

Sterke og svake sider (SDQ-Norsk)

Vennligst kryss av for hvert utsagn: stemmer ikke, stemmer delvis eller stemmer helt.

Prøv å svare på alt selv om du ikke er helt sikker eller synes utsagnet virker rart. Svar på grunnlag av hvordan du har hatt den de siste 6 månedene.

	Stemmer ikke	Stemmer delvis	Stemmer helt
Jeg prøver å være hyggelig mot andre. Jeg bryr meg om hva de føler	☐	☐	☐
Jeg er rastløs. Jeg kan ikke være lenge i ro	☐	☐	☐
Jeg har ofte hodepine, vondt i magen eller kvalme	☐	☐	☐
Jeg deler gjerne med andre (mat, spill, andre ting)	☐	☐	☐
Jeg blir ofte sint og har kort lunte	☐	☐	☐
Jeg er ofte for meg selv. Jeg gjør som regel ting alene	☐	☐	☐
Jeg gjør som regel det jeg får beskjed om	☐	☐	☐
Jeg bekymrer meg mye	☐	☐	☐
Jeg stiller opp hvis noen er såret, lei seg eller føler seg dårlig	☐	☐	☐
Jeg er stadig urolig eller i bevegelse	☐	☐	☐
Jeg har en eller flere gode venner	☐	☐	☐
Jeg slåss mye. Jeg kan få andre til å gjøre det jeg vil	☐	☐	☐
Jeg er ofte lei meg, nedfor eller på gråten	☐	☐	☐
Jeg blir som regel likt av andre på min alder	☐	☐	☐
Jeg blir lett distrahert, jeg synes det er vanskelig å konsentrere meg	☐	☐	☐
Jeg blir nervøs i nye situasjoner. Jeg blir lett usikker	☐	☐	☐
Jeg er snill mot de som er yngre enn meg	☐	☐	☐
Jeg blir ofte beskyldt for å lyve eller jukse	☐	☐	☐
Andre barn eller unge plager eller mobber meg	☐	☐	☐
Jeg tilbyr meg ofte å hjelpe andre (foreldre, lærere, andre barn/unge)	☐	☐	☐
Jeg tenker meg om før jeg handler (gjør noe)	☐	☐	☐
Jeg tar ting som ikke er mine hjemme, på skolen eller andre steder	☐	☐	☐
Jeg kommer bedre overens med voksne enn de på min egen alder	☐	☐	☐
Jeg er redd for mye, jeg blir lett skremt	☐	☐	☐
Jeg fullfører oppgaver. Jeg er god til å konsentrere meg	☐	☐	☐

Har du andre kommentarer eller bekymringer?

NASJONALT INSTITUTT
FOR ERNÆRINGS- OG
SJØMATFORSKNING

Samlet, synes du at du har vansker på ett eller flere av følgende områder:
med følelser, konsentrasjon, oppførsel eller med å komme overens med andre mennesker ?

Nei	Ja-små vansker	Ja-tydelige vansker	Ja-alvorlige vansker
☐	☐	☐	☐

Hvis du har svart "Ja", vennligst svar på følgende spørsmål:

- Hvor lenge har disse vanskene vært tilstede?

Mindre enn en måned	1-5 måneder	6-12 måneder	Mer enn ett år
☐	☐	☐	☐

- Forstyrrer eller plager vanskene deg?

Ikke i det hele tatt	Bare litt	En god del	Mye
☐	☐	☐	☐

- Virker vanskene inn på livet ditt på noen av disse områdene?

	Ikke i det hele tatt	Bare litt	En god del	Mye
HJEMME / I FAMILIEN	☐	☐	☐	☐
FORHOLD TIL VENNER	☐	☐	☐	☐
LÆRING PÅ SKOLEN	☐	☐	☐	☐
FRITIDSAKTIVITETER	☐	☐	☐	☐

- Er vanskene en belastning for de rundt deg (familie, venner, lærere osv.) ?

Ikke i det hele tatt	Bare litt	En god del	Mye
☐	☐	☐	☐

Appendix III: Questionnaire completed by participant's caregiver(s)

NASJONALT INSTITUTT
FOR ERNÆRINGS- OG
SJØMATFORSKNING

ID: _____

Spørreskjema til foresatte

I dette spørreskjema ønsker vi litt informasjon om deg og din familie, samt at vi ber deg svare på et par spørsmål knyttet til barnet ditt.

Spørsmål knyttet til deg og din familie:

Dato for utfylling (dd.mm.åååå):

Mitt barn er:

Gutt ☐

Jente ☐

Utfylt av:

❍ Mor ❍ Far ❍ Annen, spesifiser: _____

Spørsmål om inntekt og demografi:

I hvilket land er du/dere født?

	Norge	Annet land	Hvis annet land, hvilket?
Mor	❍	❍	
Far	❍	❍	
Barn	❍	❍	

NASJONALT INSTITUTT FOR ERNÆRINGS- OG SJØMATFORSKNING

ID: _____

Hva er den høyeste utdanningen dere har fullført?

Mor	❍ Grunnskole, ungdomsskole eller lign. ❍ Videregående skole med yrkesfag, yrkesskole, realskole ❍ Videregående skole med allmennfag, gymnas/artium ❍ Høgskole eller universitet, mindre enn 4 år ❍ Høgskole eller universitet, 4 år eller mer
Far	❍ Grunnskole, ungdomsskole eller lign. ❍ Videregående skole med yrkesfag, yrkesskole, realskole ❍ Videregående skole med allmennfag, gymnas/artium ❍ Høgskole eller universitet, mindre enn 4 år ❍ Høgskole eller universitet, 4 år eller mer

Hver fars hovedbeskjeftigelse for tiden?

❍ Heltidsarbeid (80 - 100%)
❍ Deltidsarbeid (50 - 79 %)
❍ Deltidsarbeid (mindre enn 50%)
❍ Trygdet/attføring/arbeidsavklaringspenger
❍ Hjemmeværende
❍ Arbeidssøkende
❍ Under utdanning

Hva er mors hovedbeskjeftigelse for tiden?

❍ Heltidsarbeid (80 - 100%)
❍ Deltidsarbeid (50 - 79 %)
❍ Deltidsarbeid (mindre enn 50%)
❍ Trygdet/attføring/arbeidsavklaringspenger
❍ Hjemmeværende
❍ Arbeidssøkende
❍ Under utdanning

Hva var den samlede inntekten i husholdningen sist år?
(samlet brutto årsinntekt inkludert overføringer og bidrag før skatt og fradrag er trukket fra)

☐ Under 200 000
☐ 200-349 999
☐ 350-549 999
☐ 550-749 999
☐ 750-999 999
☐ 1 000-1250 000
☐ 1 250-2 000 000
☐ Mer enn 2 000 000

NASJONALT INSTITUTT FOR ERNÆRINGS- OG SJØMATFORSKNING

ID: _____

Hvor mage personer bor i husholdningen hjemme hos dere?

Voksne: _____ personer

Barn: _____personer

	Svært god	God	Middels	Dårlig	Svært dårlig
Hvordan vil du beskrive familiens økonomi?	❍	❍	❍	❍	❍

Spørsmål knyttet til barnet ditt:

SØVN

Her er noen spørsmål om barnets søvn. Svar for hvordan det har vært den siste måneden.

	Hverdager:	**Helg:**
Når legger hun/han seg vanligvis?		
Når står hun/han vanligvis opp?		

Syns du barnet får nok søvn?	Ikke nok	Litt lite	Passe	Litt mye	For mye
(kryss av):	❍	❍	❍	❍	❍

NASJONALT INSTITUTT FOR ERNÆRINGS- OG SJØMATFORSKNING

ID: _____

Sykdom, funksjonshemming og medisinbruk:

Har barnet en kronisk sykdom eller funksjonshemming? Ja ❍ Nei ❍

Hvis ja, kryss av for:

- ❑ Epilepsi
- ❑ Diabetes
- ❑ Astma
- ❑ Annet (hva?) _____

Bruker din datter/sønn medisin jevnlig? Ja ❍ Nei ❍

Hvis ja:

Skriv ned hva den/de heter (evt. hva den blir brukt for), dosering og kryss av for hvor ofte.

Navn på medisin:	Dosering:	Ukentlig	Daglig	Daglig x2, eller mer	Sjeldent	Sesong
		❍	❍	❍	❍	❍
		❍	❍	❍	❍	❍
		❍	❍	❍	❍	❍
		❍	❍	❍	❍	❍
		❍	❍	❍	❍	❍

Psykisk helse:

	Ja	Nei
Har din datter/sønn fått en diagnose for psykiske vansker? (feks. ADHD, angst, depresjon, autisme)	❍	❍

Hvis ja:

Skriv inn hvilken diagnose og om den gjelder nå eller tidligere:

Diagnose:	Nå	Tidligere

NASJONALT INSTITUTT
FOR ERNÆRINGS- OG
SJØMATFORSKNING

ID: _____

Sterke og svake sider (SDQ-NOR):

Vennligst kryss av for hvert utsagn: stemmer ikke, stemmer delvis eller stemmer helt.

Prøv å svare på alt selv om du ikke er helt sikker eller synes utsagnet virker rart.
Svar på grunnlag av barnets oppførsel **de siste 6 månedene.**

	Stemmer ikke	Stemmer delvis	Stemmer helt
Omtenksom, tar hensyn til andre menneskers følelser	☐	☐	☐
Rastløs, overaktiv, kan ikke være lenge i ro	☐	☐	☐
Klager ofte over hodepine, vondt i magen eller kvalme	☐	☐	☐
Deler gjerne med andre barn (godter, leker, andre ting)	☐	☐	☐
Har ofte raserianfall eller dårlig humør	☐	☐	☐
Ganske ensom, leker ofte alene	☐	☐	☐
Som regel lydig, gjør vanligvis det voksne ber om	☐	☐	☐
Mange bekymringer, virker ofte bekymret	☐	☐	☐
Hjelpsom hvis noen er såret, lei seg eller føler seg dårlig	☐	☐	☐
Stadig urolig eller i bevegelse	☐	☐	☐
Har minst en god venn	☐	☐	☐
Slåss ofte med andre barn eller mobber dem	☐	☐	☐
Ofte lei seg, nedfor eller på gråten	☐	☐	☐
Vanligvis likt av andre barn	☐	☐	☐
Lett avledet, mister lett konsentrasjonen	☐	☐	☐
Nervøs eller klengete i nye situasjoner, lett utrygg	☐	☐	☐
Snill mot yngre barn	☐	☐	☐
Lyver eller jukser ofte	☐	☐	☐
Plaget eller mobbet av andre barn	☐	☐	☐
Tilbyr seg ofte å hjelpe andre (foreldre, lærere, andre barn)	☐	☐	☐
Tenker seg om før hun / han handler (gjør noe)	☐	☐	☐
Stjeler hjemme, på skolen eller andre steder	☐	☐	☐
Kommer bedre overens med voksne enn med barn	☐	☐	☐
Redd for mye, lett skremt	☐	☐	☐
Fullfører oppgaver, god konsentrasjonsevne	☐	☐	☐

NASJONALT INSTITUTT
FOR ERNÆRINGS- OG
SJØMATFORSKNING

ID: _____

Samlet, synes du at barnet ditt har vansker på ett eller flere av følgende områder:
med følelser, konsentrasjon, oppførsel eller med å komme overens med andre mennesker?

Nei	Ja-små vansker	Ja-tydelige vansker	Ja-alvorlige vansker
☐	☐	☐	☐

Hvis du har svart "Ja", vennligst svar på følgende spørsmål:

- Hvor lenge har disse vanskene vært tilstede?

Mindre enn en måned	1-5 måneder	6-12 måneder	Mer enn ett år
☐	☐	☐	☐

- Blir barnet selv forstyrret eller plaget av vanskene?

Ikke i det hele tatt	Bare litt	En god del	Mye
☐	☐	☐	☐

- Påvirker vanskene barnets dagligliv på noen av de følgende områdene?

	Ikke i det hele tatt	Bare litt	En god del	Mye
HJEMME / I FAMILIEN	☐	☐	☐	☐
FORHOLD TIL VENNER	☐	☐	☐	☐
LÆRING PÅ SKOLEN	☐	☐	☐	☐
FRITIDSAKTIVITETER	☐	☐	☐	☐

- Er vanskene en belastning for deg eller familien som helhet?

Ikke i det hele tatt	Bare litt	En god del	Mye
☐	☐	☐	☐

Tusen takk for hjelpen