Seafood intake and hair mercury levels in infants after a randomized controlled trial with cod consumption during pregnancy.

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Lise Rådmannsøy

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Abstract

Background: Nutrition during pregnancy and infancy has been associated with long-term health effects, and seafood in the diet contributes with nutrients essential for optimal growth and development of the fetus and later the child. However, seafood is also a source of unwanted contaminants such as methylmercury (MeHg), which is shown to be neurotoxic, particularly to the developing brain of the fetus. Fish, and especially lean fish, is currently the main source of MeHg in the Norwegian population. In Norway, few studies have investigated prenatal mercury exposure and total hair mercury (THHg) levels in infants. Seafood consumption in infants has only been investigated briefly in previous studies.

Objective: The main aims of this thesis were to investigate the effect of seafood intake during pregnancy on infant THHg levels, in addition to examine seafood intake and THHg levels during infancy.

Methods: A two-armed randomized controlled intervention trial named Mommy's Food, was conducted by the Institute of Marine Research in Bergen, Norway. A total of 133 pregnant women were randomized to either the intervention group or the control group, with the intervention period lasting 16 weeks during pregnancy (gestational week 20-36). The intervention group was instructed to consume a weekly amount of 400 grams of cod fillet provided for them, whereas the control group ought to continue their habitual diet. Seafood consumption was reported in food frequency questionnaires for both pregnant participants and later their infants. THHg levels were analyzed from hair samples obtained from the infants at 6 weeks, 6 months and 11 months of age.

Results: Total seafood consumption during the intervention period displayed no difference between the two groups, although the composition of fish species in the diet was significantly different. The estimated maternal Hg intake from seafood was significantly higher in the intervention group compared to the control group ($p = 0.002$). None of the pregnant participants exceeded tolerable weekly intake of MeHg at 1.3 µg/kg bw during the intervention period.

No difference was seen on overall THHg levels between the groups at 6 weeks, 6 months or 11 months of age. Mean THHg for all infants at 6 weeks, 6 months and 11 months of age were 332 µg/kg, 319 µg/kg and 305 µg/kg, respectively. Frequency of seafood intake from 6 to 11 months of age increased significantly ($p = 0.000$). At 6 months, 9% of infants consumed fish at least once per week, whereas this number was 98% at 11 months of age.

Conclusion: At all time points, the mean infant THHg values were found to be approximately one third of the reference dose set by the US Environmental Protection Agency at 1000 µg Hg/kg. This study population of pregnant women had a mean seafood intake in line with the recommended total seafood intake for the general population. The total seafood intake did not change during the intervention. For infants, the mean frequency of seafood intake at 11 months of age was in line with recommendations for this age group. These findings support the current seafood recommendations for pregnant women and infants, as the set limit values are not exceeded.

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Abbreviations

BMI	Body mass index
Bw	Body weight
CNS	Central nervous system
CRM	Certified reference material
DHA	Docosahexaenoic acid
dl-PCB	Dioxin-like polychlorinated biphenyls
DMA-80	Direct Mercury Analyser 80
ΔTHHg	Change in total hair mercury
EFSA	European Food Safety Authority
EtHg	Ethylmercury
FA	Fatty acid
FDA	Food and Drug Administration
FFQ	Food frequency questionnaire
g	gram
GW	Gestational week
Hg	Mercury
IAEA	International Atomic Energy Agency
IMR	Institute of Marine Research
IQR	Interquartile range
JECFA	Joint Food and Agricultural Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives
LCPUFA	Long-chained polyunsaturated fatty acid
LiN	Little in Norway cohort
LOQ	Limit of quantification
MeHg	Methylmercury
MoBa	Norwegian Mother and Child birth cohort
Ng	nanogram
NOAEL	No Observed Adverce Effect Level
NOK	Norwegian krone

p-value	Probability value
R	Spearman's rank order correlation coefficient
RCT	Randomized controlled trial
RfD	Reference dose
RKBU	Regional Centre for Child and Youth Mental Health and Child Welfare
SD	Standard deviation
SH	Thiol
SPSS	IBM Statistical Package for the Social Sciences
THg	Total mercury
THHg	Total hair mercury
TWI	Tolerable weekly intake
UIC	Urinary iodine concentration
UN	United Nations
USEPA	United States Environmental Protection Agency
VKM	Norwegian Scientific Committee for Food and Environment/ Vitenskapskomiteen for mat og miljø
WHO	World Health Organization

1 Introduction

1.1 Nutrition during pregnancy and infancy

Nutrition during pregnancy and early life are important factors for optimal growth and development for the child (1). Scientific research links long-term health consequences for the offspring with nutrition during pregnancy (2-4), perhaps as a result of alterations concerning the metabolism and physiology at this time (5, 6). These effects might not be discovered until later in life (6).

Pregnancy is a period of detrimental changes to the female's bodily functions. The entire body is affected, from circulation to renal function and hormones (7). In addition to developing the fetus, a new and large organ, the placenta, is created. One of the placenta's many tasks is to provide nutrients to the growing fetus, and is thus of great importance for optimal growth and development of the growing fetus (8). Therefore, a varied and balanced diet rich in nutrients is important to meet the nutritional requirements for both the mother and the fetus. Some nutrients, especially proteins, essential fatty acids and micronutrients such as iron, calcium, zinc, iodine and vitamin D, are required in higher demands during pregnancy (7). These nutrients are essential for development of the fetus, still some nutrients affect the growing fetus in a more detrimental way if not provided in sufficient amounts. An example is iodine, which is required for development of the central nervous system (CNS) in the first trimester of pregnancy (9). In case of severe iodine deficiency in the mother, the result can be fetal death or a condition of severe developmental retardation, called cretinism, in the child (9). Sub-optimal levels of iodine during pregnancy have also been associated with reduced IQ-scores in children as well as delayed development (10). Therefore, maternal nutrition play a major role in determining the outcomes of pregnancy.

After birth, breast milk is the optimal form of nutrition for the newborn baby (11). Breast milk is a unique source of nutrients and immunoprotective substances (11-13). Severe micronutrient deficiency in the lactating mother will to some extent be reflected in the composition of the milk, and can in turn affect the infant's nutritional status and development (7).

One of the most important milestones in relation to nutrition during a life-time is the transition from a diet only containing breast milk or infant formula to a diet including a full range of solid foods (14). This process is called weaning and the weaning process usually starts at 4-6 months of age with introduction of small portions of fruit- and/or vegetable purée, porridge, or other

commercial weaning foods (15, 16). Later in the introduction of complementary feeding, different types of meat and seafood ought to be introduced. When the infant reaches 6 months of age, breast milk is no longer sufficient to ensure the nutrient requirements of the growing child (17-19). Therefore, the composition and nutrient density of the weaning foods is important as the nutrient requirement in relation to body weight is very high for infants. Nutrient dense foods are important to secure the growth and development of the child, as well as avoiding nutrient deficiencies (20, 21).

1.1.1 The role of fish and seafood in maternal and infant diet

It is well recognized that fish and seafood are good sources of a variety of nutrients. Essential nutrients such as long-chained polyunsaturated fatty acids (LCPUFAs), vitamins D and B12, as well as the minerals selenium and iodine are abundant in seafood (22, 23). All this, together with the fact that seafood also contains protein with high biological quality including all essential amino acids, makes seafood a suitable dietary component in a healthy, nutritious diet (23, 24).

In recent years, attention has been focused towards the effect of omega-3 fatty acids (FAs) from fish and fish oils and its importance in child development (25-28). Omega-3 FAs, and particularly the marine docosahexaenoic acid (DHA) has been suggested to explain the observed beneficial effect fish consumption has on developmental outcomes in epidemiological research (29, 30). However, studies have shown that consumption of fish oils may not have the same effect on these outcomes (31). The positive health effects after fish consumption may thus be attributable to fish as a whole food with several essential nutrients in an interactive response (32). Randomized clinical trials have, until now, not used fish as a whole food during pregnancy to investigate infant development, although positive health outcomes after seafood consumption during pregnancy have been observed in several observational studies (33-35). A review by Leventakou et al. (36) investigated fish consumption and birth outcomes in 19 European birth cohort studies, including the Norwegian Mother and Child birth (MoBa) cohort (37), concluded that there was a link between a lower risk of preterm deliveries and moderate consumption of fish during pregnancy. For infants of mothers with moderate intake of fish, there was also observed a significantly higher birth weight, although this difference was small (36). The importance of these findings is high, as negative long-term effects on physical and cognitive abilities are increased by preterm delivery (38-41), and low birth weight has been associated with disease in later life (2, 4).

As mentioned above in section 1.1, the diet of infants in the second half of the first year of life should include nutrient dense foods, to ensure optimal development (20). Fish is a good source of proteins, essential fatty acids, vitamins and minerals, and inclusion of fish in the infant diet is therefore considered to be advantageous (20). However, fish and seafood are also a source of undesirable contaminants, including methylmercury (MeHg). This substance may potentially affect early life development in an adverse way (1). Extensive research has been performed in this field to investigate effects of MeHg from fish consumption, especially on fetal development (42).

Dietary guidelines from Norway, as well as the rest of Europe, USA and Australia encourage pregnant women to include fish in their diet (24, 43-47), after careful considerations on the risk and benefit of fish consumption (48). However, some restrictions should be considered during pregnancy.

1.1.2 Norwegian recommendations on consumption of fish

In Norway, the Directorate of Health regularly publish dietary recommendations for the Norwegian population (49). These recommendations are based on summaries of knowledge attained from systematic scientific research. The current recommendations are presented as 13 dietary and health advice that include recommendations on fruit and vegetables, whole grain products, fish, lean meat and meat products, dairy products, oils, salt, sugar, water and physical activity. The Norwegian Directorate of Health (49) recommend that fish should be eaten for dinner two to three times per week, corresponding to a total amount of 300-450 grams of fish for dinner per week. In addition, it is recommended that a minimum of 200 grams should originate from fatty fish (49). Alternatively, the equal amount of fish as spread can replace fish as dinner. Other seafood is not included in these recommendations.

1.1.2.1 Seafood recommendations for pregnant and infants

In Norway, pregnant women are advised to follow the same recommendations as the general population regarding fish intake, with a few exceptions (47, 48). Certain types of fish and seafood should be avoided during pregnancy due to the possibility of containing high amounts of contaminants (47). This includes large freshwater fish, exotic fish such as shark, fresh tuna and swordfish, fish liver, Greenland halibut, and some parts of crab and mussels (47). These recommendations are in agreement with the Food and Drug Administrations (FDA) advice for

pregnant women, nursing mothers and young children to avoid eating fish and seafood with mercury content higher than 0.5 mg/kg (50).

No specific guidelines on fish and seafood intake are given for children in the Norwegian dietary recommendation. However, fish is presented as a source of essential nutrients in the diet when the child is 6 to 11 months of age, and thus should be introduced during this period (51). Results from Spedkost 6 months and Spedkost 12 months (52, 53), two Norwegian diet surveys from 2006 and 2007 investigating infants dietary habits, showed that 8% of infants at 6 months and 82% of infants at 12 months consumed fish for dinner. The proportion of infants who ate fish for dinner increased from the previous Spedkost survey in 1999, at both 6 and 12 months (52, 53). There has not been conducted any new dietary surveys on infant's fish consumption in Norway in recent years, but a new Spedkost survey is being will be carried out in 2018-2019 (54).

Even though risks and benefits of fish and seafood consumption have been well investigated in observational research, undesirable substances are still an important aspect to remember when conducting new research. As this thesis is based on an intervention with cod, and cod is one of the main sources of MeHg in the Norwegian diet (48), this thesis will focus on mercury.

1.2 Mercury

Mercury, element number 80 in the periodic table with the symbol Hg, is classified as a heavy metal, has a silvery colour and is the only element that appears as a liquid in room temperature (55). Mercury exists naturally in water, air and soil, and occurs in different inorganic and organic states.

Inorganic forms of mercury include mercurous (Hg^{+}) and mercuric (Hg^{2+}) compounds, in addition to the elemental form; metallic mercury (Hg^{0}). Metallic mercury is volatile and easily evaporates into a gas, commonly called mercury vapor. This vapor is very toxic and can cause brain damage after inhalation due to its ability to cross the blood-brain barrier (55).

Mercury vapor is released into the atmosphere from both natural and anthropogenic sources (56, 57). An important natural source of mercury emission from the earth's surface, are volcanic outbreaks, whereas anthropogenic sources of mercury vapor come from human activities like burning coal, gold mining activities and recycling of cars (58, 59). Throughout history, mercury has been used in a variety of industrial products, such as thermometers, batteries, fungicides,

production of felt hats, in dental amalgam fillings, etc. (55). Mercurous and mercuric compounds were also used in medicinal practice, but its use has now been discontinued in most industrialized countries (55). However, in developing countries, some cosmetic products still contain mercury as an active ingredient (60).

Mercury vapor is a relatively stable gas but converts to inorganic mercury in the atmosphere and returns to the earth's surface with rain. Inorganic mercury in the sediments can be converted to organic mercury by aquatic microorganisms. Organic mercury then biomagnifies in the aquatic food chain (61).

Organic mercury occurs mainly in the form of MeHg, although other forms, like ethyl mercury (EtHg), exist. EtHg has been used as a conservative in vaccines for many years (61). However, several industrialized countries, including Norway, have banned the use of EtHg in standard vaccines for children (62).

Hg is considered by the World Health Organization (WHO) to be one of the top ten substances of foremost concern to the public health (63). Hg, in all forms, has harmful effects on human health if exposed to at high doses (61). Among these, MeHg is thought as the Hg-compound of most concern to human health, as a result of its presence in the food chain, its bioavailability and high affinity to the brain (64). Especially fetuses and children are vulnerable to the toxic effects of MeHg, because of the processes during brain development is highly affected by this substance (65). The main source of human exposure to organic mercury is from the diet through MeHg in fish and seafood (61).

1.2.1 Toxicokinetics of methylmercury

1.2.1.1 Absorption and distribution in the human body

Different forms of mercury are absorbed at different extents in the gastrointestinal tract. Inorganic mercury is poorly absorbed whereas MeHg is almost completely taken up into the blood. After ingestion, MeHg from fish is absorbed from the gastrointestinal tract, although the precise location of absorption is not recognized. Approximately 90 - 100% of ingested MeHg has been found to be absorbed from human intestines and exist in the body as a water-soluble substance (55, 66-68). The mechanisms of MeHg transport and mobility in the body is due to its ability to bind to sulphur atoms and make thiol (SH) complexes (69). These complexes, often containing cysteine, resembles the structure of L-methionine, a large neutral amino acid, and

thus, this MeHg-cysteine complex entering cells through the large neutral amino acid carrier positioned in the cell membranes (66). This is also thought to be the mechanism behind absorption from the gastrointestinal tract (70). Because of its ability to readily cross the cell membranes, MeHg is distributed evenly throughout different tissues in the body, and tissue concentrations therefor closely follows mercury concentrations in the blood (71).

The ability of MeHg to make thiol-complexes is also thought to be the reason for its harmful effects (55). As the sulphur containing amino acid cysteine is present in most proteins, the binding of MeHg to SH-groups of cysteine can alter the structure of these proteins, and thus the function of the protein (72). This may lead to changes in membrane permeability and cell structure, oxidative stress, damage to DNA and dysfunction in mitochondria (72). The fact that cells in the CNS and the kidneys contain high amounts of SH-groups in their membranes, may stand as an explanation to why these tissues are more susceptible to MeHg-induced damage than other tissues (73).

The MeHg-cysteine complex also enters the endothelial cells of the blood-brain barrier in the same way as in other cells, resulting in MeHg reaching the brain, where it is deposited and accumulates (74). The brain is recognized as the primary target for MeHg in the body, and mercury concentrations have been found to be about 5 times higher in the brain versus blood concentrations (55).

In human infants the absorption of ingested mercury is unknown, but there have been indications from animal studies that suckling infants have a higher rate of absorption than in adults (75). Studies performed on rats has shown different absorption of inorganic mercury in suckling animals than older animals. The results have found that more inorganic mercury is absorbed in this stage of life (76, 77). The mechanisms behind this is unclear, but it is suggested that the cause may be the immaturity of the intestine and that mechanisms of excretion through bile has not yet been initiated (69, 78).

1.2.1.2 Excretion

Excretion of mercury happens through feces, urine, hair or breast milk (79). Most of the mercury is excreted in the feces, and less than 10 % is excreted via urine. As mentioned earlier, inorganic mercury is poorly absorbed in the human gut, and is therefore excreted with the feces (55). However, as most MeHg is absorbed into the body, the excretion route is different compared to inorganic mercury. MeHg, which exits the liver cells as a glutathione complex, is

secreted into the bile and goes through enterohepatic cycling (78). When bile is released into the gastrointestinal tract, demethylation happens as biliary mercury come into contact with the microbiota, where microorganisms can break the mercury-carbon bond (69). This only happens to a fraction of the mercury, whereas the rest is reabsorbed with the bile into the portal circulation and is transported back to the liver (69). This glutathione secretion pathway has in animal studies been indicated not to start before the end of the suckling period, but this has not been confirmed in human infants (78).

1.2.2 Transfer of mercury from mother to child

MeHg has the ability to cross the placenta and pass through the umbilical cord (80). In consequence, the MeHg levels in cord blood is thought to follow the levels in maternal blood quite closely (55). At the time of birth, the mercury levels in cord blood has been measured to be proportional to levels in maternal blood, although almost twice as high (81, 82). The Hg-ratio between brain and blood is about 5-7 in adults, and this is also seen to be the case for the ratio between fetal brain and maternal blood, suggesting similar distribution of MeHg in the fetal body (83). Elevated levels of MeHg during pregnancy may have fetotoxic and teratogenic effects, causing DNA-damage and in some cases miscarriage (72, 84).

After delivery, mercury is still transferred from the mother to the infant via breast milk although in a more indirect way than through the placenta(85). Breast milk is a complete and unique source of nutrients and other health beneficial substances for the child (11), but it may also contain harmful contaminants if the mother has been exposed to these kind of substances (86). Contrary to MeHg transferred to the fetus during pregnancy, the mercury transferred to the infant during breastfeeding consist mainly of inorganic mercury (55, 87). The mammillary glands are responsible for restricting the transmission of MeHg to breastmilk (88). However, the MeHg content of breast milk increases with the mothers increased exposure to this type of mercury (86). In European studies, where analyzes of both total mercury (THg) and MeHg content of breast milk were performed, there has been reported a range of MeHg concentration in breast milk between 26 % and 63% of THg. This indicate a large variation of the MeHg contribution to THg in breastmilk (71). However, a very limited number of studies have investigated this, thus little information about postnatal mercury exposure in infants is yet available. The European Food Safety Authority (EFSA) reported in 2012 (71) that these data were too insufficient to perform an exposure assessment for intake of mercury among breastfed infants. Despite the relatively low Hg content in breastmilk, some studies have indicated that

breastfeeding for a lengthy period may increase the risk of exceeding the safe limit for intake of mercury (89) Even so, the risk of this is significantly lower than the exposure during gestation, and potential negative effects of mercury exposure might be outweighed by the positive health effects of breast feeding (12).

1.2.3 Dietary sources of methylmercury

Approximately 80-100 % of Hg in fish is present as MeHg , although calculations on dietary intake of MeHg from fish and seafood often use 100% as a conservative approach (71). Some aquatic species generally contain more MeHg than others, but there are also large variation in mercury content amongst individuals within a specie. The MeHg content in fish is influenced by age, fat content, locality, and its position in the food chain (71). The higher up in the food chain, the longer MeHg have had the chance to biomagnify. Thus, predatory fish contain higher levels of mercury than fish positioned lower in the food chain (64), confirmed in table 1.1. Also, older animals have had the possibility to accumulate MeHg over a longer period of time (71).

Most of the MeHg in fish is attached to proteins in fish muscle. Therefore, the quantity of MeHg in fish is dependent upon the amount of protein in the fish (48, 90). According to the Norwegian Scientific Committee for Food and Environment (Vitenskapskommiteen for mat og miljø, VKM) (48) lean fish contribute with approximately 80% of dietary exposure to MeHg in Norwegian adults and pregnant women. Table 1.1 shows the mean Hg levels in a selection of fish and seafood, mainly those covered in the food frequency questionnaire (FFQ) used in this thesis, in addition to some predatory fish. The numbers are retrieved from Seafood data (91) and FDA (92).

Table 1.1 - Mercury content in a selection of seafood obtained from Seafood data (91) and FDA (92), reported in mg/kg

Fish and seafood species	Mean mg Hg/kg (year analyzed)
Fatty fish:	
Atlantic salmon, farmed	0.017 (2016)
Mackerel, free	0.030 (2016)
Herring, free	0.052 (2014)
Atlantic halibut, free	0.11 (2016)
Lean fish:	
Atlantic cod, free	0.069 (2016)
Atlantic cod liver, free	0.028 (2016)
Atlantic cod roe, free	0.03* (2006)
Pollock, free	0.14 (2014)
Saithe, free	0.059 (2016)
Ling, free	0.18 (2016)
Wolffish	0.13 (2014)
Shellfish:	
Shrimp, free	0.040 (2016)
Crab (claw)	0.082 (2015)
Crab (tripe)	0.075 (2015)
Lobster (white meat), free	0.22 (2011)
Blue mussels, free	0.016 (2015)
Scallop (muscle and roe), free	0.018 (2016)
Predatory fish:	
Swordfish	0.995 (1990-2010)[x]
Tuna, fresh	0.689 (1993-2005)[x]
Shark	0.979 (1991-2007)[x]

*median; [x]data obtained from FDA (92), specific analysis year not reported. Abbreviations: FDA, U. S. Food and Drug Administration; Hg, mercury

Mercury is also found in other foods, such as meat, meat products, vegetables and cereals, but in lower levels than in fish and seafood (71). A general agreement is that mercury found in these foods consist mostly in the form of inorganic mercury (44, 71). Both the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) and EFSA have examined concentrations of THg in food groups other than fish and seafood (44, 71). The concentration of THg in these foods were mostly low, ranging from 0.0001 – 0.05 mg/kg. From the 6183 samples reviewed by JECFA (44), around 80 %

contained THg concentrations less than the limit of quantification (LOQ). The food group with the highest concentration of mercury other than fish and seafood, was fungi.

Samples of infant formula and weaning foods in the UK were analyzed to detect THg concentration. The results from these analyses showed that mercury concentration in approximately one fourth of the samples were at or above the limit of detection (LOD), with an average mercury concentration of 0.001 mg/kg. These samples usually originated from weaning products containing fish (93).

Dietary supplements with fish oil or fishmeal may also contain MeHg as they are derived from fish. However, minimal amounts of MeHg are found when general fish oils have been analyzed (94, 95). Therefore, these supplements are regarded as safe to consume in the recommended doses.

1.2.3.1 Maximum levels of mercury in fish

In Europe, the maximum level of Hg in fish is generally set to 0.5 mg/kg wet weight (96). Some predatory fish species, however, are accepted to contain mercury levels as high as 1 mg MeHg/kg wet weight. This includes fish species such as halibut, tuna, perch, pike, char, trout, etc. (96). The reason for allowing higher mercury levels in certain species is because these species are usually less consumed in the population. However, the limit is set specifically for every specie and is continually updated by the EU (96). If fish is found to contain Hg at levels higher than the maximum level, the fish is not allowed for sale. As a result of restrictions in the amount of mercury allowed in fish feed, which is set to 0.2 mg Hg/kg, farmed fish generally contain small amounts of MeHg (97). In Norway, the food authorities advise against eating fish and seafood from certain fjords that are known to be contaminated with mercury (98).

1.2.4 Human exposure to methylmercury

Due to the presence of MeHg in all aquatic species, exposure to MeHg happens to humans all over the world (55). There has not been reported any clinical cases of MeHg poisoning by ingestion of fish where the source of MeHg in the food was due to the natural biomethylation process (55). However, severe poisoning after eating fish from MeHg-polluted waters have been reported. One of the first reports came from a dramatic event in Minamata Bay in Japan in the 1950's (99). The outbreak involved fishermen´s families who had consumed fish from

waters contaminated with MeHg from a factory producing acetaldehyde. MeHg was a byproduct in the production, and ended up in the aquatic environment, and hence the fish (99).

Another outbreak of MeHg poisoning, not involving fish or seafood, happened in rural areas of Iraq during the winter in 1971-1972 (100). Through many centuries the Iraqi population had relied on wheat production, but in 1970 the crop failed, and seed grains had to be ordered from other countries to secure next year's crops (100). The seeds imported had been treated with fungicide containing MeHg, but the typical Western warning signs not to use the grains in cooking was not known to the Iraqi population. Consequently, the contaminated grains were used to bake bread, and the consumption of this resulted in poisoning and variable degree of neurological damage (100).

These cases are examples of severe accidental poisonings of MeHg caused by contaminated food. As mentioned, humans are exposed to natural sources of this substance, especially through seafood in our diet. Therefore, several studies have been conducted in populations consuming vast amounts of fish and seafood, to investigate if there are any dangers related to MeHg from intake of seafood. The longitudinal, large-scale, prospective cohort studies conducted in the Faroe Islands, Seychelles and in New Zealand are especially well-known (101-103). These studies were initiated in the 1970s and 1980s, and have focused on developmental outcomes in children after in utero exposure to MeHg from seafood (104-106). Results from New Zealand and the Faroe Islands have shown a link between prenatal MeHg exposure and negative neurodevelopment in children of various age groups (103, 107). Although prenatal mercury exposure in the Seychelle study resembled the exposure in the two abovementioned studies, no adverse effects were seen here (102, 108). It has been suggested that this could be due to the difference in type of seafood consumed on these locations (55, 109). In the Seychelles the main exposure of MeHg is through fish, whereas in the Faroe Islands the consumption of whale meat contributes to a large portion of MeHg exposure (110). Whale meat and blubber are known to also contain other contaminants, which may have contributed to the undesirable developmental outcomes in the Faroe Islands study (110).

1.2.5 Adverse effects of MeHg

As the central nervous system, particularly the brain, is the primary target of circulating MeHg, this is also where the damaging effects of this contaminant mainly occur (72). The harmful properties of this substance differ in the fully developed brains of adults versus the developing

brain in prenatal infants (55, 69). Developing brains are more susceptible to damage at lower doses of MeHg and the type of damage to the cells are also different and distinct (68). From the outbreaks in Minamata and Iraq, we have learned much about the effect of both low and high doses of ingested MeHg, and in which life stage the brain is most damaged by exposure (68, 100, 111, 112). Lower exposure to MeHg in intrauterine life is associated with delayed development, while high exposure ended in severe neurological brain damage and developmental retardation (113, 114). Findings from these historical outbreaks demonstrated that the fetal brain is particularly sensitive to the toxic properties of MeHg, affecting the most basic and highly regulated processes in the brain development, such as cell division, proliferation and migration (68, 69). This leads to disorganization of the neuronal cells arrangement in the brain, and the cortical layers of these cells are deformed.

1.2.6 Health based guidance values on Hg intake

1.2.6.1 Tolerable weekly intake

EFSA is regularly requested by the European Commission to evaluate the risk on human health linked to the amounts of mercury in food. The latest scientific opinion from EFSA on this topic was published in 2012 (71), where adjustments were made to the previously set tolerable weekly intake (TWI) on MeHg from 2006. TWI is estimated for potentially harmful substances and is a value of the amount per kilo bodyweight that can be consumed every week during a lifetime, without risk of adverse effects to human health (71).

Knowledge on the beneficial effects from nutrients in fish led to the lowering of the previous TWI for MeHg, from 1.6 µg/kg bw/week to 1.3 µg/kg bw/week (71). Results from cohort studies on the Faroe Islands (115, 116), that were used to estimate the previous TWI, may have been confounded by the effect of these beneficial nutrients, underestimating the adverse effects of MeHg in the previous evaluation. TWI for inorganic mercury was kept at the same value as in the 2006 scientific opinion, respectively at 4 µg/kg bw/week (71).

JECFA is the authority of the United Nations (UN) that have the same task as EFSA regarding evaluation of contaminants in food. This committee evaluated MeHg in 2007 and inorganic Hg in 2011. Based on the knowledge at these timepoints, the TWIs were set at 1.6 µg/kg bw/week for MeHg and 4 µg/kg bw/week for inorganic Hg (117, 118), the latter in agreement with EFSA's conclusion. JECFA also concluded that the TWI for inorganic mercury

at 4 µg/kg bw/week was regarded suitable for THg exposure from other foods than fish and shellfish (118).

EFSA has points of contact, called focal points, in many countries in Europe (119). These focal points operate as a contact between EFSA and the different national food safety authorities, research institutes and others. In Norway, EFSA's focal point is the Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) (120). Its task in relation to EFSA is to report on results from risk and benefit evaluations, promote and advice on scientific collaborations between EFSA Member States, and assist in exchanging scientific information and knowledge (119). The evaluations conducted by VKM do not specifically focus on mercury, but rather on the risk and benefit of fish and seafood consumption, which represents the food group where humans mainly are exposed to dietary Hg (71). The latest report from VKM on this topic was published in 2014 (48), where it was concluded that there was no risk of consuming toxic levels of Hg from the present amount of fish eaten by the Norwegian population, nor from the recommended fish intake in the Norwegian dietary guidelines.

1.2.7 Methods for measuring exposure to MeHg

As MeHg is present in several biological fluids and tissues, different biomarkers are available for measuring MeHg exposure (121). Blood, hair, breastmilk, urine, toe nails and cord blood can all be used, but some are better suited and more practical than others to be used as biomarker (90, 121). Even though there are both benefits and disadvantages to all types of biomarkers, the preferred materials to measure mercury exposure are blood and hair (122). For individual exposure to mercury, blood is primarily used as biomarker (123), whereas hair is normally used to examine mercury exposure in a population (124). Blood mercury levels reflects recent mercury exposure, whereas hair mercury levels indicate mercury exposure over longer periods of time (124). Thus, hair can be used to assess long term mercury exposure, e.g. throughout pregnancy.

1.2.7.1 Accumulation of Hg in hair

MeHg accumulates in hair and is thought of as a pathway for mercury excretion from the body. As seen in other type off cells, the cysteine-mercury complex is regarded as the way in which MeHg is transported into the hair cells, via large neutral amino acid carriers (121, 125). The

keratinocytes trap MeHg which accumulates over time, resulting in increased concentration of MeHg in hair compared to concentration in the blood (86). As scalp hair grows slowly, concentrations of mercury in hair has been found to be proportional to blood concentrations, however approximately 250 times higher (126). Also, hair Hg levels is found to correlate well with Hg in the brain (126), which also applies between maternal total hair Hg levels (THHg) and infant brain (83).

More than 80 %, of mercury in hair consist as MeHg, while the rest is present as inorganic mercury and appears to stay at a constant level (127). Inorganic mercury circulating in the body do not seem to accumulate to any significant extent in hair, suggesting that the inorganic mercury present in hair has been demethylated from MeHg in the hair follicle (127).

Hair grows at an average of about 1 cm per month in adults (124, 128), consequently segments of 1 cm of hair reflects the mean mercury concentrations in blood during a month (55). However, individual variations occur, as different factors influence the hair growth (121, 125). These factors may be age, gender, season, hair treatment, pregnancy and hormones. From this, growth rate variation in human hair can range from 0.65 cm to 2.2 cm per month (128). When taken into account that 0,5 cm of the hair is located under the scalp in addition to the difficult task of cutting the hair as close to the scalp as possible, calculations show that the first 2 cm of a hair sample represents formation of hair 1.3 to 3.1 months prior to sampling (128). Thus, the growth rate of hair has to be adjusted for if used to assess individual exposure. In addition to individual growth rate variations of hair, there may also be individual differences in the blood-to-hair ratio of Hg (68, 125). It has been suggested that this ratio is higher in children than in adults (71). If this is the case, effects after exposure to mercury may be underestimated in children.

1.2.7.2 Reference levels for mercury in hair

The United States Environmental Protection Agency (USEPA) has set a reference dose (RfD) at 1000 µg/kg (1 µg/g) for total level of mercury in hair (THHg) (129). The reference dose is often used for toxic substances and refers to an estimated level of daily mercuric exposure that is not likely to cause negative effects on human health. The estimated reference dose from USEPA is set for women at fertile age. It is based on studies on brain development in prenatal life, the most sensitive life stage for exposure to mercury. Thus, the dose is applicable to the entire population and at any life stage (55).

A No Observed Adverse Effect Level (NOAEL), the level to which there has not been seen any harmful effects on human health, is set by USEPA at 10 000 µg/kg (129). In populations where people eat large amounts of fish, like on the Faroe Islands, there is a risk of exceeding this level. Based on data from the Iraqi outbreaks the WHO concluded that a peak maternal hair mercury level between 10 000 to 20 000 µg/kg during pregnancy indicate a 5 % risk of negative neurologic effects in the child. This risk increases to over 30 % (high risk) at peak levels in maternal hair of 70 000 µg/kg (68). WHO states that a risk evaluation should be conducted if the average hair Hg level in a population exceeds 2000 µg/kg (90).

1.3 Dietary assessment methods

Assessment of dietary intake is essential in research investigating impact of diet on different outcomes, especially in clinical settings in relation to disease (130). It has been widely debated which dietary assessment method that should be used in epidemiological research (131-134). A simple answer to this does not seem to exist. All methods of dietary assessment are prone to errors when measuring dietary exposure in populations or individuals. Evaluation of the best suitable dietary assessment method should be conducted relating to each specific research objective, available resources and study design (135).

Different dietary assessment methods can be characterized as qualitative or quantitative (130). Among the quantitative methods, the 24-hour dietary recall is well recognized as a good method to be used in research settings. This method can also be implemented at several time points for the same subject, referred to as repeated 24-hour recalls (135). Other quantitative methods are estimated food records, weighed food records and duplicate diet approach, whereas the qualitative dietary assessment methods comprise dietary history and the well-recognized FFQ (135). Since the 1990s, the FFQ has been used extensively in epidemiological research as a method of assessing dietary intake (135). The FFQ is used to collect retrospective dietary information on the intake frequencies during a specific period of time, assessing different food groups or food items. The questionnaire is self-administered and has a relatively low subjects' burden, is time-efficient and has low economical costs (130, 136). An FFQ can be made semi-quantitative if portion sizes are implemented in the questionnaire (135). The advantage of this is linked to the ability to estimate daily intakes of different foods on an individual level. For this reason, semi-quantitative FFQs have been widely exploited in epidemiological research (130). However, the research objective and study population should be considered when

developing FFQs, as dietary intake might be affected by culture, religion, age, economic status, ethnicity, etc. (137).

1.4 Aims for this thesis

This thesis is part of a comprehensive intervention study called "Mommy's Food" (138) conducted at the Institute of Marine Research (IMR).

Based on this intervention, the aims for this thesis were to:

- Investigate if there is any difference in infant THHg levels reflecting prenatal Hg exposure between the intervention group and the control group after an intervention with cod
- Study the correlation between prenatal hair mercury levels and maternal fish and seafood intake during pregnancy
- Investigate infant THHg levels during the first year of life, and compare them with the current reference dose
- Explore frequency of seafood consumption in infants during the first year of life

2 Methods

2.1 Mommy's Food – Design

This thesis is part of a bigger project, a two-armed randomized controlled intervention study with pregnant women, and later their infant, in Bergen, Norway (138). The study is organized and conducted by IMR, and is a collaboration between IMR and the Regional Centre for Child and Youth Mental Health and Child Welfare (RKBU). The study was named "Mommy's Food" and the purpose of the study was to investigate changes in the iodine status of pregnant women after intervention with fish, and if this potential difference in iodine status would have any effects on development in the offspring.

The participants were allocated into two groups, either receiving fish or continuing with their habitual diet for 16 weeks during pregnancy. Both before and at several time points after the intervention period, biological data were collected. In addition, dietary intake was registered with FFQs that were filled out by the participants at several occasions.

In the Mommy's Food study, urinary iodine concentration (UIC) in week 36 of pregnancy (post intervention) was one of the primary outcomes, in addition to neurodevelopment of the infants at 11 months of age. However, in this thesis the main outcomes are THHg levels and seafood consumption in infants, thus the focus will be on hair samples and FFQs for infants at 6 weeks, 3 months, 6 months and 11 months of age, as well as FFQ from the mothers pre- and post-intervention (Table 2.1).

2.2 Ethics

The study was approved by the Regional Committees for Medical Health Research Ethics West (2015/879) and follows the ethical guidelines stated in the Declaration of Helsinki. The trial was also registered in ClinicalTrials.gov, a clinical trial registry, with the identification code NCT02610959. All participants had to give written informed consent when agreeing to enter the study. However, they were able to drop out of the study at any time with no explanation. They could also choose not to grant biological samples as this was voluntary. As infants were included in the study and for obvious reasons could not give informed consent to participate, special care must be taken. Trained phlebotomists with practice in taking blood samples from

infants were hired to sample venous blood from the infants. This was the only invasive sample obtained from the infants.

Confidentiality was strictly upheld on all collected data and information acquired from the participants. All data was unidentifiable when used for assessment. Biological samples, analytical results and information from the participants were, after consent from the participants, stored in a biobank at IMR. The data material in this biobank will be anonymized when the biobank expires, which is due to take place in 2025.

2.3 Participants and recruitment

The recruitment period started in December 2015 and lasted until April 2017. The recruitment was mainly conducted through the Women's Clinic at Haukeland University Hospital in Health region west in Norway, but information and invitation to join the study was also broadcasted on Facebook, Instagram and in a magazine for pregnant women in Norway. The Women's Clinic has procedures for sending out notice for the routine ultrasound, taking place in gestational week (GW) 17-19, to all pregnant women in the region. During the recruitment period, information about the Mommy's Food study was also sent out with this notice. Pregnant women who wanted to participate in the study had to contact the Mommy's Food secretariat.

The study's inclusion criteria were that the pregnant women contacted the secretariat before gestational week 19, it should be their first-time pregnancy and a single fetus (prim parous singleton pregnancy), in addition to the ability to understand, write and/or speak Norwegian as all validated tests of the infant were conducted in Norwegian. The exclusion criteria were fish allergies and known chronic diseases that affect iodine status.

In recent years the number of births in the Women's Clinic at Haukeland University Hospital has been approximately 5000 births per year (139). However, as the inclusion and exclusion criteria reduce the number of possible participants, the final number of pregnant women eligible for participation would be considerably smaller, although the exact number is not known.

2.3.1 Randomization and blinding

After the first visit in gestational week 18 where informed consent was written and instructions given out, individual randomization to either the intervention group or the control group was performed by lottery. The randomization was performed in blocks of 10, where a box was filled

with 10 pieces of paper, 5 indicating intervention and 5 indicating control, and each participant drew one piece of paper from the box. When the box was empty, 10 new pieces of paper were put into it. This was done to make sure that approximately the same number of participants were allocated to both study groups. The participants were handed a study ID-number that contained a random number that ranged between 1 and 200. Both participants and investigators were blinded until the end of allocation and baseline testing.

Blinding of the participating pregnant women was impossible as some participants received fish, and some did not. The infants, however, were blinded through the entire study. To ensure that study investigators were blinded while analyzing data, dummy-ID numbers replaced the study ID-numbers. Thus, study investigators were blinded during all statistical analysis except calculation of compliance to the intervention diet.

2.3.2 Sample size and power calculations

Sample size estimation is usually based on detecting differences in the primary outcome between groups in a study. In this case the primary outcome was UIC, and with the use of previous data from the "Little in Norway" (LiN) cohort (140), power calculation found that a sample size of 60 women in each of the two groups had a power of 95 % to detect 30 % higher UIC in the intervention group compared to the control group. Considering a possible 20 % drop out rate, each group should consist of 72 participants, with the total sample size of 144. However, it is important to be aware that this estimation of sample size is not based on detecting differences in hair mercury levels between the groups, which is the topic for this thesis.

2.4 The intervention

Participants were recruited continuously during the whole recruitment period, thus the intervention period lasted from February 2016 until September 2017. However, the actual intervention lasted 16 weeks for each participant. From this, it is clear that different participants went through the intervention at different times.

After randomization to the different groups at the second visit in gestational week 19, the intervention group were given frozen fillets of cod (bought and delivered from Lerøy A/S, Bergen, Norway). Each fillet weighed approximately 200 grams, and participants in the intervention group were instructed to eat two meals with 200 grams of this fish per week,

equaling a total of 400 grams of cod weekly. For reasons regarding compliance, the participants also received fish fillets for their partner. The participants received a pamphlet with recipes they could use to prepare the fish meals, but were free to make whatever meal they wanted. They were also instructed to weigh the fish before preparing the meal, and also weighing any left overs of the fish after finishing the meal. For this they were provided with a kitchen scale (ClasOhlson.com, article no. 34-1207-16). The weight of the fish before and after the meal, in addition to the cooking method that had been used, was recorded in a weight scheme by the participants.

Participants who were randomized to the control group were asked to continue following their habitual diet, and did not need to register any food consumption until the end of the intervention period.

2.4.1 Safety of the intervention diet

Cod is a source of unwanted contaminants such as mercury, dioxins and dioxin-like polychlorinated biphenyls (dl-PCBs) (48, 71). Therefore, researchers at IMR needed to calculate the amount of these undesirables the fish used in this study would provide to the participating pregnant women. Then they compared their estimates against the TWI for these substances. This was done to ensure that the amount of fish handed out was safe to consume. In these calculations, the amount of cod handed out per week, the average content of Hg present in cod filet, and the 5-percentile weight (56 kg) of the women in the LiN cohort (unpublished data, cited in (138)), was used to calculate weekly intake per kg body weight for these contaminants. The conclusion was that the intake of mercury, dioxins and dl-PCBs from cod provided in the trial contributed with 22 % and 4 % respectively, of TWI set by EFSA and JECFA (44, 71) for these substances in this especially vulnerable population (138).

2.5 Data collection

An overview of the study schedule, only including data relevant for this thesis, is shown in Table 2.1. For an overview of the full study schedule, see Markhus et al. (138).

Table 2.1 - Overview of the study schedule in Mommy's Food, modified from Markhus et al. (138)

	Recruitment	Enrolment	Allocation	Post-allocation					
Timepoint	Pregnancy					Infancy			
	GW < 18	GW 18	GW 19	GW 20	GW 36	6 weeks	3 months	6 months	11 months
Enrolment									
Eligability screen	X								
Informed consent		X							
Instructions		X							
Allocation			X						
Intervention									
Intervention group				X	X				
Control group									
Biological data									
THHg infant						X		X	X
Questionnaire									
FFQ		X[a]			X[a]		X[b]	X[b]	X[b]

[a]pregnant participants; [b]infant participants. Abbreviations: FFQ, food frequency questionnaire; GW, gestational week; THHg, total hair mercury

2.5.1 Dietary registration – mother and infant

Information about the mother's and the infant's diets was obtained using FFQ. For the mothers, the FFQ was a semi-quantitative short questionnaire, developed especially for this study after revision of an FFQ previously validated (141, 142). Questions about consumption of selected food categories, including fish and seafood, in addition to questions on demographic and socioeconomic factors, were included in this questionnaire.

Questions on seafood intake in pre- and post-intervention FFQ were divided into summary and detailed questions on fish and seafood consumption as dinner, warm lunch and as spread, in salads or as snacks. From this point the latter category with seafood as spread, snacks or in salad will be referred to as "spread". The questions on seafood were answered in frequency intervals ranging from "never" to "more than 5 times per week" for summary questions, whereas for detailed questions the range went from "never" to "more than 3 times per week". For both summary and detailed questions, portions eaten per meal had to be determined, ranging from "less than half a portion" to "3 portions", using predetermined portion sizes as guidance (143). See Appendix I for the complete post-intervention FFQ.

A short, non-validated FFQ was used to retrieve dietary information from the infants at 3, 6 and 11 months of age. The questions in the FFQs were custom to the age of the child and included consumption of breast milk and formula, supplements, liquids, and a selection of food categories, including seafood, commonly consumed by infants. For infant FFQ the answers on seafood consumption were only given in frequency intervals per week, and thus did not include portion sizes. The frequency intervals ranged from "never/rare" to "daily". For an overview of dietary questions in the infant FFQ at 3, 6 and 11 months, see Appendix II, III and IV.

In this thesis the main focus will be on fish and seafood consumption in the latest part of pregnancy for the mothers, and the first year of the infant's life, based on information from the FFQs, as shown in the Table 2.1.

2.5.2 Hair samples

The hair samples were collected from the infants at 6 weeks, 6 months and 11 months postpartum (Table 2.1). Hair was cut as close to the scalp as possible from the back of the head (the occipital area). Further, a thread of dental floss was tied around the sample closest to the

end nearest the scalp. The hair samples were kept in zip-lock bags marked with the project number, time of sampling (6 w, 6 m or 11 m) and ID-number. At 6 weeks of age, the hair samples were cut by the parents, after receiving instructions on how this should be done. These hair samples were kept in the home of the participants and later retrieved by study researchers and transported to IMR. The hair samples taken at 6 and 11 months were collected by the study researchers when participants came to IMR and RKBU for testing. The hair samples were then stored in a safe at IMR pending analysis by the Direct Mercury Analyser 80 (DMA-80, Milestone Srl, Italy), see section 2.7.3.

2.6 Data processing

2.6.1 Seafood index

From the FFQ, results on seafood consumption were reported as ordinal variables, and thus had to be translated to numerical data for the use in statistical analysis to estimate average weekly seafood consumption of the participants. Therefore, a seafood index developed and validated by Markhus and colleagues (142), was used as basis for the indexes applied in this thesis. Interpretation of the seafood index is quite simple; an index of 1 represents 1 portion per week of the seafood in question, an index of 2 represents 2 portions per week, and so on.

To calculate the seafood index for summary questions regarding seafood intake from the FFQ, the average frequency of seafood per week was used if the reported answer included an interval, see Table 2.2. As an example, if a participant reported an intake of 2-3 portions of seafood as dinner per week, the numerical interval would be 2-3 and the seafood index would be 2.5, the average of the numerical interval. The seafood index determined from the detailed questions, was estimated in a different way. The reason for this is that detailed questions often are prone to overestimation, especially when the reported intake is in the lower range (144, 145). Consequently, the seafood index for detailed questions on seafood intake was based on the lowest value if the reported answer contained an interval. Thus, if a participant recorded a frequency of intake equal to 1-2 times per week for a specific fish species, the numerical interval would be 1-2, but the seafood index would be 1, Table 2.3.

Seafood questions in the infant FFQs were treated in the same way regarding seafood index as summary question in the pre- and post-intervention FFQ, using the average frequency in an interval, see Table 2.4.

Table 2.2 – Seafood index and numerical interval per week converted from reported frequencies in summary questions on seafood consumption as dinner, lunch and spread in pre- and post-intervention FFQ. Modified from Markhus et al. (142)

Reported frequency	Numerical interval per week	Seafood index dinner/lunch	Seafood index spread
Never	0	0	0
<1 time/month or rare	0-0.25	0.15	0.15
1-3 times/month	0.25-0.75	0.5	0.5
1 time/week	1	1	-
1-2 times/week	1-2	-	1.5
2-3 times/week	2-3	2.5	-
3-5 times/week	3-5	-	4
≥ 4 times/week	≥ 4	4	-
≥ 5 times/week	≥ 5	-	5

Abbreviations: FFQ, food frequency questionnaire

Table 2.3 - Seafood index and numerical interval per week converted from reported frequencies in detailed questions on seafood consumption in pre- and post-intervention FFQ. Modified from Markhus et al. (142)

Reported frequency	Numerical interval per week	Seafood index
Never	0	0
< 1 time/month	0-0.25	0.1*
1-3 times/month	0.25-0.75	0.25
1-2 times/week	1-2	1
≥ 3 times/week	≥ 3	3

*Seafood index set to 0.1 to separate this category from the categorical frequency "Never".
Abbreviations: FFQ, food frequency questionnaire

Table 2.4 - Seafood index and numerical interval per week converted from reported frequencies on seafood consumption in infant FFQ at 6 months and 11 months of age. Based on seafood indexing by Markhus et al. (142)

Reported frequency	Numerical interval per week	Seafood index
Never/rare	0	0
1 time/week	1	1
2-3 times/week	2-3	2.5
4-6 times/week	4-6	5
Daily	7	7

Abbreviations: FFQ, food frequency questionnaire

The seafood index was further used to calculate portions of fish consumption per week. This was done by calculating the seafood index with number of portions eaten at every meal. The size of one portion was already specified in grams or amount in the FFQ (146).

Processed fish products like fish cakes, fish balls, fish fingers etc. roughly contains 40-60 % fish (48) depending on the brand and product. Therefore, calculating the fish intake from these types of food, the portions size was multiplied with 50 %, before calculating fish portions per week. For fish soup, the calculating factor was 20 %, as this is the fish content in fish soup currently used by VKM (48). The same calculations must be done for sushi, although the factor used for calculation is 33%, as the fish content is approximately as little as one third in this dish (48). The same factor was used on shrimps (not peeled), considering that 33 % is defined as edible part of this food (146).

2.6.2 Categorizing fish and seafood in groups

Results on seafood consumption from the post-intervention FFQ were divided into different categories to make the results easier to review. Salmon/trout, mackerel, herring and halibut was categorized as fatty fish, with fat content higher than 5 g per 100 g, thus, cod, saithe, haddock, ling and wolfish was categorized as lean fish with a fat content lower than 5g per 100 g (48). Processed fish included fish cakes/balls/pudding, fish fingers, fish gratin, fish soup, and dried and salted cod, whereas shrimps, crab, lobster, blue mussels and scallops were reported as shellfish. The last category was labeled “spread” and constituted all fish and seafood eaten as spread, in salad or as snack, covering canned mackerel, salmon, sardines and tuna, smoked salmon, cured salmon, pickled herring, caviar, peppered mackerel, peeled shrimps, anchovies, crabsticks, and pate made of cod liver and roe. Seafood consumption from the infant FFQ was already categorized as fatty fish or lean fish, and categorization was for that reason not necessary.

2.6.3 Calculating mercury intake

Mean mercury intake from all seafood in the habitual diet during the intervention (GW 20-36) was calculated for both intervention and control group. These calculations were based on answers given in the post-intervention FFQs. The average mercury content of the different fish species registered in the FFQ were retrieved from Seafood data (91) and FDA (129). The calculated portions per week of each species was multiplied with specific portion sizes in grams

(146) to estimate grams of each specie consumed per week. Further, this quantity was multiplied with the retrieved mercury concentration of the particular specie in question, resulting in a mean Hg consumption per week specified for each species. An example on calculation of Hg intake from salmon: 0.67 portions of salmon per week x 150 gram per portion for fatty fish x 0.017 µg/g Hg in salmon = 1.7 µg Hg from salmon per week. This Hg intake was also compared to the TWI, after calculations of the mean weekly intake per kg body weight using the average pre-pregnancy body weight reported in each group.

In conjunction with a processing experiment performed at IMR, we were able to analyze samples of the fish used in the intervention to determine Hg content in freeze dried samples from fresh cooked and baked cod. The analysis was conducted using the DMA-80 (section 2.7.3).

2.7 Analysis of hair samples with DMA-80

2.7.1 Hair sample preparation

Infant hair samples obtained at 6 weeks of age represents the hair that has grown intrauterine from approximately gestational week 28 until birth (147), thus reflecting metabolic activity in the fetus during the last trimester of pregnancy. As this was what we wanted to investigate, the whole hair sample was used for analysis, regardless of the length of the hair. This differs from infant hair samples collected at 6 months and 11 months of age, where only 2 cm of the sample closest to the scalp was analyzed. For these samples we wanted to investigate a limited time period, and approximately the same time period for all infants in the study. Therefore, 2 cm of the hair samples were cut at the end nearest to the scalp. This part of the hair represents hair grown approximately 3.1 months to 1.3 months prior to sampling. Thus, hair samples collected 6 months postpartum represent mercury accumulation in hair from 2.9 (± 0.2) to 4.7 (±0.4) months of age, and 11 month hair samples represent mercury accumulation from 7.9 (±0.2) to 9.7 (±0.4) months of age. These estimates are based on calculations made by LeBeau et al. (128), and with one month equal to 28 days.

Prior to analysis, the hair was cut into 2 cm samples with stainless steel scissors after precise measuring of the desired length. Small metal boats, appropriate for use in the DMA-80, were set on a calibrated four-decimal scale, and the cut hair samples were placed in separate metal boats and weighed. To ensure detection of THHg well within the calibrated range of the

machine, the weight of hair samples should optimally be within the range of 10-20 mg. However, most hair samples analyzed for use in this thesis were very small and did not reach this weight target, the lightest only weighing 0.7 mg. The weight of each sample was registered, along with its ID-number, into the computer system of the DMA-80. Then, each of the metal boats were placed in one of the 40 positions in the machine's auto sampler, in the position corresponding to the one registered in the computer system. To make sure that no contamination of mercury was present in the machine, and to discover analytical errors, each series of analysis contained two empty metal boats (blanks) and six metal boats with reference material. A total of 32 positions in the auto sampler were then free to contain hair samples, in one round of analysis. An overview of the placement of samples in the DMA-80 during analysis is shown in Table 2.5.

Table 2.5 - An overview of the position and content of the metal boats during one round of Hg analysis in the DMA-80

Position	**1-2**	**3-4**	**5-19**	**20-21**	**22-38**	**39-40**
Content	Blanks	Reference material	Hair sample	Reference material	Hair sample	Reference material

Abbreviations: DMA-80, direct mercury analyzer 80; Hg, mercury

2.7.2 Quality of analysis

Calibration of the DMA-80 was conducted in October 2017 before the start of hair sample analysis. Reference materials used in the calibration was Bovine Liver 1577, Skimmed Milk Powder, Tort-3, Fish Muscle 422, Dolt-4 and Tuna 464. The area of calibration ranged from 1.5 ng -1000 ng.

The certified reference material (CRM) used in Hg analysis of hair samples was Human hair IAEA-086 (powder, International Atomic Energy Agency (IAEA), Austria). This reference material has a certified reference value of 573 µg Hg/kg (148). All results after Hg analysis of this reference material were within the ± 20 % limit of uncertainty (458 µg Hg/kg – 688 µg Hg/kg), and these results were plotted into the control chart at IMR. The accuracy of results from analysis of Human hair IAEA-086 was on average 85 %.

A muffle furnace, Carbolite ELF 11/14B, was used to cleanse the metal boats of any contamination. In this process the metal boats were burned at 650ºC for 30 minutes between every analysis.

Hair samples from different time points, and from both intervention- and control group were analyzed at the same time, to take into account deviations in the method.

2.7.3 Principles of Hg analysis in DMA-80

The principles behind Hg analysis in the DMA-80 are quite simple (149), and a schematic overview of the DMA-80 is displayed in Figure 2.1. After placing the samples in the autosampler inside the machine, connecting the oxygen gas and starting the machine, the metal boats are retrieved one by one from its position in the autosampler and imported into a drying and decomposition furnace. Here the sample is dried and then burned into ash at 450ºC. In this process mercury vapour is released and the vapour is transported by a flow of oxygen into the release furnace containing a golden trap, the amalgamator. As Hg has high affinity to gold, the golden trap binds Hg from the vapour, where it is detained until the entire sample is burned to ashes. When all Hg from the sample has reached the golden trap, the trap is heated to 650ºC causing the Hg to be released from it. Then, the Hg vapour is transported out of the release furnace through one cuvette which is long and thin (cell 1), and afterwards through a shorter and thicker cuvette (cell 2). Light with wavelength of 254 nanometers, which is specific to Hg, is emitted from the Hg lamp through both cuvettes and registered by the detector. The two cuvettes have different purposes, the long and thin one being more sensitive to low Hg concentrations, whereas the shorter one is more suitable for higher Hg concentrations. Hg passing through the cuvettes absorbs light from this wavelength, hence the quantity of absorbed light is proportional to the amount of mercury present in the sample. However, this method, called atomic absorption spectrophotometry, does not distinguish between organic and inorganic Hg, as it measures the total amount of Hg from the sample. The DMA-80 computer system use the calibration curve and the registered weight of the sample to calculate and present the results in concentrations of µg Hg/kg sample (149). After the analysis is completed, Hg vapour is collected in a coal trap behind the instrument.

The minimum amount of Hg the DMA-80 is able to detect, called the Level of Detection (LOD), is estimated to 0.02 nanogram (ng). The LOQ is somewhat higher, at 0.08 ng. The area between LOQ and 20xLOQ (0.08 ng – 1.5 ng) is not validated, meaning that samples with Hg

concentrations within this range may be less accurately measured than samples with concentrations within the calibrated area (see section 2.7.2). In the calibrated area, the estimated uncertainty for the method is set to ± 20 %.

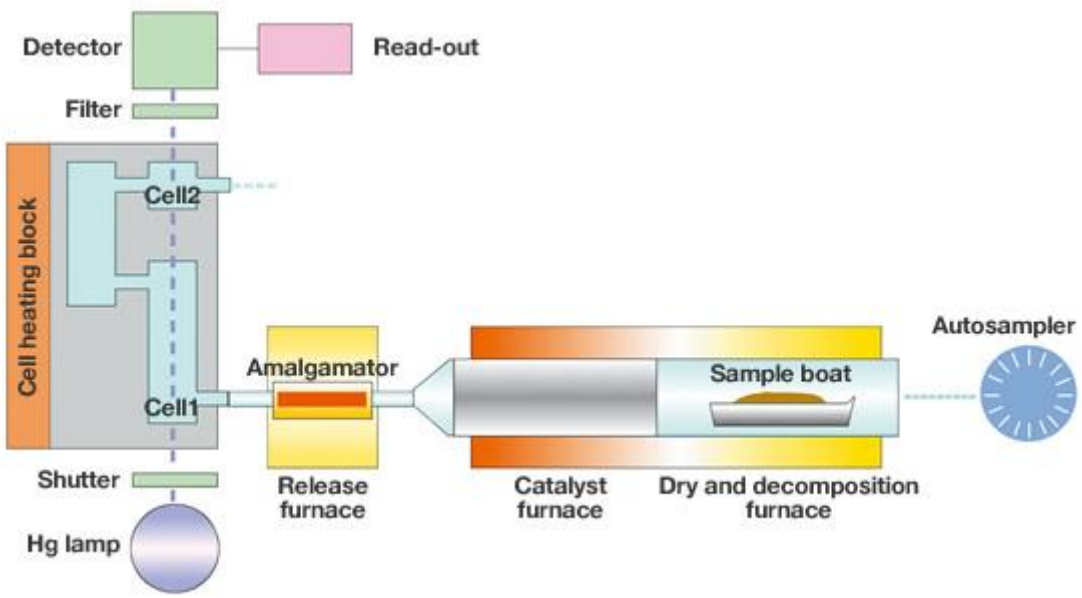


Figure 2.1 - Schematic overview of the DMA-80 (150)

Abbreviations: DMA-80, direct mercury analyzer 80; Hg, mercury

2.8 Statistical analysis

In the statistical analyses, continuous variables were mainly presented as mean and standard deviation (SD), whereas categorical variables were presented as count and percent. However, median and interquartile range (IQR) were also presented for infant THHg levels.

Normal distribution of variables was visually assessed with the use of histograms and QQ-plots. Additionally, this was confirmed with the Shapiro-Wilk test for normality. Non-parametric tests were chosen when violation of normality occurred, whereas parametric tests were used when normality was confirmed. Mann-Whitney U test or independent t-test was applied to test difference between groups for continuous variables. Chi-square test was chosen to test difference between the groups when results were reported as categorical variables, e.g. household income. Wilcoxon signed-rank test was used when examining difference in THHg levels within the groups at different time points. When evaluating correlation between maternal

seafood intake and infant THHg levels, Spearman's rank-order correlation test was used, as these variables did not comply with the normality assumption and also included outliers. The presence of outliers makes the Spearman's rank-order correlation preferable as this test is robust to extreme values (151).When performing correlation analysis, the Spearman's rank order correlation coefficient (r) is reported as a number between -1 and 1, representing the effect size of the correlation. A correlation coefficient between 0 and 1 indicated a positive correlation, whereas a correlation coefficient between 0 and -1 indicated a negative correlation. The absolute value of the effect size is classified as poor if lower than 0.3, moderate if lower than 0.5, and strong if equal to or higher than 0.5 (152).

For the statistical analyses and construction of figures, IBM Statistical Package for the Social Sciences (SPSS) version 25 was used. Two-sided statistical tests were considered statistically significant when the probability value (p-value) was < 0.05.

3 Results

3.1 Study population

As shown in the flow chart (Figure 3.1), 137 pregnant women were enrolled to participate in the trial in gestational week 18, whereas 133 participants were allocated in gestational week 19, as 4 participants resigned from the study. 68 participants were randomized to the intervention group and 65 were randomized to the control group. 9 participants were lost to follow-up during the intervention period before gestational week 36, and another 4 participants dropped out of the study before the 6-month postpartum follow-up. Up until April 2018, no further participants resigned from the study between 6- and 11-month follow-up, making the total number of drop outs 16, with 8.8 % drop out in the intervention group and 10.2 % in the control group. This left 121 participants remaining in the study, 62 in the intervention group and 58 in the control group. However, the study is not fully completed until September 2018.

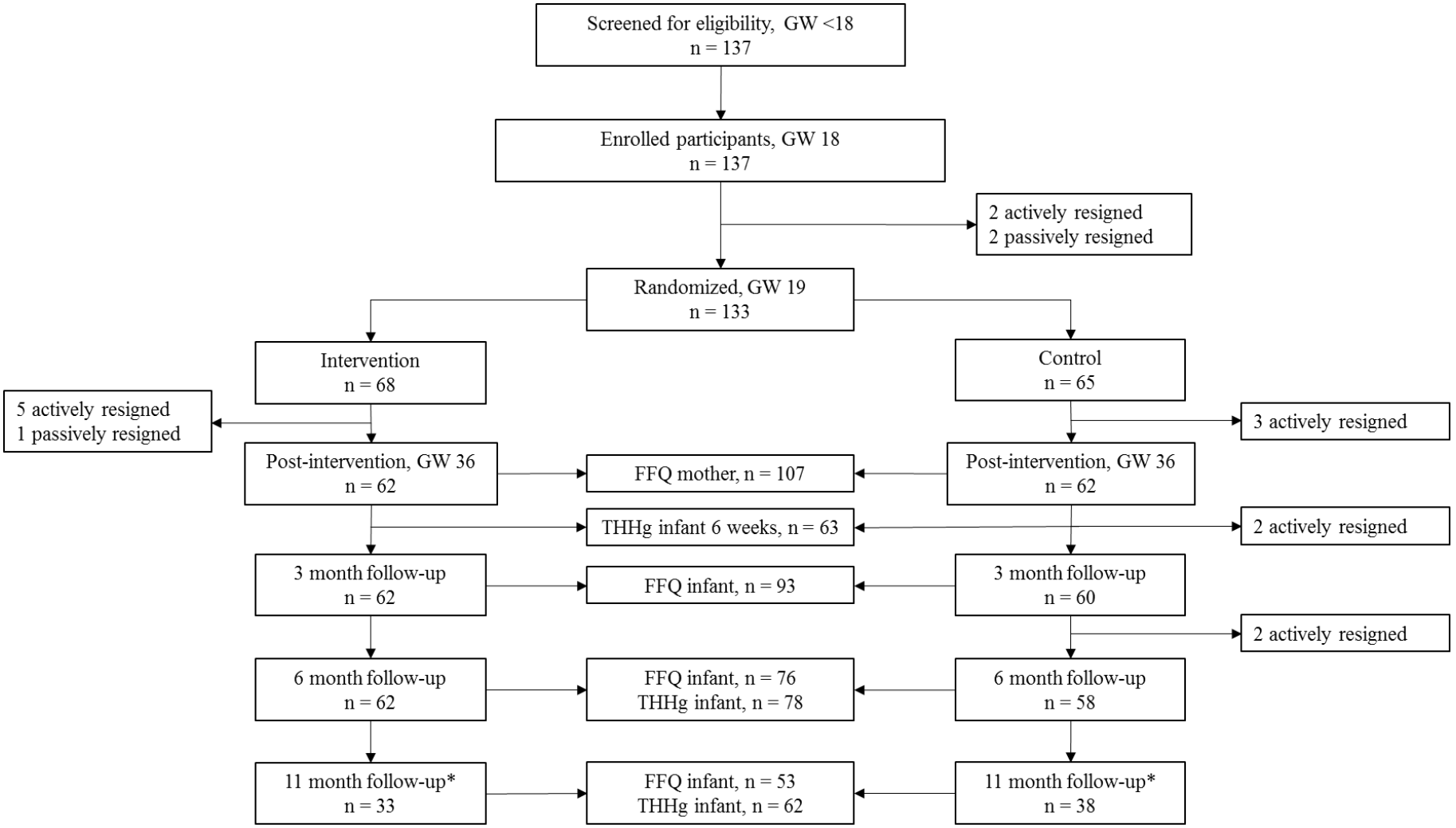


Figure 3.1 - Flow chart of participants in the Mommy's Food RCT, including main data used in this thesis

*11 month follow-up not completed until September 2018, participants attending 11 month follow-up before April 2018 are included in this thesis.
Abbreviations: FFQ, food frequency questionnaire; GW, gestational week; RCT, randomized controlled trial; THHg, total hair mercury

3.2 Characteristics

3.2.1 Baseline characteristics - pregnant participants

Baseline characteristics for pregnant women in this study are displayed in Table 3.1. There were no significant differences for any of the baseline characteristics between the groups, see Table 3.1. Education and household income were skewed towards higher values, with approximately 86% of the participants educated in university or university college and 63 % having household income of 750 000 NOK or higher.

Table 3.1 - Baseline characteristics of pregnant participants enrolled in the Mommy's Food trial. Obtained from pre-intervention FFQ in GW 18. Results presented as mean (SD) or count (%).

Characteristics Mother	n	All	Intervention group	Control group	P
Age, in years	125	29.4 (3.8)	29.7 (3.9)	29.1 (3.6)	0.39[b]
Pre-pregnancy weight, in kg	122	65 (13)	65 (12)	66 (14)	0.67[a]
Pre-pregnancy BMI, in kg/m^2	122	23 (4.1)	23 (3.9)	23 (4.4)	0.76[a]
Cohabitation status	127				0.89[c]
Cohabiting		123 (97)	63 (97)	58 (97)	
Not cohabiting		4 (3.1)	2 (3.1)	2 (3.3)	
Education	127				0.87[c]
Lower secondary school		2 (1.6)	1 (1.5)	0 (0)	
Higher secondary school		16 (13)	9 (14)	7 (12)	
<4 years university education		33 (26)	18 (28)	15 (25)	
≥4 years university education		76 (60)	37 (57)	38 (63)	
Household income, in NOK	127				0.29[c]
< 200 000 – 549 999		36 (28)	21 (32)	14 (23)	
550 000 – 999 999		44 (35)	20 (31)	23 (38)	
1 000 000 – >2 000 000		47 (37)	24 (37)	23 (38)	

[a]Mann-Whitney U test; [b]Independent samples t-test; [c]Chi-square test. Abbreviations: BMI, body mass index; FFQ, food frequency questionnaire; GW, gestational week; NOK, Norwegian krone; p, probability value; SD, standard deviation

3.2.1.1 Seafood consumption at baseline

Mean (SD) seafood consumption in portions per week among participating pregnant women at baseline was 1.6 (1.0) as dinner, 0.4 (0.5) as lunch and 2.1 (2.9) for spread (data not shown). No differences in seafood intake were seen between the groups, although there was a tendency of increased consumption of lean fish in the intervention group compared to the control group ($p = 0.06$) (data not shown).

3.2.2 Infant characteristics

Characteristics for the infants at birth, 3 months, 6 months and 11 months of age are displayed in Table 3.2. Most characteristics showed no significant difference between the two groups of infants at any time point, however length at birth was significantly higher in the intervention group versus the control group ($p = 0.022$). Infants in the intervention group had a mean (SD) length of 51.3 (3.0) cm at birth versus 50.0 (2.2) cm in the control group, a mean difference of 1.3 cm.

Table 3.2 - Characteristics of infants at birth, 3 months, 6 months and 11 months of age, presented as mean (SD) or count (%)

Infant characteristics	n	All	Intervention group	Control group	P
At birth					
Born in gestational week	91	39.9 (1.9)	39.9 (1.8)	39.9 (2.1)	0.89[a]
Sex (count, %)	88				0.68[c]
Boys		41 (47)	22 (49)	19 (44)	
Girls		47 (53)	23 (51)	24 (56)	
Weight, in grams	92	3491 (536)	3540 (478)	3442 (589)	0.18[a]
Length, in cm	88	50.6 (2.7)	51.3 (3.0)	50.0 (2.2)	0.022[a]*
Head circumference, in cm	89	35.1 (1.6)	35.1 (1.5)	35.0 (1.6)	0.92[a]
3 months					
Weight, in grams	93	6125 (891)	6236 (852)	6016 (923)	0.69[b]
Length, in cm	85	61.7 (2.7)	62.1 (2.6)	61.3 (2.8)	0.16[a]
Head circumference, in cm	87	40.5 (1.6)	40.8 (1.7)	40.2 (1.5)	0.08[b]
6 months					
Weight, in grams	77	7896 (923)	7939 (940)	7858 (918)	0.70[b]
Length, in cm	77	67.7 (2.5)	67.4 (2.7)	67.9 (2.4)	0.37[b]
Head circumference, in cm	68	43.6 (1.4)	43.7 (1.3)	43.5 (1.4)	0.72[b]
11 months					
Weight, in grams	52	9079 (2011)	9061 (2133)	9093 (1948)	0.93[a]
Length, in cm	52	73.9 (3.1)	73.9 (3.5)	74.0 (2.8)	0.82[a]
Head circumference, in cm	46	46.0 (1.4)	46.1 (1.4)	45.9 (1.5)	0.62[b]

[a]Mann-Whitney U test; [b]Independent samples t-test; [c]Chi-square test, *statistically significant difference between intervention and control group ($p<0.05$). Abbreviations: p, probability value; SD, standard deviation

3.3 Seafood consumption during the intervention period – pregnant women

Mean seafood intake for the participating pregnant women during the intervention period (GW 20-36) is displayed as portions per week in Table 3.3 and as grams per week in Table 3.4. From the summary questions, dinner is the meal where most of the weekly seafood intake is consumed for both groups (Table 3.3). For specific fish species, cod as dinner was significantly more consumed in the intervention group compared to the control group ($p = 0.000$), whereas salmon and trout as dinner were consumed more in the control group versus the intervention group ($p = 0.000$) (Table 3.3).

Table 3.3 - Seafood intake during intervention for participating pregnant women in Mommy's Food. Presented in mean (SD) portions per week. Specified for the most consumed fish species, salmon/trout and cod.

Seafood categories		**All**	**Intervention group**		**Control group**		**p[a]**
Summary questions	**n**	**Mean (SD)**	**n**	**Mean (SD)**	**n**	**Mean (SD)**	
Seafood as dinner	107	2.3 (1.4)	56	2.6 (0.8)	51	1.9 (1.7)	0.000**
Seafood as warm lunch	107	0.5 (1.3)	56	0.4 (0.8)	51	0.5 (0.4)	0.33
Seafood as spread	107	1.5 (2.3)	56	1.3 (1.9)	51	1.8 (2.7)	0.70
Total seafood	107	4.3 (4.0)	56	4.3 (3.0)	51	4.2 (4.9)	0.12
Detailed questions							
Dinner							
Fatty fish[b]	106	0.5 (0.6)	55	0.3 (0.3)	51	0.8 (0.7)	0.000**
Salmon/trout as dinner	106	0.4 (0.4)	55	0.2 (0.3)	51	0.6 (0.5)	0.000**
Lean fish[c]	106	0.9 (0.6)	55	1.2 (0.6)	51	0.5 (0.4)	0.000**
Cod as dinner	107	0.8 (0.6)	56	1.1 (0.6)	51	0.4 (0.3)	0.000**
Warm Lunch							
Fatty fish[b]	106	0.2 (0.5)	55	0.2 (0.5)	51	0.2 (0.6)	1.00
Salmon/trout as lunch	106	0.06 (0.1)	55	0.07 (0.2)	51	0.05 (0.1)	1.00
Lean fish[c]	104	0.08 (0.2)	54	0.1 (0.2)	50	0.05 (0.09)	0.12
Cod as lunch	106	0.06 (0.2)	55	0.09 (0.2)	51	0.03 (0.05)	0.05
Sushi	107	0.1 (0.1)	56	0.1 (0.1)	51	0.1 (0.2)	0.88
Processed fish[d]	106	0.7 (0.6)	55	0.6 (0.5)	51	0.8 (0.6)	0.06
Shellfish[e]	107	0.2 (0.2)	56	0.2 (0.2)	51	0.2 (0.2)	0.25
Spread[f]	106	2.0 (2.8)	55	1.7 (2.4)	51	2.3 (3.2)	0.55
Fish liver	107	0	56	0	51	0	NA
Fish roe	107	0.002 (0.01)	56	0.001 (0.004)	51	0.003 (0.02)	0.56
Total seafood[g]	102	4.8 (3.8)	52	4.5 (3.2)	50	5.1 (4.3)	0.55

[a]Mann-Whitney U Test; [b]Includes salmon/trout, mackerel, herring and halibut; [c]includes cod, saithe, pollock, ling and wolfish; [d]includes fish cakes/ bolls/pudding, fish gratin, fish fingers, fish soup and dried cod; [e]includes shrimps, claw meat from crab, brown meat from crab, lobster, mussels and scallops; [f]includes canned mackerel, canned salmon, canned tuna, smoked salmon/trout, pickled herring, caviar, peppered mackerel, peeled shrimps, canned sardines, anchovy, crabsticks and cod liver pate; [g]includes all seafood from detailed questions displayed above in the table; **statistically significant difference between intervention and control group ($p<0.001$). Abbreviations: NA, not applicable; p, probability value; SD, standard deviation

Table 3.4 - Estimated seafood intake grams during the intervention period (GW 20-36) for participating pregnant women in Mommy's Food. Results reported as mean (SD) per week

Fish/seafood	Mean (SD) seafood intake in gram per week		p[a]
	Intervention group	Control group	
Fatty fish[b]	71 (93)	148 (180)	0.000*
Salmon/Trout	43 (56)	101 (78)	0.000*
Lean fish[c]	262 (134)	122 (91)	0.000*
Cod	238 (122)	85 (71)	0.000*
Sushi[d]	14 (15)	14 (17)	0.88
Processed fish[d,e]	56 (53)	68 (60)	0.19
Shellfish[d,f]	20 (20)	24 (26)	0.38
Spread[g]	47 (67)	59 (80)	0.47
Total seafood[h]	477 (233)	439 (278)	0.21

[a]Mann-Whitney U Test; [b]Includes salmon/trout, mackerel, herring and halibut; [c]includes cod, saithe, pollock, ling and wolfish; [d]accounted for percent of fish/seafood in products; [e]includes fish cakes and bolls, fish gratin, fish fingers, fish soup and dried cod; [f]includes shrimps, claw meat from crab, brown meat from crab, lobster, mussels and scallops; [g]includes canned mackerel, canned salmon, canned tuna, smoked salmon/trout, pickled herring, caviar, peppered mackerel, peeled shrimps, canned sardines, anchovy, crabsticks and cod liver pate; [h]includes all seafood displayed above in the table in addition to fish roe; *statistical significant difference between the groups ($p<0.001$) . Abbreviations: GW, gestational week; p, probability value; SD, standard deviation

When examining seafood intake pre- and post-intervention the intervention group had a significant increase in consumption of lean fish ($p = 0.000$) and seafood as dinner ($p = 0.000$), and a decrease in consumption of fatty fish ($p = 0.001$), processed fish ($p = 0.011$), and seafood as spread ($p = 0.018$) (data not shown). The control group had not changed their intake of seafood during the intervention (data not shown).

3.3.1 Intervention – compliance

Participants in the intervention group consumed a mean (SD) of 306 (62) grams of the received cod fillets per week, with a mean (SD) total intake of cod of 4897 (992) grams during the 16-weeks intervention period. With a 100 % compliance to the intervention, the total amount of cod intake would be 6400 grams. The average dietary compliance was therefore 76.5 % in this study. 50 % of the participants ate more than 80 % of the fish.

3.3.2 Dietary intake of Hg from fish and seafood during pregnancy

Based on post-intervention FFQ Hg intake during the intervention period was significantly higher in the intervention group versus the control group. Mean (SD) weekly Hg intake from fish and seafood was 27 (12) µg in the intervention group versus 21 (13) µg in the control group. Mean dietary intake of Hg from seafood during the intervention period is shown in Table 3.5.

The mean (SD) weekly intake of Hg per kg body weight was 0.4 µg/kg (0.2) in the intervention group and 0.3 (0.2) µg/kg in the control group and the difference was significant (p = 0.004) (data not shown). These calculations were based on the pre-pregnancy weight of the participants. Comparing this to the TWI of 1.3 µg/kg bw for MeHg set by EFSA (71), Hg intake was 33 % and 25 % of TWI, respectively. None of the participants exceeded the set TWI during the intervention period (data not shown).

Table 3.5 – Estimated Hg intake from seafood during the intervention period (GW 20-36) for participating pregnant women in Mommy's Food. Reported as mean (SD) µg per week.

Fish/seafood	Mean (SD) Hg intake in µg per week		p[a]
	Intervention group	Control group	
Fatty fish[b]	1.9 (2.6)	4.1 (7.5)	0.000**
Salmon/Trout	0.7 (1.0)	1.7 (1.3)	0.000**
Lean fish[c]	19 (9.8)	9.0 (6.8)	0.000**
Cod	16 (8.7)	5.9 (4.9)	0.000**
Sushi[d]	0.2 (0.2)	0.2 (0.3)	0.88
Processed fish[d,e]	3.6 (3.6)	4.3 (3.8)	0.19
Shellfish[d,f]	1.0 (1.1)	1.2 (1.8)	0.65
Spread[g]	1.4 (1.6)	1.7 (2.6)	0.50
Total seafood[h]	27 (12)	21 (13)	0.002*

[a]Mann-Whitney U Test; [b]Includes salmon/trout, mackerel, herring and halibut; [c]includes cod, saithe, pollock, ling and wolfish; [d]accounted for percent of fish/seafood in products; [e] includes fish cakes and bolls, fish gratin, fish fingers, fish soup and dried cod; [f]includes shrimps, claw meat from crab, brown meat from crab, lobster, mussels and scallops; [g]includes canned mackerel, canned salmon, canned tuna, smoked salmon/trout, pickled herring, caviar, peppered mackerel, peeled shrimps, canned sardines, anchovy, crabsticks and cod liver pate; [h]includes all seafood displayed above in the table in addition to fish roe; *statistical significant difference between the groups (p<0.01); **statistical significant difference between the groups (p<0.001). Abbreviations: GW, gestational week; Hg, mercury; p, probability value; SD, standard deviation

Cod was the main source of Hg from total Hg intake in both groups, accounting for 64 % in the intervention group and 30 % in the control group, respectively. Mean (SD) Hg intake from cod

was significantly higher in the intervention group compared to the control group with 16 (8.7) µg versus 5.9 (4.9) µg (Table 3.5). Consumption of other fish species accounted for the remaining part of THg intake, but the contribution by each species was small.

3.3.2.1 Subgroup analysis - mothers

When restricting the analysis of maternal seafood and Hg consumption during the intervention period to the group of participants who provided infant hair samples at 6 weeks postpartum (n = 63), there was found no significant differences on total seafood and Hg intake between the intervention group compared to the control group (data not shown). However, in this subgroup the intervention group still had a higher consumption of lean fish whereas the control group consumed more fatty fish, with p = 0.000 for lean fish and p = 0.001 for fatty fish, respectively (data not shown).

3.4 Seafood consumption during infancy

The mean total fish intake in infants was not significantly different in either of the groups, neither at 6 months or 11 months of age. All participating infants had started with solid foods at 6 months of age, of which 9 % consumed fish at least once per week. The highest percentage of participants consuming fish had fish for dinner once per week (data not shown). The mean (SD) frequency of total fish intake was 0.3 (0.8) times per week for both the intervention group and the control group, and this was mainly consumed as dinner. There was no significant difference in consumption of lean and fatty fish between the groups (data not shown).

Fish consumption, as dinner and spread, increased from a mean (SD) of 0.3 (0.8) times per week at 6 months to a mean (SD) of 4.6 (3.0) times per week at 11 months, resulting in a mean increase in fish consumption of 4.3 times per week for all infants. This increase was statistically significant (p = 0.000). At 11 months of age 98 % of the infants consumed fish for dinner at least once per week, and the frequency of seafood intake most often reported was 1 time per week for all categories, lean and fatty fish as dinner and spread (data not shown). 87 % of the 11-month old infants consumed fish for dinner at least two times per week. The results showed a significant difference in fish intake between the two groups. A higher mean (SD) frequency of fatty fish consumption as dinner was seen in the control group compared to the intervention group at age 11 months (p = 0.017), (Figure 3.2). Conversely, the intervention group had a

significant higher intake of lean fish as spread compared to the control group (p = 0.033), (Figure 3.2).

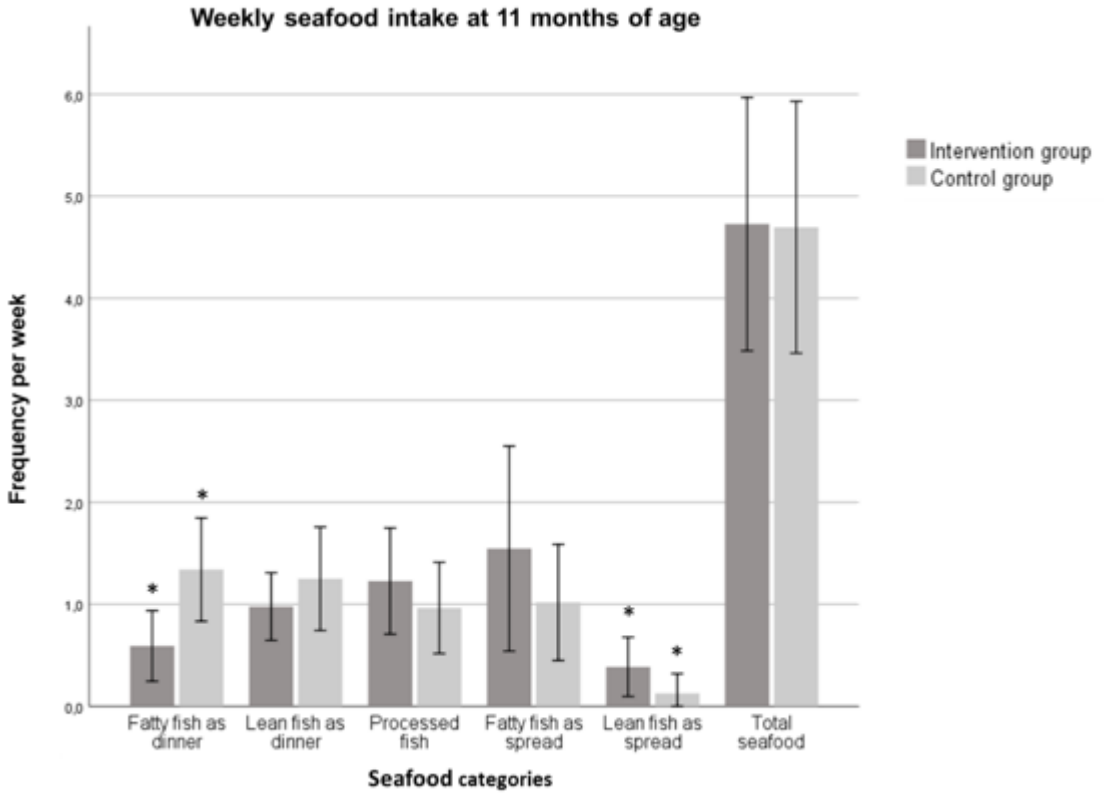


Figure 3.2 - Infant seafood intake at 11 months of age. Presented as mean frequencies per week. Error bars represent 95 % Confidence interval.

*significant difference between intervention and control group within the same seafood category, analyzed with Mann-Whitney U test.

3.5 THHg infants

Mean THHg levels in infant hair at 6 weeks, 6 months and 11 months of age are shown in

Table 3.6. Three hair samples had mercury levels below LOQ and were therefore excluded from the statistical analyses. 69 % of the hair samples had Hg levels below the validated area of the calibration curve for the analysis. The median THHg level was lower than the mean for all time points and in both groups, hence the distribution was positively skewed towards lower values. No significant differences in THHg was found when comparing the groups, and no difference in THHg levels were found when comparing boys and girls (data not shown).

Two of the hair samples (3 %) had THHg levels above the USEPAs RfD of 1000 µg/kg, both samples from 11 months of age with a value of 1040 µg/kg and 1355 µg/kg.

Table 3.6 - Measured THHg levels for infant at 6 weeks, 6 months and 11 months of age. Presented as mean (SD) and median (IQR) in µg/kg

	n	All		n	Intervention group		N	Control group		p[a]
		Mean (SD)	Median (IQR)		Mean (SD)	Median (IQR)		Mean (SD)	Median (IQR)	
THHg 6 weeks	63	332 (184)	293 (198, 432)	32	336 (173)	292 (221, 428)	31	328 (198)	297 (180, 435)	0.81
THHg 6 months	78	319 (188)	272 (173, 421)	37	334 (293)	276 (174, 422)	41	305 (184)	269 (156, 425)	0.60
THHg 11 months	62	305 (262)	199 (151, 375)	30	286 (209)	203 (163, 333)	32	323 (306)	179 (138, 447)	0.56

[a] Mann-Whitney U test. Abbreviations: IQR, interquartile range; p, probability value; SD, standard deviation; THHg, total hair mercury

3.5.1 Subgroup analysis - infants

When investigating the change in THHg throughout the infant period, the statistical analysis was restricted to the number of 33 hair sample pairs from both 6 weeks and 11 months of age. There was seen no significant difference between the two groups in the samples from 6 weeks, see Figure 3.3. As displayed in Figure 3.3, a decrease in mean THHg was visible for both groups, although only the reduction in THHg in the control group was significant (p = 0.003). The mean level of THHg at 11 months was significantly higher in the intervention group compared to the control group (p = 0.023). There was no difference in frequency of seafood intake at 11 months between the groups in this subgroup of participants.

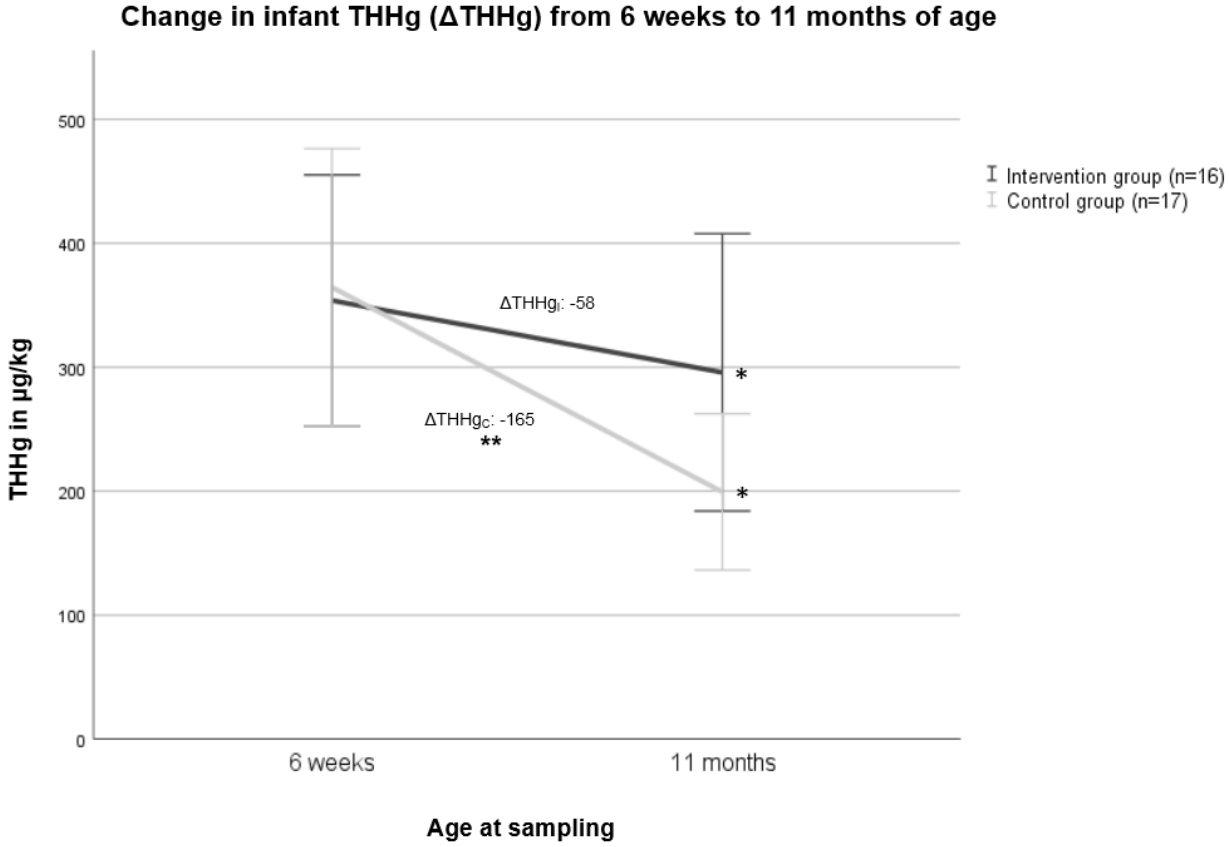


Figure 3.3 - Change in infant THHg levels between 6 weeks and 11 months of age in Mommy's Food (n = 33). Presented as µg/kg. Error bars represent 95 % Confidence interval.

*significant difference in THHg level between groups at the same time point; **significant different change in THHg level between the two time points within the same group. Abbreviations: THHg, total hair mercury; $\Delta THHg_I$, change in total hair mercury in the intervention group; $\Delta THHg_C$, change in total hair mercury in the control group

3.6 Correlation between maternal seafood intake and infant THHg

Correlations between maternal seafood intake and infant THHg 6 weeks postpartum are presented in Table 3.7. A moderate correlation was observed on total seafood, both from summary ($p = 0.011$) and detailed questions ($p = 0.022$) for all participants and also for the control group. When correlating infant THHg at 6 weeks with consumption of different groups of seafood consumed by the mothers, the results show moderate correlation with sushi ($p = 0.009$) and shellfish ($p = 0.002$) when all participants are considered. When considering only the control group, a moderate correlation was seen on seafood as spread ($p = 0.041$), from both summary and detailed questions. Total lean fish ($p = 0.049$) and shellfish ($p = 0.022$) correlated moderately with 6 weeks infant THHg in the control group. Low correlation was seen for seafood as spread ($p = 0.029$) when taking into account all participants. No correlation was seen between maternal seafood consumption and THHg from 6 weeks old infants in the intervention group.

Table 3.7 – Spearman's rho coefficient (r) comparing portions per week of maternal seafood intake and infant THHg levels 6 weeks postpartum

	All			Intervention group			Control group		
	n	**r**	**p**	**n**	**r**	**p**	**n**	**r**	**P**
Summary Questions									
Seafood as dinner	56	.251	.06	30	.244	.19	26	.327	.10
Seafood as lunch	56	.089	.51	30	-.038	.84	26	.195	.34
Seafood as spread	56	.288	.031*	30	.047	.80	26	.479	.013*
Total seafood	56	.339	.011*	30	.156	.41	26	.475	.014*
Detailed questions									
Fatty fish	56	.142	.30	30	-.005	.98	26	.326	.10
Salmon/trout	56	.102	.45	30	-.043	.82	26	.263	.20
Lean fish	54	.198	.15	29	.089	.65	25	.398	.049*
Cod	56	.175	.20	30	.056	.77	26	.326	.10
Sushi	56	.344	.009**	30	.332	.07	26	.316	.12
Processed fish	55	.164	.23	29	.180	.35	26	.109	.60
Shellfish	56	.398	.002**	30	.294	.12	26	.449	.022*
Spread	56	.293	.029*	30	.214	.26	26	.403	.041*
Total seafood	53	.315	.022*	28	.219	.26	25	.418	.037*

*statistical significant correlation ($p<0.05$); **statistical significant correlation ($p<0.01$). Abbreviations: p, probability value; r, Spearman's rho coefficient

4 Discussion

The aims of this study were to investigate prenatal mercury exposure after maternal seafood consumption in a randomized controlled trial (RCT) with cod during pregnancy, and also to examine THHg levels and intake of fish and seafood during the first year of life. Prenatal mercury exposure was investigated by estimating the mercury intake from seafood consumption registered in an FFQ completed by the mothers post-intervention in addition to measuring THHg in infants 6 weeks postpartum. Intake of seafood by infants from both 6 months and 11 months of age was reported by parents in FFQs. Results from this study provides new data on THHg levels and seafood consumption by Norwegian infants. To my knowledge, this is the first RCT investigating fish as a wholefood and its effect on THHg levels. In the following sections, findings form this thesis are discussed, assessing both strengths and weaknesses of the study.

4.1 General findings

4.1.1 Seafood intake during pregnancy

The average total seafood intake during pregnancy in this study is found to be in line with the upper tier of the recommended seafood intake from the Norwegian Directorate of Health (49), both pre- and post-intervention. However, the mean intake of fatty fish was lower than the recommended 200 g/week in both groups and at both time points. An intake of seafood in line with the recommendations is thought to be beneficial for the development of the child (33, 35). Studies have observed a decreased risk of preterm birth as well as increased birth weight after moderate fish consumption during pregnancy (36). In turn this has been associated with beneficial health outcomes later in life (2, 4), although fish consumption higher than the recommendations have been associated with increased risk of obesity (153). In this study however, there was not found any difference on birth weight and gestational length between the two groups, possibly as a result of high fish intake in both groups or lack of power due to a relatively low number of participants. However, the infant characteristics at birth revealed a small, but significant increase in length at birth in the intervention group compared to the

control group. The importance of this is questionable, especially since the length is not significantly different between the two groups at any of the follow-up time points.

The average seafood consumption in Mommy's Food was considerably higher compared to seafood consumption among pregnant women participating in MoBa (n = 67 007), where the mean total seafood intake was 255 g/week, with 85 g fatty fish and 142 g lean fish (37).

The high consumption of fish in our study may be a result of higher sosio-economic status among the study participants compared to the general Norwegian population, in respect to both education and household income. In this study 63 % of participants had a household income higher than 750 000 NOK, which is the median household income for couples with young children in Norway (154). Also, the level of education amongst women in this study are higher than for women from the general Norwegian population, with 86 % versus 37 % educated in university or university college, respectfully (155). A high socio-economic status has been associated with increased consumption of foods perceived as healthy, such as fruits, vegetables and fish (156-158). The result from this study may therefore not be applicable to the general Norwegian population.

Analysis of maternal seafood consumption from pre- to post-intervention in our study found that total seafood consumption did not change, showing a high seafood consumption already before the intervention started. However, we found a change in composition of fish species in the diet of participants in the intervention group from pre- to post-intervention. There was a reduction in fatty fish consumption in this group, that is likely to be caused by the increased intake of lean fish received during the intervention. When receiving cod during the study, participants reduced amounts of other fish and seafood species in the diet. This was also the case for intake of processed seafood and seafood as spread in the same group. This is a common problem with dietary interventions, as an increased intake of one type of food might lead to a decrease in other types of food. An interesting effect from this might be that the intake of nutrients could change. As fatty fish is especially rich in omega-3 fatty acids, the intake of these may decrease when replacing fatty fish with lean fish. Similarly, an increased intake of lean fish can lead to a raised intake of iodine. However, lean fish is also an adequate source of omega-3 FAs, with 200 g cod providing approximately 0.5 gram of these fatty acids (159), accounting for two times the general recommended daily intake on 0.25 g marine omega-3 FA set by EFSA (160). Cod consumption in addition to a possible high intake of omega-3 supplements by the participants, hopefully keeps the blood levels of DHA at a desirable level. A consequence of a low intake of fatty fish may be decreased DHA levels in blood (142). High

DHA levels in blood during pregnancy have been shown to be positively associated with infant development (42). In addition, a possible effect of low omega-3 fatty acids on increased risk of postpartum depression has been observed in several studies (161-163). Increased risk of cognitive and socio-emotional delay in children have been linked to mothers suffering from depression during early life of the child (164). Analyzes of FA status in participants from this study are being examined, but at this point we do not know whether there is a difference in FA status between the groups in this study. A possibly altered nutrient intake from this study also inquires investigation regarding its influence on infant development.

Results from post-intervention FFQ in terms of portions of seafood consumption per week made it possible to estimate the weekly Hg intake from seafood. However, the estimations were based on predetermined portion sizes and average Hg content from different fish species, introducing numerous sources of error which increase uncertainties in the calculations. Keeping this in mind, the results show a significantly higher intake of Hg in the intervention group, owing to the different distribution of fish species in the diet between the two groups. Lean fish generally contain more Hg than fatty fish, and lean fish was consumed at a higher quantity in the intervention group compared to the control group. Even though seafood consumption among the participants were high compared to the general pregnant population in Norway, estimated weekly Hg intake did not exceed the TWI of 1.3 µg MeHg/kg bw/week set by EFSA (71).

The overall estimated Hg intake was significantly higher in the intervention group compared to the control group. However, no difference was seen between the groups on Hg intake during the last half of pregnancy in subgroup analysis of Hg intake only including participants providing hair samples from infants at 6 weeks postpartum. This may explain why there is not seen any difference in THHg from infants at 6 weeks of age, which reflect the mercury exposure from approximately week 28 of pregnancy. An interesting observation is that even as this subgroup had a significantly higher maternal intake of cod and total lean fish in the intervention group compared to the control group, the fetus' exposure to Hg appear to not differ between the two groups. This suggests that the lean fish and cod consumed during the intervention may have had low concentrations of Hg. This was confirmed when a selection of cod fillets from the intervention was analyzed. However, the lack of difference can also be caused by inadequate power due to a low number of participants in this subgroup.

4.1.2 Newborn THHg reflecting maternal seafood consumption in pregnancy

To my knowledge there has only been one other Norwegian study investigating prenatal Hg exposure with THHg from infants. This was the LiN cohort, where they also analyzed THHg levels in hair samples from a selection of infants at 6 weeks of age (n = 374) (165). The result from our study supports the findings in analyses of THHg from 6 weeks hair samples in LiN, with a mean (SD) THHg level of 332 (184) µg/kg in our study compared to 330 (258) µg/kg in LiN.

No significant correlations, between maternal seafood intake and infant THHg at 6 weeks, were seen in the intervention group. The reason for this is not clear, however there are several factors to take into account when interpreting these correlations. The time-span reflected in the FFQ does not completely overlap the time of exposure represented by the hair samples, misreporting by the participants, over- or underestimation with the seafood index, uncertainty regarding the samples and analysis, and unknown factors about hair growth and accumulation in the fetus, are all factors that could influence the correlation analysis. As a consequence, the THHg levels at this time point reflect intrauterine mercury exposure, and might be a good indicator for maternal seafood consumption during pregnancy.

When investigating correlations in the control group between maternal seafood intake in the second half of pregnancy with THHg levels in infants at 6 weeks of age in our study, there were seen low to moderate correlations with seafood. The observed correlation on some groups of seafood such as shellfish, spread and sushi are presumably a reflection of an increased consumption of these seafood groups for participants also consuming other types of seafood rather than of its large contribution to Hg in the diet. One can speculate that sushi can be a major source of Hg intake in the diet as tuna and halibut are often used in sushi. However, pregnant women are usually quite careful about what they eat, and therefore it is likely that they would avoid consuming sushi containing these fish species.

4.1.3 Seafood intake and THHg in infants during the first year of life

After birth and during the first year of life, the infant is exposed to mercury mainly through breastfeeding as well as from consumption of fish and seafood when this is introduced as a solid food to the infant.

THHg from hair samples obtained at 6 months of age represent mercury exposure from approximately 3 months to 4.5 months of age. During this period, the infant diet mainly consist

of breast milk and/or infant formula. Therefore, this will be the only source of Hg intake for the infants. Nevertheless, our results from Hg analyses show that THHg levels at 6 months of age are maintained at approximately the same level as fetal THHg levels. This has also been observed in a group of children from the Faroe Islands cohort, where ingestion of mercury through breastmilk was adequate in keeping the infant's mercury levels in hair and blood in correspondence with the mother's levels (106). These findings are interesting, as mercury content in breast milk mainly consist of inorganic mercury which is thought to be poorly absorbed and to not accumulate well in hair (55, 127). This may suggest that the management of mercury is different in infants compared to adults, as implied in previous animal studies (77). Evidence from animal studies show increased absorption and retention of Hg combined with decreased biliary excretion of Hg in suckling offspring compared to older animals (78). Additionally, demethylating microorganisms in the intestines are thought to not be established until after the start of weaning (75, 76, 166).

The consumption of breast milk is likely to decrease during the first year of life, which is in line with the recommendations (51). This has its natural explanation as the infants expand their intake of other foods, both in variety and quantity. Consequently, other sources of Hg are introduced in the diet, with fish and seafood thought as the most prominent source of exposure (57, 71).

Our findings on the average frequency of seafood intake in infants show a significant increase between 6 and 11 months of age. Significant differences between the groups in intake of fatty fish for dinner and lean fish products as spread was seen at 11 months. The intervention group consumed more lean fish as spread compared to the control group, whereas the opposite applied to fatty fish for dinner. However, an important aspect to remember is that this only involves frequencies and not amount of seafood consumed, as this was not reported.

The percentage of infants receiving fish for dinner at least once a week in the current study is comparable to results from the Spedkost surveys (52, 53). This was found among 9 % of infants at 6 months of age in our study compared to 8 % in the Spedkost 6 months survey (52). At 11 months of age, 98 % of infants in our study consumed fish for dinner minimum one time per week compared to 82 % in Spedkost 12 months (53). The Norwegian Directorate of Health recommends that children should follow the same dietary advice as the general population when reaching 12 months of age (51), including the advice on eating fish for dinner 2-3 times per week and as spread. The average frequencies of fish intake for infants at 11 months of age in

this study are in agreement with these recommendations, with 87 % of the infants having fish for dinner at least two times per week.

Even when the frequency of seafood intake increased considerably from 6 months to 11 months of age, the average THHg remained at the same level as previous hair samples. However, when only including results from participants delivering hair samples at both 6 weeks and 11 months (n=33), a significant decrease in THHg is evident for all these participants. When comparing the groups, only the control group had a significant decrease in THHg levels from 6 weeks to 11 months, although a trend in lower THHg levels was also apparent for the intervention group. This contradicts our knowledge about fish and seafood being the main source of mercury, as the THHg levels are reduced when the fish intake increases. A reason could be a decrease in consumption of breast milk. Besides, mercury in breast milk has been seen to be higher in colostrum compared to mature milk, as the protein concentration in colostrum is higher, and possibly also as a result of volume dilution (11). It is also plausible to think that a decrease in THHg during infancy could be a result of the rapid increase in body weight. If the intake of mercury is fairly stable during this period, the rapid growth of the infant, including increased blood volume, would lead to a decrease in mercury concentration in the blood, and therefore a decrease in THHg levels, as this is a reflection of Hg concentration in blood (126). However, there was no difference in growth from 6 weeks to 11 months of age between the two groups, failing to explain why a difference in ΔTHHg is observed between the two groups.

There are reasons to speculate that the influence of other components in the diet may affect mercury absorption. Components in food, such as phytochemicals and dietary fibers from fruits and grains, have been suggested to alter the bioavailability of Hg, either directly by interfering with mechanisms like absorption and transport, or indirectly by affecting microorganisms in the intestines (167, 168). These findings are of interest, as fruit and grains are highly consumed by infants (52, 53). These effects may also be of greater importance in infants as their microbiota is not completely established at this stage (76, 166). However, these are only speculations, as a great deal is still not known about infants' mechanisms of handling Hg.

From the Faroe Islands study, increased levels of THHg in infants at 12 months of age were seen to be associated with enhanced accomplishment of milestones related to development. It was also observed that the THHg positively correlated with the duration of breastfeeding (106). In conclusion, the authors suggested that the apparent advantageous effect of mercury on development could be explained by the beneficial effects of breastfeeding (106). This is an

interesting finding, especially as the mean THHg level in infants from the Faroe Islands was considerably higher than THHg levels in our study.

4.2 Methodological discussion

4.2.1 Study design

When conducting scientific studies it is crucial to select the most suitable study design in relation to the research question and the resources available. The Mommy's Food study was primarily designed to investigate iodine status in pregnant women as well as child development (138). Thus the study design may impose some limitations in regards to the aims of this thesis, investigating seafood intake and THHg in infants. However, when scientific research is performed it is important to assess all available data, as research requires vast amounts of effort, considering both labor and economical resources.

In epidemiological research the RCT design is recognized as good in terms of the hierarchy of evidence when ranked by effectiveness, appropriateness and feasibility when evaluating interventions on health care (169). The RCT design has the advantage of reducing risk of certain bias and of the impact from confounding factors (170, 171). This is done by randomizing the participants to separate groups, so that possible confounding factors hopefully are equally distributed in the groups (170). Results from an RCT study also have the possibility to demonstrate causality, in contrast to observational studies (172). Consequently, the RCT design is a strength for this study.

Blinding of participants and researchers should as far as possible be achieved, to reduce potential differences in treatment from the researchers as well as reducing bias related to expected effects from participants and researchers (173). This presents a challenge and a common limitation in nutritional research (174), for obvious reasons, as food is difficult to conceal without changing its properties. Infants in the Mommy's Food study were blinded, although blinding of the participating pregnant women in our study were not possible. As a consequence, it is reasonable to speculate that the lack of blinding could have an impact on the diet of the control group, especially in relation to fish consumption. However, this aspect was investigated in our study and the results demonstrated that seafood intake did not change in the control group during the intervention period, thus strengthening our results. In addition, most

statistical analyses and analyses of THHg levels were performed blinded, except for calculations of intake of cod from the intervention which only involved the intervention group.

The time-span covered in the FFQs at 6 months and 11 months of age were not comparable to the period of exposure represented by the hair samples, therefore correlations between seafood consumption and THHg could not be conducted. The results from analyzes of 11 month hair samples show an average Hg level throughout the period represented by the hair samples, from about 8 to 9.5 months of age. As this is a period of major changes in the diet of the infant, the average Hg exposure over such a long period can give an inappropriate representation of the actual exposure of Hg from seafood. One can speculate whether a steady state level of mercury in blood might have been better suited to investigate Hg exposure from the seafood intake reported in the FFQ. This was not an option as it would be too invasive to draw excessive amounts of blood from the infants.

4.2.2 Intervention diet

As the intervention diet involved fish consumption, it is presumed that people who do not consume fish will most likely not volunteer to participate in a study where they may need to eat fish. To properly investigate the effect of seafood intake on Hg exposure and THHg in this thesis, an ideal control group would be a group completely eliminating fish from their diet during the intervention period. This is not ethically accepted when participants include pregnant women, as it is known that nutrients from fish are important in fetal development (9, 28). Another alternative control group could be one where participants received other types of food, e.g. chicken filets, in the same quantity as participants receiving fish. This would perhaps prevent the control group in consuming large amounts of fish, although this is only a speculation.

Unfortunately, we could not compare THHg between infants of mothers consuming versus not consuming fish. This would have been interesting to investigate to assess differences in Hg exposure. However, we got to investigate THHg exposure in pregnant women with a mean seafood intake corresponding to the recommended intake of seafood.

A strength of this study is the high compliance to the intervention diet. For reasons regarding compliance fish fillets were also provided for the participants' partner. This was closely monitored as the cod fillets were weighed both prior to cooking as well as possible leftovers after the meal. However, a source of error is introduced when the fish was used in fish soup or

fish gratin, as it is not possible to calculate the exact amount of fish eaten by the participant as it is shared with her partner.

4.2.3 Dietary assessment

There are both strengths and weaknesses related to using FFQ for dietary assessment in scientific research. Recall bias is always a problem with retrospective dietary assessment methods, although people with higher level of education have been seen to recall past dietary intake with reasonable reliability (175). Also, under- and over-reporting of foods, is a problem with FFQ, although this is also the case for other dietary assessment methods (176). For this reason, FFQs should be validated for the specific research question investigated. The pre- and post-intervention FFQs in our study was revised from a previously validated FFQ (141, 142). Biomarkers for omega-3 and vitamin D was used to validate the seafood index for seafood consumption reported in FFQ (142). This strengthens the results from the FFQ used to acquire maternal seafood intake. However, the FFQ has not been validated in relation to estimating Hg intake from the diet, hence introducing a limitation to the results. Infant FFQs were not validated, which may be a limiting factor when assessing seafood intake from infants.

One can speculate if another dietary assessment method, such as the repeated 24-hour recall, would be a better alternative to the FFQ. However, as this study has the intention to discover regular fish intake, the FFQ is seen as a better alternative. Fish is not consumed daily and also not by everyone, with less than half of the Norwegian adult population consuming the recommended amount of fish (49, 177). Even if performing a repeated 24-hour recall, the separate days investigated might still have a low chance of detecting a day when the participant is consuming fish. In addition, the FFQ used in our study is quite detailed and covers a wide range of different seafood and fish species, which is a strength for this thesis as it covers Hg intake from different sources of fish. To account for overreporting, which is more likely to occur when the questionnaire is very detailed (144, 145), the seafood index developed from the detailed questions was quite strict. Therefore, a miscalculation may be present, especially for cod in the intervention group as these participants are instructed to eat cod two times per week. This will be registered in the FFQ as 1-2 times per week, and then converted to a seafood index of 1, indicating an intake of one portion of cod per week. A mean difference of 65 grams of cod per week is observed between the calculated intake from the seafood index (262 grams) and the registered weekly cod intake in the weight scheme (306 grams). This is a source of error when

calculating maternal Hg intake from seafood, however it does not influence results on THHg levels and is therefore of limited importance to the conclusion of this thesis.

4.2.4 Hair sampling and analysis

There was a relatively low number of hair samples, as only around half of the study population contributed with hair samples at all three time points. There were several reasons for this. Babies are a challenging study population to work with, in regard to biological sampling. Difficulties regarding the hair samples included that many of the babies had little hair and the babies were at times very curious and uneasy during the sampling, resulting in small and few hair samples. When the number of hair samples is low, each sample has a higher impact on the mean value, resulting in a bigger influence by individual variations on the result. From the results in Table 3.6, we also see that the SD is high, suggesting large variation in the results. However, the advantage with hair samples, especially when involving infant participants, is that it is a non-invasive technique, and it is a good method to explore mercury exposure in a population (124).

As a result of small hair samples and/or low amount of mercury in the samples, 69 % of hair samples had mercury content below the validated area of the calibrated curve for analyses in the DMA-80 machine. The normal estimated uncertainty of this method was ± 20 % for results in the validated area, however this level of uncertainty is not known when results are outside this validated area. Thus, the THHg results in this thesis may have an increased uncertainty. A second source of error involving the analysis of THHg, is the fact that results after analyzing the reference material showed a systematic pattern of underestimating the mercury content of 10-20 %. This may also apply to the hair samples, implying an underestimation of mercury content in hair from the infants. However, this will apply to results in both groups and at all time points, and will presumably not affect comparison of THHg levels between the groups. Still, mercury analyzes with DMA-80 is a respectable method for measuring mercury in hair.

Studies investigating hair growth and accumulation of substances in hair have to my knowledge only been conducted on adults. Therefore, it may not be applicable to use the same calculations relating to hair growth or assume that the accumulation factor on mercury from blood to hair of 250 also applies to children, when investigating hair samples from infants. Individual variations on hair growth and accumulation have also been shown in adults (68), however we do not know if such variations may have a different impact in children. Therefore, results on THHg must be

interpreted with care, and one should be careful to draw conclusions from such results until new knowledge on hair growth and handling of Hg in infants is obtained.

4.3 Conclusion

There were found no significant differences in infant THHg levels, between the intervention group and the control group, after an intervention with cod during pregnancy as a source of mercury exposure. Prenatal exposure to mercury was reflected by infant hair samples at 6 weeks postpartum. The increased intake of cod in the intervention group during pregnancy did not seem to influence THHg levels in the fetus differently than other fish species. Infant THHg levels reflecting mercury exposure during the third trimester of pregnancy were moderately correlated with total seafood intake, although only for participants in the control group.

Overall, the infant THHg levels did not change during the study period. However, a significant decrease in THHg from 6 weeks to 11 months of age was observed in a subgroup within the control group even though there was a significant increase in frequency of infant seafood consumption during this period.

This study population of pregnant women had a mean seafood intake in line with the recommended total seafood intake for the general population. For infants, the mean total seafood intake at 11 months of age was also in line with recommendations for this age group. At all time points, the mean infant THHg values were found to be approximately one third of the RfD set by USEPA. In conclusion, when evaluating these results on THHg against the current limit values, we find that there is no need to change the dietary recommendations on seafood intake neither for pregnant women or infants.

4.4 Future perspectives

This thesis provides new information on prenatal mercury exposure in Norway, as well as THHg levels and seafood consumption for infants during their first year of life. However, there is a need for more studies investigating this, especially in populations with a moderate fish intake in agreement with the recommendations from health authorities. Most of the current knowledge on the effects of MeHg have been observations from populations affected by poisoning or with a higher intake of fish and other seafood than most populations. It is important to also measure low exposure groups and investigate long-term effects of MeHg. In regard to this it will be highly beneficial to include a larger study group as small studies may have lower power to detect differences.

There are many uncertainties concerning the subject of infants and Hg, whether it is infant exposure to Hg, or management of Hg in the infant body. Although some of these aspects might be unethical to examine directly in infants, exposure to Hg, both from seafood and breast milk, should be investigated further, preferably in relation to infant development.

Even as this study indicates low prenatal Hg exposure in a population group with seafood consumption in agreement with the recommendations, it will still be important to monitor this in the future. The use of mercury in industries is regulated in many countries, however additional sources of mercury may emerge, e.g. mercury emissions from melting permafrost due to climate change (178). In turn, the consequence of this may be increased human exposure to Hg.

References

1. Emmett PM, Jones LR, Golding J. Pregnancy diet and associated outcomes in the Avon Longitudinal Study of Parents and Children. Nutr Rev. 2015;73 Suppl 3:154-74.

2. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. The New England journal of medicine. 2008;359(1):61-73.

3. Koletzko B, Symonds ME, Olsen SF, Early Nutrition Programming P, Early Nutrition A. Programming research: where are we and where do we go from here? Am J Clin Nutr. 2011;94(6 Suppl):2036S-43S.

4. Barker DJ. The fetal and infant origins of adult disease. BMJ. 1990;301(6761):1111.

5. Lucas A. Long-term programming effects of early nutrition -- implications for the preterm infant. J Perinatol. 2005;25 Suppl 2:S2-6.

6. Symonds ME, Sebert SP, Hyatt MA, Budge H. Nutritional programming of the metabolic syndrome. Nat Rev Endocrinol. 2009;5(11):604-10.

7. Langley-Evans S. Pregnancy. Nutrition: A Lifespan Approach. United Kingdom: Wiley-Blackwell; 2009. p. 47-74.

8. Williamson CS. Nutrition in pregnancy. Nutrition Bulletin. 2006;31(1):28-59.

9. Zimmermann MB. Iodine deficiency. Endocr Rev. 2009;30(4):376-408.

10. Bath SC, Steer CD, Golding J, Emmett P, Rayman MP. Effect of inadequate iodine status in UK pregnant women on cognitive outcomes in their children: results from the Avon Longitudinal Study of Parents and Children (ALSPAC). Lancet. 2013;382(9889):331-7.

11. Ballard O, Morrow AL. Human milk composition: nutrients and bioactive factors. Pediatr Clin North Am. 2013;60(1):49-74.

12. Oddy WH. Breastfeeding protects against illness and infection in infants and children: a review of the evidence. Breastfeed Rev. 2001;9(2):11-8.

13. Field CJ. The immunological components of human milk and their effect on immune development in infants. J Nutr. 2005;135(1):1-4.

14. Alvisi P, Brusa S, Alboresi S, Amarri S, Bottau P, Cavagni G, et al. Recommendations on complementary feeding for healthy, full-term infants. Ital J Pediatr. 2015;41:36.

15. NHS Health Scotland. Fun First Foods: An easy guide to intoducing solid foods. Edinburgh: NHS Health Scotland; 2018.

16. Helsedirektoratet. Mat for spedbarn. 16th ed. Oslo: Helsedirektoratet; 2011.

17. Agostoni C, Decsi T, Fewtrell M, Goulet O, Kolacek S, Koletzko B, et al. Complementary feeding: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr. 2008;46(1):99-110.

18. EFSA Panel on Dietetic Products Nutition and Allergies. Scientific Opinion on the appropriate age for introduction of complementary feeding of infants. Wiley Online Library; 2009. Report No.: 1831-4732 Contract No.: 12.

19. Birch LL, Gunder L, Grimm-Thomas K, Laing DG. Infants' consumption of a new food enhances acceptance of similar foods. Appetite. 1998;30(3):283-95.

20. Carstairs SA, Marais D, Craig LC, Kiezebrink K. Seafood inclusion in commercial main meal early years' food products. Matern Child Nutr. 2016;12(4):860-8.

21. Domellof M, Braegger C, Campoy C, Colomb V, Decsi T, Fewtrell M, et al. Iron requirements of infants and toddlers. J Pediatr Gastroenterol Nutr. 2014;58(1):119-29.

22. Simpson JL, Bailey LB, Pietrzik K, Shane B, Holzgreve W. Micronutrients and women of reproductive potential: required dietary intake and consequences of dietary deficiency or excess. Part II--vitamin D, vitamin A, iron, zinc, iodine, essential fatty acids. J Matern Fetal Neonatal Med. 2011;24(1):1-24.

23. Dahl L, Bjørkkjær T, Graff IE, Malde MK, Klementsen B. Fisk - ikke bare omega-3. Tidsskrift for Den norske legeforening. 2006;126(3):309-11.

24. EFSA Dietetic Products Nutrition Allergies. 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).

25. Strain JJ, Yeates AJ, van Wijngaarden E, Thurston SW, Mulhern MS, McSorley EM, et al. Prenatal exposure to methyl mercury from fish consumption and polyunsaturated fatty acids: associations with child development at 20 mo of age in an observational study in the Republic of Seychelles. Am J Clin Nutr. 2015;101(3):530-7.

26. Helland IB, Smith L, Saarem K, Saugstad OD, Drevon CA. Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. Pediatrics. 2003;111(1):e39-44.

27. Campoy C, Escolano-Margarit MV, Anjos T, Szajewska H, Uauy R. Omega 3 fatty acids on child growth, visual acuity and neurodevelopment. The British journal of nutrition. 2012;107 Suppl 2:S85-106.

28. Makrides M, Smithers LG, Gibson RA. Role of long-chain polyunsaturated fatty acids in neurodevelopment and growth. Nestle Nutr Workshop Ser Pediatr Program. 2010;65:123-33; discussion 33-6.

29. Koletzko B, Cetin I, Brenna JT. Dietary fat intakes for pregnant and lactating women. The British journal of nutrition. 2007;98(5):873-7.

30. Cohen JT, Bellinger DC, Connor WE, Shaywitz BA. A quantitative analysis of prenatal intake of n-3 polyunsaturated fatty acids and cognitive development. Am J Prev Med. 2005;29(4):366-74.

31. Dziechciarz P, Horvath A, Szajewska H. Effects of n-3 long-chain polyunsaturated fatty acid supplementation during pregnancy and/or lactation on neurodevelopment and visual function in children: a systematic review of randomized controlled trials. J Am Coll Nutr. 2010;29(5):443-54.

32. Jacobs DR, Jr., Tapsell LC. Food, not nutrients, is the fundamental unit in nutrition. Nutr Rev. 2007;65(10):439-50.

33. Hibbeln JR, Davis JM, Steer C, Emmett P, Rogers I, Williams C, et al. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. Lancet. 2007;369(9561):578-85.

34. Oken E, Osterdal ML, Gillman MW, Knudsen VK, Halldorsson TI, Strom M, et al. Associations of maternal fish intake during pregnancy and breastfeeding duration with attainment of developmental milestones in early childhood: a study from the Danish National Birth Cohort. Am J Clin Nutr. 2008;88(3):789-96.

35. Daniels JL, Longnecker MP, Rowland AS, Golding J, Health ASTUoBIoC. Fish intake during pregnancy and early cognitive development of offspring. Epidemiology (Cambridge, Mass). 2004;15(4):394-402.

36. 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. Am J Clin Nutr. 2014;99(3):506-16.

37. Brantsaeter AL, Englund-Ogge L, Haugen M, Birgisdottir BE, Knutsen HK, Sengpiel V, et al. Maternal intake of seafood and supplementary long chain n-3 poly-unsaturated fatty acids and preterm delivery. BMC Pregnancy Childbirth. 2017;17(1):41.

38. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371(9606):75-84.

39. Boyle JD, Boyle EM. Born just a few weeks early: does it matter? Arch Dis Child Fetal Neonatal Ed. 2013;98(1):F85-8.

40. Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. Lancet. 2008;371(9608):261-9.

41. Platt MJ. Outcomes in preterm infants. Public Health. 2014;128(5):399-403.

42. Oken E, Wright RO, Kleinman KP, Bellinger D, Amarasiriwardena CJ, Hu H, et al. Maternal fish consumption, hair mercury, and infant cognition in a U.S. Cohort. Environmental health perspectives. 2005;113(10):1376-80.

43. Weichselbaum E, Coe S, Buttriss J, Stanner S. Fish in the diet: A review. Nutrition Bulletin. 2013;38(2):128-77.

44. World Health Organization. Report of the joint FAO/WHO expert consultation on the risks and benefits of fish consumption : Rome, 25-29 January 2010. Geneva: World Health Organization; 2011.

45. U.S. Department of Health and Human Services and U.S. Department of Agriculture. 2015 – 2020 Dietary Guidelines for Americans. 8th ed. 2015.

46. National Health and Medical Research Council. Australian Dietary Guidelines. Canberra, Australia2013.

47. Helsedirektoratet. Kostråd til gravide: Matportalen; 2018 [Accessed 27.05.18]. Available from: http://www.matportalen.no/rad_til_spesielle_grupper/tema/gravide/.

48. Vitenskapskomiteen for mattrygghet (VKM). Benefit-risk assessment of fish and fish products in the Norwegian diet - an update. Oslo, Norway; 2014.

49. Helsedirektoratet. Kostråd for å fremme folkehelsen og forebygge kroniske sykdommer : metodologi og vitenskapelig kunnskapsbidrag. Oslo: Helsedirektoratet; 2011.

50. U.S. Food & Drug Administration. Technical Information on Development of Fish Consumption Advice - FDA/EPA Advice on What Pregnant Women and Parents Should Know about Eating Fish: U.S. Food & Drug Administration; 2017 [Accessed 23.08.17] Available from: https://www.fda.gov/Food/FoodborneIllnessContaminants/Metals/ucm531136.htm.

51. Helsedirektoratet. Nasjonal faglig rettningslinje for spedbarnsernæring. Oslo, Norway; 2017.

52. Øverby NC, Helsedirektoratet. Spedkost - 6 måneder : landsomfattende kostundersøkelse blant 6 måneder gamle barn. Oslo: Helsedirektoratet; 2008.

53. Øverby NC, Helsedirektoratet. Spedkost 12 måneder : landsomfattende kostholdsundersøkelse blant 12 måneder gamle barn : spedkost 2006-2007. Oslo: Helsedirektoratet; 2009.

54. Universitetet i Oslo. Spedkost 3: Universitetet i Oslo; 2017 [Accessed 23.02.18] Available from: http://www.med.uio.no/imb/forskning/prosjekter/spedkost/.

55. Clarkson TW, Magos L. The toxicology of mercury and its chemical compounds. Critical reviews in toxicology. 2006;36(8):609-62.

56. Selin NE. Global Biogeochemical Cycling of Mercury: A Review. Annual Review of Environment and Resources. 2009;34(1):43-63.

57. Clifton JC, 2nd. Mercury exposure and public health. Pediatr Clin North Am. 2007;54(2):237-69, viii.

58. Fitzgerald WF, Clarkson TW. Mercury and monomethylmercury: present and future concerns. Environmental health perspectives. 1991;96:159-66.

59. European Commission (DG ENV). Review of the Community Stategy Concerning Mercury. 2010.

60. Chan TY. Inorganic mercury poisoning associated with skin-lightening cosmetic products. Clin Toxicol (Phila). 2011;49(10):886-91.

61. Clarkson TW, Magos L, Myers GJ. The toxicology of mercury--current exposures and clinical manifestations. The New England journal of medicine. 2003;349(18):1731-7.

62. Statens legemiddelverk. Tiomersal i vaksiner: Statens legemiddelverk; 2016 [Accessed 24.09.17]. Available from: https://legemiddelverket.no/bivirkninger-og-sikkerhet/rad-til-helsepersonell/vaksiner-til-mennesker/tiomersal-i-vaksiner#er-det-tiomersal-i-vaksiner-som-brukes-i-norge?

63. World Health Organization. Mercury and health: World Health Organization (WHO); 2017 [Accessed 29.05.18] Available from: http://www.who.int/news-room/fact-sheets/detail/mercury-and-health.

64. Liu G, Cai Y, O'Driscoll N. Environmental Chemistry and Toxicology of Mercury. New Jersey: John Wiley & Sons, Inc.; 2012. 596 p.

65. Antunes Dos Santos A, Appel Hort M, Culbreth M, Lopez-Granero C, Farina M, Rocha JB, et al. Methylmercury and brain development: A review of recent literature. Journal of trace elements in medicine and biology : organ of the Society for Minerals and Trace Elements (GMS). 2016;38:99-107.

66. Kerper LE, Ballatori N, Clarkson TW. Methylmercury transport across the blood-brain barrier by an amino acid carrier. The American journal of physiology. 1992;262(5 Pt 2):R761-5.

67. Langford N, Ferner R. Toxicity of mercury. J Hum Hypertens. 1999;13(10):651-6.

68. World Health Organization. IPCS Environmental Health Criteria 101: Methylmercury. Geneva, Switzerland: World Health Organization; 1990.

69. Clarkson TW. The three modern faces of mercury. Environmental health perspectives. 2002;110 Suppl 1:11-23.

70. Leaner JJ, Mason RP. Methylmercury accumulation and fluxes across the intestine of channel catfish, Ictalurus punctatus. Comparative biochemistry and physiology Toxicology & pharmacology : CBP. 2002;132(2):247-59.

71. EFSA Panel on Contaminants in the Food Chain. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal. 2012;10(12).

72. Rice KM, Walker EM, Jr., Wu M, Gillette C, Blough ER. Environmental mercury and its toxic effects. Journal of preventive medicine and public health = Yebang Uihakhoe chi. 2014;47(2):74-83.

73. Kimura H. Signaling molecules: hydrogen sulfide and polysulfide. Antioxid Redox Signal. 2015;22(5):362-76.

74. Davis LE, Kornfeld M, Mooney HS, Fiedler KJ, Haaland KY, Orrison WW, et al. Methylmercury poisoning: long-term clinical, radiological, toxicological, and pathological studies of an affected family. Annals of neurology. 1994;35(6):680-8.

75. Jugo S. Metabolism of toxic heavy metals in growing organisms: a review. Environmental research. 1977;13(1):36-46.

76. Kostial K, Simonovic I, Rabar I, Blanusa M, Landeka M. Age and intestinal retention of mercury and cadmium in rats. Environmental research. 1983;31(1):111-5.

77. Kostial K, Kello D, Jugo S, Rabar I, Maljkovic T. Influence of age on metal metabolism and toxicity. Environmental health perspectives. 1978;25:81-6.

78. Ballatori N, Clarkson TW. Biliary secretion of glutathione and of glutathione-metal complexes. Fundamental and applied toxicology : official journal of the Society of Toxicology. 1985;5(5):816-31.

79. Agency for Toxic Substances and Disease Registry. Toxicological profile for mercury. Atlanta, Georgia; 1999.

80. Kajiwara Y, Yasutake A, Adachi T, Hirayama K. Methylmercury transport across the placenta via neutral amino acid carrier. Archives of toxicology. 1996;70(5):310-4.

81. Vahter M, Akesson A, Lind B, Bjors U, Schutz A, Berglund M. Longitudinal study of methylmercury and inorganic mercury in blood and urine of pregnant and lactating women, as well as in umbilical cord blood. Environmental research. 2000;84(2):186-94.

82. Sakamoto M, Murata K, Domingo JL, Yamamoto M, Oliveira RB, Kawakami S, et al. Implications of mercury concentrations in umbilical cord tissue in relation to maternal hair segments as biomarkers for prenatal exposure to methylmercury. Environmental research. 2016;149:282-7.

83. Cernichiari E, Brewer R, Myers GJ, Marsh DO, Lapham LW, Cox C, et al. Monitoring methylmercury during pregnancy: maternal hair predicts fetal brain exposure. Neurotoxicology. 1995;16(4):705-10.

84. Aune T. Kontaminanter. Næringsmiddeltoksikologi : tilsetningsstoffer, miljøgifter og naturlige toksiner. 2. utg. ed. Kristiansand: Høyskoleforl.; 2007. p. 170-276.

85. Sakamoto M, Kubota M, Matsumoto S, Nakano A, Akagi H. Declining risk of methylmercury exposure to infants during lactation. Environmental research. 2002;90(3):185-9.

86. Vieira SM, de Almeida R, Holanda IB, Mussy MH, Galvao RC, Crispim PT, et al. Total and methyl-mercury in hair and milk of mothers living in the city of Porto Velho and in villages along the Rio Madeira, Amazon, Brazil. International journal of hygiene and environmental health. 2013;216(6):682-9.

87. Sandborgh-Englund G, Ask K, Belfrage E, Ekstrand J. Mercury exposure in utero and during infancy. Journal of toxicology and environmental health Part A. 2001;63(5):317-20.

88. Dorea JG. Mercury and lead during breast-feeding. The British journal of nutrition. 2004;92(1):21-40.

89. Grandjean P, Jorgensen PJ, Weihe P. Human milk as a source of methylmercury exposure in infants. Environmental health perspectives. 1994;102(1):74-7.

90. World Health Organization/United Nations Environmental Programme (WHO/UNEP). Guidance for Identifying Populations at Risk from Mercury Exposure. Geneva, Switzerland; 2008.

91. Havforskningsinstituttet. Kvikksølv (Hg): Sjømatdata; 2017 [Accessed 26.02.18] Available from: https://sjomatdata.nifes.no/#/substance/395/-2.

92. U.S. Food & Drug Administration. Mercury Levels in Commercial Fish and Shellfish (1990-2012): U.S. Food & Drug Administration; 2017 [Accessed 16.04.18] Available from: https://www.fda.gov/Food/FoodborneIllnessContaminants/Metals/ucm115644.htm.

93. Baxter M. Survey of metals in weaning foods and formulae for infants. London; 2006.

94. 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.

95. Levine KE, Levine MA, Weber FX, Hu Y, Perlmutter J, Grohse PM. Determination of mercury in an assortment of dietary supplements using an inexpensive combustion atomic absorption spectrometry technique. J Autom Methods Manag Chem. 2005;2005:211-6.

96. European Commission. Commission regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs: The Commission of the European Communities; 2006 [Accessed 19.05.18] Available from: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32006R1881.

97. Havforskningsinstituttet. Kvikksølv (Hg): Havforskningsinstituttet; 2014 [Accessed 20.02.18] Available from: https://nifes.hi.no/uonsket-stoff/kvikksolv-hg/.

98. Mattilsynet. Unngå fisk og skalldyr fra forurensede havner, fjorder og innsjøer: Matportalen; 2011 [Accessed 22.02.18] Available from: http://www.matportalen.no/verktoy/advarsler/unngaa_fisk_og_skalldyr_fra_forurensede_havner_fjorder_og_innsjoer.

99. Harada M. Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. Critical reviews in toxicology. 1995;25(1):1-24.

100. 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.

101. Grandjean P, Weihe P, Jorgensen PJ, Clarkson T, Cernichiari E, Videro T. Impact of maternal seafood diet on fetal exposure to mercury, selenium, and lead. Archives of environmental health. 1992;47(3):185-95.

102. 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.

103. 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 : an official publication of the Society for Risk Analysis. 1998;18(6):701-13.

104. Axelrad DA, Bellinger DC, Ryan LM, Woodruff TJ. Dose-response relationship of prenatal mercury exposure and IQ: an integrative analysis of epidemiologic data. Environmental health perspectives. 2007;115(4):609-15.

105. Axtell CD, Cox C, Myers GJ, Davidson PW, Choi AL, Cernichiari E, et al. Association between methylmercury exposure from fish consumption and child development at five and a half years of age in the Seychelles Child Development Study: an evaluation of nonlinear relationships. Environmental research. 2000;84(2):71-80.

106. Grandjean P, Weihe P, White RF. Milestone development in infants exposed to methylmercury from human milk. Neurotoxicology. 1995;16(1):27-33.

107. 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. Neurotoxicology and teratology. 1997;19(6):417-28.

108. Myers GJ, Davidson PW. Prenatal methylmercury exposure and children: neurologic, developmental, and behavioral research. Environmental health perspectives. 1998;106 Suppl 3:841-7.

109. Golding J, Gregory S, Iles-Caven Y, Hibbeln J, Emond A, Taylor CM. Associations between prenatal mercury exposure and early child development in the ALSPAC study. Neurotoxicology. 2016;53:215-22.

110. Grandjean P, Weihe P, White RF, Debes F. Cognitive performance of children prenatally exposed to "safe" levels of methylmercury. Environmental research. 1998;77(2):165-72.

111. Tamashiro H, Arakaki M, Akagi H, Futatsuka M, Roht LH. Mortality and survival for Minamata disease. Int J Epidemiol. 1985;14(4):582-8.

112. Myers GJ, Davidson PW, Cox C, Shamlaye C, Cernichiari E, Clarkson TW. Twenty-seven years studying the human neurotoxicity of methylmercury exposure. Environmental research. 2000;83(3):275-85.

113. Marsh DO, Clarkson TW, Cox C, Myers GJ, Amin-Zaki L, Al-Tikriti S. Fetal methylmercury poisoning. Relationship between concentration in single strands of maternal hair and child effects. Archives of neurology. 1987;44(10):1017-22.

114. Harada Y, Miyamoto Y, Nonaka I, Ohta S, Ninomiya T. Electroencephalographic studies of Minamata disease in children. Dev Med Child Neurol. 1968;10(2):257-8.

115. Budtz-Jorgensen E, Keiding N, Grandjean P, Weihe P, White RF. Consequences of exposure measurement error for confounder identification in environmental epidemiology. Stat Med. 2003;22(19):3089-100.

116. Grandjean P, Budtz-Jorgensen E, Keiding N, Weihe P. Underestimation of risk due to exposure misclassification. Int J Occup Med Environ Health. 2004;17(1):131-6.

117. The Joint FAO/WHO Expert Committee on Food Additives (JECFA). Methylmercury: World Health Organization; 2007 [Accessed 24.03.18] Available from: http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=3083.

118. The Joint FAO/WHO Expert Committee on Food Additives (JECFA). Mercury: World Health Organization; 2011 [Accessed 24.03.18] Available from: http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=1806.

119. European Food Safety Authority (EFSA). EU Member States: European Food Safety Authority; 2018 [Accessed 24.03.18] Available from: https://www.efsa.europa.eu/en/partnersnetworks/eumembers.

120. Vitenskapskomiteen for mat og miljø (VKM). Hva er EFSA og hvordan er VKM tilknyttet? : Vitenskapskomiteen for mat og miljø (VKM); 2018 [Accessed 24.03.18] Available from: https://vkm.no/efsa/omefsa/hvaerefsaoghvordanervkmtilknyttet.4.175083d415c86c573b5c1e82.html.

121. Branco V, Caito S, Farina M, Teixeira da Rocha J, Aschner M, Carvalho C. Biomarkers of mercury toxicity: Past, present, and future trends. J Toxicol Environ Health B Crit Rev. 2017;20(3):119-54.

122. Berglund M, Lind B, Bjornberg KA, Palm B, Einarsson O, Vahter M. Inter-individual variations of human mercury exposure biomarkers: a cross-sectional assessment. Environ Health. 2005;4:20.

123. Nuttall KL. Interpreting mercury in blood and urine of individual patients. Ann Clin Lab Sci. 2004;34(3):235-50.

124. Nuttall KL. Interpreting hair mercury levels in individual patients. Ann Clin Lab Sci. 2006;36(3):248-61.

125. Bartell SM, Ponce RA, Sanga RN, Faustman EM. Human variability in mercury toxicokinetics and steady state biomarker ratios. Environmental research. 2000;84(2):127-32.

126. Clarkson TW, Vyas JB, Ballatori N. Mechanisms of mercury disposition in the body. American journal of industrial medicine. 2007;50(10):757-64.

127. Lindberg A, Bjornberg KA, Vahter M, Berglund M. Exposure to methylmercury in non-fish-eating people in Sweden. Environmental research. 2004;96(1):28-33.

128. 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 science international. 2011;210(1-3):110-6.

129. United States Environmental Protection Agency (USEPA). Mercury Study Report to Congress. Volume VII: Characterization of Human Health and Wildlife Risks from Mercury Exposure in the United States. Washington DC; 1997.

130. Gibson RS. Measuring food consumption of individuals. Principles of nutritional assessment. 2nd ed. ed. Oxford: Oxford University Press; 2005. p. 41-64.

131. 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.

132. Willett WC, Hu FB. Not the time to abandon the food frequency questionnaire: point. Cancer Epidemiol Biomarkers Prev. 2006;15(10):1757-8.

133. Kelemen LE. Food frequency questionnaires: not irrelevant yet. Cancer Epidemiol Biomarkers Prev. 2006;15(5):1054.

134. Kristal AR, Potter JD. Not the time to abandon the food frequency questionnaire: counterpoint. Cancer Epidemiol Biomarkers Prev. 2006;15(10):1759-60.

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

136. Subar AF, Thompson FE, Smith AF, Jobe JB, Ziegler RG, Potischman N, et al. Improving food frequency questionnaires: a qualitative approach using cognitive interviewing. J Am Diet Assoc. 1995;95(7):781-8; quiz 9-90.

137. Teufel NI. Development of culturally competent food-frequency questionnaires. Am J Clin Nutr. 1997;65(4 Suppl):1173S-8S.

138. Markhus MW, Kvestad I, Midtbø LK, Nerhus I, Ødegaard ER, Graff IE, et al. Effects of cod intake in pregnancy on iodine nutrition and infant development: study protocol for Mommy's Food - a randomized controlled trial. BMC Nutrition. 2018;4(1):7.

139. Helse Bergen. Kvinneklinikken KK: Helse Bergen; n.d. [Accessed 30.05.18] Available from: https://helse-bergen.no/avdelinger/kvinneklinikken.

140. Dahl L, Wik Markhus M, Sanchez PVR, Moe V, Smith L, Meltzer HM, et al. Iodine Deficiency in a Study Population of Norwegian Pregnant Women-Results from the Little in Norway Study (LiN). Nutrients. 2018;10(4).

141. 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.

142. 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 Nutr Res. 2013;57.

143. Dalane JØ, Bergvatn TAM, Kielland E, Carlsen MH. Mål, vekt og porsjonsstørrelser for matvarer = Weights, measures and portion sizes for foods. Oslo: Mattilsynet Universitetet i Oslo Helsedirektoratet; 2015.

144. Krebs-Smith SM, Heimendinger J, Subar AF, Patterson BH, Pivonka E. Using food frequency questionnaires to estimate fruit and vegetable intake: Association between the number of questions and total intakes. Journal of Nutrition Education. 1995;27(2):80-5.

145. Serdula M, Byers T, Coates R, Mokdad A, Simoes EJ, Eldridge L. Assessing consumption of high-fat foods: the effect of grouping foods into single questions. Epidemiology (Cambridge, Mass). 1992;3(6):503-8.

146. Dalane JØ, Bergvatn TAM, Kielland E, Carlsen MH. Mål, vekt og porsjonsstørrelser for matvarer. Mattilsynet, Universitetet i Oslo og Helsedirektoratet; 2015. p. 72.

147. Gareri J, Koren G. Prenatal hair development: implications for drug exposure determination. Forensic science international. 2010;196(1-3):27-31.

148. International Atomic Energy Agency (IAEA). IAEA-086 , Human Hair (Methyl Mercury): International Atomic Energy Agency; 1997 [Accessed 30.05.18] Available from: https://nucleus.iaea.org/rpst/referenceproducts/referencematerials/Trace_Elements_Methylmercury/IAEA-086.htm.

149. Unites States Environmental Protection Agency. Method 7473 (SW-846): Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry. Washington, DC; 1998.

150. Milestone General. Full automatisert kvikksølvmåler DMA-80,. Milestone General; n.d.

151. Masson LF, McNeill G, Tomany JO, Simpson JA, Peace HS, Wei L, et al. Statistical approaches for assessing the relative validity of a food-frequency questionnaire: use of correlation coefficients and the kappa statistic. Public Health Nutr. 2003;6(3):313-21.

152. Cohen J. The Significance of a Product Moment r_s. Statistical power analysis for the behavioral sciences. 2nd ed. ed. Hillsdale, N.J.: L. Erlbaum Associates; 1988. p. 75-108.

153. 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.

154. Statistisk sentralbyrå (SSB). Inntekts- og formuesstatistikk for husholdninger: Statistisk sentralbyrå; 2017 [Accessed 06.04.18] Available from: https://www.ssb.no/inntekt-og-forbruk/statistikker/ifhus/aar.

155. Statistisk sentralbyrå (SSB). Befolkningens utdanningsnivå: Statistisk sentralbyrå; 2017 [Accessed 06.04.18] Available from: https://www.ssb.no/utdanning/statistikker/utniv/aar.

156. Johansson L, Thelle DS, Solvoll K, Bjorneboe GE, Drevon CA. Healthy dietary habits in relation to social determinants and lifestyle factors. The British journal of nutrition. 1999;81(3):211-20.

157. Holmboe-Ottesen G, Wandel M, Mosdøl A. Sosiale ulikheter og kosthold. Tidsskrift for Den norske legeforening. 2004;124(11):1526-8.

158. Lallukka T, Laaksonen M, Rahkonen O, Roos E, Lahelma E. Multiple socio-economic circumstances and healthy food habits. European journal of clinical nutrition. 2007;61(6):701-10.

159. Havforskningsinstituttet. Sum omega-3 (n-3): Sjømatdata; 2016 [Accessed 16.05.18] Available from: https://sjomatdata.nifes.no/#/substance/459/-1.

160. EFSA Panel on Dietetic Products Nutition and Allergies. Scientific Opinion on the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). EFSA Journal. 2012;10(7).

161. De Vriese SR, Christophe AB, Maes M. Lowered serum n-3 polyunsaturated fatty acid (PUFA) levels predict the occurrence of postpartum depression: further evidence that lowered n-PUFAs are related to major depression. Life sciences. 2003;73(25):3181-7.

162. Judge MP, Beck CT, Durham H, McKelvey MM, Lammi-Keefe CJ. Pilot trial evaluating maternal docosahexaenoic acid consumption during pregnancy: Decreased postpartum depressive symptomatology. International Journal of Nursing Sciences. 2014;1(4):339-45.

163. Markhus MW, Skotheim S, Graff IE, Froyland L, Braarud HC, Stormark KM, et al. Low omega-3 index in pregnancy is a possible biological risk factor for postpartum depression. PLoS One. 2013;8(7):e67617.

164. Kingston D, Tough S, Whitfield H. Prenatal and postpartum maternal psychological distress and infant development: a systematic review. Child Psychiatry Hum Dev. 2012;43(5):683-714.

165. Høgden HAR. Sammenheng mellom sjømatinntak under svangerskap og nivå av kvikksølv i hår fra mor og spedbarn: Univeritetet i Bergen; 2014.

166. Rowland IR. Interactions of the gut microflora and the host in toxicology. Toxicol Pathol. 1988;16(2):147-53.

167. Passos CJ, Mergler D, Gaspar E, Morais S, Lucotte M, Larribe F, et al. Eating tropical fruit reduces mercury exposure from fish consumption in the Brazilian Amazon. Environmental research. 2003;93(2):123-30.

168. Rowland IR, Mallett AK, Flynn J, Hargreaves RJ. The effect of various dietary fibres on tissue concentration and chemical form of mercury after methylmercury exposure in mice. Archives of toxicology. 1986;59(2):94-8.

169. Evans D. Hierarchy of evidence: a framework for ranking evidence evaluating healthcare interventions. J Clin Nurs. 2003;12(1):77-84.

170. Schulz KF, Grimes DA. Generation of allocation sequences in randomised trials: chance, not choice. Lancet. 2002;359(9305):515-9.

171. Roberts C, Torgerson D. Randomisation methods in controlled trials. BMJ. 1998;317(7168):1301.

172. Ferreira JC, Patino CM. Choosing wisely between randomized controlled trials and observational designs in studies about interventions. Jornal brasileiro de pneumologia : publicacao oficial da Sociedade Brasileira de Pneumologia e Tisilogia. 2016;42(3):165.

173. Gluud LL. Bias in clinical intervention research. American journal of epidemiology. 2006;163(6):493-501.

174. Staudacher HM, Irving PM, Lomer MCE, Whelan K. The challenges of control groups, placebos and blinding in clinical trials of dietary interventions. The Proceedings of the Nutrition Society. 2017;76(3):203-12.

175. Cade J, Thompson R, Burley V, Warm D. Development, validation and utilisation of food-frequency questionnaires - a review. Public Health Nutr. 2002;5(4):567-87.

176. Freedman LS, Schatzkin A, Midthune D, Kipnis V. Dealing with dietary measurement error in nutritional cohort studies. J Natl Cancer Inst. 2011;103(14):1086-92.

177. Totland TH, Helsedirektoratet. Norkost 3 : en landsomfattende kostholdsundersøkelse blant menn og kvinner i Norge i alderen 18-70 år, 2010-2011. Oslo: Helsedirektoratet; 2012.

178. Rydberg J, Klaminder J, Rosen P, Bindler R. Climate driven release of carbon and mercury from permafrost mires increases mercury loading to sub-arctic lakes. The Science of the total environment. 2010;408(20):4778-83.

Appendix

Appendix I

Post-intervention FFQ

Ultralyddato

Hei! Takk for at du har **deltatt i første del** av prosjektet "Mammas mat".

I denne undersøkelsen vil vi spørre deg blant annet om kostholdet ditt og hvordan du har hatt det de siste 16 ukene siden vårt forrige møte.

Vi setter veldig stor pris på din deltakelse!

Hvilken dato har du ultralyd-termin?

	Dag	Måned	År
	▼	▼	▼

Om deg / demografi

Hvilken svangerskapsuke er du i idag?

▼

Hvor fikk du først informasjon om studien "Mammas mat"?

- [ ] Brosjyre i posten
- [ ] Facebook
- [ ] Babyverden.no
- [ ] Via bekjente
- [ ] Via KK
- [ ] Nifes.no
- [ ] Annet, beskriv:

Din alder (år):

Hva er din sivilstatus?

- ○ Gift
- ○ Samboer
- ○ Enslig
- ○ Skilt
- ○ Separert
- ○ Enke
- ○ Annet

Hvilken utdannelse har du? (Sett ett kryss for den høyeste utdannelsen du har fullført.)

- ○ Ni- eller tiårig grunnskole
- ○ Videregående skole
- ○ Universitet/høyskole/fagskole, inntil fire år
- ○ Universitets/høyskole, fire år eller mer

Hva var din arbeidssituasjon før du ble gravid?

Her kan du sette flere kryss.

- [ ] Heltidsarbeid (80 - 100%)
- [ ] Deltidsarbeid (50 – 79 %)
- [ ] Deltidsarbeid (mindre enn 50 %)
- [ ] Student på heltid
- [ ] Student på deltid
- [ ] Hjemmeværende
- [ ] Arbeidsledig
- [ ] Uføretrygdet
- [ ] Sykemeldt

Hva er din arbeidssituasjon nå?

Her kan du sette flere kryss.

- Heltidsarbeid (80-100%)
- Deltidsarbeid (50-79%)
- Deltidsarbeid (mindre enn 50%)
- Student på heltid
- Student på deltid
- Hjemmeværende
- Arbeidsledig
- Uføretrygdet
- Sykemeldt

Hva var den samlede inntekten (før skatt) i husholdningen sist år?

- Ingen inntekt
- Under 200 000
- 200 - 349 999
- 350 - 549 999
- 550 - 749 999
- 750 - 999 999
- 1 000 000 -1 249 999
- 1 250 000 - 2 000 000
- Mer enn 2 000 000

Hvordan vil du beskrive familiens økonomi?

Svært god	God	Middels	Dårlig	Svært dårlig
○	○	○	○	○

Sjømat

Sjømat

Her vil vi gjerne få informasjon om deler av kostholdet ditt. Ha **de siste 16 ukene** 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 matinntak når det spørres om det.

I de første spørsmålene ønsker vi informasjon om ditt inntak av fisk, fiskeprodukter og annen sjømat de siste 16 ukene.

Hvor ofte har du spist fisk, fiskeprodukter eller annen sjømat som varmt måltid de siste 16 ukene (gjelder ikke pålegg)? Inkluder torsken du eventuelt fikk utlevert av oss.

Vennligst sett 1 kryss per linje.

	Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger/måned	1 gang/uke	2-3 ganger/uke	4 ganger eller mer/uke
Middag	○	○	○	○	○	○
Lunsj	○	○	○	○	○	○

Hvis du har spist fisk, fiskeprodukter eller annen sjømat til middag/varm lunsj, hvor mye har du vanligvis spist de siste 16 ukene? Inkluder torsken de eventuelt fikk utlevert av oss.

1 porsjon tilsvarer 150 gram laks, 200 gram torsk, 12 sushibiter, tre fiskekaker, seks fiskeboller, syv fiskepinner eller 2 dl reker u/skall

Vennligst sett 1 kryss per linje.

	Aldri	½ porsjon eller mindre	1 porsjon	1 ½ porsjon	2 porsjoner	3 porsjoner eller mer
Middag	○	○	○	○	○	○
Lunsj	○	○	○	○	○	○

Hvor ofte og hvor mye har du vanligvis spist av følgende sjømat som middag og/eller varm lunsj de siste 16 ukene? Inkluder torsken du eventuelt fikk utlevert av oss.

NB Sushi og fiskemat (fiskekaker, fiskeboller o.l.) er egne spørsmål og kommer senere.

Vennligst sett 1 kryss per linje.

	Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger/ måned	1-2 ganger/uke	3 ganger eller mer/uke
Laks, ørret - middag	○	○	○	○	○
Laks, ørret – lunsj	○	○	○	○	○
Torsk - middag	○	○	○	○	○
Torsk - lunsj	○	○	○	○	○
Sei - middag	○	○	○	○	○
Sei - lunsj	○	○	○	○	○
Makrell – middag	○	○	○	○	○
Makrell - lunsj	○	○	○	○	○

	Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger/ måned	1-2 ganger/uke	3 ganger eller mer/uke
Sild - middag	○	○	○	○	○
Sild - lunsj	○	○	○	○	○
Lyr - middag	○	○	○	○	○
Lyr – lunsj	○	○	○	○	○

Du har svart at du spiser laks/ørret til middag. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 150 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser laks/ørret til lunsj. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 150 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser torsk til middag. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 200 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser torsk til lunsj. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 200 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser sei til middag. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 200 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser sei til lunsj. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 200 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser makrell til middag. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 150 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser makrell til lunsj. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 150 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser sild til middag. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 150 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser sild til lunsj. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 150 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser lyr til middag. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 200 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser lyr til lunsj. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 200 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Vennligst sett 1 kryss per linje.

	Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger/ måned	1-2 ganger/uke	3 ganger eller mer/uke
Lange - middag					
Lange - lunsj					
Kveite - middag					
Kveite - lunsj					
Steinbit - middag					
Steinbit - lunsj					

Du har svart at du spiser lange til middag. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 200 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser lange til lunsj. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 200 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser kveite til middag. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 150 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser kveite til lunsj. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 150 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser steinbit til middag. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 200 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser steinbit til lunsj. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 200 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Sushi og fiskemat (fiskekaker, fiskeboller o.l.)

Vennligst sett 1 kryss per linje.

	Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger/ måned	1-2 ganger/uke	3 ganger eller mer/uke
Sushi - middag					
Sushi - lunsj					
Fiskekaker/-boller/-pudding - middag					
Fiskekaker/-boller/-pudding - lunsj					
Fiskegrateng					
Fiskepinner					
Fiskesuppe					
Klippfisk					

Du har svart at du spiser sushi til middag. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 12 biter.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser sushi til lunsj. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 12 biter.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser fiskekaker/-boller/-pudding til middag. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 3 fiskekaker, 6 fiskeboller eller 3 skiver fiskepudding.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser fiskekaker/-boller/-pudding til lunsj. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 3 fiskekaker, 6 fiskeboller eller 3 skiver fiskepudding.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser fiskegrateng. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 275 gram**.

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser fiskepinner. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 7 biter.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser fiskesuppe. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 350 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon

- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser klippfisk. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 150 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Vennligst sett 1 kryss per linje.

	Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger/ måned	1-2 ganger/uke	3 ganger eller mer/uke
Reker					
Krabbe, klokjøtt					
Krabbe, brunmat					
Hummer					
Blåskjell					
Kamskjell					

Du har svart at du spiser reker. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 250 gram reker med skall.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser klokjøtt av krabbe. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 150 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon

- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser brunmat av krabbe. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 150 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser hummer. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 150 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser blåskjell. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 115 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser kamskjell. Hvor stor porsjon spiser du vanligvis? **Én porsjon = 115 gram.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Har du spist annen sjømat som middag eller varm lunsj siden du ble gravid?

- Nei
- Ja

Vennligst oppgi hva slags fisk du har spist som middag og som varm lunsj siden du ble gravid

1 porsjon tilsvarer 150 gram laks, 200 gram torsk, 12 sushibiter, tre fiskekaker, seks fiskeboller, syv fiskepinner eller 2 dl reker u/skall

	Sjeldnere enn 1 gang/måned	1-3 ganger/måned	1-2 ganger/uke	3 ganger eller mer/uke	½ porsjon eller mindre	1 porsjon	1 ½ porsjon	2 porsjoner	3 porsjoner
1.									
2.									
3.									

Hvor ofte har du brukt fisk, fiskeprodukter eller annen sjømat som pålegg, i salat, mellommåltid, snacks eller lignende de siste 16 ukene?

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

Hvis du bruker fisk, fiskeprodukter eller annen sjømat som pålegg, i salat, mellommåltid, snacks eller lignende, hvor mye har du vanligvis spist?

1 porsjon tilsvarer for eksempel èn skive røkelaks, makrell i tomat til èn skive, kaviar til èn skive, èn fiskekake eller 2 dl reker u/skall

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon

2 porsjoner

3 porsjoner

Hvor ofte og hvor mye har du vanligvis spist av følgende fisk, fiskeprodukter eller annen sjømat som pålegg, i salat, mellommåltid, snacks eller lignende de siste 16 ukene?

Vennligst sett 1 kryss per linje.

	Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger/ måned	1-2 ganger/uke	3 ganger eller mer/uke
Makrell på boks (alle typer)					
Laks på boks					
Tunfisk på boks					
Røkt laks, ørret					
Gravet laks, ørret					
Sild (sursild, rømmesild, kryddersild el.lign.)					
Kaviar					
Peppermakrell					
Reker (ikke rekesalat)					
Sardin på boks					
Ansjos					
Crabsticks					
Svolværpostei					
Lofotpostei					

Du har svart at du spiser makrell på boks. Hvor stor porsjon spiser du vanligvis? **Én porsjon = makrell på boks til én brødskive.**

½ porsjon eller mindre

1 porsjon

1 ½ porsjon

2 porsjoner

3 porsjoner

Du har svart at du spiser laks på boks. Hvor stor porsjon spiser du vanligvis? **Én porsjon = laks på boks til én brødskive.**

½ porsjon eller mindre

1 porsjon

1 ½ porsjon

2 porsjoner

3 porsjoner

Du har svart at du spiser tunfisk på boks. Hvor stor porsjon spiser du vanligvis? **Én porsjon = én spiseskje tunfisk.**

½ porsjon eller mindre

1 porsjon

1 ½ porsjon

2 porsjoner

3 porsjoner

Du har svart at du spiser røkt laks/ørret. Hvor stor porsjon spiser du vanligvis? **Én porsjon = én oppskåret skive laks/ørret.**

½ porsjon eller mindre

1 porsjon

1 ½ porsjon

2 porsjoner

3 porsjoner

Du har svart at du spiser gravet laks/ørret. Hvor stor porsjon spiser du vanligvis? **Én porsjon = én skive gravet laks/ørret.**

½ porsjon eller mindre

1 porsjon

1 ½ porsjon

2 porsjoner

3 porsjoner

Du har svart at du spiser sild. Hvor stor porsjon spiser du vanligvis? **Én porsjon = sild til én brødskive.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser kaviar. Hvor stor porsjon spiser du vanligvis? **Én porsjon = kaviar til én brødskive.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser peppermakrell. Hvor stor porsjon spiser du vanligvis? **Én porsjon = pepper-/kaldrøkt/varmrøkt makrell til én brødskive.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser reker som pålegg. Hvor stor porsjon spiser du vanligvis? **Én porsjon = reker til én brødskive.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser sardiner på boks. Hvor stor porsjon spiser du vanligvis? **Én porsjon brisling = brisling til én brødskive.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser ansjos. Hvor stor porsjon spiser du vanligvis? **Én porsjon ansjos = ansjos til én brødskive.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser crabsticks. Hvor stor porsjon spiser du vanligvis? **Én porsjon crabsticks = 4 stk crabsticks.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har svart at du spiser svolværpostei. Hvor stor porsjon spiser du vanligvis? **Én porsjon = postei til én brødskive.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner

- 3 porsjoner

Du har svart at du spiser lofotpostei. Hvor stor porsjon spiser du vanligvis? **Én porsjon = postei til én brødskive.**

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Er det andre typer fisk, fiskeprodukter eller sjømat som du har spist som pålegg, i salat, mellommåltid, snacks eller lignende siden du ble gravid?

- Nei
- Ja

Vennligst spesifiser hvilke typer fisk du har spist hvor ofte og hvor mye

1 Porsjon tilsvarer for eksempel èn skive røkelaks, makrell i tomat til èn skive, kaviar til èn skive, èn fiskekake

	Sjeldnere enn 1 gang/måned	1-3 ganger/ måned	1-2 ganger/uke	3 ganger eller mer/uke	½ porsjon eller mindre	1 porsjon	1 ½ porsjon	2 porsjoner	3 porsjoner
1.									
2.									
3.									

Har du spist fiskerogn eller fiskelever?

- Nei
- Ja

Hvor mange ganger per år spiser du fiskeinnmat?

Vennligst sett 1 kryss per linje.

	Aldri	1-3 ganger/år	4-6 ganger/år	7-9 ganger/år	≥ 10 ganger/år
Fiskerogn					
Fiskelever					

Eventuelle kommentarer til spørsmålene om fisk, fiskeprodukter og sjømat

Melk og Meieriprodukter

Melk og meieriprodukter

I de neste spørsmålene ønsker vi informasjon om ditt inntak av melk og meieriprodukter de siste 16 ukene siden vårt forrige møte.

Hvor ofte har du spist og/eller drukket meieriprodukter (melk, yoghurt, ost e.l.) de siste 16 ukene? Ta med alternative melkedrikker som ikke er kumelk.

- Aldri
- Sjeldnere enn 1 gang/uke
- 1-3 ganger/uke
- 4-6 ganger/uke
- 1 gang hver dag
- 2 ganger/dag
- 3-4 ganger eller mer/dag

Hvor ofte og hvor mye har du drukket av følgende melke- og meieriprodukter, og/eller brukt det i frokostblandinger/grøt de siste 16 ukene?

Ta med laktosefri og laktosereduserte produkter.

NB Ikke ta med bruk av melk i kaffedrikker (kommer som eget spørsmål).

Vennligst sett 1 kryss per linje.

	Aldri	Sjeldnere enn 1 gang/uke	1-3 ganger/uke	4-6 ganger/uke	1 gang/dag	2 ganger/dag	3-4 ganger eller mer/dag
Helmelk	○	○	○	○	○	○	○
Lettmelk	○	○	○	○	○	○	○
Ekstra lett melk	○	○	○	○	○	○	○
Skummet melk	○	○	○	○	○	○	○
Melk med smak (f.eks sjokomelk, jordbærmelk)	○	○	○	○	○	○	○
Syrnet melk naturell	○	○	○	○	○	○	○
Syrnet melk med smak	○	○	○	○	○	○	○
Yoghurt (alle typer)	○	○	○	○	○	○	○
Drikkeyoghurt	○	○	○	○	○	○	○
Smoothie med melk	○	○	○	○	○	○	○
Geitemelk	○	○	○	○	○	○	○

Du har krysset av for at du har drukket helmelk. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer f.eks. 1,5 dl (lite glass) eller et lite beger yoghurt.

- ○ ½ porsjon eller mindre
- ○ 1 porsjon
- ○ 1 ½ porsjon
- ○ 2 porsjoner
- ○ 3 porsjoner

Du har krysset av for at du har drukket lettmelk. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer 1,5 dl (lite glass), et lite beger yoghurt.

- ○ ½ porsjon eller mindre
- ○ 1 porsjon
- ○ 1 ½ porsjon
- ○ 2 porsjoner
- ○ 3 porsjoner

Du har krysset av for at du har drukket ekstra lett melk. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer 1,5 dl (lite glass), et lite beger yoghurt.

- ○ ½ porsjon eller mindre
- ○ 1 porsjon
- ○ 1 ½ porsjon
- ○ 2 porsjoner
- ○ 3 porsjoner

Du har krysset av for at du har drukket skummet melk. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer 1,5 dl (lite glass), et lite beger yoghurt.

- ○ ½ porsjon eller mindre
- ○ 1 porsjon
- ○ 1 ½ porsjon
- ○ 2 porsjoner
- ○ 3 porsjoner

Du har krysset av for at du har drukket melk med smak. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer 1,5 dl (lite glass), et lite beger yoghurt.

- ○ ½ porsjon eller mindre
- ○ 1 porsjon
- ○ 1 ½ porsjon
- ○ 2 porsjoner
- ○ 3 porsjoner

Du har krysset av for at du har drukket syrnet melk naturell. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer 1,5 dl (lite glass), et lite beger yoghurt.

- ○ ½ porsjon eller mindre
- ○ 1 porsjon

- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har krysset av for at du har drukket syrnet melk med smak. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer 1,5 dl (lite glass), et lite beger yoghurt.

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har krysset av for at du har spist yoghurt. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer 1,5 dl (lite glass), et lite beger yoghurt.

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har krysset av for at du har drukket drikkeyoghurt. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer 1,5 dl (lite glass), et lite beger yoghurt.

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har krysset av for at du har drukket smoothie med melk. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer 1,5 dl (lite glass), et lite beger yoghurt.

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har krysset av for at du har drukket geitemelk. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer 1,5 dl (lite glass), et lite beger yoghurt.

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Har du drukket eller brukt andre typer melke- og meieriprodukter i frokostblandingen/grøt de siste 16 ukene (f.eks. melk fra ris, havre, soya)?

NB Ikke ta med bruk av melk i kaffedrikker (kommer som eget spørsmål).

- Nei
- Ja

Vennligst spesifiser

1 porsjon tilsvarer 1,5 dl (lite glass), et lite beger yoghurt. Ta med laktosefri og laktosereduserte produkter.

NB Ikke ta med bruk av melk i kaffedrikker (kommer som eget spørsmål).

	Sjeldnere enn 1 gang/uke	1-3 ganger/uke	4-6 ganger/uke	1 gang/dag	2 ganger/dag	3-4 ganger eller mer/dag	½ porsjon eller mindre	1 porsjon	1 ½ porsjon	2 porsjoner	3 porsjoner

1.											
2.											
3.											

Hvor ofte har du drukket kaffe de siste 16 ukene?

- Aldri
- Sjeldnere enn 1 gang/uke
- 1-3 ganger/uke
- 4-6 ganger/uke
- 1 gang/dag
- 2 ganger/dag
- 3-4 ganger eller mer/dag

Hvor ofte har du drukket te de siste 16 ukene?

- Aldri
- Sjeldnere enn 1 gang/uke
- 1-3 ganger/uke
- 4-6 ganger/uke
- 1 gang/dag
- 2 ganger/dag
- 3-4 ganger eller mer/dag

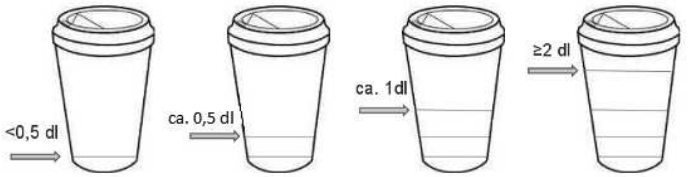
Bruker du melk i kaffe/te (gjelder kun kumelk)?

- Nei
- Ja

Hvor mye melk har du vanligvis brukt i hver kopp kaffe/te?

	Drikker ikke	< 0,5dl	ca 0,5dl	ca 1dl	≥ 2dl
Kaffe					

	Drikker ikke	< 0,5dl	ca 0,5dl	ca 1dl	≥ 2dl
Te					



Hvor ofte spiser du følgende meieriprodukter? Gjelder også økologiske og laktosefri og/eller – reduserte varianter. Ta med det du bruker i taco, i lasagne, på pizza og i annen matlaging.

Vi minner om at du skal ha **de siste 16 ukene** i tankene når du svarer på spørsmålene.

Vennligst sett 1 kryss per linje.

	Aldri	Sjeldnere enn 1 gang/uke	1-3 ganger/uke	4-6 ganger/uke	1 gang/dag	2 ganger/dag	3-4 ganger eller mer/dag
Hvitost (f.eks. Jarlsberg, Norvegia, Synnøve Finden gulost)							
Hvit geitost (f.eks Chevre, Ekte hvit geitost, Snøfrisk)							
Brunost (f.eks Gudbrandsdals-, Fløtemys-, Millom, Heidalsost)							
Brun geitost (Ekte Geitost)							
Myke oster (f.eks Brie, Camembert)							
Smøreoster (f.eks Kremost, Tubeost, Philadelphia)							
Osteprodukter på boks (f.eks Cottage cheese, Kesam/Kvarg)							
Meieriprodukter på boks (rømme, crème fraiche)							
Melkebasert mat som saus, suppe, gryte el.							
Melkebasert mat som pannekaker, vafler, sveler el.							
Is, vaniljesaus e.l (fløte/yoghurt/melkebasert)							

Du har krysset av for at du har spist hvitost. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer skivet ost til én brødskive.

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har krysset av for at du har spist hvit geitost. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer skivet ost til én brødskive eller smøreost til én brødskive.

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har krysset av for at du har spist brunost. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer skivet ost til én brødskive eller smøreost til én brødskive.

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har krysset av for at du har spist brun geitost. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer skivet ost til én brødskive eller smøreost til én brødskive.

- ½ porsjon eller mindre

- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har krysset av for at du har spist myke oster. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer skivet ost til én brødskive, smøreost til én brødskive, én mozerella.

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har krysset av for at du har spist smøreoster. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer smøreost til én brødskive.

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har krysset av for at du har spist osteprodukter på boks. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer én dl cottage cheese/kesam.

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har krysset av for at du har spist meieriprodukter på boks. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer én spiseskje rømme / crème fraiche.

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har krysset av for at du har spist melkebasert mat som saus, suppe, gryte el.. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer én dl melkebasert saus/suppe/gryte.

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har krysset av for at du har spist melkebasert mat som pannekaker, vafler, sveler el.. Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer én pannekake eller én vaffel.

- ½ porsjon eller mindre
- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Du har krysset av for at du har spist is, vaniljesaus e.l (fløte/yoghurt/melkebasert). Hvor stor er porsjonen vanligvis?

1 porsjon tilsvarer én dl melkebasert saus/suppe/gryte eller én kule is.

- ½ porsjon eller mindre

- 1 porsjon
- 1 ½ porsjon
- 2 porsjoner
- 3 porsjoner

Eventuelle kommentarer til spørsmålene om melke- og meieriprodukter

Økologiske alternativer

Økologiske alternativer
Vi minner om at du skal ha **de siste 16 ukene** i tankene når du svarer på spørsmålene.

Dersom det finnes økologiske alternativer, velger du disse?

Vennligst sett 1 kryss per linje.

	Aldri/sjelden	Noen ganger	Ofte	For det meste
Melk, melkeprodukter og ost				
Brød og kornprodukter (f.eks mel, müsli)				
Egg				
Grønnsaker				
Frukt				
Kjøtt				

Aldri/sjelden Noen ganger Ofte For det meste

Andre deler kosthold

Andre deler av kostholdet ditt

Vi minner om at du skal ha **de siste 16 ukene** i tankene når du svarer på spørsmålene.

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

- Aldri

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

Hvor ofte har du spist retter med hvitt kjøtt (kylling, kalkun, annen fjærkre) som middagsmat?

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

Hvilke brød/knekkebrødtype har du vanligvis spist de siste 16 ukene?

- Jeg spiser ikke brød eller knekkebrød
- 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)

Hvor mange porsjoner grønnsaker eller frukt/bær har du vanligvis spist de siste 16 ukene?

1 porsjon kan for eksempel være én middels stor frukt (eple, pære, banan eller annet), eller en håndfull druer, eller ett glass juice. 1 porsjon grønnsaker kan for eksempel være én gulrot eller tre buketter brokkoli eller én porsjonsbolle med salat.

Poteter regnes ikke med.

Vennligst sett 1 kryss per linje.

	Mindre enn 1-3 porsjoner/uke	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							

Hvor mange egg har du spist per uke de siste 16 ukene? (Stekt, kokt, eggerøre, omelett)

NB Egg i bakverk skal ikke tas med.

- Mindre enn 1 egg/uke
- 1 egg/uke
- 2-3 egg/uke
- 4-5 egg /uke
- 6-7 egg/uke
- 8 eller flere egg/uke

Hvor ofte har du spist sjokolade, kaker, kjeks, snop eller lignende de siste 16 ukene?

- Aldri/sjelden
- 1-2 ganger/uke
- 3-4 gang/uke
- Hver dag

Hvor ofte har du drukket følgende drikker de siste 16 ukene?

Vennligst sett 1 kryss per linje.

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

Dersom du bruker salt som tilsetning i mat og matlaging, velger du vanligvis salt tilsatt jod?

- Aldri/sjelden
- Noen ganger
- For det meste
- Vet ikke

Spisevaner

Spisevaner

De neste spørsmål handler om spisevanene dine. Vi minner om at du skal ha **de siste 16 ukene** i tankene når du svarer på spørsmålene.

Er det matvarer (mat, drikke, annet) du har spist/drukket spesielt mye av i svangerskapet (”cravings”)?

- Nei
- Ja, men bare deler av svangerskapet
- Ja, i hele svangerskapet

Vennligst spesifiser:

Er det matvarer du har unngått å spise i svangerskapet på grunn av aversjon?

- Nei
- Ja, men bare deler av svangerskapet
- Ja, i hele svangerskapet

Vennligst spesifiser:

Er det matvarer du har unngått å spise i svangerskapet av frykt for å skade barnet?

- Nei
- Ja

Vennligst spesifiser:

Er det matvarer du har begynt å spise fordi det kan være gunstig for barnet?

- Nei
- Ja

Vennligst spesifiser:

Har du vært plaget med svangerskapskvalme?

- Nei
- Ja, men bare deler av svangerskapet
- Ja, i hele svangerskapet hittil

Hvilken svangerskapsuke opphørte svangerskapsrelatert kvalme?

▼

Har du kastet opp grunnet svangerskapskvalme?

- Nei
- Ja, men bare deler av svangerskapet
- Ja, i hele svangerskapet hittil

Hvilken svangerskapsuke opphørte svangerskapsrelatert oppkast?

▼

Kosttilskudd

Kosttilskudd

I den siste delen av spørsmål om kostholdet ønsker vi informasjon om eventuelle kosttilskudd. Vi minner om at du skal ha **de siste 16 ukene** i tankene når du svarer på spørsmålene.

Tar du et komplett tilskudd for gravide (med omega-3, vitaminer og mineraler)**?**

- Nei
- Ja

Hvor ofte tar du kosttilskudd for gravide?

	Bruker ikke	1-3 ganger/uke	4-6 ganger/uke	Daglig	**Når du tar tilskudd, hvor mye tar du vanligvis ift. anbefalt mengde på flasken/pakken?** Bruker ikke	Mindre enn anbefalt mengde	Anbefalt mengde	Mer enn anbefalt mengde
Lifeline Care Gravid								
Nycoplus Care Gravid								
Annet, spesifiser:								

Bruker du annet kosttilskudd?

Kryss av på aktuelle alternativer (maks. 1 kryss per linje)

	Bruker ikke	1-3 ganger/måned	1-3 ganger/uke	4-6 ganger/uke	Daglig
Tran/flytende fiskeolje					

	Bruker ikke	1-3 ganger/måned	1-3 ganger/uke	4-6 ganger/uke	Daglig
Omega-3-kapsler					
Jern (tilskudd med kun jern)					
B-vitaminer (inkl. folsyre)					
Multivitamin og mineral					

Du har svart at du tar tran/flytende fiskeolje. Hvor mye tar du vanligvis ift. anbefalt mengde på flasken/pakken?

- Mindre enn anbefalt mengde
- Anbefalt mengde
- Mer enn anbefalt mengde

Du har svart at du tar Omega-3-kapsler. Hvor mye tar du vanligvis ift. anbefalt mengde på flasken/pakken?

- Mindre enn anbefalt mengde
- Anbefalt mengde
- Mer enn anbefalt mengde

Du har svart at du tar jern. Hvor mye tar du per gang ift. anbefalt mengde på flasken/pakken?

- Mindre enn anbefalt mengde
- Anbefalt mengde
- Mer enn anbefalt mengde

Du har svart at du tar B-vitaminer. Hvor mye tar du vanligvis ift. anbefalt mengde på flasken/pakken?

- Mindre enn anbefalt mengde
- Anbefalt mengde
- Mer enn anbefalt mengde

Du har svart at du tar multivitamin og mineraler. Hvor mye tar du vanligvis ift. anbefalt mengde på flasken/pakken?

- Mindre enn anbefalt mengde

- Anbefalt mengde
- Mer enn anbefalt mengde

Bruker du annet kosttilskudd som ikke ble nevnt?

- Nei
- Ja

Vennligst spesifiser:

					Når du tar tilskudd, hvor mye tar du vanligvis ift. anbefalt mengde på flasken/pakken?		
	1-3 ganger/måned	1-3 ganger/uke	4-6 ganger/uke	Daglig	Mindre enn anbefalt mengde	Anbefalt mengde	Mer enn anbefalt mengde
1.							
2.							
3.							

Kryss av for feltene under som eventuelt gjelder for deg:

	Nei	Ja
Har melkeallergi		
Har melkeintoleranse		
Har cøliaki/glutenallergi		
Spiser ikke meieriprodukter		
Spiser ikke egg		

SCOFF

Nå kommer noen spørsmål om dine holdninger og vaner knyttet til mat og vekt.

Er du bekymret fordi du mister kontroll over hvor mye du spiser?

- Nei
- Ja

Synes du at du er tykk selv om andre sier at du er for tynn?

- Nei
- Ja

Vil du si at mat har en dominerende plass i livet ditt?

- Nei
- Ja

EPDS

Hvordan føler du deg?

Her vil vi gjerne få vite hvordan du føler deg. Vennligst velg svaret som passer best med **hvordan du har følt deg de siste 7 dagene**, ikke bare slik du har det i dag. Ikke tenk for lenge på svaret - de spontane svarene er best.

I de siste syv dagene...

Vennligst sett 1 kryss per linje.

	Ikke i det hele tatt	Mye mindre enn vanlig	Noe mindre enn vanlig	Like mye som vanlig
Jeg har kunnet se lyst på tilværelsen og le				
Jeg har gledet meg til ting som skulle skje				

I de siste syv dagene...

Vennligst sett 1 kryss per linje.

	Nei, aldri	Nei, sjelden	Ja, ganske ofte	Ja, svært ofte
Jeg har bebreidet meg selv unødvendig når ting gikk galt				
Jeg har følt meg bekymret og engstelig uten grunn				

	Nei, aldri	Nei, sjelden	Ja, ganske ofte	Ja, svært ofte
Jeg har følt meg redd og fått panikk uten god grunn				
Det har blitt for mye for meg og jeg mestrer situasjonen dårlig				
Jeg har vært så ulykkelig at jeg har hatt vansker med søvnen				
Jeg har følt meg lei eller nedfor				
Jeg har vært så ulykkelig at jeg har grått				
Jeg har hatt tanker om å skade meg selv				

HADS

På de neste spørsmålene ber vi deg vennligst om å velge svaret som passer best med hvordan du har følt deg **de siste 7 dagene**:

Jeg føler meg nervøs og urolig

- Ikke i det hele tatt
- Fra tid til annen
- Mye av tiden
- Mesteparten av tiden

Jeg gleder meg fortsatt over tingene slik jeg pleide før

- Avgjort like mye
- Ikke fullt så mye
- Bare lite grann
- Ikke i det hele tatt

Jeg har en urofølelse som om noe forferdelig vil skje

- Ikke i det hele tatt
- Litt, bekymrer meg lite
- Ja, ikke så veldig ille
- Ja, og noe svært ille

Jeg kan le og se det morsomme i situasjoner

- Like mye nå som før
- Ikke like mye nå som før
- Avgjort ikke som før
- Ikke i det hele tatt

Jeg har hodet fullt av bekymringer

- En gang i blant
- Av og til
- Ganske ofte
- Veldig ofte

Jeg er i godt humør

- For det meste
- Ganske ofte
- Noen ganger
- Aldri

Jeg kan sitte i fred og ro og kjenne meg avslappet

- Ja, helt klart
- Vanligvis
- Ikke så ofte
- Ikke i det hele tatt

Jeg føler meg som om alt går langsommere

- Ja, helt klart
- Vanligvis
- Ikke så ofte
- Ikke i det hele tatt

Jeg føler meg urolig som om jeg har sommerfugler i magen

- Ikke i det hele tatt
- Fra tid til annen
- Ganske ofte
- Svært ofte

Jeg bryr meg ikke lenger om hvordan jeg ser ut

- Bryr meg som før
- Kan hende ikke nok
- Ikke som jeg burde
- Ja, jeg har sluttet å bry meg

Jeg er rastløs som om jeg stadig må være aktiv

- Ikke i det hele tatt
- Ikke så veldig mye
- Ganske mye
- Uten tvil svært mye

Jeg ser med glede frem til hendelser og ting

- Like mye som før
- Heller mindre enn før
- Avgjort mindre enn før
- Nesten ikke i det hele tatt

Jeg kan plutselig få en følelse av panikk

- Ikke i det hele tatt
- Ikke så veldig ofte
- Ganske ofte
- Uten tvil svært ofte

Jeg kan glede meg over gode bøker, radio og TV

- Ofte
- Fra tid til annen
- Ikke så ofte
- Svært sjelden

Sosial støtte

Om sosial støtte

Er du i et parforhold?

- Nei
- Ja

Hvor enig er du i disse beskrivelsene av ditt parforhold?

Vennligst sett 1 kryss per linje.

	Svært enig	Enig	Litt enig	Litt uenig	Uenig	Svært uenig
Det er et nært samhold mellom meg og min ektefelle/samboer/partner						
Min partner og jeg har problemer i parforholdet						
Jeg er svært lykkelig i mitt parforhold						
Min partner er generelt forståelsesfull						
Jeg tenker ofte på å avslutte vårt parforhold						
Jeg er fornøyd med forholdet til min partner						
Vi er ofte uenige om viktige avgjørelser						
Jeg har vært heldig med valg av partner						
Vi er enige om hvordan barn bør oppdras						
Jeg tror min partner er fornøyd med forholdet						

Har du noen utenom din ektefelle/samboer/partner som du kan søke råd hos i en vanskelig situasjon?

- Nei
- Ja, 1-2 personer
- Ja, flere enn to personer

Hvor ofte treffer du eller snakker i telefonen med familie (utenom husholdningen) eller nære venner?

- 1 gang i måneden eller sjeldnere
- 2-8 ganger i måneden
- Mer enn 2 ganger i uken

Føler du deg ofte ensom?

- Nesten aldri
- Sjelden
- Av og til
- Som regel
- Nesten alltid

Fysisk aktivitet, medikamenter, røyk

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

Vi minner om at du skal ha **de siste 16 ukene** i tankene når du svarer på spørsmålene

- 0-30 min
- > 30 min – 1 time
- > 1 time – 2 timer
- > 2 timer – 3 timer
- > 3 timer

Hva var din vekt før du ble gravid (kg)?

▼

Hva er din vekt nå (kg)?

▼

Hvor høy er du?

▼

Har du brukt foreskrevne medikamenter de siste 6 ukene?

- Nei
- Ja

Vennligst beskriv medikament og hvor ofte du bruker det:

	Månedlig eller sjeldnere	2-4 ganger i måneden	2-3 dager i uken	4-6 dager i uken	Daglig

Røyker og/eller snuser du nå mens du er gravid?

- Nei
- Ja

Hvor mye snus eller røyk bruker du?

Sigaretter per uke

Snusporsjoner/-poser per uke

Har du røyket og/eller snust mens du var gravid? Ta også med «festrøyk» / «festsnus».

- Nei
- Ja

I hvilken svangerskapsuke sluttet du?

Sigaretter per uke før du sluttet

Snusporsjoner/-poser per uke før du sluttet

Søvn

Søvn

Her ber vi deg om å ha **de siste 7 dagene** i tankene når du svarer:

Hvor mange dager per uke ....

	0	1	2	3	4	5	6	7
...bruker du mer enn 30 minutter for å sovne etter at lysene ble slukt?								
...er du våken mer enn 30 minutter innimellom søvnen?								
...våkner du mer enn 30 minutter tidligere enn du ønsker uten å få sove igjen?								
...føler du deg for lite uthvilt etter å ha sovet?								
...er du så søvnig/trett at det går ut over skole/jobb eller privatlivet?								
...er du misfornøyd med søvnen din?								

Om du har søvnplager, hvor lenge har de vart?

Når legger du deg vanligvis?

Hverdager

Helger

Når står du vanligvis opp?

Hverdager

Helger

Hvor lang tid går det vanligvis fra du legger deg til du sovner?

Timer

Minutter

Appendix II

Excerpt of dietary questions from infant FFQ at 3 months

Meta_text

Hei! Takk for at du har deltatt i første del av prosjektet "Mammas mat"

I denne undersøkelsen vil vi spørre deg blant annet om kostholdet ditt og hvordan du har hatt det de siste 3 månedene siden fødsel. Vi setter veldig stor pris på din deltakelse

Demografi

Hva er din sivilstatus?

- Gift
- Samboer
- Enslig
- Skilt
- Separert
- Enke
- Annet

Hvilken utdannelse har du? (Sett ett kryss for den høyeste utdannelsen du har fullført.)

- Ni- eller tiårig grunnskole
- Videregående skole
- Universitet/høyskole/fagskole, inntil fire år
- Universitets/høyskole, fire år eller mer

Hva var den samlede inntekten (før skatt) i husholdningen sist år?

- Ingen inntekt
- Under 200 000

- 200 - 349 999
- 350 - 549 999
- 550 - 749 999
- 750 - 999 999
- 1 000 000 -1 249 999
- 1 250 000 - 2 000 000
- Mer enn 2 000 000

Hvordan vil du beskrive familiens økonomi?

Svært god	God	Middels	Dårlig	Svært dårlig
○	○	○	○	○

Kosthold for spedbarn ved 3 mnd

Vi vil nå stille deg noen spørsmål om ditt barns kosthold. Hvis du ikke klarer å avgi et helt nøyaktig svar, så fyll ut etter beste skjønn.

Dato i dag

	Dag	Måned	År
	▼	▼	▼

Ultralydtermin

	Dag	Måned	År
	▼	▼	▼

Når ble barnet født?

	Dag	Måned	År
	▼	▼	▼

Hvor mange uker er barnet i dag?

▼

Barnets vekt

Fødselsvekt (gram)

Vekt ved siste veiing (gram)

Dato ved siste veiing

	Dag	Måned	År
	▼	▼	▼

Barnets lengde

Lengde ved fødsel (cm)

Lengde ved siste måling (cm)

Dato ved siste måling av lengde

	Dag	Måned	År
	▼	▼	▼

Barnets hodeomkrets

Hodeomkrets ved fødsel (cm)

Hodeomkrets ved siste måling (cm)

Dato ved siste måling av hodeomkrets

	Dag	Måned	År
	▼	▼	▼

Får barnet morsmelk *nå?*

- Ja, barnet får kun morsmelk
- Ja, barnet får både morsmelk og morsmelkerstatning
- Nei, men barnet har fått morsmelk tidligere
- Nei, barnet har aldri fått morsmelk

Hvor mange uker var barnet da det sluttet med morsmelk?

▼

Hvor mange uker var barnet da det begynte med morsmelkerstatning i tillegg til eller i stedet for morsmelk?

▼

Hvor ofte gir du vanligvis barnet ditt morsmelkerstatning i tillegg til eller istedenfor morsmelk?

	Aldri/sjeldnere enn hver uke	1-3 ganger/uke	4-6 ganger/uke	1 gang/døgn	2 ganger/døgn	3 ganger/døgn	4 ganger/døgn	5 eller flere ganger/døgn
NAN 1								
NAN H.A. 1								
HIPP Baby Combiotik 1								
Semper Allomin 1								
Annen morsmelkerstatning (spesifiser)								

Du har svart at du gir barnet ditt NAN 1. Hvor mye drikker barnet vanligvis per gang?

- ca 60 ml/per gang
- ca 120 ml/per gang
- ca 180 ml/per gang
- ca 240 ml/per gang
- Annen mengde:

Du har svart at du gir barnet ditt NAN H.A. 1. Hvor mye drikker barnet vanligvis per gang?

- ca 60 ml/per gang
- ca 120 ml/per gang
- ca 180 ml/per gang
- ca 240 ml/per gang
- Annen mengde:

Du har svart at du gir barnet ditt HIPP Baby Combiotik 1. Hvor mye drikker barnet vanligvis per gang?

- ca 60 ml/per gang
- ca 120 ml/per gang
- ca 180 ml/per gang
- ca 240 ml/per gang
- Annen mengde:

Du har svart at du gir barnet ditt Semper Allomin 1. Hvor mye drikker barnet vanligvis per gang?

- ca 60 ml/per gang
- ca 120 ml/per gang
- ca 180 ml/per gang
- ca 240 ml/per gang

- Annen mengde:

Du har svart at du gir barnet ditt en annen morsmelkerstatning. Hvor mye drikker barnet vanligvis per gang?

- ca 60 ml/per gang
- ca 120 ml/per gang
- ca 180 ml/per gang
- ca 240 ml/per gang
- Annen mengde:

Får barnet kosttilskudd (tran, vitaminer og/eller mineraler) nå?

- Ja
- Nei, men barnet har fått kosttilskudd tidligere
- Nei, barnet har aldri fått kosttilskudd

Hvilke(t) kosttilskudd har barnet fått tidligere og hvor ofte fikk barnet dette?

Skriv inn kostilskudd(ene) i de åpne tekstfeltene nedenfor, og angi hvor ofte barnet fikk dette

	Sjeldnere enn hver uke	1-2 ganger/uke	3-4 ganger/uke	5-7 ganger/uke

Hvilke typer kosttilskudd bruker barnet og hvor ofte?

	Aldri/sjeldnere enn hver uke	1-2 ganger/uke	3-4 ganger/uke	5-7 ganger/uke
Tran/omega-3 **med** vitamin D				
Tran/omega-3 **uten** vitamin D				
Vitamin D-dråper				
Folat				

	Aldri/sjeldnere enn hver uke	1-2 ganger/uke	3-4 ganger/uke	5-7 ganger/uke
Vitamin B12				
Biovit				
Jern				
Sanasol				
Annet kosttilskudd (spesifiser):				

Kryss av for feltene under som gjelder for barnet ditt nå (mulig å krysse av på flere alternativer)

	Aldri/sjelden	1 gang/uke	2-3 ganger/uke	4-6 ganger/uke	Daglig
Vann					
Te/juice/saft					
Kumelk					
Ris-/mandel-/havremelk					
Yoghurt					
Annen drikke (spesifiser):					
Industrifremstilt grøt/velling					
Hjemmelaget grøt/velling					
Frukt/fruktmos					
Grønnsaker/grønnsaksmos					
Annen fast føde (spesifiser):					

ASQ-SE 6 mnd

Les hvert spørsmål nøye og
1. kryss av i ruten som best beskriver barnet *og*
2. kryss av i sirkelen dersom atferden skaper bekymring

	Mesteparten av tiden	Av og til	Sjelden eller aldri	Kryss av hvis dette skaper bekymring
1. Når barnet er urolig, kan det roe seg innen en halv time?				
2. Smiler barnet til deg og andre i familien? 				
3. Liker barnet å bli løftet opp og holdt?				

Appendix III

Excerpt of dietary questions from infant FFQ at 6 months

Meta_text

Hei! Takk for at du har deltatt i første del av prosjektet "Mammas mat"

I denne undersøkelsen vil vi spørre deg blant annet om kostholdet ditt og hvordan du har hatt det de siste 3 månedene. Vi setter veldig stor pris på din deltakelse

Demografi

Hva er din sivilstatus?

- Gift
- Samboer
- Enslig
- Skilt
- Separert
- Enke
- Annet

Hvilken utdannelse har du? (Sett ett kryss for den høyeste utdannelsen du har fullført.)

- Ni- eller tiårig grunnskole
- Videregående skole
- Universitet/høyskole/fagskole, inntil fire år
- Universitets/høyskole, fire år eller mer

Hva var den samlede inntekten (før skatt) i husholdningen sist år?

- Ingen inntekt
- Under 200 000

- 200 - 349 999
- 350 - 549 999
- 550 - 749 999
- 750 - 999 999
- 1 000 000 -1 249 999
- 1 250 000 - 2 000 000
- Mer enn 2 000 000

Hvordan vil du beskrive familiens økonomi?

Svært god	God	Middels	Dårlig	Svært dårlig
○	○	○	○	○

Kosthold for spedbarn ved 6 mnd

I dette spørreskjemaet vil vi spørre deg noen spørsmål om ditt barns kosthold den siste uken. Hvis du ikke klarer å avgi et helt nøyaktig svar, så fyll ut etter beste skjønn.

Barnets vekt

Vekt ved siste veiing (gram) _____

Dato ved siste veiing

	Dag	Måned	År
	▼	▼	▼

Barnets lengde

Lengde ved siste måling (cm) _____

Dato ved siste måling av lengde

	Dag	Måned	År
	▼	▼	▼

Barnets hodeomkrets

Hodeomkrets ved siste måling (cm)

Dato ved siste måling av hodeomkrets

	Dag	Måned	År
	▼	▼	▼

Får barnet morsmelk og/eller morsmelkerstatning?

- Ja, barnet får kun morsmelk
- Ja, barnet får både morsmelk og morsmelkerstatning
- Nei, men barnet har fått morsmelk tidligere
- Nei, barnet har aldri fått morsmelk

Hvor mange uker var barnet da det sluttet med morsmelk?

▼

Hvor mange uker var barnet da det begynte med morsmelkerstatning i tillegg til eller i stedet for morsmelk?

▼

Hvor ofte gir du vanligvis barnet ditt morsmelkerstatning i tillegg til eller istedenfor morsmelk?

	Aldri/sjeldnere enn hver uke	1-3 ganger/uke	4-6 ganger/uke	1 gang/døgn	2 ganger/døgn	3 ganger/døgn	4 ganger/døgn	5 eller flere ganger/døgn
NAN pro 1								
NAN pro 2								
NAN H.A. 1								
NAN H.A. 2								
HIPP Baby Combiotik 1								
HIPP Baby Combiotik 2								
Semper Allomin 1								
Semper Allomin 2								
Annen morsmelkerstatning (spesifiser)								

Du har svart at du gir barnet ditt NAN pro 1. Hvor mye drikker barnet vanligvis per gang?

- ca 60 ml/per gang
- ca 120 ml/per gang
- ca 180 ml/per gang
- ca 240 ml/per gang
- Annen mengde:

Du har svart at du gir barnet ditt NAN pro 2. Hvor mye drikker barnet vanligvis per gang?

- ca 60 ml/per gang
- ca 120 ml/per gang
- ca 180 ml/per gang
- ca 240 ml/per gang
- Annen mengde:

Du har svart at du gir barnet ditt NAN H.A. 1. Hvor mye drikker barnet vanligvis per gang?

- ca 60 ml/per gang
- ca 120 ml/per gang
- ca 180 ml/per gang
- ca 240 ml/per gang
- Annen mengde:

Du har svart at du gir barnet ditt NAN H.A. 2. Hvor mye drikker barnet vanligvis per gang?

- ca 60 ml/per gang
- ca 120 ml/per gang
- ca 180 ml/per gang
- ca 240 ml/per gang
- Annen mengde:

Du har svart at du gir barnet ditt HIPP Baby Combiotik 1. Hvor mye drikker barnet vanligvis per gang?

- ca 60 ml/per gang
- ca 120 ml/per gang
- ca 180 ml/per gang
- ca 240 ml/per gang
- Annen mengde:

Du har svart at du gir barnet ditt HIPP Baby Combiotik 2. Hvor mye drikker barnet vanligvis per gang?

- ca 60 ml/per gang
- ca 120 ml/per gang
- ca 180 ml/per gang
- ca 240 ml/per gang
- Annen mengde:

Du har svart at du gir barnet ditt Semper Allomin 1. Hvor mye drikker barnet vanligvis per gang?

- ca 60 ml/per gang
- ca 120 ml/per gang
- ca 180 ml/per gang
- ca 240 ml/per gang
- Annen mengde:

Du har svart at du gir barnet ditt Semper Allomin 2. Hvor mye drikker barnet vanligvis per gang?

- ca 60 ml/per gang
- ca 120 ml/per gang
- ca 180 ml/per gang
- ca 240 ml/per gang
- Annen mengde:

Du har svart at du gir barnet ditt en annen morsmelkerstatning. Hvor mye drikker barnet vanligvis per gang?

- ca 60 ml/per gang
- ca 120 ml/per gang
- ca 180 ml/per gang
- ca 240 ml/per gang
- Annen mengde:

Får barnet kosttilskudd (tran, vitaminer og/eller mineraler)?

- Ja
- Nei, men barnet har fått kosttilskudd tidligere
- Nei, barnet har aldri fått kosttilskudd

Hvilke(t) kosttilskudd har barnet fått tidligere og hvor ofte fikk barnet dette?
Skriv inn kostilskudd(ene) i de åpne tekstfeltene nedenfor, og angi hvor ofte barnet fikk dette

	Sjeldnere enn hver uke	1-2 ganger/uke	3-4 ganger/uke	5-7 ganger/uke

Hvilke typer kosttilskudd bruker barnet og hvor ofte?

	Aldri/sjeldnere enn hver uke	1-2 ganger/uke	3-4 ganger/uke	5-7 ganger/uke
Tran/omega-3 **med** vitamin D				
Tran/omega-3 **uten** vitamin D				
Vitamin D-dråper				
Folat				
Vitamin B12				
Biovit				
Jern				
Sanasol				
Annet kosttilskudd (spesifiser):				

Hvor gammel var barnet da det fikk fast føde for første gang? Trykk på listen og velg antall uker

▼

Kryss av for feltene under som gjelder for barnet ditt nå (mulig å krysse av på flere alternativer)

	Aldri/sjelden	1 gang/uke	2-3 ganger/uke	4-6 ganger/uke	Daglig
Vann					
Te/juice/saft					

	Aldri/sjelden	1 gang/uke	2-3 ganger/uke	4-6 ganger/uke	Daglig
Kumelk					
Ris-/mandel-/havremelk					
Yoghurt					
Annen drikke (spesifiser):					
Industrifremstilt grøt/velling					
Hjemmelaget grøt/velling					
Frukt/fruktmos/bær/smoothie					
Grønnsaker/grønnsaksmos					
Fet fisk til middag (f.eks. laks, ørret, makrell, sild)					
Mager fisk til middag (f.eks. torsk, sei, hyse)					
Fiskegrateng, fiskepudding, fiskepinner, fiskekaker					
Kylling til middag					
Rødt kjøtt til middag (f.eks. biff, svin, vilt, lam, pølser, kjøttdeig)					
Vegetarmiddag					
Fet fisk som pålegg (f.eks. makrell, ørret, laks, kaviar)					
Magre fiskeprodukter som pålegg (reker, fiskekaker etc.)					
Kjøtt som pålegg					
Vegetarpålegg					
Prim original/lett					
Prim Sprett					
Brunost (f.eks. Gudbrandsdals-, Fløtemys-, Millom, Heidalsost)					
Brun geitost (Ekte Geitost)					
Hvitost (f.eks. Norvegia, Jarlsberg, smøreoster)					
Annen fast føde (spesifiser):					

Dersom barnet får grøt, hva slags væske tilsettes vanligvis grøten ved tilberedning?

- Vann
- Morsmelk
- Morsmelkerstatning
- Melk (kumelk)
- Annet (spesifiser):

Spiser barnet egg (stekt, kokt, eggerøre, omelett)**?**

- Ja
- Nei

Omtrent hvor mange egg spiser barnet per uke, vanligvis?

- <1
- 1
- 2
- 3
- ≥4

Har barnet vært på sydenreise de siste 3 månedene?

- Nei
- 1 uke
- 2 uker
- 3 uker
- ≥4 uker

ASQ-SE 6 mnd

Les hvert spørsmål nøye og
1. kryss av i ruten som best beskriver barnet og
2. kryss av i sirkelen dersom atferden skaper bekymri

	Mesteparten av tiden	Av og til	Sjelden eller aldri	Kryss av hvis dette skaper bekymring
1. Når barnet er urolig, kan det roe seg innen en halv time?				
2. Smiler barnet til deg og andre i familien? 				
3. Liker barnet å bli løftet opp og holdt?				
4. Stivner barnet og spenner ryggen når det blir løftet opp?				
5. Når du snakker til barnet, ser det på deg og ser ut til å lytte?				
6. Gir barnet uttrykk for at det er sultent eller sykt?				

Appendix IV

Excerpt of dietary questions from infant FFQ at 11 months

Meta_text

Hei! Takk for at du har deltatt i første del av prosjektet "Mammas mat"

I denne undersøkelsen vil vi spørre deg blant annet om kostholdet ditt og hvordan du har hatt det de siste 3 månedene. Vi setter veldig stor pris på din deltakelse

Demografi

Hva er din sivilstatus?

- ○ Gift
- ○ Samboer
- ○ Enslig
- ○ Skilt
- ○ Separert
- ○ Enke
- ○ Annet

Hvilken utdannelse har du? (Sett ett kryss for den høyeste utdannelsen du har fullført.)

- ○ Ni- eller tiårig grunnskole
- ○ Videregående skole
- ○ Universitet/høyskole/fagskole, inntil fire år
- ○ Universitets/høyskole, fire år eller mer

Hva var den samlede inntekten (før skatt) i husholdningen sist år?

- ○ Ingen inntekt
- ○ Under 200 000
- ○ 200 - 349 999
- ○ 350 - 549 999
- ○ 550 - 749 999
- ○ 750 - 999 999
- ○ 1 000 000 -1 249 999

- ○ 1 250 000 - 2 000 000
- ○ Mer enn 2 000 000

Hvordan vil du beskrive familiens økonomi?

Svært god	God	Middels	Dårlig	Svært dårlig
○	○	○	○	○

Kosthold for spedbarn ved 11 mnd

I dette spørreskjemaet vil vi spørre deg noen spørsmål om ditt barns kosthold de 3 siste ukene. Hvis du ikke klarer å avgi et helt nøyaktig svar, så fyll ut etter beste skjønn.

Barnets vekt

Vekt ved siste veiing (gram)

Dato ved siste veiing

Dag	Måned	År

Barnets lengde

Lengde ved siste måling (cm)

Dato ved siste måling av lengde

Dag	Måned	År

Barnets hodeomkrets

Hodeomkrets ved siste måling (cm)

Dato ved siste måling av hodeomkrets

Dag	Måned	År

Får barnet morsmelk og/eller morsmelkerstatning?

- ○ Ja, barnet får morsmelk (i tillegg til annen mat og drikke)
- ○ Ja, barnet får både morsmelk og morsmelkerstatning (i tillegg til annen mat og drikke)
- ○ Nei, men barnet har fått morsmelk tidligere
- ○ Nei, barnet har aldri fått morsmelk

Hvor mange uker var barnet da det sluttet med morsmelk?

Hvor mange uker var barnet da det begynte med morsmelkerstatning i tillegg til eller i stedet for morsmelk?

Hvor ofte gir du vanligvis barnet ditt morsmelkerstatning i tillegg til eller istedenfor morsmelk?

	Aldri/sjeldnere enn hver uke	1-3 ganger/uke	4-6 ganger/uke	1 gang/døgn	2 ganger/døgn	3 ganger/døgn	4 ganger/døgn	5 eller flere ganger/døgn
NAN pro 1	○	○	○	○	○	○	○	○
NAN pro 2	○	○	○	○	○	○	○	○
NAN pro 3	○	○	○	○	○	○	○	○
NAN H.A. 1	○	○	○	○	○	○	○	○
NAN H.A. 2	○	○	○	○	○	○	○	○
NAN H.A. 3	○	○	○	○	○	○	○	○
HIPP Baby Combiotik 1	○	○	○	○	○	○	○	○
HIPP Baby Combiotik 2	○	○	○	○	○	○	○	○
HIPP Baby Combiotik 3	○	○	○	○	○	○	○	○
Semper Allomin 1	○	○	○	○	○	○	○	○
Semper Allomin 2	○	○	○	○	○	○	○	○
Semper Allomin 3	○	○	○	○	○	○	○	○

	Aldri/sjeldnere enn hver uke	1-3 ganger/uke	4-6 ganger/uke	1 gang/døgn	2 ganger/døgn	3 ganger/døgn	4 ganger/døgn	5 eller flere ganger/døgn
Annen morsmelkerstatning (spesifiser)	○	○	○	○	○	○	○	○

Du har svart at du gir barnet ditt NAN pro 1. Hvor mye drikker barnet vanligvis per gang?

- ○ ca 60 ml/per gang
- ○ ca 120 ml/per gang
- ○ ca 180 ml/per gang
- ○ ca 240 ml/per gang
- ○ Annen mengde:

Du har svart at du gir barnet ditt NAN pro 2. Hvor mye drikker barnet vanligvis per gang?

- ○ ca 60 ml/per gang
- ○ ca 120 ml/per gang
- ○ ca 180 ml/per gang
- ○ ca 240 ml/per gang
- ○ Annen mengde:

Du har svart at du gir barnet ditt NAN H.A. 1. Hvor mye drikker barnet vanligvis per gang?

- ○ ca 60 ml/per gang
- ○ ca 120 ml/per gang
- ○ ca 180 ml/per gang
- ○ ca 240 ml/per gang
- ○ Annen mengde:

Du har svart at du gir barnet ditt NAN H.A. 2. Hvor mye drikker barnet vanligvis per gang?

- ○ ca 60 ml/per gang
- ○ ca 120 ml/per gang
- ○ ca 180 ml/per gang
- ○ ca 240 ml/per gang
- ○

Annen mengde:

Du har svart at du gir barnet ditt HIPP Baby Combiotik 1. Hvor mye drikker barnet vanligvis per gang?

- ca 60 ml/per gang
- ca 120 ml/per gang
- ca 180 ml/per gang
- ca 240 ml/per gang
- Annen mengde:

Du har svart at du gir barnet ditt HIPP Baby Combiotik 2. Hvor mye drikker barnet vanligvis per gang?

- ca 60 ml/per gang
- ca 120 ml/per gang
- ca 180 ml/per gang
- ca 240 ml/per gang
- Annen mengde:

Du har svart at du gir barnet ditt Semper Allomin 1. Hvor mye drikker barnet vanligvis per gang?

- ca 60 ml/per gang
- ca 120 ml/per gang
- ca 180 ml/per gang
- ca 240 ml/per gang
- Annen mengde:

Du har svart at du gir barnet ditt Semper Allomin 2. Hvor mye drikker barnet vanligvis per gang?

- ca 60 ml/per gang
- ca 120 ml/per gang
- ca 180 ml/per gang
- ca 240 ml/per gang
- Annen mengde:

Du har svart at du gir barnet ditt en annen morsmelkerstatning. Hvor mye drikker barnet vanligvis per gang?

- ca 60 ml/per gang
- ca 120 ml/per gang
- ca 180 ml/per gang
- ca 240 ml/per gang
- Annen mengde:

Får barnet kosttilskudd (tran, vitaminer og/eller mineraler)?

- Ja
- Nei, men barnet har fått kosttilskudd tidligere
- Nei, barnet har aldri fått kosttilskudd

Hvilke(t) kosttilskudd har barnet fått tidligere og hvor ofte fikk barnet dette?
Skriv inn kostilskudd(ene) i de åpne tekstfeltene nedenfor, og angi hvor ofte barnet fikk dette

	Sjeldnere enn hver uke	1-2 ganger/uke	3-4 ganger/uke	5-7 ganger/uke
	○	○	○	○
	○	○	○	○
	○	○	○	○
	○	○	○	○
	○	○	○	○

Hvilke typer kosttilskudd bruker barnet og hvor ofte?

	Aldri/sjeldnere enn hver uke	1-2 ganger/uke	3-4 ganger/uke	5-7 ganger/uke
Tran/omega-3 **med** vitamin D	○	○	○	○
Tran/omega-3 **uten** vitamin D	○	○	○	○
Vitamin D-dråper	○	○	○	○
Folat	○	○	○	○
Vitamin B12	○	○	○	○
Biovit	○	○	○	○
Jern	○	○	○	○
Sanasol	○	○	○	○
Annet kosttilskudd (spesifiser):	○	○	○	○

Hvor gammel var barnet da det fikk fast føde for første gang? Trykk på listen og velg antall uker

Kryss av for feltene under som gjelder for barnet ditt nå (mulig å krysse av på flere alternativer)

	Aldri/sjelden	1 gang/uke	2-3 ganger/uke	4-6 ganger/uke	Daglig
Vann	○	○	○	○	○
Te/juice/saft	○	○	○	○	○
Kumelk	○	○	○	○	○
Ris-/mandel-/havremelk	○	○	○	○	○
Yoghurt	○	○	○	○	○
Annen drikke (spesifiser):	○	○	○	○	○
Industrifremstilt grøt/velling	○	○	○	○	○
Hjemmelaget grøt/velling	○	○	○	○	○
Frukt/fruktmos/bær/smoothie	○	○	○	○	○
Grønnsaker/grønnsaksmos	○	○	○	○	○
Fet fisk til middag (f.eks. laks, ørret, makrell, sild)	○	○	○	○	○
Mager fisk til middag (f.eks. torsk, sei, hyse)	○	○	○	○	○
Fiskegrateng, fiskepudding, fiskepinner, fiskekaker	○	○	○	○	○
Kylling til middag	○	○	○	○	○
Rødt kjøtt til middag (f.eks. biff, svin, vilt, lam, pølser, kjøttdeig)	○	○	○	○	○
Vegetarmiddag	○	○	○	○	○
Fet fisk som pålegg (f.eks. makrell, ørret, laks, kaviar)	○	○	○	○	○
Magre fiskeprodukter som pålegg (reker, fiskekaker etc.)	○	○	○	○	○
Kjøtt som pålegg	○	○	○	○	○
Vegetarpålegg	○	○	○	○	○
Prim original/lett	○	○	○	○	○
Prim Sprett	○	○	○	○	○
Brunost (f.eks. Gudbrandsdals-, Fløtemys-, Millom, Heidalsost)	○	○	○	○	○
Brun geitost (Ekte Geitost)	○	○	○	○	○
Hvitost (f.eks. Norvegia, Jarlsberg, smøreoster)	○	○	○	○	○
Annen fast føde (spesifiser):	○	○	○	○	○

Dersom barnet får grøt, hva slags væske tilsettes vanligvis grøten ved tilberedning?

- ○ Vann

- Morsmelk
- ○ Morsmelkerstatning
- ○ Melk (kumelk)
- ○ Annet (spesifiser):

Spiser barnet egg (stekt, kokt, eggerøre, omelett)**?**

- ○ Ja
- ○ Nei

Omtrent hvor mange egg spiser barnet per uke, vanligvis?

- ○ <1
- ○ 1
- ○ 2
- ○ 3
- ○ ≥4

Har barnet vært på sydenreise de siste 3 månedene?

- ○ Nei
- ○ 1 uke
- ○ 2 uker
- ○ 3 uker
- ○ ≥4 uker

ASQ-SE 6 mnd

Les hvert spørsmål nøye og
1. kryss av i ruten som best beskriver barnet og
2. kryss av i sirkelen dersom atferden skaper bekymring

	Mesteparten av tiden	Av og til	Sjelden eller aldri	Kryss av hvis dette skaper bekymring
1. Når barnet er urolig, kan det roe seg innen en halv time?	○	○	○	○
2. Smiler barnet til deg og andre i familien? 	○	○	○	○
3. Liker barnet å bli løftet opp og holdt?	○	○	○	○
4. Stivner barnet og spenner ryggen når det blir løftet opp?				