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Vitamin A consumption in pregnancy and risk of offspring type 1 diabetes

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Summary

Background and aim: Vitamin A have been shown to influence immunity, and animal studies have shown that maternal vitamin A intake could have immunomodulatory effects that may influence offspring type 1 diabetes (T1D) development. There are few human studies that have investigated the association between vitamin A consumption and T1D. This study aimed to examine the association between maternal vitamin A intake during pregnancy and the risk of childhood T1D in the Norwegian, Mother, Father and Child Cohort study (MoBa).

Methods: This study includes 82,605 children born between 2000 and 2009. Children were followed to April 15, 2018. T1D diagnosis was obtained from the Norwegian Childhood Diabetes Registry (NCDR). Maternal vitamin A intake was estimated through a semi-quantitative food frequency questionnaire (FFQ) and divided into quintiles and as well as modeled as a continuous exposure measure (per 100 retinol equivalents, RE/day). Hazard ratios (HR) were estimated using Cox proportional hazard regression, with adjustment for the maternal factors: T1D, pre-pregnant body mass index, smoking, vitamin D intake, education level, and age at delivery. Further analysis was performed to examine if vitamin A from supplements and food sources separately, as well as vitamin A intake below or above the Nordic Nutrition Recommendations (NNRs), was associated with risk of offspring T1D.

Results: During a mean follow-up time of 12.2 years, 345 children developed T1D. The mean intake was 1,665 RE/day. Compared to the mid-quantile, the lowest fifth of total vitamin A intake (HR=1.17, 95% Cl: 0.83, 1.67) and highest fifth (HR=1.11, 95% Cl: 0.78, 1.59) was not statistically significant associated with the risk of offspring T1D. Total vitamin A was not log-linearly associated with T1D (HR per 100 RE/day: 1.00, 95% Cl: 0.99, 1.01). Intake below (HR=1.17, 95% Cl: 0.84, 1.62) or above (HR=1.11, 95% Cl: 0.73, 1.68) the NNRs daily recommended intake (DRI) was not associated with offspring T1D.

Conclusion: Results in this large prospective cohort suggests no overall association between vitamin A consumption in pregnancy and the risk of offspring T1D.

Sammendrag

Bakgrunn og formål: Vitamin A har innvirkning på immunsystemet og dyrestudier har vist at mors vitamin A-inntak kan ha immunmodulerende effekter som kan påvirke utvikling av type 1 diabetes (T1D) hos barnet. Det er få studier på mennesker har undersøkt om vitamin A -inntak er assosiert med T1D. Formålet med denne studien var å undersøke sammenhengen mellom mors vitamin A inntak under svangerskapet og risikoen for T1D hos barnet i Den norske mor, far og barn-undersøkelsen (MoBa).

Metode: 82,605 barn født mellom 2000 og 2009 ble inkludert. Barn ble fulgt til 15. april 2018. T1D diagnose ble hentet fra Nasjonalt medisinsk kvalitetsregister for barne- og ungdomsdiabetes. Mors vitamin A-inntak ble estimert med et semi-kvantitativt matfrekvensspørreskjema (FFQ) og delt inn i kvintiler, samt modellert som en kontinuerlig eksponeringsvariabel (per 100 retinol ekvivalenter, RE/day). Hazard ratio (HR) ble estimert ved bruk av Cox proporsjonal hazard regresjon, med justering for de maternelle faktorene: T1D, kroppsmasseindeks før graviditeten, røyking, vitamin D inntak, utdanningsnivå og alder ved fødsel. Videre analyser ble utført for å undersøke om mors vitamin A-inntak fra tilskudd og mat separat, eller om vitamin A inntak over eller under de nordiske næringsstoffanbefalinger (NNR) var assosiert med T1D hos barnet.

Resultater: I løpet av oppfølgingstiden på 12.2 år, utviklet 345 barn T1D. Gjennomsnittlig inntak var 1,665 RE/dag. Sammenlignet med den midterste kvintil, var det ingen signifikant assosiasjon mellom totalt vitamin A inntak i den laveste kvintil (HR=1.17, 95% Cl: 0.83, 1.67) og den høyeste kvintil (HR=1.11, 95% Cl: 0.78, 1.59) og risiko for T1D hos barnet. Det var ingen signifikant assosiasjon mellom totalt vitamin A inntak (HR per 100 RE/dag 1.00, 95% Cl: 0.99, 1.01) og T1D. Inntak under (HR=1.17, 95% Cl: 0.84, 1.62) eller over (HR=1.11, 95% Cl: 0.73, 1.68) NNR sitt anbefalte daglige inntak (ADI) var ikke assosiert med T1D hos barnet.

Konklusjon: Resultatet i denne store prospektive kohortstudien viser at vitamin A inntak i svangerskapet ikke er assosiert med risiko for T1D hos barnet.

Abbreviations

BMI: Body mass index

DKA: Diabetic ketoacidosis

DP-BB rat: T1D-prone BioBreeding rat

FFQ: Food Frequency Questionnaire

HLA: Human leucocyte antigen

HR: Hazard ratio

MBRN: Medical birth Registry of Norway

MoBa: Norwegian acronym for the Norwegian Mother, Father and Child Cohort Study (Den norske mor, far og barn-undersøkelsen)

NCDR: Norwegian Childhood Diabetes Registry

NIEHS: Norwegian Ministry of Education and Research

NIH: Norwegian Ministry of Health and Care Services

NIPH: Norwegian Institute of Public Health

NOD mouse: Non-Obese Diabetic Mouse

NPR: Norwegian Patient Registry

PAGE: Prediction of Autoimmune diabetes and celiac disease in childhood by Genes and perinatal Environment

RA: Retinoic Acid

RE: Retinol equivalents

STATA: Software for Statistics and Data Science

UL: Tolerable upper intake level

VAD: Vitamin A Deficiency

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1 Background

1.1 Type 1 diabetes

Type 1 diabetes (T1D) is a severe, chronic, autoimmune disease that results from destruction of the insulin producing beta cells in the pancreas (1-3). Pancreatic destruction of beta cells leads eventually to absolute insulin deficiency. Insulin secretion failure results in dangerously high blood sugar levels that need to be controlled, and a lifetime requirement for insulin injections for those affected. Historically, T1D was predominantly considered a disease of children and adolescent, but today it is known that T1D can occur at any age. Age at symptomatic onset is no longer a restricting factor (4), although the incidence of T1D is highest during childhood and adolescence and is the most common metabolic disorder among this group (5). The age of onset is typically 6 months to 25 years. The rate of beta cell destruction is variable and usually proceeds rapidly in infants and children and slowly in adults.

People with T1D are at risk of both acute and chronic complications (3, 6, 7). Without the discovery of insulin over hundred years ago, and treatment with daily insulin injections, T1D would still be a deadly disease (1, 8). Although T1D mortality has declined dramatically over the past decades, there is still a greater relative risk of death compared to the non-diabetic population, particularly in people with late diabetes complications such as cardiovascular disease, nephropathy, retinopathy, and neuropathy (9, 10). According to intensive research efforts over the past century T1D is caused by a complex interaction between genetic predisposition, the immune system, and many possible environmental factors (11-13). It is presently not possible to prevent T1D because the etiology is still unclear and there is also no known cure for T1D (1, 2, 14). The treatment is demanding and has a considerable impact on daily life. Although technologies have developed at an accelerated rate in the last three decades to assist T1D patients in managing lifelong insulin therapy, there is still a long way to go in terms of optimizing diabetes management (7, 15, 16).

1.1.1 Incidence

The incidence of T1D is rising across all age groups worldwide, although there are wide variations in disease incidence between countries (7, 12). The World Health Organization (WHO) estimated that there were 9 million people with T1D in 2017; the majority of T1D

individuals live in high-income countries (17). In Norway, the prevalence of diagnosed T1D at all ages in 2020 was estimated to 23,100 (0.43%) (18).

Since T1D is most common to be diagnosed during childhood and puberty (10, 19, 20), most epidemiologic studies have concentrated extensively on this age group (20). Finland, Sweden, and Norway are high incidence countries (19, 20). Nationwide epidemiological studies in these three countries covering children with T1D aged 0 to 14 years revealed incidences of 62.5, 43.9, and 32.7 per 100,000 person-years, respectively (21-23). During approximately 1970-2010s, there was a doubling of the T1D incidence in high incidence countries. Following 2010, it was reported that countries with low incidences had modest increase, and countries with high incidences tend to have a modest increase or even a stabilization in T1D incidence (20). The reasons for the shifting incidence remain unknown (10, 19). Furthermore, within-country variability and marked ethnic variation in T1D incidence has also been documented in studies (19, 20). For example, in Chile (24) and New Zealand (25), higher incidences were detected for children of European origin compared with non-Europeans. According to data from the EURODIAB study, the incidence of T1D in Europe declines from north to south and from west to east (26). In China (27) and Germany (28), such a north-south and west-east decreasing trend of incidence has been observed. In contrast, regional variations measured between 2004-2012 reported that Aust-Agder, one of Norway's southernmost counties, had the highest incidence, while Finnmark, Norway's northernmost county, had the lowest (23). Moreover, seasonal differences in T1D diagnosis have also been documented in various studies. In both the northern and southern hemispheres, there is a peak in the number of cases diagnosed in the fall and winter, and a lesser proportion of cases diagnosed in the spring and summer (19, 26, 29). It is thought that viral or other periodic variables have a role in the timing of the T1D onset. Because diverse approaches, data covering relatively short periods of time, and a small number of cases were used, the results of the seasonal variation analysis are not always comparable (19).

The global average rate of increase in the incidence has been 3-4% every year over the last few decades (2). Finland, Sardinia, and Sweden have the highest incidences, followed by Kuwait, some other northern European countries, Saudi Arabia, Algeria, Australia, New Zealand, the United States, and Canada. East and South-East Asia have the lowest incidences (10, 20). Furthermore, there is a steady increase in the incidence rate with age up to around

10-15 years (19). Data from Finland has indicated an increase in 0- to 4-year-olds that is approximately as high as that in 10- to 14-year-olds (30). Most studies show that the incidence rate is lower in 15- to 29-year-olds compared to 0- to 14-year-olds (29, 31-33). There is also suggested a second rise in incidence after the age of 25-30 years (19, 34, 35). In addition, there is suggested an influence of puberty because the peak in incidence rate occurs slightly earlier in girls than in boys (19). Overall, there is a slightly male excess in high incidence countries, while in low incidence countries there is a female excess among children (19, 20). In Norway, recently updates from the Norwegian Childhood Diabetes Registry (NCDR) revealed that T1D accounts for 98% of all diabetes cases in children aged 0-17 years (36). In 2020, the Norwegian incidence of T1D in children aged 0-14.9 years was 48.8 per 100,000 person-years in males and 38.7 per 100,000 person-years in females (36).

1.1.2 History of type 1 diabetes

In 2021, the one hundredth anniversary of insulin's discovery was celebrated. Insulin's discovery was a revolution that saved thousands of lives around the world. Some argue that the discovery of insulin was one of the greatest medical breakthroughs of the twentieth century, as it was one of the first occasions that modern medical research was able to deliver lifesaving medicine (1, 8).

Since antiquity, T1D has been described, and the earliest known documentation of the disease is an Egyptian papyrus dating from around 1500 Before Christ. Thomas Willis is suggested as the first to describe the sweet taste of the urine in T1D patients in 1679. Earlier Indian medical documents, however, stated that ants had a special interest in the urine of T1D patients (1). Further, an important milestone was Paul Lagerhans's discovery in 1869 of the hormone-producing cells in the pancreas, now known as the islet of Langerhans (37). Next, in 1889 Oskar Minkowski and Joseph von Mering removed the pancreas from dogs and reproduced the diabetic state. They hypothesized that this was due to the loss of an important substance synthesized and secreted by the pancreas (8, 38). At the beginning of the twentieth century, an American pathologist, showed a pathological connection between a diabetic state and damage to the islet of Langerhans in the pancreas. This led to the idea that specific cells in the pancreas produced a substance that is essential for normal metabolism (39).

The most significant therapeutic event in T1D history was the discovery of exogenous insulin replacement by Dr. Fredrick Banting and Charles Best in 1921 (1, 3, 8). They injected the extract from pancreas into a diabetic dog without insulin producing beta cells and saw that she restored her health. Their research demonstrated that beta cells in the pancreas are the only cells in the body that make insulin, which is required for optimal metabolism and survival. As a result of Banting and Best's medical discovery, pancreatic extract was synthesized from the pancreas of animals and administered in the treatment of T1D patients. Leonard Thompson, a 14 year old boy, was the first human to receive treatment with synthetic insulin derived from animal cells, and it saved his life (8). Norway obtained insulin in 1923 and used it to treat the first patients. Even though T1D no longer was considered a deadly disease due to lifesaving insulin treatment, the lives spared by insulin remained vulnerable. Early insulin was primitive, difficult to create, painful to inject, and frequently caused allergic responses. Acute hypoglycemia owing to a lack of frequent injections was a limitation (1). However, technologies have advanced at a rapid pace in the last three decades, with the goal of enhancing glycemic control and T1D management (15).

1.1.3 Pathophysiology

T1D results in most cases from cell-mediated autoimmune destruction of beta cells in the islet of Langerhans within the pancreas. Autoimmune diseases occur when the natural immune system in the body fails to distinguish between its own cells and foreign cells and starts attacking healthy cells. This attack leads to insulitis lesions, meaning inflammation of the islets of Langerhans and selective loss of beta cells (19, 40). Biomarkers of beta cell immune destruction include islet autoantibodies. These autoantibodies are those reactive to insulin, glutamic acid decarboxylase, insulinoma-associated autoantigen 2, and zinc transporter 8 (7, 41). In newly diagnosed T1D cases, more than 90% have one or more of the mentioned autoantibodies (42). Islet autoantibodies are hypothesized to arise once a genetically susceptible individual is exposed to a putative environmental factor that causes a breakdown of immune tolerance (19). It has been demonstrated that being genetically susceptible is insufficient to develop T1D and that unknown non-genetic factors must play a role in development (43). However, detection of islet autoantibodies in first-degree relatives or in the general population can help identify individuals with an increased risk for developing T1D (14, 42). Furthermore, autoantibodies can occur from 6 months of age and can be present moths to years before symptomatic onset and T1D diagnosis (42, 44). While most individuals

have immune-mediated T1D, a minority of individuals have an unclear pathogenesis, referred to as idiopathic T1D. A small subset of diabetes cases is monogenic, such as Maturity-Onset Diabetes of the Young (MODY) (3, 7).

According to a staging classification model, T1D develops at three stages in genetically susceptible individuals (Figure 1). Stage 1 is defined as the presence of two or more types of islet autoantibodies with normoglycemia and no symptoms. Stage 2 is presymptomatic defined as the presence of beta cell autoimmunity and inadequate insulin secretion (dysglycemia). When individuals are diagnosed in stage 3 defined as the symptomatic onset (hyperglycemia), then insulin therapy is initiated. The model is useful in framing much of the known and unknown pathophysiology in T1D development (45, 46).

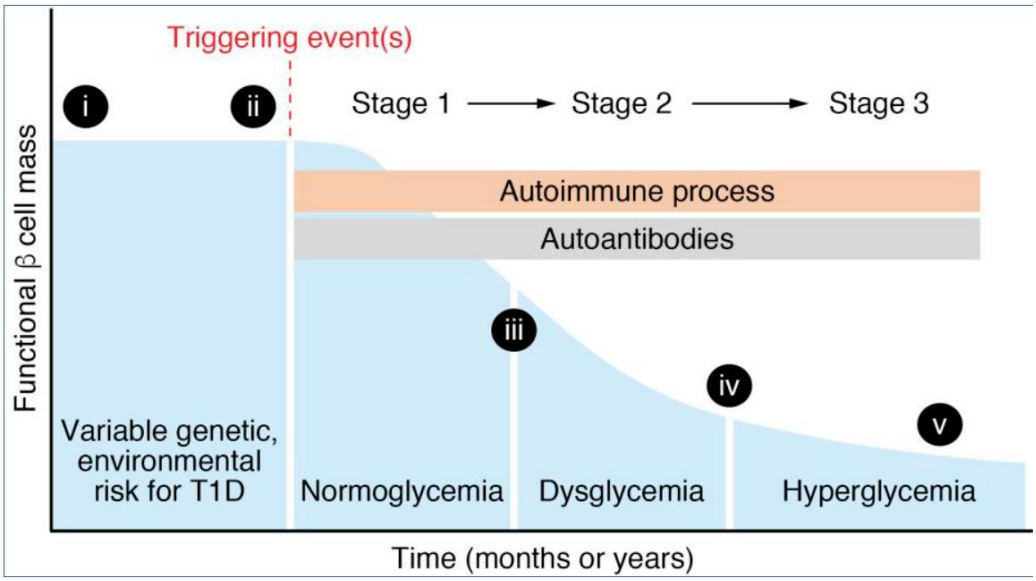

Figure 1. Staging classification model for type 1 diabetes. Original figure from Powers AC. Type 1 diabetes mellitus: much progress, many opportunities. J Clin Invest. 2021;131(8) (45). Reprinted with a CC BY 4.0 license. Copyright: American Society for Clinical Investigation. [1]

[1] The functional beta cell mass is illustrated by the blue shaded area through the stages of T1D. The x axis reflects time to T1D development that could be months or years. Roman numerals on the graph refer respectively to questions about the unknown pathogenesis which cannot yet be answered (i-v); e.g., i) What is the beta cell mass at each stage of T1D? ii) What event(s) sets off beta cell autoimmunity? iii) Do all stages have the same amount of autoimmunity? iv) Is the loss of beta cells progressive or can it be stopped? v) Why do some beta cells in T1D patients survive?

The inflammation-cell composition within the insulitis lesions consists predominantly of T-cells, macrophages, autoantibody-producing B lymphocytes, and plasma cells. It is not well defined how much loss of beta cells must occur before symptomatic onset (stage 3). Loss of approximately 90% is often associated with symptomatic onset (47). Two essential animal models of immune mediated T1D have been utilized to research the genetics, pathophysiology, and environmental impact of T1D. These animal models are the T1D-prone BioBreeding (DP-BB) rat (48) and the non-obese diabetic (NOD) mouse (49). Studies on these animal models, consistent with studies on human T1D, have demonstrated that the disease occurs by beta cell loss due to a breakdown in immune regulation and expansion of T cells and B lymphocytes (6, 50). However, biopsies from human pancreas with proven autoimmunity indicate that some have no insulitis lesions and inflammation-cell composition. The pancreatic size has also been shown to be smaller in some individuals with newly discovered T1D (51, 52). This indicates that there are several mechanisms that can lead to loss of beta cells (47, 51, 52).

1.1.4 Insulin, symptoms, and diagnosis

Insulin is the most important anabolic hormone in the body, promoting cellular uptake, storage, and synthesis of nutrients within the cell (53, 54). The hormone is synthesized in and exported from the beta cells and distributed to the rest of the body through the arterial circulation. Insulin binds to receptors on cell surfaces called glucose transporters (GLUT) and stimulates glucose uptake in body cells where glucose is converted to energy. Stimulation of glucose uptake keeps the blood glucose concentrations within a normal range (53, 54).

Insulin deficiency results in symptoms such as high blood glucose concentrations (hyperglycemia), excessive thirst (polydipsia), increased appetite (polyphagia), excessive urination volume (polyuria) and electrolyte disturbance (7, 46, 55). Weight loss is common and blurred vision from lens swelling caused by the osmotic effects of hyperglycemia may occur (46). In worst cases diabetic ketoacidosis (DKA) occur due to marked breakdown of stored fuels, presence of ketone bodies, non-esterified fatty acid, and extreme hyperglycemia and glycosuria. DKA is fatal if left untreated (7, 56).

The clinical T1D diagnosis relies primarily on two main factors: the need for exogenous insulin replacement and presence of islet autoantibodies (3, 7, 55, 57). Furthermore, the

diagnosis is made based on characteristic symptoms as well as a fasting blood glucose concentration of more than 7.0 mmol/L (126 mg/dL) or a random blood glucose concentration of more than 11.1 mmol/L (200 mg/dL). Glycated hemoglobin (HbA1c) concentration at or above 48 mmol/mol (6.5%) can also be used, although it is not commonly used as diagnostic criteria in children present with typical symptoms (57). In cases of doubt about the clinical diagnosis, measurement of autoantibodies, C-peptide, and genetic testing are used in children. C-peptide is a measurement of insulin production (36).

Diagnosis of T1D among children and adolescents is considered relatively simple compared to adults where the typical symptoms may not be present (45, 46). Misclassification of other types of diabetes is more common among adults. This emphasizes the need for additional criteria in adults such as genetic risk score, immunological markers, other autoimmune diseases, and the integration of clinical features to diagnose T1D (45). Other autoimmune diseases such as thyroid disease, adrenal insufficiency, or celiac disease is present in up to 20% of individuals with T1D and should be considered in T1D diagnosis (58).

1.1.5 Management

The primary goal for individuals with T1D is to achieve glycemic control. Exogenous insulin replacement may not always provide enough glycemic control to prevent T1D-related complications. Acute complications include hyperglycemia, whereas late complications include cardiovascular and cerebrovascular disease, nephropathy, retinopathy, and neuropathy (5, 7, 15, 59, 60). Both acute and late complications result in increased morbidity and early death in T1D patients. Mortality is especially higher in people with T1D complications such as diabetic nephropathy (10). There is a ten-times higher risk of cardiovascular events in individuals with T1D compared to the age-matched non-diabetic populations (61). However, numerous innovations and technical advances have made T1D management easier for individuals with T1D. Continuous glucose monitoring, flash glucose monitoring, insulin pens, and insulin pump therapy are at the forefront of these technical advances, and they have helped to lower the frequency of problematic hypo-and hyperglycemia (5, 15). Insulin pump therapy is the most physiological treatment since it comprises of continuous insulin administration for the individual's basal insulin requirements, as well as additional insulin corrections for elevated glucose levels and boluses at meals (5). Having affordable access to vital insulin, medications, and supplies is crucial to reducing mortality and morbidity in T1D.

Intensive training of health personnel in the field and in hospitals is required. These factors, together with good education of patients and their families, can reduce acute and long-term T1D complications (59, 62).

T1D management in children is challenging and further complicated by cognitive, behavioral, and social-emotional development. Due to heightened insulin sensitivity, hypoglycemia susceptibility, and potentially long-term neuropsychological consequences, children and adolescents have more difficulty fulfilling treatment goals (60, 62). Parental concern and stress are common reactions to the burden of T1D management in children (60). Maintaining a HbA1c concentrations below 58 mmol/mol (7.5%), monitoring blood glucose levels at least four times per day, and participating in healthy eating habits with proper vitamin and mineral consumption are the goals in T1D management in children (5, 62). It is critical that the treatment is individualized with frequent adjustments and holistic overall care so that the T1D management goals can be met (5).

1.1.6 Genetic risk factors

Having a first-degree relative with T1D increases the lifetime risk of developing the disease. The risk depends on whether it is the mother (3%), father (5%), or sibling (8%) that has the disease (12). Islet autoimmunity is markedly increased if both parents or one parent and one sibling have T1D compared with a single diagnosed family member (63). Individuals with siblings diagnosed at age 7 or earlier have a higher risk of developing T1D compared to those with siblings diagnosed in later life (64). There is a much higher concordance rate for T1D in monozygotic twins than in dizygotic twins (65). In monozygotic twins the concordance rate is suggested between 40-50%. Because monozygotic twins share all their genomic DNA, this shows that genetic predisposition is not enough to promote T1D development (19). Overall, familial T1D accounts for less than 10% of T1D cases in the general population (66).

The increased risk found in family members can be linked to shared genes as well as shared environment. Human leukocyte antigen (HLA) genotype known to be strongly associated with beta cell autoimmunity. HLA linkage to the DQA, DQB, and DRB genes creates alleles. These HLA-DQ/DR alleles can be either predisposing or protective against T1D development. Individuals with the combination of HLA classes DR3-DQ2 and DR4-DQ8 have a high risk, whereas those who carry one of the classes have moderately increased risk

of T1D (7, 12). In Scandinavia, it is suggested that about 90% of children diagnosed with T1D had one or both haplotypes (12, 67).

The frequency of HLA susceptibility genotypes may explain some of the variation in T1D incidence between countries. Genes alone are not sufficient to explain variation between countries (19). Evidence shows that the proportion of newly diagnosed T1D patients who carry both the HLA classes have decreased, whereas the proportion of those with moderate genetic risk has increased (68). It has been speculated whether increased exposure to potential risk factors or decreased exposure to protective factors have caused the development of T1D in patients with moderate genetic risk (19).

1.1.7 Non-nutritional environmental factors

Changes in the environment or lifestyle are suggested responsible for the global rise in T1D cases (13, 19, 69). Childhood obesity, birthweight, cesarean section delivery, preterm birth, preeclampsia, viral-and bacterial infections, and maternal-child interaction, are some of the many factors thought to trigger development of T1D (13, 20, 68). The beta cell stress hypothesis proposes that factors causing increased insulin demand might play a role in development of T1D in children (13). Such factors could be foods with high glycemic indexes causing glucose overload, velocity of growth and related stressors in puberty, low physical activity, and psychological stress or trauma (13, 68, 70, 71).

Excess weight gain in infancy and early childhood may lead to increased insulin resistance and could initiate beta cell destruction and T1D development (72, 73). There has been suggested a correlation between increasing weight and height in childhood and incidence of T1D (73-75). Several studies have shown an association between excess weight gain and rapid growth and earlier age at T1D diagnosis in children (76-78), whereas other studies have not found this association (79, 80). Furthermore, a systematic review and meta-analysis have indicated that high birthweight is a risk factor for T1D (81). Another meta-analysis supported this and revealed that children who are heavier at birth have a significant and consistent, but small, increase in T1D risk (82). In contrast, it has been proposed that lower birthweight may accelerate T1D diagnosis, and that low birthweight could be a potential marker of fetal exposure that reduces beta cell mass development (76).

Older maternal age at delivery has been reported as a risk factor for T1D in the child (13, 83). Preterm birth has been reported as significantly associated with increased risk of T1D in a systematic review and meta-analysis (84). A cohort study conducted in Sweden found an inverted U-shaped relation between gestational age and T1D. Children born before the 33rd week or after the 40th week had the lowest risk of T1D, whereas those born between the 33rd and 36th weeks had the highest risk, compared to those born at term. While these factors are unlikely to directly cause the development of T1D in the offspring, they may direct future research toward causal exposures (85).

Numerous studies have drawn attention to viral infections as causative agents in T1D. Several viruses have been implicated (13, 68). Infection with enteroviruses, which are transmitted via a fecal-oral route, have emerged as associated with T1D and have the strongest evidence as potential risk factors compared to other viruses (86-88). It has been detected enteroviruses in the pancreas of patients recently diagnosed with T1D (87, 89, 90). The Diabetes Virus Detection study (DiViD) was the first to examine pancreatic tissue at the diagnosis of T1D for the presence of viruses and they found enteroviruses in pancreatic islets (90). Additionally, it has been reported that those diagnosed with T1D have more frequent enteroviral immunoreactivity in the beta cells compared with healthy controls. Enteroviral immunoreactivity in the beta cells show higher risk of the cells to undergo cell death (90). Furthermore, there are studies reporting that enteroviral infections during pregnancy may play a role in offspring T1D development (91-94). Increased frequency of enterovirus infections has been observed in mothers of children with T1D compared to children without the diagnosis. It is, however, unclear if maternal enteroviral infections are triggers of offspring islet autoimmunity, promoters of T1D development, or non-specific triggering stressors (91-94). In addition to viral infections, bacterial infections and the intestinal microbiota might modulate the risk of T1D (95). It has been reported lower microbial diversity in children with beta cell autoimmunity, compared with healthy controls (95, 96). Outside the intestine, gut microbes have an impact on immunology and systemic inflammation, as well as their impact on lipid and glucose metabolism. Some environmental factors that are associated with the risk of T1D, such as cesarean section delivery and use of antibiotics, are intertwined with the microbial diversity (13).

1.1.8 Nutritional risk factors

Several types of nutritional and dietary exposures may be connected to the development T1D. There are especially nutritional factors during the fetal period, infancy and childhood that have received increased attention in the etiology of T1D (11). Maternal diet during pregnancy may influence beta cell autoimmunity and T1D development in the offspring (97). Intake of root vegetables, potato, berries, coffee, and low-fat margarines in pregnancy has been associated with a decreased risk of T1D in the offspring (97, 98). It has been reported that daily consumption of vegetables during pregnancy may prevent the infant against beta cell autoimmunity (99). In contrast, a study has observed no relationship between maternal intake of selected antioxidants, vitamins and minerals during pregnancy and the risk of islet autoantibodies in the child (100). Vitamin D is an exception. Fronczak et al. reported that exposure to vitamin D *in utero* from the maternal diet protect against beta cell autoimmunity (101). In prospective studies, maternal vitamin D intake has emerged as a possible protective factor for T1D development in the offspring, but the research findings are not conclusive (11). Whereas a Norwegian study found that high maternal vitamin D concentration during the last trimester was inversely associated with the risk of offspring T1D (102), a comparable Finnish study found no association (103). Also, vitamin D status in mid-pregnancy did not have a clinically important effect on risk of childhood T1D in a large Scandinavian study (104).

A Norwegian population-based case-control study suggested that maternal intake of the omega-3 fatty acids eicosatetraenoic acid (EPA) and docosahexaenoic acid (DHA) from cod liver oil have a protective effect against offspring T1D (105). The Finnish Type 1 Diabetes Prediction and Prevention (DIPP) study did not find any conclusive associations between maternal fatty acid intake and T1D (106). Another Norwegian study did not observe a significant association between serum omega-3 fatty acid consumption (EPA or DHA) during the final trimester and risk of offspring T1D (106). In addition, intake of different types of milk products has been investigated as a risk factor of T1D (106). Whereas consumption of cheese and fresh milk has been associated with decreased risk, consumption of sour milk products during pregnancy has been related to increased risk of offspring T1D. The observed difference in risk between milk products is thought to be related to processing changes and influence of immunological properties in the fetus (106). Furthermore, maternal consumption of processed red meat products, vegetable oils, and foods that may affect the composition of the mother's gut microbiota, could increase the risk of T1D development in the child.

Potential mechanisms could be that these foods have proinflammatory effect, creates oxidative stress reactions, and gut flora abnormalities in the mother's body which induce beta cell autoimmunity (106-110).

In infancy and childhood, several potential risk factors have been suggested, such as short duration of breastfeeding and early introduction of cow's milk (111, 112). Breastfeeding has been suggested as a possible protective factor against beta cell autoimmunity, owing to the presence of biologically active chemicals in the milk that supports the infant's immune system (113). Furthermore, several studies have found that the timing of exposing children with an increased genetic risk of T1D to a variety of foods may impact beta cell autoimmunity (11, 114, 115). Infants who were early introduced (<3 months) to cereals were associated with higher risk of T1D (115). The Diabetes Autoimmunity Study in the Young (DAISY) shows that timing of introduction of any type of cereal (gluten and non-gluten) was associated with an increased risk of pancreatic islet autoimmunity (114). Results from the DIPP study reported that children introduced to root vegetables by the age of 4 months doubled the risk of islet autoimmunity compared with later introduction. Exposure to egg before 8 months of age was also associated with increased risk of T1D (116).

1.2 Vitamin A

Vitamin A is a naturally occurring vitamin and is crucial for most types of life and plays an important role in several physiologic processes. By definition, vitamin A refers to the fat-soluble compound all-*trans*-retinol, hereafter referred to as retinol. Furthermore, vitamin A is commonly used to collectively represent substances with similar biological effects as retinol, including the retinol active metabolites retinal and retinoic acid (RA), and provitamin A carotenoids (117). Retinol is the most physiologically active form of vitamin A. In foods, vitamin A is mainly present retinol or retinyl esters from animal product or as provitamin A carotenoids from fruit and vegetables, which are converted to retinol in the body. In addition, retinoids are the collective term for all the naturally occurring forms of vitamin A, as well as all synthetic analogues of retinol with or without vitamin A activity (118-120). In this thesis, I use the term vitamin A in contexts where there is retinol activity or similar biological activity to retinol. The term retinoids are used in other contexts when both natural substances, retinol metabolites, and synthetic compounds designed as pharmacologic agents are included.

Vitamin A is fat-soluble, essential, compounds that must be obtained from the diet or supplements. Humans cannot synthesize vitamin A and is therefore dependent on external supply through food or supplemental sources. These sources are primarily derived from animal products that provide retinol or retinyl ester consumed as preformed vitamin A or converted to retinol from provitamin A carotenoids in the body. Beta-carotene is quantitatively the most important provitamin A carotenoid and one of the main vitamin A precursors from plants in the human diet (118, 119). Beta-carotene have antioxidant properties and are thought to protect against disease development in form of inhibit oxidative stress and radical attack in the body (121).

A high intake of dietary vitamin A is seen in many Western populations. Westernized diets may provide excess vitamin A from intake of animal products, fortified foods, and supplements (122). As a result, vitamin A deficiency (VAD) rarely occur in developed countries such as Norway (123). In contrast, VAD has been a public health priority in developing countries for more than six decades by the World Health Organization (WHO) (124, 125). In developing countries where VAD is widespread, it has been shown that dietary rich sources of vitamin A are deficient (126). VAD especially affects children, women of reproductive age, and pregnant women. It is estimated that nearly 20 million pregnant women are affecting by VAD (125, 127). The most common symptom of VAD is Xerophthalmia, a condition characterized by eye dryness. If left untreated, xerophthalmia causes night blindness and could eventually lead to total blindness. Another major health consequence of VAD includes reduced immune function, which leads to an increased susceptibility to infectious diseases (120, 125). Despite the recognition of VAD for decades, many elements of VAD remain unknown, including its epidemiology, classification, metabolism, and pathogenesis (120, 124).

1.2.1 Dietary vitamin A sources

Vitamin A is especially found in liver, fish, dairy fat, eggs, and cod liver oil, as well as in fruit and vegetables with strong colors. Fruit and vegetables are thought to supply around a third of the vitamin A in the human diet (119). Fatty fish, especially cod liver, is rich in vitamin A. Milk and dairy products such as cheese, butter, fermented milk, yoghurt, and cream are also good sources of vitamin A (128). The main sources to retinol in the Nordic countries are liver and liver products, edible fat, milk, and dairy products including retinol-fortified margarine

spreads. Vegetables and some fruits are the main sources to beta-carotene (129). The traditionally Norwegian diet contains relatively high amounts of vitamin A due to fatty fish, cod liver oil, cow's milk and milk products, red and processed meat, and liver pate (130, 131). The Norwegian Scientific Committee for Food Safety (VKM) reported in 2013 that the main sources to vitamin A in the diet among Norwegian adults was butter, margarine and oils, meat products such as liver pate and food supplements (132). Moreover, owing to modern processing and cleaning of high quantities of retinol in cod liver oil, vitamin A intakes in Nordic countries decreased between 2002 and 2010/2011 (129).

In contrast to the Nordic countries were fortification in staple foods is not commonly used, fortified dairy and cereal products is some of the richest sources of preformed vitamin A in the United States (120). Vitamin A fortification in staple foods such as sugar, flour, and vegetable oil are used in developing countries where VAD is a public health problem (120, 126). Furthermore, human milk supplies adequate amounts of vitamin A to the infant when maternal nutrition status is good. Poor maternal nutritional status results in milk with insufficient vitamin A concentration, which can place the infant at risk for complication (133). Breastmilk of well-nourished mothers in the Nordic countries usually contains enough vitamin A to cover the infant's needs (129, 134).

1.2.2 Physiological function of vitamin A

Vitamin A is crucial for most types of life as it is required in tissues to maintain normal cell proliferation, differentiation, and cell death (118). In addition, vitamin A is essential for visual transduction, immune function, reproduction, and embryonic development (118-120). Vitamin A modulates immune processes and is important for adaptive immunity by being involved in helper T cell and B cell development, as well as for functioning of the innate immunity (135, 136). Most of the vitamin A effects on cells are due retinoic acid (RA), a metabolite of retinol, and its ability to modulate the rate of transcription of genes and regulate expression on proteins (118, 137). By interacting to receptors in the body, RA regulates the transcription of approximately 500 genes involved in cell development and differentiation (120, 138). Furthermore, studies have reported, primarily animal studies involving BB rats and NOD mice, that retinoids are required for maintaining energy metabolism and normal endocrine activities (139). It is also observed that vitamin A are needed for normal pancreas and beta cell development *in utero* (140, 141).

1.2.3 Vitamin A metabolism

Vitamin A metabolism is specialized, involving several different chemical species (118, 142, 143). However, retinol enters the intestinal lumen mainly as retinyl ester and before intestinal absorption, retinyl ester is enzymatic converted to retinol within the intestinal lumen and absorbed in enterocytes. Then, retinol is re-esterified with long-chain fatty acids within the enterocytes to form retinyl ester. In addition, beta-carotene is absorbed in the intestinal lumen and into the enterocytes by passive diffusion. In the enterocytes, beta-carotene is enzymatic converted to retinol and then, in the same way as for retinol, re-esterified to form retinyl esters (118, 119, 142, 143). Next, retinyl esters from both enzymatic conversions is packaged into chylomicrons and secreted into the lymphatic system and transported to the liver for primary storage. Chylomicron remnants are taken up by liver cells, in which the retinyl esters are hydrolyzed into retinol. Furthermore, the liver stores, under normal dietary conditions, 70-90% of vitamin A in specific liver cells called hepatic stellate cells (119). Hepatic stellate cells have the capacity to store large quantities of vitamin A in the form of retinol, where it accumulates in lipid droplets (142). Additionally, extrahepatic tissues also play an important role in the overall metabolism and storage. A person with normal vitamin A status will have a reserve that last for several months. In contrast to water-soluble vitamins, vitamin A has a long half-life and is slowly excreted from the body (118).

The homeostatic concentrations of vitamin A in the body are maintained by mobilizing the lipid droplets (142). Bound to its specific transport protein, retinol-binding protein (RBP) synthesized in the liver, retinol is released into the circulation from the liver to surrounding tissue to meet the requirements (135, 142). The concentration of retinol bound to RBP in plasma is kept between 1 to 2 µmol/L, except in cases of VAD. Cells express several retinol- and RA-binding proteins in different tissues in the body. Retinol is oxidized to retinal and RA in the tissues, which are essential for vision and gene regulation, respectively (118, 142). RA is also considered the active form of vitamin A during embryogenesis as it serves as a ligand of retinoic acid receptors in embryonic tissues, and further regulate and expressions in genes that are important in organ development (144).

The body’s ability to absorb vitamin A varies. The bioavailability of retinol and beta-carotene can be affected by several factors, such as protein-energy malnutrition, zinc deficiency, alcohol consumption, dietary fat, infections, preparation method, and the degree of food

processing (119, 129). Retinol absorption is efficient if fat metabolism is normal and 70-90% of what we eat is absorbed in the intestine. The uptake of beta-carotene is lower, and it is estimated that people on a normal diet absorb and convert about 1/6 of beta-carotene if fat metabolism is normal (119).

1.2.4 Recommendations on dietary vitamin A intake and supplements

Retinol equivalents (RE) is used to convert all sources of preformed retinol and beta-carotene in the diet into a single unit (129). Nutritionist often quantify vitamin A intake in terms of retinol equivalents (REs): 1 RE is defined as 1 µg of retinol or 6 µg of beta-carotene. The international unit (IU) is sometimes used (1RE=3.3 IU) (117, 134). Based on differences in bioavailability the Institute of Medicine, expresses vitamin A requirements as retinol activity equivalents (RAEs) where 1 RAE is equivalent to 1µg retinol or 12µg beta-carotene (118, 129). However, there are still uncertainties in how to express vitamin A due to differences in bioavailability and bioconversion of retinol and beta-carotene, as well as the factors previously mentioned that could affect absorption of vitamin A (119, 129). In this thesis, RE is used to express the total amount of vitamin A, from retinol and/or beta-carotene, consumed during pregnancy.

Recommendations for vitamin A are based on studies that assured adequate body stores and plasma levels of retinol and where no clinical signs of deficiency, such as reduced plasma retinol, reduced dark adaption, dryness of the skin and eye discomfort, were observed (129). The Nordic Nutrition Recommendation from 2012 are the basis for the Norwegian recommendations for vitamin A (132). The NNRs are in line with the US Dietary Reference Intakes for vitamin A (145). The term recommended intake refers to the quantity of a nutrient that, based on current research, may satisfied nutritional requirements in all healthy people of a certain gender, age, life stage, or physiological state such as pregnancy or lactation (129, 132, 146). The daily recommended intake (DRI) of vitamin A according to the NNR is 900 RE/day for men and 700 RE/day for women. Pregnant and lactating women have increased daily requirements. The DRI for pregnancy is set to 800 RE/day to cover individual variation. It is common in Nordic countries to advise the use of cod liver oil especially during pregnancy when the need for nutrients has increased to some extent. The use of cod liver oil provides vitamin D, vitamin E, vitamin A and omega-3 fatty acids. The recommendation of cod liver oil is manly to prevent vitamin D deficiency (129, 132). It should be emphasized

that vitamin A supplement should not be administrated to pregnant women if there is no documented reason to assume VAD (119).

The average requirement (AR) is defined as that an intake below the AR is associated with the probability of not meeting the requirement, whereas an intake between the AR and DRI does not exclude the probability of inadequate intake (129). Experimental data to estimate an AR during pregnancy are lacking, mostly because it is not ethical to conduct experimental studies on pregnant women. However, the AR for pregnant women is estimated to 550 RE/day, based on using the retinol accumulation in fetal liver as a criterion. Hence about 50 µg vitamin A per day would be needed in addition to the AR for non-pregnant women. The lower recommended intake (LI) is defined as an intake with a high probability of not meeting the nutritional requirement. The LI is 400 RE/day (129).

The Nordic Council of Ministers (129), the Institute of Medicine (IOM) (145), and the VKM (132), consider retinol toxicity as an important issue in the Nordic countries due to the relatively high retinol intake. The NNR 2012 have established a tolerable upper intake level (UL) for vitamin A, especially retinol. Because of observed adverse effects of preformed consumption of retinol, the ULs are stated in terms of vitamin A or retinol intake (129). Adverse effects of beta-carotene supplementation have been recorded, although they are unrelated to its conversion to retinol (129, 132). The tolerable upper intake level (UL) is not recommended levels of intake but is an estimate of maximum levels of daily chronic intakes that is unlikely to pose a risk of adverse health effects in humans. The UL level are derived for the normal healthy adult population. Setting a UL for vitamin A intake is considered problematic due to the narrow margin between the DRI value and doses that may be of concern to different groups of the population. It should be emphasized that the UL should be used carefully at the individual level. Teratogenicity in women of reproductive age is recognized as the most serious adverse effect on which to establish vitamin A ULs. Liver abnormalities are regarded as a major adverse impact for establishing ULs for other groups of the population. In NNR 2012 the recommended maximum consumption of retinol supplements of 3,000µg/day for women of reproductive age was chosen as the UL for the entire adult population (118, 129). No increased risk of birth defects has been observed among pregnant women consuming retinol in doses between 800 to 3,000µg/day (129, 132). In addition, since epidemiological data indicate that the threshold for teratogenicity is higher

than 3,000µg/day of retinol, it is thought that this level offers protection against teratogenic effects (147).

1.2.5 Consequences of excess or vitamin A deficiency in pregnancy

Animal models has been valuable for studying vitamin A requirements during embryogenesis (148). These models have demonstrated that both excess or VAD can give rise to embryonic malformation and that a single dose of retinol or RA can be teratogenic if given at a specific state of early important development (129, 148). In humans, retinoid treatment of severe acne can be teratogenic and is contraindicated during pregnancy (118, 129). It has been reported that women who consumed more than 4,500µg retinol per day had a 3.5-fold higher incidence of birth defects in cranial neural crest tissue in their offspring compared to those women consuming 1,500µg or less per day (118). However, several epidemiological studies show conflicting results and suggests different doses of vitamin A supplements that could lead to birth defects. One main limitation in these studies is that they do not differentiate between the physical form of the vitamin A preparation. For instance, aqueous vitamin A emulsion is more teratogenic compared to oil-based vitamin A supplements (119, 143). Overall, many aspects of teratogenicity due to excess vitamin A consumption is unclear (142, 148, 149).

VAD is possible one of the major reasons that causes fetal growth restriction (118, 119). Additionally, it has been reported that VAD during pregnancy can cause impairment in endocrine pancreas development in rats; these findings have been proposed as a possible explanation for why VAD impacts fetal islet development (149). In addition, insufficient vitamin A status in the fetus results in abnormal development of the heart, which can lead to fetal loss, as well as abnormalities in structures from the skeletal, respiratory, urogenital, and central nervous systems (150). Moreover, direct limitation of vitamin A *in utero* nutrient levels could affect immunity in the fetus (151).

Serum retinol, serum RBP, and breast-milk retinol have been used to identify populations at risk for VAD. However, there are some limits to utilizing biomarkers to assess an individual's vitamin A status. Retinol serum concentrations, for instance, are homeostatically controlled and stay stable throughout a wide range of vitamin A intake or liver storage. Consequently, a considerable decrease in serum retinol concentration may not be apparent unless liver stores of vitamin A are critically low. In addition, factors such as infection and inflammation,

pregnancy, and other nutritional deficits affect circulating retinol concentrations. The measurement of vitamin A reserves in the liver using isotope dilution methods is regarded the most reliable and noninvasive marker of vitamin A status, however it is rather expensive and labor-intensive (120, 125).

1.2.6 Maternal-Fetal Transfer of Vitamin A

The embryo cannot synthesize vitamin A and is completely reliant on maternal circulating forms of vitamin A absorbed in the placenta and passage through the umbilical cord (144). Vitamin A is critical to ensure proper embryonic development as it supports cell division, immune function, and affects the development of organs (136). During pregnancy, maternal circulating vitamin A is delivered to the placenta, and there are several possible mechanisms in maternal-fetal transfer of vitamin A. However, the mechanism(s) remain unknown, and future research is needed to answer questions about the transfer of vitamin A to the fetus, especially at which stage in development the transfer begins and which forms of vitamin A that is transferred (144, 152). Furthermore, the placenta is a distinctive feature of mammalian embryonic development and a temporary organ that forms during pregnancy. It allows for effective exchange of gases, nutrients and embryonic wastes between the mother and fetus. These functions are fulfilled by the establishment of a blood-placenta barrier. The barrier enables the fetal blood vessels to be paired to the maternal blood network, allowing nutrients and gas to be transferred without direct contact between the maternal and fetal circulations. During embryonic development, the mammalian fetus is supported by the umbilical cord. Almost all nutrients, respiratory gases, and excretion products to pass through the umbilical cord (144).

From the maternal bloodstream, the most abundant vitamin A available to the embryo is retinol bound to RBP, retinyl esters, and beta-carotene, that are incorporated into chylomicrons and lipoproteins (144, 152, 153). The growing embryo’s availability to maternal circulating retinoids and provitamin A carotenoids (beta-carotene) is illustrated in Figure 2.

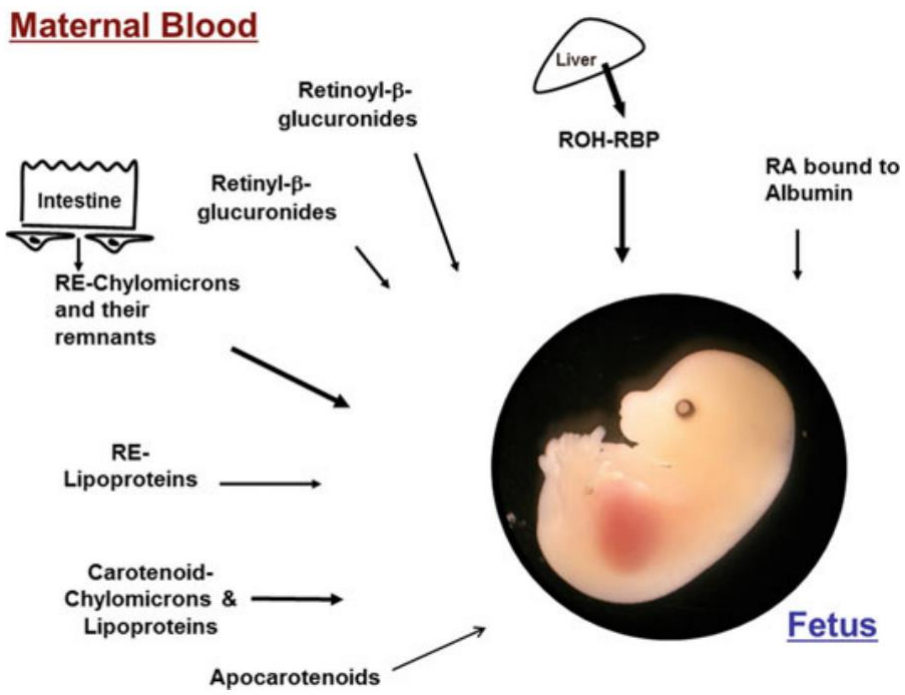

Figure 2. Circulating forms of retinoids in maternal bloodstream available for the growing embryo. Figure reprinted from Quadro et.al (2020)(144) with permission from Springer Nature.[2]

[2] The vitamin A supply of the embryo is dependent on maternal circulating retinoids. In the maternal bloodstream, two major retinoid forms can be identified: 1. Retinol bound to RBP, which is secreted into the circulation from maternal liver stores, and 2. Retinyl esters packaged in chylomicrons, which account for most circulating retinoids upon dietary vitamin A intake. At lower maternal concentrations, other forms of retinoids circulate in the bloodstream, such as beta-carotene in chylomicrons and lipoprotein particles, as well as its metabolites (apocarotenoids). ROH, retinol; RBP, retinol-binding protein; RE, retinyl ester; RA, retinoic acid.

Vitamin A and type 1 diabetes

It has been hypothesized whether vitamin A metabolism can affect beta cell autoimmunity and the onset of T1D (141, 154, 155). Most of the studies performed are on animal models; for instance, DP-BB rats or NOD mice. In humans, case-control studies have been conducted to examinate serum vitamin A levels in T1D patients compared to controls. However, these studies have been few, with small study samples, lack in consistency of exposure measurements, and there is a high probability of selection bias due to unsatisfactory selection of cases and controls (141, 155-157). It has been indicated that cohort studies with a bigger research sample are required (141, 155). Nevertheless, most of the case-control studies have demonstrated that, compared to non-diabetics, individuals with T1D have decreased serum levels of vitamin A or RBP in their circulation at the onset of beta cell autoimmunity (141, 155-157). Thereby, it has been suggested that impaired vitamin A metabolism can affect autoimmunity and T1D development (141, 156, 157).

Experimental studies of T1D in rodents have been largely in agreement with human studies (141). In DP-BB rats and induced T1D mice which spontaneously develop T1D, it has been demonstrated that serum vitamin A levels and the major storage form of vitamin A in the liver were reduced, as well as reduction in enzymes responsible for mobilization of hepatic vitamin A stores (154, 156). Early evidence showed that excess vitamin A diet did not raise serum vitamin A concentrations in DP-BB rats, indicating a failure in vitamin A metabolism rather than enhanced catabolism of vitamin A is associated with T1D (141). However, VAD diet has been observed as protective against T1D in DP-BB rats, whereas dietary vitamin A promoted T1D (158). The reasons behind these contradictory results are not known (141).

More consistent data is to be found in studies of RA, vitamin A in its more bioactive form. Evidence shows that administration of RA to NOD mice, which like DP-BB rats spontaneously develop autoimmune T1D, delayed the onset of T1D and protected against loss of beta cell mass (159). In addition, it has been reported that disruption of RA signaling impairs beta cell differentiation in and thereby leads to reduced insulin secretion in the mouse (160). *In vitro* studies, using human and mouse pancreatic islets, indicates that RA, by activating a special receptor in pancreatic cells, may play a role in stimulating and maintaining functional beta cell mass (160, 161). Furthermore, maternal VAD is suggested to impair fetal beta cell growth and development in offspring mice (149, 162). The number of

beta cells per islet were reduced in fetuses exposed to low marginal amount of retinol *in utero* compared to those fed with vitamin A sufficient diet. These findings suggests that maternal vitamin A consumption *in utero* affected offspring islet development, the beta cell function and insulin secretion in later life (162). Moreover, it has been demonstrated that RA increased the numbers tolerogenic immunosuppressive Treg cells, that act to suppress immune response which react to self-antigens and maintaining self-tolerance in NOD mice. Destruction of beta cells is largely due to defect in tolerance toward self-antigens (163).

1.2.7 Potential mechanisms: vitamin A and type 1 diabetes development

The capacity of vitamin A, specifically RA, to enhance immunotolerance have brought most attention to vitamin A in relation to T1D development. The evidence points in the direction that vitamin A plays a potential role in strengthening immune tolerance, which inhibits inflammation in the beta cells and progression to T1D (141, 163, 164). Specifically, by inducing Treg cell proliferation that suppresses the cells (T lymphocytes) that causes autoimmune reaction and attacks the beta cells in the pancreas (163). Genetically susceptible children to T1D have reduced immune tolerance to islet antigen (14). Insufficient vitamin A supply have been demonstrated to impaired fetal differentiation of B lymphocytes, cells that produces antibodies and are responsible for early phase of protection against pathogens (165). Both T and B lymphocytes can be activated against self-antigens and trigger an immunological response that leads to beta cell destruction (164). All factors mentioned above, indicates that vitamin A could have a potential impact on T1D development through affecting beta cell differentiation and/or acting on immune functions (140, 141, 155, 163, 164). It is, however, pointed out the requirements of more vigorous studies with larger cohorts to investigate whether vitamin A is associated with the T1D development (141).

Furthermore, the incidence peak in islet autoimmunity in the second year of life indicates that potential environmental factors influencing the initiation of the autoimmune T1D process are likely to operate very early in life (166). This implies that maternal exposure to maternal dietary intake *in utero* could have a role in offspring T1D development (97, 101). As mentioned earlier, red meat products (106), cod liver oil (105), milk and milk products (106), and eggs (116) has been associated with T1D. These foods items are all rich in retinol (119). It is plausible that maternal retinol intake from these foods could be the main player in these

observed associations and that it may influence development of beta cell autoimmunity *in utero* and T1D onset.

Intake of vitamin A, specifically retinol, during pregnancy has been linked to decreased alpha diversity and a larger number of bacteria from the proteobacteria family, which contains numerous pathogens and has a pro-inflammatory effect on the microbiota of the mother (167). It has been established that the microbiota of the mother influences the offspring microbiota and health outcomes (168, 169). Gut microbes have an impact on lipid and glucose metabolism, as well as immunology and systemic inflammation (169). Decreased microbial diversity is observed in children with preclinical T1D, compared to healthy controls (95, 96). It is possible that this is attributable to the mother's retinol consumption during pregnancy. This proposes another potential mechanism; that vitamin A consumption during pregnancy may modify intestinal bacteria and could influence the risk of offspring T1D acting via intestinal microbes.

2 Aim

The overall aim for this thesis was to investigate the relationship between maternal vitamin A intake and the risk of childhood type 1 diabetes in one of the world's largest birth cohorts: The Norwegian Mother, Father and Child Cohort Study (MoBa). Specifically:

i) Assess whether maternal intake of vitamin A during pregnancy is associated with the risk of T1D in the offspring.

ii) Examine if vitamin A from supplements and vitamin A from dietary sources separately is associated with the risk of offspring T1D.

iii) Assess if total maternal vitamin A intake below or above the Nordic Nutrition Recommendations (NNRs) daily recommended intake (DRI) is associated with the risk of offspring T1D.

3 Methods

3.1 The MoBa study

The MoBa study is an ongoing, prospective, long-term, population-based cohort study conducted and maintained by the Norwegian Institute of Public Health (NIPH). The objective of MoBa is to test specific aetiological hypotheses by estimating degree of the association between potential causal factors (exposures) and diseases (outcomes), aiming at prevention. MoBa is supported by the Norwegian Ministry of Health and Care Services and the Ministry of Education and Research (NIH/NIEHS). The target population was all women giving birth in Norway. MoBa started recruiting women in 1999 throughout the country (41% participation) at around week 18 of gestation when a prenatal screening is offered to all pregnant women (170, 171). Eligible individuals for participation were women who had received a referral for an ultrasound examination and had appointments registered in the Medical Birth Registry (MBR) and who were fluent in Norwegian (172). By 2009, over 100,000 pregnancies were included in the pregnancy cohort. MoBa builds on questionnaires during pregnancy and childhood, and biological material from both mother, father, and child. Participants have been connected to several health registries using the personal identification number provided to all Norwegian citizens, allowing a more complete follow-up for many diseases (171, 172).

3.2 Design and study cohort

This study has a prospective cohort design and is a sub-study of one of the projects in MoBa called Prediction of Autoimmune diabetes and celiac disease in childhood by Genes and perinatal Environment (PAGE) initiated by the NIPH. The PAGE project investigates how hereditary factors interact with environmental factors on the risk of developing celiac disease and T1D.

The study cohort in this thesis are derived from the latest updated version of the quality-assured data file, MoBa 12, completed in January 2019. Data were linked to the Medical Birth Registry of Norway (MBRN), the Norwegian Childhood Diabetes Registry (NCDR), and the Norwegian Patient Register (NPR). MoBa 12 contains a total of 115,249 children, and of these, 106,964 were live-born children between 2000 and 2009 with available follow-up data (Figure 3). This thesis utilized data from questionnaires distributed in week 18 and around

week 20 of gestation. Further, eligible children for this thesis were those with available data on the mother's nutritional intake during pregnancy from the food frequency questionnaire (FFQ) and the questionnaire covering demographic characteristics, reproductive history, and exposures during pregnancy, including smoking, alcohol, medication, and occupational and household exposures.

3.3 Exclusion criteria

Of the 106,964 live births between 2000-2009 with follow-up data, children of mothers with no questionnaire at baseline were excluded (n children=5,577) (Figure 3). Twelve cases of MODY or type 2 diabetes (n children = 12), and twins and triplets (n children=3,560), were excluded (Figure 3). Further, children of mothers with incomplete FFQ during pregnancy (n = 13,861), missing information on maternal vitamin A intake or missing information on maternal energy intake (n children =1,349), were excluded. The final study sample consisted of 82,605 children with valid data on maternal dietary intake in pregnancy from the MoBa FFQ (Figure 3).

Compared to those included in the study, total excluded children (n=32,644) were more likely to have a birthweight below 2,500gram and more likely to be born with cesarean section delivery (Appendix B Supplemental Table 1). In addition, compared to included children, excluded children were less likely to have mothers with a pre-pregnant body mass index (BMI) above 30, more likely to be daily smokers, less likely to have more than 16 years of education, less likely to have breastfed their children for 12 months or more, and more likely to have mothers who were older at delivery. There was no difference in maternal T1D (Appendix B Supplemental Table 1).

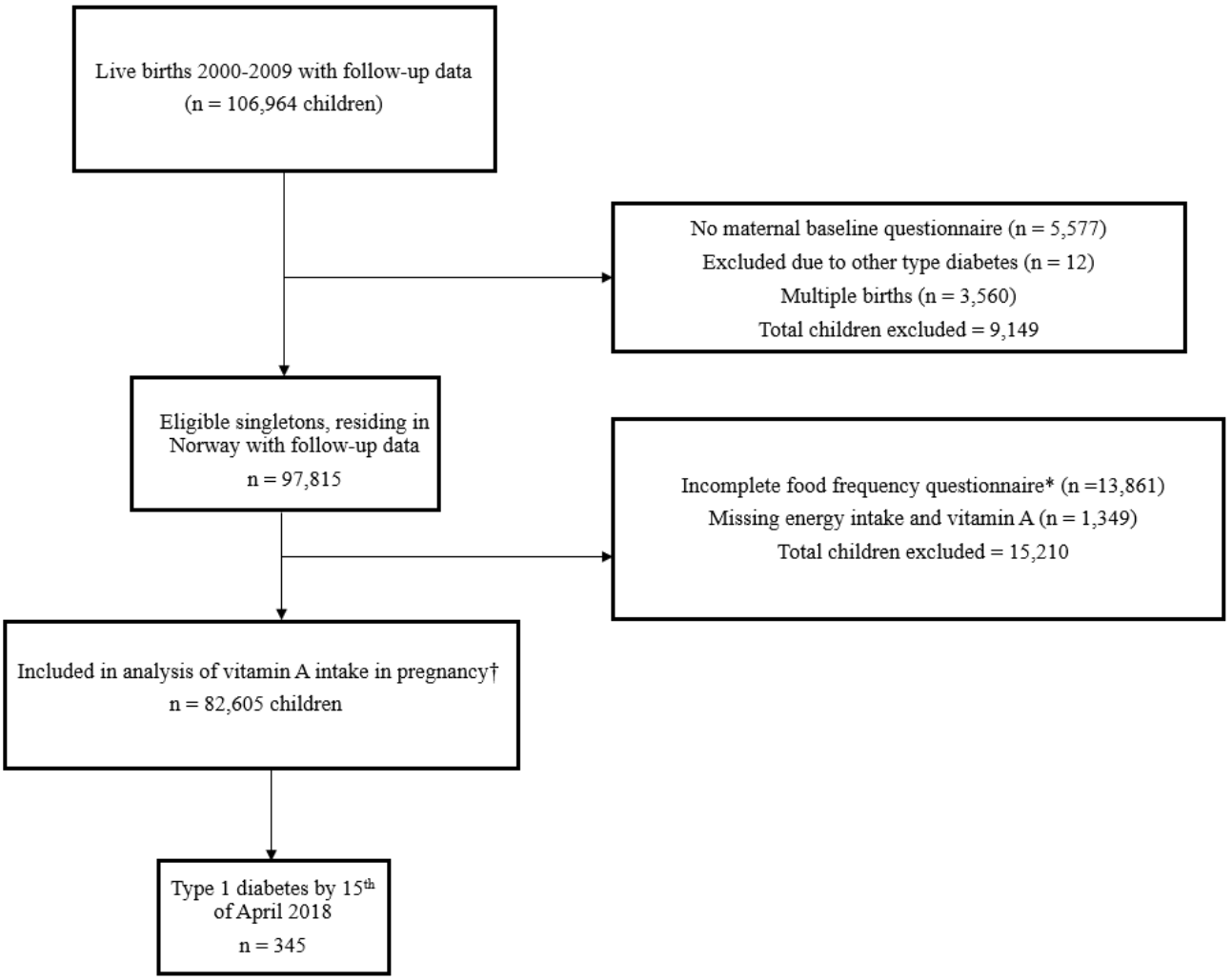

Figure 3. Flowchart of participants eligible for inclusion, exclusion criteria, and type 1 diabetes cases. †Participants included in the analysis of vitamin A intake during pregnancy. The MoBa 12 cohort 2000-2009.

3.4 Exposure

The exposure, vitamin A intake during pregnancy, was measured by the MoBa FFQ. The MoBa FFQ is a semiquantitative questionnaire that covers 255 food items and was designed to capture detailed information on dietary habits and intake of supplements since becoming pregnant and the first 4 or 5 months of pregnancy. It was sent out per mail to all participants around gestational week 22. The response rate was 92% and average time point for filling in the questionnaire was weeks 23-24 of pregnancy (168, 173). Participants filled in the mean nutritional intake since becoming pregnant and answered in total 263 questions organized into 40 groups according to the Norwegian meal pattern (174). Depending on the food item, the frequency of intake was given per day, week, and/or month. Frequency intervals on food

items ranged from never to more than eight times a day. Portion size was only given for units of fruit, slices of bread and cups/glasses of liquids. Other than fruit, bread, and liquids, all other food item frequencies were converted into food amounts (grams/day) using standard Norwegian portion sizes for women (173, 175). The FFQ has been extensively validated against a four-day weighed food dairy and with selected biomarkers (168, 176).

The last page in the FFQ contains questions about use of food supplements. Commonly used liquid supplements, followed by listed capsules and tablets, were pre-coded. In total thirteen vitamin, mineral and cod liver oil/fish oil supplements were listed, followed by open spaces where participants were asked to fill inn name and manufacturer of supplement(s) used but not listed. Liquid supplements were reported as weekly used, ranging from never, <1 and 1-7 times per week. Amount per time was given in 1 teaspoon, 1 child spoon or 1 tablespoon. Capsules/tablets were reported with the same frequencies as liquid supplements, and amounts were reported as number of capsules/tablets taken per time (174, 176).

All the questionnaires have been optically read. Daily food, energy, and nutrient intakes have previously been calculated using a food database and software system created at the University of Oslo's Department of Nutrition. For determining energy and nutritional intake, the Norwegian food composition table has been utilized (168, 176). Missing answers in the FFQ have in previous work been interpreted as not eaten and imputed with zero (177). Further, an access database for more than 1,000 different food supplements was constructed by Brantsæter et.al (168) and continuously updated and used by the MoBa team to generate a complete datafile with dietary intake. All food supplements recoded by the women and unknown supplements were registered and continuously added to the database (168, 176).

In this study, the already generated MoBa datafile on dietary sources was merged with the previously collected MoBa datafile on supplements to get a complete dataset with all the vitamin A information needed to perform the analysis. The total amount of vitamin A from both supplement and dietary sources was generated in a new variable and used as the main exposure variable in the statistical analyses. The sum of total retinol and total beta-carotene, from both diet and supplements, was expressed as REs per day by using the conversion factor: 1 RE equals to 1 µg retinol or 6 µg beta-carotene to account for differences in

bioavailability (117). In total, nine new variables on maternal vitamin A intake were generated in the datafile and used in the analyses.

3.5 Covariates and other variables

All variables were created based on data from health registers and self-reported data collected from MoBa questionnaires. Information mode of delivery, sex of the child, prematurity, birthweight, and maternal age was obtained from the MBRN. Mode of delivery was classified as caesarean section yes/no. Preterm birth, defined as born at less than 37 completed weeks of gestation, was categorized as yes/no. The child's birthweight was divided into four groups: <2,500 gram, 2,500-3,499 gram, 3,500-4,499 gram, and ≥4,500 gram. Maternal age was divided into three groups: <25 years, 25-34 years, and ≥35 years. Further, information on smoking, body mass index (BMI), and education level was collected from the recruitment questionnaire filled out by participants approximately in week 18th of gestation. Information on smoking during pregnancy was divided into three groups: never, occasionally, and daily. Maternal BMI was divided into four groups: underweight (< 20 kg/m^2), normal weight (20-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obesity (≥ 30 kg/m^2). Maternal education level was divided into three groups: ≤12 years, 13-15 years, and ≥16 years. Information regarding duration of breastfeeding was obtained from the questionnaire completed at 6 and 18 months after the women gave birth. Data on maternal T1D from the NPR was merged with the main data-file to be able to adjust for maternal T1D in the analysis. Vitamin D intake was included as a continuous variable (µg/day).

3.5.1 Identification of confounding factors

A confounding factor influences both the exposure and the outcome and is not a variable on the path between exposure and outcome (178). Potential confounding factors were selected based on previous T1D literature and research. A directed acyclic graph (DAG) was drawn to illustrate potential confounding factors (Figure 4). Factors included in the DAG were the following maternal factors: T1D, BMI before pregnancy, age, education, vitamin D intake, and smoking, and the following offspring factors: premature birth, caesarean section, sex, birthweight, and breastfeeding duration.

It is known that the risk in children born by mothers with T1D is about 3% during lifetime compared with the risk of about 0.5% in children born by mothers without T1D (12).

Individuals with T1D may have a higher or lower intake of vitamin A compared to those without T1D due to diet changes as a consequent of the disease and their need for blood sugar control (7). Thus, maternal T1D is a confounding factor.

Pre-pregnancy obesity and early pregnancy obesity, and higher maternal age is associated with increased risk of T1D in the child (13). An inverse relationship has been observed between overall dietary beta-carotene intakes and BMI, particularly in women (122). Maternal age and education level has been linked to the total vitamin A intake among women (122), and older maternal age and low parental education level has been associated with increased offspring T1D risk (70), thereby identifying BMI, age, and education level as confounding factors.

Caesarean section delivery (179), birthweight (76, 81), and preterm birth (84), have all been associated with increased risk of T1D (180). Preconceptional and periconceptional intake of supplements or dietary intake of such nutrient in accordance with the recommendations may reduce the risk of adverse outcomes such as low birthweight (168). Vitamin A status in the mother is thought to affect fetal growth and has been associated with birthweight in the offspring (181). Thus, identifying caesarean section delivery, birthweight, and preterm birth, as mediating, and not confounding factors. Further, breastmilk composition reflects maternal vitamin A intake and stores and intake in the mother may affect the duration of breastfeeding (11). Breastfeeding is suggested to have a protective effect against T1D development (115), thereby identifying breastfeeding as a mediating, and not confounding factor.

Additionally, since both vitamin D intake and smoking during pregnancy has been associated with reduced risk of offspring T1D they were included in the DAG (182). Former smokers have been observed to have a lower retinol and beta-carotene intake compared to non-smokers or current smokers (122). An earlier study based on MoBa data has suggested that maternal smoking during pregnancy has a protective effect against childhood T1D (182). Vitamin A and D both bind to Retinol Responsive Elements on the DNA, and there may be competitive binding (183). It has also been implied that there is increased sensitivity for retinol toxicity among subjects with vitamin D insufficiency (129). Thus, vitamin D intake and smoking during pregnancy were included as confounding factors.

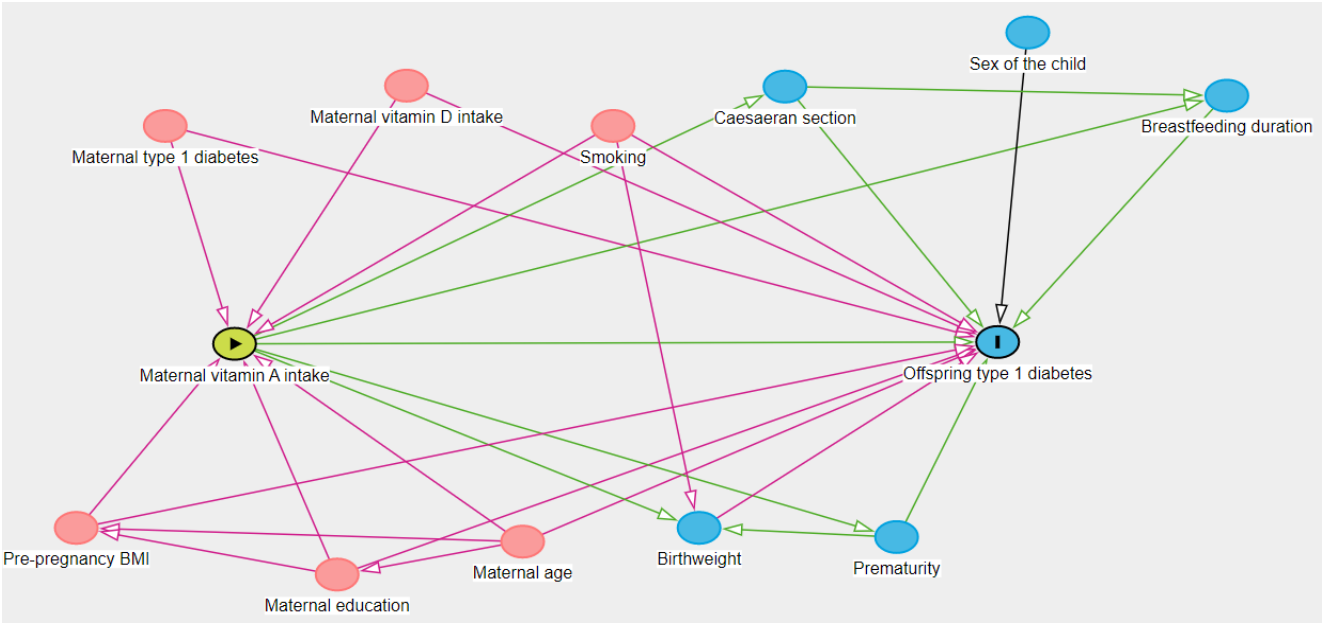

Figure 4. A directed acyclic graph (DAG) showing exposure (maternal vitamin A intake), outcome (offspring type 1 diabetes), and possible confounding variables for which adjustments are done in the analyses (red nodes). Potential mediators and predictors not related to exposure (blue nodes) were not adjusted for in the analyses. Graph was drawn with DAGitty version 2.3 created from http://dagitty.net/.

Green circle: exposure variable/ Green lines: causal path/ Red circles: adjusted variables/ Blue circles: precursor variables of outcome variable/ Blue circle with I: outcome variable.

3.6 Outcome

The outcome was the time to clinical diagnosis of T1D in the child, defined as the first day of insulin treatment in accordance with the ERODIAB criteria (19). Data on offspring T1D were obtained from the NCDR and the Norwegian Patient Registry (NPR). The NCDR contains all new cases of childhood-onset diabetes, aged 0-17.9 years, reported from all pediatric departments in Norway, with informed consent from the child after the age of 12 and the child's parents (184). Identified during follow-up, by the end of April 15th, 2018, and following the completion of the exclusion criteria, 345 children with valid data were enrolled in this study as incident T1D cases (Figure 3).

3.7 Statistical analyses

Software for Statistics and Data Science, STATA 16, was used for all the statistical analyses in the study (College Station, Tx, USA). The association between the maternal intake of vitamin A and risk of childhood T1D diagnosis was analyzed using Cox proportional hazards

analysis to estimate Hazard ratios (HRs) with 95% Confidence intervals (Cls). Predefined statistical significance set as p-values of 0.05. The follow-up time was counted from birth to T1D diagnosis or end of follow-up on April 15, 2018. Observations with missing value were excluded, thus complete case analyses were performed. The probability of T1D in the study cohort was estimated using the Kaplan-Meier method.

Total vitamin A, the sum of total retinol and beta-carotene from both foods and supplements, was expressed as RE/day. In addition, total vitamin A intake during pregnancy was divided into quintiles (Q1-Q5) to assess potential nonlinear associations with childhood T1D. Cox proportional hazards regression models estimated HRs and 95% CIs across quintiles of vitamin A intake. Quintile 3 (Q3) was set as the reference category. The overall statistical significance of vitamin A variables was tested with a Wald test. I also modeled the total vitamin A variable, as well as the vitamin A variable from foods, as a continuous exposure measure (per 100 RE/day) and adjusted for the same potential confounders. In addition, the supplement variable was modeled as a continuous exposure and estimated HR with 95% Cl per increase in 50 RE/day with the same adjustments.

To examine vitamin A from supplements and foods separately, each respective measure was classified into quintiles (Q1-Q5). The same statistical procedure was used as for total vitamin A. Furthermore, to test if there was an association between maternal vitamin A intake above or below the recommended level in the NNR during pregnancy and risk of offspring T1D the variable for total vitamin A intake was categorized into four categories according to the NNR. The first category was maternal vitamin A intake less than the lower intake level (LI) <400 RE/day, the second category was within average requirements (AR) 400 to <800RE/day, the third category was within the daily recommended intake (DRI) 800 to 3,000 RE/day, and the fourth category was above the tolerable upper intake level (UL) >3,000 RE/day. The DRI category was set as reference in the Cox proportional hazards regression model.

Sensitivity analyses were performed that included multiple births (twins and triplets) in the study cohort (n children= 85,724) (Appendix C).

3.8 Ethical consideration and data safety

The master thesis is based on previously collected data without any patient involvement in development of the research question or outcome measures. All participant in MoBa have

provided written informed consent. The PAGE Study has been approved by the Regional Committee for Medical and Health Research Ethics (REC) of South-East Norway. In collaboration with the research group in PAGE, I was registered as a project investigator and a change notification was sent to REC. After REC's approval I gained access to the data material used in this master thesis. The data was remotely accessed at NIPH's research server.

3.9 Reporting

Checklist of Strengthening the Reporting of Observational studies in Epidemiology (STROBE) is used to help in the presentation of study findings (Appendix B). The STROBE checklist consists of 22 items which are filled out to contribute to ascertain critical appraisal of the research (185). STOBE standards were developed to assist in presenting epidemiological studies and research findings, not as a validation mechanism for the performed study or as a foundation on which to conduct an observational study (186).

4 Results

The Kaplan Meier plot illustrate increasing probability of T1D with age, and that most of the children in the study cohort developed T1D before they reached 15 years of age (Figure 5). Among the 82,605 children included in the study with complete exposure data, 345 (0.42%) were diagnosed with T1D during a mean observational time of 12.2 years (range 0.68-16.0 years) (Figure 3).

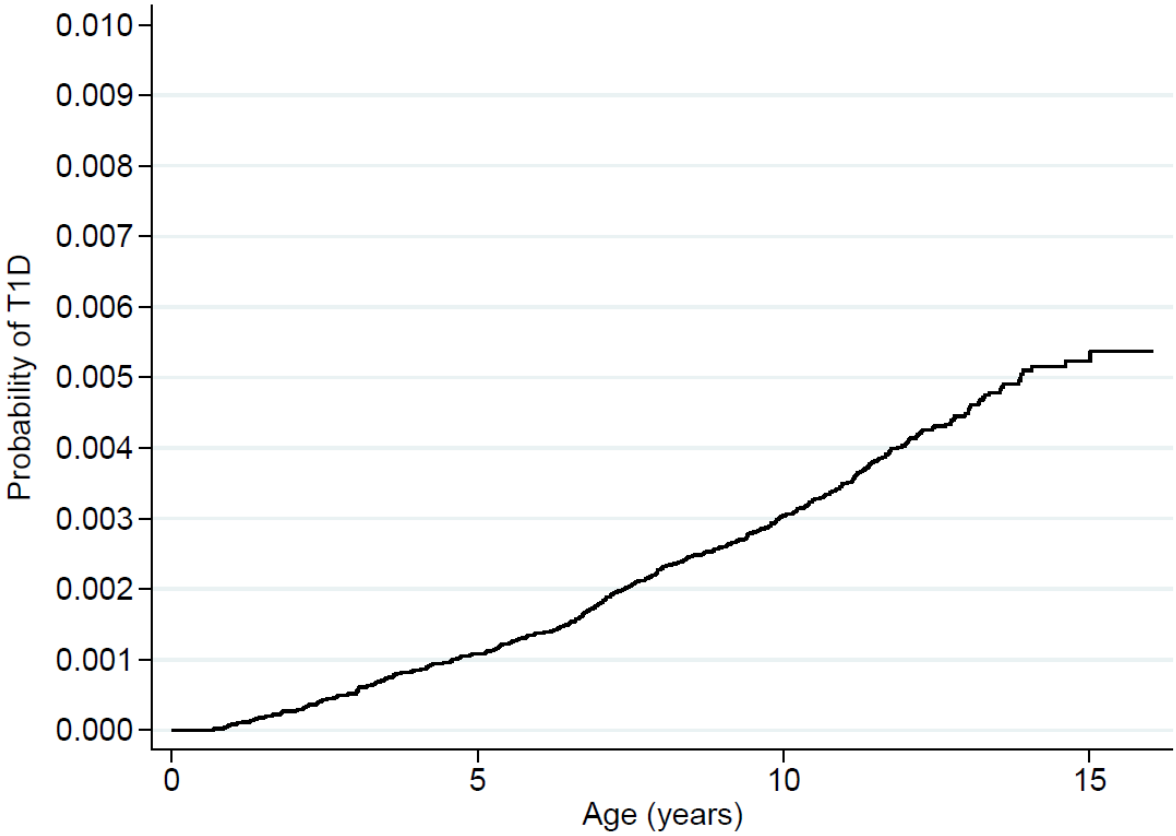

Figure 5. Probability of type 1 diabetes in the study cohort.

4.1 Characteristics of the study cohort

Of the 345 children diagnosed with T1D, 48.2% (n = 166) was boys and 51.8% (n = 179) was girls (Table 1). Most of the children weighed between 3500-4499 grams at birth. Children with T1D were more likely to be delivered through caesarean section and to be born prematurely than those without T1D. They were also more likely to have mothers who were overweight or obese during pregnancy, had T1D, lower education level, or were younger than mothers of children who did not have T1D. There were no differences in prevalence of smoking or breastfeeding duration. The mean age at delivery was 29.8 (SD 4.5) years, and mean intake of total maternal vitamin A intake was 1,666 RE/day (SD 920) (Table 1). The mean intake of retinol and beta-carotene from both foods and supplements was, respectively, 1,206 µg/day (SD 797) and 2,756 µg/day (SD 2,228). Mothers of children with T1D had a slightly higher intake of total vitamin A (RE/day) and beta-carotene (µg/day) compared to those without T1D (Table 1), although differences in vitamin A between those with T1D compared to those without were small.

Of the total study sample, 66,117 (80%) mothers had vitamin A intake within the NNRs DRI. Those with an intake below the DRI were 10,234 (12.4%) and those with an intake above the UL was 6,254 (7.6%) (Table 1). There was a slightly lower proportion of children with T1D who had mothers that consumed the recommended intake of vitamin A compared to mothers of children without T1D. There was no difference in vitamin D intake during pregnancy between the groups (Table 1).

Table 1. Characteristics of the study participants and variables included in the analyses

Variables Offspring characteristics	Participants (n = 82,605 children)	Type 1 diabetes (n = 345 children)	Not type 1 diabetes (n = 82,260 children)
Sex of the child, n (%)			
Boys	42,330 (51.2)	166 (48.2)	42,164 (51.1)
Girls	40,275 (48.8)	179 (51.8)	40,096 (48.9)
Birthweight, grams n (%)			
<2,500	2,264 (2.7)	9 (2.6)	2,255 (2.7)
2,500-3,499	31,152 (37.7)	134 (38.8)	31,018 (37.7)
3,500-4,499	45,555 (55.2)	189 (54.8)	45,366 (55.1)
≥4,500	3,628 (4.4)	13 (3.8)	3,615 (4.4)
Missing data	6	0	6
Caesarean section, n (%)			
Yes	11,652 (14.1)	65 (18.8)	11,587 (14.2)
No	70,963 (85.9)	280 (81.2)	70,673 (85.9)
Prematurity, n (%)			
Yes	3,901 (4.7)	27 (7.8)	3,874 (4.7)
No	78,648 (95.2)	317 (91.9)	78,331 (95.2)
Missing data	56	1	55
Maternal characteristics			
Pre-pregnancy BMI, n (%)			
<20	10,046 (12.2)	34 (9.9)	10,012 (12.2)
20-24.9	45,003 (54.5)	164 (47.5)	44,839 (54.5)
25-29.9	17,733 (21.5)	96 (27.8)	17.637 (22.4)
≥30	7,707 (9.3)	43 (12.5)	7,664 (9.3)
Missing data	2,116	8	2,163
Smoking			
Never	75,058 (90.9)	318 (92.2)	74,740 (90.9)
Occasionally	1,408 (1.7)	6 (1.7)	1,402 (1.7)
Daily	5,696 (6.9)	18 (5.2)	5,678 (6.9)
Missing data	443	3	440
Education			
≤12 years	29,555 (35.9)	137 (39.7)	29,418 (35.8)
13-15 years	33,721 (40.8)	143 (41.5)	33,578 (40.2)
≥16 years	18,960 (23.0)	63 (18.4)	18,897 (23.0)
Missing data	369	2	387
Breastfeeding duration			
≤6 months	10,288 (17.7)	47 (13.6)	10,241 (17.7)
6-12 months	26,888 (32.6)	110 (38.9)	26,778 (32.6)
≥12 months	23,805 (28.8)	105 (30.4)	23,700 (28.8)
Missing data	21,624	83	21,541
Age (years), mean (SD)	29.8 (4.5)	29.7 (4.5)	29.8 (4.5)
n (%)			
<25	10,105 (12.2)	39 (11.3)	10,066 (12.2)
25-34	55,091 (66.7)	236 (68.4)	54,855 (66.7)
≥35	12,572 (15.2)	47 (13.6)	12,525 (15.2)
Missing data	4,837	23	5,046
Type 1 diabetes n (%)			
Yes	327 (0.4)	13 (3.8)	314 (0.4)
No	82,278 (99.6)	332 (96.3)	81,946 (99.6)
Total vitamin A (RE/day), mean (SD)	1,665.7 (919.8)	1,678.8 (925.9)	1,665,7 (919.8)
Retinol (µg/day), mean (SD)	1,206.4 (796.6)	1,207.4 (769.2)	1,206.4 (796.7)
Beta-carotene (µg/day), mean (SD)	2,755.7 (2,228.2)	2,828.7 (2,628.4)	2,755.4 (2,226.4)
Nordic Nutrition Recommendation (NNR), n (%)			
Below (LI, AR)	10,234 (12.4)	48 (13.9)	10,186 (12.4)
Recommended (DRI)	66,117 (80.0)	270 (78.3)	65,847 (80.0)
Above (UL)	6,254 (7.6)	27 (7.8)	6,227 (7.6)
Vitamin D intake (µg/day), mean (SD)	3.5 (2.3)	3.5 (2.1)	3.5 (2.3)

Little variations in cesarean section were observed in the different quintiles of vitamin A intake but the highest proportion (14.9%) was observed in Q1 (Table 2). Also, no differences were observed in birthweight throughout the quintiles. Although there were little variations in preterm birth, children of mothers who consumed the most vitamin A (Q5) during pregnancy were slightly more likely to be delivered prematurely (Table 2). Compared to mothers within recommended intake of vitamin A during pregnancy (Q3), those with low intake (Q1) were younger, more likely to have obesity and be current smokers, more likely to have the lowest education level (Table 2). When comparing to mothers who had greater vitamin A intakes during pregnancy, mothers who had the lowest intake (Q1) were more likely to breastfeed for shorter durations of time. Mothers who consumed the highest level of vitamin A also consumed the highest levels of vitamin D in the study cohort (Table 2). The highest proportion incidence (0.45%, n=75) of offspring T1D was observed in the lowest quintile of maternal vitamin A intake (Q1), although the differences were small (Table 2 and 3).

Table 2. Distribution of characteristics according to the quintiles of total vitamin A consumption in pregnancy

Total vitamin A intake by percentile median (range) in RE/day	Total included (n = 82,605 children)	Q1 750 (150-943) (n = 16,521)	Q2 1,117 (944-1,293) (n = 16,521)	Q3 1,480 (1,294-1,683) (n=16,521)	Q4 1,923 (1684-2,258) (n=16,521)	Q5 2,785 (2,259-29,504) (n=16,521)
Offspring characteristics						
Type 1 diabetes n (%)	345 (0.4)	75 (0.5)	60 (0.4)	64 (0.4)	73 (0.4)	73 (0.4)
Cesarean section n (%)	11,652 (14.1)	2,466 (14.9)	2,233 (13.5)	2,254 (13.6)	2,265 (13.7)	2,434 (14.7)
Birthweight (g)						
<2500	2,264 (2.7)	489 (3.0)	499 (3.0)	410 (2.5)	415 (2.5)	451 (2.7)
2,500-3,499	31,152 (37.7)	6,435 (39.0)	6,261 (37.9)	6,154 (37.3)	6,173 (37.7)	6,129 (37.1)
3,500-4,499	45,555 (55.2)	8,864 (53.7)	9,095 (55.1)	9,211 (55.8)	9,182 (55.6)	9,203 (55.7)
≥4,500	3,628 (4.4)	732 (4.4)	665 (4.0)	745 (4.5)	751 (4.6)	735 (4.5)
Missing data	6	1	1	1	.	3
Prematurity	3,901 (4.7)	797 (4.8)	779 (4.7)	749 (4.5)	771 (4.7)	805 (4.9)
Missing data	56	17	12	12	6	9
Maternal characteristics						
Age (years), mean (SD)	29.8 (4.5)	29.4 (4.6)	29.8 (4.5)	30.0 (4.4)	30.1 (4.5)	29.9 (4.6)
n (%)						
<25	10,105 (12.2)	2,407 (14.6)	1,955 (11.8)	1,854 (11.2)	1,772 (10.7)	2,117 (12.8)
25-34	55,091 (66.7)	11,012 (66.7)	11,092 (67.1)	11,114 (76.3)	11,081 (67.1)	10,792 (65.3)
≥35	12,572 (15.2)	2,249 (13.6)	2,460 (14.9)	2,552 (15.5)	2,664 (16.1)	2,650 (16.0)
Missing data	4,837	856	1,014	1,001	1,004	962
Pre-pregnancy BMI, mean (SD)						
n (%)	24.1 (4.3)	24.5 (4.5)	24.1 (4.3)	24.0 (4.2)	23.9 (4.2)	23.8 (4.2)
<20	10,046 (12.2)	1,766 (10.7)	1,966 (11.9)	2,006 (12.1)	2,077 (12.6)	2,231 (13.5)
20-25	45,003 (54.5)	8,499 (51.4)	8,988 (54.4)	9,168 (55.5)	9,178 (55.5)	9,170 (55.5)
25-29.99	17,733 (21.5)	3,962 (24.0)	3,565 (21.6)	3,493 (21.1)	3,429 (20.8)	3,284 (19.9)
≥30	7.707 (9.3)	1,833 (11.1)	1,551 (9.4)	1,461 (8.8)	1,430 (8.7)	1,432 (8.7)
Missing data	2,116	461	451	393	407	404
Type 1 diabetes n (%)	327 (0.4)	54 (0.3)	67 (0.4)	58 (0.4)	63 (0.3)	85 (0.5)
Smoking						
Never	75,058 (90.9)	14,840 (89.8)	14,979 (90.7)	15,108 (91.5)	15,180 (91.9)	14,951 (90.5)
Occasionally	1,408 (1.7)	301 (1.8)	289 (1.8)	256 (1.6)	247 (1.5)	315 (1.9)
Daily	5,696 (6.9)	1,284 (7.8)	1,156 (7.0)	1,074 (6.5)	1,017 (6.2)	1,165 (7.1)
Missing data	443	96	97	83	77	90
Education n (%)						
≤12 years	30,613 (35.9)	6,777 (40.3)	6,049 (35.1)	5,816 (33.7)	5,774 (33.5)	6,197 (36.9)
13-15 years	35,044 (41.1)	6,573 (39.1)	7,058 (40.9)	7,310 (42.4)	7,221 (42.0)	6,882 (40.9)
≥16 years	19,678 (23.1)	3,479 (20.7)	4,132 (24.0)	4,122 (23.9)	4,213 (24.5)	3,732 (22.2)
Missing data	389	91	73	71	74	80
Breastfeeding duration n (%)						
≤6 months	10,288 (12.5)	2,319 (14.1)	2,095 (12.7)	1,995 (12.1)	1,853 (11.2)	2,026 (12.3)
6-12 months	26,888 (32.6)	5,533 (33.5)	5,526 (33.6)	5,360 (32.4)	5,407 (32.7)	5,062 (30.6)
≥12 months	23,805 (28.8)	4,047 (24.5)	4,715 (28.5)	4,956 (30.0)	5,105 (30.9)	4,982 (30.2)
Missing data	21,624	4,622	4,185	4,210	4,156	4,451
Vitamin D intake (µg/day)	3.5	2.5	3.2	3.5	3.9	4.6

4.2 Associations between maternal vitamin A intake and risk of offspring type 1 diabetes

4.2.1 Total vitamin A intake

Maternal vitamin A intake in the reference group, mid-quintile (Q3), was comparable to, or slightly higher than, the NNR for pregnant women of 800 RE/day, but below the UL of 3,000 RE/day. Overall, there was no statistically significant association between total vitamin A (RE/day) intake during pregnancy and the risk of offspring T1D (Wald test, p=0.57). The adjusted HR for the highest quintile (≥2,259RE/day) compared with the reference category of total vitamin A intake was 1.11 (95% Cl: 0.78, 1.59). Further, the adjusted HR for the lowest quintile (Q1) compared to the reference category (Q3) was 1.17 (95% Cl: 0.83, 1.67).

Total vitamin A was not log-linearly associated with T1D (adjusted HR per 100 RE/day: 1.00, 95% Cl: 0.99, 1.01).

Table 3. Associations between total maternal vitamin A intake during pregnancy and risk of type 1 diabetes among 82,605 children in MoBa

			Hazard ratio (95% Cl) of type 1 diabetes						
Total maternal vitamin A intake (RE/day)	Unadjusted Cases/total (n children)	Incidence proportion (%)	Unadjusted	p*	Adjusted Cases/ total (n children)	Incidence proportion (%)	Adjusted†	p*	p‡
Q1 ≤943	75/16,521	0.45	1.16 (0.83,1.61)	0.39	70/15,070	0.46	1.17 (0.83, 1.67)	0.37	0.57
Q2 944-1293	60/16,521	0.36	0.94 (0.66, 1.34)	0.74	41/14,942	0.27	0.88 (0.60, 1.28)	0.51	
Q3 1,294-1,683	64/16,521	0.38	Ref.		58/15,020	0.39	Ref.		
Q4 1,684-2,258	73/16,521	0.44	1.14 (0.81, 1.59)	0.45	64 /15,018	0.42	1.09 (0.77, 1.56)	0.62	
Q5 ≥2,259	73/16,521	0.44	1.13 (0.81, 1.59)	0.47	66/15,020	0.43	1.11 (0.78, 1.59)	0.56	
Per 100 (RE/day)	345/82,605	0.42	1.00 (0.91, 1.01)	0.79	309/75,070	0.41	1.00 (0.99, 1.01)	0.67	

* p-value refers to the reported Hazard ratio for the comparison between the mid-quintile and respective quintile.

†Adjusted for the following maternal factors: type 1 diabetes (yes/no), pre-pregnant body mass index, smoking (yes, sometimes, no), vitamin D intake in pregnancy (continuous, µg/day), maternal education level (<high school, high school, up to 4 years of college/university, > 4 years of college/university) and maternal age (<25, 25-34, ≥35 years).

‡Wald test 4 degrees of freedom (d.f.) to test if all regression coefficients equaled 0.

4.2.2 Vitamin A from supplements

Overall, there was show no statistically significant associations between vitamin A from supplements, with sources both from beta-carotene or retinol, and offspring T1D (Wald test p=0.51). The lowest incidence proportion of T1D was observed in the second unadjusted quintile (Q2) (0.34%, n=50). Compared to the mid-quintile (Q3), the second quintile of supplement intake of 0-150 RE/day (adjusted HR=0.88, 95% Cl: 0.59, 1.30) was not statistically significant associated with the risk of offspring T1D (Table 4).

Vitamin A from supplements was not log-linearly associated with T1D (adjusted HR per 50 RE/day: 1.00, 95% Cl: 0.99, 1.01) (Table 4).

Table 4. Associations between vitamin A from supplements and risk of offspring type 1 diabetes among 82,605 children in MoBa

			Hazard ratio (95% Cl) of type 1 diabetes						
Maternal vitamin A intake from supplements (RE/day)	Unadjusted Cases/total (n children)	Incidence proportion (%)	Unadjusted	p*	Adjusted Cases/total (n children)	Incidence proportion (%)	Adjusted †	p*	p‡
Q1 ≤0	94/20,913	0.44	1.06 (0.78, 1.44)	0.71	87/18,866	0.46	1.12 (0.80, 1.57)	0.50	0.51
Q2 0-150	50/14,533	0.34	0.91 (0.64, 1.31)	0.45	43/13,149	0.33	0.88 (0.59, 1.30)	0.52	
Q3 151-300	69/17,457	0.39	Ref.		59/15,948	0.37	Ref.		
Q4 301-643	52/13,356	0.39	1.16 (0.82, 1.62)	0.41	56/12,126	0.46	1.23 (0.85, 1.77)	0.27	
Q5 ≥644	70/16,346	0.43	1.05 (0.76, 1.47)	0.76	64/14,981	0.43	1.12 (0.78, 1.59)	0.54	
Per 50 RE/day	345/82,605	0.42	1.00 (0.99, 1.00)	0.59	309/75,070	0.41	1.00 (0.99, 1.00)	0.40	

*p-value refers to the reported Hazard ratio for the comparison between the mid-quintile and respective quintile.

† Adjusted for the following maternal factors: type 1 diabetes (yes/no), pre-pregnant body mass index, smoking (yes, sometimes, no), vitamin D intake in pregnancy (continuous, µg/day), maternal education level (<high school, high school, up to 4 years of college/university, > 4 years of college/university) and maternal age (<25, 25-34, ≥35 years).

‡ Wald test 4 d.f. to test if all regression coefficients equaled 0.

4.2.3 Vitamin A from foods

Overall, there was no statistically significant association between maternal vitamin A intake from foods, with sources both from beta-carotene and retinol, and offspring T1D (Wald test p = 0.58) (Table 5). The adjusted HR for the lowest quintile (≤716 RE/day) compared to the reference quintile of vitamin A (972-1,276) was 1.16 (95% Cl: 0.79, 1.68).

Vitamin A from foods was not log-linearly associated with T1D (adjusted HR per 100 RE/day: 1.00, 95% Cl: 0.99, 1.01) (Table 5).

Table 5. Associations between vitamin A from foods and risk of offspring type 1 diabetes among 82,605 children in MoBa

			Hazard ratio (95% Cl) of type 1 diabetes						
Vitamin A from diet (RE/day)	Cases/total (n children)	Incidence proportion (%)	Unadjusted	p*	Cases/ total (n children)	Incidence proportion (%)	Adjusted	p*	p‡
Q1 ≤716	68/16,521	0.41	1.19 (0.83, 1.69)	0.33	61/15,115	0.40	1.16 (0.79, 1.68)	0.45	0.58
Q2 717-971	76/16,521	0.46	1.34 (0.95, 1.88)	0.10	69/15,038	0.46	1.34 (0.93, 1.91)	0.12	
Q3 972-1,276	57/16,521	0.34	Ref.		52/15,000	0.35	Ref.		
Q4 1,277-1,743	68/16,521	0.41	1.19 (0.84, 1.69)	0.33	60/14,946	0.40	1.16 (0.80, 1.68)	0.45	
Q5 ≥1,744	76/16,521	0.46	1.31 (0.93, 1.85)	0.12	67/14,976	0.45	1.26 (0.88, 1.83)	0.21	
Per 100 RE/day	345/82,605	0.42	0.99 (0.98, 1.01)	0.93	309/75,070	0.41	1.00 (0.98, 1.01)	0.92	

* p-value refers to the reported Hazard ratio for the comparison between the mid-quintile and respective quintile.

†Adjusted for the following maternal factors: type 1 diabetes (yes/no), pre-pregnant body mass index, smoking (yes, sometimes, no), vitamin D intake in pregnancy (continuous, µg/day), maternal education level (<high school, high school, up to 4 years of college/university, > 4 years of college/university) and maternal age (<25, 25-34, ≥35 years).

‡Wald test 4 d.f. to test if all regressions coefficient equaled 0.

4.2.4 Vitamin A intake in accordance with the Nordic Nutrition Recommendations

Intake below or above recommended level for vitamin A according to NNR in pregnancy was not statistically significant associated with offspring T1D (Wald test p = 0.79). In either of the NNR categories, no association between maternal vitamin A intake and risk of offspring T1D was detected (Table 6). The adjusted HR for the LI category (<400 RE/day) compared with the DRI category (800-3,000 RE/day) was 0.92 (95% Cl: 0.23, 3.70). The adjusted HR for the UL category (>3,000 RE/day) compared with the DRI category was 1.11 (95% Cl: 0.73, 1.68). Note that in the LI group there were only two children (Table 6).

Table 6. Associations between vitamin A according to Nordic Nutrition Recommendation in pregnancy and the risk of offspring type 1 diabetes among 82,605 children in MoBa

			Hazard ratio (95% Cl) of type 1 diabetes*						
Nordic Nutrition Recommendation (RE/day)	Unadjusted Cases/total (n children)	Incidence proportion (%)	Unadjusted	p*	Adjusted Cases/total (n children)	Incidence proportion (%)	Adjusted†	p*	p‡
LI <400	2/569	0.35	0.81 (0.20, 3.28)	0.76	2/523	0.38	0.92 (0.23, 3.70)	0.90	0.79
AR 400-<800	46/9,617	0.48	1.15 (0.84, 1.57)	0.39	42/8,796	0.48	1.17 (0.84, 1.62)	0.37	
DRI 800-3,000	270/65,847	0.41	Ref.		239/60,048	0.40	Ref.		
UL >3,000	27/6,227	0.43	1.05 (0.70, 1.56)	0.82	26/5,703	0.46	1.11 (0.73, 1.68)	0.61	

AR, average requirement; DRI, daily recommended intake; LI, lower intake level; UL, tolerable upper intake level.

* p-value refers to the reported Hazard ratio for the comparison between the mid-quintile and respective quintile.

†Adjusted for the following maternal factors: type 1 diabetes (yes/no), pre-pregnant body mass index, smoking (yes, sometimes, no), vitamin D intake in pregnancy (continuous, µg/day), maternal education level (<high school, high school, up to 4 years of college/university, > 4 years of college/university) and maternal age (<25, 25-34, ≥35 years).

‡Wald test 4 d.f. to test if all regressions coefficients equaled 0.

4.2.5 Sensitivity analysis: multiple births

Inclusion of multiple births (n children = 85,724) in the sensitivity analysis (Appendix C) showed similar results; overall no statistical significant associations between total vitamin A intake (Wald test, p=0.46), vitamin A intake from supplements (Wald test, p=0.65) or food sources (Wald test, p=0.70), and the risk of offspring T1D. Additionally, an intake below or above the NNRs DRI (800-3,000 RE/day) during pregnancy was overall not associated with T1D after accounting for multiple births (Wald test, p=0.72) (Appendix C).

5 Discussion

In this thesis, I have assessed consumption of maternal vitamin A intake during pregnancy and the risk of offspring T1D in MoBa, a prospective, long-term, nationwide population-based cohort study. In addition, I have examined if vitamin A from foods or supplements separately, or if an intake below or above the NNRs, is associated with the risk of offspring T1D. The main findings were that there is no association between any of the measures of maternal vitamin A consumption during pregnancy and the risk of offspring T1D. Nor was vitamin A intake below or above the recommended level in the NNRs during pregnancy associated with the risk of T1D in the child. Given the limited number of human studies in the field, and the fact that it is unknown whether vitamin A consumption during pregnancy is associated with the offspring's T1D risk, this study fills an important knowledge gap.

Despite the importance of vitamin A in cell differentiation, particularly during embryological development, and immune cell maintenance (118, 122), as well as the current global increase in T1D incidence among children and adolescents (19), the findings of this study show no link between maternal vitamin A intake during pregnancy and the risk of T1D development in the child.

5.1 Research in context

5.1.1 Comparison with other studies

Although I observed no association between vitamin A intake and T1D, there are data from animal research and observational studies suggesting that vitamin A may affect beta cell autoimmunity and the onset of T1D (140, 141, 154-156, 161). However, several studies on the topic of vitamin A and T1D are not comparable to my study because T1D occurred before vitamin A was measured (141, 155-157).

In line with my findings of no evidence of any association between vitamin A and T1D, a nested case-control study within the DIPP study cohort revealed no association between beta-carotene concentration and risk of beta cell autoimmunity in 108 children with HLA-conferred susceptibility compared to 216 matched control children (187). In contradiction to my findings, they did, however, discovered a modest inverse association between beta-carotene concentrations and beta cell autoimmunity when present with islet autoantibodies in genetic susceptibility children compared to controls. The researchers emphasized that their

findings need to be interpreted with caution because of the number of tests made, the lack of consistency of results and the sensitivity of results to the choice of time interval (187). Further, in contrast to my study sample they used 4,297 children with HLA-conferred susceptibility to T1D and used the present of autoantibodies as their outcome variable, as well as not including maternal vitamin A intake as an exposure (187). Therefore, the result in the case-control is not comparable to my findings. Furthermore, another cohort study using data from the DIPP study cohort, supports my findings of no association between maternal vitamin A, both from retinol or beta-carotene, intake in pregnancy and beta cell autoimmunity (100). On the other hand, their outcome was the presence of islet autoantibodies and not T1D diagnosis. Maternal vitamin A intake was also measured by an FFQ designed to assess the diet during the past month before beginning of maternity leave (100). Because of the many unknown mechanisms behind the maternal-fetal transfer of vitamin A, it is difficult to draw comparisons between which periods for estimating vitamin A consumption by the FFQ during pregnancy are best in relation to the outcome (144).

Selected metabolites in umbilical cord blood have recently been studied as potential predictors of T1D in MoBa. The research findings revealed no predictive role of 27 selected metabolites in cord blood concentrations in the development of T1D (188). These finding, also from the MoBa cohort, could indicate that vitamin A metabolites concentrations in cord blood may not have a predictive role in offspring T1D development.

In contradiction to my findings, several animal studies have found an association between vitamin A, especially RA in its more bioactive form, and beta cell autoimmunity or T1D in rodents (141, 158, 160, 162, 164). One downside of animal research is that there are many divergent animal species and strains, each with a specific metabolic route, resulting in variations in efficacy and toxicity. The findings may not be valid due to the different breed models for inducing T1D, with varying similarity to the human condition. In addition, before any comparisons can be made between animal models and humans, the quantity of vitamin A supplied used in the animal research must be translated to a comparable amount in humans. Moreover, animal studies often have small experimental groups with inadequate statistical power. In comparison to the methodologies used in this study, rodent studies have a varied length of follow-up, which may not match to illness latency in people (189).

5.1.2 Vitamin A consumption

This thesis reveals that the majority of MoBa women was consuming the appropriate amount of vitamin A throughout pregnancy. Mothers who consumed vitamin A within the lowest quintile (Q1) were more likely to be younger, have a lower education, have a higher BMI, and be current smokers (Table 2). In line with my findings, Parr et.al (170) used MoBa data in a study to examine child asthma in relation to maternal vitamin A intake and observed that high intakes (≥2,031 REs/day) were associated with older age, higher education, lower BMI, and less smoking during pregnancy (170). In contrast to this study, they measured serum retinol levels in a single nonfasting venous blood sample drawn around week 18 of gestation. Compared with the estimated vitamin A levels from the FFQ, serum concentrations of retinol varied little with vitamin A intake during pregnancy among the MoBa women, but it was as expected due to strict homeostatic control of retinol (170).

Contrary to the observation of a mean intake of total vitamin A (1,666 RE/day) and retinol (1,206 µg/day) in this thesis, the Nordic councils of ministers estimated that the mean intake of total vitamin A in the Nordic countries varied from 960 to 1,240 RE/day, and retinol from 740 to 1,100 µg/day (129). These intakes are slightly lower compared with the observed intakes among MoBa women in this study (129). VKM estimated that mean intake of total vitamin A among Norwegian women between 18-70 years of age were 1,499 RE/day, and mean intake of total retinol was 983µg/day (132). However, both NNR and VKMs estimated these intakes for the entire adult populations and all adult women, respectively, not only pregnant women which normally has a higher intake due some increased nutritional requirements in pregnancy (142).

A European study has conducted a comparative analysis of the dietary intake levels of retinol and beta-carotene among the whole adult population, with considerations of food sources, lifestyle confounders and seasonal variations, using data obtained from a cohort from ten European countries. Their findings revealed that, for women, intake of retinol ranged from 241 (Ragusa, Italy) to 1219 µg/day (Umeå, Sweden). There was observed a statistically significant geographical gradient of increased retinol intakes from Southern to Northern Europe. Intake of retinol showed no difference by season, whereas beta-carotene showed little variation by season (122). By comparison, this geographical gradient in increased retinol intake from southern to northern Europe contradicts with the observed north-south decreasing

trend in T1D incidence (26). Although it is speculations, this indicates no association between VAD and T1D and is in line with my findings of no observed association.

Additionally, data from the same European cohort, showed that vitamin A, both from beta-carotene and retinol, was one of the most often consumed supplement components in the UK general population, as well as among Swedish and Norwegian women. Vitamin A components in supplements was reported as almost as frequently used as vitamin D in European countries. Several European consumers took the same ingredient from two or more supplements daily. For fat-soluble vitamins, which accumulate in the body, this indicates potential overdosing and adverse health effects and may have teratogenic effects if taken during pregnancy. Oil-based supplements were popular in Norway, Denmark, and the United Kingdom. For example, the traditionally use of cod liver oil in Norway is considered as a part of the diet, particularly in the northern coast areas (190). This suggests that MoBa women from coastal regions may have a greater intake than participants from Norway's interior.

This thesis reveals that 12.4% of MoBa participant were consuming below the DRI recommended NNR, 80% were consuming between DRI and UL, and 7.6% were consuming above the UL during pregnancy. A Nordic population-based cohort conducted in 2019 who aimed to explore the intake of food and nutrients in pregnant women in Norway and their adherence to the NNRs, found that 90.4% of the women had a maternal vitamin A intake above 800 RE/day (130). This shows that most pregnant Norwegian women follow the recommendations and may not be at risk of VAD. By contrast, in the United States 15% of pregnant women were consuming less than 550 RE/day, even with the use of dietary supplements (191). The reported prevalence of women consuming below the recommendations was determined to be 40% (192) and 74% (193) in Indonesia, 10% in Canada (194), and 69% in Scotland (195). By comparison, this emphasize MoBa women in my study sample have a high intake compared to other countries.

It should be emphasized that the given values, NNRs, are intended for use only in assessing results from dietary surveys. Long-term intakes below LI are associated with an increased risk of VAD symptoms but there is no guarantee that deficiency symptoms will be avoided with an intake above LI. This means that NNRs values can never determine if an intake is adequate or not, but indicate the likelihood that it is (129). Although the findings in this observational

study showed no association between an intake below the LI group, it does not offer a rationale for considering revisions to the NNRs on vitamin A during pregnancy based on the offspring T1D risk.

5.2 Strengths

A major strength of this master thesis was the use of a large, population-based cohort with a well-defined study population. Another strength is the linkages to national registries with high quality data and level of ascertainment. The large sample and prospectively collected data reduces the risk of recall bias seen in case-control studies. More specifically, the exposure, vitamin A intake during pregnancy, was measured before the outcome, offspring T1D, leading to more accurate measurements and less recall bias than asking after the disease has occurred. Furthermore, the availability of detailed data that enabled to adjust for other factors improves the credibility of the findings in this thesis.

Norway has special prerequisites for effectively following up on a health survey like MoBa. In addition, a well-ordered society, individuals disclose truthful information about themselves, and there is a high level of social stability (171). Because of the generally high consumption of vitamin A from food sources as well as the widespread use of cod liver oil as a dietary supplement, MoBa data materials are beneficial for the study of high intakes of vitamin A during pregnancy. But for the same reason, the MoBa cohort may not be well suited to studying low intake (129, 170).

Another strength of this study is the accuracy of the outcome variable T1D. There is an accurate diagnosis of T1D in Norway among children. In Norway diabetes autoantibodies are analyzed in all children with childhood-onset diabetes, and any doubt about the clinical diagnosis is resolved by autoantibodies, C-peptide, and genetic testing (36). In addition, MoBa is linked to several health registries, allowing a more complete follow-up of diseases (172). Specifically, the link between the MoBa study cohort and NCDR in this study allows a more complete follow-up of childhood T1D (36).

5.3 Limitations

5.3.1 Selection bias

Potential selection bias cannot be excluded due to the initial participation rate, loss to follow-up, or selective recruitment to the MoBa cohort. The participation rate was 41% (172), and considering this, there is a concern as to what extent the results in this study are valid for the total pregnant population. Nilsen et.al (196) have evaluated potential bias due to self-selection in MoBa when comparing study participants and all women giving birth in Norway in 2000-2006 using data from the MBRN. It was found that MoBa women were older, less likely to be single, less likely to have had more than two previous deliveries, and less likely to smoke, whereas use of folic acid and multivitamin supplements was over-represented. This suggests that MoBa participant may have healthier life-style behaviors, thus leading to a high risk of healthy-user bias in the study cohort that may have implicated my results (196). Additionally, in comparison to the Danish National Birth Cohort (DNBC), MoBa women were slightly older and substantially less likely to smoke or use alcohol during pregnancy (177). Therefore, the possibility that participants characteristics could influence the generalizability of my results to some underrepresented high-risk groups cannot be overruled.

Bias due to loss to follow-up might threaten the internal validity of the exposure-outcome association and is the most often caused by an overrepresentation of participants with high education levels and socioeconomic status (197). Vejrup et al. (198) has described the characteristics of drop out in the MoBa study cohort. Their findings showed that participants who continued to respond in questionnaires were older, higher educated, less likely to smoke and had lower BMI. Through an eight-year period, there was a gradual decline in the inverse relationship between maternal smoking during pregnancy and offspring birthweight with increasing follow-up information. This indicates selection bias due to drop out. The inverse association between parental education level and BMI increased with amount of follow-up information, indicating higher effect estimates due to selection bias. The researcher concluded that users of large cohorts should be aware of selective loss to follow-up and consider imputation or weighting (198). Therefore, the results may be biased in this study due to loss to follow-up and it could be a limitation that a complete case analysis was conducted (199). However, it is difficult to know which direction loss to follow-up in MoBa potentially could have affected my results.

Furthermore, economic restrictions impacted MoBa decisions, such as the choice of Norwegian as the primary language. As a result, the chances of enrolling many non-ethnic Norwegians as participants were considered low. Consequently, the FFQ focuses on a nutritional profile that is typical of the general Norwegian population (174), which may have led to selection bias in the study cohort used in this thesis.

5.3.2 Information bias

Information bias refers to measurement errors when collecting information about exposures or outcomes (200). In this section, I discuss potential measurement errors in the exposure variable, vitamin A, based on the FFQ collection method.

The choice of utilizing semi-quantitative FFQ as a measurement method in MoBa was based on the strategy to collect as many relevant exposure and health outcomes as feasible, taking the large number of participants into account as well as covering as many aspects of the diet as possible (168, 173, 174). Other methods were considered by MoBa researchers, such as diet records and repeated 24-h recalls, but they concluded that there was no realistic alternative to a FFQ in assessing the habitual diet among pregnant MoBa women (174). For assessing diet in studies among pregnant women, FFQ have been shown to be an appropriate method (173, 201). FFQ can be self-administrated, is highly cost-effective, and requires less nutrition expertise compared to other dietary intake methods (202, 203). However, FFQ have received much scientific criticism. The main limitations are systematic errors and biases in estimates (202). Self-reported FFQ data are prone to systematic bias and methodological challenges (130). FFQ challenges respondents with rather cognitive tasks and could be stressful and time consuming (174, 202). People willing to participate in a cohort study may be more interested in health and health habits than the average person. Thus, respondents may have eating behaviors that differ from those of the general population (174). Additionally, social desirability bias can occur, which means that that study participant tends to answer questions in a way that will be viewed favorably by others and leads to overreporting of good habits and underreporting of unhealthy dietary habits (130). Moreover, there are uncertainty factors related to over- or under-estimation of consumption of foods and supplements, as well as uncertainty related to the calculated values of vitamin A in foods and supplements (174). To obtain an accurate estimate of individual vitamin A intake, repeated measurements are needed, since day-to-day variation is large (132).

The MoBa FFQ only contained information about the first 4-5 month of pregnancy and therefore dietary information were limited throughout pregnancy (174). The approach is not considered appropriate for assessing the vitamin A consumption at the individual level. It could, however, be used in epidemiological research to rank individuals along the range of consumption, separating those with low intakes from those with high intakes (173, 174, 201, 202). The semi-quantitative FFQ contains reference portion sizes, and respondents are asked how often they eat the specified portion of a specific food or beverage, or to estimate their normal portion size based on a specific measure. The inclusion of portion size questions in FFQs has been criticized because it is known to increase the responder burden as it relies on memory and the ability to estimate portion sizes (173, 202). Reporting portion size might lead to imprecise estimations and quantification of nutrient intake (202). Moreover, assessments of nutrition during pregnancy poses challenges because of the considerable intra-individual variances caused by pregnancy difficulties. Some of the challenges associated with measuring dietary habits in pregnancy are the impact on eating habits, such as nausea, vomiting, constipation, and bed rest (174).

The MoBa semi-quantitative FFQ has been validated against a 4-day weighted food diary (173). The findings revealed an overall level of agreement between the FFQ and the 4-day weighted food diary. Significant correlations were observed for all major food groups and for nutrients, including vitamin A but not for vitamin E. The average correlation coefficient between the FFQ and the food diary was 0.36 for daily intake of nutrients and 0.48 for foods. For total beta-carotene (µg) the correlation coefficient was 0.34 and for total retinol (µg) it was 0.32. On category level, 68% of the participants were classified into the same adjacent quintile (correct classification) by the two methods. There was no difference in the incidence of pregnancy nausea between the FFQ and food diary study sample (173). Additionally, another study has validated the MoBa FFQ for measuring the intake of fruit and vegetables. The results showed that the FFQ correlated with specific biomarkers on these intakes, as well as the 4-day weighted food dairy. Most participant fell into the same adjacent quintiles when classified by FFQ and biomarkers. Significant correlations between the FFQ and the food dairy were found for vegetables (r=0.34) and for fruit (r=0.39) (204). Overall, these validation studies indicates that the MoBa FFQ produced valid intake estimates and is a valid measurement to rank women according to low and high vitamin A intakes of food and nutrients among pregnant women. MoBa nutrient intake data are not absolute. Biochemical

measurements and thorough dietary assessments are required at the individual level to determine if vitamin A consumption is adequate or not (173, 204).

Furthermore, a potential information bias may be related to collecting nutritional data on vitamin A from cod liver oil. It would be useful to know if it was considered during data collection that cod liver oil was purified for high levels of vitamin A in 2002 and that vitamin A intake in the Nordic countries decreased as a result between 2002 and 2010/2011 (129). If this were not considered in the MoBa nutritional data collection, it is likely that those who responded to FFQ before 2002 had a higher intake compared to those who responded after the modern methods of cod liver purification were adopted.

5.3.3 Generalization

Given the considerations discussed above, included the self-selection bias and participant rate, it is unclear to what extent the MoBa results are applicable to the entire pregnant population (168, 172). The dietary data may not be representable for pregnant women in other countries or other ethnicities, and therefore might limit the generalizability of my findings and the implications in other setting. Moreover, many of the children in the study cohort are still young, and their follow-up time is rather short, but most children were followed to or beyond the age peak T1D incidence. Some of the children may still develop T1D in the future (205). Consequently, the findings in this study cannot be generalized to adult onset T1D.

5.3.4 Confounding

Recognizing confounding and controlling for its effects are important because confounding variables may hide an actual association or falsely reveal an apparent association between the exposure and outcome when there is none. Detailed information on maternal and offspring characteristics in this prospective cohort made it possible to consider and adjust for many potential confounders. However, the possibility of confounding by unmeasured variables cannot be ruled out (178).

Identification of potential confounding were identified *a priori* using a DAG (206). In constructing the DAG, assumptions of causation between variables were made based on existing T1D research and findings on variables that previously have been thought to influence beta cell autoimmunity or T1D. Further, since a confounding factor influences both the exposure and the disease, and because there are no established environmental etiological

factors that are obvious confounders in T1D studies, the assumptions may be wrong and could have led to misspecification in the model. However, adjusting for the confounding variables did not change the results and had little impact on risk estimates in the analysis of maternal vitamin A intake and T1D. Some participants were, however, lost during the adjustments, which in turn may have led to the small changes in the observed risk estimates.

5.4 Other limitations

It is worth noting that even my lowest intake group (Q1) in the quintile categorization of total vitamin A intake (Table 3) included individuals who meet the DRI, i.e., 800 RE per day, implying that the lowest group contains a mix of people who meet the DRI and those who do not. On the other hand, the results were complemented and supported by the NNR grouping, which also found no association between vitamin A and the risk of offspring T1D in this thesis.

Furthermore, although this study used maternal vitamin A intake during the first 4 to 5 months of pregnancy as the exposure, it is reasonable to speculate whether it is the lifelong vitamin A intake in the mother before her pregnancy that could have an impact on fetal immune system development or beta cell autoimmunity in the offspring. Furthermore, it might have been an advantage if the vitamin A intake during the entire pregnancy was measured.

In addition to T1D diagnosis as the outcome, it would be an advantage if islet autoantibodies were available in MoBa so that onset of beta cell autoimmunity could be a secondary outcome in this study. Moreover, it would have been beneficial if the children had been screened for HLA-conferred susceptibility to the disease. In the MoBa data material, we do not know which of the children that could have had a higher genetic risk for developing the disease. It may have been an advantage to exclude those with high or moderate genetic risk or to include only children with the same genetic risk.

6 Conclusions

In conclusion, maternal vitamin A consumption in pregnancy was not associated with the risk of offspring T1D in this large nationwide cohort study. Overall, there was no statistically significant association between maternal vitamin A intake either from supplements or foods or and intake below or above the recommended level in the NNRs and offspring T1D. Thus, this study suggests that maternal vitamin A intake during pregnancy is not a risk factor of offspring T1D.

6.1 Future perspectives

Although the findings in this study shown no association between vitamin A consumption during pregnancy and offspring T1D, the body of evidence suggests an association. However, there is still much we do not know, and further research on various aspects of vitamin A intake and T1D is needed (141, 155, 164). For instance, the child's own vitamin A intake during infancy or childhood should be highlighted as a potential exposure in T1D studies. Another possible approach for future research could be to study serum concentrations of vitamin A in breastmilk and risk of offspring T1D. Available serum concentrations in the milk could provide information on how much vitamin A the child is being exposed to during lactation (120, 124, 133). It is possible that exposure to vitamin A in breastmilk has an impact on the development of beta cell autoimmunity than exposure *in utero*, but this is speculations.

When investigating potential prospective dietary risk factors for the development of T1D, it is critical to avoid raising unnecessary concern among families before these factors are recognized as causal T1D risk factors. Nevertheless, the search of other aspects of the effect of *in utero* environment on the risk for islet autoimmunity and T1D should continue. Researchers may be able to determine the nature of these linkages over time in the effort to one day be able to avoid development of T1D.

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Appendix A. Checklist of Strengthening the Reporting of Observational studies in Epidemiology

	Item No	Recommendation	Page number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	III, IV
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	III, IV
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	1-23
Objectives	3	State specific objectives, including any prespecified hypotheses	III, IV, 24
Methods			
Study design	4	Present key elements of study design early in the paper	III, 25
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	25, 33
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	25-31, Fig 3
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N.A.
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Fig 4, 27-31
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	27-29
Bias	9	Describe any efforts to address potential sources of bias	31-32
Study size	10	Explain how the study size was arrived at	25-27, Fig 3
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	31-32
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	29-32
		(*b*) Describe any methods used to examine subgroups and interactions	N.A.
		(*c*) Explain how missing data were addressed	N.A
		(*d*) If applicable, explain how loss to follow-up was addressed	N.A.
		(*e*) Describe any sensitivity analyses	32
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Fig 3
		(b) Give reasons for non-participation at each stage	26-27, Fig 3
		(c) Consider use of a flow diagram	Fig 3
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	29-31, Tables 1-2,
		(b) Indicate number of participants with missing data for each variable of interest	Tables 1-2
		(c) Summarise follow-up time (eg, average and total amount)	31
Outcome data	15*	Report numbers of outcome events or summary measures over time	31, Fig 5
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	30, Figure 4, Tables 3-6
		(*b*) Report category boundaries when continuous variables were categorized	Tables 2-6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N.A.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	68-71
Discussion			
Key results	18	Summarise key results with reference to study objectives	43
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	48-52
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	43, 44
Generalisability	21	Discuss the generalisability (external validity) of the study results	51
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	N.A.

*Give information separately for exposed and unexposed groups

Appendix B. Supporting material

Supplemental Table 1 Characteristics of the included versus the excluded participants (n=32,644 children)

Variables Offspring characteristics	Included (n children= 82,605)	Excluded (n children= 32,644)
Sex of the child, n (%)		
Boys	42,330 (51.2)	16,111 (49.4)
Girls	40,275 (48.8)	15,214 (46.6)
Missing	0	1,319
Birthweight, grams n (%)		
<2,500	2,264 (2.7)	2,999 (9.2)
2,500-3,499	31,152 (37.7)	12,234 (37.5)
3,500-4,499	45,555 (55.2)	14,876 (45.6)
>4,500	3,628 (4.4)	1,325 (4.1)
Missing data	6	1,210
Caesarean section, n (%)		
Yes	11,652 (14.1)	5,666 (17.4)
No	70,963 (85.9)	25,874 (79.3)
Missing	0	1,104
Prematurity, n (%)		
Yes	3,901 (4.7)	3,884 (4.7)
No	78,648 (95.2)	27,622 (84.6)
Missing data	56	1,138
Maternal characteristics		
Pre-pregnancy BMI, n (%)		
<20	10,046 (12.2)	2,786 (8.5)
20-25	45,003 (54.5)	11,439 (35.0)
25-29.99	17,733 (21.5)	4,387 (13.4)
≥30	7,707 (9.3)	2,058 (6.3)
Missing data	2,116	11,974
Smoking		
Never	75,058 (90.9)	17,744 (54.4)
Occasionally	1,408 (1.7)	579 (1.7)
Daily	5,696 (6.9)	2,477 (7.6)
Missing data	443	11,844
Education		
<12 years	29,555 (35.9)	9,351 (28.7)
13-15 years	33,721 (40.8)	7,801 (23.9)
≥16 years	18,960 (23.0)	4,065 (12.5)
Missing data	369	11,427
Breastfeeding duration		
≤6 months	10,288 (17.7)	2,690 (8,3)
6-12 months	26,888 (32.6)	5,412 (16.6)
≥12 months	23,805 (28.8)	4,472 (13.7)
Missing data	21,624	20,070
Age (years), mean (SD)	29.8 (4.5)	29.7 (4.8)
n (%)		
<25	10,105 (12.2)	2,989 (9.2)
25-34	55,091 (66.7)	13,843 (42.4)
≥35	12,572 (15.2)	14,545 (44.6)
Missing data	4,837	1,267
Type 1 diabetes n (%)		
Yes	327 (0.4)	121 (0.4)
No	82,278 (99.6)	32,523 (99.6)

Supplemental Table 2 Total vitamin A intake during pregnancy in relation food and supplements of retinol and beta-carotene (n=82,605 children)

	Total vitamin A intake (retinol equivalents, RE[3]/day) in quintiles (Q1 to Q5)					
	Q1 (≤943)	Q2 (944-1293)	Q3 (1294-1682)	Q4 (1683-2258)	Q5 (≥2259)	Total
N	16,521	16,521	16,521	16,521	16,521	82,605
Median (interquartile range) intake						
Food retinol (µg/day)	381	567	740	945	1641	663 (13, 8,521)
Supplemental retinol (µg/day)	82	184	305	470	715	223 (0, 6,582)
Total retinol (µg/day)	463	751	1,045	1,416	2,356	1,023 (642, 1,552)
Food beta-carotene (µg/day)	1,533	2,146	2,555	3,040	3,810	2,034 (573, 9,856)
Supplemental beta-carotene (µg/day)	22	54	72	99	446	0 (0, 3085)
Total beta-carotene	1,555	2,200	2,626	3,139	4,257	2,104 (578, 10,571)
Vitamin A from food (RE/day), retinol & beta-carotene	637	925	1,166	1,452	2,276	1,114 (360, 3,830)
Vitamin A from supplements (RE/day), retinol & beta-carotene	86	193	317	487	790	250 (0, 2,050)
Total vit A (RE/day)	723	1,118	1,483	1,939	3,065	1,481 (429, 4,685)

[3] Retinol equivalents calculated using the following conversion factor: 1 ug retinol= 6 ug beta-carotene

Appendix C. Sensitivity analyses

Associations between total vitamin A intake during pregnancy and risk of offspring type 1 diabetes (n=85,724)

	Hazard ratio (95% Cl) of type 1 diabetes						
Total maternal vitamin A intake (RE/day)	T1D cases/total (n children)	Incidence proportion (%)	Unadjusted	p*	Adjusted†	P*	p‡
Q1 ≤944	79/17,145	0.50	1.11 (0.80,1.55)	0.51	1.11 (0.79,1.57)	0.52	0.46
Q2 945-1,295	60/17,145	0.35	0.86 (0.61,1.21)	0.39	0.79 (0.55,1.14)	0.21	
Q3 1,296-1,686	70/17,145	0.41	Ref.		Ref.		
Q4 1,687-2,264	72/17,145	0.42	1.02 (0.7,1.4)	0.87	0.97 (0.69,1.38)	0.89	
Q5 ≥2,265	74/17,144	0.43	1.04 (0.7,1.4)	0.77	1.02 (0.72,1.45)	0.89	

* p-value refers to the reported Hazard ratio for the comparison between the mid-quintile and respective quintile.

†Adjusted for the following maternal factors: type 1 diabetes (yes/no), pre-pregnant body mass index, smoking (yes, sometimes, no), vitamin D intake in pregnancy (continuous, µg/day), maternal education level (<high school, high school, up to 4 years of college/university, > 4 years of college/university) and maternal age (<25, 25-34, >35 years).

‡Wald test 4 degrees of freedom (d.f.) to test if all regression coefficients equaled 0.

Associations between vitamin A from supplements and risk of offspring type 1 diabetes (n=85,724)

	Hazard ratio (95% Cl) of type 1 diabetes						
Maternal vitamin A intake from supplements (RE/day)	T1D cases/total (n children)	Incidence proportion (%)	Unadjusted	p*	Adjusted†	p*	p‡
Q1 ≤0	96/21,569	0.44	1.06 (0.78,1.44)	0.71	1.10 (0.80,1.54)	0.53	0.65
Q2 0-150	54/15,050	0.36	0.91 (0.64,1.31)	0.63	0.93 (0.63,1.36)	0.71	
Q3 151-300	71/18,123	0.43	Ref.		Ref.		
Q4 301-643	64/13,868	0.46	1.16 (0.82,1.62)	0.38	1.23 (0.86,1.76)	0.25	
Q5 ≥644	70/17,114	0.40	1.02 (0.73,1.41)	0.92	1.06 (0.75,1.51)	0.71	

* p-value refers to the reported Hazard ratio for the comparison between the mid-quintile and respective quintile.

†Adjusted for the following maternal factors: type 1 diabetes (yes/no), pre-pregnant body mass index, smoking (yes, sometimes, no), vitamin D intake in pregnancy (continuous, µg/day), maternal education level (<high school, high school, up to 4 years of college/university, > 4 years of college/university) and maternal age (<25, 25-34, >35 years).

‡Wald test 4 degrees of freedom (d.f.) to test if all regression coefficients equaled 0.

Associations between vitamin A from foods and the risk of offspring type 1 diabetes (n=85,724)

			Hazard ratio (95% Cl) of type 1 diabetes				
Total maternal vitamin A intake (RE/day)	T1D cases/total (n children)	Incidence proportion (%)	Unadjusted	p*	Adjusted†	p*	p‡
Q1 ≤716	71/17,145	0.41	1.78 (0.83,1.68)	0.36	1.14 (0.79,1.64)	0.50	0.70
Q2 717-972	77/17,146	0.45	1.29 (0.92,1.80)	0.14	1.27 (0.89,1.81)	0.18	
Q3 973-1,276	60/17,144	0.35	Ref.		Ref.		
Q4 1,277-1,743	69/17,145	0.40	1.15 (0.81,1.62)	0.44	1.11 (0.77,1.59)	0.57	
Q5 ≥1,744	78/17,144	0.45	1.28 (0.92,1.79)	0.15	1.23 (0.86,1.76)	0.25	

* p-value refers to the reported Hazard ratio for the comparison between the mid-quintile and respective quintile.

†Adjusted for the following maternal factors: type 1 diabetes (yes/no), pre-pregnant body mass index, smoking (yes, sometimes, no), vitamin D intake in pregnancy (continuous, µg/day), maternal education level (<high school, high school, up to 4 years of college/university, > 4 years of college/university) and maternal age (<25, 25-34, >35 years).

‡Wald test 4 degrees of freedom (d.f.) to test if all regression coefficients equaled 0.

Associations between vitamin A according to the Nordic Nutrition Recommendation in pregnancy and the risk of offspring type 1 diabetes (n=85,724)

			Hazard ratio (95% Cl) of type 1 diabetes				
Nordic Nutrition Recommendation (RE/day)	T1D cases/total (n children)	Incidence protortion (%)	Unadjusted	p*	Adjusted†	p*	p‡
LI <400	2/597	0.33	0.79 (0.20,3.17)	0.74	0.87 (0.21,3.50)	0.84	0.72
AR 400-800	49/9,999	0.49	1.19 (0.86,1.65)	0.28	1.20 (0.86,1.68)	0.27	
DRI 800-3,000	277/68,592	0.40	Ref.		Ref.		
UL ≥3,000	27/6,536	0.41	1.01 (0.68,1.50)	0.95	1.07 (0.70,1.64)	0.74	

AR, average requirement; DRI, daily recommended intake; LI, lower intake level; UL, tolerable upper intake level.

* p-value refers to the reported Hazard ratio for the comparison between the mid-quintile and respective quintile.

†Adjusted for the following maternal factors: type 1 diabetes (yes/no), pre-pregnant body mass index, smoking (yes, sometimes, no), vitamin D intake in pregnancy (continuous, µg/day), maternal education level (<high school, high school, up to 4 years of college/university, > 4 years of college/university) and maternal age (<25, 25-34, >35 years).

‡Wald test 4 degrees of freedom (d.f.) to test if all regression coefficients equaled 0.