UiT The Arctic University of Norway

Faculty of Health Sciences

Intake of marine fatty acids in early life and risk of celiac disease

Mari Thesen
Master's thesis in Clinical Nutrition (ERN-3009), May 2021

Main supervisor: Nicolai A. Lund-Blix, the Norwegian Institute of Public Health
Co-supervisor: Guri Skeie, the Arctic University of Norway

Acknowledgments

First of all, I would like to thank my supervisor Nicolai A. Lund-Blix for all the long discussions, constructive feedback and for reassuring me in moments of doubt. It has been inspiring to have a supervisor with such knowledge and interest in this field. I am also very grateful for the valuable feedback from co-supervisor Guri Skeie.

I would also like to thank the others from the Norwegian Institute of Public Health (NIPH) that has been involved, Lars C. Stene, German Tapia and Ketil Størdal, for all their help. I am very grateful that I got opportunity to write this thesis at the NIPH, it has been exciting to gain some insight into a large research environment. Even though most of this year unfortunately had to be spent on home office.

Last, but not least, a well-deserved thanks goes to family and friends for supporting me throughout this year. Specially thanks to the two Eirin`s in my life for motivating me and making the days in a locked-down Oslo a bit brighter.

Mari Thesen
Oslo, Mai 2021

Summary

Background: About 1% of the European population are affected by celiac disease (CD). Intake of omega-3 fatty acids in early life has been associated with lower risk of other immune-mediated diseases in children, but there are limited studies on CD. The primary aim of this thesis was to investigate if maternal intake of EPA and DHA during pregnancy, from food and/or supplements, was associated with risk of CD in the offspring. The secondary aim was to investigate if children`s use of cod liver oil, at 6 months and at 18 months, was associated with risk of CD.

Material and methods: In the analysis of maternal intakes, 85,592 children from The Norwegian Mother, Father and Child Cohort Study (MoBa) were included. Intakes of EPA and DHA had been calculated from a food frequency questionnaire in mid-pregnancy. In the analyses of children`s use of cod liver oil, at 6 months and at 18 months, 87,056 and 72,188 children from MoBa were included, respectively. Information about use of cod liver oil was obtained from questionnaires. CD status was obtained from the Norwegian Patient Registry and questionnaires. Binary log-linear regression was used to estimate relative risks (RR) with 95% confidence intervals (CI) for associations with CD.

Results: Of the children included in the analysis of maternal intakes, 965 (1.1%) were diagnosed with CD. Median maternal intakes of EPA and DHA were 0.22 and 0.34 g/day, respectively, and 68% used supplements containing EPA and DHA. Use of supplements was significantly associated with increased risk of CD in the offspring (adjusted RR: 1.18, 95% CI: 1.02–1.37). Intake of EPA and DHA from food was significantly associated with reduced risk of CD in the offspring, per g/d increase in intake, in the unadjusted analysis (RR: 0.82, 95% CI: 0.68-0.99), but the association did not remain significant after adjustment. Total intake (food and supplements) of EPA and DHA was not associated with risk of CD in the offspring. Of the children included in the analyses of use of cod liver oil, at 6 months and at 18 months, 976 (1.1%) and 832 (1.2%) were diagnosed with CD, respectively. At 6 months of age, 53% used cod liver oil, and 56% used it at 18 months of age. No associations were observed between children`s use of cod liver oil and risk of CD.

Conclusion: Maternal use of supplements containing EPA and DHA during pregnancy was significantly associated with increased risk of CD in the offspring. However, the observed effect was small and due to this, the clinical relevance is limited. Further research on the potential relationship between intake of EPA and DHA in early life and risk of CD are needed.

Keywords*:* Celiac disease, fish, omega-3, EPA, DHA, cod liver oil, MoBa, prospective

Sammendrag

Bakgrunn: Omtrent 1% av befolkningen i Europa har cøliaki (CD). Inntak av omega-3 fettsyrer tidlig i livet har blitt assosiert med redusert risiko for andre immun-medierte sykdommer hos barn, men det er begrenset med studier på CD. Hovedmålet med denne oppgaven var å undersøke om mors inntak av EPA og DHA fra mat og/eller kosttilskudd, under svangerskapet, var assosiert med risiko for CD hos barnet. Sekundærmålet var å undersøke om barns bruk av tran, ved 6 måneders alder og ved 18 måneders alder, var assosiert med risiko for CD.

Material og metode: I analysen av mors inntak var 85,592 barn fra Den norske mor, far og barn-undersøkelsen (MoBa) inkludert. Inntak av EPA og DHA hadde blitt beregnet fra et matvarefrekvensskjema midt i svangerskapet. I analysene av barn bruks av tran, ved 6 måneders alder og ved 18 måneders alder, ble henholdsvis 87,056 og 72,188 barn fra MoBa inkludert. Informasjon om bruk av tran ble hentet fra spørreskjemaer. CD status ble hentet fra Norsk pasientregister og spørreskjemaer. Binær log-lineær regresjon ble brukt til å estimere relativ risiko (RR) med 95% konfidensintervall (KI) for assosiasjoner med CD.

Resultater: Blant barna inkludert i analysen av mors inntak ble 965 (1.1%) diagnostisert med CD. Median inntak av EPA og DHA blant mødrene var henholdsvis 0.22 og 0.34 g/dag, og 68% brukte tilskudd som inneholdt EPA og DHA. Bruk av tilskudd var signifikant assosiert med økt risiko for CD hos barnet (justert RR: 1.18, 95% KI: 1.02–1.37). Inntak av EPA og DHA fra mat var signifikant assosiert med redusert risiko for CD hos barnet, per g/d økning i inntak, i ujustert analyse (RR: 0.82, 95% KI: 0.68-0.99), men assosiasjonen var ikke signifikant etter justering. Blant barna inkludert i analysene av bruk av tran, ved 6 måneders alder og ved 18 måneders alder, ble henholdsvis 976 (1.1%) og 832 (1.2%) diagnostisert med CD. Ved 6 måneders alder brukte 53% tran og 56 % brukte det ved 18 måneders alder. Ingen assosiasjoner ble funnet mellom barns bruk av tran og risiko for CD.

Konklusjon: Mors bruk av tilskudd som inneholdt EPA og DHA under svangerskapet var signifikant assosiert med økt risiko for CD hos barnet. Den observerte effekten var imidlertid liten, og den kliniske relevansen er derfor begrenset. Videre forskning på det potensielle forholdet mellom inntak av EPA og DHA i tidlig liv og risiko for CD er nødvendig.

Table of contents

Acknowledgments III

Summary IV

Sammendrag VI

List of abbreviations X

List of tables XII

List of figures XII

1 Background 1

1.1 Celiac disease 1

1.1.1 Clinical presentation 1

1.1.2 Prevalence and incidence 1

1.1.3 Genetics 2

1.1.4 Pathogenesis 2

1.1.5 Diagnostic criteria 4

1.1.6 Treatment and consequences 4

1.2 EPA and DHA 4

1.2.1 Sources 5

1.2.2 In early life 5

1.2.3 Recommendations and intakes in Norway 6

1.3 Celiac disease and EPA and DHA 7

1.3.1 Potential mechanisms 8

1.3.2 Omega-3 fatty acids and immune-mediated diseases 9

2 Aims and hypotheses 10

3 Material and methods 11

3.1 Study design 11

3.2 Study population 11

3.2.1 Inclusion and exclusion criteria 12
3.3 Exposures 13
3.3.1 Maternal intakes of EPA and DHA during pregnancy 13
3.3.2 Children`s use of cod liver oil at 6 and 18 months 13
3.4 Outcome 14
3.5 Covariates 14
3.6 Statistical analyses 15
3.6.1 Main analysis 15
3.6.2 Sensitivity analysis 16
3.6.3 Correlation analysis 16
3.7 Ethics 16
4 Results 17
4.1 Pregnancy 17
4.1.1 Characteristics 17
4.1.2 Intakes of EPA and DHA 21
4.1.3 Correlation between intakes of EPA and DHA 23
4.1.4 Maternal intake of EPA and DHA during pregnancy and CD in the offspring 23
4.2 6 months 27
4.2.1 Children`s use of cod liver oil at 6 months and CD 30
4.3 18 months 32
4.3.1 Children`s use of cod liver oil at 18 months and CD 35
5 Discussion 37
5.1 Main findings 37
5.2 Results in context 38
5.2.1 EPA and DHA intakes compared with other countries 40
5.3 Methodological considerations 40
5.3.1 Strengths 40

5.3.2 Information bias 41
5.3.3 Selection bias 41
5.3.4 Residual confounding 42
5.3.5 Dietary assessment 43
5.3.6 Other limitations 45
5.4 Potential negative effects due to intake of EPA and DHA 46
5.4.1 Environmental contaminants 46
5.4.2 High intakes of EPA and DHA 47
6 Conclusion 49
References 50

Appendix 1
Excerpts of questionnaires
Appendix 2
Supplementary Table 1. Characteristics of the participants included in the analysis of maternal EPA and DHA intakes during pregnancy by the child`s CD status
Appendix 3
Supplementary Table 2. Maternal intakes of EPA and DHA and supplement use by maternal CD status

List of abbreviations

ALA	Alpha-linolenic acid
BMI	Body mass index
CD	Celiac disease
CI	Confidence interval
CRP	C-reactive protein
DHA	Docosahexaenoic acid
dl-PCBs	Dioxin-like polychlorinated biphenyls
EFSA	European Food Safety Authority
EMA	Endomysial antibodies
EPA	Eicosapentaenoic acid
ESPGHAN	European Society of Paediatric Gastroenterology, Hepatology and Nutrition
EU	European Union
FA	Fatty acid
FD	Food diary
FFQ	Food frequency questionnaire
HLA	Human leukocyte antigen
IELs	Intraepithelial lymphocytes
Ig	Immunoglobulin
IL-15	Interleukin-15
IL-6	Interleukin-6
MBRN	Medical Birth Registry of Norway
MoBa	The Norwegian Mother, Father and Child Cohort Study (Den norske mor, far og barn-undersøkelsen)
n-3	Omega-3
n-6	Omega-6
NF-kB	Nuclear factor-kappa B
NIPH	Norwegian Institute of Public Health
NPR	Norwegian Patient Registry
PAGE	Prediction of Autoimmune diabetes and celiac disease in childhood by Genes and perinatal Environment
PUFA	Polyunsaturated fatty acid

RR	Relative risk
SD	Standard deviation
TEDDY	Environmental Determinants of Diabetes in the Young study
TNF-α	Tumor necrosis factor alpha
tTG	Tissue transglutaminase
TWI	Tolerable weekly intake
T1D	Type 1 diabetes
ULN	Upper limit of normal
VKM	Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø)

List of tables

Table 1. Characteristics of the participants included in the analysis of maternal EPA and DHA intakes during pregnancy by consumption quartiles and supplement use 18
Table 2. Characteristics of included participants and of participants excluded due missing exposure data in the pregnancy cohort 20
Table 3. Maternal intakes of EPA and DHA during pregnancy 21
Table 4. Maternal intake of EPA and DHA from food by supplement intake category 21
Table 5. Maternal use of supplements during pregnancy by the child`s birth year 22
Table 6. Spearman`s correlation between maternal EPA and DHA intakes 23
Table 7. Associations between maternal intakes of EPA and DHA during pregnancy and risk of CD in the offspring 25
Table 8. Characteristics of the study participants included in the analysis of children`s use of cod liver oil at 6 months by the child`s CD status and use of cod liver oil 28
Table 9. Characteristics of included participants and of participants excluded due to missing exposure data in the 6 months cohort 29
Table 10. Children`s use of cod liver oil at 6 months by birth year 30
Table 11. Association between children`s use of cod liver oil at 6 months and risk of CD.... 31
Table 12. Characteristics of the study participants included in the analysis of the children`s use of cod liver oil at 18 months by the child`s CD status and use of cod liver oil 33
Table 13. Characteristics of included participants and of participants excluded due to missing exposure data in the 18 months cohort 34
Table 14. Children`s use of cod liver oil at 18 months by birth year 35
Table 15. Association between children`s use of cod liver oil at 18 months and risk of CD.. 36

List of figures

Figure 1. Adaptive and innate immune responses involved in celiac disease. 3
Figure 2. Flow chart 12

1 Background

1.1 Celiac disease

Celiac disease (CD) is an immune-mediated disease with a prevalence of about 1% in Europe (1). It is characterized by an inappropriate immune response following ingestion of gluten that leads to villous atrophy and crypt hyperplasia in the small intestine (2, 3). Both environmental factors and genetics are involved in the development of CD (4). It was previously considered a childhood disease, but it can occur at any age (5, 6). Evidence suggests that the diagnosis rate has increased among adults (5), but the incidence is still considerably higher among children (7). Women are diagnosed with CD more often than men (3).

1.1.1 Clinical presentation

The clinical presentation of CD varies between individuals and with age of disease onset. Classical symptoms such as diarrhea, malnutrition and failure to thrive are typical for pediatric CD (3, 8). Adults often have milder and more diffuse symptoms, such as abdominal bloating and discomfort (5, 8). CD is also associated with extraintestinal symptoms such as arthritis, neurological symptoms, and dermatitis herpetiformis (5, 9). Since some patients have very few symptoms or are asymptomatic, CD is considered to be heavily underdiagnosed, and this has been confirmed in screening studies (1, 10, 11).

1.1.2 Prevalence and incidence

The prevalence of CD varies greatly between European countries, from 2.0% in Finland to 0.3% in Germany (1). In Scandinavia, the prevalence and incidence of CD have increased considerably over the last decades. The incidence increased from 2.8 to 10.0 per 100,000, while the prevalence increased from 43.2 to 83.6 per 100,000, from 2000-2010, in Denmark (12). In the same period, the incidence of pediatric CD increased from 15.9 to 45.4 per 100,000 person-years in South-Eastern Norway (13). In Finland, the prevalence nearly doubled from 1.1% in 1978-80 to 2.0% in 2000-2001 (14). Some of the observed increase in incidence and prevalence may be explained by increased disease awareness and improved diagnostic tools, but studies have emphasized that it also has been a true increase in CD incidence and prevalence (12-14).

1.1.3 Genetics

Genetic susceptibility is necessary for development of CD. The most important genetic factors identified are the human leukocyte antigen (HLA) molecules DQ2 and DQ8 (4). HLA molecules are proteins that present foreign proteins to the immune systems T-cells. They are encoded by a group of genes that has a large number of alleles, so different HLA molecules can be expressed in different individuals (15). Most people who develop CD express the HLA molecules DQ2 and/or DQ8. However, only a small proportion of individuals with these HLA molecules develops CD (2, 9). It has also been identified several non-HLA genes that may influence the risk of CD. They have been estimated to explain about 15% of the heritability, while the HLA genes explains about 40% (9, 16). Due to the heritability, CD tends to cluster in families, and a systematic review and meta-analysis found that about 8% of first-degree relatives have CD (16). There is also higher prevalence of CD among people with other immune-mediated diseases, such as type 1 diabetes (T1D) that also is associated with HLA DQ2 and DQ8 (17). About 1-10% of patients with T1D also have CD (10).

1.1.4 Pathogenesis

Ingestion of gluten triggers development of CD (18). Gluten is a mixture of proteins mainly found in wheat, rye and barley. The gluten proteins are called gliadins and glutenins in wheat, secalin in rye and hordein in barley. Enzymes in the small intestine usually break down dietary proteins to small peptides and amino acids that are transported across the epithelium (19). Due to high content of the amino acids glutamine and proline, gluten proteins are not completely degraded, and long gliadin peptides are generated after ingestion of gluten (3, 9). These peptides are accumulated in the small intestine, where they can exert toxic effects in those who are genetically predisposed to CD (19). The peptides activate both adaptive and innate immune responses (Figure 1).

T- cells in the lamina propria are mediators for the adaptive immune response.(3, 9). These T-cells are called CD4-positive because they express the co-receptor protein CD4, which is important for the T-cells recognition of HLA II molecules (20). The gliadin peptides enter the lamina propria where they are deaminated by the enzyme tissue transglutaminase (tTG) (3, 9). As a result, the peptides affinity to HLA DQ2 and DQ8 is increased (9). Antigen-presenting cells take up the modified peptides and present them on their surface, bound to HLA DQ2 or DQ8 (3, 9). The T-cells recognize the peptides, gets activated and produce pro-inflammatory

signal molecules (cytokines), such as interferon-γ (3, 9, 21). This leads to an inflammatory process that eventually causes villous atrophy (shortened villi) and crypt hyperplasia (elongated crypts) (9). The T-cells also trigger activation of B-cells that differentiate into plasma cells secreting autoantibodies against tTG, including endomysial antibodies (EMA), and antibodies against deamidated gliadin peptides (3, 9, 22). The innate immune response involves, among other things, increased number of intraepithelial lymphocytes (IELs) and overexpression of interleukin-15 (IL-15), a cytokine (3, 9). IL-15 activates IELs, which makes them kill intestinal epithelial cells.

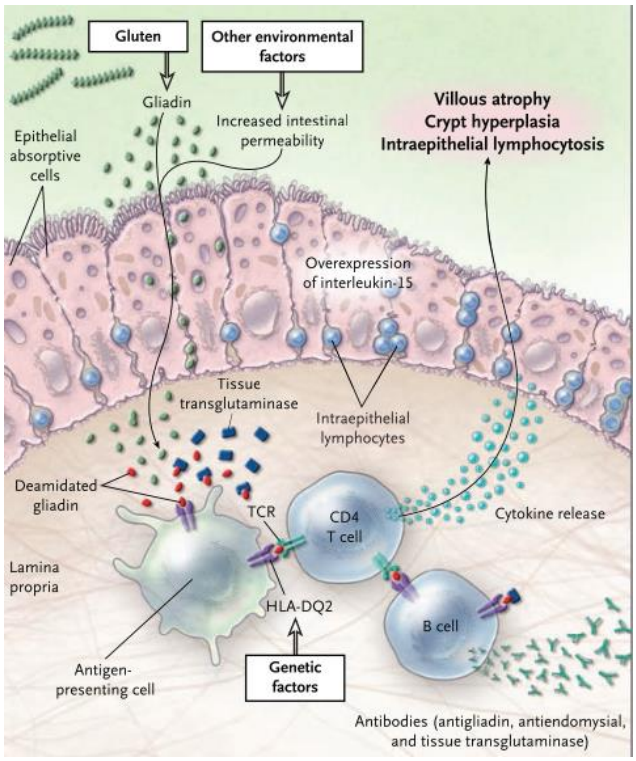

Figure 1. Adaptive and innate immune responses involved in celiac disease.

Gliadin peptides enter the lamina propria where they are deaminated by tissue transglutaminase. They are further bound to HLA DQ2 (or DQ8) on antigen-presenting cells and presented to CD4+ T cells through a T-cell receptor. The T-cells produce cytokines and trigger activation of B-cells that differentiate into plasma cells secreting antibodies. This leads to an inflammatory process that eventually causes villous atrophy and crypt hyperplasia. The number of intraepithelial lymphocytes is increased, and when they are activated by interleukin-15, which is overexpressed, they kill intestinal epithelial cells. Abbreviations: HLA, human leucocyte antigen; TCR, T-cell receptor
Figure reproduced with permission from Green PH, Cellier C. Celiac disease. The New England journal of medicine. 2007;357(17):1731-43, Copyright Massachusetts Medical Society.

1.1.5 Diagnostic criteria

CD diagnosis can be established through duodenal biopsies to detect villous atrophy and crypt hyperplasia, and/or serological testing (3, 23). The European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) published new diagnostic guidelines for CD in children and adolescents in 2020 (24, 25). For serological testing, they recommend measurement of total serum immunoglobulin (Ig) A and IgA antibodies against tTG. Testing for IgG antibodies against deaminated gluten peptides, EMA or tTG is only indicated if total IgA concentration is low. The diagnosis can be established without biopsies if serum levels of anti-tTG are ≥10 times the upper limit of normal (ULN), as long as EMA-IgA is positive in a second blood sample. Biopsies should be taken when anti-tTG levels are low (< 10 times ULN). They recommend ≥ 4 biopsies from the distal duodenum and ≥ 1 from the duodenal bulb.

1.1.6 Treatment and consequences

Currently, no strategies for primary prevention of CD are known, and the only available treatment is lifelong avoidance of gluten (3, 9). This can be challenging since gluten is present in a wide range of foods, and gluten-free foods can be contaminated (26). Some CD patients continue to experience symptoms and/or have intestinal inflammation, even though they follow a strict gluten-free diet (9, 27). Both treated and untreated CD have been associated with reduced quality of life and increased mortality (9). Meta-analyses have shown that patients with CD have increased risk of cancers, such as non-Hodgkin lymphoma, esophageal cancer and small intestinal carcinoma (28, 29).

1.2 EPA and DHA

Fatty acids (FA) can be classified based on the length of, and the number of double bonds in, their hydrocarbon chain, in addition to the position of the first double bond (30). Long-chain polyunsaturated fatty acids (PUFAs) have more than 12 carbon atoms, and more than two double bonds, in their chain. FAs with the first double bond located at the third carbon atom from the methyl end are termed omega-3 (n-3). n-3 PUFAs, especially the very-long eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are generally considered to be beneficial for health (30, 31).

1.2.1 Sources

EPA (20:5n-3) and DHA (22:5n-3) can, to some extent, be generated in the body through conversion of alpha-linolenic acid (ALA), an essential n-3 PUFA (30). ALA (18:3n-3) is present in plant sources, such as seeds of chia and flax, green leafy vegetables, and vegetable oils. The conversion of ALA to EPA and DHA involves enzymatic elongation and desaturation, in addition to beta-oxidation for DHA (32). However, reported conversion rates are relatively low, up to 21% in women and 8% in men (33). Consequently, dietary intakes of EPA and DHA are required to achieve sufficient levels (34). The main dietary sources are fish and other seafood (35). Their content of EPA and DHA varies, not only depending on the species, but also their feed, among other things (30). Oily fish species, such as herring, mackerel and farmed salmon, are particularly good sources (36). Recent analyses have found that median combined content of EPA and DHA in these species are 2.2, 5.2 and 1.2 g/100 g, respectively. In some countries, including Norway, fish oil supplements, such as cod liver oil, are also an important source of EPA and DHA (37). One teaspoon (5 ml) of cod liver oil, the recommended daily dose in Norway, provides about 0.4 g EPA and 0.6 g DHA (38)

1.2.2 In early life

n-3 PUFAs are important for fetal growth and development (39). A systematic review and meta-analysis found that maternal supplementation with n-3 PUFAs, from pregnancy through infancy, had significant benefit on cognitive development and visual acuity in infants (40). The fetal brain grows rapidly during the third trimester of pregnancy (41, 42), and from that trimester to 18 months after birth, DHA is accumulated there at high rates (39, 43). Due to lack of desaturase enzymes required for conversion from ALA to DHA in the placenta, and limited fetal desaturase activity, fetal DHA requirements must be met by placental transfer (41, 42). The mechanisms of placental transfer of FAs are not fully understood, but several membrane proteins, such as fatty acid translocase, are thought to be involved (39, 41). Studies have reported that there is a preferential transfer of DHA relative to other fatty acids (39, 42). After birth, DHA is transferred from the mother to the infant through breastmilk. The amount of DHA that is transferred, during both gestation and lactation, depends, to a large extent, on maternal DHA status (43).

1.2.3 Recommendations and intakes in Norway

Norway has long traditions for fish consumption, and compared with many other countries, both the total consumption and the percentage of lean fish are high. It is also more common to eat fish in the form of cold cuts and spreads due to the Norwegian custom of eating several meals with bread daily (44). It is also long traditions for use of cod liver oil, and the percentage of users is considerably higher in Norway than in many other countries. In a Nordic survey from 2009, 30% of the Norwegians, and only 2% of the participants from the other Nordic countries, reported use of cod liver oil (2).

The Norwegian health authorities recommend eating fish for dinner two to three times per week (45). For adults, this equals about 300-450 g pure fish per week, of which at least 200 g should be fatty fish. Pregnant and lactating women are advised to limit or avoid intake of fish species that may contain high levels of environmental toxins (46). For adults and children over two years old, it is recommended that n-3 PUFAs constitute at least 1% of the total energy intake, of which 200 mg/day should be DHA in pregnant and lactating women (47).

According to national dietary surveys, most one- and two-year-olds consume some fish, with an average intake of 19 and 33 g/day, respectively (48, 49). Data on intake of fish and seafood among pregnant women in Norway are based on The Norwegian Mother, Father and Child Cohort Study (MoBa). During the period 2002-2008, the average intake of fish and seafood among pregnant women was 36 g/day, and 67% used fish oil supplements (50). The median total intake of fish and seafood remained stable over the entire period at around 31 g/d (51). It was a small increase in intake of medium-oily fish and salmon/trout at the expense of lean fish over time. The percentage of fish oil supplement users increased from 54% in 2002-2003 to 77% in 2008 (37).

It has been a long tradition in Norway to recommend cod liver oil from the age of four weeks, as a source of vitamin D for breastfed infants, and of DHA for formula-fed infants (52). In the latest national dietary surveys, from 2018 and 2019, 40% of children aged 6 months (53) and about 30% of one- and two-year-olds used cod liver oil (48, 49). However, since September 2020, use of cod liver oil is no longer recommended for children under the age of one year, for either breastfed or formula-fed infants (52, 54). This is due to new European Union (EU) regulations concerning infant formula that includes mandatory addition of DHA and increased minimum level of vitamin D (52). In addition, the previously recommended daily dose of cod

liver oil did not provide enough vitamin D to meet the requirements for infants under the age of 6 months, while analyses have indicated that that breastmilk from most Norwegian women contains more than sufficient amounts of DHA to meet infant requirements (52). Thus, cod liver oil will not provide enough vitamin D for breastfed infants and can lead to an intake of DHA that is considerably higher than recommended, in both breastfed and formula-fed infants. The current recommendations are that infants fed exclusively on formula should not receive any nutrient supplements, while breastfed infants should receive vitamin D drops from the age of one week (52, 54).

Results from national dietary surveys have shown that use of cod liver oil among children has been reduced over time, even before the new recommendations came. From 1999 to 2007, the percentage of users was reduced from 55% to 40% among children aged 6 months (55, 56), from 45% to 39% among one-year-olds (57, 58) and from 47% to 28% among two-year-olds (59, 60).

1.3 Celiac disease and EPA and DHA

The increased incidence and prevalence of CD the last decades suggest that environmental factors, in addition to gluten, are involved in the disease development (13, 61). Although CD can occur at any age, it has been observed that many develop antibodies against tTG already when they are two-three years old (62). This suggest that exposures in early life, meaning exposures in utero and the first years of life, could be very important (61). The hypothesized risk factors include nutritional exposures, childhood infections, delivery methods and drug use, among others (2, 9, 63). For many of the environmental factors, the exact mechanisms of potential action are unknown. Potential mechanisms include inflammation, increased intestinal permeability, altered composition of the gut microbiota and direct toxic effects (63). Intake of n-3 PUFAs has been hypothesized to have therapeutic potential, and even a preventive effect, on the development of immune-mediated diseases (34, 64).

Potential mechanisms

1.3.1.1 Anti-inflammation

The exact mechanisms behind the hypothesized beneficial effects of n-3 PUFAs on immune-mediated diseases are not known. However, the main hypothesis is that they are due to the n-3 PUFAs anti-inflammatory effects, which are mainly attributable to EPA and DHA (32, 34, 64). Nuclear factor-kappa B (NF-κB) is a transcription factor that regulates expression of pro-inflammatory cytokines (19, 65). It has been reported that EPA and DHA can inhibit activation of NF-κB and thus reduce the production of these cytokines. A meta-analysis including 68 studies showed that supplementation with marine-derived n-3 PUFAs had a lowering effect on levels of the cytokines interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α), in addition to the acute phase protein C-reactive protein (CRP) (66). Concentration of CRP in the blood is used as a marker for inflammation (67). It has been observed that patients with CD have significantly higher levels of IL-6 and TNF-α than healthy controls (19, 68). It has also been observed that mothers of children who have developed CD had higher level of certain cytokines, including TNF-α, during pregnancy, than mothers of children without CD (69).

n-3 PUFAs can also affect the production of eicosanoids, biologically active compounds involved in inflammation and regulation of immune function (65). Eicosanoids can be produced from both omega-6 (n-6) and n-3 PUFAs, but those produced from n-3 PUFAs, especially marine n-3 PUFAs, are less pro-inflammatory (31). When intake of n-3 PUFAs is increased they partially replace n-6 PUFAs in cell membranes, and production of eicosanoids from n-3 PUFAs will increase at the expense of those from n-6 PUFAs (70). In addition are EPA and DHA precursors of a type of eicosanoids called resolvins, which have been found to be anti-inflammatory and inflammation resolving in animal studies (70).

1.3.1.2 Composition of gut microbiota

Studies have also indicated that n-3 PUFAs may affect the composition of the gut microbiota by, among other things, increasing the bacterial diversity and the level of bacteria considered as health-promoting (71). It has been observed that patients with CD have a different composition of the gut microbiota than healthy controls (61, 72, 73). This includes higher concentration of potentially pro-inflammatory bacteria, and lower concentration of bacteria considered as health-promoting. However, since most of the studies where this has been

observed are observational, it is not clear whether the altered gut microbiota is a cause or consequence of CD (61).

1.3.2 Omega-3 fatty acids and immune-mediated diseases

In a Norwegian case-control study, both maternal use of cod liver oil during pregnancy, and children`s use of cod liver oil during the first year of life, were associated with lower risk of T1D in the child (74). In a later, larger study, an association was only found for children`s use of cod liver oil (75). In a study among children at increased risk of T1D, both higher intake and higher proportion in erythrocyte membranes of total n-3 PUFAs were associated with lower risk of T1D autoimmunity (76). However, the study found no associations for marine n-3 PUFAs alone. A randomized controlled trial found that maternal supplementation with EPA and DHA, during the third trimester of pregnancy, was associated with reduced risk of asthma in the offspring (77).

Few prospective studies have investigated whether there is an association between intake of n-3 PUFAs in early life and risk of CD. Yang et al. (78) and Størdal et al. (79) investigated use of supplements with n-3 PUFAs during pregnancy. Both studies found no association with risk of CD in the offspring. These studies did not examine intake of n-3 PUFAs from dietary sources, the individual effects of different n-3 PUFAs, or intake in infancy and early childhood. In a prospective analysis, a “prudent” dietary pattern, of which fish formed an essential part, in children around one year of age, was significantly associated with lower odds of CD autoimmunity (80).

2 Aims and hypotheses

A better understanding of the environmental factors involved in onset of CD may provide strategies for prevention of the disease in the future (3). If proven effective, increased intake of EPA and DHA, through food and/or dietary supplements, could be an easy administered part of prevention.

The primary aim of this thesis was to investigate if maternal intake of EPA and DHA during pregnancy, from food and/or supplements, was associated with risk of childhood onset CD in the offspring.

Secondary aim:

- Investigate if children`s use of cod liver oil, at 6 months and at 18 months, was associated with risk of childhood onset CD

Hypotheses:

- Higher maternal intakes of EPA and DHA, during pregnancy, is associated with lower risk of childhood onset CD in the offspring.
- Children`s use of cod liver oil, at 6 months and at 18 months, is associated with lower risk of childhood onset CD

3 Material and methods

3.1 Study design

Prospective nationwide pregnancy cohort study with linkage to registry data.

3.2 Study population

Participants from The Norwegian Mother, Father and Child Cohort Study (MoBa) were included in this study. MoBa is a prospective population-based pregnancy cohort that aims to detect causes of diseases (81). It is conducted by the Norwegian Institute of Public Health (NIPH). Pregnant women were recruited from across Norway from 1999-2008 and 41% agreed to participate. Over 114,000 children, 95,000 mothers and 75,000 fathers are included, making it one of the largest pregnancy cohorts in the world. Data is obtained through collection and analyses of biological material, regular questionnaires, and by linkage to national health registries.

The present study is based on version XII of the quality-assured data files released for research in 2020. Information from questionnaires administered at 18 gestational weeks (questionnaire 1) (82), 22 gestational weeks (questionnaire 2) (83), 30 gestational weeks (questionnaire 3) (84), child age 6 months (questionnaire 4) (85) and child age 18 months (questionnaire 5) (86) was used in this study. Questionnaire 1 and 3 covers general background information, in addition to the women's previous and present health problems and exposures (81). Child nutrition, health and development are the main focus in questionnaire 4 and 5. Questionnaire 2 is a validated food frequency questionnaire (FFQ) that was developed specially for use in MoBa (87, 88). It was introduced in the study in 2002 and covers maternal diet, including use of dietary supplements, the first four-five months of the pregnancy (89). Questions about intake of 255 food items are included in the FFQ (87). Additional data were obtained by linkage to the Medical Birth Registry of Norway (MBRN) and the Norwegian Patient Registry (NPR), with the use of unique personal identification numbers. The MBRN contains information about all births in Norway (90), while the NPR contains activity data from all Norwegian government-owned hospitals and outpatient clinics (79).

3.2.1 Inclusion and exclusion criteria

Three different cohorts were included in this study, one for maternal intake during pregnancy, one for intake at 6 months and one for intake at 18 months. For the children to be eligible for the cohorts, the mothers had to have answered questionnaire 1 and children with unconfirmed CD diagnosis were also excluded. For the children to be included in the analysis of maternal intake during pregnancy, the mothers had to have answered questionnaire 2 and have a daily energy intake between 4.5 and 20 MJ/day. The cutoff for energy intake is standard in MoBa studies and have been chosen based on plausibility (87). Questionnaire 4 had to be answered for the children to be included in the analysis of use of cod liver oil at 6 months. Both questionnaire 4 and 5 had to be answered for the children to be included in the analysis of use of cod liver oil at 18 months. An overview of the exclusion process is presented in Figure 2 (flow chart).

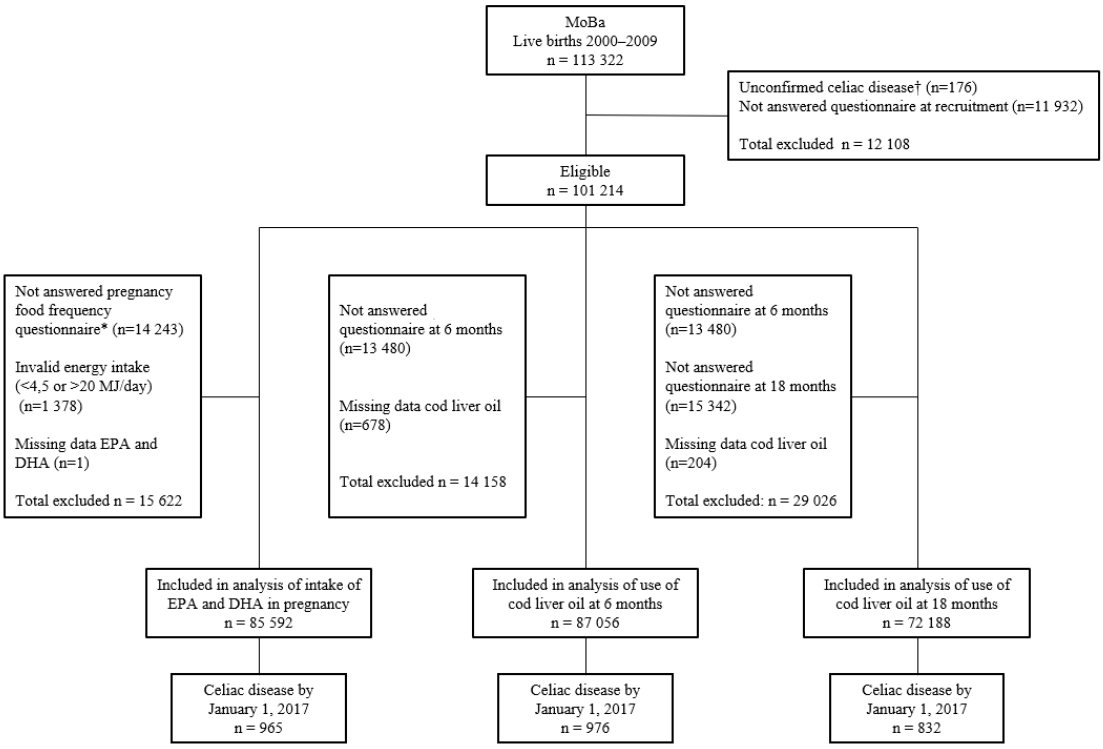

Figure 2. Flow chart showing selection of the study participants from the Norwegian Mother, Father and Child Cohort Study (MoBa)

†Children registered with celiac disease in the Norwegian Patient Registry only once (without questionnaire confirmation) were regarded to have unconfirmed celiac disease and were excluded
*The food frequency questionnaire was introduced in 2002
Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid

3.3 Exposures

3.3.1 Maternal intakes of EPA and DHA during pregnancy

Maternal intakes of EPA and DHA during pregnancy, from food and supplements, were obtained from the MoBa database. They had previously been calculated from the FFQ in mid-pregnancy. The FFQ included 10 questions that covered use of fish and other seafood as cold cuts or spreads on bread, and 15 questions that covered fish and other seafood eaten for dinner (Appendix 1). Use of supplements containing EPA and DHA was covered by four questions. The Norwegian food composition table and the program FoodCalc had been used to calculate intakes from food (50). Standard Norwegian portion sizes had been used to convert consumption frequencies of fish into food amounts (grams per day) (91). It has been developed a database with details on the declared content of supplements used by MoBa participants, and this had been used to calculate intakes of EPA and DHA from supplements (50).

3.3.2 Children`s use of cod liver oil at 6 and 18 months

Information about children`s use of cod liver oil at 6 and 18 months was derived from the questionnaires administered at these ages. The questionnaires included a general question of whether the child was given any dietary supplements (including cod liver oil), followed by a question regarding cod liver oil asking how often (daily or sometimes) it was given to the child (Appendix 1). If the general question was answered with “yes” and the question about cod liver oil was unanswered, the child was considered as a non-user. If neither of the questions were answered it was considered as missing data on use of cod liver oil and the child was excluded in the exclusion process. In the analyses, use of cod liver oil was both treated as a dichotomous variable separating non-users and users, and as a variable for frequency of use with three categories: never, sometimes, daily. Some had answered both “sometimes” and “daily” when asked how often the child was given cod liver oil, and their children were excluded from the analysis with the variable for frequency of use.

3.4 Outcome

The outcome of interest was clinically diagnosed CD. Parental questionnaires and linkage to the NPR were used to identify children diagnosed with CD by the end of 2016. It is mandatory to report to the NPR (79), and children suspected of having CD may be registered with the diagnosis before it is histologically confirmed. Thus, to reduce the risk of false-positive cases, case definition was restricted to individuals registered at least two times. The NPR was not launched before January 2008, so children diagnosed before this and not followed as recommended in the outpatient clinic may not be identified there (92). Due to this, questionnaires were also used to identify CD cases. A specific question of whether the child has been diagnosed with CD was included in the parental questionnaires administered when the children were seven and eight years old.

3.5 Covariates

Parental and child characteristics that may be associated with both maternal intake of EPA and DHA/children`s use of cod liver oil and development of CD based on previous studies were included as covariates in regression models. Pre-pregnancy weight and height reported in questionnaire 1 were used to calculate pre-pregnancy body mass index (BMI) that was divided into four categories: < 20, 20–24,9, 25-29.9, ≥30 kg/m^2. Maternal education was obtained from questionnaire 1 and divided into three categories: ≤12 years (high school or less), 13-15 years (3 years of college/university), ≥ 16 years (4 years or more of college/university). Maternal smoking during pregnancy was obtained from questionnaire 1, 3 and 4, and divided into three categories: never, sometimes and daily. Individuals smoking seven or more cigarettes per week were considered daily smokers. The NPR provided information on maternal and paternal CD status. Information about maternal parity, maternal age at delivery, mode of delivery, child`s sex and child`s birth year were obtained from the MBRN. Maternal parity was divided into three categories: nulliparous, one previous birth, two or more previous births. Maternal age at delivery and child`s birth year were treated as continuous variables. Gestational duration and child`s birth weight were obtained from the MBRN and questionnaire 4. Children born before gestational week 37 were defined as premature. Birth weight was divided into four categories: <2500, 2500-3499, 3500-4499, ≥4500 g.

3.6 Statistical analyses

All analyses were performed using Stata Release 16 (College Station, Tx, USA).

3.6.1 Main analysis

Binary log-linear regression was used to estimate relative risks (RR) with 95% confidence intervals (CI). Cluster sandwich estimator for variance was used to account for potential clustering among siblings. P-values below 0.05 were considered statistically significant.

In the analysis of maternal EPA and DHA intake during pregnancy, we hypothesized log-linear associations and treated all exposure variables as continuous variables (g/day):

- Total (food and supplements) intake of EPA, intake of EPA from food, intake EPA from supplements
- Total (food and supplements) intake of DHA, intake of DHA from food, intake of DHA from supplements
- Total (food and supplements) combined intake of EPA and DHA, combined intake of EPA and DHA from food, combined intake of EPA and DHA from supplements

We also explored potential nonlinear associations by studying maternal intakes in quartiles or categories. The combined intake of EPA and DHA from food and supplements, and the combined intake of EPA and DHA from food, were divided into quartiles. Use of supplements was both analyzed as a dichotomous variable, separating non-users and users, and as a variable with three categories, with non-users as one group and users ranked into two groups, divided by the median.

Children's use of cod liver oil, at 6 months and at 18 months, was studied both as dichotomous variables and frequency variables with three categories (never, sometimes, daily).

In addition to an unadjusted model, we had two adjusted models, a main model (model 1) and a sensitivity model (model 2). The variables included in the main adjusted model were maternal age at delivery, maternal education, smoking during pregnancy, parity, pre-pregnancy BMI, caesarean section, maternal and paternal CD status, child`s sex, child`s birth weight, and prematurity.

3.6.2 Sensitivity analysis

As mentioned in the introduction, it has previously been observed that maternal use of fish oil supplements and intake of fatty fish have increased, while children`s use of cod liver oil has been reduced, over time. In addition, the risk of CD increases with increased follow-up time. Due to this, we adjusted for the child`s birth year, in addition to the variables included in the main adjusted model, as a preplanned sensitivity analysis (model 2).

3.6.3 Correlation analysis

Since EPA and DHA tend to come from the same dietary sources and n-3 FA supplements usually contain both FAs, the association between intakes of EPA and DHA was examined. Spearman`s correlation analysis was used since intakes of EPA and DHA were not normally distributed.

3.7 Ethics

This study was as part of a project in MoBa called Prediction of Autoimmune diabetes and celiac disease in childhood by Genes and perinatal Environment (PAGE). All participants in MoBa have provided written informed consent. PAGE has been approved by the Regional Committee of Medical and Health Research Ethics of South-East Norway and the Norwegian Data Inspectorate. The study data was only accessed through the NIPHs research server.

4 Results

4.1 Pregnancy cohort

4.1.1 Characteristics

In the analysis of maternal intakes of EPA and DHA, 85,592 children from MoBa born between 2002 and 2009, followed to January 1st, 2017, were included. After a mean of 11.5 years (range 0.75–14.8) of follow-up, 965 children (1.1%, 62% girls) had developed CD at a mean age at diagnosis of 6.3 years (range 0.8 – 13.7).

Parental and child characteristics of the included participants are presented in Table 1. Characteristics differed across increasing quartile of total EPA and DHA intake and between users and non-users of supplements. Women in the lowest consumption quartiles had higher BMI, included fewer first-time mothers, more women with low education and more smokers than those in the higher consumption quartiles. There was a higher percentage of first-time mothers and mothers with high education among supplement users, while the percentage of daily smokers and women with high BMI was higher among non-users.

Besides parental CD and female sex, there were small differences in parental and child characteristics between those who developed CD and those who did not (Appendix 2, Supplementary Table 1). Mothers of children with CD were less likely to smoke, to have two or more previous births, and to have had caesarean section. They were also more likely to have high education and the children with CD had lower birth weight.

Characteristics of participants excluded due to missing exposure data are presented in Table 2. The prevalence of CD among the excluded participants was similar to the prevalence among those included (0.9% vs. 1.1%). The percentage of daily smokers and women with low education was higher among excluded participants, while the percentage of first-time mothers was higher among included participants.

Table 1. Characteristics of the participants included in the analysis of maternal EPA and DHA intakes during pregnancy by consumption quartiles and supplement use

	Total intake of EPA and DHA, n (%)				EPA/DHA supplement use, n (%)	
	1st quartile n = 21,244	2nd quartile n = 21,517	3rd quartile n = 21,455	4th quartile n = 21,376	No (32%)	Yes (68%)
Maternal						
Age at delivery* (years), mean (SD)	29.5 (4.6)	30.2 (4.4)	30.5 (4.5)	30.8 (4.6)	29.9 (4.8)	30.4 (4.4)
Parity						
0	8,793 (41.4)	9,599 (44.6)	10,353 (48.3)	10,361 (48.5)	9,616 (35.1)	29,490 (50.7)
1	8,161 (38.4)	7,902 (36.7)	7,191 (33.5)	7,044 (33.0)	10,874 (39.7)	19,424 (33.4)
≥2	4,290 (20.2)	4,016 (18.7)	3,911 (18.2)	3,971 (18.6)	6,911 (25)	9,277 (15.9)
Caesarean section	3,202 (15.1)	3,159 (14.7)	3,330 (15.5)	3,322 (15.5)	4,178 (15.3)	8,835 (15.2)
Education*						
≤12 years	9,705 (45.7)	7,320 (34.0)	6,867(32.0)	6,674 (31.2)	13,130 (47.9)	17,436 (30.0)
13-15 years	8,029 (37.8)	9,040 (42.0)	9,116 (42.5)	8,804 (41.2)	9,913 (36.2)	25,076 (43.1)
≥ 16 years	3,421 (16.1)	5,038 (23.4)	5,393 (25.1)	5,796 (27.1)	4,227 (15.4)	15,241 (26.5)
Smoking during pregnancy*						
Never	18,563 (87.4)	19,720 (91.7)	19,806 (92.3)	19,736 (92.3)	23,541 (85.9)	54,284 (93.3)
Sometimes	417 (2.0)	338 (1.6)	353 (1.7)	330 (1.5)	586 (2.1)	852 (1.5)
Daily	2,153 (10.1)	1,356 (6.3)	1,183 (5.5)	1,177 (5.5)	3,111 (11.4)	2,758 (4.7)
CD*	84 (0.4)	79 (0.4)	100 (0.5)	100 (0.5)	97 (0.4)	266 (0.5)
Pre-pregnancy BMI* (kg/m^2), mean (SD)	24.6 (4.6)	24.2 (4.3)	23.9 (4.2)	23.6 (4.0)	24.7 (4.7)	23.8 (4.1)
>20	2,339 (11.0)	2,339 (11.8)	2,653 (12.4)	2,814 (13.2)	2,905 (10.6)	7,435 (12.8)
20-24.9	10,602 (49.9)	11,591 (53.9)	12,059 (56.2)	12,382 (57.9)	13,434 (49.0)	33,200 (57.1)
25-29.9	5,074 (23.9)	4,792 (22.3)	4,385 (20.4)	4,136 (19.4)	6,697 (24.4)	11,690 (20.1)
≥30	2,602 (12.3)	2,058 (9.6)	1,856 (8.7)	1,546 (7.2)	3,497 (12.8)	4,565 (7.8)

Table 1. Continued

	Total intake of EPA and DHA, n (%)				EPA/DHA supplement use, n (%)	
	1st quartile n = 21,244	2nd quartile n = 21,517	3rd quartile n = 21,455	4th quartile n = 21,376	No (32%)	Yes (68%)
Paternal						
CD	71 (0.3)	76 (0.4)	86 (0.4)	75 (0.4)	92 (0.3)	215 (0.4)
Child						
Female sex	10,334 (48.6)	10,610 (49.3)	10,501 (49.0)	10,297 (48.2)	13,306 (48.6)	28,436 (48.9)
Birth weight* (g), mean (SD)	3561 (595)	3567 (583)	3561 (590)	3560 (587)	3584 (596)	3552 (585)
<2500	904 (4.3)	842 (4.0)	932 (4.3)	902 (4.2)	1,119 (4.1)	2,461 (4.2)
2500-3499	8,127 (38.3)	8,233 (38.3)	8,230 (38.4)	8,190 (38.3)	10,071 (36.8)	22,709 (39.0)
3500-4499	11,282 (53.1)	11,513 (53.5)	11,390 (53.1)	11,418 (53.4)	14,887 (54.3)	30,716 (52.8)
≥4500	930 (4.4)	926 (4.3)	902 (4.2)	865 (4.1)	1,322 (4.8)	2,301 (4.0)
Prematurity* (<37 weeks)	1,385 (6.5)	1,352 (6.3)	1,358 (6.3)	1,337 (6.3)	1,676 (6.1)	3,756 (6.5)

Cutoff for quartiles: 1st quartile: < 0.32 g/d, 2nd quartile: 0.32-0.56 g/d, 3rd quartile: 0.56–1.07 g/d, 4th quartile: 1.07-8.93 g/d

*Missing information maternal age at delivery: 219, maternal education: 389, smoking during pregnancy: 460, maternal CD: 32, pre-pregnancy BMI: 2,169, birth weight: 6, prematurity: 62

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; CD, celiac disease; BMI, body mass index; SD, standard deviation

Table 2. Characteristics of included participants and of participants excluded due missing exposure data in the pregnancy cohort

	Included participants, n (%) n = 85,592	**Excluded participants, n (%) n = 15,622**
Maternal		
Age at delivery (years), mean (SD)	30.3 (4.5)	29.7 (4.8)
Missing data	219 (0.3)	46 (0.3)
Parity		
0	39,106 (45.7)	6,332 (40.5)
1	30,298 (35.4)	5,748 (36.8)
≥2	16,188 (18.9)	3,541 (22.7)
Missing data	-	1 (0.0)
Caesarean section	13,013 (15.2)	2,225 (14.2)
Missing data	-	1 (0.0)
Education		
≤12 years	30,566 (35.7)	7,213 (46.2)
13-15 years	34,989 (40.9)	5,565 (35.6)
≥ 16 years	19,648 (23)	2,729 (17.5)
Missing data	389 (0.5)	115 (0.7)
Smoking during pregnancy		
Never	77,825 (90.9)	12,220 (78.2)
Sometimes	1,438 (1.7)	431 (2.8)
Daily	5,869 (6.9)	1,854 (11.9)
Missing data	460 (0.5)	1,117 (7.2)
CD	363 (0.4)	58 (0.4)
Missing data	32 (0.04)	46 (0.3)
Pre-pregnancy BMI (kg/m^2), mean (SD)	24.1 (4.3)	23.9 (4.3)
>20	10,340 (12.1)	2,133 (14.2)
20-24.9	46,634 (54.5)	8,315 (55.3)
25-29.9	18,387 (21.5)	3,188 (20.4)
≥30	8,062 (9.4)	1,409 (9.0)
Missing data	2,169 (2.5)	577 (3.7)
Paternal		
CD	307 (0.4)	26 (0.2)
Missing data	-	-
Child		
Female sex	41,742 (48.8)	7,590 (48.6)
Missing data	-	1 (0.0)
Birth weight (g), mean (SD)	3562 (589)	3591 (602)
<2500	3,580 (4.2)	654 (4.2)
2500-3499	32,780 (38.3)	5,659 (36.2)
3500-4499	45,603 (53.3)	8,513 (54.5)
≥4500	3,623 (4.2)	785 (5.0)
Missing data	6 (0.0)	11 (0.1)
Prematurity (<37 weeks)	5,432 (6.4)	927 (6.0)
Missing data	62 (0.1)	50 (0.3)
CD	965 (1.1)	145 (0.9)

Abbreviations: CD, celiac disease; SD, standard deviation; BMI, body mass index

4.1.2 Intakes of EPA and DHA

The median intakes (interquartile range) of EPA and DHA were 0.22 (0.12 – 0.46) and 0.34 (0.20 – 0.61) g/day, respectively (Table 3). The relative contribution of food intake was 49% for EPA and 57% for DHA. Women in the highest category of intake of EPA and DHA from supplements also had the highest intake of EPA and DHA from food (Table 4). Use of supplements containing EPA and DHA was reported by 68% of the women. The median combined intake of EPA and DHA from supplements among supplement users was 0.29 g/d.

Table 3. Maternal intakes of EPA and DHA during pregnancy

	Mean, g/d	SD, g/d	Median, g/d	% of total EPA and DHA	Mean, g/d	
					EPA/DHA supplement use	
					No (32%)	Yes (68%)
Total						
EPA and DHA	0.81	0.71	0.56	100	0.42	1.00
EPA	0.34	0.33	0.22	42.2	0.16	0.43
DHA	0.47	0.39	0.34	57.8	0.26	0.57
Food						
EPA and DHA	0.43	0.41	0.32	53.6	0.42	0.44
EPA*	0.17	0.17	0.12	20.5	0.16	0.17
DHA*	0.27	0.24	0.20	33.1	0.26	0.27
Supplements						
EPA and DHA	0.38	0.56	0.16	46.4	-	0.55
EPA	0.18	0.27	0.07	21.7	-	0.26
DHA	0.20	0.29	0.09	24.7	-	0.29

*The relative contribution of food intake was 49% for EPA and 57% for DHA
Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SD, standard deviation

Table 4. Maternal intake of EPA and DHA from food by supplement intake category

	Intake of EPA and DHA from food					
	n (%)				Mean (SD), g/d	Median, g/d
	1st quartile	2nd quartile	3rd quartile	4th quartile		
	<0.20 g/d	0.20-0.32 g/d	0.32-0.52 g/d	0.52 -8.12 g/d		
Supplement use						
No supplement	7,979 (37.6)	6,727 (31.2)	6,432 (29.9)	6,263 (29.4)	0.42 (0.42)	0.30
<0.29 g/d	7,177 (33.8)	7,716 (35.8)	7,427 (34.5)	6,770 (31.8)	0.42 (0.37)	0.31
≥ 0.29 g/d	6,076 (28.6)	7,098 (33.0)	7,663 (35.6)	8,264 (38.8)	0.47 (0.43)	0.35

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SD, standard deviation

There was a small increase in intake of EPA and DHA over time. Median combined intake of EPA and DHA from food increased from 0.31 g/d (mean: 0.44 g/d) in 2002 to 0.38 g/d (mean 0.46 g/d) in 2009. The median intake from supplements increased from 0 g/d (mean: 0.29 g/d) in 2002 to 0.19 g/d from 2006-2009 (mean 2009: 0.41 g/d). The percentage of women that used supplements increased over the study period, from 48% in 2002 to 80% in 2009 (Table 5).

Table 5. Maternal use of supplements during pregnancy by the child`s birth year

	EPA/DHA supplement use, n (%)	
	No	**Yes**
Child`s birth year		
2002	2,298 (52.2)	2,107 (47.8)
2003	5,100 (43.5)	6,617 (56.5)
2004	5,133 (41.4)	7,269 (58.6)
2005	4,454 (33.0)	9,036 (67.0)
2006	3,706 (24.4)	11,502 (75.6)
2007	3,410 (24.5)	10,525 (75.5)
2008	2,715 (23.5)	8,851 (76.5)
2009	585 (20.4)	2,284 (79.6)
Total	27,401 (32.0)	58,191 (68.0)

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid

4.1.3 Correlation between intakes of EPA and DHA

The participants intakes of EPA and DHA were strongly correlated ($r = 0.98$, $p < 0.001$) (Table 6).

Table 6. Spearman`s correlation between maternal EPA and DHA intakes

	Correlation coefficient (r)	p-value
EPA and DHA		
Total (food and supplements)	0.98	<0.001
Food	0.98	<0.001
Supplements	0.99	<0.001

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid

4.1.4 Maternal intake of EPA and DHA during pregnancy and CD in the offspring

4.1.4.1 Total intake (food and supplements)

In the analyses of total intake from food and supplements as continuous variables, neither combined intake of EPA and DHA nor intake of one FA separately, was associated with risk of CD (Table 7). When combined intake of EPA and DHA from food and supplements was divided into quartiles, the cutoff for the quartiles was: 1st quartile: < 0.32 g/d; 2nd quartile: 0.32-0.56 g/d; 3rd quartile: 0.56–1.07 g/d; and 4th quartile: 1.07-8.93 g/d. Compared with intake in the lowest quartile, intake in the third quartile was significantly associated with increased risk of CD in the unadjusted analysis (RR: 1.20, 95% CI: 1.00 - 1.44), but the association did not remain significant after adjustment (Table 7).

4.1.4.2 Intake from food

In the analyses of intake from food as continuous variables, combined intake of EPA and DHA (RR: 0.82, 95% CI: 0.68 – 0.99), intake of DHA (RR: 0.71, 95% CI: 52 – 0.98) and intake of EPA (RR: 0.64, 95% CI: 0.41 - 0.99), were significantly associated with reduced risk of CD, per g/d increase in intake, in the unadjusted analysis (Table 7). However, the associations did not remain significant after adjustment. When combined intake of EPA and DHA from food was divided into quartiles, the cutoff for the quartiles was: 1st quartile: < 0.20 g/d; 2nd quartile: 0.20-0.32 g/d; 3rd quartile: 0.32–0.52 g/d; 4th quartile: 0.52-8.12 g/d. In the analysis, with the lowest quartile as reference category, no association with CD was found (Table 7).

4.1.4.3 Intake from supplements

In the analyses of intake from supplements as continuous variables, combined intake of EPA and DHA was significantly associated with increased risk of CD per g/d increase in intake (RR:1.12, 95% CI: 1.00 – 1.23), but the association did not remain significant after adjustment (Table 7). When the two FAs were examined as separate variables, intake of EPA, but not DHA, was significantly associated with increased risk of CD per g/d increase in intake, and the association remained significant after adjustment (aRR: 1.23, 95% CI: 1.00 - 1.52, Table 7). When analyzed as a dichotomous variable, maternal use of supplements was significantly associated with increased risk of CD in the offspring, even after adjustment (aRR: 1.18, 95% CI: 1.02 – 1.37). When supplements users were divided into two groups by the median (0.29 g/d), combined intake of EPA and DHA was between 0.01 g/d–0.28 g/d (median 0.14 g/d) in the low group and between 0.29 g/d–8.79 g/d (median 0.80 g/d) in the high group. Compared with no intake from supplements, both intake under the median (RR: 1.23, 95% CI: 1.04 - 1.44) and intake over the median (RR: 1.22, 95% CI: 1.04 – 1.43) was significantly associated with increased risk of CD in the unadjusted analysis (Table 7). However, after adjustment, the association only remained significant for intake over the median (aRR: 1.20, 95% CI: 1.02 - 1.42).

4.1.4.4 Sensitivity analysis

Results from the sensitivity analysis are presented as model 2 in Table 7. Additional adjustment for the child`s birth year had minimal effect on the effect measures. Combined intake of EPA and DHA from supplements, as a continuous variable (g/d), became borderline significant (p-value reduced from 0.050 to 0.049), but the effect measure was unchanged (aRR: 1.11, 95% CI: 1.00 - 1.23).

Table 7. Associations between maternal intakes of EPA and DHA during pregnancy and risk of CD in the offspring

	CD, n (%) n = 965	No CD, n (%) n = 84,627	Unadjusted RR (95% CI)	p-value	Adjusted RR[a] (95% CI) Main analysis (model 1)	p-value	Adjusted RR[b] (95% CI) Sensitivity analysis (model 2)	p-value
Total (food and supplements)								
EPA and DHA								
Continuous, per g/d increase			1.01 (0.93 – 1.10)	0.802	1.01 (0.93 - 1.11)	0.74	1.02 (0.93 - 1.11)	0.73
By quartile								
1st (<0.32 g/d)	215 (22.3)	21,029 (24.9)	1		1		1	
2nd (0.32-0.56 g/d)	251 (26.0)	21,266 (25.1)	1.15 (0.96 - 1.39)	0.13	1.10 (0.92 - 1.33)	0.30	1.11 (0.92 - 1.33)	0.20
3rd (0.56-1.07 g/d)	261 (27.0)	21,194 (25.0)	1.20 (1.00 - 1.44)	0.048	1.15 (0.95 - 1.38)	0.15	1.15 (0.96 - 1.40)	0.14
4th (1.07-8.93 g/d)	238 (24.7)	21,138 (25.0)	1.1 (0.91 - 1.32)	0.31	1.09 (0.90 - 1.32)	0.36	1.09 (0.91 - 1.32)	0.35
DHA								
Continuous, per g/d increase			1.00 (0.86 – 1.17)	0.98	1.01 (0.86 - 1.19)	0.89	1.01 (0.86 - 1.19)	0.88
EPA								
Continuous, per g/day increase			1.05 (0.87 – 1.26)	0.62	1.05 (0.87 - 1.27)	0.59	1.05 (0.87 - 1.27)	0.58
Food								
EPA and DHA								
Continuous, per g/day increase			0.82 (0.68 - 0.99)	0.039	0.83 (0.69 - 1.01)	0.059	0.83 (0.69 - 1.01)	0.060
By quartile								
1st (<0.20 g/d)	249 (25.8)	20,983 (24.8)	1		1		1	
2nd (0.20-0.32 g/d)	256 (26.5)	21,285 (25.2)	1.01 (0.85 – 1.21)	0.88	0.99 (0.83 - 1.17)	0.87	0.99 (0.83 - 1.17)	0.87
3rd (0.32-0.52 g/d)	245 (25.4)	21,277 (25.1)	0.97 (0.81 – 1.16)	0.74	0.96 (0.80 - 1.15)	0.67	0.96 (0.80 - 1.15)	0.68
4th (0.52 -8.12 g/d)	215 (22.3)	21,082 (24.9)	0.86 (0.72 – 1.04)	0.11	0.86 (0.71 - 1.03)	0.11	0.86 (0.71 - 1.03)	0.11

Table 7. Continued

	CD, n (%) n = 965	No CD, n (%) n = 84,627	Unadjusted RR (95% CI)	p-value	Adjusted RR[a] (95% CI) Main analysis (model 1)	p-value	Adjusted RR[b] (95% CI) Sensitivity analysis (model 2)	p-value
Food								
DHA								
Continuous, per g/d increase			0.71 (0.52 – 0.98)	0.037	0.73 (0.53 - 1.01)	0.057	0.73 (0.52- 1.01)	0.058
EPA								
Continuous, per g/d increase			0.64 (0.41 - 0.99)	0.044	0.66 (0.42 - 1.03)	0.065	0.66 (0.42 - 1.03)	0.065
Supplements								
EPA and DHA								
Continuous, per g/d increase			1.12 (1.00 – 1.23)	0.047	1.11 (1.00 - 1.23)	0.050	1.11 (1.00 - 1.23)	0.049
By category								
No use (32%)	268 (27.8)	27,133 (32.1)	1		1		1	
Use (68%)	697 (72.2)	57,494 (67.9)	1.22 (1.06 - 1.41)	0.005	1.18 (1.02 - 1.37)	0.025	1.20 (1.03 - 1.39)	0.020
No supplement	268 (27.8)	27,133 (32.1)	1		1			
<0.29 g/d[c] (median 0.14 g/d)	349 (36.2)	28,741 (34.0)	1.23 (1.04 - 1.44)	0.013	1.17 (0.99 - 1.38)	0.068	1.18 (1.00 - 1.39)	0.057
≥0.29 g/d[d] (median 0.80 g/d)	348 (36.1)	28,753 (34.0)	1.22 (1.04 - 1.43)	0.014	1.20 (1.02 - 1.42)	0.028	1.21 (1.02 - 1.43)	0.025
DHA								
Continuous, per g/d increase			1.21 (0.99 – 1.47)	0.059	1.21 (0.99 - 1.48)	0.059	1.21 (0.99 - 1.49)	0.058
EPA								
Continuous, per g/d increase			1.24 (1.01 – 1.52)	0.042	1.23 (1.00 - 1.52)	0.049	1.24 (1.00 - 1.52)	0.047

[a]Model 1 adjusted for maternal age at delivery, maternal education, smoking during pregnancy, parity, pre-pregnancy BMI, caesarean section, maternal CD, paternal CD, child`s sex, child`s birth weight and prematurity
[b]Model 2 adjusted for the same covariates as model 1 in addition to child`s birth year
[c]Intake ranged from 0.01 g/d–0.28 g/d
[d]Intake ranged from 0.29 g/d–8.79 g/d
Abbreviations: CD, celiac disease; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; RR, relative risk; CI, confidence interval; BMI, body mass index

4.2 6 months cohort

In the analysis of use of cod liver oil at 6 months, 87,056 children in MoBa born between 1999 and 2009, followed to January 1st, 2017, were included. After a mean of 11.4 years (range 0.75 – 17.2) of follow-up, 976 children (1.1%, 61% girls) had developed CD at a mean age at diagnosis of 6.6 years (range 0.8 – 15.1).

Parental and child characteristics of the included participants are presented in Table 8. Maternal characteristics differed between users and non-users of cod liver oil. Mothers of non-users had higher BMI, while the percentage of first-time mothers and women with high education was higher among mothers of users. Besides parental CD and female sex, there were small differences characteristics between those who developed CD and those who did not. Mothers of children with CD were less likely to smoke, to have two or more previous births, and to have had caesarean section. They were also more likely to have high education and the children with CD had lower birth weight.

Characteristics of participants excluded due to missing exposure data are presented in Table 9. The prevalence of CD among the excluded participants was similar to the prevalence among those included (1.0% vs**.** 1.1%). The percentage of daily smokers and women with low education was higher among excluded participants, while the percentage of first-time mothers was higher among included participants.

Table 8. Characteristics of the study participants included in the analysis of children`s use of cod liver oil at 6 months by the child`s CD status and use of cod liver oil

	No CD, n (%)	CD, n (%)	Use of cod liver oil, n (%)	
	n = 86,080	n = 976	No (47%)	Yes (53%)
Maternal				
Age at delivery* (years), mean (SD)	30.3 (4.5)	29.9 (4.4)	30.2 (4.5)	30.3 (4.5)
Parity*				
0	39,338 (45.7)	457 (46.8)	17,056 (41.4)	22,739 (49.6)
1	30,287 (35.2)	360 (36.9)	15,604 (37.9)	15,043 (32.8)
≥2	16,454 (19.1)	159 (16.3)	8,503 (20.7)	8,110 (17.7)
Caesarean section*	12,643 (14.7)	122 (12.5)	6,391 (15.5)	6,374 (13.9)
Education*				
≤12 years	30,761 (35.7)	335 (34.3)	15,956 (38.8)	15,140 (33.0)
13-15 years	35,430 (41.2)	393 (40.3)	16,430 (39.9)	19,393 (42.3)
≥ 16 years	19,531 (22.7)	244 (25.0)	8,627 (21.0)	11,148 (24.3)
Smoking during pregnancy*				
Never	77,082 (89.6)	901 (92.3)	36,602 (88.9)	41,381 (90.2)
Sometimes	1,544 (1.8)	11 (1.1)	752 (1.8)	803 (1.8)
Daily	6,120 (7.1)	48 (4.9)	3,261 (7.9)	2,907 (6.3)
CD*	315 (0.4)	36 (3.7)	164 (0.4)	187 (0.4)
Pre-pregnancy BMI* (kg/m^2), mean (SD)	24.0 (4.2)	23.9 (4.3)	24.3 (4.4)	23.8 (4.0)
>20	10,473 (12.2)	117 (12.0)	4,808 (11.7)	5,782 (12.6)
20-24.9	47,218 (54.9)	559 (57.3)	21,555 (52.3)	26,222 (57.1)
25-29.9	18,449 (21.4)	189 (19.4)	9,439 (22.9)	9,199 (20.0)
≥30	7,826 (9.1)	94 (9.6)	4,345 (10.6)	3,575 (7.8)
Paternal				
CD	242 (0.3)	47 (4.8)	134 (0.3)	155 (0.3)
Child				
Female sex*	41,886 (48.7)	598 (61.3)	20,546 (49.9)	21,938 (47.8)
Birth weight* (g), mean (SD)	3573 (583)	3546 (567)	3555 (604)	3589 (562)
<2500	3,399 (4.0)	34 (3.5)	1,988 (4.8)	1,445 (3.2)
2500-3499	32,615 (37.9)	402 (41.2)	15,541 (37.8)	17,476 (38.1)
3500-4499	46,261 (53.7)	501 (51.3)	21,836 (53.1)	24,926 (54.3)
≥4500	3,795 (4.4)	39 (4.0)	1,791 (4.3)	2,043 (4.5)
Prematurity* (<37 weeks)	5,171 (6.0)	59 (6.1)	2,942 (7.5)	2,288 (5.0)

*Missing information: maternal age at delivery: 217, parity: 1, caesarean section: 1, maternal education: 362, smoking during pregnancy: 1350, maternal CD: 68, pre-pregnancy BMI: 2131, sex: 1, birth weight: 10, prematurity: 40
Abbreviations: CD, celiac disease; BMI, body mass index; SD, standard deviation

Table 9. Characteristics of included participants and of participants excluded due to missing exposure data in the 6 months cohort

	Included participants, n (%) n = 87,056	**Excluded participants, n (%) n = 14,158**
Maternal		
Age at delivery (years), mean (SD)	30.3 (4.5)	29.8 (5.0)
Missing data	217	48
Parity		
0	39,795 (45.7)	5,643 (39.9)
1	30,647 (35.2)	5,399 (38.1)
≥2	16,613 (19.1)	3,116 (22.0)
Missing data	1 (0)	-
Caesarean section	12,765 (14.7)	2,473 (17.5)
Missing data	1 (0)	-
Education		
≤12 years	31,096 (35.7)	6,683 (47.2)
13-15 years	35,823 (41.2)	4,731 (33.4)
≥ 16 years	19,775 (22.7)	2,602 (18.4)
Missing data	362 (0.4)	142 (1.0)
Smoking during pregnancy		
Never	77,983 (89.6)	12,062 (85.2)
Sometimes	1,555 (1.8)	314 (2.2)
Daily	6,168 (7.1)	1,555 (11.0)
Missing data	1350 (1.2)	227 (1.6)
CD	351 (0.4)	70 (0.5)
Missing data	68 (0.1)	10 (0.1)
Pre-pregnancy BMI (kg/m^2), mean (SD)	24.0 (4.2)	24.2 (4.6)
>20	10,590 (12.2)	1,883 (13.3)
20-24.9	47,777 (54.9)	7,172 (50.7)
25-29.9	18,638 (21.4)	2,937 (20.7)
≥30	7,920 (9.1)	1,551 (11.0)
Missing data	2131 (2.5)	615 (4.3)
Paternal		
CD	289 (0.3)	44 (0.3)
Missing data	-	-
Child		
Female sex	42,484 (48.8)	6,848 (48.4)
Missing data	1 (0)	-
Birth weight (g), mean (SD)	3573 (583)	3525 (638)
<2500	3,433 (3.9)	801 (5.7)
2500-3499	33,017 (37.9)	5,422 (38.3)
3500-4499	46,762 (53.7)	7,354 (51.9)
≥4500	3,834 (4.4)	574 (4.0)
Missing data	10 (0)	7 (0.1)
Prematurity (<37 weeks)	5,230 (6.0)	1,129 (8.0)
Missing data	40 (0.1)	72 (0.5)
CD	976 (1.1)	134 (1.0)

Abbreviations: CD, celiac disease; SD, standard deviation; BMI, body mass index

It was reported that 53% of the children used cod liver oil, 33% used it daily and 20% sometimes. Information about frequency of use was missing for 92 children. The percentage of supplement users was relatively stable over the study period (Table 10).

Table 10. Children`s use of cod liver oil at 6 months by birth year

	Use of cod liver oil, n (%)	
	No	**Yes**
Birth year*		
1999	16 (45.7)	19 (54.3)
2000	661 (38.2)	1,071 (61.8)
2001	1,659 (47.9)	1,804 (52.1)
2002	3,267 (44.8)	4,034 (55.2)
2003	4,866 (45.6)	5,802 (54.4)
2004	5,206 (46.0)	6,106 (54.0)
2005	5,691 (44.9)	6,997 (55.1)
2006	6,695 (48.0)	7,259 (52.0)
2007	6,536 (51.6)	6,134 (48.4)
2008	5,302 (50.0)	5,292 (50.0)
2009	1,264 (48.0)	1,374 (52.0)
Total	41,163 (47.3)	45,892 (52.7)

*Information about birth year missing for one child

4.2.1 Children`s use of cod liver oil at 6 months and CD

Use of cod liver oil at 6 months was not associated with risk of CD (aRR: 0.91, 95% CI: 0.80-1.03, Table 11). Results from the sensitivity analysis are presented as model 2 in Table 11. Additional adjustment for the child`s birth year did not affect the effect measures.

Table 11. Association between children`s use of cod liver oil at 6 months and risk of CD

Use of cod liver oil	CD, n (%) n = 976	No CD, n (%) n = 86,080	Unadjusted RR (95% CI)	p-value	Adjusted RR[a] (95% CI) Main analysis (model 1)	p-value	Adjusted RR[b] (95% CI) Sensitivity analysis (model 2)	p-value
No use (47%)	489 (50.1)	40,674 (47.3)	1		1		1	
Use (53%)	487 (49.9)	45,406 (52.8)	0.89 (0.79 - 1.01)	0.081	0.91 (0.80 - 1.03)	0.14	0.91 (0.80 - 1.04)	0.15
Never[c] (47%)	489 (50.3)	40,674 (47.3)	1		1		1	
Sometimes[c] (20%)	189 (19.4)	17,286 (20.1)	0.91 (0.78 - 1.08)	0.28	0.90 (0.75 - 1.06)	0.21	0.90 (0.76 - 1.07)	0.22
Daily[c] (33%)	295 (30.3)	28,031 (32.6)	0.89 (0.76 - 1.01)	0.077	0.91 (0.78 - 1.05)	0.19	0.91 (0.78 - 1.05)	0.21

[a]Model 1 adjusted for maternal age at delivery, maternal education, smoking during pregnancy, parity, pre-pregnancy BMI, caesarean section, maternal CD, paternal CD, child`s sex, child`s birth weight and prematurity
[b]Model 2 adjusted for the same covariates as model 1, in addition to child`s birth year
[c]Information about frequency of use missing for 92 children
Abbreviations: CD, celiac disease; RR, relative risk; CI, confidence interval; BMI, body mass index

4.3 18 months cohort

In the analysis of use of cod liver oil at 18 months, 72,188 children in MoBa born between 1999 and 2009, followed to January 1st, 2017, were included. After a mean of 11.3 years (range 0.83 - 17.2) follow-up, 832 children (1.2%, 61% girls) had developed CD at mean age at diagnosis of 6.6 years (range 0.8 – 15.1).

Parental and child characteristics of the included participants are presented in Table 12. Maternal characteristics differed between users and non-users of cod liver oil. Mothers of non-users had higher BMI, while the percentage of first-time mothers and women with high education was higher among mothers of users. Besides parental CD and female sex, there were small differences characteristics between those who developed CD and those who did not. Mothers of children with CD were less likely to smoke, to have two or more previous births, and to have had caesarean section. They were also more likely to have high education and the children with CD had lower birth weight.

Characteristics of participants excluded due to missing exposure data are presented in Table 13. The prevalence of CD among the excluded participants was similar to the prevalence among those included (1.0% vs. 1.2%). The percentage of daily smokers and women with low education was higher among excluded participants, while the percentage of first-time mothers was higher among included participants.

Table 12. Characteristics of the study participants included in the analysis of the children`s use of cod liver oil at 18 months by the child`s CD status and use of cod liver oil

	No CD, n (%)	CD, n (%)	Use of cod liver oil, n (%)	
	n = 71,356	n = 832	No (44%)	Yes (56%)
Maternal				
Age at delivery* (years), mean (SD)	30.4 (4.5)	30.1 (4.3)	30.1 (4.5)	30.6 (4.4)
Parity*				
0	33,324 (46.7)	397 (47.7)	13,747 (43.7)	19,974 (49.1)
1	24,655 (34.6)	301 (36.2)	11,266 (35.8)	13,690 (33.6)
≥2	13,376 (18.8)	134 (16.1)	6,461 (20.5)	7,049 (17.3)
Caesarean section*	10,363 (14.5)	101 (12.1)	4,708 (15.0)	5,756 (14.1)
Education*				
≤12 years	24,436 (34.3)	263 (31.6)	12,558 (39.9)	12,141 (29.8)
13-15 years	29,925 (41.9)	344 (41.4)	12,576 (40.0)	17,693 (43.5)
≥ 16 years	16,721 (23.4)	222 (26.7)	6,222 (19.8)	10,721 (26.3)
Smoking during pregnancy*				
Never	64,346 (90.2)	770 (92.6)	27,975 (88.9)	37,141 (91.2)
Sometimes	1,224 (1.7)	11 (11.3)	577 (1.8)	678 (1.7)
Daily	4,644 (6.5)	36 (4.3)	2,458 (7.8)	2,222 (5.5)
CD*	248 (0.4)	31 (3.7)	114 (0.4)	165 (0.4)
Pre-pregnancy BMI* (kg/m^2), mean (SD)	24.0 (4.2)	23.9 (4.2)	24.4 (4.5)	23.6 (3.9)
>20	8,673 (12.2)	102 (12.3)	3,463 (11.0)	5,312 (13.1)
20-24.9	39,569 (55.5)	471 (56.6)	16,358 (52.0)	23,682 (58.2)
25-29.9	15,164 (21.3)	167 (20.1)	7,338 (23.3)	7,993 (19.6)
≥30	6,316 (8.9)	78 (9.4)	3,546 (11.3)	2,848 (7.0)
Paternal				
CD	199 (0.3)	37 (4.5)	104 (0.3)	132 (0.3)
Child				
Female sex*	34,746 (48.7)	511 (61.4)	15,745 (50.0)	19,512 (47.9)
Birth weight* (g), mean (SD)	3575 (579)	3544 (566)	3566 (593)	3581 (568)
<2500	2,745 (3.9)	29 (3.5)	1,375 (4.4)	1,399 (3.4)
2500-3499	27,110 (38.0)	345 (41.5)	11,879 (37.7)	15,576 (38.3)
3500-4499	38,363 (53.8)	426 (51.2)	16,776 (53.3)	22,013 (54.1)
≥4500	3,130 (4.4)	32 (3.9)	1,442 (4.6)	1,720 (4.2)
Prematurity* (<37 weeks)	4,208 (5.9)	52 (6.3)	2,067 (6.7)	2,193 (5.4)

*Missing information: maternal age at delivery: 169, parity: 1, caesarean section: 1, maternal education: 277, smoking during pregnancy: 1157, maternal CD: 56, pre-pregnancy BMI: 1648, sex: 1, birth weight: 8, prematurity: 27
Abbreviations: CD, celiac disease; BMI, body mass index; SD, standard deviation

Table 13. Characteristics of included participants and of participants excluded due to missing exposure data in the 18 months cohort

	Included participants, n (%) n = 72,188	**Excluded participants, n (%) n = 29,026**
Maternal		
Age at delivery (years), mean (SD)	30.4 (4.5)	29.8 (4.9)
Missing data	169	96
Parity		
0	33,721 (46.7)	11,717 (40.4)
1	24,956 (34.6)	11,090 (38.2)
≥2	13,510 (18.7)	6,219 (21.4)
Missing data	1 (0)	-
Caesarean section	10,464 (14.5)	4,774 (16.5)
Missing data	1 (0)	-
Education		
≤12 years	24,699 (34.2)	13,080 (45.1)
13-15 years	30,269 (41.9)	10,285 (35.4)
≥ 16 years	16,943 (23.5)	5,434 (18.7)
Missing data	277 (0.4)	227 (0.8)
Smoking during pregnancy		
Never	65,116 (90.2)	24,929 (85.9)
Sometimes	1,235 (1.7)	634 (2.2)
Daily	4,680 (6.5)	3,043 (10.5)
Missing data	1,157 (1.6)	420 (1.5)
CD	279 (0.4)	142 (0.5)
Missing data	56 (0.1)	22 (0.1)
Pre-pregnancy BMI (kg/m^2), mean (SD)	24.0 (4.2)	24.2 (4.5)
>20	8,775 (12.2)	3,698 (12.7)
20-24.9	40,040 (55.5)	14,909 (51.4)
25-29.9	15,331 (21.3)	6,244 (21.5)
≥30	6,394 (8.9)	3,077 (10.6)
Missing data	1,648 (2.3)	1,098 (3.8)
Paternal		
CD	236 (0.3)	97 (0.3)
Missing data	-	-
Child		
Female sex	35,257 (48.8)	14,075 (48.5)
Missing data	1 (0)	-
Birth weight (g), mean (SD)	3574 (579)	3546 (619)
<2500	2,774 (3.8)	1,460 (5.0)
2500-3499	27,455 (38.0)	10,984 (37.8)
3500-4499	38,789 (53.7)	15,327 (52.8)
≥4500	3,162 (4.4)	1,246 (4.3)
Missing data	8 (0)	9 (0)
Prematurity (<37 weeks)	4,260 (5.9)	2,099 (7.2)
Missing data	27 (0)	85 (0.3)
CD	832 (1.2)	278 (1.0)

Abbreviations: CD, celiac disease; SD, standard deviation; BMI, body mass index

It was reported that 56% of the children used cod liver oil, 33% used it daily and 23% sometimes. Information about frequency of use was missing for 40 children. The percentage of supplement users was relatively stable over the study period, with a small increase the last years (Table 14).

Table 14. Children`s use of cod liver oil at 18 months by birth year

	Use of cod liver oil, n (%)	
	No	**Yes**
Birth year*		
1999	13 (46.4)	15 (53.6)
2000	674 (44.8)	831 (55.2)
2001	1,366 (46.0)	1,606 (54.0)
2002	2,878 (45.8)	3,409 (54.2)
2003	4,085 (45.4)	4,911 (54.6)
2004	4,028 (42.3)	5,503 (57.7)
2005	4,369 (41.9)	6,061 (58.1)
2006	4,999 (43.8)	6,411 (56.2)
2007	4,596 (44.5)	5,731 (55.5)
2008	3,608 (42.0)	4,983 (58.0)
2009	858 (40.7)	1,252 (59.3)
Total	31,474 (43.6)	40,713 (56.4)

*Information about birth year missing for one child

4.3.1 Children`s use of cod liver oil at 18 months and CD

Use of cod liver oil at 18 months was not associated with risk of CD (aRR: 0.95, 95% CI: 0.82–1.09, Table 15). Results from the sensitivity analysis are presented as model 2 in Table 15. Additional adjustment for the child`s birth year did not affect the effect measures.

Table 15. Association between children`s use of cod liver oil at 18 months and risk of CD

Use of cod liver oil	CD, n (%) n = 832	No CD, n (%) n = 71,356	Unadjusted RR (95% CI)	p-value	Adjusted RR[a] (95% CI) Main analysis (model 1)	p-value	Adjusted RR[b] (95% CI) Sensitivity analysis (model 2)	p-value
No use (44%)	374 (45.0)	31,100 (43.6)	1		1		1	
Use (56%)	458 (55.0)	40,256 (56.4)	0.95 (0.82 – 1.09)	0.44	0.95 (0.82 - 1.09)	0.44	0.95 (0.82 - 1.09)	0.45
Never[c] (44%)	374 (45.0)	31,100 (43.6)	1		1		1	
Sometimes[c] (23%)	174 (20.9)	16,471 (23.1)	0.88 (0.73 - 1.06)	0.17	0.88 (0.73 - 1.05)	0.16	0.88 (0.73 - 1.05)	0.16
Daily[c] (33%)	283 (34.1)	23,746 (33.3)	0.99 (0.85 - 1.16)	0.91	0.99 (0.84 - 1.17)	0.93	0.99 (0.84 - 1.17)	0.93

[a]Model 1 adjusted for maternal age at delivery, maternal education, smoking during pregnancy, parity, pre-pregnancy BMI, caesarean section, maternal CD, paternal CD, child`s sex, child`s birth weight and prematurity
[b]Model 2 adjusted for the same covariates as model 1, in addition to child`s birth year
[c]Information about frequency of use missing for 40 children
Abbreviations: CD, celiac disease; RR, relative risk; CI, confidence interval; BMI, body mass index

5 Discussion

5.1 Main findings

The primary aim of this thesis was to investigate if maternal intake of EPA and DHA during pregnancy, from food and/or supplements, was associated with risk of childhood onset CD in the offspring. Maternal use of supplements containing EPA and DHA was significantly associated with increased risk of CD in the offspring. We found no significant association between maternal intake of EPA and DHA from food and risk of CD in the offspring. Total maternal intake (food and supplements) of EPA and DHA was not associated with risk of CD in the offspring.

When intake of the EPA and intake of DHA were analyzed separately, their effect measures were similar in all analyses and had the same directions of the associations with risk of CD as the analyses on combined intake of EPA and DHA. However, since intake of the two FAs were strongly correlated (r=0.98), i.e. increased intake of one of the FAs also leads to increased intake of the other, it is difficult to separate their individual effects. Due to this, we focus on the results from the analyses on combined EPA and DHA intake.

When combined intake of EPA and DHA from supplements was analyzed in three categories, only intake over the median remained significantly associated with increased risk of CD after adjustment. However, the effect measure was similar for intake under (aRR: 1.17, 95% CI: 0.99-1.38) and intake over (aRR: 1.20, 95% CI: 1.02-1.42) the median. This indicates that there is no dose-response relationship. Combined intake of EPA and DHA from food was significantly associated with reduced risk of CD in the offspring, per g/d increase in intake, in the unadjusted analysis (RR: 0.82, 95% CI: 0.68-0.99). The association did not remain significant after adjustment for covariates, however, the effect measure remained virtually unchanged (aRR: 0.83, 95% CI: 0.69-1.01). Thus, the study findings suggest that intake of EPA and DHA from food and intake of EPA and DHA from supplements may have opposite effects on the risk of CD. To our knowledge, there is no obvious biological explanation that makes this plausible. Therefore, we cannot exclude the possibility that the findings could be random or caused by confounding factors not adjusted for.

The observed association between maternal use of supplements and increased risk of CD in the offspring could have been hypothesized to be due to lower intake of EPA and DHA from food among supplement users, had we not already observed that those who use supplements also are those with the highest intake of EPA and DHA from food. Many supplements with EPA and DHA also contain other nutrients, and EPA/DHA supplement users could be more likely to use other nutrient supplements. Thus, the observed increased risk of CD in the offspring could be due to intake of other nutrients from supplements, not necessarily EPA and DHA. Mothers with health-seeking behavior could be more likely to both use supplements and get their children investigated for CD. If having, or being at increased risk of, an immune-mediated disorder underlie the health-seeking behavior, their children could be at increased risk of CD as well. On the other hand, mothers with health-seeking behavior could also be more likely to have a high intake of EPA and DHA from food. To further explore this, we compared the percentage of supplement users and intake of EPA and DHA among mothers with and without CD and found no major differences (Appendix 3, Supplementary Table 2).

Overall, for the associations that were found, the observed effects were small, especially compared with the large increase in CD incidence and prevalence that has been observed over the last decades. For the continuous variables the observed effects are per g/d increase in intake. In this study, the median combined intake of EPA and DHA from supplements was 0.16 g/day (mean: 0.38 g/d), while the median intake from food was 0.32 g/d (mean: 0.43 g/d). Compared with the median intake levels, a change in intake of 1 g/d is relatively large. Therefore, the clinical relevance of the study findings is limited.

The secondary aim of this thesis was to investigate if children`s use of cod liver oil, at 6 months and at 18 months, was associated with risk of childhood onset CD. No associations were observed between use of cod liver oil and risk of CD.

5.2 Results in context

In line with our weak findings of limited clinical relevance for maternal use of supplements, the Environmental Determinants of Diabetes in the Young (TEDDY) study found no association between maternal use of n-3 PUFA supplements during pregnancy and risk of CD in the offspring (78). There are several differences between TEDDY and the present study. The present study only included participants from Norway, while TEDDY is international

with participants from four countries: the USA, Finland, Germany and Sweden. TEDDY only included children at increased genetic risk of CD, while the participants in the present study were recruited from the general population. While the present study only included cases with clinically diagnosed CD, repeated screening was used to identify cases in TEDDY. Compared with the 87,056 children that were included in the analysis of maternal intake in this study, the number of participants in TEDDY was small (n=6608). Only 17% of the mothers in TEDDY reported use of supplements containing n-3 PUFAs during pregnancy, compared to 68% in the present study.

A previous study from MoBa also found no association between maternal use of n-3 PUFA supplements during pregnancy and risk of CD in the offspring (93). The study mainly investigated maternal use of iron supplements and only included a small supplementary analysis on use of n-3 PUFA supplements without any further investigation. The number of participants in that study (n=78,846) was somewhat lower than in the analysis of maternal intakes in the present study (n=85,592), and only 0.4% of the children had developed CD at that time, compared to 1.1% in the present study. In the previous study, maternal use of supplement during pregnancy was derived from the questionnaire administered around 30 gestational weeks, instead of the FFQ from 22 gestational weeks that was used in the present study. Although, the percentage of participants that had reported use of supplements (72 %) was similar to the percentage of users in the present study (68 %).

The present study complements the previous studies in several ways. The present study also examines intakes of n-3 PUFAs from food, not only intake from supplements. Second, the study examines the individual effect of different n-3 PUFAs. Third, the study also examines the effect of children`s use of supplements containing n-3 PUFAs on risk of CD, not only maternal use during pregnancy.

A study from the Netherlands examined associations between children's dietary patterns around one year of age and CD autoimmunity (80). It found that high adherence to a dietary pattern named "prudent" was associated with reduced risk of CD autoimmunity. This dietary pattern was characterized by moderate consumption of fish, in addition to high consumption of vegetables and grains, and low consumption of sugar and refined cereals, among other things. The study did not determine the participants` CD status, only their concentration of antibodies against tTG. Both the total number of participants (n=1997) and the number of

anti-tTG positive cases (n=27) were small. Since the observed association was with a dietary pattern, of which fish was only a part, it is not possible to determine whether the association was due to intake of fish, and specially not if it was due to intake of EPA and DHA. It is likely that the observed reduced risk of CD autoimmunity could be due to other elements in the diet or other healthy characteristics among those with this dietary pattern.

5.2.1 EPA and DHA intakes compared with other countries

The average intake of EPA (0.34 g/day) and DHA (0.47 g/day), from food and supplements, in this study is larger than reported around the same time among pregnant women in Denmark (0.06 g/day for EPA, 0.32 g/day for DHA) (94), but lower than reported in pregnant women in Iceland (0.7 g/day for both EPA and DHA) (95). The percentage of pregnant women that used n-3 PUFA supplements in this study (68%) is somewhat lower than reported in Northern Norway (75%) (96), but higher than reported in Iceland (44%) (95) and Denmark (4%) (94). It is also higher than reported in the different countries included in the TEDDY study: 7% in Sweden, 9% in Finland, 26% in the USA and 34% in Germany. This is consistent with the long traditions for fish consumption and use of cod liver oil in Norway.

5.3 Methodological considerations

5.3.1 Strengths

Among the main strengths of this study are the large sample size and the prospective design, with assessment of dietary intakes before development of the outcome. Another strength is that we had detailed information on maternal EPA and DHA intakes and on other maternal and offspring characteristics. The FFQ that was used to assess maternal intakes was developed and validated especially for use in MoBa, and the women in MoBa were recruited from all over Norway. Data on the outcome, and several other variables, were derived from national registries that are considered reliable. Data from the NPR are routinely analyzed and have generally been found to have a high level of completeness (97). Several variables in the MBRN have been validated against patient records, and most of them are found to be satisfactory (98-101).

5.3.2 Information bias

The possibility that misclassification of outcome status has occurred cannot be ruled out. If we had access to clinical, biochemical, and histological information, we might have been able to confirm the diagnosis for some of those registered in NPR only once. The participants in MoBa have not undergone screening for CD and as mentioned in the introduction, CD is heavily underdiagnosed. Thus, it likely that screening would have led to identification of more cases. The exclusion of those registered in the NPR only once was done to avoid false positive cases, which are of greater concern than false negatives when it comes to validity since it is unlikely that the prevalence of undiagnosed CD is more than 1-2% (1, 11). This method for case identification has previously been validated in the MoBa cohort (102). Parents confirmed the diagnosis for 92% in the validation sample, and most of these children had undergone small intestinal biopsy prior to diagnosis. Since this study only included clinically diagnosed, childhood onset CD, the findings are not necessarily generalizable to screening detected CD or CD developed later in late.

5.3.3 Selection bias

Both low response rate and loss to follow-up can introduce selection bias. Those who agree to participate in health studies, such as MoBa, may be more health conscious than the average individual, and therefore have different health characteristics and dietary habits. The response rate in MoBa was rather low, 41% of the invited women agreed to participate (81). Thus, it can be questioned whether the findings in MoBa can be generalized to all pregnant women in Norway. A study that compared the participants in MoBa with all women who gave birth in Norway in the same period found significant differences between the two groups for most of the variables that were investigated (103). Among other things, the MoBa participants are older, more likely to use folic acid supplements and less likely to smoke. However, the eight exposure-outcome associations investigated in that study did not appear to be biased due to self-selection.

As previously shown, the prevalence of CD among those from MoBa that was included in this study was similar to the prevalence among those from MoBa who were excluded due to missing exposure data. However, those who were included had higher education and were less likely to smoke, compared to those who were excluded. Higher education might lead to a slightly higher intake of EPA and DHA than in the whole pregnant population. Adjustment

for maternal age, education, and smoking during pregnancy had little impact on the estimates in this study, and it is thus considered unlikely that selection bias has affected the results considerably. However, the findings may be less generalizable to some underrepresented groups, such as those with low socioeconomic status.

Compared to the national dietary surveys conducted in the same period as the data used in this study was collected, the percentage of children who used cod liver oil at 6 months in this study (53%) was similar to that reported in 1999 (55%) (55), but higher than reported in 2007 (40%) (56). The percentage of children who used cod liver oil at 18 months in this study (56%), was higher than that reported among one-year-olds in 1999 (45%) (57) and 2007 (39%) (58), and among two-year-olds in 1999 (47%) (59) and 2007 (2%) (60). This suggest that those who participate in MoBa may be more health conscious than the average individual.

5.3.4 Residual confounding

Detailed information on maternal and offspring characteristics made it possible to adjust for a large number of potential confounders. However, since the study is observational, the possibility of confounding by unmeasured variables cannot be ruled out. The observational nature of the study also precludes the possibility to determine whether the found associations are causal.

5.3.4.1 Vitamin D

Most sources, both food and supplement, of EPA and DHA also contain vitamin D.
Low vitamin D intake/status has been associated with increased risk of other immune-mediated diseases, such as T1D (104, 105), and has also been hypothesized to be associated with increased risk of CD (106, 107). However, a previous study from MoBa found no association between maternal 25-hydroxyvitamin D concentration or vitamin D intake during pregnancy and CD in the offspring (107). Children`s use of vitamin D supplements during the first 18 months of life was not associated with CD either. In addition, Yang et al. (78) found no association between maternal use of vitamin D supplements during pregnancy and CD in the offspring.

5.3.5 Dietary assessment

It is several limitations with FFQs that may have affected the accuracy of the calculated intakes of EPA and DHA. FFQs are better suited for ranking individuals according to their consumption level than for estimation of precise intakes (108). Filling out an FFQ can be cognitively challenging, and misinterpretation of questions may lead to misreporting (109). Since FFQs rely on the respondent`s memory, recall bias may occur. In this study, the risk of recall bias is reduced since the recall periods in the questionnaires were relatively short (four-five months for the pregnancy FFQ) and the questionnaires were filled out short time after exposure. However, over- and under-reporting may have occurred. Social desirability bias, including over-reporting of foods considered as healthy, such as fish, is not uncommon in nutritional research (110).

Compared with other dietary assessment methods, FFQ is associated with low costs and low participant burden (108). Due to this, it is often the preferred method in large epidemiological studies. Alternative dietary assessment methods that could have been used are food records and 24-hour recall (24HR), among others. An advantage with food records, compared with both 24HR and FFQs, is that recall bias is avoided, but on the other hand, participants may alter their diet since they know they are being observed (108). As both 24HR and food records are best suited to assess short term intake, they must be repeated when the goal is to assess usual intake. These methods may not capture intake of foods that are not usually eaten every day, such as fish, if not repeated enough times. As in FFQs, under-reporting of intakes may occur in both 24HR and food records (108). Previously, only food records and FFQs could be performed self-administered, while 24HR required a trained interviewer (108, 111). However, programs for self-administered 24HR, such as Automated Self-Administered 24HR, have been developed over the last years (111), but these programs were not yet available when MoBa was conducted.

5.3.5.1 Standard portion sizes

The questions in the FFQ that covered intake of fish only asked about frequency of consumption, not average portion sizes. Standard Norwegian portion sizes were used to calculate amounts in grams per day, and this may have affected the accuracy of calculated intakes from food. The average portion sizes among pregnant women may be different than the average for the whole population, and it may be large between-person variation due to

various degree of pregnancy complications that may influence dietary intakes, such as nausea and vomiting. However, inclusion of questions about portion size leads to increased participant burden due to increased number of questions (87). In addition can it be challenging for participants to estimate their usual portion size, under- or overestimation may occur (108). It has been reported that the improvement in validity, when questions about portion sizes are included, is small (87), and that there is bigger variation in frequency of consumption than in portion sizes (111). In MoBa, they chose not to include portion sizes for most foods in the FFQ to be able to include questions on a larger number of various food items (87).

5.3.5.2 Supplement database

Over- and under-reporting, in addition to reporting of rare supplements difficult to identify, may have occurred for use of supplements. Besides this, the calculated maternal intakes of EPA and DHA from supplements are considered reliable since they were calculated with use of the MoBa database, which contains details of the declared content in supplements. The content, including the FA composition, in dietary supplements may change over time. However, it is unlikely that this has affected the accuracy of the intakes considerably since such changes have been taken into account continuously in the database (87).

5.3.5.3 Validation of the FFQ

The pregnancy FFQ has previously been validated in a study with 119 participants from MoBa (88, 91, 112). Dietary intakes estimated from the FFQ were compared with intakes assessed by a 4-day weighed food diary (FD) and biomarkers in blood and urine. For total intake (food and supplements) of n–3 FAs (%) the two dietary methods were significantly correlated ($r = 0.49$, $p<0.01$) (112). When total intakes of EPA and DHA, estimated from the FFQ, were compared with erythrocyte membrane levels, there was a significant correlation for DHA ($r = 0.25$, $p = 0.014$), but not for EPA (91). Use of n-3 FA supplements was reported by 82 women in the FFQ and by 79 women in the FD. The agreement (Kappa) was 0.64 ($p<0.001$) (112).

5.3.6 Other limitations

The diet reported in the pregnancy FFQ may not be representative for the entire pregnancy, or even the entire period it was designed to capture (the first four-five months of the pregnancy). Nausea, which may affect dietary intakes considerably, is most common in the first part of the pregnancy. It has been observed that women change their diet, not only when they get pregnant, but also over the course of a pregnancy (113, 114). It is known that diet in early pregnancy may be more important in regard to organ development, while diet later in pregnancy could be more important in regard to overall fetal growth (114). However, it is not known when the most optimal period for prevention strategies for CD is, and it could also be that exposures later in life are more important than exposures in utero.

Studies on other immune-mediated diseases and n-3 PUFAs have also assessed maternal intake during lactation (115). However, the questionnaire in MoBa administered at child age 6 months only assessed maternal use of supplements in the first 6 months after the child`s birth, not intake of food items. In this study, we chose to focus on the time point when maternal intake was reported from both food and supplements.

It was originally planned, when the study protocol was developed, to not only assess children`s use of cod liver oil, but also their intakes of EPA and DHA (from both food and supplements). However, the questions about intake of fish and seafood, in the questionnaires administered at child age 6 and 18 months, are not detailed enough to get reliable estimates on EPA and DHA intakes. Studies on children`s use of cod liver oil may not be so relevant for countries where its use is not as widespread as in Norway. It is also possible that children in Norway will be less likely to use cod liver oil later in the childhood if they, in line with the new national recommendation, are not introduced to it during the first year of life.

The data on the children`s diet overall is too limited to get reliable estimates on their total energy intakes. Due to this, we did not exclude children with implausible energy intakes from the 6- and 18-months cohorts, as was done for implausible maternal energy intakes in the pregnancy cohort. Another limitation with the analyses of children`s use of cod liver is that only intake frequency, and not actual intake, was investigated. This was due to limited information about the amount given, especially at 18 months age. However, it is considered unlikely that this has affected the results considerably.

5.4 Potential negative effects due to intake of EPA and DHA

5.4.1 Environmental contaminants

Fish and other seafood are not only the main dietary source of EPA and DHA, but also of environmental contaminants, such as dioxins and dioxin-like polychlorinated biphenyls (dl-PCBs) (116). These contaminants may have adverse health effects and can be transferred from the mother to the child, both through the placenta in utero and through breastmilk during lactation (51). A study from MoBa found a negative association between maternal intakes of dioxins and dl-PCBs during pregnancy and fetal growth (116). In a risk-benefit assessment of fish in the Norwegian diet from 2014, the Norwegian Scientific Committee for Food and Environment (VKM) concluded that:

> …the benefits clearly outweighs the negligible risk presented by current levels of contaminants and other known undesirable substances in fish. Furthermore, adults including pregnant women with fish consumption less than one serving per week may miss the beneficial effects on cardiovascular diseases and optimal neurodevelopment in the foetuses and infants. (37)

However, new data has become available since then, and in 2018 the European Food Safety Authority (EFSA) set a tolerable weekly intake (TWI) level for dioxins and dl-PCBs that is seven-times lower than the previous one from 2001 (117). VKM is currently performing a new risk-befit assessment of fish in the Norwegian diet (118). Despite purification, fish oil supplements also contain environmental contaminants. The Institute of Marine Research has performed analyses on the level of different environmental contaminants in several fish oils available in Norway (119, 120). It was found that there is large variation in the level of contaminants between different oils, but that none of the oils have a level that exceeds Norway`s and EU's upper limit values. However, in young children, use of the fish oils supplements with the highest content of dioxins and dl-PCBs could possibly lead to an intake of these contaminants that is above the new TWI (121). VKM is also performing a risk assessment of dioxins and dl-PCBs in food in the Norwegian diet, including marine oils taken as supplements (122).

Limited research has been conducted on the relationship between environmental pollutants and CD. A pilot study from the USA, with 88 participants, examined the association between

different persistent organic pollutants and CD in children (123). The study found that serum P,p'-dichlorodiphenyldichloroethylene was associated with increased odds of CD (OR: 2.04, 95% CI: 1.08, 3.84). However, these findings must be considered with caution due to the study`s small sample size and cross-sectional design.

5.4.2 High intakes of EPA and DHA

As previously mentioned, cod liver oil is no longer recommended for children under the age of one year, partly since most children will receive considerably higher intakes of DHA than recommended if they get it from cod liver oil in addition to the intake they already have from breastmilk and/or formula. High intakes of EPA and DHA could potentially be harmful, but there are limited studies in infants. Studies in adults have found associations between high levels of n-3 PUFAs and adverse health outcomes (124).

6 Conclusion

In this prospective cohort study, maternal use of supplements containing EPA and DHA during pregnancy was significantly associated with increased risk of CD in the offspring. We found no significant association between maternal intake of EPA and DHA from food and risk of CD in the offspring. Total maternal intake (food and supplements) of EPA and DHA was not associated with risk of CD in the offspring. Children`s use of cod liver oil, at 6 months and at 18 months, was not associated with risk of CD.

For the associations that were found, the observed effects were small, even those observed for a large change in intake in analyses per g/d increase of EPA and DHA. The findings also suggest that maternal EPA and DHA intake from food and intake from supplements may have opposite effects on risk of CD in the offspring. Due to this, the clinical relevance of the study findings is limited. The findings do not provide basis for specific recommendations regarding intake EPA and DHA in early life, from either food or supplements, for prevention of CD in children.

Future research

More studies are needed before any conclusions on the potential relationship between intake of EPA and DHA in early life and risk of CD can be drawn. Future research on maternal intakes should also assess intakes during lactation. For investigation of children`s intakes, future studies should have more detailed data and assess their intakes of EPA and DHA from both food and supplements. It could also be useful to assess intakes in children older than 18 months and not only investigate intake at one time point, but also intake over time using repeated measures with more detailed information.

References

1. Mustalahti K, Catassi C, Reunanen A, Fabiani E, Heier M, McMillan S, et al. The prevalence of celiac disease in Europe: results of a centralized, international mass screening project. Ann Med. 2010;42(8):587-95.

2. Abadie V, Sollid LM, Barreiro LB, Jabri B. Integration of genetic and immunological insights into a model of celiac disease pathogenesis. Annu Rev Immunol. 2011;29:493-525.

3. Green PH, Cellier C. Celiac disease. N Engl J Med. 2007;357(17):1731-43.

4. Lionetti E, Catassi C. The Role of Environmental Factors in the Development of Celiac Disease: What Is New? Diseases. 2015;3(4):282-93.

5. Rashtak S, Murray JA. Celiac disease in the elderly. Gastroenterol Clin North Am. 2009;38(3):433-46.

6. Popp A, Mäki M. Changing Pattern of Childhood Celiac Disease Epidemiology: Contributing Factors. Front Pediatr. 2019;7:357.

7. King JA, Jeong J, Underwood FE, Quan J, Panaccione N, Windsor JW, et al. Incidence of Celiac Disease Is Increasing Over Time: A Systematic Review and Meta-analysis. Am J Gastroenterol. 2020;115(4):507-25.

8. Stamnaes J, Sollid LM. Celiac disease: Autoimmunity in response to food antigen. Semin Immunol. 2015;27(5):343-52.

9. Lindfors K, Ciacci C, Kurppa K, Lundin KEA, Makharia GK, Mearin ML, et al. Coeliac disease. Nat Rev Dis Primers. 2019;5(1):3.

10. Størdal K, Bakken IJ, Surén P, Stene LC. Epidemiology of coeliac disease and comorbidity in Norwegian children. J Pediatr Gastroenterol Nutr. 2013;57(4):467-71.

11. Myléus A, Ivarsson A, Webb C, Danielsson L, Hernell O, Högberg L, et al. Celiac disease revealed in 3% of Swedish 12-year-olds born during an epidemic. J Pediatr Gastroenterol Nutr. 2009;49(2):170-6.

12. Dydensborg S, Toftedal P, Biaggi M, Lillevang ST, Hansen DG, Husby S. Increasing prevalence of coeliac disease in Denmark: a linkage study combining national registries. Acta Paediatr. 2012;101(2):179-84.

13. Beitnes AR, Vikskjold FB, Jóhannesdóttir GB, Perminow G, Olbjørn C, Andersen SN, et al. Symptoms and Mucosal Changes Stable During Rapid Increase of Pediatric Celiac Disease in Norway. J Pediatr Gastroenterol Nutr. 2017;64(4):586-91.

14. Lohi S, Mustalahti K, Kaukinen K, Laurila K, Collin P, Rissanen H, et al. Increasing prevalence of coeliac disease over time. Aliment Pharmacol Ther. 2007;26(9):1217-25.

15. Vartdal F. HLA [Internet]. Store medisinske leksikon [updated 2020 Jan 17; cited 2020 June 7]. Available from: https://sml.snl.no/HLA.

16. Singh P, Arora S, Lal S, Strand TA, Makharia GK. Risk of Celiac Disease in the First- and Second-Degree Relatives of Patients With Celiac Disease: A Systematic Review and Meta-Analysis. Am J Gastroenterol. 2015;110(11):1539-48.

17. Moheb-Alian A, Forouzesh F, Sadeghi A, Rostami K, Aghamohammadi E, Rostami-Nejad M, et al. Contribution of HLA-DQ2/DQ8 haplotypes in type one diabetes patients with/without celiac disease. J Diabetes Complications. 2019;33(1):59-62.

18. Biesiekierski JR. What is gluten? J Gastroenterol Hepatol. 2017;32 Suppl 1:78-81.

19. Ferretti G, Bacchetti T, Masciangelo S, Saturni L. Celiac disease, inflammation and oxidative damage: a nutrigenetic approach. Nutrients. 2012;4(4):243-57.

20. Wang JH, Reinherz EL. The structural basis of αβ T-lineage immune recognition: TCR docking topologies, mechanotransduction, and co-receptor function. Immunol Rev. 2012;250(1):102-19.

21. Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations. Annu Rev Immunol. 2010;28:445-89.

22. Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr. 2012;54(1):136-60.

23. Caio G, Volta U, Sapone A, Leffler DA, De Giorgio R, Catassi C, et al. Celiac disease: a comprehensive current review. BMC Med. 2019;17(1):142.

24. The European Society of Paediatric Gastroenterology Hepatology and Nutrition. New Guidelines for the Diagnosis of Paediatric Coeliac Disease: The European Society of Paediatric Gastroenterology. Hepatology and Nutrition; [cited 2020 Des 09]. Available from: https://www.espghan.org/knowledge-center/publications/Clinical-Advice-Guides/2020_New_Guidelines_for_the_Diagnosis_of_Paediatric_Coeliac_Disease.

25. Husby S, Koletzko S, Korponay-Szabó I, Kurppa K, Mearin ML, Ribes-Koninckx C, et al. European Society Paediatric Gastroenterology, Hepatology and Nutrition Guidelines for Diagnosing Coeliac Disease 2020. J Pediatr Gastroenterol Nutr. 2020;70(1):141-56.

26. Selimoğlu MA, Karabiber H. Celiac disease: prevention and treatment. J Clin Gastroenterol. 2010;44(1):4-8.

27. Penny HA, Baggus EMR, Rej A, Snowden JA, Sanders DS. Non-Responsive Coeliac Disease: A Comprehensive Review from the NHS England National Centre for Refractory Coeliac Disease. Nutrients. 2020;12(1).

28. Han Y, Chen W, Li P, Ye J. Association Between Coeliac Disease and Risk of Any Malignancy and Gastrointestinal Malignancy: A Meta-Analysis. Medicine (Baltimore). 2015;94(38).

29. Tio M, Cox MR, Eslick GD. Meta-analysis: coeliac disease and the risk of all-cause mortality, any malignancy and lymphoid malignancy. Aliment Pharmacol Ther. 2012;35(5):540-51.

30. Saini RK, Keum YS. Omega-3 and omega-6 polyunsaturated fatty acids: Dietary sources, metabolism, and significance - A review. Life Sci. 2018;203:255-67.

31. Jiang J, Li K, Wang F, Yang B, Fu Y, Zheng J, et al. Effect of Marine-Derived n-3 Polyunsaturated Fatty Acids on Major Eicosanoids: A Systematic Review and Meta-Analysis from 18 Randomized Controlled Trials. PLoS One. 2016;11(1).

32. Galli C, Calder PC. Effects of fat and fatty acid intake on inflammatory and immune responses: a critical review. Ann Nutr Metab. 2009;55(1-3):123-39.

33. Nordic Council of Ministers. Nordic Nutrition Recommendations 2012 - Integrating nutrition and physical activity. 5 th edition. Nord 2014:002. Copenhagen: Nordic Council of Ministers; 2014. Available from: http://dx.doi.org/10.6027/Nord2014-002.

34. Li X, Bi X, Wang S, Zhang Z, Li F, Zhao AZ. Therapeutic Potential of ω-3 Polyunsaturated Fatty Acids in Human Autoimmune Diseases. Front Immunol. 2019;10:2241.

35. Swanson D, Block R, Mousa SA. Omega-3 fatty acids EPA and DHA: health benefits throughout life. Adv Nutr. 2012;3(1):1-7.

36. Nøstbakken OJ, Rasinger JD, Hannisdal R, Sanden M, Frøyland L, Duinker A, et al. Levels of omega 3 fatty acids, vitamin D, dioxins and dioxin-like PCBs in oily fish; a new perspective on the reporting of nutrient and contaminant data for risk-benefit assessments of oily seafood. Environ Int. 2021;147:106322.

37. Norwegian Scientific Committee for Food Safety. Benefit-risk assessment of fish and fish products in the Norwegian diet - an update. Scientific Opinion of the Scientific Steering Committee. Oslo: Norwegian Scientific Committee for Food Safety; 2014. VKM Report 2014:15.

38. Rimestad AH, Borgejordet A, Vesterhus KN, Sygnestveit K, Løken EB, Trygg K, Pollestad ML, Lund-Larsen K, Omholt-Jensen G, Nordbotten A. Den store matvaretabellen [The Norwegian Food Composition Table]. Oslo: Norwegian Food Safety Authority; Norwegian Directorate of Health; University of Oslo, Department of Nutrition; 2001.

39. Duttaroy AK. Transport of fatty acids across the human placenta: a review. Prog Lipid Res. 2009;48(1):52-61.

40. Shulkin M, Pimpin L, Bellinger D, Kranz S, Fawzi W, Duggan C, et al. n-3 Fatty Acid Supplementation in Mothers, Preterm Infants, and Term Infants and Childhood Psychomotor and Visual Development: A Systematic Review and Meta-Analysis. J Nutr. 2018;148(3):409-18.

41. Hanebutt FL, Demmelmair H, Schiessl B, Larqué E, Koletzko B. Long-chain polyunsaturated fatty acid (LC-PUFA) transfer across the placenta. Clin Nutr. 2008;27(5):685-93.

42. Larqué E, Demmelmair H, Gil-Sánchez A, Prieto-Sánchez MT, Blanco JE, Pagán A, et al. Placental transfer of fatty acids and fetal implications. Am J Clin Nutr. 2011;94(6 Suppl):1908-13.

43. Carlson SE, Cheatham CL, Colombo J. Long-Chain Polyunsaturated Fatty Acids in the Developing Central Nervous System. In: Polin RA, Rowitch DH, Fox WW, Abham SH, Benitz WE, editors. Fetal and Neonatal Physiology. 5 ed: Elsevier 2017. p. 380-9.

44. Norwegian Scientific Committee for Food Safety. A comprehensive assessment of fish and other seafood in the Norwegian diet. Oslo: Norwegian Scientific Committee for Food Safety; 2006. VKM report 2006:16.

45. National Council for Nutrition (Norway). Kostråd for å fremme folkehelsen og forebygge kroniske sykdommer. Metodologi og vitenskapelig kunnskapsgrunnlag. Report. Oslo: Norwegian Directorate of Health; 2011. IS-1881.

46. Matportalen. Kostholdsråd for gravide/Dietary advice for pregnant women (in Norwegian) [Internet]. Norwegian Food Safety Authority, Norwegian Directorate of Health, Norwegian Institute of Public Health [updated 2020 Jun 26; cited 2020 Nov 25]. Available from: http://www.matportalen.no/rad_til_spesielle_grupper/tema/gravide/#tabs-12-anchor.

47. Norwegian Directorate of Health. Anbefalinger om kosthold, ernæring og fysisk aktivitet. Report. Oslo: Norwegian Directorate of Health; 2014. IS-2170.

48. Astrup H, Myhre JB, Andersen LF, Kristiansen AL. Småbarnskost 3. Landsomfattende undersøkelse av kostholdet blant 2-åringer i Norge. [Småbarnskost 3. Nationwide dietary survey among 2-year-olds in Norway]. Report. Oslo: Norwegian Institute of Public Health, University of Oslo; 2020.

49. Paulsen MM, Myhre JB, Andersen LF, Kristiansen AL. Spedkost 3. Landsomfattende undersøkelse av kostholdet blant spedbarn i Norge, 12 måneder [Spedkost 3. Nationwide dietary survey among infants in Norway, age 12 months]. Report. Oslo: Norwegian Institute of Public Health, University of Oslo; 2020.

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

51. Caspersen IH, Knutsen HK, Brantsæter AL, Haugen M, Alexander J, Meltzer HM, et al. Dietary exposure to dioxins and PCBs in a large cohort of pregnant women: results from the Norwegian Mother and Child Cohort Study (MoBa). Environ Int. 2013;59:398-407.

52. Hay G, Fadnes L, Holven KB, Øverby NC, Madar AA, Henriksen C. New advice on vitamin D supplements and cod liver oil for infants. Tidsskr Nor Legeforen. 2020;140(16).

53. Myhre JB, Andersen LF, Kristiansen AL. Spedkost 3. Landsomfattende undersøkelse av kostholdet blantspedbarn i Norge, 6 måneder [Spedkost 3. Nationwide dietary survey among infants in Norway, age 6 months]. Report. Oslo: Norwegian Institute of Public Health, University of Oslo; 2020.

54. Norwegian Directorate of Health. Nasjonal faglig retningslinje for spedbarnsernæring. Norwegian Directorate of Health; 2016 Oct 11 [updated 2021 Jan 07; cited 2021 May 05]. Available from: https://www.helsedirektoratet.no/retningslinjer/spedbarnsernaering

55. Lande B. Spedkost 6 måneder. Landsomfattende kostholdsundersøkelse blant spedbarn i Norge [Spedkost 6 months. Nationwide dietary survey among infants in Norway, age 6 months]. Report. Oslo: Norwegian Directorate of Health; 2003. IS-1074.

56. Øverby NC, Kristiansen AL, Andersen LF, Lande B. Spedkost 6 måneder. Landsomfattende kostholdsundersøkelse blant 6 måneder gamle barn. Spedkost 2006-2007 [Spedkost 6 months : Nationwide dietary survey among children in Norway, age 6 months. Spedkost 2006-2007]. Report. Oslo: Norwegian Directorate of Health; 2008. IS-1535.

57. Lande B, Andersen LF. Spedkost 12 måneder. Landsomfattende kostholdsundersøkelse blant spedbarn i Norge [Spedkost 12 months. Nationwide dietary survey among infants in Norway, age 12 months]. Report. Oslo: Norwegian Directorate of Health; 2005. IS-1248.

58. Øverby NC, Kristiansen AL, Andersen LF, Lande B. Spedkost 12 måneder. Landsomfattende kostholdsundersøkelse blant 12 måneder gamle barn. Spedkost 2006-2007 (Spedkost 12 months: Nationwide dietary survey among infants in Norway, age 12 months. Spedkost 2006-2007). Report. Oslo: Norwegian Directorate of Health; 2009. IS-1635.

59. Lande B, Andersen LF. Småbarnskost . Kosthold blant 2-åringer. Landsomfattende kostholdsundersøkelse. [Småbarnskost. Diet among 2-year-olds. Nationwide dietary survey]. Report. Oslo: Norwegian Directorate of Health; 2005. IS-1299.

60. Kristiansen AL, Andersen LF, Lande B. Småbarnskost 2 år. Landsomfattende kostholdsundersøkelse blant 2-åringer. Småbarnskost 2007. [Spedkost 2 years: Nationwide dietary survey among infants in Norway, age 2 years]. Report. Oslo: Norwegian Directorate of Health; 2009. IS-1731.

61. Størdal K, Kahrs C, Tapia G, Agardh D, Kurppa K, Stene LC. Review article: exposure to microbes and risk of coeliac disease. Aliment Pharmacol Ther. 2021;53(1):43-62.

62. Andrén Aronsson C, Lee HS, Hård Af Segerstad EM, Uusitalo U, Yang J, Koletzko S, et al. Association of Gluten Intake During the First 5 Years of Life With Incidence of Celiac Disease Autoimmunity and Celiac Disease Among Children at Increased Risk. JAMA. 2019;322(6):514-23.

63. Pes GM, Bibbo S, Dore MP. Coeliac disease: beyond genetic susceptibility and gluten. A narrative review. Ann Med. 2019;51(1):1-16.

64. Simopoulos AP. Omega-3 fatty acids in inflammation and autoimmune diseases. J Am Coll Nutr. 2002;21(6):495-505.

65. Calder PC. n-3 fatty acids, inflammation and immunity: new mechanisms to explain old actions. Proc Nutr Soc. 2013;72(3):326-36.

66. Li K, Huang T, Zheng J, Wu K, Li D. Effect of marine-derived n-3 polyunsaturated fatty acids on C-reactive protein, interleukin 6 and tumor necrosis factor α: a meta-analysis. PLoS One. 2014;9(2):e88103.

67. Sproston NR, Ashworth JJ. Role of C-Reactive Protein at Sites of Inflammation and Infection. Front Immunol. 2018;9:754.

68. Manavalan JS, Hernandez L, Shah JG, Konikkara J, Naiyer AJ, Lee AR, et al. Serum cytokine elevations in celiac disease: association with disease presentation. Hum Immunol. 2010;71(1):50-7.

69. Lindehammer SR, Björck S, Lynch K, Brundin C, Marsal K, Agardh D, et al. Early human pregnancy serum cytokine levels predict autoimmunity in offspring. Autoimmunity. 2011;44(6):445-52.

70. Calder PC. Omega-3 fatty acids and inflammatory processes. Nutrients. 2010;2(3):355-74.

71. Costantini L, Molinari R, Farinon B, Merendino N. Impact of Omega-3 Fatty Acids on the Gut Microbiota. Int J Mol Sci. 2017;18(12).

72. Nadal I, Donant E, Ribes-Koninckx C, Calabuig M, Sanz Y. Imbalance in the composition of the duodenal microbiota of children with coeliac disease. J Med Microbiol. 2007;56(12):1669-74.

73. Transeth EL, Dale HF, Lied GA. Comparison of gut microbiota profile in celiac disease, non-celiac gluten sensitivity and irritable bowel syndrome: A systematic review. Turk J Gastroenterol. 2020;31(11):735-45.

74. Stene LC, Ulriksen J, Magnus P, Joner G. Use of cod liver oil during pregnancy associated with lower risk of Type I diabetes in the offspring. Diabetologia. 2000;43(9):1093-8.

75. Stene LC, Joner G. Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: a large, population-based, case-control study. Am J Clin Nutr. 2003;78(6):1128-34.

76. Norris JM, Yin X, Lamb MM, Barriga K, Seifert J, Hoffman M, et al. Omega-3 polyunsaturated fatty acid intake and islet autoimmunity in children at increased risk for type 1 diabetes. J Am Med Assoc. 2007;298(12):1420-8.

77. Bisgaard H, Stokholm J, Chawes BL, Vissing NH, Bjarnadottir E, Schoos AM, et al. Fish Oil-Derived Fatty Acids in Pregnancy and Wheeze and Asthma in Offspring. N Engl J Med. 2016;375(26):2530-9.

78. Yang J, Tamura RN, Aronsson CA, Uusitalo UM, Lernmark A, Rewers M, et al. Maternal use of dietary supplements during pregnancy is not associated with coeliac disease in the offspring: The Environmental Determinants of Diabetes in the Young (TEDDY) study. Br J Nutr. 2017;117(3):466-72.

79. Størdal K, Haugen M, Brantsaeter AL, Lundin KE, Stene LC. Association between maternal iron supplementation during pregnancy and risk of celiac disease in children. Clin Gastroenterol Hepatol. 2014;12(4):624-31.

80. Barroso M, Beth SA, Voortman T, Jaddoe VWV, van Zelm MC, Moll HA, et al. Dietary Patterns After the Weaning and Lactation Period Are Associated With Celiac Disease Autoimmunity in Children. Gastroenterology. 2018;154(8):2087-96.

81. Magnus P, Birke C, Vejrup K, Haugan A, Alsaker E, Daltveit AK, et al. Cohort Profile Update: The Norwegian Mother and Child Cohort Study (MoBa). Int J Epidemiol. 2016;45(2):382-8.

82. The Norwegian Institute of Public Health. Questionnaire 1. English translation of the baseline questionnaire used in the Norwegian Mother and Child Cohort Study. Available from: https://www.fhi.no/globalassets/dokumenterfiler/studier/den-norske-mor-far-og-barn--undersokelsenmoba/sporreskjemaer/questionnaire---week-15-of-pregnancy-to-mother.pdf.

83. The Norwegian Institute of Public Health. Questionnaire 2, Your diet. English translation of the food Frequency Questionnaire used in the Norwegian Mother and Child Cohort Study. Available from: https://www.fhi.no/globalassets/dokumenterfiler/studier/den-norske-mor-far-og-barn--undersokelsenmoba/sporreskjemaer/questionnaire---week-22-of-pregnancy.pdf.

84. The Norwegian Institute of Public Health. Questionnaire 3. English translation of the questionnaire used at 30 gestational weeks in the Norwegian Mother and Child Cohort Study. Available from: https://www.fhi.no/globalassets/dokumenterfiler/studier/den-norske-mor-far-og-barn--undersokelsenmoba/sporreskjemaer/questionnaire-3---30-weeks-of-gestation.pdf.

85. The Norwegian Institute of Public Health. Questionnaire 4. English translation of the questionnaire used at child age 6 months in the Norwegian Mother and Child Cohort Study. Available from: https://www.fhi.no/globalassets/dokumenterfiler/studier/den-norske-mor-far-og-barn--undersokelsenmoba/sporreskjemaer/questionnaire---6-months-after-birth.pdf.

86. The Norwegian Institute of Public Health. Questionnaire 5. English translation of the questionnaire used at child age 18 months in the Norwegian Mother and Child Cohort Study. Available from: https://www.fhi.no/globalassets/dokumenterfiler/studier/den-norske-mor-far-og-barn--undersokelsenmoba/sporreskjemaer/questionnaire---18-months-after-birth.pdf.

87. Meltzer HM, Brantsaeter AL, Ydersbond TA, Alexander J, Haugen M. Methodological challenges when monitoring the diet of pregnant women in a large study: experiences from the Norwegian Mother and Child Cohort Study (MoBa). Matern Child Nutr. 2008;4(1):14-27.

88. Brantsaeter AL, Haugen M, Alexander J, Meltzer HM. Validity of a new food frequency questionnaire for pregnant women in the Norwegian Mother and Child Cohort Study (MoBa). Matern Child Nutr. 2008;4(1):28-43.

89. Brantsaeter AL, Haugen M, Rasmussen SE, Alexander J, Samuelsen SO, Meltzer HM. Urine flavonoids and plasma carotenoids in the validation of fruit, vegetable and tea intake during pregnancy in the Norwegian Mother and Child Cohort Study (MoBa). Public Health Nutr. 2007;10(8):838-47.

90. Norwegian Institute of Public Health. Medical Birth Registry of Norway [Internet]. 2016 Oct 18 [updated 2019 Des 16; cited 2021 April 24]. Available from: https://www.fhi.no/en/hn/health-registries/medical-birth-registry-of-norway/medical-birth-registry-of-norway/.

91. Brantsaeter AL, Haugen M, Thomassen Y, Ellingsen DG, Ydersbond TA, Hagve TA, et al. Exploration of biomarkers for total fish intake in pregnant Norwegian women. Public Health Nutr. 2010;13(1):54-62.

92. Marild K, Kahrs CR, Tapia G, Stene LC, Stordal K. Infections and risk of celiac disease in childhood: a prospective nationwide cohort study. Am J Gastroenterol. 2015;110(10):1475-84.

93. Størdal K, Haugen M, Brantsaeter AL, Lundin KE, Stene LC. Association between maternal iron supplementation during pregnancy and risk of celiac disease in children. Clin Gastroenterol Hepatol. 2014;12(4):624-31.e1-2.

94. Olsen SF, Mikkelsen TB, Knudsen VK, Orozova-Bekkevold I, Halldórsson TI, Strøm M, et al. Data collected on maternal dietary exposures in the Danish National Birth Cohort. Paediatr Perinat Epidemiol. 2007;21(1):76-86.

95. Thorsdottir I, Birgisdottir BE, Halldorsdottir S, Geirsson RT. Association of fish and fish liver oil intake in pregnancy with infant size at birth among women of normal weight before pregnancy in a fishing community. Am J Epidemiol. 2004;160(5):460-5.

96. Veyhe AS, Hansen S, Sandanger TM, Nieboer E, Odland J. The Northern Norway mother-and-child contaminant cohort study: implementation, population characteristics and summary of dietary findings. Int J Circumpolar Health. 2012;71:18644.

97. Bakken IJ, Ariansen AMS, Knudsen GP, Johansen KI, Vollset SE. The Norwegian Patient Registry and the Norwegian Registry for Primary Health Care: Research potential of two nationwide health-care registries. Scand J Public Health. 2020;48(1):49-55.

98. Baghestan E, Børdahl PE, Rasmussen SA, Sande AK, Lyslo I, Solvang I. A validation of the diagnosis of obstetric sphincter tears in two Norwegian databases, the Medical Birth Registry and the Patient Administration System. Acta Obstet Gynecol Scand. 2007;86(2):205-9.

99. Engeland A, Bjørge T, Daltveit AK, Vollset SE, Furu K. Validation of disease registration in pregnant women in the Medical Birth Registry of Norway. Acta Obstet Gynecol Scand. 2009;88(10):1083-9.

100. Thomsen LC, Klungsøyr K, Roten LT, Tappert C, Araya E, Baerheim G, et al. Validity of the diagnosis of pre-eclampsia in the Medical Birth Registry of Norway. Acta Obstet Gynecol Scand. 2013;92(8):943-50.

101. Vikanes Å, Magnus P, Vangen S, Lomsdal S, Grjibovski AM. Hyperemesis gravidarum in the Medical Birth Registry of Norway - a validity study. BMC Pregnancy Childbirth. 2012;12:115.

102. Kahrs CR, Magnus MC, Stigum H, Lundin KEA, Størdal K. Early growth in children with coeliac disease: a cohort study. Arch Dis Child. 2017;102(11):1037-43.

103. Nilsen RM, Vollset SE, Gjessing HK, Skjaerven R, Melve KK, Schreuder P, et al. Self-selection and bias in a large prospective pregnancy cohort in Norway. Paediatr Perinat Epidemiol. 2009;23(6):597-608.

104. Hyppönen E, Läärä E, Reunanen A, Järvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet. 2001;358(9292):1500-3.

105. Sørensen IM, Joner G, Jenum PA, Eskild A, Torjesen PA, Stene LC. Maternal serum levels of 25-hydroxy-vitamin D during pregnancy and risk of type 1 diabetes in the offspring. Diabetes. 2012;61(1):175-8.

106. Moos C, Duus KS, Frederiksen P, Heitmann BL, Andersen V. Exposure to the Danish Mandatory Vitamin D Fortification Policy in Prenatal Life and the Risk of Developing Coeliac Disease-The Importance of Season: A Semi Ecological Study. Nutrients. 2020;12(5).

107. Mårild K, Tapia G, Haugen M, Dahl SR, Cohen AS, Lundqvist M, et al. Maternal and neonatal vitamin D status, genotype and childhood celiac disease. PLoS One. 2017;12(7):e0179080.

108. Slimani N, Freisling H, Illner A, Huybrechts. Methods to Determine Dietary Intake. I: Lovegrove JA, Hodson L, Sharma S, Lanham-New SA, red. Nutrition Research Methodologies. First edition. John Wiley & Sons, Ltd; 2015. S.48-70.

109. Food and Agriculture Organization of the United Nations (FAO). Dietary Assessment: A resource guide to method selection and application in low resource settings. Rome: FAO; 2018. Available from: http://www.fao.org/3/i9940en/I9940EN.pdf.

110. Widmar NJ, Byrd ES, Dominick SR, Wolf CA, Acharya L. Social desirability bias in reporting of holiday season healthfulness. Prev Med Rep. 2016;4:270-6.

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

112. Brantsaeter AL, Haugen M, Hagve TA, Aksnes L, Rasmussen SE, Julshamn K, et al. Self-reported dietary supplement use is confirmed by biological markers in the Norwegian Mother and Child Cohort Study (MoBa). Ann Nutr Metab. 2007;51(2):146-54.

113. Olafsdottir AS, Skuladottir GV, Thorsdottir I, Hauksson A, Steingrimsdottir L. Maternal diet in early and late pregnancy in relation to weight gain. Int J Obes (Lond). 2006;30(3):492-9.

114. Rifas-Shiman SL, Rich-Edwards JW, Willett WC, Kleinman KP, Oken E, Gillman MW. Changes in dietary intake from the first to the second trimester of pregnancy. Paediatr Perinat Epidemiol. 2006;20(1):35-42.

115. Klemens CM, Berman DR, Mozurkewich EL. The effect of perinatal omega-3 fatty acid supplementation on inflammatory markers and allergic diseases: a systematic review. BJOG. 2011;118(8):916-25.

116. Papadopoulou E, Caspersen IH, Kvalem HE, Knutsen HK, Duarte-Salles T, Alexander J, et al. Maternal dietary intake of dioxins and polychlorinated biphenyls and birth size in the Norwegian Mother and Child Cohort Study (MoBa). Environ Int. 2013;60:209-16.

117. Knutsen HK, Alexander J, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, et al. Risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food. EFSA J. 2018;16(11):e05333.

118. Norwegian Scientific Committee for Food and Environment. Risk-benefit assessment of fish in the Norwegian diet [Internet]. [cited 2021 April 24]. Available from: https://vkm.no/english/riskassessments/allpublications/riskbenefitassessmentoffishinthenorwegiandiet.4.7b65040716afa427d7ec5d3a.html. .

119. Nilsen BM, Sanden M. Miljøgifter i fisk og fiskevarer 2018. Organiske miljøgifter, tungmetaller, 3-MCPD og glysidylestere i marine oljer til humant konsum [Contaminants in fish and

fish products 2018. Organic contaminants, heavy metals, 3-MCPD and glycidyl esters in marine oils for human consumption]. Rapport fra havforskningen nr. 2019-33.

120. Nilsen BM, Wiech M, Sanden M. Miljøgifter i fisk og fiskevarer 2019. Organiske miljøgifter, tungmetaller, 3-MCPD og glysidylestere i marine oljer til humant konsum. [Contaminants in fish and fish products 2019. Organic contaminants, heavy metals, 3-MCPD and glycidyl esters in marine oils for human consumption]. Rapport fra Havforskningen nr. 2020-25.

121. Bjørke-Monsen AL, Huber S, Averina M, Brox J, Bolann BJ. Tran inneholder også miljøgifter. Tidsskr Nor Laegeforen. 2021;141(4).

122. Norwegian Scientific Committee for Food and Environment. Risk assessment of dioxins and dl-PCBs in food [Internet]. [cited 2021 April 24]. Available from: https://vkm.no/english/riskassessments/allpublications/riskassessmentofdioxinsanddlpcbsinfood.4.267f797a16a4e7cde952a29.html.

123. Gaylord A, Trasande L, Kannan K, Thomas KM, Lee S, Liu M, et al. Persistent organic pollutant exposure and celiac disease: A pilot study. Environ Res. 2020;186:109439.

124. Fenton JI, Hord NG, Ghosh S, Gurzell EA. Immunomodulation by dietary long chain omega-3 fatty acids and the potential for adverse health outcomes. Prostaglandins Leukot Essent Fatty Acids. 2013;89(6):379-90.

Appendix 1

Excerpts of questionnaire at week 22 of pregnancy

Dietary Supplements

39. Do you use, or have you used supplements during this pregnancy? ☐Yes ☐No

40. If yes, we ask you to name and quantify the supplements you have used/are using
(ts = 3.5 ml (teaspoon), bs = 5 ml (2 x teaspoon), ss = 10 ml (3 x teaspoon)

Liquid supplements	Times per week									Amount		
	7	6	5	4	3	2	1	<1	0	1 ts	1bs	1ss
1. Cod liver oil	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
2. Omega-3 cod liver oil	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Capsules/tablets	Times per week									Number(s) per time			
	7	6	5	4	3	2	1	<1	0	1	2	3	4+
10. Cod liver oil capsules	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
11. Fish oil capsules	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Spreads on bread, crispbread, crackers

8. How many slices of bread with the following spreads have you eaten on average since you became pregnant? (Mark only one box per line).

Number of slices with this food item

	per day						or per week			or per month			
	6+	5	4	3	2	1	5-6	3-4	1-2	3	2	1	0
Fish													
7. Roe spread	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
8. Mackerel/sardines in tomato sauce	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
9. Sardines in oil	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
10. Smoked salmon/trout/mackerel	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
11. Herring, pickled	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
12. Shrimp, (prawn)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
13. Crab	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
14. Tuna	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
15. Svolværpostei (spread of fish liver/roe)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
16. Other kinds of fish	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Hot meals

14. How often have you on average had the following types of hot food since you became pregnant?

	per week						or per month			
	6+	5	4	3	2	1	3	2	1	0
Seafood										
33. Cod, saithe, haddock, pollack (boiled/fried/smoked)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
34. Mackerel, herring	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
35. Salmon, trout	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
36. Halibut, plaice, flounder	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
37. Tuna fish	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
38. Perch, pike, pikecake	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
39. Other fish	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
40. Fish cake, fish pudding, fish balls	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
41. Fish fingers, breaded fish	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
42. Fish casserole, soup, fish au gratin	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
43. Shrimps	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
44. Mussels	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
45. Crab	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
46. Roe	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
47. Fish liver	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Excerpt of questionnaire at child age 6 months

21. Do you give your child cod liver oil, vitamins, iron or any other dietary supplement?

☐ No ☐ Yes +

22. If you give your child cod liver oil, vitamins, iron or another dietary supplement, specify how much you give your child each time and how often. How old was your child in months and weeks when you gave him/her the product for the first time?

Name of product	How many teaspoons each time?	How often do you give your child this?	How old was your child when you started giving the product?
1. Cod liver oil	☐ teaspoons	☐ daily ... ☐ sometimes	☐ months and ☐ weeks

Excerpt of questionnaire at child age 18 months

11. Do you give your child cold liver oil, vitamins, iron or any other dietary supplement?

☐ No

☐ Yes +

+

12. If yes, specify which product(s) and how often you give them to your child. How old was your child when you first started giving him/her the product?

+

	How often do you give it to your child?		How old was your child when you first gave him the product?
	Every day	sometimes	Number of months
1. Cod liver oil	☐	☐	☐☐

Appendix 2

Supplementary table 1. Characteristics of the participants included in the analysis of maternal EPA and DHA intakes during pregnancy by the child`s CD status

	No CD, n (%) n = 84,627	**CD, n (%) n = 965**
Maternal		
Age at delivery* (years), mean (SD)	30.3 (4.5)	30.0 (4.4)
Parity		
0	38,659 (45.7)	447 (46.3)
1	29,936 (35.4)	362 (37.5)
≥2	16,033 (18.9)	156 (16.17)
Caesarean section	12,895 (15.2)	118 (12.2)
Education*		
≤12 years	30,240 (35.7)	326 (33.8)
13-15 years	34,597 (40.9)	392 (40.6)
≥ 16 years	19,405 (22.9)	243 (25.2)
Smoking during pregnancy*		
Never	76,920 (90.9)	905 (93.8)
Sometimes	1,426 (1.7)	12 (1.2)
Daily	5,824 (6.9)	45 (4.7)
CD*	325 (0.4)	38 (3.9)
Pre-pregnancy BMI* (kg/m^2), mean (SD)	24.1 (4.3)	24.0 (4.4)
>20	10,223 (12.1)	117 (12.1)
20-24.9	46,088 (54.5)	546 (56.6)
25-29.9	18,202 (21.5)	185 (19.2)
≥30	7,963 (9.4)	99 (10.3)
Paternal		
CD	255 (0.3)	52 (5.5)
Child		
Female sex	41,148 (48.6)	594 (61.6)
Birth weight*(g), mean (SD)	3562 (589)	3542 (574)
<2500	3,546 (4.2)	34 (3.5)
2500-3499	32,378 (38.2)	402 (41.7)
3500-4499	45,115 (53.3)	488 (50.6)
≥4500	3,582 (4.2)	41 (4.3)
Prematurity* (<37 weeks)	5,378 (6.4)	54 (5.6)

*Missing information maternal age at delivery: 219, maternal education: 389, smoking during pregnancy: 460, maternal CD: 32, pre-pregnancy BMI: 2,169, birth weight: 6, prematurity: 62
Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; CD, celiac disease; BMI, body mass index; SD, standard deviation

Appendix 3

Supplementary Table 2. Maternal intakes of EPA and DHA and supplement use by maternal CD status

	Maternal CD status	
	No (n=85,197)	**Yes (n=363)**
Intake of EPA and DHA, g/d		
Food		
Mean (SD)	0.43 (0.41)	0.43 (0.40)
Median	0.32	0.32
Supplements		
Mean (SD)	0.38 (0.56)	0.42 (0.57)
Median	0.17	0.19
Supplement use, n (%)		
No use	27,292 (32.0)	97 (26.7)
Use	57,905 (68.0)	266 (73.3)

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; CD, celiac disease; SD, standard deviation