Pregnant women's seafood intake and fatty acid composition in red blood cells – a randomized controlled trial with dietary cod

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Abstract

Background: Seafood is the predominant source of the dietary long-chain omega-3 polyunsaturated fatty acids (LCn-3PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Consumption of these fatty acids (FAs) during pregnancy are associated with increased neural and visual development in fetus, and decreased risk of postpartum depression in mothers. The proportions of DHA in the fetus is positively correlated with the mothers, making the maternal intake crucial for fetal levels. There are dietary guidelines for fatty fish consumption in relations to its content of EPA and DHA. However, such guidelines does not exist for lean fish and cod which is a great source of LCn-3PUFAs, as 200g of cod contributes with 480 mg EPA and DHA, double of the daily adequacy level set by the European Food Safety Authority (EFSA).

Objective: To investigate if an increased intake of cod during pregnancy have an impact on FA content in red blood cells (RBC), with focus on the marine associated LCn-3PUFAs, in addition to evaluating seafood and omega-3 (n-3) supplement intakes.

Methods: A total of 137 women with prim parous, singleton pregnancy from Bergen, Norway, were enrolled in the dietary two-armed randomized controlled trial "Mommy's Food". The pregnant women were randomized into intervention group consuming 400 gram of cod per week, or the control group continuing their habitual diet. The intervention lasted for 16 weeks, from gestational week 20 to gestational week 36. A Gas Chromatograph–Flame Ionization Detector was used to measure FA content in RBC. Seafood and cod and intake were calculated using a food frequency questionnaires (FFQ) and a weight registration form.

Results: There were no significant differences in the FA content at post-intervention between the intervention- and control- group. The intervention group had a significant increase in the relative amount of DHA ($p<.002$) and stability in the n-3 index, with no correlation to cod intake. Both groups had a decrease in Σ omega-6 (n-6) and stable Σ n-3 FAs, resulting in an increase of the n-3:n-6 ratio. During intervention 71% reported eating seafood for dinner $\geq$2-3 times/week, and 82% consumed n-3 supplements at baseline and post intervention.

Conclusion: There was no significant difference for the FA content between the groups at post intervention. Therefore, in this study, an intervention with dietary cod did not have a significant impact on the marine associated LCn-3PUFA in RBC of pregnant women with a high intake of seafood and a large percentage taking n-3 supplements.

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Abbreviations

AA	Arachidonic acid
ALA	α-linoleic acid
BMI	Body mass index
CHD	Coronary heart disease
CNS	Central nervous system
DHA	Docosahexaenoic acid
dl-PCB	dioxin-like polychlorinated biphenyls
EDTA	Ethylenediaminetetraacetic acid
EFA	Essential fatty acid
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic acid
FA	Fatty Acid
FFQ	Food frequency questionarie
GW	Gestational week
Hg	Mercury
HHS	Heater-shaker
HMSL	Hamilton Microlab Star Line
IMR	Institute of Marin Research
IS	Internal Standard
IQR	Interquartile range
JECFA	Joint FAO/WHO expert Committee on Food safety
LA	Linoleic acid
LCn-3PUFA	Long-chain omega-3 poly unsaturated fatty acid
LiN	Little in Norway study
MoBa	Norwegian Mother and Child Cohort study
n-3	Omega-3
n-6	Omega-6
NDH	Norwegian Directorate of Health
PA	Palmitic acid
PL	Phospholipids
PUFA	polyunsaturated fatty acids
RBC	Red blood cells
RCT	Randomized control trial

REK	Regional Committees for Medical and Health Research Ethics West
RKBU	Regional Centre for Child and Youth Mental Health and Child Welfare
TAG	Triacylglycerides
TWI	Tolerable weekly intake
SCFA	Short-chain fatty acid
SD	Standard deviation
SFA	Saturated fatty acids
UFGC-FID	Ultra Fast Gas Chromatograph–Flame Ionization Detector
UIC	Urinary iodine concentration

1. Introduction

A nutritious, well-balanced diet during pregnancy is essential for optimal health and growth of the fetus, therefore the quality and nutritional content of the maternal diet is of the utmost importance [1]. Seafood is an important source of essential nutrients such as the long-chain omega-3 poly unsaturated fatty acids (LCn-3PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [2]. Higher levels of DHA from seafood intake are in epidemiological studies associated with increased neural and visual development in infants, and a decreased risk of poor child performance on standardized IQ-test [3-8]. In adults, omega-3 (n-3) from seafood is found to have protective quality from coronary heart disease (CHD), and the recommendation of approximately 1 portion of fatty fish per week is based on this [4, 9, 10]. The majority of studies concerning seafood and pregnancy are observational, or randomized control trials (RCTs) with n-3 fish oil supplementation and not seafood [5, 11]. There is a lack of RCTs, the gold standard of studies, on maternal seafood intakes impact on fetal health. The RCT Mommy's food and this thesis could potentially provide new information or support existing evidence on seafood intake and pregnancy.

1.1 Seafood and omega-3 supplements, recommendations and intake

In this thesis the term seafood denotes invertebrate or vertebrate aquatic animals including fish, shellfish and crustacean from marine or fresh water, whether wild or farmed. It excludes aquatic mammals, reptiles echinoderms and jellyfish [7]. Seafood is a source of energy, proteins with high biological value, and the LCn-3PUFAs; EPA and DHA. It contributes with the intake of essential nutrients such as vitamins A, vitamin E, vitamin D, vitamin B12, iodine, zinc, calcium and selenium [7, 12]. The nutritional content varies between the types of fish and seafood, a fatty fish like salmon contains up to 18 times more n-3 than lean fish such as cod [13].

1.1.1 Recommendations for seafood and omega-3

Health authorities recommends a regular intake of fish and seafood to ensure a healthy balanced diet, and according to The European Food Safety Authority (EFSA) the recommendations are ranging from 100g per week to 200g per day [7]. In Norway fish is the most frequently consumed seafood product. The Norwegian Directorate of Health (NDH) recommend eating fish for dinners 2-3 times per week and encourage the usage of fish/fish-products as spread. The 2-3 fish dinners equal 300-450g of pure fish per week, and 200g or 1.3 portions of this should come from fatty fish. Non-fish seafood have no specific recommendations other than it is favorable to include it in a balanced diet [9, 10].

In Norway pregnant women are recommended to follow the general guidelines for seafood consumption, with some limitation to seafood containing higher levels of mercury and other undesirable contaminants [14]. EFSA reported that consuming 1-2 and up to 3-4 portions of fish/seafood per week during pregnancy is associated with better functional outcomes of neural development in children and protection against CHD in adulthood [4, 7].

There are several recommendations for daily intake of EPA and DHA from different health organizations. EFSA set an adequate intake of 250 mg/day of EPA and DHA, which is equivalent to 1-2 fish meals per week and based on the protective effect of CHD. An additional 100-200 mg are recommended for pregnant and lactating women, this is in accordance with guideline from the FAO/WHO [6, 7]. The American Heart Association, American Dietetic Association and The American Psychiatric Association agreed on a DHA and EPA recommendation of 1 g/day. Norway does not have recommendations for dietary intake of EPA and DHA for the general population, and the available data is insufficient to set an average requirement or a clear tolerable upper intake level [8]. However, the NDH has recommended a daily intake of 200 mg of DHA for pregnant and breastfeeding women based on the Nordic Nutrition Recommendation [15]. The optimal intake of EPA and DHA is not established, however all the aforementioned organization agrees that the n-3 in seafood or supplementation is linked to beneficial health outcomes [6-8, 12, 15].

1.1.2 Health outcomes from seafood and omega-3 supplements

The influence of seafood in the maternal diet and the fetal development is linked to many beneficial health outcomes, though more high-quality studies are needed to establish the relationships [12]. Seafood or marine n-3 supplementation during pregnancy is associated with positive health outcomes for post-partum depression, pregnancy related nausea, pre-term birth, infant sleeping pattern, birth weight, and decreased risk of poor neural and visual development in children [3, 6, 8, 16-21]. Pregnant women in Norway, even highly educated, decrease their seafood intake during pregnancy [22]. The lower mean intake of seafood amongst pregnant women compared to than the general population may be due to a fear of exposure to contaminants, or a possible misunderstanding of guidelines [8, 12, 23, 24]. However, FAO/WHO agrees that the benefits of consuming seafood during pregnancy outweigh the possible risk posed by contaminants [6].

The positive health benefits associated with seafood is largely attributed to the LCn-3PUFAs, especially DHA [12, 15, 25]. DHA from seafood is suggested to be the main influencer of the improvement in fetal brain and visual development that has been associated with higher intakes of seafood [26, 27]. The fetus obtains all of its DHA from the mother trough placental transfer and the maternal levels are therefore the main factor of fetal DHA status [28]. Studies with mothers consuming n-3 supplements have shown conflicting results on the improvement of neural development, and other benefice health outcomes associated with seafood intake. This shows that further testing is needed, or there might be other nutrients in seafood contributing to the associated health outcomes [18, 29-31]. Iodine is an example of another nutrient that is linked to neural development and seafood is found to be a great source of it [8, 32-34].

1.1.3 Seafood and omega-3 supplement intake

The mean fish consumption in Norway is higher than most other European countries such as the Netherland, Belgium and Ireland, but lower than countries such as Island, Italy, Spain and Portugal [18, 35, 36]. This is shown in the Norwegian Mother and Child (MoBa) Cohort study, where the median intake of total seafood per week was 234g, this is 1.6 portions per week based on the NDH portions sizes [10, 37]. Of the total seafood intake lean fish constituted 56% and fatty fish 34%, the remaining 10 % was form shellfish. The total seafood intake contributed with 248 mg LCn-3PUFA per day [13, 37].

In the average Norwegian population 34% reported using n-3 supplementation, with a mean of 3 g/day. Pregnant women had a much higher intake of 77%, and the pregnant women using n-3 supplements had a higher intake of fish than the non-supplement users [8]. Though research have shown conflicting results in the efficacy of n-3 supplements to achieve some of the health benefits associated with high seafood intake [18, 31]. It is still an effective way of increasing the n-3 status of maternal diets low in fish and other DHA and EPA rich foods [38-40].

1.1.4 Cod nutrition value

Fatty fish such as salmon, trout and mackerel are good dietary sources of LCn-3PUFAs, however, lean fish including cod is also a good source of DHA and EPA[13]. The cod's highest concentration of n-3 is found in the liver, with 19-29% of the total liver fat content consisting of EPA and DHA. There are minor seasonal variations in the fat content, and more so in females than males [41]. The cod liver is generally not eaten as it is, but is instead used to make supplements such as cod liver oil and n-3 capsules which is an important source to marine n-3 FAs in the Norwegian diet [12, 38, 42, 43].

A serving of 200g of cod fillet contains 480 mg EPA and DHA, even though the fat content in cod fillet is below 2% the n-3 content is double of what EFSA considers to be the daily adequate intake [7, 13]. Cod is also a good source of other nutrients, it consists mainly of high-quality protein. A serving of 200g of a cod fillet covers about 40% of the daily requirement for vitamin B6, vitamin B12, phosphor, and 120% of selenium which some evidence suggest might have protective properties against mercury toxicity. 200 gram of cod also contains more than double the daily requirement of 250 µg/g of iodine, which many pregnant women have inadequate levels of [34, 44, 45]. Cod is therefor a very nutrient rich food to consume in pregnancy.

1.2 Lipids and fatty acids

Lipids are essential components of our body composition, and the NDH recommends that 25-40% of our daily calorie intake come from fats [46]. These hydrophobic organic compounds are comprised of hydrogen atoms attached to a long carbon backbone with a small number of oxygen substitution [39, 47, 48]. The dietary fat is broken down in the small intestine to lipids that are utilized as energy, structure in cell membrane, hormone production and vitamin storage. Lipids are commonly classified into three major groups; triacylglycerides (TAG), phospholipids (PL) and steroids [39, 47-50].

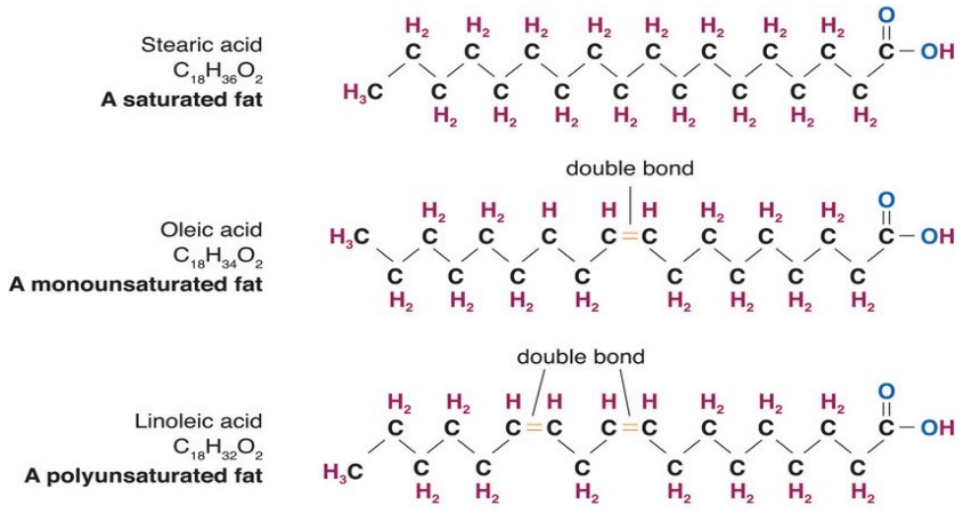
TAG is built up of three FAs that are esterified to a glycerol backbone and it is the most energy efficient molecule in relations to its size and make up 95% of out dietary fats. PL are structural lipids made up of two hydrophobic fatty acid tails connected to the hydrophilic head at the glycerol base with its phosphate group. In cod fillet 83.6% of lipids are structural PL [39, 51-53]. Sterols include cholesterol and steroid hormones, unlike TAG and PL they do not have FA tails. [49].

1.2.1 Structure and saturation of fatty acids

FAs are long aliphatic chains with a carboxylic acid at one end as shown in figure 1.1. They can be up to 36 carbons long, however most common dietary lipids have between 16-22 carbons. The FAs are classified as either saturated or unsaturated, depending on the presences of the carbon-carbon double bonds (figure 1.1)[39]. The saturated fatty acids (SFAs) have no double bonds, while monounsaturated FAs have one double bond and the polyunsaturated fatty acids (PUFAs) have between two and six cis double bonds [50, 54]. The unsaturated FAs take up more space and makes the cellular membrane more flexible, this is because the double bonds cause the FAs to have a kink, meaning they are in a “cis” configuration and cannot be packed as tightly as the saturated FAs [39, 47, 53, 55, 56].

FAs are characterized by the length and number of carbons. A short-chain fatty acid (SCFA) is less than eight carbons long, they are water soluble, and found primarily in dietary products containing milk fats [39]. Medium-chains are between 8-14 carbons long, and are found in the body as intermediates in FA synthesis or from consumption of coconut oil or dairy fat. The long-chain FAs are 15 carbons or more and are the most common ones in the human body, the n-3 and omega-6 (n-6) FAs fall in this category [39, 52].

Figure 1.1 *Chain structure of fatty acid as saturated, monounsaturated and polyunsaturated [57].*



1.3 Essential fatty acids, omega-3 and omega-6.

Essential fatty acids (EFAs) are needed to maintain normal development and function, they cannot be synthesized by the human body and must be supplied through the diet. Traditionally there are two types of FAs that are deemed essential: Linoleic acid (LA, 18:2n-6), the precursor for the biologically active arachidonic acid (AA, 20:4n-6) in the n-6 PUFA metabolic pathway. The second is α-linoleic acid (ALA, 18:3n-3) a precursor for biologically active EPA (20:5n-3) and DHA (22:6n-3) which are the marine associated FAs (figure1.2) [23, 39, 52]. Though only LA and ALA are deemed essential, deficiencies of EFAs can be reversed or avoided by consuming AA [39, 52]. Also DHA and AA are not made in sufficient amounts by newborn to guarantee a normal development, and are considered essential in infant nutrition, pragmatics might therefor include these as EFAs [26, 58, 59]. In this thesis AA, and DHA are included as EFAs.

1.3.1 Synthesis, elongating and desaturation of fatty acids

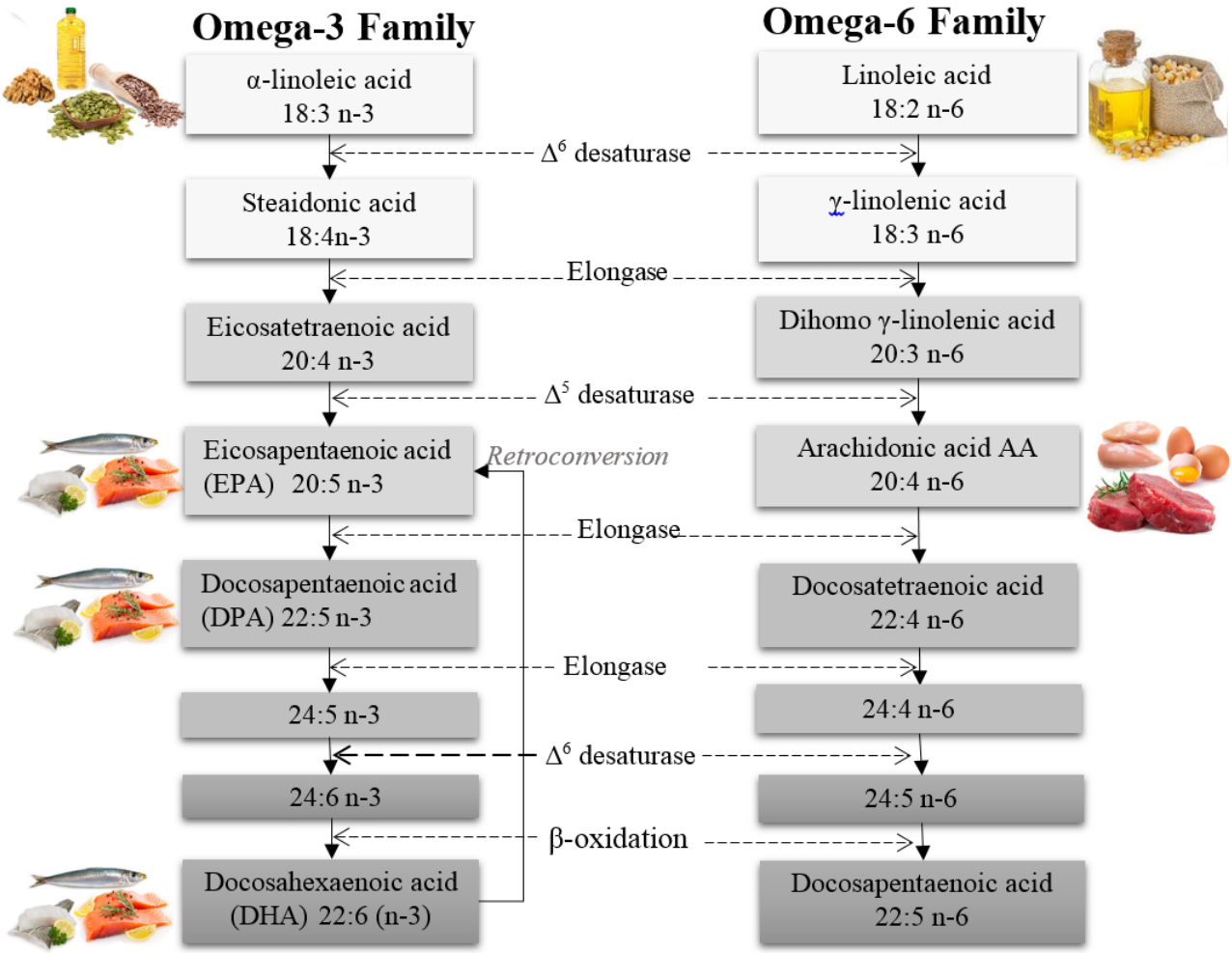
Through the fatty acid synthesis in the cytoplasm, the body turn acetyl CoA into a FA through a series of enzymatic reactions. The end-product of this process is normally palmitic acid (PA) (16:0). PA can be converted into other FA except for EFAs, through the process of elongating and/or desaturation [55, 56]. During elongation, the enzyme "elongase" attaches two new carbons to the existing FA. The process takes place in the mitochondria using acetyl CoA as a substrate, or in the endoplasmic reticulum using malonyl CoA, the most important process is elongation of EFAs [55, 60]. ALA and AA is converted into EPA, DHA and AA (and DHA is converted back to EPA through retroconversion) in the enzymatic pathway taking place in the endoplasmic reticulum utilising β-oxidation, elongase and desaturase enzymes. The reaction pathway with intermediate metabolites and enzymes are shown in figure 1.2 [52, 60, 61]. Desaturation uses different enzymes depending on the FA, and removes hydrogens to create double bonds [55, 60].

Humans lack the necessary Δ^{12}- or Δ^{15}- desaturase to add a double bond at the 12th and15th carbon of a long chain fatty acid to synthesize LA or ALA *de novo,* and do not have n-3 desaturase converting enzymes that can convert n-6 to n-3 [3, 62-64]. Subsequently ALA needs to come from the diet and be desaturated and elongated by the same enzymes as AL and AA in order to be turned into the EPA and DHA. This usage of the same enzyme is the reason for the for the competition between n-3 and n-6 in the body (figure 1.2) [3, 39, 65, 66]. Getting n-3 in form of EPA and DHA is more efficient than conversion from ALA. In men <6% of ALA is converted to EPA and only 0,5% to DHA, women have higher rates with 21% to EPA and 9% to DHA [67-69]. The Δ^6 desaturase preferred ALA over LA, however abundance of ALA has been shown to suppress the conversion of ALA to DHA [70, 71]. Supplementing with the intermediate FA sardonic acid to be converted into DHA is found to be more efficient, suggesting that Δ^6 desaturase could be a limiting factor in the conversion to DHA [71, 72].

1.3.2 Dietary sources of omega-3 and omega-6

The dietary sources (table 1.2) of LA are vegetable oils such as sunflower and corn oil. The dietary sources of AA are animal products such as meat, game, poultry and eggs. Dietary sources of ALA are found in oils from nuts and seeds such as flaxseed, chia, pumpkin seeds and walnuts. EPA and DHA are mainly found in seafood especially fatty fish such as salmon, mackerel, and sardine, and oils from fish liver, krill and algae [39, 65, 73, 74]. The highly insufficient conversion of ALA to DHA is one of the reasons why it is important and recommended to consume food containing DHA and EPA such as fish [75]

Figure 1.2 *Flowchart of the metabolic pathways of omega-3 and omega-6 polyunsaturated fatty acids in human, and its dietary sources. Modified from* [76]



1.3.3 Ratio of omega-3:omega-6

The genetic patterns of humans were established on a very different diet than what is consumed today. The hunter-gatherer diet was lower in n-6 and much higher in n-3 from structural fats found in animal brain and fish, subsequently the ratio of n-3:n-6 was close to 1:1 [77]. The western diet, consumed in the USA, Europe and Australia, is low in n-3 and excessive in n-6 FA. The n-3:n-6 ratio of the western diet is between 1:10-1:25, which is much higher than the original 1:1 ratio the was biologically intended [65, 78]. A higher level of n-6:n-3 have been linked to diseases such as CHD, cancer, allergies, inflammatory and autoimmune diseases, while increased levels of n-3 show a suppressive effect [79].

Norway have recommendation for the ALA and AL intake, but no recommendation for the ratio of n-3:n-6 [8]. However, a panel of lipid experts, where Norway were represented recommend a ratio of 1:2. This ratio is in high contrast to the average USA ratio of 1:25 [78, 80, 81]. To my knowledge there are no measures of the average Norwegian ratio, but the US, UK, and northern European countries are found to have the diets highest in LA and lower levels of n-3 [77, 82]. Norway are among the countries with high seafood and n-3 supplementation intake, it is therefore possible that the Norwegian population have a higher n-3:n-6 ratio compared to the US, the UK, and other northern European countries with western diets [8, 35, 79].

1.3.4 Associated biological impact of omega-3 and omega-6 fatty acids

n-3 and n-6 have biological functions in the human body throughout life, impacting the immune system, oxygen transport, cell membrane function and neurotransmitter metabolism [23]. DHA is one of the most studied FAs in association to fetal development, as DHA deficiencies seems to affect brain and eye development [39, 83, 84]. The role of DHA in the retina has not been established in its entirety. However, in times of body DHA deficit the retina still contains high amounts due to its recycling mechanism, suggesting that DHA is of importance for optimal function. Inadequate levels of DHA in human infants are associated with low vision acuity, and numerous animal studies found DHA necessary for normal development and retina function. DHA is important in the photoreceptor function in retinoid transport and membrane properties, as well as regeneration of rhodopsin; a visual pigment in the visual transduction system that is responsible for turning the light that hits the retina into the images in the brain [73, 84-86].

DHA and AA are fundamental components in the brain and central nervous system (CNS). The maternal transfer of DHA is suspected to be higher in the third trimester to meet the fetus's high demands of DHA during the brain growth spurt, the fetal DHA brain concentration increases dramatically at this time [87]. The brain consists of 35 % of PUFAs and DHA make up 90% of all the n-3, and almost all FAs in the brain is in the form of structural phospholipids [87, 88] DHA is concentrated in the frontal lobes which is important in short-term memory, association, sustained attention and problem solving [88, 89]. DHA can affect cellular characteristics such as membrane fluidity, neurotransmitter release, myelination and neuronal differentiation. DHA is a component of myelin sheets which assures that nerve impulse move quickly and efficiently, and is in high concentration in the membrane of the synaptic terminals [88]. DHA is found in large amount in the gray matter in the brain, and is especially high during development while it decreases with age[88]. Numerus studies have found that low levels of n-3 during pregnancy, infancy and are associated with poor neurodevelopment and CNS function [3, 73, 84].

The first postpartum year has the higher risk of depression amongst women with 45-65% of the women experiencing depression having their first depressive episode [90]. Postpartum depression affects both mother and child, as children of mothers with postpartum depression are found to have both somatic and psychiatric problems [91-93]. Lower seafood intake and DHA levels in RBC is associated with higher frequency of perinatal and postpartum depression, though it is EPA that seems most promising in treatment of depression [20, 94-96].

EPA and AA is the parent compounds of eicosanoids, which are a large group of cell-signaling molecules. They act upon many different physiological systems and play a large role in immune and inflammatory responses [39, 77]. Eicosanoids derived from EPA tend to be less pro-inflammatory, while those from AA tend to be more pro-inflammatory. Many of EPA derived eicosanoids have an anti-inflammatory effect, and therefore less potent inducers of blood vessel constriction and coagulation than those from AA. However, some of the AA derived eicosanoids are anti-inflammatory as well, so it is an over simplification to label them all as pro-inflammatory [39, 73]. Due to the anti-inflammatory effect of n-3 derived eicosanoids, consuming n-3 PUFAs are associated with a positive effect on inflammatory diseases such as asthma, allergies and rheumatoid arthritis [39, 65, 73, 79]. Thus, a diet high in AA and other n-6 FAs and low in n-3 can shift the physiological state to one that is pro-inflammatory [77]. Inflammation during pregnancy have been associated with negative health outcomes for gestational diabetes, perinatal depression, preterm delivery and preeclampsia [97-99]

1.3.5 Deficiencies of omega-3 and omega-6

In 1927, Evans and Burr were the first to establish that a deficiency of fat severely affected growth and reproduction [52]. Two years after that, they found that some FAs were more important or essential, and that consuming LA or ALA reversed the fat deficiency [39, 52]. EFA deficiency is characterized by dry scaly skin, growth retardation, impaired neural development, increased susceptibility to infection, poor wound healing, reproductive failure and impaired vision. All these symptoms can be complete cured with n-6 alone [87]. Having lower levels than recommended does not necessarily lead to presentation of clinical symptoms of deficiencies. The cases with deficiency are usually seen in clinical disorders, trauma from surgery and some forms of parenteral nutrition [39, 52, 73, 84]. Deficiency or low levels of LCn-3PUFA during third trimester brain development have been implicated in the pathophysiology of different psychiatric disorders, such as depression [100, 101].

1.4 Fatty acids in red blood cells

Red blood cells (RBC) or erythrocytes accounts for about 36- 53% of the total blood volume and are produced in the bone marrow. They have a flattened biconcave disc shape due to the lack of mitochondria and nuclei. The lack of these cellular organelles is the reason for the shorter circulation life span of only 120 days [102]. RBC membranes is of particular interest as it contains lipids and FAs that are highly affected by the dietary lipids from the diet. Already after 10 days of consuming corn oil, an extensive change in the erythrocytes FA composition can be observed. Due to RBC life span of 120 days their FA composition is a good indication of the dietary FAs consumed the past 4 months. Hence, FA levels in RBC is a good biomarker for evaluating the amount of EFA consumed in the past 4 months [103, 104].

1.4.1 Omega-3 index

The n-3 index is a biomarker reflecting the relative amount (%) of sum EPA and DHA within the RBC, in research and clinical settings it can be used to document compliance of increased LCn-3PUFA intake [105]. A level of >8 means that >8% of the total lipid content in the RBC are EPA and DHA, and is considered an n-3 index with greater CHD protection. An n-3 index of 4-8% is intermediate protection, and <4 is associated with lower protection [106, 107]. The n-3 index varies between dietary cultures more so than ethnicity, as shown in the n-3 index of the American and Japanese Americans with an average of 3.2%, while Japanese people living in japan had an average of 8.5 % [108]. In a Norwegian study of pregnant women were found to have an average n-3 index of 6.4%, this is higher than the US average of 3-6% [20, 105, 108].

1.5 Changes in mothers to sustain fetal growth, and impact on fetus

During pregnancy the female body goes through several changes to accommodate and sustain the growth of a fetus. There is an increase in energy requirements, blood volume and renal plasma flow [39, 52]. The body also have to sustain the creation of a new organ, the placenta. It serves as a site of nutrient exchange between the maternal and fetal blood. It has a high metabolic rate utilizing about a third of all the glucose supplied by the maternal blood, and the rate of protein synthesis is higher than in the liver. It is even more dependent on maternal FA contribution due to its poor synthesis of FA. [109, 110]. The health and development of the unborn child depends on the habits, health, and the lifestyle of the mother. As the fetus in entirely dependent on the mother for all its nourishment through the umbilical cord. Therefore, the nutrient composition of the diet is of importance during pregnancy, especially consuming nutrient such as n-3 FAs that the fetus in unable to produce itself [23, 52, 84].

1.5.1 Fatty acid changes during pregnancy

Women's fat deposit increases during pregnancy, together with a change in the homeostasis of the fat-soluble and water- soluble nutrients in the plasma, resulting in an increase of fat-soluble nutrients and a relative decrease of the water-soluble [52, 54, 111]. The exact physiological reasons behind this is not established in its entirety, but are potentially beneficial to the fetal development. It takes place in gestational week (GW) 10-12 before the fetus's needs are too demanding on the body and could cause maternal depletion of nutrients [39, 111]. TAG do not cross the placenta, but the FAs does and when resembled into TAG in the fetus, it mimics that of the mother's adipose tissue. The fetus get all its EFAs form the mother through the umbilical cord and the mother gets her EFAs from the diet. Because of this relationship there is a positive correlation between the mothers EFA intake and the fetus's EFA status [28, 54, 111, 112].

During pregnancy there is a steady decline in the maternal DHA and AA status due to a selective transfer of these of these FAs to the developing fetus. The transfer is highest in the third trimester during the fetal brain spurt [113, 114]. That DHA status is found to be higher among first times mothers together with the evidence of mobilization of maternal DHA stores, suggesting that the maternal DHA the fetus receives comes from a maternal pool that is not easily replenished[114]. N-3 supplementation during pregnancy have been shown to prevent decrease and enhancing the maternal DHA status, and the effect of the supplementation have been shown to last to 6 weeks postpartum [115].

1.5.2 Essential fatty acids in fetus

Although the fetus can synthesize FAs except EFAs, evidence suggest that as much as 50% of the FA requirement are maternally derived [110]. DHA and AA is important structural component of the CNS and retina during fetal development, however, they also accumulate in other organs. FAs are transferred across the placenta by specific binding of transfer proteins or by simple diffusion [28, 54]. These membrane-associated and cytosolic FA binding proteins favor n-6 and n-3 fatty acids over non-EFAs and prefers DHA and AA over other forms of the EFAs. The transfer proteins facilitate the fetal concentration of higher levels of DHA and AA, and lower amounts of LA and ALA [28, 54, 112]. Studies show that supplementing mothers with n-3 in form of ALA did not result in higher levels of DHA in fetus, compared to n-3 supplements or a diet rich in DHA which increases the level in the fetus [116]. Concentration of DHA and AA in the fetus is positively correlated with the mothers, it is therefore important to have adequate levels of n-3 during pregnancy [3, 28, 54, 112]

1.6 Aim.

This thesis is part of the larger study "Mommy´s Food" at the Institute of Marine Research (IMR). The main aim is to investigate if an increased intake of cod during pregnancy have an impact on maternal iodine status and infant development, in a two-armed RCT with cod for 16 weeks in the 2nd and 3rd trimester [117]. The aim of this thesis was to investigate if an increased intake of cod during pregnancy have an impact on FA content in RBC, with focus on the marine associated LCn-3PUFAs, in addition to evaluating seafood and n-3 supplement intakes at baseline and post-intervention.

2 Methods

The intervention study Mommy's Food was a two-armed randomized controlled trial. It was led and organized by the section of Food Security and Nutrition at the IMR in collaboration with the Regional Centre for Child and Youth Mental Health and Child Welfare (RKBU)[117]. In this thesis, RBC were utilized to investigate the differences and changes in FA profiles between the intervention-group and the control-group in gestational week (GW) 18 (baseline) and GW 36 (post-intervention). The weight registration forms of cod intake and food frequency questionaries' (FFQs) were used to investigate seafood intake.

2.1 Ethics

The Mommy's Food trial was registered in ClinicalTrials.gov (NCT02610959) and approved by the Regional committees for Medical and Health Research Ethics west (REK Vest, 2015/879), and complied with the Declaration of Helsinki [117]. Participation in the trial was voluntary, and participants could withdraw at any time without stating a reason. A written informed consent was obtained from all the participants. Infants do not have the ability to consent and therefore have special protection in research, which this study adhered to. All the biological samples were optional for both mother and child, and were stored in the IMR biobank. Strict confidentiality was upheld for the biological samples and the data collected as well as it being de-identified during evaluations, with anonymity for all participants in analyses of the data and in any publication of the study [117].

2.2 Participants and recruitments

Participant recruitment was between December 2015 and February 2017. The main recruitment site was the Women's Clinique at Haukeland University Hospital Bergen, where approximately 5000 women give birth each year. The pregnant women received study information and invitation to participate together with their routine ultrasound appointment taking place between GW 17 and 19. Appointments were sent out by the Women's Clinic by postal mail to pregnant women in Bergen and greater Bergen area in Health Region West. To ensure sufficient recruitment, information and invitation to join the study were broadcasted on common social media sites such as Facebook, Instagram and women's pregnancy magazines. The women interested in participating contacted the study secretariat at IMR before GW 19.

2.2.1 Inclusion and exclusion criteria

The inclusion criteria was first time biological mothers with a singleton pregnancy, and had to be less than 19 weeks pregnant with Norwegian speaking and writing skills (information, forms and validated test of the child were in Norwegian). Exclusion criteria were fish allergies and diseases affecting iodine status (Thyroid Nodules, Graves' disease, know hypo- or hyperthyroidism and Thyroiditis) [117]. Chronic disease affecting fatty acid composition and not iodine status (E.g. hyperlipidemia) was not in the exclusion criteria, as changes in iodine status was the main aim and focus of the study.

2.2.2 Sample size

The power calculation for sample size was based on the cohort study "Little in Norway" (LiN) with data of urinary iodine concentration (UIC) and daily iodine intake [118]. The power calculation is in based on iodine, and not FAs. An intervention-group of 60 participants would have a 95% power to detect an iodine content that is 30% higher than that of the control-group. Mommy's food aimed for a total sample size of 144 participants, with a 20% dropout rate expected. Enrolment closed with 137 participants where 4 women dropped out before allocation, making the intervention-group 68 women and the control-group 65.

2.2.3 Study schedule and group allocation

Participants were recruited and started intervention at separate times between February 2016 and September 2017. Each participant followed the same study schedule with the intervention period lasting 16 weeks (Table 2.1).

To achieve randomization and equal distribution in intervention- and control- groups, participants blindly drew group allocation by lottery during their second visit in GW 19 (table 2.1). The lottery box contained 10 notes, 5 control and 5 intervention. Blinding of the participating mothers of group allocation was impossible due to the nature of the study. Participant were given a random ID number between 1-200 during baseline sampling, data collection and input. A dummy ID during statistical analysis was utilized to ensure further blinding of investigators, due to possible exposure of original ID and group allocation during data input. The infants were blinded throughout the duration of the trial.

Table 2.1 *Overview of the study schedule from Mommy's food, and the main activities that are included in the thesis, modified from [117].*

	GW<18	GW 18	GW 19	GW 20	GW 36
ENROLLMENT					
Eligibility screening	X				
Informed consent		X			
Instructions		X			
Allocation			X		
INTERVENTION					
Control					
Intervention				←	→
Weight registration forms				←	→
BILOGICAL DATA					
RBC-FA status		X			X
QUESTIONAIR					
FFQ		X			X

Abbreviations: GW; gestational week, RBC; red blood cells, FA; fatty acid
FFQ; food frequency questionnaire

2.3 Intervention and food safety

In the GW 19 visit, the intervention- group received cod fillets for themselves and their live-in partner, while the control- group was instructed to follow their habitual diet (table 2.1). The cod fillets, skin- and boneless and weighing approximately 200g each, were stored at -30 °C at the IMR. The cod was purchased after tender from Lerøy A/S Bergen. The intervention- group was given 400g of cod fillets to consume each week and the preparation method was optional. For compliance, participants were instructed to weigh (Kitchen Scale, article no. 34–1207-16, ClasOhlson.com) the cod fillets raw after thawing and any leftovers from the meal. Preparation method, side dish and cod weight were noted in the weight registration form received together with the scale and a recipe booklet.

2.3.1 Safety of cod

The safety of the food is especially important in vulnerable populations, such as low contaminant levels in the food consumed during pregnancy. 400g of cod was calculated to have a maximum contribution of 22% of the tolerable weekly intake (TWI) for mercury (Hg), and 4% for dioxin and dioxin-like polychlorinated biphenyls (dl-PCB) [117, 119]. The TWI was established by the CONTAM panel at EFSA and established to be 1.3 µg/kg for Hg and 14 pg-TEQ/kg for dioxin and dl-PCB. The maximum intake of unwanted contaminants any one participants would consume were calculate from the LiN cohort study 5-percential weight [118].

2.4 Data collection

2.4.1 Blood sampling

Blood sampling was done by a qualified bioengineer from IMR or Betanien, the first sample was drawn in GW 18, and again in GW 36, for all participants. The two Ethylenediaminetetraacetic acid (EDTA) vials drawn were left for 30-120 minutes. Than centrifuged for 10 minutes at 3000 rpm and separate into tubes of serum, plasma, buffy coat and RBC. The two RBC sample were labeled with project and participant number, and frozen at -80 °C until analysis.

2.4.2 Food frequency questionnaires

All participants answered an electronical FFQ in GW 18 and GW 36 about their habitual diet during pregnancy. The FFQs were developed to capture iodine rich food and was based on a validated semi-quantitative short seafood FFQ that analyses seafood habits in pregnant and post-partum women and was developed by Markhus *et al* [120]. The FFQs consisted of six sections; Seafood, milk and dairy products, other parts of diet, food habits, supplements and personal social economic questions (appendix I). In the GW 36 FFQ the control- group were asked to include consumption of the intervention cod in their answers.

Seafood question were divided into warm dinner and lunch referred to in the thesis as “warm meals”, and spread, salads and snacks referred to as “spread”. The warm meal and spread sub-sections consisted of questions regarding portions size, and summary- and detailed-consumption questions about seafood, fish species and seafood products (table 2.2). Participants were asked to answer based on their habitual diet during the past 3 months. Portion sizes are stated in standardized portion descriptions for the detail questions. The warm meal portion sizes were 150g salmon, 200g cod, 12 sushi pieces, 3 fishcakes, 6 fish balls, 7 fish fingers or 2 dl peeled shrimp. The spread portion sizes were one sandwich with either caviar, mackerel, smoked salmon, or 1 fish cake. The portion sizes were derived from Norwegian Food Safety Authority report “Weight measures and portion sizes” [121].

Participants were asked whether they consumed a complete pregnancy supplement containing omega-3. If yes, a pop question of brand, frequency and dosage appeared. The supplement section also included a question regarding other omega-3 supplements together with or instead of the complete pregnancy supplements, and the frequency and dosage.

2.4.3 Converting from seafood frequency and portion to seafood index

Seafood frequency intake was reported in ordinal data from the FFQ, then converted to numerical interval data and further to a seafood index (E.g.: ≤**1 time/month** = *ordinal data* → **0,25** = *numerical interval* → **0,25** = *seafood index*) (table 2.2, 2.3 and 2.4). This enables statistical analysis and aggregation of different types and quantity estimations of seafood consumption. The seafood index is based on a validated method developed by Markhus *et al.* [120], and is an estimation of seafood consumption per week, with a seafood index of 1 representing one portion of seafood per week [121].

The seafood index for summary questions of warm meals and spreads were based on the frequency average per week (table 2.3). There is a tendency of over-reporting low intakes in detail questions about individual fish species and seafood consumption [122, 123]. The seafood index is therefore the lowest possible weekly intake for warm meals and spread detailed questions (table 2.2). Processed fish products has a typical fish fillet content of 40-60% and sushi 33%, therefore calculating the seafood index is half or ⅓ of the lowest possible weekly intake of processed fish product and sushi (table 2.3)[8]. The seafood index for omega-3 supplement were based on the average weekly intake, same as the summary questions for warm meals and spread (table 2.4).

Table 2.2 *The reported seafood intake frequencies from the FFQs in numerical interval and the corresponding seafood index for the warm meals and spread [120].*

Reported frequency	***Numerical interval per week*** [a]	***Seafood index (warm meals)***[b]	***Seafood index (spread)***[c]
Never	0	0	0
< 1 time/month, rarely	≤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

[a] Numerical interval based on the average consumption frequency of summery question of seafood intake per week as warm and cold meals

[b] The seafood index assigned the average weekly intake frequency of seafood as warm meals

[c] The seafood index assigned the average weekly intake frequency of seafood as spread

Table 2.3. *The reported seafood intake frequencies from the FFQs in numerical interval, and the corresponding seafood index for fish and seafood, fish products and sushi [120].*

Reported frequency	***Numerical interval per week[a]***	***Seafood index*** **Fish and seafood [b]**	***Seafood index Fish products*** [c]	***Seafood index Sushi*** [d]
Never	0	0	0	0
< 1 time/month	< 0.25	0.1	0.05	0.033
1-3 times/month	0.25-0.75	0.25	0.125	0.083
1 time/ week	1	1	0.5	0.33
2 times/week	2	2	1	0.66
≥3 times/week	≥ 3	3	1.5	1

Abbreviations: FFQ, food frequency questionnaire

[a] Numerical interval based on the average consumption frequency per week of detailed question about fish, processed products and sushi as warm and cold meals

[b] The seafood index assigned the lowest possible weekly consumption frequency for detailed questions about fish and seafood species as warm meals and cold meals

[c] The seafood index assigned the lowest possible weekly consumption frequency halved for detailed questions about processed fish as warm meals and cold meal. It is halved because processed fish products typically contain 40-60% fish[8].

[d] The seafood index assigned the lowest possible weekly consumption frequency divided by 3 for detailed questions of sushi as warm meals and cold meal. Because ⅓ of the sushi bites are fish[8].

Table 2.4 *The reported omega-3 supplement frequencies from the FFQs in numerical interval, and the corresponding seafood index [120].*

Reported frequency	***Numerical interval per week*** [a]	***Seafood index Omega-3 supplements*** [b]
Never	0	0
1-3 times/month	0,25-0,75	0,5
1-3 times/week	1-3	2
4-6 times/week	4-6	5
Daily	7	7

Abbreviations: FFQ, food frequency questionnaire

[a] Numerical interval based on the average weekly intake frequency of omega-3 supplements.

[b] The seafood index based on the average weekly intake frequency of omega- 3 capsules, liquid omega-3 and cod-liver oil.

The portions consumed per week were calculated by multiplying seafood index with the reported potion sizes. E.g. if a participant consumed 2 portions of fish cakes 1 time/week (50% fish, seafood index = **0.5**) and one portion of cod fillet 2-3 times/ week (100% fish, seafood index = **2.5**) the seafood portions per week is **3.5** = (2 potions ***0.5 seafood index) + (**1 portion *** 2.5 seafood index)**. The total seafood intake was the sum of participants frequency and portions size for warm meals and spread.

2.4.4 Establishing seafood categories

The types of seafood consumption in the warm meals category was put into groups depending on the fat content. Fatty fish had a fat content higher than 5g per 100g = 5%, and the other categories had fat content lower than 5% fat or fish containing less than 5% fat, shellfish was all <5% [8] (table 2.5). All types of spread were put into one category to simplify analysis.

Table 2.5 *Overview of seafood and fish categories derived from the FFQ, in Mommy's Food.*

Seafood Category	***Type of seafood***
Fatty fish >5% , warm meal	Salmon/trout, mackerel, herring, halibut
Lean fish ≤5%, warm meal	Cod, pollock, pollack, haddock, common ling and catfish
Processed fish product warm meals ≤5% fish fat content	Fishcakes/balls/pudding, fish pie, fish fingers, fish soup,
Spread	Tinned mackerel, sardine, herring, tuna and salmon, smoked or cured salmon and trout, anchovies, peppered mackerel lofotpostei, svolværpostei, caviar, crabsticks
Shellfish and crustaceans ≤5% fat content	Shrimp, crab claw meat, crabmeat brown, lobster, mussels, scallops
Total fish	Salmon/trout, mackerel, herring, halibut, cod, pollock, pollack, haddock, common ling and catfish
Total seafood	All fish, processed fish, spread and shellfish listed in this table

Abbreviations: FFQ, food frequency questionnaire

2.4.5 Intervention compliance

Dietary compliance was calculated from weight registration forms filled out by the participants in the intervention-group showing in grams the amount of the received cod that was consumed. Participants registered the weighed of the defrosted cod in grams, which was then subtracted by the registered weight of any leftovers to establish the weight of the consumed cod for every meal. If the full 200g serving were eaten participants registered cod leftovers as 0g or leaved the column blank. Any cod registered that was not supplied by the IMR were changed into zero, as other participant might also have eaten non-IMR cod without registered this as per instruction, and the weight registration form need to be filled out compatibly for all participants.

The intervention period lasted for 16 weeks with 400g as the intended weekly intake, making the maximum intake of cod 6400g (=16 weeks x 400g) with a compliance score of 100. Compliance scores were calculated by dividing the total intake by the maximum intake and multiplying it with 100. E.g. a participant with a total cod intake of 4800g or average weekly intake of 300g would have a compliance score of **75** (4800g/6400g=0.75 x 100= **75**) .

2.5 Determining fatty acid composition in red blood cells

2.5.1 Extraction of fatty acids from red blood cells

The Hamilton Microlab Star Line (HMSL) robot was used to extract the FAs from the RBC. It was operated by a qualified bioengineer with a user-manual course, according to the IMR method-description "435-FA with Hamilton Robot and Ultra-GC-FID" (appendix). RBC from baseline and post- intervention were taken out of -80 ºC freezer and defrosted on the Gyromini Nutating mixer. Participants' baseline and post-intervention RBC samples were analyzed at the same time to ensure there were no method variances or discrepancies. Cleansing water (H_20), Heptane (C_7H_{16}) and Sulfuric acid (H_2SO_4) were filled into three separate containers and placed in its designated spots in the HMSL. Next, four test tubes containing 2 ml of Internal Standard (IS) containing 19:0 Methyl, were placed in the robot. A sequence contained 32 RBC samples, that were placed into the machine together with a serum with known FAs values and a blank (distilled water).

The HMSL added 60 µl of the RBC samples to the startplate and weighed it to ensure no errors occurred, 60 µl of sample serum and the blank was included for quality control. Next, 60 µl of the IS and 750 µl of H_2SO_4 2% in methanol were added. The startplate was sealed and shaken at 1200 rpm before being moved to heater-shaker (HHS). Where samples were heated at 105 ºC and shaken at 100 rpm for 40 min, the last 20 seconds it was shaken at 1000 rpm. The plate was cooled down for 15 min, than 250 µl water and 500 µl of hepthan was added and mixed. The startplate was than centrifuged for 2 mins at 3000 rpm. A hepthan layer with the extracted FA formed, and 300 µl was pipetted by hand into small glass tubes to be analyzed in the Ultra Fast Gas Chromatograph–flame ionization detector (UFGC-FID).

2.5.2 Analysis and integration of fatty acid

The extracted samples of FAs were analyzed in a UFGC-FID to determine the mg/g sample for each individual FA. For quality control 1 sample of heptane, 1 of standard cod liver oil solution, 2 blanks of distilled water and 2 control samples, were run together with 1 µl of each finished sample of FAs in every sequence. The UFGC-FID used gas chromatography to separate the FAs compounds and flame ionization to detect the amounts. It gave a readout of the individual FA in a chromatogram, where each FA was represented by a peak. The area of the peak indicated the amount, and the placement the type of FA. The FAs were decided using an integrator (Chromelon 6.80, Dionex Corperation, California, USA) by a bioengineer.

Table 2.6 *An overview of the fatty acids detected in the UFGC-FID*

	Saturated fatty acid	***Unsaturated fatty acid***	***Omega-3 PUFA***	***Omega-6 PUFA***	***Others polyunsaturated***
Types of Fatty acid Detected In the UFGC	14:00	16:1	16:3n-3	18:2n-6 (LA)	16:2n-4
	15:00	18:1	18:3n-3(ALA)	18:3n-6	20:3n-9
	16:00	20:1	18:4n-3	20:2n-6	
	17:00	22:1	20:3n-3	20:3n-6	
	18:00	24:1n-9	20:4n-3	20:4n-6(AA)	
	20:00		20:5n-3(EPA)	22:4n-6	
	22:00		22:5n-3(DPA)	22:5n-6	
			22:6n-3(DHA)		

Abbreviations: UFGC; Ultra Fast Gas Chromatograph-flame ionization detector, PUFA; polyunsaturated fatty acid,

2.5.3 Quality control of fatty acid analysis

For every sequence of RBC analyzed in the HMSL a control-solution with a known FA concentration were analyzed (human plasma). The control-card was made from analyzing samples of control-solution ten times, and the FA concentration of 16:0, 18:2n-6 and 22:6n-3 were the main focus. The readout for the same three FA from the control-solution were put into the control-card to get an average of what the FA should be. The control-solution and the whole sequence were approved if the average laid within +/-2 standard deviations (SD) of the control-card average. The IS, 19:0 Methyl, was added in such a concentration that it made up 10-30% of the total FA amount. The absolute amount (mg/g) of FAs in the sample was found by calculating the area of the individual FA against the area of 19:0 (IS) in the sample that has a known value.

To ensure a correct readout in the UFGC, for a new analysis, a standard solution and Nu-Check standard is analyzed first. It has to have a value that varies <5% from the theoretical values and sufficient space between the FA peaks for the biological samples to be analyzed. Limit of detection or limit of quantification (LOQ) for the method was sat at 0,01 mg/g or 10 µg/g, and are the smallest concentration of a quantity that can be reliably measured by this analytical procedure [124, 125]. FAs below the LOQ was not included in the results.

2.6 Statistical analysis

IBM SPSS version 25 (IBM Corporation) was utilized for all statistical analyses. Microsoft Office Excel or Microsoft Office Word 2013 were used to create tables and figures. P-values < .05 were considered statistically significant. Normality was tested for using visual inspection with Q-Q plots and histograms.

The mean and (SD) were used when the numbers met parametric assumptions. Median and interquartile range (IQR) were used when the numbers were non-parametric, interquartile range is stated as one number IQR = Q3 − Q1 (Q3=75th percentile and Q1=25th percentile)[126].

For the baseline characteristics the mean +/- SD, or participant numbers and percentages were found. For the mean (SD) the Mann-Whitney U was used for the non-parametric numbers and the independent sample t-test for the parametric numbers. The Chi-square test was used to find the n (%). The p-value was not presented as the groups was randomized and there should be no statistical significance.

The FAs were presented in relative amount (%), which was the percentage content of the FA out of the total FAs content. The FAs were also given in absolute-amount (µg/g). The FAs were non-parametric and was therefore stated in median (IQR). The non-parametric test Related Wilcoxon signed rank was used to test for differences within the intervention- group and control- group between baseline and post-intervention. While the non-parametric Mann-Whitney U test were used for differences between the intervention- and control- group at post-intervention.

For the FFQ the numbers were non-parametrical and median (IQR) was used or percentages where appropriate. The frequency of seafood intake at baseline and post-intervention was stated in percentage of participants in each consumption frequency for spread, lunch and dinner. The media (IQR) were used for the FFQ seafood intakes with a seafood index as they were non parametric. The non-parametric test Related Wilcoxon signed rank was used to test for differences within the control- group and intervention- group between baseline and post-intervention. While the non-parametric Mann-Whitney U tested for differences between the control- and intervention- group at post-intervention.

For the compliance test both mean (SD) and median (IQR) was used together with the minimum and maximum intake of cod in grams for each category.

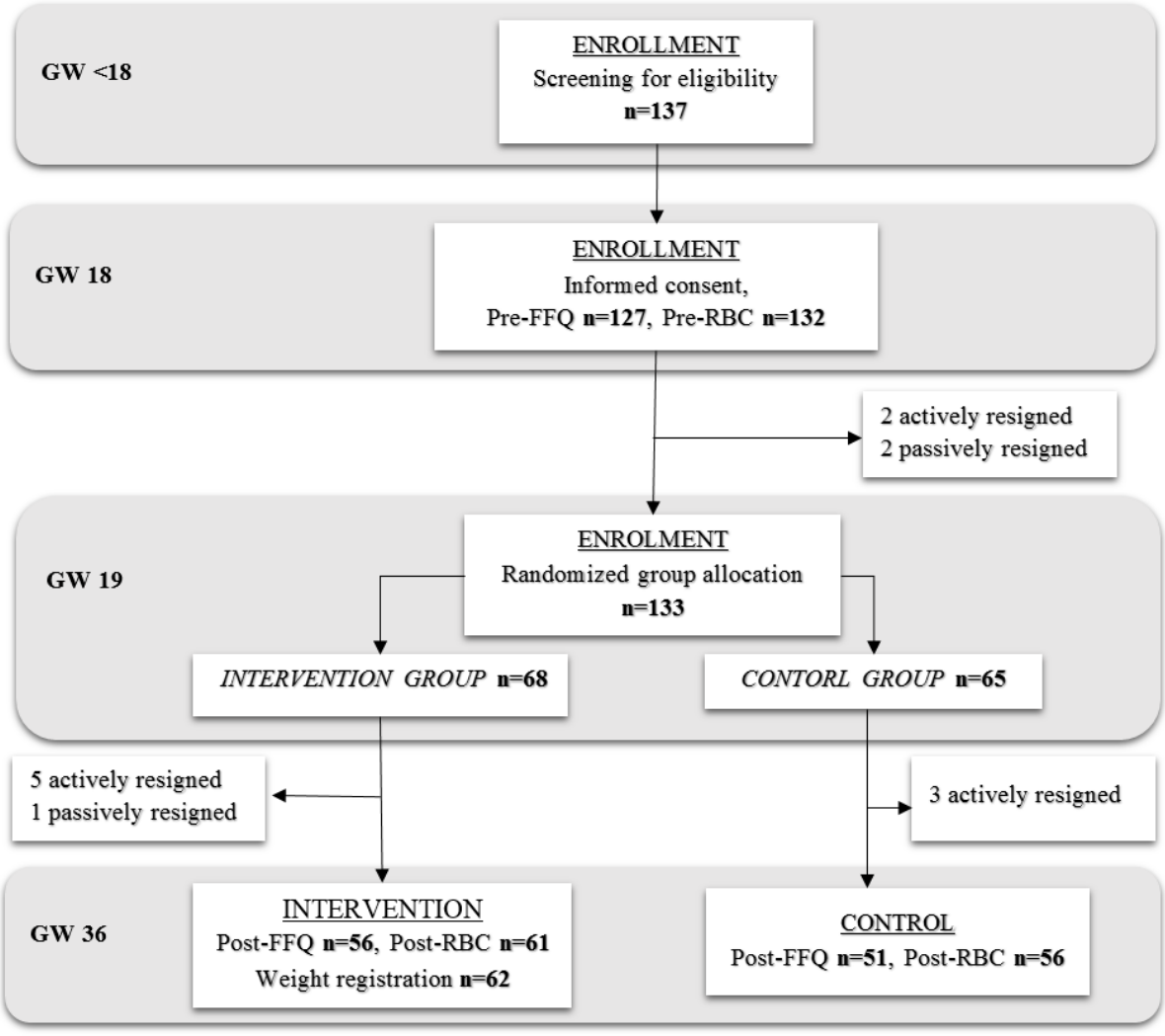
The non-parametric Spearman's rank-order correlation was used to test for correlations between FAs and seafood or cod intakes from FFQ or weight registration form. There was considered very weak correlation with an r_s =.00-.199, a weak correlation between r_s =.200-.399, moderate correlation between r_s =.400-.599, strong correlation between r_s =.600-.799 and very strong correlation for rs =.800-1.000 [126, 127].

3 Results

3.1 Participation rate

Figure 3.1 shows an overview of the study population with the dropout and participation rates at different stage of data collection. There was a dropout rate of 9.5% (n=13) during the intervention period, with RBC- samples collected from 99.2% (n=132) of participants at baseline and from 94.4% (n=117) at post-intervention. For the baseline FFQ 92% (n=127) answered, with 86% (n=107) out of the remaining 124 participants answered the post-intervention FFQ.

Figure 3.1 *Flowchart of participant numbers and the data at different GWs in the Mommy's Food trial.*



Abbreviations: FFQ, food frequency questionnaire, GW, gestational week, RBC; red blood cell

3.2 Baseline characteristics

3.2.1 Demographic of participants at pre-intervention

Baseline characteristics of the participants are shown in table 3.1. The average age in the study population was 29.3 (3.8) years. 86.4% of participants had a higher education at university/college level, and 60% attended university/college for more than four years. Household income was skewed to the left towards higher values and 63.2% had a combined household income of 750 000 NOK or higher. The average body mass index (BMI) for pre-pregnancy was 23.2 kg/m^2 which is within the normal range (18.5-25.0) for adults [128, 129].

Table 3.1 *Baseline characteristics of the pregnant study population from pre-intervention FFQ, randomized to intervention- or control- group, in pregnant women in Mommy's Food.*

Demographics Characteristics	All (n=127)	Intervention (n=65)	Control (n=60)
Physical attributes mean (SD)			
Age (years)	29.3 (3.8)	29.6 (3.6)	29.1 (3.9)
Gestational Age (weeks)	19 (1.3)	19.1 (0.1)	19 (0.1)
Pre-pregnancy BMI (kg/m^2)	23.2 (4,3)	23.2 (4.0)	23.3 (4.6)
Cohabitation, n (%)			
Cohabitation	121 (96.8)	63 (96.9)	58(65)
Single or Other	4 (3.2)	2(3.4)	2 (3.4)
Education level, n (%)			
Lower secondary school	1 (0.8)	1(1.5)	0 (0)
Higher secondary school	16 (12.8)	9 (13.8)	7 (11.7)
<4 years university/college	33 (26.4)	18 (27.7)	15 (25)
≥4 years university/college	75 (60)	37 (56.9)	38 (63.3)
Total household income (NOK), n (%)			
≤200,000-349,000	11 (8.8)	5 (7.7)	6 (10)
350,000-749,999	35 (28)	19 (29.2)	16 (26.6)
750,000-1,249,000	62 (49.6)	32 (49.3)	30 (50)
1,250,000->2,000,000	17 (13.6)	9 (13.8)	8 (13.4)

3.3 Fatty acid composition in relative- and absolute- amounts

Table 3.2 and 3.3 shows an overview of median (IQR) of the individual and Σ FAs in relative- and absolute-amount at baseline and post-intervention. There was no statistically significant changes between the groups at post-intervention for either relative- nor absolute-amount.

There were statistically significant increases in Σ saturated FA when comparing baseline with post-intervention for the relative amount in the intervention- group, and relative- and absolute-amount for the control- group. There was also a statistically significant increase in Σ unsaturated FA for the relative- and absolute-amount in both groups. The 16:0 (PA), 18:1 and 24:1n-9 all had statistically significant increased in relative- and absolute-amount between baseline and post-intervention for both groups.

The Σ polyunsaturated FAs had a statistically significant decrease in relative- and absolute-amount within in the control-group, and absolute-amount within the intervention-group when comparing baseline to post-intervention ($p<.000$).

There was a statistically significant decrease in Σ n-6, 20:4n-6 (AA) and 22:4n-6 for both intervention- and control- group in both relative- and absolute-amounts. The Σ n-3 was stable with no statistically significant increase in either group. The intervention-group had a statistically significant increase in absolute amount of 22:6 n-3 (DHA) at post-intervention. The control-group had a statistically significant decrease in the 22:5 n-3 (DPA) in both relative- and absolute- amount.

There was a statistically significant change in the n-3:n-6 ratio, the Σ n-3 was stable and Σ n-6 decreased between baseline and post-intervention for both groups. The intervention group had higher levels of n-3:n-6 (0.44) in median relative- and absolute-amount, than the control- group ratio (0.40). However, there was no statistically significant difference between the groups.

The n-3 index was a median of 8.4% at baseline and 8.5% at post-intervention for all. However, there was no changes within the groups between baseline and post-intervention, and no significant difference between the groups.

Table 3.2 *The absolute amount (µg/g) of the individual fatty acids in red blood cells, for the control- and intervention- group at baseline and post-intervention, in pregnant women in Mommy's Food.*

	All median (IQR)		*Intervention group median (IQR*			*Control group median (IQR*			*In Both* [c]
	Pre	Post	Pre	Post	p [a]	Pre	Post	p [a]	p [b]
Fatty acid	n = 132	n = 117	n = 68	n = 61	in	n = 64	n = 58	in	btwn
14:0	10 (10)	10 (10)	10 (10)	10 (10)	.180	10 (10)	10(10)	.052	.252
16:0 PA	440 (100)	500 (95)	460 (97)	500 (100)	**.003**	440 (100)	489 (88)	**.000**	.666
18:0 SA	290 (70)	300 (65)	300 (68)	300 (80)	.436	290 (70)	302 (50)	.431	.956
22:0*	10 (0)	10 (10)	10 (10)	10 (10)	.480	10 (10)	10 (10)	.655	.282
Σ Saturated	772 (175)	828 (165)	793 (165)	846 (192)	.118	766 (177)	824 (147)	**.003**	.752
18:1 OA	260 (50)	270 (65)	260 (60)	280 (70)	**.007**	265 (45)	270 (60)	**.012**	.991
24:1n-9	30 (10)	40 (20)	30 (10)	40 (20)	**.017**	30 (10)	40 (18)	**.000**	.952
Σ Unsaturated	308 (65)	323 (77)	306 (71)	322 (80)	**.003**	306 (57)	327 (75)	**.002**	.900
18:2n-6, LA	190 (50)	190 (60)	190 (47)	180 (55)	.411	200 (40)	190 (68)	.734	.545
20:3n-6	30 (10)	30 (10)	30 (10)	30 (15)	.103	30 (10)	30 (10)	.678	.305
20:4n-6, AA	260 (40)	220 (55)	260 (50)	230 (50)	**.000**	260 (38)	220 (60)	**.000**	.904
22:4n-6	40 (20)	40 (10)	40 (20)	40 (10)	**.000**	40 (10)	40 (10)	**.000**	.939
Σ n-6	533 (107)	483 (112)	535(122)	481 (110)	**.024**	533 (87)	489 (118)	**.003**	.923
20:5n-3, EPA	20 (20)	20 (20)	20 (20)	120 (20)	.271	20 (20)	20 (20)	.946	.853
22:5n-3, DPA	40 (10)	40 (20)	40 (20)	40 (15)	.813	40 (10)	40 (20)	**.010**	.270
22:6n-3, DHA	140 (40)	150 (40)	140 (58)	150 (50)	.056	140 (30)	150 (38)	.176	.189
n-3 index	155 (52)	167 (53)	155 (64)	170 (54)	.137	155 (47)	166 (55)	.218	.300
Σ n-3	203 (66)	216 (72)	203 (76)	225 (65)	.258	202 (54)	212 (72)	.697	.216
n-3/n-6	0.38 (10)	0.43 (125)	0.39(20)	0.44(10)	**.000**	0.38 (10)	0.40 (10)	**.005**	.085
Σ Poly-unsaturated	741 (124)	718 (160)	747 (140)	721 (171)	.272	743 (122)	716 (156)	**.023**	.727

Abbreviations: n-6: omega 6, n-3: omega 3,PA: palmitic acid, SA: Stearic acid, OA: oleic acid, AA: Arachidonic acid, EPA: Eicosapentaenoic acid, DPA: Docosapentaenoic acid, DHA: Docosahexaenoic acid, n-3 index: Σ EPA and DHA, btwn: between, IQR: interquartile range, p: p-value, in: within.

Fatty acids bellow the limit of quantification <10 µg/g was 15:00, 20:00, 16:1, 20:1, 22:1, 18:3n-6, 20:2n-6, 22:5n-6 ,16:3n-3, 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 16:2n-4, 20:3n-9, and was therefore not included.

[a] Related Wilcoxon signed rank for comparison for differences pre- and post-intervention within the control- and intervention- group

[b]Mann-Whitney U Test for comparison for differences between the control- and intervention- group post-intervention

[c] Difference between groups at post-intervention

[d] Diffrences within groups between baseline and post-intervention.

Table 3.3 *The relative amount (%) of the individual fatty acids in the total fatty acid content in red blood cells, for the control- and intervention- group at baseline and post-intervention, in pregnant women in Mommy's Food.*

	All median (IQR)		*Intervention group median (IQR*			*Control Group median (IQR)*			*Both* [c]
	Pre	Post	Pre	Post	p [a]	Pre	Post	p [a]	p [b]
Fatty acid	n = 132	n = 117	n = 68	n = 61	In [d]	n = 64	n = 58	in [d]	btwn
14:0	0.7 (0.2)	0.7 (0.3)	0.7 (0.2)	0.7 (0.3)	**.010**	0.7 (0.3)	0.7 (0.3)	**.023**	.239
16:0 PA	23.3 (2.1)	24.9 (1.8)	23.5(2.3)	25 (1.8)	**.000**	23 (1.8)	24.8 (2.1)	**.000**	.416
18:0 SA	15.4 (1.8)	15.3 (1.9)	15.5 (1.6)	15.2 (1.6)	**.005**	15.5 (1.8)	15.5 (2.2)	.935	.326
22:0*	0.4 (0.2)	0.5 (0.2)	0.4 (0.2)	0.5 (0.2)	.097	0.5 (0.2)	0.5 (0.1)	**.002**	.102
Σ Saturated	40.2 (3.7)	42.1 (3.1)	40.8 (3.5)	42.0 (3.1)	**.001**	40.0 (4.4)	42.3 (3.6)	**.000**	.974
18:1 OA	13.5 (2.0)	13.9 (2.1)	13.3 (1.4)	13.8 (2.1)	**.000**	13.8 (2.1)	13.9 (2.3)	**.001**	.516
24:1n-9	1.8 (0.5)	2.1 (0.6)	1.9 (0.5)	2.1 (.6)	**.003**	1.8 (0.5)	2.2 (0.7)	**.000**	.563
Σ Unsaturated	16.0 (2.0)	16.6 (2.1)	15.9 (1.6)	16.6 (2.2)	**.000**	16.1 (1.9)	16.7 (2.3)	**.000**	.451
18:2n-6, LA	10.1 (1.7)	9.9 (1.9)	10.0 (1.7)	9.6 (1.8)	.344	10.3 (1.5)	10.2 (1.9)	**.030**	.247
20:3n-6	1.4 (0.4)	1.4 (0.5)	1.4 (0.5)	1.5 (.5)	.672	1.4 (0.4)	1.4 (0.3)	.411	.147
20:4n-6, AA	13.3 (1.7)	11.6 (1.6)	13.2 (1.7)	11.4 (1.5)	**.000**	13.4 (1.7)	11.7 (2.0)	**.000**	.106
22:4n-6	2.2 (0.7)	1.8 (.6)	2.2 (0.9)	1.8 (.5)	**.000**	2.2 (0.7)	1.8 (.09)	**.000**	.922
Σ n-6	27.9 (2.5)	25.5 (2.4)	27.6 (2.6)	25.2 (2.6)	**.000**	28.3 (2.4)	26 (2.6)	**.000**	.124
20:5n-3, EPA	1.1 (0.8)	1.0 (0.7)	1,1 (0.8)	1.0 (0.7)	.190	1.1 (0.8)	1.0 (0.7)	.203	.961
22:5n-3, DPA	2.2 (0.6)	2.1 (0.4)	2.2 (0.6)	2.1 (0.5)	.349	2.2 (0.5)	2.1 (0,5)	**.000**	.651
22:6n-3, DHA	7.3 (1.9)	7.5 (1.7)	7.5 (1.6)	7.7 (1.6)	**.002**	7.2 (2.2)	7.4 (1.8)	.080	.917
n-3 index	8.4 (2.5)	8.5 (2.4)	8.6 (2.5)	8.6 (2.2)	.056	8.3 (2.8)	8.3 (2.7)	.309	.202
Σ n-3	10.9 (2.8)	10.9 (2.9)	10.9 (2.6)	11.1 (2.5)	.117	10.8 (3.3)	10.7 (3.3)	.796	.211
n-3/n-6	0.38 (0.1)	0.43 (0.1)	0.39 (0.2)	0.44 (0.1)	**.000**	0.38 (0.1)	0.40 (0.1)	**.005**	.085
Σ Poly-unsaturated	38.8 (2,5)	36.7 (2.4)	38.6 (2.2)	36.4 (2.2)	**.000**	39.2 (3.0)	36.7 (2.8)	**.000**	.698

Abbreviations: n-6: omega 6, n-3: omega 3, PA: palmitic acid, SA: Stearic acid, OA: oleic acid AA: Arachidonic acid, EPA: Eicosapentaenoic acid, DPA: Docosapentaenoic acid, DHA: Docosahexaenoic acid, n-3 index: Σ EPA and DHA, btwn: between. IQR: interquartile range, P: p-value, in: within.

Fatty acids bellow limit of quantification <10 µg/g was 15:00, 20:00, 16:1, 20:1, 22:1, 18:3n-6, 20:2n-6, 22:5n-6 ,16:3n-3, 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 16:2n-4, 20:3n-9, and was therefore not included .

[a] Related Wilcoxon signed rank for comparison for differences pre- and post-intervention within the control- and intervention- group

[b]Mann-Whitney U Test for comparison for differences between the control- and intervention- group post-intervention

[c] Difference between groups at post-intervention

[d] Differences within groups between baseline and post-intervention.

3.4 Reported Seafood intake baseline and post- intervention

3.4.1 Summary question from FFQ.

The summary questions for dinner, warm lunch and spread at baseline and post-intervention are shown in figure 3.2 and figure 3.3 as frequency (%) of intake. The highest frequency of seafood as dinner were the 43% that reported eating seafood 2-3 times per week, this increased to 69% at post-intervention. At baseline, spread was the only category that had a frequency intake of ≥4 times a week. At post-intervention all categories were represented in the ≥4 times/week with 2.8% for spread, and both dinner and warm lunch went from 0% to 1.9%. The lowest frequency for dinner at post-intervention was 1-3 times/month, compared to the 4% at baseline that reported consuming seafood for dinner 'never' or '<1 time/month'. The percentage of spread went up for the three lowest frequencies at post-intervention.

Figure 3.2 *Frequency of seafood intake as dinner, lunch and spread at baseline in percentage from the food frequency questionair, in pregnant women in Mommy's Food.*

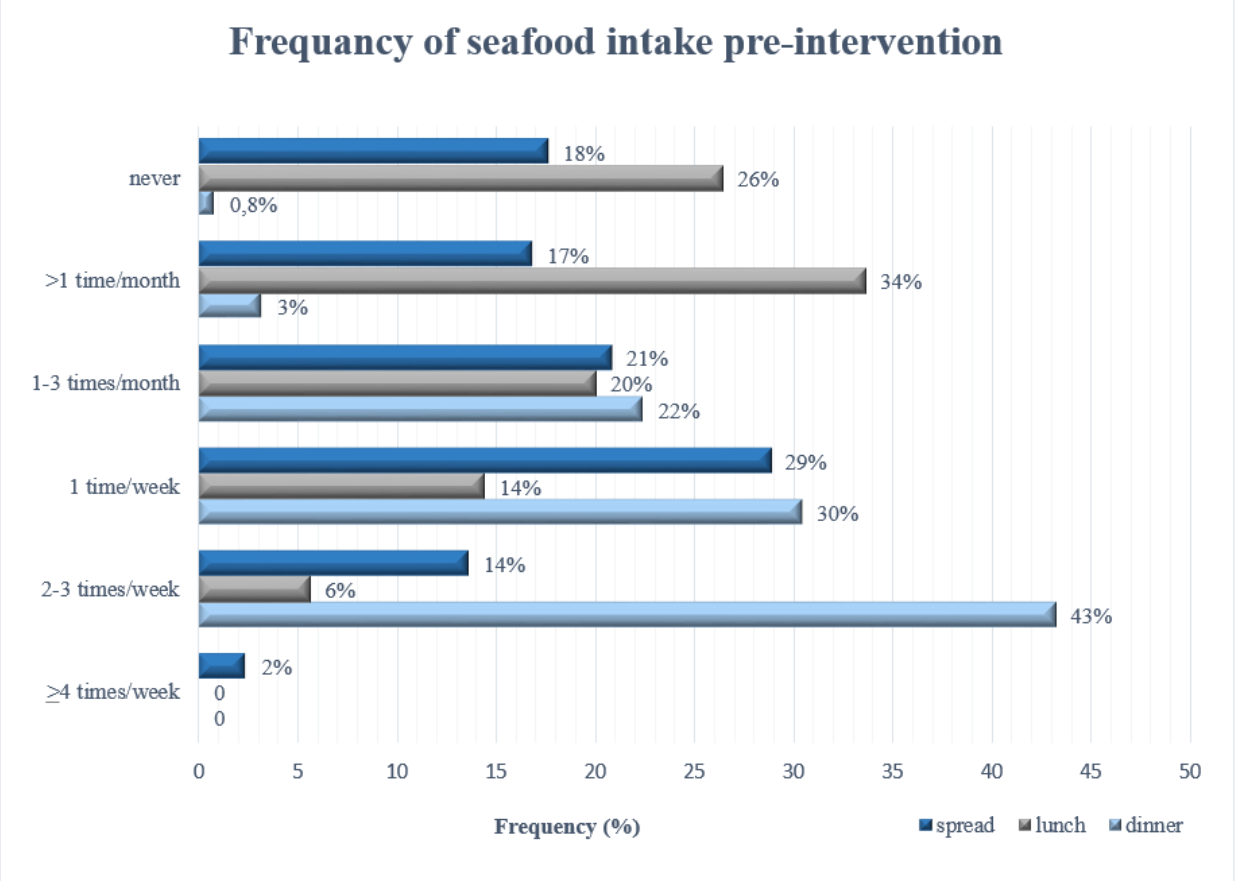
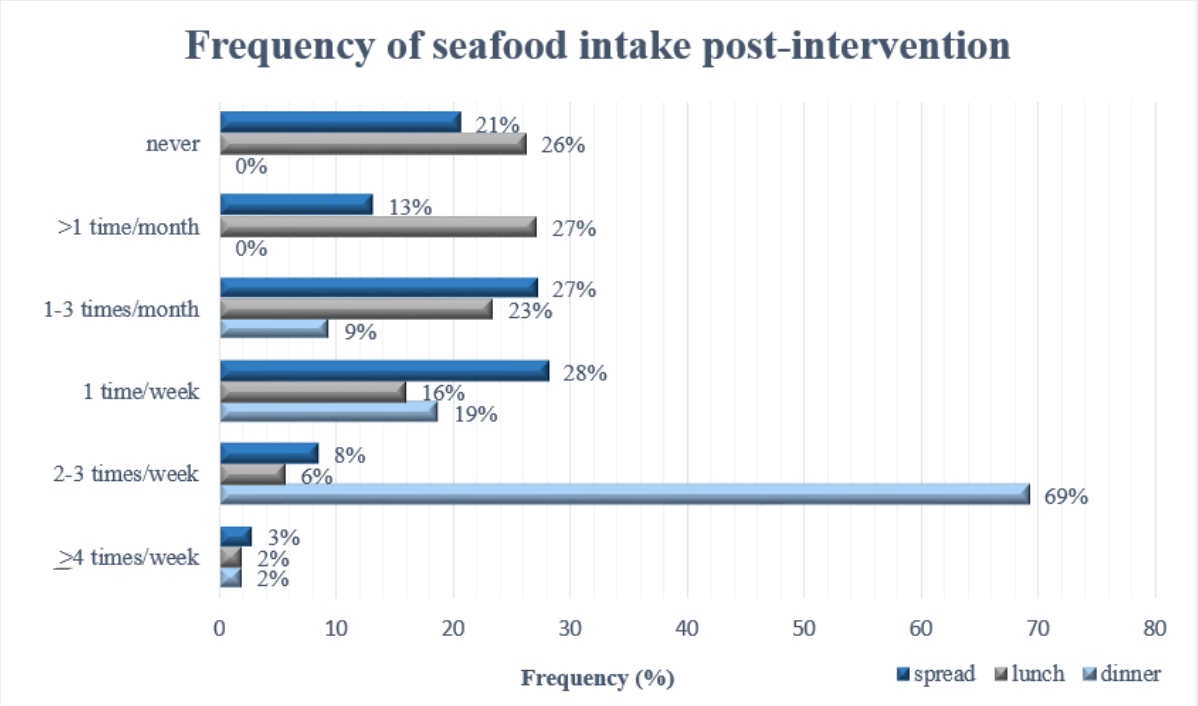


Figure 3.1 *Frequency intake of seafood as dinner, lunch and spread at post-intervention in percentage from the food frequency questionair, in pregnant women in Mommy's Food.*



3.4.2 Seafood and omega-3 supplement intake

The seafood intake in portions per week from the baseline and post-intervention FFQ is given in table 3.4. The intervention-group had a statistically significant higher intake of seafood as dinner, lean fish, total fish, and lower fatty fish than the control-group at post-intervention.

There was no statistically significant difference in the seafood intake in the control-group between baseline and post-intervention. The intervention-group had statistically significant increase of lean fish, seafood for dinner and total fish. There was a statistically significant decrease of fatty fish, seafood as spread and processed fish. There was however no significant change in the total seafood intake either between or within groups. Among the participants 82.8% reported taking a form of n-3 supplement at baseline, with no change at post-intervention, and no statistically significant difference between the groups.

Table 3.4 *The reported seafood intake from the FFQs at baseline and post-intervention, given in portions per week, in pregnant women in Mommy's food trial.*

Seafood categories	All Median (IQR)				Intervention group Median (IQR)		P [a]	Control Group Median (IQR)		P [a]	P [b]
Summary question	n	Pre interv.	n	Post, interv.	Pre interv.	Post interv.	in.	Pre interv.	Post interv.	In.	Btw
Seafood dinner	127	1.3 (1.8)	107	2.50 (1.25)	1.5 (2.0)	2.5 (0.0)	**.000**	1.0 (1.7)	1.5 (1.5)	.288	**.000**
Seafood lunch	127	0.2 (0.5)	107	0.15 (0.5)	0.2 (0.5)	0.3 (0.5)	.764	0.2 (.50)	0.08 (.50)	.976	.328
Seafood spread	127	1.0 (1.9)	106	0.5 (1.4)	1.0 (1.9)	0.5 (0.9)	**.025**	1.0 (1.9)	0.8 (1.55)	.761	.632
Detailed questions											
Warm meal											
Fatty fish	121	0.6 (0.9)	104	0.4 (0.8)	0.5 (.85)	0.3 (0.4)	**.001**	0.9 (.9)	0.8 (0.9)	.630	**.000**
Lean fish	123	0.5 (0.8)	105	1.0 (0.7)	0.6 (.90)	1.1 (0.5)	**.000**	0.5 (0.5)	0.5 (0.5)	.459	**.000**
Cod	123	0.3 (0.4)	106	1.0 (0.8)	0.3 (0.8)	1.0 (0.1)	**.000**	0.3 (0.3)	0.3 (0.3)	.566	**.000**
Processed fish	113	0.5 (0.3)	106	0.3 (0.3)	0.5 (0.3)	0.2 (0.2)	**.000**	0.4 (0.5)	0.3 (0.3)	.003	.118
Spread											
Total spread	121	0.9 (2.4)	106	0.8 (1.9)	0.6 (2.3)	0.8 (1.8)	.105	1.0 (2.6)	0.6 (2.4)	.137	.586
Warm meal & spread											
Total shellfish	124	0.1 (0.2)	107	0.08 (0.2)	0.1 (0.2)	0.07 (0.2)	.176	0.1 (0.2)	0.08 (0.2)	.538	.493
Total fish	120	1.4 (1.2)	104	1.4 (1.1)	1.3 (1.4)	1.5 (0.9)	**.001**	1.5 (0.9)	1.2 (1.3)	.513	**.029**
Total seafood	114	3.1 (3.9)	100	2.8 (2.8)	3.0 (4.1)	2.70 (2.3)	.258	3.1 (3.7)	2.9 (3.4)	.163	.825
Supplements n-3, n (%)	n=122		n=106		n=63	n=56		n=59	n=50		
Yes	101 (82.8)		87 (82.1)		53 (84.1)	46 (82.1)	1.00	48 (81.4)	41 (82.0)	1.00	.985
No	21 (17.2)		19 (17.9)		10 (15.9)	10 (17.9)		11(18.6)	9 (18.0)		

Abbreviations: interv: intervention, btw: between, in= within.

[a] Related Wilcoxon signed rank for comparison for differences pre- and post-intervention within the control and intervention group.

[b] Mann-Whitney U Test for comparison for differences between the control and intervention group post-intervention

3.4.3 Compliance of intervention

The mean compliance score was 76 and ranging from 35 to 102 as shown in table 3.5. That gives a mean total intake of 4884 g out of maximum of 6400 g of cod, the mean weekly intake was 305 g. About 50% of the group had a compliance score of 80 or more, and less than 10% had a compliance score under 50 (data not shown).

Table 3.5 *The intervention-group's compliance in consumption of cod from the weight registration form during the 16-week intervention period, in the Mommy's Food trial.*

Compliance of intervention	Mean (SD)	Median (IQR)	Min-max
Total intake of cod (grams)	4884 (987.1)	5084 (1279)	2254-6556
Weekly intake of cod (grams)	305 (62)	318 (80)	141-410
Compliance score	76 (15)	79 (20)	35-102

3.5 Correlation between fatty acids and seafood, cod and omega-3

A Spearman's rank order correlation was run to assess the relationship between the total seafood and fish intake, and the relative- or absolute- amount of n-3 index in both groups at post-intervention. In the intervention-group there was a statistically significant, weak positive correlation in the relative-amount, r_s = .295, ($p<.032$), and the absolute-amount r_s = .458, ($p<.001$), There was no statistically significant correlation in the control group for the total seafood and fish intake and the n-3 index in the relative amount, r_s = .245, ($p<.109$) or the absolute amount r_s= .280 ($p<.065$).

The Spearman's rank order correlation was also run to assess the relationship between the cod intake from the FFQ and the absolute-amount of the Σ n-3 in the groups at post-intervention. In the intervention-group there was a statistically significant, weak positive correlation, r_s = .359, ($p<.007$). There was no statistically significance correlation in the control group, r_s = .206, ($p<.165$). Running a Spearman's rank order, there was no statistically significant correlation between the cod intake from the FFQ and the relative-amount of the Σ n-3 at post-intervention in the intervention-group, r_s= .111 ($p<.420$), or the control-group r_s= .097 ($p<.517$).

A Spearman's rank order correlation was run to assess the relationship between the cod intake from the FFQ and the relative- or absolute- amount of DHA in both groups at post-intervention. In the intervention-group there was a statistically significant, weak positive correlation between the cod intake from the FFQ and the absolute- amount of DHA, $r_s = 0.334$ ($p<.013$). However, this correlation was not observed between the cod intake from the FFQ and the relative- amount of DHA, $r_s = 0.098$ ($p<.477$). For the control group there was no statistically significant correlations between the cod form the FFQ and DHA absolute-amount $r_s = 0.199$ ($p<.179$) or DHA relative-amount $r_s= 0.060$ ($p<.688$).

Spearman's rank order correlations were run again to assess the relationship between the total cod intake from weight registration forms and relative- or absolute- amounts of DHA or Σ n-3 for both groups at post-intervention. There was found no statistically significant correlation between any combination or groups, $r_s <.200$ ($p>.05$).

4 Discussion

Seafood is a good source of the LCn-3FAs EPA and DHA, and during pregnancy higher levels of DHA has been linked to beneficial health outcomes for mother and child [130, 131]. There is currently lacking in RCT studies of seafood intake during pregnancy and FAs, and the existing ones does not focus specifically on FAs in relation to lean fish [5, 11]. Consequently, this thesis may shed some light on what impact lean fish consumption in pregnancy have on marine associated LCn-3FAs. The overall aim of this thesis was to investigate if an increased intake of cod during pregnancy have an impact on FA content in RBC, with focus on the marine associated LCn-3PUFAs, in addition to evaluating seafood and n-3 supplement intakes at baseline and post- intervention. In the two-armed RCT Mommy's Food study the FA content was measured by UFGC-FID, and seafood intake reported in FFQs at baseline and post-intervention. The intervention-group were instructed to consume 400g of cod between GW 20-36, while the control-group continued their habitual diet.

4.1 Discussion of main findings

There were no significant changes between the intervention- and control-group FA statuses at post-intervention, respectively. There were however significant changes within both groups, between baseline and post-intervention. There were significant decrease in Σ n-6 FAs seen in both groups for the relative- and absolute- amounts, this is likely due to the maternal decrease in n-6 FAs commonly seen in the last trimester [115]. Women are also known to have this progressive decline in DHA and other n-3 FAs during pregnancy due to the increased demands of the fetus in the 3rd trimester [114, 115, 132]. However, this DHA, Σ n-3 and n-3 index decline was not present in either the control- or intervention-group. The n-3 index levels stayed desirable and stable without significant changes within, or between the groups. The intervention group had a statistically significant increase in the relative-amount of DHA between baseline and post-intervention. The n-3:n-6 increased for both groups, likely linked with the decrease in Σ n-6 and stability in Σ n-3.

4.1.1 Increase in relative-amount of DHA in the intervention group

During pregnancy there is a decrease in the maternal DHA due to a selective transfer of this FA to the developing fetus, and transfer is highest in the third trimester when the post-intervention blood sample was drawn (GW 36) [113, 114]. In the current study this pattern was not observed. The intervention group had a significant increase in the relative-amount of DHA from 7.5% to 7.7% ($p<0.002$), though the increase was not significant in absolute-amount.

The DHA levels of the control group did not have a statistically significant increase, and there was no significant difference between the groups. The increase in the maternal DHA indicates that the intervention group had a higher n-3 intake than reported in other studies where maternal decline is present. The higher n-3 intake enabled the mother to meet the fetus' increased demand without depleting the maternal DHA storage [113, 115]. The maternal EPA and DHA levels are found to be reflected in the n-3 status of the fetus. Therefore, a good maternal DHA level without decline would indicate that the fetus' status is good and it is getting adequate DHA levels during development [28]. High maternal DHA in pregnancy is associated with better vision acuity and brain development in the fetus, as large levels of DHA resided in both the brain, CNS and the retina[133]. Having an increase in the 3rd trimester is especially good as this is the time of increased fetal demands due to the brain growth spurt. Children of mother with good DHA levels are seen to have a higher birth weight, and higher scores on standardized IQ tests [3, 84-88]. In the mothers, higher DHA levels are associated with lower levels of preterm delivery, inflammation, and postpartum depression [2, 5, 61, 84, 130]. The increase in DHA levels in the intervention group, and stability in DHA levels of the control group is therefore a favorable outcome.

Cod is a good source of LCn-3PUFAs, as one portion of 200g contains double the daily adequate level of EPA and DHA set by EFSA [7, 13]. The increase in DHA levels seen only in the intervention group suggested that cod could be an influence, as it was the only reported DHA source to significantly increased during the intervention-period. The cod intake from the FFQ or the cod intake from the weight registration form, and relative- or absolute- amount DHA level was tested for a correlation. There was no correlation between the cod intake from weight registration forms or the FFQ and the significant increase in the relative-amount of DHA. There was a weak correlation between the absolute-amount of DHA and FFQ cod intake. However, this correlation was not seen between the absolute-amount of DHA and the actual cod intake from the weight registration forms. As the cod intake from the weight registration forms are more accurate than the FFQ there is likely no relationship between the cod intake and the absolute amount of DHA. The cod intake was therefore not correlated with the increase seen in the relative-amount of DHA or the stability in the absolute-amount, in the Mommy's Food study. The n-3 supplementation can be a possible explanation for the increase in the relative-amount of DHA. As it is found to be an effective means to retain maternal DHA levels during pregnancy, and ≈82% of the participants reported taking n-3 supplements throughout the study [115].

4.1.2 Stability of the Σ omega-3 and omega-3 index in both groups

An n-3 index of >8% is associated with greater cardio protection and is set as the desirable n-3 index. While 8%-4% is considered to offer intermediate protection and <4 is considered to have lower protection [134]. Though an index of ≤5% is still found to reduce the risk of primary cardiac arrest with 70% [107, 135]. The intervention- and control- group had the same median n-3 index at both baseline and post-intervention, with no significant difference between or within the groups. Both groups had an n-3 index >8%, meaning they had an optimal level of EPA and DHA in relation to CHD and other n-3 associated health benefits [134].

The stability and high n-3 index present in both groups indicates a supplementation habit or a diet rich in EPA and DHA. When testing for a relationship between the n-3 index and seafood intake from the FFQ the intervention-group had a weak positive correlation. However, no correlation was seen in the control group. The intervention-group's n-3 index was than tested for a correlation with the cod intake. The same correlation pattern seen in cod intake and DHA was present. There was no relationship between the increase in n-3 index relative-amount and the cod intake from the FFQ or the cod intake from the weight registration form. There was however a weak positive correlation between the FFQ cod intake and the n-3 index absolute-amount. This indicates the same as with DHA, that there is no correlation between the dietary cod intake and the stability in n-3 index. The intervention groups had a weak correlation unlike the control group, indicating that the seafood consumed in the intervention group have a large impact on the n-3 index than the seafood in the diet of the control group, despite the fact the intervention cod was not correlated. The control group's EPA and DHA was not correlated with the seafood intake and therefore suggesting that the n-3 supplementation is responsible for the high n-3 index in this group.

The study population had a median n-3 index of 8.4% at baseline and 8.5% at post intervention which was higher than the average previously found in Norwegian pregnant women of 6.4%, and US average between 3%-6% [20, 105, 108, 136, 137]. An n-3 index can be used as a biomarker to check for compliance of n-3 intakes from supplement or diet [105]. A higher than average n-3 index such as in this study population would therefore indicate intakes of LCn-3PUFAs that was higher than average population, at both baseline and post intervention [20]. The lack of decline in Σ n-3 FAs and a desirable stable n-3 index such as the study group had throughout their pregnancy, is associated with the same beneficial health outcomes as aforementioned with a higher level of DHA [87].

4.1.3 Increase in the omega-3:omega-6 ratio

AA, like DHA is important for brain and retinal development during gestation, though a much higher levels of n-6:n-3 is not beneficial for the health as the humans evolved on a ratio closer to 1:1 [77, 138]. There was a significant increase in the n-3:n-6 ratio for both groups between baseline and post-intervention. The main influencer of the change in ratio is likely owing to the significant decrease of the Σ n-6 FAs. When the diet is rich n-3 the n-6 levels in the body have been known to decrease, though in this study the n-6 decrease is likely due to the maternal decline commonly seen in the last trimester [113, 114]. Since there were significant increases for the n-3:n-6 ratio seen in both groups, and no significant difference between the two it is likely due to metabolic and dietary factors present in both groups. Therefore the dietary cod is unlikely to be the reason for the significant increase in the n-3:n-6 ratio. The study population had a n-3:n-6 ratio at baseline and post-intervention that was close to 1:2 (= 0.5 μg/g, 0.5%). A ratio of 1:2 is higher in n-3 than that associated with an average western diets, which ranges from 1:10 up to the American average of 1:25 [78, 80]. The study population's ratio is desirable as it is the recommended ratio of n-3:n-6 set by a panel of lipid experts [81]. Higher levels of n-3:n-6, like the ones in the study population, is associated with beneficial health outcomes for childhood obesity, neural development and CHD. As well as being less inflammatory due to higher levels of n-3 derived anti-inflammatory eicosanoid and cytokines, which is associated with lower levels of autoimmune diseases, asthma, eczema and allergies [2, 77, 79, 139-141].

4.2 Discussion of findings in seafood intake and study population

4.2.1 Changes in seafood intake reported from the food frequency questionnaire

The significant differences seen in seafood intake at post-intervention between the intervention- and control- group can all be linked to the intervention group's increased intake of cod. The control group followed their habitual diet during the intervention period and had no significant differences between their baseline and post-intervention seafood intakes. The intervention group's increased intake of lean fish resulted in a significantly lower intake of fatty fish, they went from consuming 0.5 portion of fatty fish per week to 0.3 portions per week. This is not desirable as the NDH recommends half of the of 2-3 portions of fish per week to come from fatty fish, in order to achieve the recommended amount of EPA and DHA [10]. However, there was no reduction in Σ n-3 FAs or the n-3 index despite the decrease in the fatty fish intake. This means that the intervention group was getting adequate levels of EPA and DHA and would still have protection against CHD despite reduction in fatty fish intake.

EFSA concluded that a fish/seafood intake of 1-2 up to 3-4 portions per week during pregnancy was associated with better neural development and protection against CHD [7]. The majority of the study population followed this advice, as 73% at baseline and 91% at post-intervention reported eating seafood for dinner ≥1 time/week. The reported frequency of seafood for dinner increased at post intervention, as 71% reported consumed seafood for dinner ≥ 2-3 times/week compared the 43% at baseline. In addition at post intervention 38% reported eating seafood as spread ≥ 1 time/week, and 24% eating seafood as warm lunch ≥ 1 time/week.

4.3 Discussion of method, limitation and strengths

4.3.1 High intake seafood and omega-3 supplement in the study population

The women of study population had a high intake of seafood and n-3 supplements as shown in the n-3 index, and the higher than average social-economic status is a possible explanation for this. As 86% of participants had a higher education, this was more than double of the national average 37% for women [142]. The national average household income for couples with children of 0-6 year is 743 000 NOK, and couples without children under the age of 45 is 586 000 NOK. This falls within the household income category of 350 000-749 999 NOK, where only 28% of the study population reported their combined household income to be [143]. In addition, 63% reported a household income between 750 000 NOK and up, which is above the national average in both categories. The average age was the same as the Norwegian average age for first time mothers of 29 year [144]. A higher social-economic status is associated with a healthy habits such as taking recommended supplements and higher intake of healthy foods such as fruit, vegetable and fish, and this may explain the high baseline intake of seafood and n-3 supplements in this study population [145, 146].

Another possible explanation for the high seafood intake is that the study required participants in the intervention group to consume cod twice a week. This requirement would not have the same appeal to women who never eat fish compared to the women regularly consumed fish. This is shown in the 99.2% of women who reported eating seafood for dinner at baseline. The NDH have no n-3 recommendation for the general population, but recommends pregnant women to consume 200 mg/day of DHA, possibly explaining why the pregnant study population have a high n-3 intake [147]. Having a baseline population with high intake of seafood and n-3 supplements containing LCn-3PUFA makes it hard to assess the impact of dietary LCn-3PUFA from cod.

4.3.2 Presentation of relative- and absolute amount of fatty acids.

FAs were presented in both relative- and absolute- amount. When there is no clear methodology of which to use it can be beneficial to present both [148, 149]. Presenting FA as relative- or absolute- amounts can yield different results. When Mocking (2012) analyzed the difference between the two using a Pearson's correlation, he found the correlation to vary from $r_s = 0.3$ to $r_s = 1.0$ for the same FA. The variation is greatest when the FA is strongly correlated to the total FA concentration [148]. This is seen in the results for the intervention group's 18:0 (stearic acid) which makes up a large part of the concentration (15% and 300 μg/g) and have a large variation in the p-values at post-intervention for the relative amount ($p<.005$) and absolute amount ($p<.436$).

4.3.3 The impact of omega-3 supplementation.

Throughout the study ≈82% of the study population were taking n-3 supplementation. This was 7% higher than the national average for pregnant women, and 47% higher than the national average for adults [8]. There were no changes in the amount of women taking supplement between baseline and post-intervention ($p<1.000$) or between the groups ($p<.985$). However, the amount and frequency of n-3 supplementation was not taken into consideration during statistical analysis, only whether participants were taking supplements or not. Due to this the actual amount of n-3 provided from supplements could vary between participants, groups, and between baseline and post-intervention. It is therefore difficult to say how large an impact the n-3 supplementation has on the n-3 FA status. As n-3 supplements have been found to increase the n-3 status during pregnancy, and the control group's n-3 index was not correlated with seafood intake, the high n-3 supplement intake in the study population might be responsible for the stability observed in n-3 FAs [115]. Optimally, in order to find the true impact of cod and seafood on marine associated n-3 FAs, neither group would take supplementation, but that is not ethically possible as pregnant women are recommended by EFSA to get 350-450 mg/day of EPA+DHA per day, and 200mg/day of DHA by the NDH [7, 147].

4.3.4 Seafood-index calculation of dietary cod

Getting self-reported intake from an FFQ is a relatively inexpensive method as, it is easier for participants to undertake and does not require trained interviewers [150]. However, FFQs are also prone to over- and under- reporting [151]. It is therefore important to use a validated FFQ which is a strength of this study, as the FFQ is based on a validated seafood-FFQ [120].

The lowest consumption frequency was used when calculating the seafood index in the detailed questions, because of a tendency among participants of over reporting intakes when asked detailed questions about specific seafood species [121]. This was in accordance with the method [120]. When participants reported eating cod 1-2 times per week that was be calculated as 1 time per week, and one portion of cod equals 200g. The intervention group's reported median intake of lean fish was 1.1 portion per week equaling a maximum of 220 grams of cod. The median intake of cod was also calculated from the weight registration forms to be 318 g or 1,6 portions of cod per week. The difference seen between the reported intake from the FFQ, and the median intake from the weight registration forms indicated that the method underestimates the cod intake for the intervention group. This is further supported by the compliance score showing less than 10% had a compliance score under 50, which is 1 portion of cod per week. Therefore, most of the participants likely had intake closer to 2 portions per week than 1 portion per week. Cod was such a large part of the intervention group's seafood intake that an underestimation would affect the other calculations from detail question such as total seafood and fish intake. Using an average intake instead of the lowest frequency could result in a cod intake closer to the true median in this particular study.

4.4 Conclusion

In conclusion, there was no significant difference for the FA content between the groups at post intervention. Therefore, in this study, an intervention with dietary cod did not have a significant impact on the marine associated LCn-3PUFA in RBC of pregnant women with a high intake of seafood and a large percentage taking n-3 supplements. There was a significant increase in the intervention group's relative-amount of DHA, though this was not correlated with the intake of cod. A plausible explanation for the increase, is the high percentage of women taking n-3 supplement, though further testing is needed to establish this.

There was a significant decrease in the Σ n-6 within both groups between baseline and post-intervention. This was likely due to the increased demand from the fetus in the third trimester rather than dietary changes [113, 114]. The Σ n-3 was stable for both groups meaning the mothers had adequate dietary intakes to maintain the LCn-3PUFA level. The stable and higher than average n-3 index of >8% for both groups indicate a diet rich and above average in LCn-3PUFAs at both baseline and post-intervention. The increase seen in the n-3:n-6 ratio was likely due to the decrease in n-6 status, and assisted by the stable n-3 status.

The study population had a high intake of seafood and n-3 supplementation, possibly due to the higher social economic status. There was a change in the seafood intake of the intervention group; the increased cod intake resulted in a reduction in fatty fish intake, this did not negatively impact the n-3 levels. A possible underestimation of the cod intake in the intervention group for the detail question when comparing it to the weight registration forms was seen.

4.5 Future perspectives

This thesis provides new information about dietary cod's impact on marine associated LCn-3PUFAs status in pregnant women with a habitual diet relatively high in n-3. Future studies should have a larger study population that assess the impact of cod on pregnant populations with a habitual diet closer to the average n-3 intake, which would be more representative of the general Norwegian population. A future studies could also test the impact of cod consumption in other populations than pregnant women, as the impact might be different during pregnancy due to physiological changes. In a larger study it could be beneficial to calculate the exact n-3 supplementation intake, as it can have a large impact on the marine associated LCn-3PUFA status in RBC.

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Appendices

- **Appendix I:** Questionnaire (FFQ) post-intervention
- **Appendix II:** IMR Protocol 435 - FA with Hamilton robot and UFGC-FID

Appendix I: Questionnaire (FFQ) post-intervention

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, vaffer, 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:

	1-3 ganger/måned	1-3 ganger/uke	4-6 ganger/uke	Daglig	Når du tar tilskudd, hvor mye tar du vanligvis ift. anbefalt mengde på flasken/pakken? 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: IMR Protocol 435 - FA with Hamilton robot and UFGC-FID

1. ANVENDELSESOMRÅDE

Beskrivelse av metode for bestemmelse av fettsyresammensetning av totalfettsyrer i vevsvæsker, fortrinnsvis RBC, serum, plasma og fullblod. Metoden implementerer metode 354 og (metode 041 som er akkreditert) i redusert skala og med ultra fast gasskromatografi (UFGC).
Resultatene er samsvarende med metode 041, men i denne metoden får man ikke separert de enumettede fettsyrene, disse oppgis som 16:1, 18;1, 20:1 og 22:1.

Metode 041 Fettsyresammensetning benyttes for næringsmidler og fôr.

2. PRINSIPP

- Tilsetting av intern standard
- Tilsetting av metyleringsreagens
- Direkte transesterifisering ved hjelp av varmeblokk
- Ekstraksjon av metylesterene
- Gasskromatografisk seperasjon og deteksjon med flammeionisasjonsdektektor
- Integrering og beregning av de ulike fettsyrene i prøven i % og mg/g.

3. SIKKERHET

Det bør jobbes i avtrekkskap og brukes hansker ved arbeid med løsemidler. Det anbefales at det brukes vernebriller og hansker ved arbeid med syrer og baser. For å unngå klemskader ved bruk, skal roboten ikke røres når den er operativ.

Analytiker skal sette seg inn i risikovurderinger som angår metoden. Risikovurderinger er lagret her: F:\HMS\Risikovurdering. Risikovurdering er utført for gass, gasskromatograf (GC), og robot.

Ved bruk av ultralydbad følges egen sikkerhetsinstruks. HMS.13.INS-04; sikkerhetsinnstruks for ultralydbad.

Ved analyse av smitteførende prøver (humane prøver) følges egen sikkerhetsprosedyre. HMS.13.INS-02; Instruks for oppbevaring og håndtering av humane prøver. Prøvene regnes ikke lenger som smitteførende etter koking på varmeblokken. Alle prosjekter blir risikovurdert og analytiker skal sette seg inn i risikovurderingen før analysene startes. HMS.13.ARB-02; Risikovurdering av humane prøver

Roboten står i avtrekk, hele dette området pluss robot er regnet som smittefarlig og er derfor merket med gul tape. Når prøvene er overført til dypbrønnsplaten og kokt på varmeblokken, er prøvene i brønnene ikke lenger smittefarlige. Prøvene ekstraheres og overføres videre av roboten til en ny dypbrønnsplate, som dermed kan tas med til GC-en for kjøring. Reagenser og humane prøver skal fjernes fra roboten umiddelbart etter at prøvene er ferdig opparbeidet på roboten. Spisser som brukes av roboten faller ned i en pose, posen fjernes ved endt kjøring og settes i avtrekk til neste dag. Deretter lukkes posen og plasseres i søppelbøtte som vanlig boss. Roboten vaskes med 70 % sprit etter hver opparbeiding.

4. KJEMIKALIER

4.1 Metanol, (CH_3OH), HPLC
4.2 Svovelsyre, (H2SO4)
4.3 Renset vann, (H_2O)
4.4 Heptan, (C7H16), HPLC
4.5 Destillert vann, (H_2O)
4.6 Luft, komprimert
4.7 Helium, kvalitet 6.0
4.8 Hydrogen, kvalitet 5.0

4.9	Nitrogen, kvalitet 5.0	
4.10	19:0 Metyl, (methylnonadekanoate), Larodan	
4.11	Tran, Petter Møller:	14:0, 15:0, 16:0, 16:1n-x, 17:0, 18:0, 18:1n-x, 18:2n-6, 18:3n-3, 18:4n-3, 20:1n-x, 20:2n-6, 20:4n-6, 22:1n-x, 20:4n-3, 20:5n-3, 24:1n-9, 22:5n-3, 22:6n-3
4.12	Standard Nu-Chek, 06A:	16:0, 18:0, 20:0, 22:0, 24:0
	Standard Nu-Chek, 2A og B:	18:0, 18:1n-9, 18:2n-6, 18:3n-3, 20:4n-6
	Standard Nu-Chek, 20:Xn-x:	20:3n-3 (methyl 11-14-17 eicosatrienoate) 20:3n-6 (methyl homogamma linolenate) 20:2n-6 (methyl 11-14 eicosadienoate)
	Standard Nu-Chek, 14A:	13:0, 15:0, 17:0, 19:0, 21:0
	Standard Nu-Chek, 3A:	18:2n-6, 18:3n-3, 20:4n-6, 22:6n-3
	Standard Nu-Chek, 7A:	16:1n-7, 18:1n-9, 20:1n-9, 22:1n-11, 24:1n-9
	Standard Nu-Chek, 68D:	14:0, 14:1n-9, 16:0, 16:1n-7, 18:0, 18:1n-9, 18:1n-7, 18:2n-6, 20:0, 18:3n-3, 20:1n-9, 20:2n-6, 22:0, 20:3n-3, 20:4n-6, 22:1n-9, 20:5n-3, 24:0, 24:1n-9, 22:6n-3.
4.13	Standard enkelt fettsyrer:	16:2n-4, 16:3n-3, (16:4n-3) 20:3n-9, 21:5n-3, 18:3n-6, 22:4n-6, 22:5n-6, 24:5n-3, 24:6n-3

Standarder på enkeltfettsyrer kjøpes enkeltvis hos Larodan eller Sigma i ulike konsentrasjoner. 14:1n-9 og 16:4n-3 kan ikke kjøpes separat. 14:1n-9 finnes i blanding i Nu-Chek 68D. Standarder som oppbevares på frys (-20°C til -80°C), er holdbar i minst 10 år.

5. LØSNINGER OG STANDARDER

5.1 <u>2 % Svovelsyre i Metanol:</u>
Fyll en 100ml målekolbe halvfull med metanol (4.1) og tilsett 2 ml svovelsyre (4.2). Fyll opp til merket med metanol og bland godt.

5.2 <u>Intern standardløsning 0.4 mg/ml:</u>
Vei inn, med 4 desimaler, 40 mg 19:0 Metyl (4.10) i en 100 ml målekolbe, fyll opp til merket med metanol (4.1). Bland godt, sett løsningen på ultralydbad i 15 minutter og se etter at standarden er fullstendig oppløst og har klar farge. Ved bruk sjekk at det ikke har oppstått utfelling.

Standarden merkes med innveid mengde, fortynning, konsentrasjon, laget dato og initialer. Skjemaet Intern Standard Fettsyrer (LAB.NÆR.SKJ.ANAJ-22) fylles ut og det skrives en merknad i kontrollkortet når ny standard er tatt i bruk. Standardløsningen skal være godkjent før den er tatt i bruk (testes med kontrollprøve).

Standardløsningen fordeles i fire rør på roboten, overskuddet av standarden i disse rørene blir tømt i avfallsfaske merket med tallkode 7042. Etter bruk av intern standardløsning, settes en merke på målekolben der det viser hvor mye standard det er igjen i målekolben, merk også med dato og initial.

Fettprosenten i humane prøver i plasma, serum og RBC ligger rundt 0.2 til 0.4 %.
19:0 Metyl bør tilsettes i en slik konsentrasjon at den utgjør 7 %<>30 % av total fettsyremengde.
60 ul av 0.4 mg/ml standardløsning tilsettes hver prøve, mengde tilsatt 19:0 blir da 0,024 mg. Dette utgjør12 % når fettprosenten er 0.2.

Løsninger av intern standard som oppbevares ved romtemperatur er holdbar i minst 1 år.

5.4 Blandingsforhold Standard Tranblanding:

Nøyaktig konsentrasjon på standard Tran blanding er ikke nødvendig, da denne bare brukes til identifisering av fettsyrene.

Løsning 1, me-estre 5 mg/ml - lages fra tran, veies, forsåpes, metyleres og fortynnes.
Løsning 2, 4A 2,5 mg/ml – ampulle løses i hexan, fortynnes.
Løsning 3, 06A 0.1 mg/ml - ampulle løses i hexan, fortynnes.
Løsning 4, 2B 0.1 mg/ml - ampulle løses i hexan, fortynnes.
Løsning 5, 20:Xn-x 0.1 mg/ml - en ampulle av hver løses i hexan, blandes, fortynnes.
Løsning 6,14A 0.1 mg/ml - ampulle løses i hexan, fortynnes.

Videre blandes:
2ml av løsning 1
100µl av løsning 2
4 ml av løsning 3
1 ml av løsning 4
2ml av løsning 5
4ml av løsning 6.
Denne blandingen kaller vi stamløsning og den fortynnes 2:8.

For å få identifisert alle ønskede fettsyrer må det også tilsettes noen enkelt fettsyrer.
Ta ut 2 ml av stamløsningen tilsett deretter de ulike fettsyrene slik at de utgjør en ca sluttkonsentrasjon i henhold til tabell under når total volumet på Standard Tran blanding er 10 ml.

16:2n-4 0,01 mg/ml, (tilsett for eksempel 200µl av 0,5 mg/ml 16:2n-4)
16:3n-3 0,01 mg/ml, (tilsett for eksempel 200µl av 0,5 mg/ml 16:3n-3)
20:3n-9 0,005 mg/ml, (tilsett for eksempel 250µl av 0,2 mg/ml 20:3n-9)
21:5n-3 0,005 mg/ml, (tilsett for eksempel 20µl av 2,5 mg/ml 21:5n-3)
18:3n-6 0,025 mg/ml, (tilsett for eksempel 250µl av 1 mg/ml 18:3n-6)
22:4n-6 0,025 mg/ml, (tilsett for eksempel 100µl av 2,5 mg/ml 22:4n-6)
22:5n-6 0,025 mg/ml, (tilsett for eksempel 100µl av 2,5 mg/ml 22:5n-6)
24:5n-3 0,025 mg/ml, (tilsett for eksempel 50µl av 5 mg/ml 24:6n-3)
24:6n-3 0,025 mg/ml, (tilsett for eksempel 50µl av 5 mg/ml 24:6n-3)

6. INSTRUMENTER OG UTSTYR

Oversiktsbilde av Hamilton Microlab Star Line robot

6.1 Hamilton Microlab Star Line robot

Analysevekt, 5 desimaler
Sentrifuge
Varmeblokk/ristemaskin (heatershaker)
Nitrogen inndampingsenhet
Sealer til forsegling av platene med aluminiums-papir
300ul tips med filter til pipettering av prøver og intern standard
300ul/1000ul tips uten filter til overføring og fortynning
Robotprogram: Venus

6.2 Trace GC Ultra, Thermo Corporation
SSL- injektor
Flamme Ionisasjon Detektor (FID)
Kolonne, Wax kolonne (P/N UFMC00001010501) 5 m lang, 0.1 mm. Id., 0.1 µm filmtykkelse
Labdataprogram Chromeleon

6.3 Analysevekt, 4 desimaler.

6.4 Whirlimixer

6.5 Ultralydbad

6.6 Vannrenseanlegg

6.7 Glassutstyr:
10 ml sovirelrør, 4 ml, 2 ml, 1 ml og 100 µl prøverør, målekolber, glassflasker

6.8 Pipetter: pasteurpipetter, gummismukker, fullpipetter, automatpipetter, rainin stempelpipette

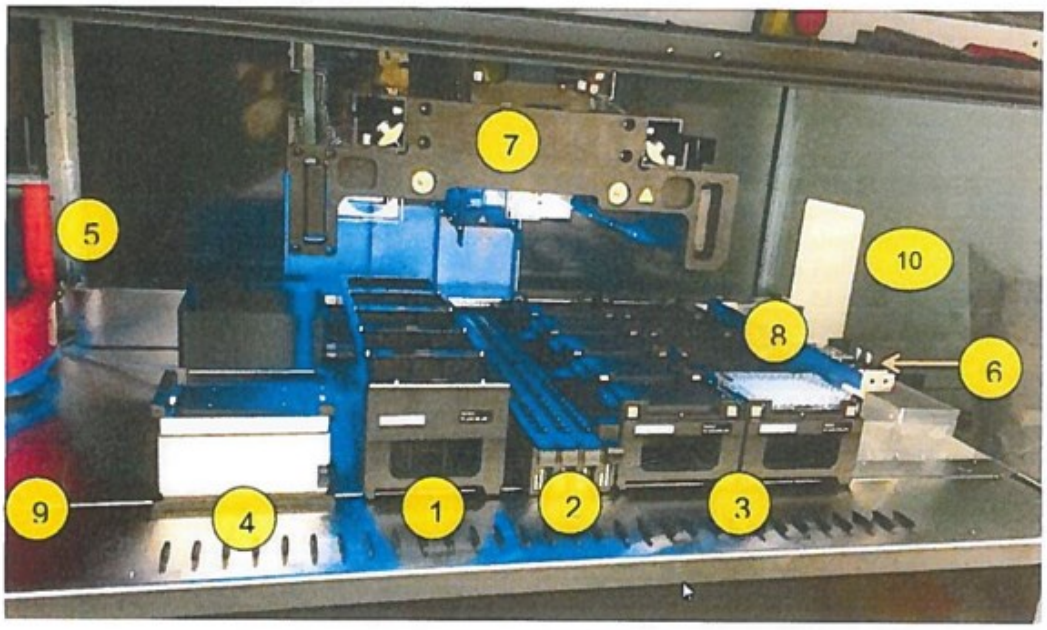
6.9 Engangsutstyr til roboten og GC: DeepWell Plate PP 2ml, pipettespisser robot 300ul/m filter, pipettespisser robot u/filter 300ul og 1000ul, thermo-seal til alps 3000, vials 2 ml og korker til GC kjøring.

6.10 Ristemaskin til blodprøver: Nutating mixer

6.11 Gass: Helium, kval. 6.0 50 liter
Hydrogen, kval. 5.0, 50 liter
Luft, kompressor.
Nitrogen, kval. 5.0, 50 liter

7. EKSPERIMENTELT

Oversikt over modulene på Hamilton robot.



7.1

#1: Tip Carrier
#2: Sample Tube Carrier 24pos & 32pos
#3: Plate Carrier 5 pos
#4: Multiflex Carrier (MFX) with Balance and HHS (HeaterShaker)
#5: HiG Centrifuge
#6: CoRe Gripper Paddles
#7: Modular Arm with Channels: 8 x 1000ul
#8: Tip-Waste with Teaching-Needles 8x1000
#9: Ultravap Evaporator
#10: ALPS300 Sealer

Oversikt over trinnene (på engelsk) i prøveopparbeidingen på roboten:

1	Init starlet	
2	GUI: select input file	
3	GUI: number of samples	
4	GUI: load the robot	
5	Move startplate to balance	

6	Pipetting: Sample 60 ul blood added to startplate, each sample is weighted	Grouping weigh samples: Moves startplate to weight if else (do part 1) submethod p03_Samples Result file handling
7	Move startplate to HHS	Grouping IS Move startplate to HHS If, else (do part 2) submethod p01_IS Layout changed moved seq_src_IS
8	Pipetting: internal standard 60 ul IS is added to startplate	
9	Pipetting: H2SO4 750 ul H2SO4 2% in methanol added to startplate	Grouping H2SO4 If else (do part 4)
12	Move startplate to sealer	
13	Sealing	
10	MIX and incubation Shake first at 1200 rpm Then 100 rpm with heating in 40 minutes at 105°C. Then shake at 1000 rpm in 20 seconds.	Added MIX step If, else (do part 5)
11	Move startplate to park Cool the plate for 15 minutes	Grouping Seal DPW If, else do part 6
14	Move startplate to park	
15	Move startplate to HHS	Grouping Pierce plate
16	Pipetting: water 250 ul of water	
17	Pipetting: Hepthan 500 ul of hepthan	
18	Mix	Grouping mix Moves plate from HHS to park
19	Move startplate from HHS to park	
20	Move plates to sentrifuge	Grouping centrifuge 1
21	Centrifuge 1	
22	Move plates from centrifuge to park	
23	Pipette : 300 ul from startplate to targetplate	
24	Move startplate to HHS	Grouping hepthan Move plate to HHS, locks plate
25	Pipette: 300 ul hepthan to startplate	
26	Mix	Grouping Mix Realease plate
27	Move startplate from HHS to park	
28	Move plate to centrifuge	Grouping Centrifuge 2 Changed to new submethod p15_centrifuge2 Disabled steps "present bucket B" and "transport counterweight to centrifuge" (counterweight already inside centrifuge)
29	Centrifuge 2	
30	Move plates from centrifuge to park	
31	Pipette: 300 ul hepthan from startplate to targetplate	
32	~~Seal targetplate~~	

7.2 GLC systemet inkluderer 6.2

For innstilling av instrumentparametere se tabell under. Temperaturprogrammet og flow hastighet på bæregass vil være avhengig av kolonne. Under er gitt eksempel på innstilling.

GC parameter	Trace GC Ultra		
Bæregass, He	Konstant flow: 0,5 ml/min med splittforhold 1:80		
Injektor:	SSL-inj		
Detektorgass luft:	350 ml/min		
Detektorgass H2:	35 ml/min		
Detektor temp:	250 °C		
Makeupgass	30 ml/min		
Injektor temp:	300 °C (konstant)		
Ovn temp:	Rate (°C/min)	Temp (°C)	Isotherm (min)
	Start	100	0,1
	50	220	1,0
	80	250	0,2
Analysetid i min.	7		

7.3 Instrumentvedlikehold

Robot vedlikehold.

Det finnes et eget program på roboten for daglig vedlikehold og et for ukentlig vedlikehold. Roboten sjekker da ut at spissene sitter skikkelig og kontrollerer diverse posisjoner og høyder. Dato og resultat blir logget automatisk av programmet

Når en tester ukentlig kontroll/vedlikehold blir programmet for daglig kontroll implementert.

Ved skifte av deler og annet større vedlikehold anbefales det å benytte veiledninger i tilhørende manualer. Disse finnes elektronisk lagret på: T:\Utstyr\Hamilton\Manuals

GC vedlikehold.

God kolonne er viktig for å få godt resultat:

Hvis separasjonen av fettsyrene blir dårlig, vurder å skifte hele kolonnen.

Ved skifte av ny kolonne

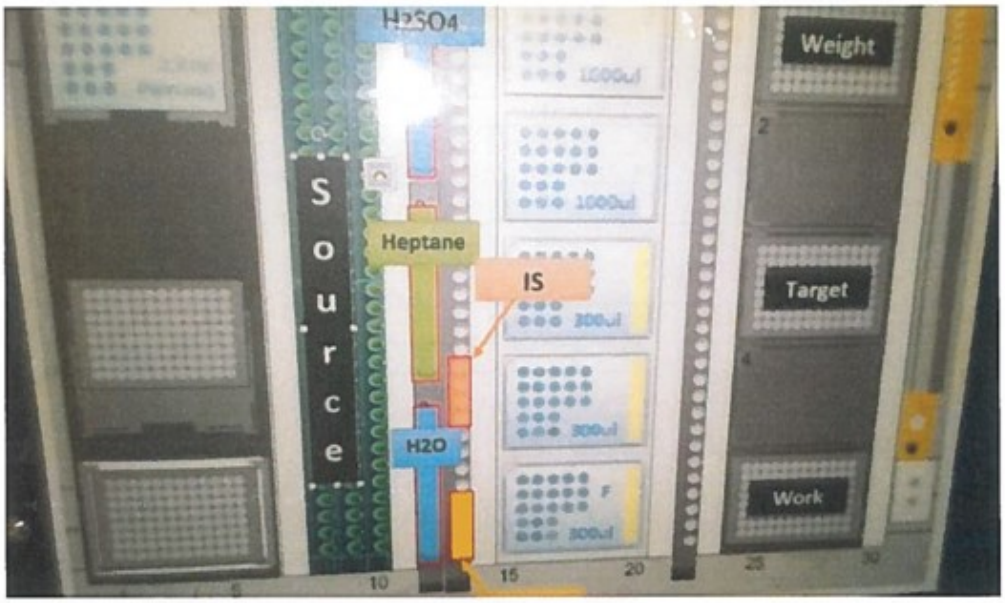
- Sjekk GLC systemet for lekkasje, se loggbok.
- Det anbefales at septum på injektor skiftes samtidig.
- Når ny kolonne settes inn, kjøres alltid noen av Nu-Chek standardene 2A, 3A, 06A, 7A og 68D og standardtranblanding (5.4). Hvis Nu-Chek standardene gir riktig resultat, (<5 % avvik fra teoretisk verdi) og separasjonen mellom fettsyren er god er kolonnen klar til kjøring. Hvis vi ikke får god separasjon mellom fettsyrene i standardtranblanding (5.4) må separasjonsparametrene endres, f.eks temperaturprogrammet. Verdier for Nu-Chek standarder føres inn i MET.NÆR.04-05; Nu-Chek Kontroll - Fettsyrer

Andre tips

- Er det tegn til at prøvene ikke blir injisert, må injektorsystemet sjekkes, se loggbok. Sjekk også injekjsjonsnål. Denne kan skiftes eller renses.
- Ved stigende baselinje kan det være lurt å kjøre kolonne på høy temperatur 220°C over natten.
- Ved behov for skift av deler og vedlikehold, benyttes veiledning i tilhørende manualer.

8. PROSEDYRE

Homogeniser prøvematerialet godt. Ref. arbeidsbeskrivelse LAB.GRU.BIL.ARB-01; instruks for opparbeiding av prøver ved NIFES



Klargjøring av robot

Bilde over viser plasseringen av reagensene (vann, heptan og svovelsyre), internstandarden, de ulike typer tips og 96-brønnsplatene.

Vann, heptan og 2% svovelsyre helles over i plastbegre (følger med roboten) og settes på sine spesifikke plasser.
Internstandard blir overført til fire 10.5 ml plastrør og satt på roboten.
Prøvene tines ved å plassere dem på en ristemaskin (Nutating rister) beregnet for humane prøver og settes så direkte i rack med eller uten innsats. (Kommer an på hvilke type prøveglass en har).
Se etter at det er nok spisser til antallet prøver som skal kjøres. Sett på avfallspose, denne må fjernes med en gang opparbeidelsen av prøvene er ferdig, dette for å redusere skadeeffekten av svovelsyredampen.

Klargjøring av sekvens. Gå til lims og opprett en batch som vanlig, legg inn journalnumre, blankprøver og kontroller.
Når veietallene er komplett i excel-fil fra Hamilton roboten, kopieres disse over i batchfilen. Batchen importeres videre til Chromeleon på Ultrafast GC-en.

Prøveopparbeidelse på roboten er oppsummert i punkter under.

- 60 ul av hver prøve blir pipettert fra originale prøveglass over i dypbrønnsplaten som er plassert på vekten. Alle prøvene i sekvensen blir veid inn før neste trinn utføres. Vekt registres i excel fil.
- 60 ul internstandard tilsettes prøvene i platen med iSWAP arm 4 channels, dvs. at det tilsettes i fire brønner samtidig.
- 750 ul 2% svovelsyre i metanol overføres til prøvene (metyleringsprosess).
- Platen forsegles i sealer-enheten.
- Platen ristes og kokes på 105°C (reell temperatur er 95 °) i 40 minutter (direkte transesterifisering).
- Platen avkjøles i 15 minutter.
- Prøvene tilsettes 250ul vann og 400ul heptan (ekstraksjon av fettsyrene).

- Platen ristes og blir så sentrifugert i 2 min.
- 350ul av heptanfasen blir overført til en ny dypbrønnsplate.
- Ekstraksjonen gjentas, men nå med 300ul heptan.
- Den nye dypbrønnsplaten blir så forseglet i sealer-enheten, og er etter dette klar til å kjøres på GC.

Det injiseres 1µl på Trace GC Ultra. Det kjøres en heptan, en standard tranblandig (5.4), 2 blanker, 2 kontrollprøver i tillegg til prøvene i hver sekvens.

9. VALIDERINGSPARAMETERE

9.1 Spesifisitet

Blindprøven viser interfererende topper for fettsyrene 16:0 og 18:0. Vi velger derfor å bruke blankkorreksjon. Bestemmelse av mengde fettsyre skjer da ved at arealet av den interfererende toppen i en blankprøve trekkes fra arealet av tilsvarende topp i prøven før utregning. Dette gjøres ved at en velger en av blankprøvene i sekvensen og bruker denne til korreksjon. En velger den blankprøven som i areal ligger nærmest gjennomsnittet en fikk under valideringen.
Det virker som om forurensingen er relativ stabil, men vil velger å opprette et kontrollkort for 16:0 og 18:0 for å ha dette under oppsikt.

I denne metoden får man ikke separert de enumettede fettsyrene, disse oppgis som 16:1, 18;1, 20:1 og 22:1.

9.2 Linearitet

Det er liten variasjon i fettnivå i prøvene vi analyserer, så nivået av tilsatt internstandard vil være relativt stabilt i prøvene. Det antas at linearíteten til internstandarden er lik som for metode 354 – Fettsyrebestemmelse vha ultrafast GC. Der er linearíteten satt slik at internstandarden er lineær når den utgjør 7- 70% av fettsyreinnholdet i prøven. Vi har likevel valgt å si at mengde 19:0 Metyl optimalt bør ligge mellom 10 - 30 %. Ved for lite 19:0 Metyl tilsatt kan vi få mer variasjon mellom parallellene og ved for mye 19:0 Metyl tilsatt kan små fettsyrer bli neglisjert.

9.3 Deteksjonsgrense/Kvantifiseringsgrense

LOD og LOQ er satt ut fra signal/støy forhold i en blank prøve. LOD = 3 x støy og LOQ = 10 x støy. Omregnet til konsentrasjon ved 0,056 gram innveid og 1 µl injeksjon gir dette:

LOD 0,003 mg/g= 3 mg/kg
LOQ 0,01 mg/g= 10mg/kg

LOQ gjelder for enkeltfettsyrer og sum av disse.

9.4 Riktighet

Riktigheten ble testet ved å analysere to ulike referansematerialer SRM 2378 humant serum på tre ulike nivåer og SRM 1950 humant serum.

SRM 2378	Robot opparbeiding			
	Dato	Resultat ug/g	Sann verdi ug/g	Riktighet %
14_0				
Serum nivå 1	06.10.2016	50,3	45	113
Serum nivå 2	06.10.2016	34,4	34	102
Serum nivå 3	06.10.2016	36,9	35	107
16_0				
Serum nivå 1	06.10.2016	952,8	833	114
Serum nivå 2	06.10.2016	839,6	715	117
Serum nivå 3	06.10.2016	739,3	642	115
18_0				
Serum nivå 1	06.10.2016	326,6	221	148
Serum nivå 2	06.10.2016	310,6	231	134
Serum nivå 3	06.10.2016	260,2	194	134
18_1				
Serum nivå 1	06.10.2016	617,3	645	96
Serum nivå 2	06.10.2016	781,3	774	101
Serum nivå 3	06.10.2016	577,4	601	96
18_2n_6				
Serum nivå 1	06.10.2016	950,7	1030	92
Serum nivå 2	06.10.2016	1093,5	1220	90
Serum nivå 3	06.10.2016	863,1	913	95
18_3n_3				
Serum nivå 1	06.10.2016	32,2	33	99
Serum nivå 2	06.10.2016	33,8	32	107
Serum nivå 3	06.10.2016	22,7	17	134
18_3n_6				
Serum nivå 1	06.10.2016	11,2	12	91
Serum nivå 2	06.10.2016	16,4	21	78
Serum nivå 3	06.10.2016	12,6	15	86
20_0				
Serum nivå 1	06.10.2016	7,1	7,6	93
Serum nivå 2	06.10.2016	8,5	8,7	98
Serum nivå 3	06.10.2016	8,8	7,9	111
20_4n_6				
Serum nivå 1	06.10.2016	207,0	196	106
Serum nivå 2	06.10.2016	241,5	235	103
Serum nivå 3	06.10.2016	237,0	228	104

20_5n_3				
Serum nivå 1	06.10.2016	89,8	84	107
Serum nivå 2	06.10.2016	21,0	21	101
Serum nivå 3	06.10.2016	18,7	19	99
22_5n_3				
Serum nivå 1	06.10.2016	23,9	22	107
Serum nivå 2	06.10.2016	15,8	17	93
Serum nivå 3	06.10.2016	12,3	11	108
22_6n_3				
Serum nivå 1	06.10.2016	109,6	104	105
Serum nivå 2	06.10.2016	51,8	55	94
Serum nivå 3	06.10.2016	56,3	55	103

SRM 1950	Robot opparbeiding		
	Dato	Resultat P1	Riktighet
		ug/g	[%]
16_0	Sann verdi	594	
1	07.04.2016	707,9	119
2	07.04.2016	719,3	121
3	07.04.2016	646,1	109
4	12.05.2016	753,7	127
Middel		**706,7**	**119**
18_0	Sann verdi	179	
1	07.04.2016	120,3	67
2	07.04.2016	118,2	66
3	07.04.2016	115,3	64
4	12.05.2016	238,4	133
Middel		**148,1**	**83**
18_2n_6	Sann verdi	780	
1	07.04.2016	718,8	92
2	07.04.2016	732,1	94
3	07.04.2016	675,1	87
4	12.05.2016	832,1	107
Middel		**739,5**	**95**
20_2n_6	Sann verdi	5,7	
1	07.04.2016	8,6	151
2	07.04.2016	9,8	173
3	07.04.2016	10,2	178
4	12.05.2016	4,5	78
Middel		**8,3**	**145**

20_3n_6	Sann verdi	41	
1	07.04.2016	33,6	82
2	07.04.2016	35,0	85
3	07.04.2016	32,6	80
4	12.05.2016	45,0	110
Middel		**36,6**	**89**
20_4n_6	Sann verdi	293	
1	07.04.2016	214,2	73
2	07.04.2016	216,4	74
3	07.04.2016	200,9	69
4	12.05.2016	215,2	73
Middel		**211,7**	**72**
20_5n_3	Sann verdi	11,4	
1	07.04.2016	10,3	90
2	07.04.2016	10,6	93
3	07.04.2016	9,6	84
4	12.05.2016	13,5	118
Middel		**11,0**	**96**
22_6n_3	Sann verdi	37,9	
1	07.04.2016	37,6	99
2	07.04.2016	40,2	106
3	07.04.2016	33,0	87
4	12.05.2016	38,2	101
Middel		**37,3**	**98**

Riktighet er også testet med kontrollkort-metode 354 mot kontrollkort –metode 435 på % og mg/g.

Fettsyrer %

Kontrollkort for metode 354 Ultra-GC n=50 analysert over 30 dager, to analytikere									
Fettsyrer %	14:0	16:0	16:1	18:0	18:1	18:2n-6	20:4n-6	20:5n-3	22:6n-3
Middel	1,70	23,16	2,27	6,78	24,37	28,74	5,47	0,73	1,79
Standardavvik	0,09	0,30	0,20	0,38	0,61	0,26	0,14	0,05	0,12
2RSD	10,02	2,59	17,35	11,08	5,02	1,78	5,27	13,01	13,71
Kontrollkort for metode 435 UFGC automatisert						n=30	robot		
Fettsyrer %	14:0	16:0	16:1	18:0	18:1	18:2n-6	20:4n-6	20:5n-3	22:6n-3
Middel	1,81	25,13	2,15	8,00	21,75	26,46	5,65	0,84	1,73
Standardavvik	0,06	0,64	0,08	0,41	0,62	0,43	0,29	0,05	0,06
2RSD	6,54	5,10	7,86	10,14	5,74	3,22	10,15	10,89	6,86

% "riktighet" mellom metode 354 og metode 435, vi antar at metode 354 resultatene er sann verdi.									
Fettsyrer %	14:0	16:0	16:1	18:0	18:1	18:2n-6	20:4n-6	20:5n-3	22:6n-3
% riktighet	107	109	95	118	89	92	103	115	97

Fettsyrer mg/g

Kontrollkort for metode 354 Ultra-GC n=50 analysert over 30 dager, to analytikere									
Fettsyrer mg/g	14:0	16:0	16:1	18:0	18:1	18:2n-6	20:4n-6	20:5n-3	22:6n-3
Middel	0,07	0,98	0,10	0,29	1,03	1,21	0,23	0,03	0,08
Standardavvik	0,01	0,07	0,01	0,02	0,08	0,08	0,01	0,00	0,01
2RSD	14,29	13,88	22,21	16,72	14,62	12,85	12,51	15,68	18,51
Kontrollkort for metode 435 UFGC automatisert						n=30	robot		
Fettsyrer mg/g	14:0	16:0	16:1	18:0	18:1	18:2n-6	20:4n-6	20:5n-3	22:6n-3
Middel	0,07	0,98	0,08	0,31	0,84	1,03	0,22	0,03	0,07
Standardavvik	0,01	0,11	0,01	0,04	0,08	0,10	0,02	0,00	0,01
2RSD	21,18	22,42	21,18	25,38	19,61	19,19	19,14	17,89	19,79
% "riktighet" mellom metode 354 og metode 435, vi antar at metode 354 resultatene er sann verdi.									
Fettsyrer mg/g	14:0	16:0	16:1	18:0	18:1	18:2n-6	20:4n-6	20:5n-3	22:6n-3
% riktighet	100	100	80	113	82	85	96	100	88

Riktigheten er også testet ved å sammenligne resultatene fra 7 RBC prøver fra WP 5 prosjektet opparbeidet med metode 354 mot resultatene opparbeidet med roboten.

Fettsyrer RBC WP5 prosjektet	Journalnr.										
Opparbeidet på robot 24.05.2016	mg/g	16:0	18:0	18:1	18:2n-6	20:3n-6	20:4n-6	20:5n-3	22:4n-6	22:5n-3	22:6n-3
Ny kolonne	2016-16-1	0,50	0,35	0,31	0,27	0,02	0,27	0,03	0,03	0,04	0,12
	2016-16-2	0,61	0,40	0,40	0,33	0,05	0,33	0,02	0,04	0,04	0,13
	2016-16-3	0,59	0,40	0,38	0,35	0,03	0,24	0,01	0,03	0,03	0,09
	2016-16-4	0,58	0,43	0,34	0,26	0,02	0,21	0,02	0,03	0,04	0,11
	2016-16-6	0,45	0,36	0,32	0,26	0,03	0,33	0,02	0,04	0,05	0,12
	2016-16-8	0,45	0,35	0,25	0,22	0,02	0,21	0,06	0,02	0,04	0,17
	2016-16-10	0,49	0,35	0,33	0,26	0,02	0,27	0,02	0,04	0,04	0,10
Fettsyrer RBC WP5 prosjektet											
manuell opparbeiding	mg/g	16:0	18:0	18:1	18:2n-6	20:3n-6	20:4n-6	20:5n-3	22:4n-6	22:5n-3	22:6n-3
Gammel kolonne	2016-16-1	0,49	0,37	0,39	0,31	0,03	0,32	0,03	0,04	0,04	0,17
	2016-16-2	0,48	0,37	0,41	0,30	0,04	0,33	0,02	0,03	0,04	0,15
	2016-16-3	0,53	0,41	0,48	0,43	0,04	0,33	0,02	0,04	0,04	0,13
	2016-16-4	0,44	0,37	0,34	0,26	0,03	0,30	0,02	0,03	0,05	0,15
	2016-16-6	0,46	0,38	0,35	0,26	0,02	0,38	0,02	0,04	0,05	0,13
	2016-16-8	0,44	0,37	0,29	0,23	0,02	0,25	0,06	0,02	0,06	0,24
	2016-16-10	0,47	0,36	0,39	0,28	0,02	0,34	0,02	0,05	0,05	0,14
% Riktighet	mg/g	16:0	18:0	18:1	18:2n-6	20:3n-6	20:4n-6	20:5n-3	22:4n-6	22:5n-3	22:6n-3
	2016-16-1	103	95	81	87	88	85	99	82	93	71
	2016-16-2	128	108	99	108	120	100	121	112	106	84
	2016-16-3	111	100	79	80	83	73	81	76	80	72
	2016-16-4	132	117	102	100	75	69	84	92	76	74
	2016-16-6	97	96	89	98	122	88	101	97	97	88
	2016-16-8	103	94	84	93	86	83	87	86	78	71
	2016-16-10	103	97	85	94	99	81	86	83	85	77

Riktighet for SRM 1950 varierte fra 64 % til 178 %.
Riktighet SRM 2378 varierte fra 86 % til 148 %. Hovedtyngden av fettsyrene har en riktighet innenfor 78-134 %, bortsett fra 18:0 der vi har en riktighet på 148 % på serum nivå 1. Konsentrasjonen er forholdvis lik på 18:0 i serum nivå 1, 2 og 3. De to andre serum prøvene gir en riktighet på 134 %.
Riktighet på FINS Rbc prøver varierte fra 69 % til 132 % (antatt metode 354 gir «sann verdi»
KM 2016 Plasma varierte fra 89 -118 % på relative verdier og 82 % til 113 % på absolutte verdier.

Riktigheten er god for KM 2016 både på relative verdier og absolutte verdier, og godt innfor måleusikkerheten for de ulike nivåene. Det samme gjelder for rbc prøver fra FINS analysert med manuell og robotmetode. Dette tyder på at det er god samsvar mellom den manuelle metoden og robotmetoden i forhold til riktighet.

For SRM 2378 serum har vi god riktighet på alle fettsyrer på alle tre nivå bortsett fra 18:0 på serum nivå 1. Resultater er foreløpig basert på kun en analyse og vi bør analysere flere ganger for å bekrefte resultat. Det er ett dyrt referansemateriale, så velger å ikke bruke den som kontroll, men anbefales at den analyseres årlig.

For SRM 1950 er det veldig varierende riktighet. Mange av fettsyrene er i sertifikatet er i tillegg oppgitt som referanseverdier, mens de samme fettsyrer er oppgitt som sertifiserte verdier i SRM2387. SRM2387 består av ett sett med tre ulike serum prøver. Vi velger derfor å vektlegge SRM2387 mest og i videre arbeid vil vi bruke SRM 2378 som en årlig kvalitetsparameter på metoden, da denne har oppgitte verdier på tre ulike serumprøver.

9.5 Presisjon, som repeterbarhet og intern reproduserbarhet

Kontrollkort for metode 354 Ultra-GC n=50 analysert over 30 dager, to analytikere Intern Repoduserbarhet									
Fettsyrer %	14:0	16:0	16:1	18:0	18:1	18:2n-6	20:4n-6	20:5n-3	22:6n-3
Middel	1,70	23,16	2,27	6,78	24,37	28,74	5,47	0,73	1,79
Standardavvik	0,09	0,30	0,20	0,38	0,61	0,26	0,14	0,05	0,12
2RSD	10,02	2,59	17,35	11,08	5,02	1,78	5,27	13,01	13,71

Kontrollkort for metode 435 UFGC automatisert 10*1 analysert samme dag på robot, repeterbarhet									
Fettsyrer %	14:0	16:0	16:1	18:0	18:1	18:2n-6	20:4n-6	20:5n-3	22:6n-3
Middel	1,77	24,98	2,18	7,99	21,28	26,26	5,34	0,82	1,70
Standardavvik	0,04	0,66	0,06	0,34	0,31	0,22	0,13	0,05	0,05
2RSD	4,54	5,25	5,58	8,45	2,89	1,70	4,75	12,42	5,95

% "riktighet" mellom metode 354 og metode 435, vi antar at metode 354 resultatene er sann verdi.									
Fettsyrer %	14:0	16:0	16:1	18:0	18:1	18:2n-6	20:4n-6	20:5n-3	22:6n-3
% riktighet	104	108	96	118	87	91	98	112	95

Kontrollkort for metode 354 Ultra-GC n=50 analysert over 30 dager, to analytikere Intern Repoduserbarhet									
Fettsyrer mg/g	14:0	16:0	16:1	18:0	18:1	18:2n-6	20:4n-6	20:5n-3	22:6n-3
Middel	0,07	0,98	0,10	0,29	1,03	1,21	0,23	0,03	0,08
Standardavvik	0,01	0,07	0,01	0,02	0,08	0,08	0,01	0,00	0,01
2RSD	14,29	13,88	22,21	16,72	14,62	12,85	12,51	15,68	18,51

Kontrollkort for metode 435 UFGC automatisert	10*1 analysert samme dag på robot, repeterbarhet								
Fettsyrer mg/g	14:0	16:0	16:1	18:0	18:1	18:2n-6	20:4n-6	20:5n-3	22:6n-3
Middel	0,07	1,00	0,09	0,32	0,85	1,05	0,21	0,03	0,07
Standardavvik	0,01	0,14	0,01	0,05	0,11	0,13	0,03	0,00	0,01
2RSD	26,78	27,08	23,55	29,26	25,63	24,99	25,66	22,22	24,43

% "riktighet" mellom metode 354 og metode 435, vi antar at metode 354 resultatene er sann verdi.

Fettsyrer mg/g	14:0	16:0	16:1	18:0	18:1	18:2n-6	20:4n-6	20:5n-3	22:6n-3
% riktighet	100	102	90	110	83	87	91	100	88

Intern reproduserbarhet gitt i % og mg/g se kontrollkort kjørt over tid, under punkt 9.4 Riktighet.

9.6 Måleområde

0,1-100% relativ sammensetning

>10mg fettsyre/kg vått materiale (0,01 mg/g).

9.7 Måleusikkerhet

Metodens måleusikkerhet er vurdert ut fra intern reproduserbarhet (kontrollkort) og analyse av SRM. Måleusikkerheten gjelder for enkeltfettsyrer og sum av disse.

Relative mengder %

Nivå	Konsentrasjon %	Måleusikkerhet
Veldig Lav	0,1	100
Lav	0,2-0,5	50
Middel	0,6-100	10

Absolutte mengder mg/g

Nivå	Konsentrasjon %	Måleusikkerhet
Veldig Lav	0,1	100
Lav	0,2-0,5	50
Middel	0,6-100	20

På høyt nivå er det satt en større usikkerhet ved absolutte verdier, da tilsetting av intern standard også gir bidrag til usikkerheten. Nivå vurderes ut fra relative (%) verdiene. Måleusikkerhet på 18:0 kan være større. Denne må vi vurdere ved neste analyse av SRM2397.

Bidrag til måleusikkerhet:

Trinn i metodeprosedyre	Ubetydelig bidrag	Middels bidrag	Stort bidrag
1.Homogenisering			X (matriseavhengig)
2.Innveiing			X
3.Tilsetting av intern standard			X
4.Transesterifisering		temp X tid X	
5.Metylering/temp/tid		temp X tid X	
6.Ekstraksjon		X	
7.Tillaging av intern standard			X
8.Separasjon på GC			X
9.Integrering			X

1.Homogenisering: I denne metoden vil vi hovedsakelig analysere på rbc, plasma, serum eller fullblod. I noen tilfeller kan en få prøver som har utfelling eller er delvis størknet/klumper, det er derfor viktig å blande prøvene godt på forhånd. Dette blir gjort ved å plassere prøvene på nutating mixer fra Labnet før pipettering, og sjekke i etterkant av pipettering at prøven er tatt med av roboten.
2.Innveiing: Innveiingen er veldig viktig for riktig resultat av mg fett /g prøve. Innveiing av prøven utføres nå av roboten. Vekten på roboten er kontrollert av leverandør før levering, og blir kontrollert ved den årlige servicen av Hamilton. Likevel viktig å følge med på at den er stabil, noe som gjøres ved å analysere kontrollmateriale og referansematerialer. Det er ikke mulig å innføre månedlige kontroller som på de andre vektene.

3.Tilsetting av intern standard er også et viktig trinn for at en skal få riktig resultat på mengde mg fettsyre per gram prøve. Det ble testet at roboten holdt kravene til pipettering ved installasjon, det er også med et sertifikat fra leverandør hvor % avvik for de ulike pipetteringsmengder er oppgitt. Gjennom den årlige serviceavtalen vi har med Hamilton blir kravene til pipettering i varetatt og sertifikat utstedt.
4/5. For direkte transesterifisering og metylering. Den koketiden som ble valgt er 40 minutter på maks temperatur ved 105 grader. Dette gav best utbytte av fettmengden og ikke så mye interferens av 16:0 og 18:0. Jo lenger koketid jo mer interferens, dessuten gav lenger koketid heller ikke vesentlig større fettprosent.
7. Tillaging av intern standard er spesielt viktig, om den er feil blir hele utregningen av resultatet feil. Internstandarden 19:0 blir nå løst kun i metanol, løsningen må derfor deretter settes i ultralydbad i ca.15 minutter, og det er viktig å sjekke at alt pulveret er løst opp i væsken.
8/9. En ny, god kolonne er viktig for å få en god seperasjon av fettsyrene, og dermed også får en riktigere integrering av disse. Ved dårlig (slitt) kolonne må en passe på at de små toppene også blir integrert, og at en integrerer så likt som mulig gjennom hele sekvensen.

9.8 Robusthet

Roboten må fungere for at vi i det hele tatt kan få analysert noen prøver. Derfor viktig at alle moduler virker.
Robotens program Venus er bygget slik at metodefilen består av mange sub-program som kan kjøres selvstendig om nødvendig. Om roboten merker at et eller annet er feil i oppsettet, vil den stoppe opp og sette på en alarm. Vi kan da gå inn manuelt å rette opp eventuelle feil/mangler, det kan være seg for lite spisser, for lite væske i beholdere, satt opp feil antall prøver osv. Etter at feilen er rettet opp programmerer vi roboten til å fortsette fra der den stoppet opp eller fra neste sub-program.
Dette gir oss en god oversikt og trygghet på at prosedyren under opparbeidingen av prøvene blir riktig utført.

God kolonne er viktig for å få et godt resultat.
Hvis separasjonen av fettsyrene blir dårlig og grunnlinjen ustabil vurder å skifte kolonne. Dårlig seperasjon av fettsyrer og ulik/manglende integrering av små topper, kan gi utslag på resultatene. Minst to blankprøver og to kontroller analyseres alltid med i en serie. Disse blir også opparbeidet av roboten, og settes i begynnelsen og på slutten av en serie (evt. i midten dette er avhengig av antall prøver). Siden

blankprøven gir interfererende topper, må blankkorreksjon brukes. Arealene fra de to interferens toppene i blankprøven trekkes fra arealene for tilhørende topper i prøven. Dette gjøres ved at en velger en av blank prøvene i sekvensen og bruker denne til korreksjon. En velger den blankprøven som ligger nærmest gjennomsnittet i areal.
Det virker som om forurensingen er relativ stabil, men vil velger å opprette et kontrollkort for 16:0 og 18:0 for å ha dette under oppsikt. Dette kortet ligger under T:\Kontrollkort\435_Ultra GC_Robot\Kontrollkort blankprøver.

Det er en begrensning i antall prøver som kan settes opp om gangen med robot opparbeiding. Anbefaler 2 oppsett daglig med 32 prøver hver gang, dette fordi det viser seg at platen med væske i (svovelsyre i metanol) utvider seg under kokeprosessen og at det i tillegg utvikler seg at trykk over brønnene i platen. Aluminiumsfolien kan bli blåst av. Robotarmen klarer heller ikke å flytte platen til kjøling når denne er utvidet, da den nå sitter mye fastere i underlaget. Ved å sette opp 32 prøver har en dette under kontroll.

10. KVALITETSKONTROLL

10.1 Kontroll av metodens pålitelighet utføres ved hjelp av:

1. Dobbeltbestemmelser, ved behov. For prosjekter der vi har prøverserier med≥5 biologiske paralleller er det ikke krav til dobbeltbestemmelser.
2. Kjøring av standard.
3. Føring av 16:0, 18:2n-6, 22:6n-3 og sum fettsyrer i kontrollkort på internt kontrollmateriale (plasma).
4. Føring av blankprøve i kontrollkort.
5. Referansemateriale.

Vi godtar en differanse på 10 %, ved innhold fra 0,6-100 % på relative verdier, mens den er 15 % på absolutte verdier. På små fettsyrer kan avviket være større. Hva som er akseptabelt avvik, vurderes ut fra måleusikkerheten på de ulike nivåene, ideelt bør avviket være < enn metodens måleusikkerhet på aktuelt nivå. I denne metoden har vi for enkelhets skyld, satt samme tallverdi som måleusikkerhet-

Differanse mellom paralleller beregnes direkte i LIMS. For denne metoden er det ikke lagt ut en rød fargekode/varsel om vi er over krav. Om alle alternativer på varsel skulle legges i LIMS, ville det medført at det hadde godt meget seint i LIMS systemet å få lagt inn resultater. Analytiker må derfor være spesielt oppmerksom på å vurdere avvik fortløpende. Kontroller gjerne sum fettsyrer på avvik mellom paralleller på absolutte verdier først. Hvis denne ikke holder krav, gjenspeiler dette seg sannsynligvis også i enkeltfettsyrer.

Prøven skal reanalyseres (men sjekk først integrering, seperasjon og identifisering nøye) dersom det er store avvik fra krav til differanse mellom paralleller. Ved mindre avvik til kravet skal resultatet vurderes av prosjektleder eller avdelingsleder før reanalyse settes i gang.

Differanse mellom paralleller må tilpasses metodens måleusikkerhet på de ulike nivåer.

$$\%\ \text{differanse} = \frac{(\text{høyeste verdi} - \text{laveste verdi})}{\text{høyeste verdi}} * 100$$

Dette er basert på laboratoriets historiske data/grenseverdier.

10.2 I hver prøveserie inngår en kontrollprøve. Areal % og mg/g for 16:0, 18:2n-6, 22:6n-3 føres på kontrollkortet samt mg på total fettsyrer. For tillaging, bruk og vurdering av kontrollmateriale ref. instruks KH.MET.BIL-08; etablering av kontrollkort og internt kontrollmateriale.

11. BEREGNINGER

11.1 Beregning av mengde 19:0metyl som skal tilsettes:

$$\text{mg fett i prøven} = \frac{\text{innveid(g) prøve} * \text{antatt \% fett i prøven} * 1000\text{mg/g}}{100}$$

$$\text{mg 19:0 som skal tilsettes} = \frac{\text{\% 19:0M ønsket tilsatt} * \text{mg fett i prøven}}{100}$$

11.2 Det beregnes % fordeling av de ulike fettsyrene, mhp. areal og mengde(mg/g) av de ulike fettsyrene ved hjelp av intern standard.

12. RAPPORTERING

Det beregnes % fordeling av de ulike fettsyrene, mhp. areal og mengde(mg/g) av de ulike fettsyrene ved hjelp av intern standard. I tillegg oppgis sum av fettsyrer som angitt i tabell under punkt 14.

Det oppgis 1 desimal på alle nivå på de relative verdiene.
Det oppgis 2 desimaler for verdier <0,1mg/g og 1 desimal for verdier >0,1 mg/g på de absolutte verdiene.

13. BEMERKNINGER

13.1 Parametere som er kritisk for metoden:

Det er en begrensning i antall prøver som kan settes opp om gangen med vår metodeopparbeiding. Anbefaler 2 oppsett daglig med 32 prøver hver gang, dette fordi det viser seg at platen med væske i (svovelsyre i metanol) utvider seg under kokeprosessen og at det i tillegg utvikler seg at trykk over brønnene i platen. Aluminiumsfolien kan bli blåst av. Robotarmen klarer heller ikke å flytte platen til kjøling når den er utvidet, da den sitter mye fastere i underlaget i heat-shakeren. Ved å sette opp 32 prøver har en dette under kontroll.
Det går fint an å bruke dypbrønnsplatene to ganger ved å snu dem, dette for å spare på bruken av engangsutstyret.

13.2 Tidsforbruk: I løpet av en normal arbeidsdag kan det opparbeides og settes på Ultra fast GC 64 prøver. Roboten bruker ca. fire timer på denne opparbeidingen.
En prøve bruker ca. 6 minutter på Ultra fast GC. Det går noen minutter til kjøling av GC-en mellom hver prøve. Til gjennomgang av kromatogrammene og overføring av tallene til lims brukes ca.1dag avhengig av hvor god kolonne en har.

14. LIMS

Legge til metoder (analyse):

- Se brukerveileding LIMS.BRUK-02 Prøveflyt for oversikt over hvordan metoder (analyser) legges på prøver.

- Når FS_U_ROBOT velges, vil spørsmål FS_U_ROBOT Varition? gi deg mulighet til å velge mellom *Ultra GC-1parallell og Ultra GC2-paralleller*. Marker riktig variasjon og trykk OK (evt. dobbeltklikk på riktig variasjon).

For å starte GCen bruker en LIMS:

1) Åpne Result entry, fyll ut:
* Instrument
* Innveid mengde, kopierer excel fil fra Hamilton roboten når den er ferdig med opparbeidingen
* Internstandard tilsatt

Lage sekvens til Chromeleon:

2) File|Open Batch Manager
* Klikk New Batch - ikonet
* Template: FS_U_ROBOT
* Velg Sample View
* Dra over prøver fra ett eller flere journalnummer

3) Velg Batch|Actions
* Trekk ned "Actions" - rullegardinmenyen
* Velg "Send kjørefil til <riktig instrument>"
* Trykk "Add"

4) Åpne "S:\Laboratorier\Naeringsstoffer\LIMS\Sekvens til Instrument\"435_Ultrafast_ROBOT", velg create, velg så sequence, dobbelklikk på "<batchnavn>.wle".

Har nå laget sekvens i Chromeleon.

5) Legg evt. til heptan, tranblanding, kontroller etc.
* NB! Aldri endre prøvenavn eller Replicate ID!
* Sekvensen kan flyttes til andre mapper under instrumentet i Chromeleon.

6) Kjør sekvensen på vanlig måte. (Starte GC)

7) Kvantitér og rapportér med processing method "\ 435_Ultrafast_ROBOT <instrument>" (avhengig av hvilket instrument som benyttes)

8) Åpne rapporten i Chromeleon, stå på fane "LIMS og trykk på Chromeleon ikonet, så export, ok.
* Lagre filen under S:\Laboratorier\Naeringsstoffer\LIMS\Resultatfil til LIMS\435_Ultrafast_ROBOT
* Formatet må være excel (*.xls)

Importere data fra Chromeleon til Lims:

9) I hovedvinduet til LIMS-klienten, velg "Macros|GC Chromeleon import"
* Velg filen som nettopp ble lagret (under S:\Laboratorier\Naeringsstoffer\LIMS\ Resultatfil til LIMS\435_Ultrafast_ROBOT) og trykk OK. Data blir importert til LIMS. Under importeringen gjør LIMS omregning til mengde (mg/g).
Omregning av fettsyrene fra % til mengde (mg/g):

(tilsatt 19:0(mg))*(% fettsyre (fra inst.))
(% 19:0 (fra inst.))*(innveid (g))

Rapport av analysedata fra Lims hentes på følgende måte:

Står beskrevet i bruksanvisning MET.NÆR.05-02 Eksportering av data fra LIMS til Excel

Nærmere beskrivelse av felt som brukes til resultatene:

Det rapporteres areal % fordeling og mg/g av de ulike fettsyrene.
Det oppgis 1 desimal på de relative verdiene.
Det oppgis 2 desimaler på de absolutte verdiene.

Parametrene er satt opp i denne rekkefølgen, en for % fordeling og en for mg fettsyre/ g prøve.

Fettsyrer
6:0
8:0
10:0
12:0
14:0
14:1
15:0
16:0
16:1
16:2n-4
17:0
16:3n-3
18:0
18:1
18:2n-6
18:3n-6
18:3n-3
18:4n-3
20:0
20:1
20:2n-6
20:3n-9
20:3n-6
20:4n-6
20:3n-3
20:4n-3
20:5n-3
22:0
22:1
21:5n-3
22:4n-6
22:5n-6
22:5n-3
22:6n-3
24:0
24:1n-9
24:5n-3
24:6n-3
Sum uidentifiserte
Sum identifiserte
Sum fettsyrer
Sum mettet
Sum 16:1
Sum 18:1
Sum 20:1
Sum 22:1
Sum en-umettet
Sum EPA+DHA
Sum n-3
Sum n-6

Sum flerumettet
n-3/n-6
Innv.(g)
Tilsatt 19:0M (mg)

14. METODEREFERANSER

354 - Fettsyresammensetning av totalfettsyrer ved hjelp av Ultra-fas GC, med tilhørende referanser.

041 - Fettsyresammensetning av totalfettsyrer ved hjelp av GLC, med tilhørende referanser.

Morrison,W.R. and Smith, L.M. (1964). Preparation of fatty acid methy esters and dimethylacetals from lipids with boron-fluoride-methanol. J. Lip. Res. 1964 Oct 5, 600-608.

Høy,C.E.; Hølmer,G. (1981) Incorporation of cis-Octadecenoic Acids into the Rat Liver Mitochondrial Membrane Phospholipids and Adipose Tissue Triglycerides. Lipids 16: 102-108.

Lie, Ø. and Lambertsen, G., 1991. Fatty acid composition of glycerophospholipids in seven tissues of cod (Gadus morhua), determined by combined high-performance liquid chromatography and gas chromatography. J. Chromatogr. 1991 Apr 19, 565, 119-129.

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- NMKL (2007), Referansematerialer. NMKL-prosedyre nr. 9.
- NIFES Valideringsdokument